PREVALENCE OF MYCOTOXIGENIC FUNGI AND EFFICACY OF MULTI-SPECTRAL KERNEL SORTING AND IRRADIATION TECHNIQUES IN MANAGEMENT OF MYCOTOXIGENIC FUNGI AND MYCOTOXINS IN MAIZE

MOHAMED ASHA BAKARI

(BSc. Microbiology and Biotechnology, MSc. Microbiology, University of Nairobi)

A80/55365/2019

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN PLANT PATHOLOGY

DEPARTMENT OF PLANT SCIENCES AND CROP PROTECTION

FACULTY OF AGRICULTURE

UNIVERSITY OF NAIROBI

2023

DECLARATION AND RECOMMENDATION

This thesis is my original work and has not been submitted for award of a degree in any other University.

Mohamed Asha Bakari … ……Date…24th August 2023……

A80/55365/2019 Signature

This thesis has been submitted with our approval as university supervisors

Prof. James W. Muthomi …… Date 30/8/2023

Department of Plant Science and Crop Protection

University of Nairobi

Prof. Maina Wagacha mainawagacha Date: 24th August 2023

Department of Biology

University of Nairobi

Prof. Matthew Stasiewicz Date 24th August 2023

Department of Food Science & Human Nutrition

University of Illinois at Urbana-Champaign

Dr. Charity Mutegi Date: 24th August 2023.

Rootooba Limited

UNIVERSITY OF NAIROBI

Declaration of Originality Form

This form must be completed and signed for all works submitted to the University for examination.

Name of Student: Asha Bakari Mohamed

Registration Number: A80/55365/2019

College: Agriculture and Veterinary Sciences

Faculty/School/Institute: Faculty of Agriculture

Department: Plant Sciences and Crop Protection

Course Name: Plant Pathology

Title of the work:

Prevalence of mycotoxigenic fungi and efficacy of multi-spectral kernel sorting and irradiation techniques in management of mycotoxigenic fungi and mycotoxins in maize

DECLARATION

1. I understand what Plagiarism is and I am aware of the University's policy in this regard
2. I declare that this Thesis (Thesis, project, essay, assignment, paper, report, etc) is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work, or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.
3. I have not sought or used the services of any professional agencies to produce this work
4. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his/her own work
5. I understand that any false claim in respect of this work shall result in disciplinary action, in accordance with University Plagiarism Policy.

Signature: Asha

Date: 24th August 2023

DEDICATION

I dedicate this thesis to my late dad, mum, siblings, husband, and all farmers affected by the aflatoxin menace.

ACKNOWLEDGEMENTS

I wish to thank the International Atomic Energy Agency (IAEA) for funding this work, the University of Illinois led by Dr. Matthew Stasiewicz for guidance, support and giving me the opportunity to carry out the research in their state of art laboratories, the Texas A&M University's National Center for Electron Beam Research facility led by Dr. Suresh Pillai for letting me use their facility, the International Institute of Tropical Agriculture led by Dr. Charity Mutegi for assisting me in my field work, the University of Nairobi led by Prof. James Muthomi and Prof. Maina Wagacha for their supervision and guidance. I am also very grateful to my colleagues in Stasiewicz' s laboratory who supported me a lot in my work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX

LIST OF ACRONYMS

ACDI-VOCA:	Agricultural Cooperative Development International- Volunteers Oversees Cooperative Assistance
CGA:	Cereal Growers Association
CMA:	Cereal Millers Association
DNA:	Deoxyribonucleic Acid
EC:	European Commission
ELISA:	Enzyme linked Immunosorbent Assay
EU:	European Union
FAO:	Food and Agriculture Organization
FDA:	Food and Drug Administration
GYEP:	Glucose Yeast Extract Peptone
HPLC:	High Performance Liquid Chromatography
IARC:	International Agency for Research on Cancer
IAEA:	International Atomic Energy Agency
ICS:	Immunochromatographic Strip
IFPRI	International Food Policy Research Institute
IITA	International Institute of Tropical Agriculture
ITS	Internal Transcribed Spacer
KALRO:	Kenya Agricultural Livestock Research Organization
KEBS:	Kenya Bureau of Standards
KEPHIS:	Kenya Plant Health Inspectorate Service

LAMP: Loop Mediated Isothermal Amplification

LC: Liquid Chromatography

LCMS: Liquid Chromatography Mass Spectroscopy

MC: Moisture Content

NIR: Near Infrared

PACA: Partnership for Aflatoxin Control in Africa

PCR: Polymerase Chain Reaction

PDA: Potato Dextrose Agar

PICS: Purdue Improved Crop Storage

rRNA: Ribosomal Ribonucleic Acid

TAMU: Texas A&M University

TLC: Thin Layer Chromatography

USDA: United States Department of Agriculture

UV: Ultra-Violet

WFP: World Food Program

ABSTRACT

Maize, the main staple food in Kenya is largely affected by mycotoxin contamination mainly aflatoxin and fumonisins due to attack by toxigenic *Aspergillus* and *Fusarium* species, resulting in health and economic burdens. The aim of this study was to assess the prevalence of mycotoxigenic fungi and enhance food safety and human health by reducing mycotoxin contamination in maize using multi-spectral sorting and irradiation techniques. The study was carried out during the short rains cropping season of 2019/2020 in Eastern Kenya: Embu, Meru, Tharaka Nithi, Machakos, Makueni and Kitui Counties. Ninety-nine maize samples were collected from standing crop in farmers' fields while 97 samples were collected from farmers stores. In order to determine the effects of farmer practice on the population of fungi and mycotoxins, a structured questionnaire in a surveyCTO software was administered to the farmers during harvesting of the maize. Prior to the baseline survey, an ethical approval was obtained through the National commission of Science, Technology and Innovation. During the survey, 20 to 30 maize cobs were sampled in a zigzag manner in each farmer's field. The cobs were shelled manually by hand, mixed, and subdivided by quartering to obtain 1kg of kernels which was shipped to the Regional Mycotoxin Laboratory in Kenya Agricultural and Livestock Research Organization in Katumani for processing and shipment to the University of Illinois for microbial analysis by plate count and mycotoxin analysis using enzyme-linked immunosorbent assay method. Secondly, to determine the effect of multi-spectral sorting on reduction of fumonisin, toxigenic *Fusarium* and other microbes, maize kernels were sorted in a calibrated multi-spectral sorter and samples in the accepted and rejected streams were assayed for fumonisin levels by enzyme-linked immunosorbent assay; toxigenic *Fusarium* by qPCR; and other microbes by sequencing of ITS1F- ITS2 and V3-V5 regions for fungi and bacteria respectively. Lastly to evaluate the efficacy of E-beam irradiation in reducing *Aspergillus*, *Fusarium*, aflatoxin and fumonisins, 24 samples out of the 97 samples collected in farmers' stores and had aflatoxins greater than100 µg/kg and fumonisins greater than 1000 µg/kg were treated with E-beam irradiation dose of 5, 10 and 20 kGy. The *Aspergillus* and *Fusarium* in the sample were assayed by culture plating method on potato dextrose agar modified with antibiotics and qPCR while the aflatoxin and fumonisin levels were assayed by enzyme-linked immunosorbent assay. Both microbial populations and mycotoxin levels were compared before and after E-beam treatment. Data were analyzed by an open-source R-software. A higher proportion of the farmers in Eastern

Kenya planted improved maize varieties (45.2%); practiced intercropping (83.7%); preferred planting maize using organic manure (45.5%); tilled their fields by hand (52.6%); and after harvesting, majority of the farmers (65.6%) stored their crops on wooden racks in the house. The aflatoxin contamination levels increased with the increased use of tractors ($p= 0.03$, $r= 0.85$); a similar trend ($p= 0.02$, $r=0.87$) was also seen in use of mixed varieties (combining both improved and local varieties) with fumonisin accumulation. No relation was established between farmers' practice and fungal population. Single kernel multi-spectral sorting reduced fumonisins by an average of 88.9 % (ranged between 27.6 to 99.8% reduction) with a low median rejection rate of 1.87% (ranged 0% to 48%). The proportion of toxigenic *Fusarium* infection on the maize kernel was significantly ($p= 0.005$) lower in the accept stream (1.4%) than in the reject stream (30.1%). Similarly, there was a significant decrease ($p=0.002$) of 31% and 90 % in the total fungal and bacterial counts in accepted maize kernels respectively. E-beam irradiation at doses greater than 5 kGy reduced fungal loads (average 3.7 log CFU/g) to below limits of detection by culturing method. E-beam dose of 20 kGy caused a significant ($p = 0.03$) 6.2 ng/g reduction in aflatoxins in the maize slurry. However, the 20 kGy did not reduce fumonisin in the maize slurry. Farmers' practices influence accumulation of mycotoxins accumulation in the field. Multi-spectral sorting was effective in reducing fumonisins, toxigenic *Fusarium* and other microbes. Furthermore, E-beam irradiation was effective in reduction of preformed aflatoxins and complete elimination of microbes in maize. Since no relation was seen between farmer practices and population of fungi, there is need to investigate other factors that may affect the abundance of fungi in soil. There is also need to upscale multi-spectral sorting technique to suit large scale farmers. Lastly, emerging technologies like E-beam irradiation should be adopted in Sub-Saharan Africa and high doses should be explored to manage fumonisins.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Maize is the main staple crop in Kenya used to prepare dishes like *Ugali, Githeri,* and *Muthokoi*. Maize is important in assessing food security concerns in Kenya, although maize production rate is lower compared to consumption rate and population growth rate in Kenya (Kariuki *et alet al.*, 2020). Maize production over the last decade is estimated below 40 million bags (90kgs) annually while the demand is about 50 million bags per year (Njeru, 2019). The average annual per capita maize consumption is 80 kg (Njeru, 2022) and is mostly consumed as stiffened porridge (Khamila *et al.*, 2019).

Maize crop is susceptible to attack by toxigenic fungal species at all stages including pre-harvest and post-harvest stages, resulting in production of mycotoxins. Aflatoxins, fumonisins, ochratoxins, deoxynivalenol, zearalenone and patulin are the most implicated mycotoxins affecting human and animal health out of more than 400 known mycotoxins (Reddy *et al.*, 2010). Mycotoxins are produced as secondary waste products during growth and metabolism of the fungi. Toxigenic fungi attack and colonize maize while in the fields and remain in the crop during storage (Dura, 2022). The Food and Agriculture Organization (FAO) approximates mycotoxins contamination to affect up to 25 % of the crops globally (Reddy *et al.*, 2010). Aflatoxins are excreted by toxigenic members of *Aspergillus* section *Flavi;* These fungi grow producing green yellowish spores and dark spherical structures termed sclerotia (Varga *et al.*, 2011) and is composed of both *Aspergillus* species that are able to produce aflatoxin (toxigenic) and those that cannot produce aflatoxins (atoxigenic)(Ehrlich, 2014). Fumonisins are excreted by toxigenic *Fusarium* species which are members of the *Gibberella fujikuroi* complex which include *F. verticillioides, F. proliferatum, F. subglutinans, and F. thapsinum* which express the FUM1 gene (Bluhm *et al.*, 2004). *Fusarium* is the main causative agent for maize ear rot diseases whose symptoms appears as white to pinkish scattered tufts of molds on the maize cobs and are associated with starburst patterns on the kernels (Odjo *et al.*, 2022). Ear rots in maize has been associated with fumonisin contamination (Parsons & Munkvold, 2010).

Successful management for mycotoxins involves following an integrated approach that employs several strategies including before and after harvest measures. Pre-harvest mitigation strategies

like use of biocontrol products, practising good agricultural practices like irrigation, weeding, pest control, timely planting, and crop rotation can be employed to reduce crop stress thus managing mycotoxin contamination at field level stage. Post-harvest methods of controlling and managing mycotoxins act like last line of defence after harvest. Multi-spectral sorting is a post-harvest technique that reduces aflatoxin and fumonisins in maize kernels by use of sorting algorithms that enable the identification of visibly high risk features exhibited by infected kernels, thus this method directly improves food security (Stasiewicz *et al*., 2017; Chavez *et al*., 2023). However, the spectral sorting method is not commonly used because it requires trained personnel and material. Furthermore, the spectral sorting method requires development of algorithms for sorting that are expensive to build (Chavez *et al*., 2023).Another post-harvest technique is the use of irradiation where non-ionizing radiation such as solar, UV and microwave and ionizing radiation such as gamma radiation (Ghanem *et al*., 2008) and electronic beam (Assuncao *et al*., 2015) have also been seen to reduce toxigenic fungi and mycotoxins in food. Use of ionizing radiation in agriculture to promote food safety such as in the elimination of mycotoxins is a peaceful use of nuclear energy. Ionizing radiation may eliminate the negative effect of using chemical additives and residues (Atehnkeng *et al*., 2008) in food for preservation. E-beam irradiation is a non-thermal technique that is used to reduce food loss and improve food safety (Fan & Niemira, 2020). E-beam has been used to enhance food safety in sea food (Gautam & Venugopal, 2021); red pepper (Woldemariam *et al*., 2021); rice (Zhai *et al*., 2022); and kiwi fruit (Li *et al*., 2021). Furthermore, E-beam has been shown to be a promising technology in the simultaneous reduction of mycotoxigenic fungi and mycotoxins in food (Akhila *et al*., 2021).

1.2 Statement of the problem

Mycotoxins are a major concern in maize which is a staple for Kenya's growing population. Mitigation strategies to curb mycotoxin contamination at production levels are limited. There are currently a few innovative strategies that specifically act to reduce mycotoxins in the field. Most preventive strategies are the good agricultural practices that generally prevent plant stress which is a predisposing factor for mycotoxin contamination. However, these good agricultural practices do not guarantee elimination of mycotoxigenic fungi and mycotoxins after harvest. Farmers lack access to new technologies to specifically minimize mycotoxin contamination after harvest.

Apart from this, pre- harvest methods to prevent mycotoxins require capital which most farmers have no access to. Furthermore, Post-harvest farmer practices like drying may prevent further mycotoxin production but do not in any way destroy the already pre-formed mycotoxins. Maize is one of the cereals rich in substrates that are affected by fungi and mycotoxins (Wagacha & Muthomi, 2008). Mycotoxin contamination has led to death (Probst *et al.*, 2007) of people in Kenya after consumption of contaminated maize. During crop growth and post-harvest handling most African staples like cassava, groundnuts, sorghum, barley, and millet are susceptible to aflatoxin contamination due to infection by *Aspergillus* (Atehnkeng *et al.*, 2008). Kenya has had repeated epidemics of acute aflatoxicosis that has been associated with home grown maize (Probst *et al.*, 2011).In 2004, up to 317 cases of lethal aflatoxicosis were reported in Kenya(Probst *et al.*, 2007). Apart from causing death in humans, mycotoxins were first reported to cause death in animals (Blount, 1961). Mycotoxins also result in reduced productivity in animals (Magnoli *et al.*, 2019); increases in cases of liver cancer (Claeys *et al.*, 2020), retarded growth in children (Gong *et al.*, 2004) and adversely affect trade in rejection of maize commodities in the Kenyan market. Mycotoxin contamination results into economic losses for producers, companies and exporters that cause instability in trade (Capcarova *et al.*, 2016). The Ministry of Public Health and Sanitation in Kenya banned 2.3million bags of maize due to mycotoxin contamination (Schmidt, 2013). Kenyan Bureau of Standards (KEBS) banned more than 17 brands of locally produced Kenyan maize flour because of high levels of aflatoxins greater than10 µg/Kg (The Standard, 2020) and imported maize from Uganda which were unfit for human consumption because of high fumonisin levels (The East African, 2021). There are no proper legislations in ensuring the mycotoxin contamination is minimized at farmer level. Most surveillance is conducted for commercial end products rather than at the raw material level.

1.3 Justification

An earlier study by Njeru *et al*. (2019) showed farmer practices like the use of diammonium phosphate (DAP) at planting stage was positively associated with the accumulation of aflatoxins and fumonisins in maize. Postharvest intervention like drying, sorting and packaging technologies play an integral part in management of contamination and consequent aflatoxin production during storage (Hell and Mutegi, 2011). However, most postharvest inventions reduce mycotoxins by halting fungal growth and preventing further proliferation and toxin production, but the already formed mycotoxins are not altered in any way. There is a need to denature and eliminate the fungi and pre-formed mycotoxins in the crop originating from the field. The use of nuclear irradiation can be exploited to destroy existing fungi and denature mycotoxin molecules in the crop. The irradiation damages the DNA molecules of the microbe and chemical bonds in the mycotoxins directly by breaking the chemical bonds or indirectly by radicals arising from radiolysis of water (Lung *et al.*, 2015). The mycotoxin produced by the fungi is also reduced to a less toxic form by the irradiation energy (Wang *et al.*, 2011).

Mycotoxins are known to be heterogeneous in nature (Shephard, 2016) leading to disparity in quantification of a given batch since the toxins exist within pockets and the distribution is not normal. Current industrial methods for mycotoxin management in maize focus on the bulk level which can lead to rejection of harmless lot or acceptance of harmful lot due to heterogeneity of mycotoxin, hence this can be resolved by using single kernel spectroscopy sorting technique. A earlier study on reduction of aflatoxins and fumonisins in maize kernels showed a 98 and 60.8 % reduction respectively (Murithi, 2014). The technology uses a mathematical model relating reflectance of visual characteristics associated with mycotoxin contamination like insect damage, moldiness, brokenness to identify bad kernels. The technology selectively rejects only contaminated kernels within a batch and accepts healthy kernels therefore reducing mycotoxin contamination in a maize batch based on reflectance at nine-distinctive wavelength (470-1550 nm).

Multi-spectral sorting is useful to the farmer or aggregator as it eliminates biasness as each kernel is screened for presence of mycotoxins and eliminated unlike other methods that involve bulk mitigation. Furthermore, irradiation eliminates microbes that will eventually spoil the maize and also produce mycotoxins and levels of irradiation within recommended limits do alter the physiochemical characteristics of food neither do they cause harm to humans.

1.4 Objectives

1.4.1 Broad objective

Assessment of mycotoxin prevalence and enhance food safety and human health by reducing mycotoxin contamination using multi-spectral sorting and irradiation techniques in maize.

1.4.2 Specific objectives

i. To determine the effect of farmer practices on population of *Aspergillus* and *Fusarium* species and resulting aflatoxin and fumonisin contamination of maize
ii. To determine the effectiveness of single kernel multi-spectral sorting technique in reduction of fumonisin, toxigenic *Fusarium* and other microbes in maize
iii. To evaluate the efficacy of E-beam irradiation in reduction of *Aspergillus* section *Flavi*,*Fusarium* population and resulting aflatoxin and fumonisin in maize

1.5 Hypotheses

i. Farmer practices do not affect the population of *Aspergillus* and *Fusarium* species and resultant aflatoxin and fumonisin contamination of maize.
ii. Single kernel multi-spectral sorting technique is not effective in reduction of fumonisin, toxigenic *Fusarium* and other microbes in maize.
iii. E-beam irradiation is not effective in reducing *Aspergillus* section *Flavi*, *Fusarium* population and resulting aflatoxin and fumonisin in maize.

CHAPTER TWO: LITERATURE REVIEW

2.1 Maize production in Kenya

Almost 90% of households in Kenya plant on their farms maize for trade and subsistence use (Mbithi, 2000). It is a source of carbohydrates, fiber, vitamins, anti-oxidants and minerals (Arnarson, 2019). 75 % of maize growers are small-scale farmers growing the maize for subsistence use while 25% are large-scale farmers plant the crop for sale (Kang'ethe, 2011). Areas like Trans Nzoia, Uasin Gishu, Nakuru, Bomet, and Kakamega Counties are high potential maize production zones thusmost of the households (70 %) sell maize. Farmers also utilize the grain, leaves, stalk, tassel, and cob from maize for food and feed (FarmLINK Kenya, 2017).

Previously, annual maize production was estimated at 2.7 to 2.8 million tons (Nyoro *et al.*, 2004). Recently, an increase in maize production was documented, with the highest production realized in 2018 at 4 million tons (Njeru, 2019). Production of maize has not matched the rising demand presented by population growth (Nyoro *et al.*, 2004). Thus the imbalance between production and consumption of maize in Kenya is a policy concern issue (Masese *et al.*, 2022). Future projection shows that by 2025, Kenyans demand for maize will be 60 million bags (Njeru, 2019). There is a need to improve and limit factors that hinder maize production in order to reduce the high cost of living and ensure higher income to producers at lower production prices and consequently increase access to food. To increase maize production in Kenya, reducing the cost of production is key(Nyoro *et al.*, 2004).

2.2 Factors constraining maize production in Kenya

Maize production in Kenya is well below the consumption rate. Kenya is a maize import-dependent country that depends on its neighbours: Tanzania and Uganda for its maize deficit (FEWS NET, 2021). Kenya's neighbours have a higher maize production rate compared to Kenya because of the high cost incurred during production in Kenya (Nyoro, 2004). Furthermore, pest and diseases result in 50 - 80% losses in maize production. A majority of pest diseases in maize are due to fungal pathogens which cause 11% of maize diseases hence a reduction in the harvest (Oerke & Dehne, 2004). Other diseases caused by viruses like maize lethal necrosis has been predicted by simulation to cause up to 73% loss in maize productivity (Batchelor et al., 2020). Moreover, production of maize in Kenya is also hindered by poor soils,

unpredictable climatic patterns, and harsh environmental conditions. Small-scale farmers also lack access to inputs like fertilizers, farm and harvesting equipment and high yield grains. Furthermore, lack of efficient production, processing and storage facilities increases further post-harvest losses due to lack of capital for production (Kimeli, 2013). Size of the land owned by the farmers, quantity of planted seeds, fertilizer and pesticide used on the farm has also been documented to positively influence the maize productivity in the farm. Apart from these, other factors that affect maize productivity include: Experience of the farmer in maize planting, level of extensions service, membership in farmer groups, farmer's level of education, family size and income in the household (Mogeni, 2019). Another emerging issue affecting maize production and mycotoxin contamination is climate change (Medina *et al.*, 2017).

Maize production can be increased by tackling issues affecting farmers both at farm and government level. At farm level, early and good land preparation, timely planting, among other good agronomic practices can lead to bumper harvest (Hell & Mutegi, 2011). The government should also avail farming inputs like subsidized fertilizer to farmers to increase production of maize in the country (Simiyu, 2014). In effort increase productivity by subsidizing fertilizers due to the high cost of living affecting famers in Kenya, the government reduced the price of fertilizers recently from Kenya shilling 6500 to 3500 (Ministry of Agriculture, Livestock, Fisheries and Cooperatives, 2022). Furthermore, water harvesting techniques and irrigation technologies should be put in place especially in arid and semi-arid areas (li *et al.*, 2018).

2.3 Mycotoxins and their economic importance

Mycotoxins are naturally occurring waste products produced by filamentous moulds that affect human, animal and plant health. Mycotoxins were first associated with Turkey X-disease and toxins in peanut feed (Blount, 1961). These toxins are produced when fungi with the necessary genetic coding for the toxins colonize crops in an environment that is conducive for fungal growth characterized by high temperatures and humid conditions (Daou *et al.*, 2021). Crops in tropical and subtropical areas are mostly affected by high levels of mycotoxins contamination (Mutegi *et al.*, 2018). Climate change and Institutional challenges within the tropical and subtropical regions of Africa have also played a role in the increase in mycotoxin contamination (Ezekiel *et al.*, n.d). The primary home for these fungi is mostly soil from where they are

dispersed into host crops while searching for nutrients. The fungi mostly occur as secondary infections to stressed crops (insect or disease damaged crops). As the fungi grow, they feed saprophytically on the crop and produce mycotoxins as secondary metabolites. Mycotoxins that mostly contaminate food crops include aflatoxin, fumonisin, deoxynivalenol, ochratoxin and zearalenone (Wu, 2021). The major crops affected by mycotoxins include maize, peanuts, beans, rice, cassava, spices and pepper, among others (WHO, 2018).

2.3.1 Effect of mycotoxins to human and animal health

Mycotoxins cause adverse effects to humans, animals and plants (Ismaiel & Papenbrock, 2015). Although no literature is available to describe social factors associated with mycotoxin contamination for example occupational, dietary habits and immunity (Mehan *et al*., 1991). Aflatoxin and fumonisin are the most implicated of the mycotoxins due to their high occurrence in food crops, animal feed and products. Aflatoxin is the best studied fungal metabolite (Scheidegger & Payne, 2003). In humans, aflatoxins produced by *Aspergillus* species are known to be highly carcinogenic. Aflatoxin contamination of staples such as maize presents both health and economic burdens (Mamo *et al*., 2020). Exposure to aflatoxins consumption in Kenya starts from infancy from contaminated breast milk and continues during early childhood due to consumption of cereal based weaning formulas that are mainly made from maize or sorghum as seen in a study done in Nandi and Makueni counties (Kang'ethe *et al*., 2017). Generally, aflatoxins results in health complications which include: reduced immunity, retarded child growth with diminished intelligence quotient, hepatitis, vomiting, diarrhoea, yellowing of the skin (jaundice), abdominal swelling and even death in cases of acute poisoning (Probst *et al*., 2007). Acute aflatoxicosis occurs when the patient is exposed to high level of aflatoxin resulting into adverse symptoms and effects while chronic aflatoxicosis occurs when low levels of aflatoxins are taken for a long period with mild symptoms that accumulate over time (Mehan *et al*., 1991). Effect of aflatoxins in humans causing acute hepatitis were first reported in 1982 (Ngindu *et al*., 1982). Mycotoxins have received considerable attention across various media articles, published peer reviewed papers after the 21st century (Mutegi *et al*., 2018).

In animals, infection of poultry feeds with toxigenic *Aspergillus* results in aflatoxins in the poultry feeds thus affects the animal causing high morbidity, increased mortality and low

production in poultry birds (Ezekiel *et al.*, 2014). In Kenya, aflatoxicosis in animals was first reported in 16,000 duckling who died from contaminated peanut feed while other subsequent reported cases have also shown dogs and poultry equally affected (Mutegi *et al.*, 2018). In plants, mycotoxins majorly affect the quality of the seed, its ability to germinate and grow (Ismaiel and Papenbrock, 2015). Aflatoxin inhibits synthesis of chlorophyll and carotenoid, limit germination of seed and seedling growth in lettuce (Crisan, 1973), mung (Sinha & Kumari, 1990), wheat (Sinha & Sinha, 1993), and cowpea (Adekunle & Bassir, 1973).

Fumonisins are of equal economic importance like aflatoxin. Fumonisins have also been associated with oesophageal (Marasas, 2001) and liver cancer in Human (Sun *et al.*, 2011) as they are group 2 carcinogens (IARC, 2002). In addition to this, fumonisins also cause neural tube defects (Sadler *et al.*, 2002). In a recent study by Kimanya *et al.* (2021) in Tanzania, 38% of household involved in a study were found to be exposed to maximum tolerable daily intake of fumonisins > 2 µg/ kg body weight based on recommended consumption rate of maize of 771 g/person/day. The World Food Program threshold for fumonisin is set at 1 ppm while other Regulatory bodies worldwide have set level of 2 µg/g (Wagacha and Muthomi, 2008). Recently, the East African Bureau of Standards set 2 µg/g as the maximum threshold limit for fumonisins in maize and peanuts (Ankwasa *et al.*, 2021).

Presence of fumonisins in animal feed causes central nervous system diseases known as leucoencephalomalacia in horses (Marasas *et al.*, 1988) donkeys and mules (Mostrom, 2021). Kidney and liver cancer has also been observed in experimental rodent and pulmonary edema in pigs due to fumonisins (Harrison *et al.*, 1990). Additionally, fumonisins reduce cell viability and increases membrane leakage (Yu *et al.*, 2020).

2.3.2 Effect of mycotoxins on trade

Mycotoxins have also affected trade between and within countries. To curb aflatoxin contamination in commodities, different aflatoxin standards have been put in place by countries and regulatory bodies for food product use for instance the European Union (EU) threshold for total aflatoxins is 4 µg/kg, Kenya Bureau of Standards (KEBS) aflatoxin standard permitted in Kenya is 10 µg/kg and US Food, and Drug Administration (FDA) threshold is 20 µg/kg.

However, most African countries have not put in place thresholds for mycotoxins (Degraeve *et al.*, 2016). Although mostly, products tested for mycotoxins confirm higher levels than the recommended standards (Logrieco *et al.*, 2021). These regulatory thresholds are used for both locally and internationally manufactured food commodities and may hamper trade across regions. With the standards in place, the cost of commodities increases because of mandatory testing costs and rejection of consignment that may be above threshold and even imposed ban of commodities: (Madala *et al.*, 2018). Contaminated products lead to reduced marketable volumes or are forced into informal markets causing contamination among people living below the poverty line (Logrieco *et al.*, 2021). Countries that have stringent mycotoxin standards like the EU (4 µg/kg) make it difficult in importing products from areas that are aflatoxin prevalent. Losses in the export market in African countries has been experienced due to non-conformity to mycotoxin standards where importation of African cereals by the European countries went down by USD $670 million which was equivalent to 67% reduction in exportation of cereals (Otsuki *et al.*, 2001). Losses due to aflatoxin contamination range up to hundreds of millions of Dollars in the USA (Wu, 2015). However, most countries in Africa do not implement regular surveillance (Warth *et al.*, 2012). Aflatoxins affect many small-scale farmers who consume maize without testing, most of which is unfit for human consumption even in countries that have very strict regulatory standards surveillance is limited to trade commodities (Wu, 2015).

2.4 Factors that influence mycotoxin accumulation in maize

In most agricultural fields in the world, toxigenic microbes make majority of the pathogens (Daou *et al.*, 2021). They attack and proliferate on a variety of crops, and are able to produce mycotoxins under different environmental circumstances (Richard *et al.*, 2003). There are several factors that influence the growth of the fungus and consequent production of mycotoxins. Contamination of crops with mycotoxin occurs at different levels of the value chain, it initiates in the field and can accumulate at harvest, drying stage and eventually during storage (Richard *et al.*, 2003). The circumstances that lead to production of mycotoxins vary from the circumstance under which fungi grow therefore the availability of fungi may not necessarily reflect mycotoxin production and accumulation (Kochiieru *et al.*, 2020). Process that may destroy the fungi may not remove mycotoxin as mycotoxins are chemically resistant in nature (Daou *et al.*, 2021). Growth of fungi occurs in two phases: (i) the primary phase where for making of biomass and

production of energy required for essential growth, organic substances are used during this phase. (ii) The secondary phase occurs after growth has been maintained and may sometimes but not always lead to production of spores and secondary metabolites. Mycotoxin are example of secondary metabolites, they do not affect primary growth of the fungi but are produced when primary metabolites are in excess thus a way of regulating primary metabolite inside the fungi, the primary metabolites are used as precursors for formation of secondary metabolites (Perdoncini *et al*., 2019). Mycotoxin producing fungi and the crops that are affected are very many, a specific combination of condition that may lead to mycotoxin contamination is difficult to define (Daou *et al*., 2021). The main factors that contribute to mycotoxin production and accumulation are mainly high temperatures, water activity, high humidity, type of fungi, substrate, and pH.

The occurrence of fungi is greatly influenced by environmental climatic conditions (Smith *et al*., 2016). Environmental conditions like temperature and humidity greatly affect the extent of fungal contamination and fungal activity (Magan, 2004). Furthermore, environmental conditions also affect the frequency, ability to survive and develop, their distribution and consequently their ability to produce toxins (Doohan *et al*., 2003). In addition to this, humidity and temperature greatly influence the growth of plants, plant health, strength and determines the competition of toxigenic fungi (Marin *et al*., 2013). The optimum temperature and water activity of each fungi growth, germination and production of mycotoxin differs (Daou *et al*., 2021). The environmental conditions, requirement of growth, development of fungi and production of mycotoxin differs in each geographical area for instance aflatoxin has been reported mostly in tropical and subtropical areas (Mannaa & Kim, 2017). Favourable humidity and temperature for fungi to invade crops can occur either during pre-harvest stage in the field or after harvest during drying and storage and determining the start of the invasion and infection may not be identified distinctly (Perdoncini *et al*., 2019). *Fusarium* species are mostly found contaminating crops in the field as they are hygrophilic and thus they require high humidity greater than 90% to survive. Once the crop is harvested *Fusarium* species disappear and give way to xerophilic species like *Penicillium* and *Aspergillus* species which are able to thrive and excrete mycotoxins at less than 90 % relative humidity (Mannaa & Kim, 2017). When the surrounding humidity in the environment where the grains are stored exceeds the humidity in the grains, the grains will gain moisture, the high water activity will favour growth of the fungi and production of mycotoxins (Daou *et al*.,

2021). For temperature conditions, most fungal species growth occurs at a range of 5 -35 ^{o}C since they are mesophiles, the optimal temperature for most fungi is 25 – 30 ^{o}C (Dix & Webster, 1995). However, other fungi are psychrophiles can thrive well at low temperature while others are thermophiles and are able to survive high temperatures (Magan, 2004). Temperature influences the growth of fungi by regulating chemical substances required for the development, and at optimal range of temperature, growth occurs at a high rate. On the contrary, when the temperature deviates from the optimal range, a decline in the chemical reaction occurs resulting into growth termination (Kamil *et al.*, 2011). High temperature and humidity increase the growth of fungi that produce the mycotoxins (Daou *et al.*, 2021). *Aspergillus* and *Fusarium* grow optimally at a temperature of 25-30 ^{o}C (Giorni *et al.*, 2007) and 27 ^{o}C (Cruz *et al.*, 2019), respectively. Although, conditions that enhance growth of fungi may not necessarily favour production of mycotoxins, a water activity greater than 0.78 at a temperature range of 25 -30 ^{o}C, and relative humidity range of 88 -95 % may favour both growth of the fungi and simultaneous production of mycotoxin (Thanushree *et al.*, 2019).

Another factor that influences the production and accumulation of mycotoxins is the fungal strain. The type and amount of mycotoxin produced varies according to fungal species (Nicholson, 2004). Specificity of strain, instability and variation may affect production of mycotoxin (Greeff-Laubscher *et al.*, 2019). This is because strains within the same species may prefer different optimal conditions necessary for both growth of the fungi and production of the toxin thus different strains may produce different mycotoxin levels and type. *Aspergillus carbonarius* survives better at wide temperature range of 8 to 40 ^{o}C and excretes ochratoxin A while *A. flavus* thrives better a temperature range of 15 to 44 ^{o}C (Mannaa & Kim, 2017). Furthermore, *A. flavus* S-strain has been shown to produce copious amounts of aflatoxin B1 compared to *A. flavus* L-strain (Ehrlich, 2014).

The substrate is also an important factor that affects the production of mycotoxin. Several substrates can support fungal growth and mycotoxin production although the main reason for the affinity of fungi to certain food substrate is still unknown (Daou *et al.*, 2021). Although fungi

attack nearly all food crops they mainly require carbon and nitrogen for their growth hence substrates with carbohydrates are mostly preferred (Kokkonen *et al*., 2005). This is because most carbohydrates contain carbon which fungi are able to hydrolyze and generate energy to support growth (Duran *et al*., 2010). Fungal growth is accelerated where readily available sugars are present for breakdown by fungi while in the presence of complex sugars, growth is slowed down as more energy is required to breakdown the complex sugars. An increment of soluble sugars like sucrose, glucose, and maltose have been reported to promote mycotoxin production in cell culture. Additionally Similarly, an increase in aflatoxin B1 produced by *A. flavus* was reported in medium that has an increased content of sugar (Uppala *et al*., 2013). However, the substrate that supports growth of fungi may not necessarily support the production the mycotoxin as condition for mycotoxin production in a substrate are more specific compared to conditions for fungal growth (Daou *et al*., 2021). Production of mycotoxin in general is promoted by a combination of many factors within the substrate like the pH, sugar composition and temperature of the substrate (Özcelik & Özcelik, 1990). Growth of fungi, their germination and production of mycotoxin may be limited within a substrate by several factors interacting, the absence of any factor may hinder fungal development. Moreover, growth of fungi and mycotoxin production may also be hindered by osmotic pressure in a substrate, osmotic stress affects fungal physiology and excretion of mycotoxins (Duran *et al*., 2010). Additionally, fungi undergoing osmotic stress have the ability to change their physiology in order to adapt and survive (Duran *et al*., 2010).

Additionally, an acidic pH of 4 to 4.5 favours the growth of fungi and production of mycotoxins while alkaline conditions limit mycotoxin production (Moreno-Pedraza *et al*., 2015). The pH of the medium around the fungi plays a vital role in the growth and production of mycotoxins. The hydrogen atoms saturation or the pH value in the medium around the fungi affects the fungi in two ways: (i) directly through interaction with the cell surface (ii) indirectly by influencing the availability of nutrients (Daou *et al*., 2021). Fungi have the ability to modify the pH in their surrounding by either producing acidic or basic compounds in their surroundings for instance *Aspergillus* are *Penicillium* species have been reported to secrete citric and gluconic acids to acidify the medium (Vylkova, 2017). The ability to regulate the pH enables the fungi to adapt to the surrounding in the host, can affect the temperature and water activity reactions because pH can influence process of metabolism related to morphogenesis and spore formation (Wang *et al*., 2017). Furthermore, pH has also been reported to affect the expression of genes involved in

biosynthesis, for instance the production of ochratoxin A occurs at a pH of 8 (Brzonkalik *et al.*, 2012). It is well reported that germination and production of mycotoxins is favoured under acidic pH though not much is known on the specific pH requirement for each mycotoxin. For a few like aflatoxin, an acidic pH of 4 favours its biosynthesis (Reverberi *et al.*, 2010), for fumonisin B_1, a pH range of 4 -5 is required for its biosynthesis while Ochratoxin A and trichothecene biosynthesis is also favoured under acidic conditions (Perdoncini *et al.*, 2019).

Climate change has also brought with it increase in pest and diseases in stored grain (Moses *et al.*, 2015). Furthermore, unexpected calamities such as floods, drought, insect attack and other intrinsic factors increase mycotoxin contamination (Logrieco *et al.*, 2021). Climate change has become a hot topic in recent times as it has brought with it changes in climatic conditions, thus it has been predicted that by the end of the 21st century the temperatures around the globe will rise by 1.5-4.5 ^{o}C (Liu & Van der Fels-Klerx, 2021). As a result, there is expected extreme climatic conditions characterized by increased precipitation, heat waves, prolonged winter season and increased concentration of carbon dioxide (Medina *et al.*, 2015). Climate change and global warming have a risk of affecting food security and safety by reducing crop yield and quality thus unfit for human use especially due to mycotoxin accumulation. The European Food Safety Authority has projected that some areas in the world will benefit from climate change while other regions will be disadvantaged (Battilani *et al.*, 2012). For instance, the Mediterranean basin and southern part of Europe will face reduced crop yield and high incidences of mycotoxin contamination while North Europe will be favoured by climate change (Medina *et al.*, 2017). Furthermore, each mycotoxin will be affected in a different way for instance aflatoxins contamination will rise due to increase in *Aspergillus* infection of crops due to increased temperature as was seen in Italy in 2003 and 2004 (Giorni *et al.*, 2007). Climate change will affect mycotoxin prevalence in several ways like by increasing pests and diseases that will cause diseases to plant and act as predisposing factor for mycotoxin contamination. Climate change may also bring about early ripening and maturity of crops, reduced plant resilience and change in pathology of host due to increased carbon dioxide (Medina *et al.*, 2015).

Apart from these, poor agricultural practices like poor sanitation, lack of nutrients in soil, water, lack of weeding, lack of pest control and use of highly susceptible varieties result in the plant

stress which predisposes the crop to mycotoxin contamination. Delayed harvesting of maize in the field is also a cause of accumulation of mycotoxins in maize and has been attributed to high aflatoxin level in maize that led to 2004 outbreak of acute aflatoxicosis fatalities (Mutegi *et al.*, 2018). Improper harvesting, drying and storage methods are the predisposing factors in mycotoxin contamination by storage fungi. Furthermore, mixing of grains or old harvest with new harvest can be a predisposing factor for mycotoxin contamination and accumulation (Hamad *et al.*, 2023).

2.5 Mycotoxins producing fungi

Aflatoxin and fumonisin are the most important mycotoxin and are produced by *Aspergillus* and *Fusarium* species respectively. Genus *Aspergillus* belong to order *Eurotiales of Ascomycetes* members (Houbraken *et al.*, 2020). *Aspergillus* section *Flavi* is a category of fungal species that propagate by green to yellowish conidia and develop darkbrown to black survival structuresknown as sclerotia that enables them to survive harsh conditions in soil and other niches (Varga *et al.*, 2011). *Aspergillus* section *Flavi* is composed of both species that express genes for aflatoxin production and those that do not have the gene responsible for aflatoxin production. (Ehrlich, 2014). *Aspergillus flavus* and *A. parasiticus* are in most cases associated with infection of crops and production and accumulation of aflatoxins in the crops(Probst *et al.*, 2007). The nontoxigenic members of*Aspergillus* section *Flavi* include some of *A. flavus* L-strains, *A. pseudotamarii, A. oryzae, A. sojae* and *A. tamarii. Other members of Aspergillus section Flavi include A. arachidicola, A. bombycis, A. minisclerotigens, A. nomius and A. parvisclerotigenus* (Kumeda & Asao, 1996) .

Aspergillus flavus forms yellowish greenish conidia and exists in two forms denoted as L or S-strain depending on the sclerotia size. The *A. flavus* L-strain forms fewer, larger spherical sclerotia with a diameter greater than > 400 µm diameter and less aflatoxin B1 compared to *A. flavus* S-strain that produces many, small sclerotia with an average diameter less than 400 µm and produce copious amounts of aflatoxin B1 (Garber & Cotty, 1997). *Aspergillus flavus* exists in both toxigenic and atoxigenic forms. The ability to produce toxins is coded in the genetic material of the *Aspergillus* species and the inability is due to deletion of some codes in the genes.

An earlier study shows that *A. flavus* isolates have a deletion in the 0.8–1.5-kb aflatoxin biosynthesis gene cluster resulting to inability to produce aflatoxin G (Ehrlich *et al.*, 2004). However, in Kenyan maize *A. flavus* produces copious amounts of both aflatoxin B and G (Mohamed, 2016). Atoxigenic *A. flavus* has been widely used as a biocontrol agent in managing aflatoxin (Khan *et al.*, 2021). A biocontrol product by the trade name aflasafe is currently being used in Kenya, Nigeria, and Senegal in mitigating aflatoxin in maize and peanuts (CGIAR, Performance report, 2019).

Aspergillus parasiticus is characterized by dark green conidia on 5/2 agar (Atehnkeng *et al*, 2008) and exists in both atoxigenic and toxigenic strains and is the main cause of *Aspergillus* ear rot in maize (Nikolić *et al.*, 2021) The toxigenic strains produce both B and G aflatoxins (Mohamed, 2016). Other metabolites produced by *A. parasiticus* include: aflavines aspergillic acid, kojic acid, , oryzaechlorin, parasiticolides, paspalinine, paspaline, and other sclerotial metabolites (Pildain *et al.*, 2008). Unlike the smooth conidia formed by *A. flavus,* conidia of *A. parasiticus* are round and rough (Rodrigues *et al.*, 2009). *Aspergillus parasiticus* is mostly been found contaminating both food and feed (Al-Hmoud *et al.*, 2012). *Aspergillus parasiticus* has majorly found in peanuts (Mutegi *et al.*, 2012)but is also a contaminant in maize (Atehnkeng *et al.*, 2008).

Aspergillus tamarii produces brownish to yellow and with a double walled spores (Ito *et al.*, 1998) that makes it morphologically similar to *A. caelatus*. *Aspergillus tamarii* has been associated with human keratomycoses in India (Homa *et al.*, 2019). Most members of *A. tamarii* are atoxigenic while a few (*A. pseudotamarii*) can produce aflatoxin B1 (Mohamed, 2016). *Aspergillus tamarii* that does not produce aflatoxins have been reported in Sub-Saharan Africa (Ezekiel *et al.*, 2014). Other studies have reported the occurrence of *A. tamarii* in peanuts in Kenya (Nyirahakizimana *et al.*, 2013 Mutegi *et al.*, 2012), chicken feeds in Nigeria (Ezekiel *et al.*, 2014) and in soil in lower Eastern Kenya (Mohamed, 2016). In submerged and solid-state culture, *Aspergillus tamarii* makes tannase and gallic acid (Da Costa, 2013) and xylanase enzyme that is used for industrial degradation of xylan (Gouda & Abdel-Naby, 2002).

Fusarium species colonize maize ears causing ear rots (Logrieco *et al.*, 2021) resulting in fumonisin production and accumulation (Morales *et al.*, 2018). *Fusarium verticillioides* and *F. proliferatum* are the main causes of ear rot in maize (Pfordt *et al.*, 2020). Other members of genus *Fusarium* include*: F. graminearum* and *F. anthophilium* (Fandohan *et al.*, 2005). Culture of *F. verticillioides* is a soil and seed borne facultative endophyte that forms white colonies and changes from pink to violet as its ages (Pascual *et al.*, 2016). *Fusarium graminearum* and *F. Culmorum* are known to cause red ear rot/ fusariosis while *F. proliferatum, F. verticillioides* and *F.subglutinans* are known to cause pink ear rot/fusariosis (Logrieco *et al.*, 2021). Microconidia are club shaped and appear in clusters and long chains. *Fusarium verticillioides* does not produce chlamydospores but has swollen cells in the hyphae that appear like chlamydospores or pseudochlamydospores. Infection with *F. verticillioides* is characterized by a star bust symptom where a whitish to lavender discoloration appears on kernel or silk with white streaks diverging from the cap of the kernel where the silk attaches to the cob (Pascual *et al.*, 2016). *Fusarium* species are known to produce fumonisins, trichothecenes and zearalenone (Ekwomadu *et al.*, 2021*)* and deoxynivalenol (Ekwomadu *et al.*, 2020).

Fusarium proliferatum produces white aerial mycelia that become light orange as it ages. It also produces thin-walled, slander and straight macroconidia while the microconidia are oval to obovoid shape (Yang *et al.*, 2020). *Fusarium proliferatum* contaminates grains causing maize ear rot disease (Pfordt *et al.*, 2020) and produce fumonisins, moliniformin, beauvericin, and fusaproliferin (Bottalico, 1998).

2.6 Methods of fungal detection

To ascertain food safety, detection methods that are sensitive and have high reproducibility are very important. Detection of pathogens in food has been challenging because of presence of other non- target microbes within the same food matrix, low incidence of target microbe and the hassle of extracting microbes from food matrix (Aladhadh, 2023). Several methods have been used in detection of pathogenic fungi in food and these include the traditional culturing and identification method using morphology, use of immunoaffinity methods, nucleic acid detection method which comprises of polymerase chain reaction and sequencing. For these methods to be

successful in the identification of the pathogens, aseptic techniques should be followed during sample collection, processing, analysis, and storage. Sampling techniques chosen must be in line with the food type to be analyzed, microbial pathogen targeted, and the detection method identified to be used. It is necessary to follow standard operating procedures developed by official organization like ISO or FDA in the collection of the sample and analysis of the sample (Da Silva *et al.*, 2018). Some of the other protocols that are standardized are available for collection samples, analysis, interpretation of the data according to the kit used and manuals for the use of equipment by the manufacturer.

Mycotoxin contaminated food may contain fungi that can be ingested in the food. Detection of fungi in food using culturing method may take five to seven days for the viable fungi to grow. Traditional methods of culturing have made isolation, cultivation and enumeration of target pathogen possible with the elimination of other non-target microbes occurring in the same food matrix using selective media either in solid or liquid form (Akkina *et al.*, 2022). To identify target food pathogen, pre-enrichment, selective enrichment and selective plating may be done followed by a biochemical or serological test to confirm the results. Traditional culturing method can give both qualitative and quantitative results: For qualitative, selective media is used to determine the presence or absence of the target pathogen while for quantitative results, plate counts are done to enumerate the number of colony forming units per gram of sample (Jasson *et al.*, 2010). Culture based methods are regarded as the gold standard, are cheap and easy to do but are labour intensive and time consuming (Akkina *et al.*, 2022).

Another detection method is the use of matrix-assisted laser desorption ionization-time of flight mass spectrometry which has been used in the identification of food pathogens like *Aspergillus, Fusarium, Mucor* and *Rhizopus* (Elbehiry *et al.*, 2017). In foods like salads, tortillas and cheese, pathogenic fungi like *A. flavus, A. niger*, and other fungal contaminants like *Candida albicans, P. digitatum, Alternaria alternata* have been detected (Elbehiry *et al.*, 2017). The matrix-assisted laser desorption ionization-time of flight mass spectrometry method has also been used to detect Mucor (Quéro *et al.*, 2019), yeast (Quintilla *et al.*, 2018), and fungi (Ahmadsah *et al.*, 2018) in food. This method is however not commonly used for fungi compared to bacteria due to the lack of database for fungal spectra and usability of the method to fungi (Bader, 2017).

Another way of fungal detection is by use of immunological assays, which include tests like ELISA, serotyping, lateral flow assays, and immunofluorescence have been used in detection of fungi in food commodities. Some of these methods, for instance ELISA can be used in the detection of both the toxigenic fungi and the mycotoxin it produces. The principle used in the test is the immunological affinity that exists between antibody and antigen to form a complex (Singh *et al.*, 2020). These methods are preferred compared to traditional cultural method because they are rapid, more specific and simple to use and can also be used for toxin detection apart from microbial detection (Agriopoulou *et al.*, 2020). Recently, lateral flow devices together with ELISA test have been reviewed and are more commonly used in the detection of mycotoxigenic fungi and mycotoxins (Agriopoulou *et al.*, 2020). Further advances in use of ELISA technique involve coupling it with PCR. For instance, in the detection of pathogenic fungi, *F. verticillioides* in maize that is contaminated, PCR-ELISA was found to be more efficient in detection than conventional PCR by a 100-fold (Omori *et al.*, 2018). The PCR-ELISA based kits for detection of fungal pathogens and other microbes have been developed (Omori *et al.*, 2018). Additionally, detection of mycotoxins has also been widely done using ELISA kits, mostly in the detection of aflatoxins and fumonisins in maize (Chavez *et al.*, 2023), aflatoxin in stockfish (Ogunleye & Olaiya, 2015), in groundnuts (Sserumaga *et al.*, 2020), soy milk (Beley & Teves, 2013). Likewise, lateral flow devices have also been used to quantify fumonisins, aflatoxins and deoxynivalenol in barley and maize study (Di Febo *et al.*, 2019).

Polymerase chain reaction is another rapid method of fungal detection that involves use of nucleic acid from DNA to determine the presence of the fungi. Despite being a quick method, PCR has not been extensively used in detection of fungi as compared to bacteria (Aladhadh, 2023). However, some studies show the clinical use of PCR to detect pathogenic *Candida auris* (Sexton *et al.*, 2018) and other pathogenic fungi (Wagner *et al.*, 2018). A recent shows the different types of PCR that have been used in investigating fungi that produce mycotoxins which include: real-time PCR, nested PCR, quantitative RT-PCR, multi-plex PCR, and loop-mediated isothermal amplification (LAMP) (Rahman, *et al.*, 2020). These techniques have been used on food sample extract, food surface swabs to investigate pathogens associated with food. In Korea,

multiplex PCR has been used in fermented soybean to test the presence of toxigenic *Aspergillus* species (Kim *et al.*, 2011). Primers were designed to selectively distinguish between aflatoxigenic and non-aflatoxigenic *Aspergillus,* and to further validate this test, aflatoxin analysis was done using HPLC and TLC (Kim *et al.*, 2011). Moreover, simultaneous detection of *Fusarium, Penicillium* and *Aspergillus* in maize flour has been enabled by multiplex PCR technique (Rahman, *et al.*, 2020).

Penicillium expansum that produces patulin toxin has also been quantified successfully by RT-PCR technique in artificially innoculatedd apples (Tannous *et al.*, 2015). *Penicillium nordicum* that produces ochratoxin has also been identified in cured dry meat using LAMP technique (Ferrara *et al.*, 2015). Conventional PCR can be coupled with other techniques like denaturing gradient gel electrophoresis and can be used in detection of *Aspergillus niger* that produces ochratoxin in wine (Laforgue *et al.*, 2009). Denaturing gradient gel electrophoresis technique dissociates amplicons from PCR according to their melting properties in gel made from polyacrylamide. A fingerprint of multiplied microbial groups is generated on the gel is generated and enables characterization downstream when necessary (Laforgue *et al.*, 2009).

Another very powerful and sensitive technique in food safety in the identification of toxigenic fungi is the use of sequencing which involves bioinformatic tools. Next-generation sequencing has several uses: (i) It can be used to establish the genomic sequence of an organism (pathogen), (ii) It can be used to identify a number of organisms that exist within the same sample for example fungi and bacteria colonizing a food sample by sequencing the ITS and 16S rRNA respectively (Jagadeesan *et al.*, 2019). Sequencing has been widely used in detection of pathogens in clinical samples (Armstrong *et al.*, 2019). Sequencing is a quick, accurate and sensitive method of microbe detection (Jiang *et al.*, 2022) although it is an expensive technique that requires well trained personnel.

2.7 Methods of mycotoxin quantification

Methods of analysing mycotoxins in food and feed products have to be robust, effective and comprehensive because mycotoxins have diverse chemical structures, and high chemical stability (Janik *et al.*, 2021). Mycotoxin are also heterogenous in nature, their distribution in a sample is not even hence quantification of sample using conventional bulk methods may lead to false

positive or false negative results (Mitchell *et al.*, 2016). Several mycotoxin detection methods that range from traditional detection methods for example solid phase extraction to analytical techniques have been used in various studies. Surveillance of mycotoxins and monitoring for compliance to set legal threshold are done using several methods. Conventional analytical methods include chromatographic techniques like high performance liquid chromatography (HPLC), thin layer chromatography (TLC), liquid chromatography (LC), and gas chromatography (GC). The chromatographic techniques require trained personnel and are costly (Sapsford *et al.*, 2006). However, the HPLC is precise, sensitive, and selective (Kotani, 2012). Most of these methods use clean up column that has a solid phase matrix to remove interference, impurities and improve the detection of the mycotoxins (Zheng *et al.*, 2006). Thin layer chromatography is a powerful and low cost technique that can be used for the separation, purity assessment, and identification of organic compounds (Rahmani *et al.*, 2009).

In recent times, rapid methods of analysis are commonly used. These methods are cheaper, require less training, portable, require less equipment and labour thus help commodities to move rapidly through the market channel within a short time span (Zheng *et al.*, 2006). Rapid methods of mycotoxin analysis include enzyme linked immunosorbent assay (ELISA); fluorometric assay; flow through membrane-based immunoassay; fluorescence polarization and immuno-chromatographic assay (Zheng *et al.*, 2006).

Enzyme linked Immuno-Sorbent Assay has become more common due to the simple protocols involved and easy market accessibility. Recent studies have employed ELISA to quantify concentrations of aflatoxins, fumonisins and other mycotoxins (Mohamed *et al.*, 2022). The technology is based on antibody with specific mycotoxin antigen (Zheng *et al.*, 2006). This method is most commonly used because it is simple to use, cost effective, rapid and is more preferred than HPLC which is costly (Nesic *et al.*, 2017) despite its disadvantage of producing in false negatives and positives results (Kumari, 2021).

Membrane based immunoassays are fast, user- friendly, portable do not require equipment and skilled personnel (Burmistrova *et al.*, 2014). However, interpretation of results may be cumbersome since the method is semi-quantitative. Lateral flow tests, also known as

immunochromatographic strip (ICS) tests, are also fast and easy to use test formats, which do not require additional steps or chemicals (Goryacheva *et al*., 2007).

Immunoaffinity chromatography also referred to as immunoextraction involves a solid phase in the extraction process and uses both traditional and immunological techniques to detect and quantify mycotoxins that are regulated. It is an essential process used to clean up impurities in mycotoxin for other processes like HPLC and LC-MSMS. It is a cost effective and quick way of removing impurities that may interfere with the sample and selectively preconcentrates the sample of interest before other chromatographic analysis. This technology utilizes plastic or glass made test column that are embedded with anti-mycotoxins sorbent. The process begins with sample extraction using a solvent, then extract is added to the column and washing with a buffer is done to remove the unbound sample and impurities. The bound sample is then eluted with a suitable solvent and collected for chromatographic analysis. Procedure and protocols outlined by the supplier of the kits should be followed and normally they are single use test kits. Kits for several mycotoxins are available in the market (Wang *et al*., 2022).

In recent times, major innovations have been developed that employ the spectral technology to classify and detect kernels based on their mycotoxin content (Yao *et al*., 2010).Single kernel analysis which were first documented in 1980 and are advantageous compared to conventional methods because it allows for multiple detection of different mycotoxins that co-occur together in the kernels, it also is more accurate than bulk methods, is a non-destructive method and can allow for categorization on the level of contamination (Chavez *et al*., 2020). The hyperspectral imaging by using ultra-violet light can categorize kernels based as low, medium or high toxin content. Further advanced multi-spectral approach by use of infrared, visible and ultraviolet light can be used to detect and sort kernels based on mycotoxin (Pearson *et al*., 2001). Fluorescence and infrared imaging are among the new advancements in testing for mycotoxins in single kernels of maize (Chavez *et al*., 2020). Single kernel analysis of maize for mycotoxin involves spectral imaging, mathematical algorithms and wet chemistry (Chavez *et al*., 2020). Fluorescence under UV light that reflects in bright yellow green colour also known as black light testing is one of the first method used in detection of aflatoxins. The bright yellow colour appears due to reflectance of kojic acid which is produced by *Aspergillus* species that also

produce aflatoxins (Chavez *et al.*, 2020). However, there is limited data on detection of fumonisins in single kernels using fluorescence method (Zhu *et al.*, 2016). Despite the advantage of fluorescence detection being a cheap, non-invasive method, it is disadvantageous because low signals used can cause errors when recording high intensities and emission of fluorescence. Additionally, fluorescence detection can result in cross contamination when analyzing a bigger lot as instrument can get contaminated (Yao *et al.*, 2015). Another downside of fluorescence imaging is that maize has other constituent that may respond similarly to mycotoxin being analysed hence causing an overlap (Chavez *et al.*, 2020). Infrared spectroscopy is another technique in detection of mycotoxins and has a broad spectrum of wavelength ranging from 800 nm to 1,000,000 nm (Chavez *et al.*, 2020). Although Near infrared can distinguish fumonisins levels as high as greater than 10 µg/g or as low as less than 10 µg/g (Dowell *et al.*, 2002) and levels of aflatoxins as high as greater than 100 µg/kg or low as less than 20 µg/kg (Chu *et al.*, 2017), it has low sensitivity to mycotoxin emitted signals (Levasseur-Garcia, 2018). Near Infrared spectroscopy which is based on reflectance and transmission has also been used in quantification of mycotoxin although it is less sensitive to micro molecules (Levasseur-Garcia, 2018). A study on integration of both reflectance and fluorescence in the detection of aflatoxins in single kernels showed better results than either of the method individually (Zhu *et al.*, 2016). Furthermore, dual high speed cameras have been developed and tested in the detection of aflatoxin in maize using multi-spectral fluorescence imaging (Han *et al.*, 2018).

2.8 Approaches in mycotoxins management

Mitigation of mycotoxin contamination calls for an integrated approach (Hamad *et al.*, 2023) that involves both pre-harvest and post-harvest good agricultural practices for example, crop rotation, tilling the land, application of antifungal mulch (Nada *et al.*, 2022), favorable planting time, controlling weeds and pests, minimizing water stress and nutritional stress by use of fertilization and irrigation, ensuring proper sanitation, proper drying, and storage. The choice of mycotoxin management strategy is dependent on history of severe effects on animals and humans, cost of management strategy, knowledge of mycotoxins and their impacts, incentives for mycotoxin safe products and scope intended for the use of the mitigation strategy (Mutegi *et al.*, 2018). Farmers do not apply mycotoxin mitigation strategies because they lack knowledge about mycotoxins, have no idea about the mitigation strategies, or the strategies are unaffordable and labour

intensive to the farmer (Logrieco *et al.*, 2021). There is a need to sensitize farmers on good agricultural practices that enable them to reduce stress to the crops and maintain good sanitation. Furthermore, it is necessary for sectors like nutrition, agriculture and health to collaborate to solve mycotoxin contamination and to develop adequate policies around mycotoxin mitigation (Mutegi *et al.*, 2018). Several public and private sector organizations have been championing for mycotoxin safe food Kenya which includes KEBS, KALRO, KEPHIS, Ministry of Agriculture, Livestock, Fisheries and Cooperative, Ministry of Public Health, IITA, ACDI-VOCA, PACA, Cereal Growers Association (CGA), Cereal Millers Association (CMA), World Food Program (WFP), FAO and others.

Recent interventions in combating mycotoxin menace include the use of live fungi through competitive exclusion principle (atoxigenic *A. flavus* L-strain) has gained popularity in reduction of both aflatoxins and fumonisin. In Kenya, aflasafe KE01 (atoxigenic *A. flavus* L-strain) has been found to be effective in displacing aflatoxigenic strains of *Aspergillus* and consequently reduce aflatoxin contamination in maize fields that were inoculated (Mohamed, 2016). Similar studies have been conducted in the US (Abbas *et al.*, 2005) and Nigeria (Atehnkeng *et al* 2008) with up to 80-90%, aflatoxin reduction in aflasafe treated fields. The inability for some strains of *Aspergillus* species to produce aflatoxins is caused by gene deletion and single nucleotide polymorphism in the genes involved in aflatoxin biosynthesis (Adhikari *et al.*, 2016). In Kenya four of these atoxigenic strains have been formulated with roasted sorghum which is a source of nutrient for the fungi and a career material, a blue dye to differentiate it from edible sorghum and to prevent birds from feeding on it, and a polymer to adhere all the constituent together to make aflasafe. So far, aflasafe product development, testing and registration has taken place in Burkina Faso, Gambia, Ghana, Kenya, Nigeria, Malawi, Mozambique, Senegal, Tanzania and Zambia (Moral *et al.*, 2020). To increase adoption of the technology, IITA has transferred aflasafe production and distribution to either public or private organizations in Nigeria, Kenya, Tanzania and Senegal (Logrieco *et al.*, 2021). The use of aflasafe is a promising intervention in increasing farmer income, improving health and food security in Africa.

Another mitigation strategy against mycotoxins has been through push -pull technique which has also proved to be effective against stem borer and fall army worms (Guera *et al.*, 2021). This

technique is done by intercropping cereal for example maize or sorghum with legumes that repel insects and surrounding the two crops with *Desmodium* or napier grass (Midega *et al*., 2018). *Desmodium* produces semi chemical repugnant that repel the stemborer moths that are drawn to nappier grass or *Brachiaria* which release organic compounds but the emerging larvae is not able to survive on the grass (Khan *et al*., 2011). Additionally, parasitic weed like *Striga* is also suppressed by *Desmodium* mechanism of allelopathy. This leads to improved soil health by fixing nitrogen, increased organic matter, and preservation of moisture in the soil (Khan & Pickett, 2004). Furthermore, *Desmodium* also acts like mulch because it is able to grow across the year irrespective of rainy season (Khan *et al*., 2011). Another advantage of this plant is that it can also be used to make animal feed since it is highly nutritious (Khan *et al*., 2008). The push-pull technique is been used extensively in Eastern Africa because it well aligned with traditional intercropped farming practice (Njeru *et al*., 2020).

A more traditional method of mitigating mycotoxins is by nixtamalization which is an ancient technique that dates back to Mesoamerican civilization times (Serna-Saldivar, 2015). However a more recent study show traditional nixtamalization done practiced by indigenous people is not efficient in reduction of mycotoxins in maize (Rodríguez-Aguilar *et al*., 2020). The method is still applied in recent years and it involves using alkaline such as lime (Calcium hydroxide) or wood ash to cook maize kernels (Schaarschmidt & Fauhl-Hassek, 2019). In the process of nixtamalization, after the maize is cooked in lime it is then soaked overnight at high alkaline pH and elevated temperatures which causes the endosperm to become soft and the pericarp to detach.

Water is used to wash off the lime and detached pericarp after soaking while the aleurone layer (peripheral layer of the endosperm) remains attached to the endosperm starchy part. The outer parts of the kernels that are removed during nixtamalization contain a high concentration of mycotoxins. The reduction in aflatoxin and its binding to the lime water depends on the aflatoxin type (De Arriola *et al*., 1988). Early literature shows that the efficacy of nixtamalization is higher in reducing the levels of aflatoxin G_1 and G_2 (75% reduction) than aflatoxin B_1 and B_2 (40 to 50 %) (Ulloa-sosa & Schroeder, 1969). Similar findings were also observed by De Arriola *et al*. (1988), where kernels that were inoculated with fungi then nixtamalized showed a higher

reduction of aflatoxin G_1 (98 %) and G_2 (97 %) than the reduction of aflatoxin B_1 (93 %) and B_2 (90 %). Furthermore, aflatoxin B_1 was found to be reduced more (40%) than aflatoxin B_2 (28 %) by the process of nixtamalization of tortilla that used 2 % of calcium hydroxide.

The use of three layered hermetic bags is another post-harvest invention that reduced 55% of aflatoxin levels in stored maize compared to polypropylene bags (Maina *et al.*, 2016). The hermetic bags prevent air circulation inside the bag thus depriving mycotoxigenic fungal growth and other crop pests (Anankware *et al.*, 2012). Purdue Improved Crop Storage (PICS) bags and Zerofly® hermetic bag brands and woven polypropylene bags containing grains that have been treated with actellic gold dust reduces have been reported to control insects, molds and to maintain aflatoxins levels below KEBS threshold of 10 µg/kg in stored grains (Mutambuki & Likhayo, 2021). Adoption of hermetic bags in Africa has increased, attracting more producers of the bags and so far more than a million bags have been bought (Logrieco *et al.*, 2021).

Other efforts aimed at managing mycotoxins is the development of resistant maize inbred lines (Meseka *et al.*, 2018). A study carried out in Kenya on germ plasm resistant to *Aspergillus* also showed resistance to *F. verticillioides* and *Fusarium* ear rot (Rose *et al.*, 2017). Similarly, in another study in South Africa, maize inbred lines resistant to *F. verticillioides* have also been identified (Small *et al.*, 2012). Maize fortified with vitamin A, has also been found to show some resistance to aflatoxins (Suwarno *et al.*, 2019). Efforts by breeders to avail resistant maize varieties to farmers can be a breakthrough in mycotoxin mitigation. Although complete resistance to any pathogen is not possible, monitoring and integration with other mitigation strategies is key as stated by Logrieco *et al.* (2021). Furthermore, development of resistant genotype is cumbersome due to polygenic inheritance, lack of information on resistance principle mechanisms and unavailability of germplasm that is resistant to mycotoxins (Logrieco *et al.*, 2021).

Cold plasma treatment is another method that has been used to mitigate mycotoxin contamination. Plasma is basically 4^{th} state matter made of full or partial ionized gas where there is dissociation of gaseous molecules as a result of application of an electric current in neutral gas (Kiš *et al.*, 2020) and can be generated under different atmospheric pressure (Kamano *et al.*,

2021). When the breakdown voltage is exceeded by the applied voltage, plasma is discharged to create reactive oxygen and nitrogen species (Hojnik *et al.*, 2021). Generally, plasma is a non-thermal process that is made up of different particles in form of atoms, electrons, protons, anions, cations, neutral molecules, and other particles all in present in the same space (Kamano *et al.*, 2021). Furthermore, cold plasma can be used to eliminate microbes in seafood (Esua *et al.*, 2020), degradation deoxynivalenol in wheat (Chen *et al.*, 2022), reduction of *A. flavus* and *A. parasiticus* in peanuts (Dasan *et al.*, 2016), HT-2 and T-2 reduction in wheat grains (Iqdiam *et al.*, 2021), aflatoxin reduction in peanuts (Iqdiam *et al.*, 2020), degradation of aflatoxin B_1 in maize (Shi *et al.*, 2017), and degradation of aflatoxin M_1 in skimmed milk (Nguyen *et al.*, 2022). Despite having many potential applications in mycotoxin management, cold plasma treatment is not highly efficient in treatment of bulky and irregular shaped commodities and has a low penetration power (Kamano *et al.*, 2021).

Use of ozone is another method that has been shown to be effective in reducing both mycotoxins and mycotoxigenic fungi (Freitas-Silva & Venâncio, 2010). In corn grit, a 57 % reduction in aflatoxin was realized after gaseous ozonation (Porto et al., 2019). Despite being few studies that have focused on application of ozone in degradation of mycotoxins, ozonation has proven to be a promising intervention in microbial inactivation and degradation of toxic metabolites (Freitas-Silva & Venâncio, 2010). Ozone is safe as it has no residual effect on food commodities.

Recently, post-harvest intervention based on multi-spectral sorting has been found to be efficacious in reduction of aflatoxins and fumonisins by 83% (Stasiewicz *et al.*, 2017). Use of irradiation technologies for example, Gamma radiation (Ghanem *et al.*, 2008) and E-beam radiation (Woldemariam *et al.*, 2021) has been found to reduce aflatoxin B1 by up to 87.8% in rice and ochratoxin A by 25 % in red pepper..

2.9 Optical sorting technique in reduction of mycotoxins

The technology is based on optical detection and sorting of kernels according to mycotoxin content. By the end of the 19th Century, sorting of grains was mainly done by centrifugation and floatation based on size of particles and the weight, however in recent times, sorting has advance to optical methods (Karlovsky *et al.*, 2016). Sorting techniques have evolved over time; traditionally farmers have used hand sorting methods to reduce mycotoxins in maize. A study in

South Africa showed small scale farmers were able to reduce fumonisins contamination in maize by 84 % by using hand sorting (van der Westhuizen *et al.*, 2010). Hand sorting method has also been used to remove fungal infected maize kernels thus reducing aflatoxin B_1 (Sipos *et al.*, 2021) as well as reduction of fumonisin B_1 by > 95% (Matumba *et al.*, 2015). In most parts of Africa, food handling practices and postharvest management strategies have implemented physical sorting to remove damaged or moldy kernels to reduce fumonisin before milling processes (Neme & Mohammed, 2017). A study in Tanzania shows that manual sorting of grains that showed rotting visual characteristics, and discoloration reduce mycotoxin contamination (Nakuwa *et al.*, 2023). Sorting according to size of grain using a laboratory sieve can also reduce mycotoxins efficiently in oats (Brodal *et al.*, 2020). Size sorting can reduce aflatoxin B_1 in Brazil nuts and maize by up to 98% (Sipos *et al.*, 2021). Even though manual sorting is economical for resource poor farmers the method is unsuitable for large scale use and may be time consuming, therefore mechanizing intervention is key (Stafstrom et al., 2021a) rom *et al.*,). DropSort method using bulk density and 100 kernel weight has been shown to also reduce fumonisin in maize to under 2 µg/kg (Stafstrom *et al.*, 2021; Aoun *et al.*, 2020).

Optical sorting has been used in industrialized countries to control mycotoxin in human food (Logrieco *et al.*, 2021). The presence of aflatoxin and fumonisins in maize has been associated with a bright greenish yellow fluorescence and bright orange fluorescence respectively under UV light (Shotwell & Hesseltine, 1981). Initially use of one spectral band of light was being used which was not effective in segregating contaminated and healthy grains (Pearson *et al.*, 2009). In recent times, the newest development involves multi-spectral sorting techniques to detect mycotoxin contaminated kernels using calibrate imaging of infrared, visible, and ultraviolet (Stasiewicz *et al.*, 2017). The optical sorter is calibrated using kernels that show visible characteristics that are associated with aflatoxin and fumonisin contamination (Chavez *et al.*, 2023). The optical sorting technology devices is cost effective, approximately 300$ (Stafstrom *et al.*, 2021a). Moreover, optical methods are quick and non-destructive hence desirable compared to conventional methods (Han *et al.*, 2018).

Optical sorting can reduce aflatoxin and fumonisin in yellow maize by 81% and 85% respectively (Haff & Pearson, 2006). Furthermore, a recent study by Stasiewicz *et al.* (2017)

illustrated that multi-spectral sorting could concurrently minimize contamination of aflatoxin and fumonisin in Kenyan maize market samples by 83%. A more recent study by Chavez *et al.* (2023) showed multi-spectral sorting reduced aflatoxins by 31 µg/kg and fumonisin by 1.9 µg/g. Single kernel Near infrared spectroscopy techniques have been widely used in screening for mycotoxins in cereals though there is need to scale up this techniques for large scale users (Fox & Manley, 2014).

2.10 Irradiation technologies in reduction of mycotoxin and mycotoxigenic fungi

Irradiation is a process of exposure of food to ionizing radiation. It has been reported that every year more than 500,000 tonnes of food is irradiated including fruits, sea food, meat, spice and herbs (Mostafavi *et al.*, 2012). Food is irradiated mainly to mitigate diseases that are caused by microbial pathogens (Hallman, 2013). Radiation energy is emitted from a source and interacts with most materials and space. Radiation is classified into either ionizing or non-ionizing, where ionizing radiation occurs when an unstable atom in the process of attaining stability losses energy or a particle (Calado *et al.*, 2014). Non- ionizing is energy from a machine or instrument source that can travel at a certain wavelength in the form of electromagnetic waves (Bisht *et al.*, 2021). Non-ionizing irradiation like Ultra violet (UV) rays have been shown to have a reduction effect on both *Aspergillus flavus* and *A. parasiticus* causing aflatoxins and aflatoxin B_1 found in Iranian rice (Faraji *et al.*, 2022). Ultra Violet irradiation has been a mitigation method of controlling mycotoxins due to the photo sensitivity (Faraji *et al.*, 2022). Elimination of microbes and degradation of chemical contaminant is possible by using UV because of direct photolysis and advanced oxidation process that occur during UV treatment (EL-Saeid *et al.*, 2021). In the horticulture industry, UV has been found to have an antimicrobial effects on fresh fruits and vegetables thus eliminating microbes that cause spoilage (Yemmireddy *et al.*, 2022). Recent reviews have focused majorly on ionizing irradiation in prevention of mycotoxins in food (Calado *et al.*, 2014; and Khaneghah *et al.*, 2020). Use of Ionizing irradiation in food is also referred to as cold pasteurization because sterilization of food takes place at low temperatures where no heat is involved (Calado *et al.*, 2014). Irradiation can be used in controlling mycotoxins in maize (Mohamed *et al.*, 2022). Several International organizations consider 10 kGy effective recommended dose that is safe on food (Joint FAO/IAEA/WHO Expert Committee, 1981) and is recommended for elimination of pathogenic microbes is recommended in food (Mossel, 1979).

Ionizing radiation includes electromagnetic rays such as x and gamma rays and particles such as electrons (E-beam) and neutrons. Food irradiation is uncommon especially in Sub- Saharan Africa since the commercial irradiation facilities are not available due to the high costs of installation. Additionally, food irradiation is being perceived negatively due to nuclear irradiation involved. However, there are several efforts by the International Atomic Energy Agency (IAEA) to encourage safe use of nuclear energy in promoting food safety and security. Gamma irradiation by use of ^{60}Co, is more desired in the food industry because its high penetration power (Calado *et al*., 2014). Gamma irradiation dose of 3.5 kGy eliminated total fungi on fruits (Aziz & Moussa, 2002). However, mycotoxins are known to be more radioresistant than fungi that produce them. While low doses of gamma could kill fungi doses of gamma as high as 20 kGy were found to not cause any significant reduction in aflatoxin B_1 in maize, wheat and soybean (Hooshmand & Klopfenstein, 1995). Even higher doses of up to 60 kGy were found not completely degrade ochratoxin A and aflatoxin in black pepper (Jalili *et al*., 2010). Vegetative bacteria, fungi and yeast are known to be less resistant to gamma irradiation compared to bacterial spores which are less resistant to gamma irradiation than viruses (Harrell *et al*., 2018). More recent advancement in use of gamma irradiation has been in the use of sterilizing male fruit flies in sterile insect techniques (Bakri, *et al*., 2016). Despite the high penetration power of gamma irradiation, its use in the food industry is becoming less popular because of high cost of ^{60}Co, projected future shortage of supply of ^{60}Co, and tightening international restriction on shipment of radioactive sources. Thus, machine-based sources like E-beam are becoming more popular since they are machine sources they can be turned on and off when needed.

E-beam are generated from electricity using linear accelerators and can be used in two direction to achieve sterility within shortest time possible (Kyung *et al*., 2019). Apart from the food industry, E-beam has been tested on floriculture (Kwon *et al*., 2020), used in preservation of culture heritage materials that are biodegradable (Chmielewska-Śmietanko *et al*., 2018). Items like books, documents, and wooden artefacts can be preserved using E-beam irradiation (Capraru, *et al*., 2023). E-beam has also been used in polymer modification (Puhova *et al*., 2015) thus strengthening plastic used to case car cables. More recent use of E-beam has been in medical sector, sterilization of personal protectives equipment especially during the high demand

covid-19 period, sterilization of vaccines and medical devices. E-beam irradiation has been used in improving food safety by reducing aflatoxin levels in maize by up to 75% (Rogovschi *et al.*, 2009). In another study involving split beans, E-beam irradiation successfully eliminated fungal pathogens such as *A. niger, A. flavus* and *P. chrysogenum* and prevented formation of aflatoxin and ochratoxin after storage at a dose of 10 and 2.5 kGy respectively (Prabhavathi *et al.*, 2014). In soybean, 26 kGy of E-beam was effective in decontamination and had minor to negligible change in the physico-chemical characteristics of the soybean compared to 20 kGy of gamma irradiation which incapacitated germination of the soybean, increased the oxidation of lipids, reduced scavenging activity of radicals, decreased carotenoid level and maximized oxidation of lipids (Kikuchi *et al.*, 2003). Other studies show that when equal doses of high energy E-beam irradiation and gamma are used, gamma is more efficacious than E-beam causing one more log reduction of coliforms, total coliphage and total microflora in waste water (Farooq *et al.*, 1993). E-beam has also been used in inducing higher frequency of desired mutations than gamma irradiation in rice (Gowthami *et al.*, 2021). Furthermore, E-beam has been used extensively in controlling insect pests for phytosanitary purposes: Low E-beam doses of 0. 2 kGy have been found to inhibit egg hatching in female oriental leaf worm moth; while lower E-beam doses of 0.15 and 0.1 kGy destroyed the larva of the moth and prevented hatching of the eggs respectively (Yun *et al.*, 2014). In food safety E-beam has been used as a better substitute to reduce the effects of fungi and their toxins. In Canavalia maritima beans contaminated with *A. flavus, A. niger, A. ochraceus,* and *P. chrysogenum,* an E-beam dose of 10 kGy was effective elimination of fungal contamination and thus increased the shelf life of the beans (Supriya *et al.*, 2014). Additionally, a 6 kGy dose of E-beam was able to inhibit *Phakopsora pachyrhizi*, that causes soybean rust (Villavicencio *et al.*, 2007). A lower dose of E-beam at 3 kGy used on almonds, eliminated *Aspergillus* species on the almonds, while maintaining the sensory characteristics (Sánchez-Bel *et al.*, 2005). Similarly, elimination of *Fusarium* species has been reported in barley treated with 6 and 8 kGy of E-beam which also had minimal effect on malting of the wheat (Kottapalli *et al.*, 2006). In an experiment involving lotus seeds irradiated with an E-beam dose of 10 kGy and later sown Czapek Dox and dichloran glycerol containing media, fungal population was significantly reduced on the testa (Bhat *et al.*, 2010).

High irradiation doses may affect micro molecules of food such as vitamins and even higher E-beam doses above 10 to 30 kGy can degrade gossypol and further reduce gossypol content in poultry feed (Calado *et al.*, 2014). Furthermore, E-beam can also enhance *Macuna* seed properties like absorption capacity of oil and water, gelling and forming properties, improves linoleic acid level, digestibility of protein, carbohydrates and crude proteins (Bhat & Sridhar, 2008). The pharmaceutical and nutritional use of *Mucuna* seeds have also been enhanced by use of ionizing irradiation (Bhat *et al.*, 2007). E-beam irradiation on sorghum and *Mucuna* seeds did not result in any change in the ash, fiber, cell wall and protein content (Shawrang *et al.*, 2011). However data on use of E-beam irradiation on cereals and products derived from cereals is very scanty (Freitas-Silva *et al.*, 2014). Most studies prioritized elimination of fungi as the primary objective, mycotoxins degradation has been a secondary objective in the study of efficacy of E-beam irradiation in cereals (Calado *et al.*, 2014). Like in the study by Nemtanu *et al.* (2014) showed efficacy of 4.8, 2.5 and 1.7 kGy of E-beam in reduction of *Aspergillus*, *Fusarium* and *Penicillium* species in maize. In another study that compared the effect of E-beam and ozone in reducing zearalenone and ochratoxin A, 16 kGy of E-beam reduced ochratoxin A and zearalenone by 90% and 92.76% respectively (Yang *et al.*, 2020). Similarly, in a study by Supriya *et al.* (2014) aflatoxin B1 and B2 were completely reduced by an E-beam irradiation dose of 10 and 15 kGy while 2.5. to 5 kGy resulted in a partial reduction. In a similar study involving slight lower doses of E-beam at 1.5 kGy degradation of aflatoxins in almonds was observed (Lanza *et al.*, 2013).

E-beam technology is not common in Sub-Saharan Africa due to the initial cost in setting up a facility which ranges between 8.75 to 10.75 million US dollars. Tanzania will be the first country in East Africa to set up a commercial E-beam facility for industrial application in the food and medical industry. For a country to set up an E-beam facility, (i) there is need to carry out a feasibility study, (ii) create necessary infrastructure as outlined in the IAEA guideline, (iii) develop a business plan, (iv) secure project finance. A commercial E-beam facility in Pakistan has enabled penetration into the US market for Pakistani mangoes. E-beam has a potential in chlorophyl breakdown by suppressing chlorophyll degrading peroxidase and pheophytinase which degrade chlorophyll (Nguyen *et al.*, 2022).

CHAPTER THREE

EFFECT OF FARMER PRACTICES ON THE POPULATION OF *ASPERGILLUS* AND *FUSARIUM* SPECIES AND RESULTING AFLATOXIN AND FUMONISIN CONTAMINATION OF MAIZE

3.1 Abstract

Pre-harvest and post-harvest farmer practices of maize are an important predisposing factor to attack by *Aspergillus* and *Fusarium* resulting in accumulation of aflatoxin and fumonisin. This study evaluated the effect of farmer practices on the population of *Aspergillus* and *Fusarium* and resulting aflatoxin and fumonisin contamination in maize. The research was carried out during the short rain season of 2019/2020 in six counties in Eastern Kenya: Embu, Meru, Tharaka Nithi, Machakos, Makueni and Kitui. Ninety-nine farmers who had not received any aflatoxin mitigation training were selected uniformly in the six counties and a structured questionnaire on farmer practices was administered to each of the farmers and 99 maize samples were collected at harvest. The maize samples were shipped to the regional mycotoxin laboratory, dried under the sun for seven days while packed in kakhi bags and later moisture content was determined and those samples that had greater than 13% moisture content were further dried in an oven at 45 ^{o}C for 48 hours to reduce the moisture content to 13%. A quarter kilogram sample was subsampled from each of the 99 samples that were double packed in zip lock bags and shipped to the University of Illinois for microbial analysis by plating method and mycotoxin analysis using ELISA method. The farmers mostly planted improved varieties (45.2 %), intercropped maize with legumes (83.7 %), used organic manure (45.5 %) and preferred tilling their lands by hand (52.6 %), and after harvesting most stored their maize in the house on wooden structures (65.6 %). The maize samples were found to be more contaminated with fumonisins (mean = 2.2 µg/g) and *Fusarium* spp. (mean = 4.6 log CFU/g) than with *Aspergillus* (mean = $\leq$ 2 log CFU/g) and aflatoxins (mean = 12.7 ng/g). Farmers that used tractors for tillage had high levels of aflatoxin in maize ($p = 0.03$, $r = 0.85$). Similarly, farmers who used mixed maize varieties (improved and local) and the high levels of fumonisin ($p = 0.02$, $r = 0.87$). However, there was no significant correlation between the farmer practice and population of fungi in maize. Hence this study shows that farmer practices especially use of mixed varieties and tillage by tractors on the farm affect the accumulation of the mycotoxins during pre-harvest phase. More investigation on other factors that may affect the population of mycotoxigenic fungi is recommended. There is need for

policy interventions that facilitate farmers with necessary agricultural inputs.

3.2 Introduction

Poor agronomic practices are a contributing factor to accumulation of mycotoxins in crops in the field and at storage. Contamination of crops by toxigenic fungi starts in the field and continues during storage. Once a crop is infected by toxigenic fungi, the growth and yield of the crop is affected thus leading to loss of market value (Kumar *et al.*, 2021). Good agronomic practices like proper tillage and fertilization increase plant yield (Haarhoff & Swanepoel, 2018). Thus, depriving maize crops of these good practices can result in stress to the plant which is a pre-disposing factor to attack by toxigenic molds. Molds can enter a maize plant via roots, pollens, silk or through wounds made by insects or physical damage (Rankin & Grau, 2002). The previous crop, host cultivars, and application of fungicides have also been shown to cause accumulation of mycotoxins in cereals (Wegulo, 2012).

Most small-scale farmers tend to ignore good agricultural practices due to lack of training on sanitary practices at farm level. Furthermore, more than 70% of small-scale farmers use their produce for home consumption, therefore making it hard for mycotoxin monitoring and regulation (Garrido-Bazan *et al.*, 2018). There is a tendency for farmers to recycle planting seeds by acquiring them from neighbors, family members or a previous crop that may be infected with toxigenic molds (Centre for Food Safety, 2023). Use of farmer-saved seeds is cost effective compared to planting of certified seed although purity and quality is guaranteed with certified seeds (Furtas, 2018). In addition to this, farmers mostly prefer to recycle local varieties due to their taste, tolerance to abiotic stress and yield ability (Sibiya *et al.*, 2013).

Molds are known to remain in soil and crop residues thus causing infection and mycotoxin production in the next cropping season (Juraschek *et al.*, 2022). It is important for farmers to do tillage and crop rotation to reduce the risk of infection (Rankin & Grau, 2002). Furthermore, crop stress has been related to fungal infection and mycotoxin accumulation (Ferrigo *et al.*, 2014). Keeping the plant irrigated and ensuring nutrient supply to the crop by adding proper amendments is key. Lastly, proper sanitation at harvest, drying and storage is necessary otherwise non-infected crop can get contaminated at harvest. Some farmers tend to put cobs directly to soil without using sheets, dry the cobs on bare soil and store the maize in unkept

stores/granaries infested with insects and rodents thus can cause fungal infection.

3.3 Materials and Methods

3.3.1 Description of study area and sample collection

The survey was conducted in upper and lower Eastern Kenya in Embu, Meru and Tharaka Nithi, Machakos, Makueni and Kitui Counties (Fig.1) during the short rain season of 2019/2020. A list of villages in maize-growing sub-counties within these counties was generated with help from local agricultural extension officers and village elders based on the criteria that the farmer grows maize, has 0.5 to 5 acres and was willing to participate in the study. For sample village selection, randomization was stratified at county and subcounty level (Elfil & Negida, 2017). A total of 729 villages were stratified and 99 villages were selected. Random numbers were assigned in ascending or descending order to the farms in each village within the sub counties and one farm was selected per village based on a predetermined number. Samples were collected at harvest randomly from villages in the six counties across different agroecological zones which include: Upper midland I, II, III and IV; Lower midland III, IV, and V and low land VI. At least 100 maize cobs were collected in a uniform zigzag manner in a farm for farms up to 5 acres, this was adopted from method used by Mohamed (2016) where 20 to 25 maize cobs were collected from each acre of maize farm. The 100 cobs made approximately 20 kg of kernels after being shelled manually by hand and sub divided by quartering method to obtain 1kg sample for laboratory analysis.

3.3.2 Determination of maize farmers production practices in Eastern Kenya

A questionnaire on farmer production practices was programmed on a surveyCTO software (Appendix 1) and was administered to each farmer during harvesting of maize. A baseline survey questionnaire focused on farming practices such as use of soil amendments, ploughing method, maize variety planted, cropping system and storage facilities. Questionnaire was administered by direct questioning of the farmers in English and translation to local vernacular language was done by extension officers to farmers who did not understand English.

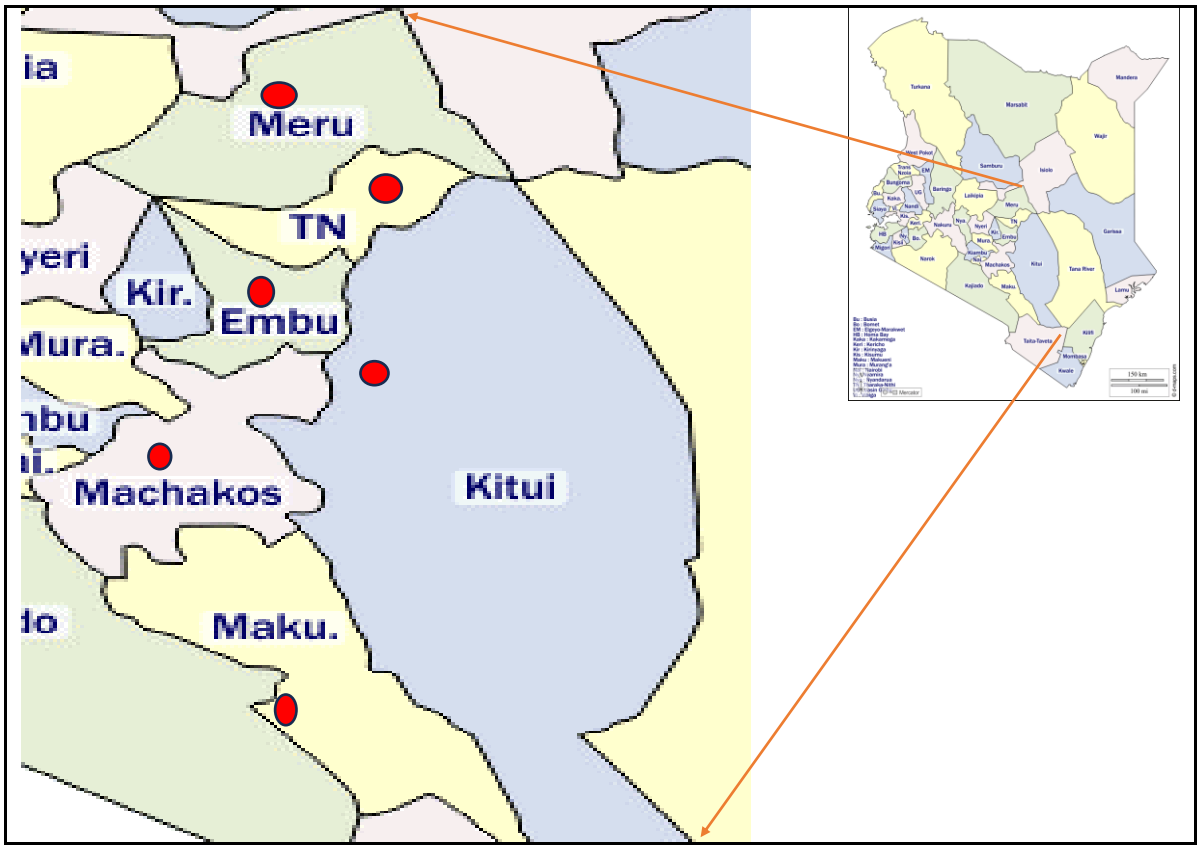

Figure 1: Map of Kenya showing six study counties (marked with a red circle) (D-map.com, 2023)

3.3.3 Preparation and shipment of maize sample for analysis

Ninety-nine shelled maize samples collected from farmers' fields were packed in kakhi bags in a cooler box and shipped to the Regional Mycotoxin Laboratory in Kenya Agricultural Livestock and Research Organization (KALRO) Katumani. Moisture content of the maize samples was measured using a moisture meter (Intratec 1241 analyzer, Foss, Denmark) with a temperature range of 0 - 42 ^{o}C. Samples with moisture content greater than 13% were oven dried at 45 ^{o}C for 48 hours. Two hundred grams of each sample selected were labeled, double packed and stored at 4 ^{o}C prior to shipping to the University of Illinois, USA, for toxin, microbial, single-kernel and irradiation analysis. Two hundred and fifty grams of each kernel sample (n = 99) was exported to University of Illinois under United States Department of Agriculture (USDA) permit according to all applicable regulations.

3.3.4Aflatoxin and fumonisin analysis of maize samples

At the University of Illinois in the Agricultural Bioprocessing Laboratory, 100 g of each sample were ground using a laboratory grinder (Perten Instruments, model 3170, USA). The sample was manually shaken to achieve homogeneity within the sample. Aflatoxin and Fumonisin levels were determined manufacturer-validated ELISA kit methods (Total aflatoxin ELISA Quantitative and fumonisin ELISA Quantitative, Helica Biosystems Inc., Santa Ana, CA). The kit method was based on competitive ELISA where antibodies mounted on the wells were sites for competition between aflatoxin in sample and aflatoxin conjugates provided in the kit. Five grams of each ground maize sample were mixed with 25 ml of 80 % methanol and mixed for 3 min in an orbital shaker (Thermo Scientific MaxQ 4450 Orbital Shaker Incubator, Florida, USA). The protocol on manufacturer's kit was adopted and samples that had concentrations above the limit of detection (> 20 ng/g for aflatoxins and 6000 ng/g for fumonisin) re-mixed in 80% methanol and analyzed again. A further dilution of sample to methanol at a ratio of 1:40 was done for fumonisin analysis. A standard curve developed for each plate was used to estimate aflatoxins and fumonisin in the samples. Standards for aflatoxin ranged from 0 -4 ng/g while for fumonisin ranged from 0 – 6 µg/g and R^2 values of 0.99 for standard curve was achieved thus validating the kit's protocol was followed accurately.

3.3.5 Isolation and identification of *Aspergillus* and *Fusarium* from maize samples

Aspergillus and *Fusarium* isolation in maize was done on Potato Dextrose Agar medium (PDA) amended with antibiotics. The isolation media was prepared by weighing 39 g of PDA into 1 liter of water and sterilized by autoclaving at 121 ^{o}C and 15 PSI pressure 20 minutes. After allowing the media to cool to about 50 ^{o}C, 2.5 ml/L streptomycin and chloramphenicol were added to restrict growth of bacteria. One gram of the ground sample was mixed with9 ml sterile deionized water in a 50 ml centrifuge tube and diluted serially up to dilution 10^{-3}. Culturing of the fungi was done by spread plate method where the sample (50 µl) was inoculated on the media, spread using glass spreaders andplates were incubated at 31 ^{o}C for five days and viable fungi counts were counted and calculated as CFU/g.

CFU/g = (Number of colonies on the plate * Dilution Factor)/ Volume inoculated

For identification of *Fusarium* spp morphological features were used (Leslie & Summerell, 2006), individual colonies were transferred to low strength PDA made by measuring 17 g of

PDA in one liter of de-ionized water, then 1 g of Potassium dihydrogen phosphate (KH_2PO_4), 1 g of Potassium nitrate (KNO_3), 0.5 g Magnesium sulphate anhydrous ($MgSO_4$), 0.5 g Potassium chloride (KCl), was added and stirred. Thereafter autoclaved at 121 ^oC, 15 PSI for 20 minutes. For *Aspergillus* spp. identification, colonies that appeared greenish yellow were transferred into 5/2 agar prepared by stirring 50 ml of V8 juice with 950 ml of de-ionized water and mixed with 20 g of agar. Thereafter, the media was autoclaved at 121 ^oC, 15 PSI for 20 minutes. The genus *Aspergillus* and *Fusarium* was identified based on cultural and morphological characteristics.

3.3.6 Data analysis

Open-source R software version 4.1.3 was used in data analysis. To assess the effect of farmer practices on population of *Aspergillus*, *Fusarium*, resulting aflatoxin and fumonisin contamination of maize, Pearson correlation was calculated among the proportion of farmers' doing a certain practice, toxin levels and fungal population. Means of aflatoxin, fumonisin and fungal population were analyzed using analysis of variance test and means separated by Tukey test at 95% confidence interval. Chi-square test of goodness of fit was also done on the proportion of farmers carrying out each of the production practices.

3.4 Results

3.4.1 Maize production practices in Eastern Kenya

A majority (45.2 %) of the farmers in Eastern Kenya preferred to plant improved maize, followed by local varieties (43.7 %) while a few (11.1 %) preferred to mix local and improved varieties (Table 1). Intercropping of maize with other crops was preferred by most farmers across Eastern Kenya compared to pure stand maize. A majority of farmers preferred using only organic manure followed by those that used inorganic only and then those that preferred to use mix both organic and inorganic manure. However, a few (16.5%) farmers did not use any soil amendment. Tilling was mainly done by hand and oxen plough while a few farmers used tractors. Most of the farmers (65.6 %) kept their crops in the house on wooden structure after harvesting, a few others used granaries while a small proportion stored their crop in the house on the floor as shown in Table 1.

Table 1:Agronomic practices carried out by small-scale farmers in Eastern Kenya

County	Embu	Kitui	Machakos	Makueni	Meru	Tharaka Nithi	Average	X^2 -value	P-value
Agroecological zone	LMIV, UMIV	LMIV &V, LVI	LMIII,IV,V, UMIV &V	LMIII&IV, UMIII&IV	UMI, II&III, LMIII	LM IV&V, UMIV			
Agronomic practices									
Maize varieties									
Local	75.0	50.0	33.3	59.0	20.0	25.0	43.7	14.4**	<0.001
Improved	25.0	50.0	38.1	28.2	80.0	50.0	45.2		
Mixed	0.0	0.0	28.6	12.8	0.0	25.0	11.1		
Cropping system									
Intercropping	100.0	83.3	95.2	79.5	44.0	100.0	83.7	26.1**	<0.001
Pure stand	0.0	16.7	4.8	20.5	56.0	0.0	16.3		
Soil amendment									
organic manure	75.0	66.7	38.1	64.1	4.0	25.0	45.5	13.3*	0.004
Inorganic manure	0.0	0.0	38.1	10.3	64.0	50.0	27.1		
Mixed	0.0	0.0	14.3	10.3	16.0	25.0	10.9		
No amendment	25.0	33.3	9.5	15.4	16.0	0.0	16.5		
Tillage method									
Hand	50.0	100.0	23.8	20.5	96.0	25.0	52.6	28.7**	<0.001
Oxen	25.0	0.0	76.2	79.5	4.0	75.0	43.3		
Tractor	25.0	0.0	0.0	0.0	0.0	0.0	4.2		
Storage method									
Granary	25.0	16.7	52.4	41.0	12.0	25.0	28.7	29.9**	<0.001
House on wooden structure	75.0	66.7	42.9	53.8	80.0	75.0	65.6		
House on the floor	0.0	16.7	4.8	5.1	8.0	0.0	5.8		

3.4.2 Aflatoxin, Fumonisin, *Aspergillus* and *Fusarium* levels in maize in Eastern Kenya

The mean aflatoxin level in Eastern Kenya was slightly above the Kenya Bureau of Standards (KEBS) regulatory threshold (< 10 ng/g). Embu County had the highest mean aflatoxin level while Meru had the least mean (Table 2). The mean aflatoxin in Embu County was significantly different from Meru and Makueni. For fumonisins, the mean level was higher than the KEBS threshold (< 1 µg/g). There was no significant difference across the six counties in the mean levels of fumonisin. Machakos County had the greatest mean fumonisin level while Tharaka Nithi had the least. Whereas for the fungal load, the means of *Fusarium* was not significantly different across the six counties while *Aspergillus* was rarely isolated as shown in Figure 2 and Table 3 because *Aspergillus* is mostly a storage fungi.

Table 2: Mycotoxin levels and fungal load in maize samples collected in Eastern Kenya

County	**Aflatoxin (ng/g)**		**Fumonisin (µg/g)**		***Aspergillus* load (log CFU/g)**		***Fusarium* load (log CFU/g)**	
	Mean	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
Embu	31.6[a]	0.7-123.3	1.8[a]	0-3.7	<2	<2-4.3	4.3[a]	3.3.-5.3
Kitui	13.6[ab]	0.7-64.9	1.1[a]	0-4.2	<2		4.3[a]	3.6-4.6
Machakos	8.8[ab]	0.8-89.4	4.1[a]	0-34.5	<2	<2-3.9	5.1[a]	5.3-5.1
Makueni	2.9[b]	0.2- 50.7	1.5[a]	0-31.7	<2		4.7[a]	3.3-5.7
Meru	2.5[b]	0.2-18.6	4.2[a]	0-53.9	<2	<2-4.3	4.8[a]	3.3-6.0
Tharaka Nithi	16.9[ab]	1.3-42.1	0.7[a]	0-1.8	<2		3.3[a]	<2 -3.3
Mean	**12.7**		**2.2**		**<2**		**4.6**	

LOD < 2 log CFU

Similar letter superscript indicates means were not significantly different at p=0.05 within same column analyzed by ANOVA and Tukey HSD test

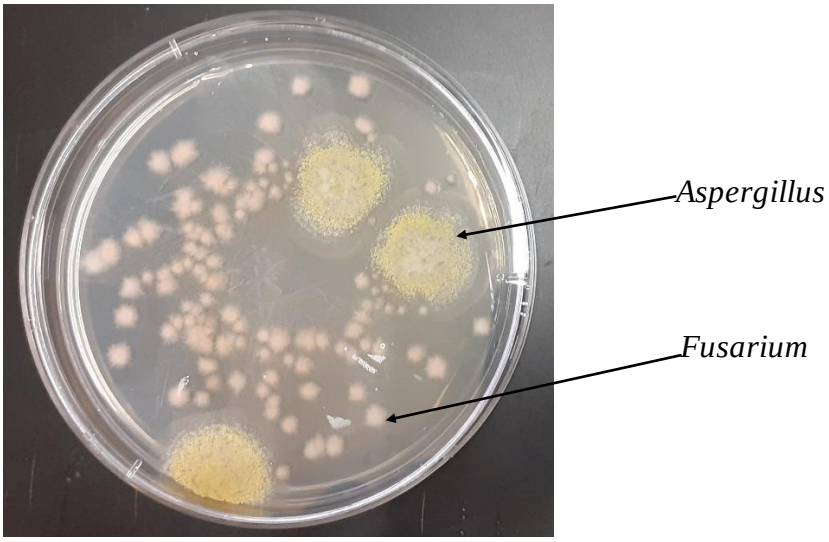

Figure 2: *Aspergillus* and *Fusarium* spp. isolated from maize on PDA (after 5 days of incubation)

3.4.3 Correlation among farmer production practices, mycotoxin levels and fungal load

The increase in the use of tractors increased as the levels of aflatoxins in the harvested maize from Eastern Kenya increased. There are two parameters correlated positive ($p= 0.03$, $r=0.85$) as shown in Table 3. Similarly, the levels of fumonisin were also directly related to the increase in use of mixed varieties of maize (planting both local and improved maize varieties in the same field at the same time). However, there was no association between the farmer practices in Eastern Kenya and population of fungi in maize as shown in Table 3.

Table 3: Correlation among agronomic practices, mycotoxins level and fungal load

Agronomic practices	Aflatoxin		Fumonisin		CFU of *Fusarium* and *Aspergillus*	
	R-value	P-value	R-value	P-value	R-value	P-value
Maize varieties						
Local	+0.58	0.22	-0.34	0.51	+0.07	0.89
Improved	-0.29	0.57	-0.21	0.69	+0.00	0.99
Mixed	-0.50	0.32	+0.87	0.02*	-0.11	0.83
Cropping system						
Intercropping	-0.06	0.91	+0.69	0.13	-0.43	0.40
Pure stand	+0.06	0.91	-0.69	0.13	+0.43	0.40
Soil amendment						
organic manure	+0.28	0.58	-0.19	0.72	+0.01	0.99
Inorganic manure	-0.26	0.62	+0.28	0.58	-0.03	0.96
Mixed	-0.44	0.38	+0.53	0.28	-0.30	0.57
No amendment	+0.30	0.56	-0.67	0.15	+0.28	0.59
Tillage method						
Hand	+0.02	0.97	-0.61	0.19	+0.12	0.83
Oxen	-0.25	0.64	+0.63	0.17	-0.09	0.87
Tractor	+0.85	0.03*	-0.14	0.79	-0.09	0.86
Storage method						
Granary	-0.08	0.88	+0.51	0.31	+0.40	0.43
House on wooden structure	+0.27	0.61	-0.34	0.51	-0.55	0.25
House on the floor	-0.43	0.40	-0.45	0.37	+0.30	0.56

*Significant correlation at P<0.05.

3.5 Discussion

3.5.1 Farmer agronomic practices in Eastern Kenya

This study found that farmers in Eastern Kenya mostly preferred using improved maize varieties compared to local varieties; and intercropped the maize with other crops. The high preference in the use of organic manure compared to inorganic manure, use of hand tillage and storage of grains in the house on wooden structures has also been reported in this study. The findings concur with a recent study conducted in Bomet, Narok, Kirinyaga, Embu, and Nakuru Counties in Kenya where 87% of farmers were found planting improved varieties (Njeru *et al.*,

2022).Earlier studies in Eastern Kenya demonstrated that 71% (Ouma & De Groote, 2011) and 66.7 % (Maina *et al.*, 2017) of the farmers planted improved maize varieties while 31% planted local varieties. Similarly, in a recent study in Western Kenya, up to 61% of the farmers planted improved varieties (Almekinders *et al.*, 2021). On the contrary, a previous study in western Kenya indicated farmers preferred planting local varieties hence underwent late harvesting and striga pest infestation (Wambugu *et al.*, 2012, Njeru *et al.*, 2019). There have been many campaigns carried out in Eastern Kenya on good agricultural practices which include the planting of improved varieties that produce high yields, resistant to pests and diseases and are tolerant to drought. Farmers have received improved seed varieties to boost their productivity (ICRISAT, 2020). Farmers prefer to use improved varieties because these varieties are commercialized and advertised on several forums that include demonstration fields, farmers' days, and agricultural shows. An earlier study in Eastern Kenya shows adoption of improved varieties is positively associated with per capita consumption expenditure (Ouma *et al.*, 2014). Farmers use improved varieties because they are easily available in agrovets, recommended by the local agricultural extension officers for their high yield and tolerance to abiotic stress. When farmers use improved varieties coupled with intercropping, they get high yields despite effect of climate change (CIMMYT, 2017).

A study by Mohamed (2016) in Lower Eastern Kenya showed that farmers preferred intercropping maize with legumes. Another study in Central Kenya, also showed a high yield in maize after intercropping with legumes (Mucheru-Muna *et al.*, 2011). Intercropping is advantageous as it maximizes the production capacity of the land and more so if legumes are used to intercrop cereals, they act as cover crops that helps to conserve soil moisture and prevent growth of weeds (Recha *et al.*, 2013). Furthermore, intercropping enables the farmer to obtain a variety of crops and in case of crop failure in one crop the farmer can obtain a harvest of the other. In addition to this, the crops that intercropped with maize are mostly consumed together with maize in Kenyan delicacies for example beans in the case of *githeri.*

In another study by Njeru *et al.*, 2019 farmers in Western Kenya were reported to mostly used

compost and farm manure. The use of organic manure improves soil structure, increases water infiltration and retention in soil, increases microbial activity (Recha *et al.*, 2013). Furthermore, it results in increased soil water and nutrient use in dryland farming while inorganic amendments cause soil organic matter loss, soil compaction, and changes in soil water and nutrients availability (Wang *et al.*, 2020). Farmers also tend not to use inorganic fertilizer due to the high cost and lack of government subsidies to bring down the cost (Njeru *et al.*, 2022). Organic manure is more commonly used because it is easily available from farm animals; is easy to use and is environmentally friendly. To increase crop yield, organic manure has been shown to be better than inorganic (Mugwe *et al.*, 2010).

Apart from producing organic manure, farm animals are also used in tilling the land although in this study, most farmers do hand ploughing because they have enough human resource assistance from family members. Furthermore, extension officers play a key role in promoting minimum tillage practices (Jena, 2022). Tilling in the current study was mainly by hand, some farmers used oxen plough while a few others used tractors. Hand tillage was also common in western Kenya (Njeru, 2019). However, the use of oxen for ploughing is more common in lower Eastern Kenya (Maina *et al.*, 2017) A study by Njeru, (2022) indicated that farmers in Narok County mainly used tractors for ploughing while farmers in Embu County preferred hand ploughing. Conventional tillage results in enlarged leaf area index of the maize plant, increased plant height, a high aboveground biomass, increased crop growth rate, and a high grain yield (Otieno *et al.*, 2020). Tillage by use of glyphosate has been shown to be effective in weed management compared to hand weeding (Ita *et al.*, 2014). Tillage by hand is selective especially where intercropping is done, the farmer removes only the weeds but no other intercropped crops.

Moreover, farmers in this study were found to mostly store their maize in the house on wooden structure while others used granaries, a few stored their maize in the house on bare floor. A previous study in Eastern Kenya also indicated a large proportion of farmers kept their maize in a room in the house (Njoroge *et al.*, 2019) while another study in Eastern Kenya indicated that majority of farmers (56.7 %) stored their maize in granaries while a few (43.3 %) stored their maize in the house (Maina *et al.*, 2016).However, in North-west Kenya farmers mainly stored

their maize in cribs in the house where there is minimized loss of maize compared to other storage structures due to close monitoring (Komen *et al.*, 2010). In traditional structures, up to 59.48% of losses in maize occurs (Costa, 2014). Storage of the grain in the house is common for easy accessibility and farmers prefer to use wooden racks because of the trainings they receive from extension officers and previous experience of a shorter shelf life when grains are put directly on the floor without wooden racks.

3.5.2 Population of *Fusarium* and *Aspergillus* and fumonisins and aflatoxins levels in mature freshly harvested maize

This study showed that the maize samples obtained from standing crop in Eastern Kenya were more contaminated with fumonisins than aflatoxins. Similarly, the population of *Fusarium* was higher than *Aspergillus* in the samples. Other studies done on mycotoxin levels in standing crop indicate similar findings where the fumonisin levels were more pronounced than aflatoxin levels in western Kenya (Njeru *et al.*, 2019) and Zambia's freshly harvested maize (Mukanga *et al.*, 2010). Similarly, a study on Tanzanian pre-harvest maize also showed a high fumonisin contamination of 52% of the samples compared to 18% contaminated with aflatoxin (Kimanya *et al.*, 2021). The two mycotoxins are of great economic importance and although they co-occur together in most cases, especially in maize that has ear rot disease, fumonisin contamination is often higher. This may be attribute to the fact that *Fusarium* are the main pathogens in maize ear rots (Rosa Junior *et al.*, 2019). However, this is always not the case, a study on prevalence of both aflatoxin and fumonisin in Nigeria indicated more samples (21%) contained more aflatoxin compared to fumonisins (13%) above the US threshold (Liverpool-Tasie *et al.*, 2019). Nevertheless, *Fusarium* spp. are more abundant compared to *Aspergillus* spp. in freshly harvested maize (Domenico *et al.*, 2015) and rice (Phan *et al.*, 2021). *Fusarium* spp. are mostly referred to as field fungi because they are abundant in the crop before harvest hence produce fumonisins in the crop while *Aspergillus* are referred to as storage fungi and mostly found contaminating crop at storage leading to accumulation of aflatoxins.

3.5.3 Correlation between farmer practice and level of fumonisin, aflatoxin, *Fusarium* and *Aspergillus*

This study shows that aflatoxins levels increased with the increase in the use of tractors for ploughing, likewise the levels of fumonisins increased with the increase in planting of mixed

varieties. However, there was no correlation between farmers' practice and population of fungi. This concurs with another study done in Tanzania, where only a few practice like crop rotation, storage practices and insect control strategies influenced the levels of mycotoxins (Degraeve *et al.*, 2016). Despite tractors being less labour intensive compared to hand ploughing, the use of tractors being a heavy machines might cause soil compaction thus causing water erosion and less absorption of water to the plant (Egrutz, 2018). The inability to access water by the plant may lead to stress thus plants become vulnerable to attack by opportunistic *Aspergillus* that cause aflatoxin production. Again, farmers normally hire tractors, and the tractor could be used in the whole village, thus when not properly washed or sterilized it could carry pathogens from one farm to another. A study by Recha *et al.* (2013) indicated that most of the farmers (95%) in lower Eastern Kenya got the local varieties of maize through informal ways for instance through exchange with neighbors, or from recycling their own reserved grains from previous harvest or obtain the seeds from local markets in the villages (Muhammad *et al.*, 2003) to cut down on the expenses incurred during production due to economic constraints. Farmers that use recycled seeds from previous season that may be infected with *Fusarium* may introduce the inoculum and thus favour persistence of *Fusarium* species across seasons. Local varieties are less vigor than improved varieties and may negatively affect the health of plants during growth. Furthermore, improved varieties of maize are more tolerable to disease and pests compared to local varieties (Sibiya *et al.*, 2013). When planted together with improved varieties, the local varieties may act as a source of inoculum for the improved varieties. Thus, farmer practices may have an impact on the toxin accumulation though not directly on the total population of fungi because the population of fungi is mainly affected by environmental conditions like temperature and humidity.

3.6 Conclusion

Farmer practices such as use of mixed varieties of maize (local and improved) and use of tractors increase fumonisin and aflatoxin production respectively. Pre-harvest farmer practices contribute to the accumulation of mycotoxins in freshly harvested maize but not the population of fungi (*Aspergillus* and *Fusarium* spp.). Other farmer agronomic practices like use of improved varieties did not correlate to mycotoxin accumulation in the grain in any way. Thus, pre-harvest

practices determine the mycotoxin accumulation produced in the grains prior to harvest. Fumonisins were the major mycotoxins compared to aflatoxins in grain prior to harvest.

CHAPTER FOUR

EFFECTIVENESS OF SINGLE KERNEL MULTI-SPECTRAL SORTING TECHNIQUES IN REDUCTION OF FUMONISIN AND TOXIGENIC *FUSARIUM* AND OTHER MICROBES IN MAIZE

4.1 Abstract

Fusarium species infect maize crops leading to production of fusaritoxins like fumonisin trichothecenes, and zearalenone by their toxigenic members. Elimination of microbes is critical in mitigating further post-harvest spoilage and toxin accumulation. Singl kernel spectral sorting technique eliminates bias in detection of mycotoxins as each kernel is screened individually to determine the level of contamination. The current study investigates the efficacy of a previously described multi-spectral sorting technique in the reduction of fumonisin and toxigenic *Fusarium* species and other microbes found contaminating maize kernels in Kenya. Maize samples (n = 99) were collected from six mycotoxin hotspot counties in Kenya (Embu, Meru, Tharaka Nithi, Machakos, Makueni and Kitui) and aflatoxin and fumonisin testing in the samples was performed using commercial ELISA kits. The levels of Aflatoxin in majority (91%) of the samples were below the 10 ng/g threshold set by the KEBS and therefore were not studied further. The 23/99 samples that had > 2,000 ng/g of fumonisin were selected for sorting. The sorter was calibrated using kernels sourced from Ghana to reject visibly high-risk kernels for fumonisin contamination using reflectance at nine distinct wavelengths (470-1,550 nm). Accepted and rejected streams were tested for fumonisin using ELISA, presence of toxigenic *Fusarium* using qPCR and other microbes by sequencing of ITS1F- ITS2 and V3-V5 regions of fungi and bacteria respectively. After sorting, there was a significant (p-value < 0.001) reduction of fumonisin, by an average 88.9 % (ranged between 27.6 to 99.8 % reduction) with a median rejection rate of 1.9 % (ranged 0 % to 48 %). The percentage infection with toxigenic *Fusarium* was significantly higher (p = 0.005) in maize kernels from the rejected streams compared to the kernels in the accept stream. Similarly, there was a significant decrease (p = 0.002) of 31 % and 90 % in total fungi and bacteria respectively in accepted maize kernels. This study demonstrates the use of a multi-spectral sorting as a potential post-harvest intervention tool for the reduction of *Fusarium* species and pre-formed fumonisin. The spectral sorting approach of this study suggests that classification algorithms developed from high-risk physical features on the kernels that are linked to mycotoxin can be applied across different sources of maize to reduce fumonisin. Furthermore, upscaling of the single kernel multispectral technique is recommended to serve large scale farmer, grain aggregators and government strategic food reserves.

4.2 Introduction

Fusarium species include plant pathogens that attack maize crops mainly while in the field (Kamle *et al*., 2019). Some *Fusarium* spp. produce mycotoxins such as trichothecene, fumonisin (Munkvold, 2017), and deoxynivalenol (DON) as secondary metabolites (Mansfield *et al*., 2005), which have negative health effects on humans and animals. They grow on maize ears and stalks and are characterized by whitish to reddish growth. The growth of *Fusarium* species is highly influenced by weather, particularly temperature and precipitation (Pfordt *et al*., 2020), and agronomic practices (Ariño *et al*., 2009). *Fusarium graminearum, F. verticillioides*, *F. proliferatum* and *F. temperatum* are the most common *Fusarium* species isolated from maize showing symptoms of ear rot, whereas *F. graminearum, F. equiseti, F. culmorum,* and *F. temperatum* are more commonly associated with rot that appears on the stalks of maize (Pfordt *et al*., 2020).

Fumonisins frequently contaminate maize and were first isolated and characterized in South Africa in 1988 (Marasas, 1995). There are more than 15 homologues of fumonisin (FB); with FB_1, FB_2 and FB_3 being the most common in food and maize (Braun & Wink, 2018). The ability to produce fumonisin is only seen within the toxigenic *Fusarium* species that have the biosynthetic gene cluster FUM, which consist of FUM_1 to FUM_{19} genes. FB_1 is the most relevant fumonisin due to it carcinogenic characteristics caused by consumption of the mycotoxin. It is categorized as group 2B carcinogen (International Agency for Research on Cancer (IARC), 1993) and has been directly associated to esophageal cancer (Yu *et al*., 2021). Furthermore, in humans, FB_1 consumption has been associated with increased risk for defects in the neural tube when consumed (Gelineau-van Waes *et al*., 2009), and growth impairment in children (Chen *et al*., 2018). FUM_1 is responsible for production of FB_1 (Glenn *et al*., 2008). There are at least ten toxigenic *Fusarium* species containing FUM_1, where *F. proliferatum* and *F. verticillioides* are the most common in food (FAO/WHO, 2001).

To minimize the health impacts of fumonisin consumption, governments and organizations have set maximum levels allowed in food. The fumonisin threshold put in place by the Joint FAO/WHO Expert Committee on Food Additives is 2 µg/g (FAO/WHO, 2002). In unprocessed maize, the threshold for fumonisin is 4 µg/g, while for processed maize flour it is 2 µg/g (Codex

Alimentarius, 1997). In Kenya, the Kenya Bureau of Standards (KEBS) has established a threshold of 1 µg/g for fumonisin in maize, a standard which is adopted from the World Food Program (Mutiga *et al.*, 2015). Recently, the East African Bureau of Standards harmonized the fumonisin threshold in East Africa for maize and peanuts and was set at 2 µg/g (Ankwasa *et al.*, 2021).

One physical approach to reduce fumonisin in feeds and food products is sorting (Deepa *et al.*, 2021; Chavez *et al.*, 2020). Mycotoxins are heterogenous in nature and therefore bulk analysis may lead to rejection of a safe consignment or acceptance of a contaminated consignment (Chavez *et al.*, 2020). Sorting techniques that employ single kernel analysis are a promising intervention; and range from traditional to mechanized methods. In South Africa, traditional hand sorting methods have been used to reduce fumonisin levels by 84 % in small-scale farmers' maize (van der Westhuizen *et al.*, 2010). Although this method is economical for resource-constrained subsistence farmers, it is time consuming and not efficient for bulk consignment. Therefore, mechanizing small-scale agricultural interventions is crucial (Stafstrom *et al.*, 2021b). The use of a relatively inexpensive optical sorter system could reduce both aflatoxin and fumonisin by 83 and 84 %, respectively, by use of reflectance of Kenyan maize kernels passing through a beam of visible and near infrared spectrum (Stasiewicz *et al.*, 2017). Furthermore, size sorting and drop sorting methods have also been shown to reduce fumonisin (Aoun *et al.*, 2020).

Earlier sorting studies have focused on reduction of pre-formed mycotoxins specifically aflatoxin and/or fumonisin, in maize. Data on efficacy of multi-spectral sorting in reduction of toxigenic fungi is scarce. The current study investigated the efficacy of a multi-spectral sorter in reducing, fumonisin, toxigenic *Fusarium* and other microbes in maize kernels from Eastern Kenya. This study serves as an advance over Stasiewicz *et al.* (2017) and Murithi (2014) where in both studies efficacy of single kernel was only tested on reduction of mycotoxins (aflatoxin and fumonisin) but not on mycotoxigenic fungi.

4.3 Materials and Methods

4.3.1 Single kernel multi-spectral sorting

Approximately, 100 g of each sample was sorted according to methods described by Stasiewicz *et al.* (2017) using a relatively low- cost multi-spectral sorter. The linear discriminant analysis

(LDA) algorithm software was used to calibrate the sorter to sort samples. The algorithm was programed to reject maize kernels that present high-risk visual characteristics and accept kernels with low risk. Kernels from Ghana were used for the reject and accept calibration set. For the reject calibration set, 150 g of each 10 samples were selected based on high levels of aflatoxin greater than 15 µg/kg and high fumonisin levels greater than 2 µg/g in their calibration pair set. From this sample set, all kernels that had Bright Green Yellow Fluorescence (BGYF) under ultraviolet (UV) light (365) were selected (about 20 kernels per sample). In addition, kernels that showed insect damage, discoloration, and signs of breakage were selected. 1000 kernels in total were selected for the reject calibration set. Likewise, for accept calibration set 1000 kernels were also selected. Kernels that had their pair with less than 15 µg/kg of aflatoxins level and less than 2 µg/g for fumonisin were randomly selected provided they did not have any physical damage or high-risk feature and that appeared to be healthy. Training of the LDA algorithm was done by using 4000 spectral observations that were generated by scanning each calibration set twice. Sensitivity (Sn, n high-risk positive kernels rejected /n high-risk positive kernels) and specificity (Sp, 1 – n high-risk positive kernels rejected /n high-risk positive kernels) were calculated. The training classification was able reject visibly high-risk (n=1000 kernels) and accept visibly low risk spectra (n=1000 kernels) at a 63% sensitivity and 90% specificity. Each 100 g sample was scanned through the multi-spectral sorter programmed in the sorting mode. The software initiated the removal of kernels with high-risk features by using compressed pressurized air at 60 psi to push out rejected kernels in accordance with the training algorithms. Out of the 99 samples tested for fumonisins and aflatoxins, 23 samples that had fumonisin level > 2 µg/g were selected and used in multi-spectral sorting analysis.

4.3.2 Fumonisin analysis of single kernel sorted samples

Individual samples from rejected and accepted stream of each sample were milled in a laboratory mill grinder for samples with mass >5 g (Mohamed *et al.*, 2022) while samples with mass <5 g were ground by using a bead beater at 30 Hz for 10 seconds to a fine flour in 2 mL centrifuge tubes using balls made of stainless steel (MM301 mill, manufacturer jars; Retsch Haan, Germany) as done by Stasiewicz *et al.* (2017). Fumonisins were extracted from the sorted 23 samples using 80% methanol, mixed with deionized water to obtain a sample to solvent ratio of 1:40. ELISA method was done according to manufacturer's instructions (Total aflatoxin ELISA

Quantitative and fumonisin ELISA Quantitative, Helica Biosystems Inc., Santa Ana, CA) as described in section 3.3.4.

4.3.3 Determination of reduction in toxigenic *Fusarium* in single kernel sorted samples

Glucose yeast extract Agar was prepared to subculture pure *Fusarium proliferatum* hyphae before DNA extraction. The pure colony of F. proliferatum was identified on PDA using its white fluffy morphology and production of a light purple pigment and confirmed by qPCR. The agar was prepared by adding 5 g of peptic digest of animal tissues, 5 g of yeast extract agar, and 2 g of glucose into 500 ml of deionized water. The pH was adjusted to 5 and the broth media autoclaved at 121 ^{o}C for 20 minutes. For toxigenic *Fusarium* quantification, qPCR method done by Bluhm *et al*. (2004) was adopted and slightly modified. FUM1 primers and probes were sourced from Life Technologies Corporation, USA Forward-

5'- CCATCACAGTGGGACACAGT-3'; reverse- 5'- CGTATCGTCAGCATGATGTAGC-3' and Taqman probe 5'- TCTCAAGGCCAGCCAAGGAGTCGGCGC-3'TAMRA which resulted in a 183 bp amplicon product after each cycle. To prepare the 20 µl of reaction mix (1X), 10 µl of TaqMan Fast Advanced Master Mix (Life Technology Corporation, USA) was mixed with 1 µl of 10 µM of each forward and reverse primer, 0.1 µl of 20 µM probe and 2.9 µl Nuclease free PCR water and later 5 µl of template DNA to be amplified was added in each tube. The reaction was carried out with a two-profile cycles: A pre-cycle of 50 °C for 2 min; 95 °C for 10 min followed by 40 cycles of 95 ^{o}C for 15 sec and 60 ^{o}C for 2 min each. Mixed sample standard curves were developed by method used in Mohamed *et al*. (2022). A mixed standard curve equation was used to extrapolate the amount of DNA of toxigenic *Fusarium* in the total extracted sample DNA. For *Fusarium proliferatum* DNA, the equation of the mixed standard curve was y=-3.374x+27.631 with an efficiency of 98%, R=0.99. To ensure inclusion of all qPCR requirements are listed the MIQE checklist was used (Bustin *et al*., 2009). The proportion of sample infected by toxigenic *Fusarium* was expressed as an infection coefficient (IC) was the percentage of fungal pathogen DNA over the total DNA in the sample.

4.3.4 Determination of reduction of other microbes in single kernel sorted samples

Nine samples (18 subsamples of reject and accept streams) out of the 23 sorted samples that had

>2 g of flour in the reject stream were used in microbiome analysis. DNA extraction was done using an IBI extraction kit protocol (IBI Scientific, Iowa, USA) with minor modification where one gram of each maize sample was used to obtain the DNA pure sample. DNA samples were analyzed by illumina sequencing for fungal and bacterial populations using ITS1F- ITS2 and V3-V5 regions respectively at the Biotechnology center in U of I. For fungal DNA sequencing for the ITS1F-ITS2 region was done using locus specific primers: 5'-CTTGGTCATTTAGAGGAAGTAA and 5'-GCTGCGTTCTTCATCGATGC while for bacterial DNA sequencing for V3_F357 -V5_R926 was done using locus specific primers: 5'-CCTACGGGAGGCAGCAG and 5'-CCGTCAATTCMTTTRAGT. To quantify the library, qPCR and sequenced on one MiSeq Nano flowcell was done for 251 cycles from each end of the fragments using a MiSeq 500-cycle sequencing kit version 2. The bcl2fastqv2.20 Coversion software (Illumina) was used to demultiplex the generated fastq files with reads of 250 nucleotides. Modern Illumina sequencing method generates high quality sequences and chances of spurious sequences is highly reduced (Edgar, 2017). During the MiSeq runs, PhiX DNA was used as a spike-in control and later removed for normal processing. PhiX genome was 3kb in length.

4.3.5 Data analysis

Open-source R software version 4.1.3 was used in analyzing the data. To determine the effectiveness of single kernel multi-spectral sorting technique in reduction of fumonisin and toxigenic *Fusarium* and other microbes in maize, rejection rate was expressed as a percentage of mass rejected over total mass of maize kernels sorted. Fumonisin levels in the bulk sample and accept stream were log transformed to cater for skewness and were compared by paired t-test where the mean difference between the fumonisin level in the bulk and accept stream was >1. For *Fusarium,* percentage infection was expressed as a percentage of toxigenic *Fusarium* DNA over total DNA extracted in each sample and a mixed standard curve was utilized for quantification as in Mohamed *et al* (2022); A paired t-test was also used to determine significant difference in percentage infection between reject stream and accept stream. Similarly, the abundance of fungal and bacterial populations was analyzed using a paired t-test in accept and reject samples. The abundance of data was derived from analyzing the raw sequence fastq files. Cutadapt was used to remove unwanted sequences like the adapters and primers were removed

using cutadapt (Martin, 2011). Dada2 algorithm was used to denoise and filter the DNA sequences (Callahan *et al.*, 2017). Trimming was done to remove unwanted sequence and improve the quality of the reads. Paired ended reads were then merged, sequences were inspected for chimeras, taxonomic assignment was done to the amplicon sequence variants then phyloseq package was used to visualize the operational taxonomic units.

4.4 Results

4.4.1 Mass of sample in sorted streams

A highly significant ($p < 0.001$) low portion of maize (average of 4.4 g) was rejected during single kernel multi-spectral sorting of a bulk sample of approximately 90 g (Figure 3). A large proportion of the sample was accepted (average of 84.4 g) translating to a median rejection rate of 1.87 %. The rejection rate ranged between 0 - 48 % depending on how contaminated with bad kernels the samples.

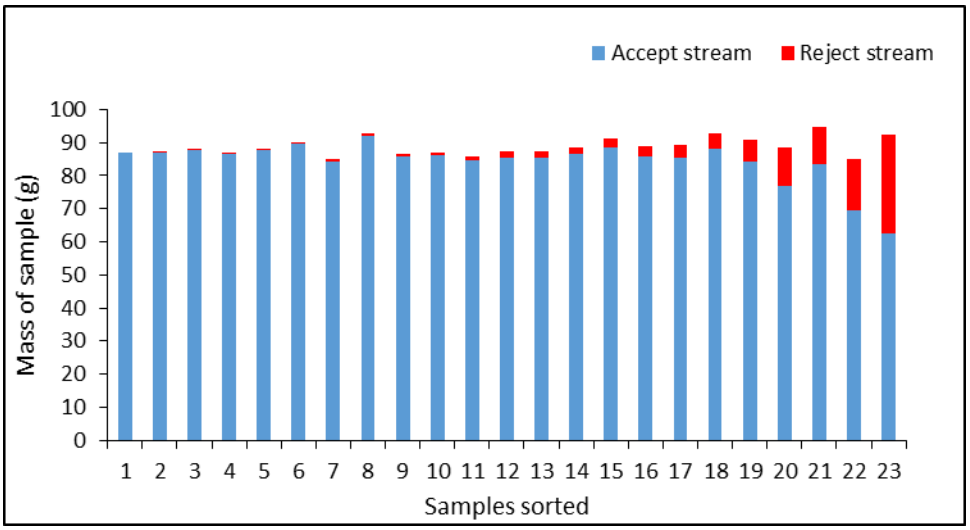

Figure 3: Mass of samples (g) in accepted and rejected stream after single kernel spectral sorting. Mass means of accepted and reject stream are significantly different at t = 28.886, df = 22, p-value < 0.001

4.4.2 Fumonisin levels in sorted streams

Fumonisin levels in the bulk sample were high before single kernel multi-spectral sorting. After single kernel multi-spectral sorting, the fumonisin levels in the accept stream were significantly reduced. Thus, showing a 2.05 log reduction in fumonisin as result of rejecting contaminated kernels that had high fumonisin level as shown in Table 4. The levels of fumonisin before sorting ranged between 3.3- 4.7 log µg/kg and the levels were reduced to a range of 0.0 -3.5 log µg/kg.

Table 4: Fumonisin levels (log µg/kg) in maize streams before and after single kernel multi-spectral sorting

Sample code	Before sorting	After sorting		Log reduction (Before sorting- Accept stream)
		Accept stream	Reject stream	
1	3.57	2.54	5.11	1.03
2	3.46	0.00	4.99	3.46
3	3.39	0.00	3.00	3.39
4	3.64	0.00	4.42	3.64
5	3.34	2.67	4.22	0.67
6	4.18	3.47	4.52	0.71
7	3.56	1.55	5.05	2.01
8	4.49	2.15	4.04	2.34
9	4.29	1.87	4.21	2.42
10	3.49	1.39	4.42	2.10
11	4.50	2.04	4.14	2.46
12	4.73	3.57	4.17	1.16
13	3.42	3.28	0.63	0.14
14	3.39	1.90	4.00	1.49
15	4.54	2.59	4.45	1.95
16	3.73	1.91	4.63	1.82
17	3.31	2.91	0.00	0.40
18	3.60	0.00	3.03	3.60
19	3.50	2.25	4.60	1.25
20	3.56	0.00	0.00	3.56
21	3.71	0.00	4.28	3.71
22	3.38	3.22	4.99	0.16
23	3.74	0.00	0.00	3.74
Mean	**3.76*** **	**1.71**	**3.60**	**2.05**

***Significant FUM reduction (2.05 log reduction, t = 8.3, df = 22, p-value = < 0.001) in the accept streams. Levels of fumonisins were determined by ELISA method and were log transformed..Acceptable fumonisin level in East Africa is log 3.3

4.4.3 Population of toxigenic *Fusarium* and other microbes in single kernel multi-spectral sorted samples

4.4.3.1 Population of toxigenic *Fusarium* in sorted streams

The proportion of toxigenic *Fusarium* in the accept stream were significantly reduced ($p = 0.005$) compared to the accepted stream as shown in Table 5. The proportion of toxigenic *Fusarium* correlated positively ($p < 0.001$, $R = 0.7$) to the level of fumonisin in the sorted streams.

Table 5: Toxigenic *Fusarium* biomass percentage infection in sorted maize streams.

Sample code	Toxigenic *Fusarium* percentage infection (%)		Percentage reduction (%)
	Accept stream	Reject stream	
MS13	2.59	22.49	19.9
MS16	0.17	76.51	76.34
MS25	0.93	11.36	10.43
MS31	2.13	70.41	68.28
MS34	5.19	41.17	35.98
MS36	0.34	20.19	19.85
MS39	0.32	28.44	28.12
MS119	0.23	3.79	3.56
MS122	0.26	3.30	3.04
Mean	1.35	30.85**	29.5

** Means of percentage infection of maize with toxigenic *Fusarium* in the accept and reject stream were significantly different (t = 3.3, df = 8, p-value = 0.005). Percentage infection (pathogen/Total DNA ratio) of toxigenic *Fusarium* DNA in maize DNA(n=18)

4.4.3.2 Population of other microbes in the sorted streams

The total fungal abundance (Table 6) was significantly lower (p-value = 0.002) in the accepted stream compared to rejected stream. Fungi were fewer in the accepted maize kernels, while in the rejected stream the fungi were significantly higher. The difference in the fungal abundance in accepted stream was lower than in the reject stream by a mean of 31 %. Similarly, the total bacterial abundance was significantly lower (p-value = 0.002) in the accepted stream than rejected stream. Bacteria were fewer in accepted maize kernels than in the rejected maize kernels. The total bacterial abundance was reduced in accepted stream by a mean reduction of 90 %, the reduction ranged from 85.5 to 100 %.

Fusarium, Aspergillus and *Candida* were the most abundant fungi in both reject maize stream. The abundance of total *Fusarium* was significantly lower (p = 0.001) in the accept compared to reject streams. The abundance of other fungi between the reject and accept streams did not differ significantly. For bacteria, *Enterobacteriaceae Yersiniaceae* and *Burkholderiaceae* were the most abundant bacterial families in the rejected maize stream. There was significant difference in the population of *Enterobacteriaecea, Erwiniaceae* and *Xanthomonadaceae* ($p < 0.05$) between the accept and reject stream as shown in Table 7.

Table 6: Abundance of total fungi and bacterial reads in accepted and rejected streams

Sample code	Number of reads			
	Fungal		Bacterial	
	Accept stream	Reject stream	Accept stream	Reject stream
MS13	12,872	14,447	8	1,931
MS16	6,373	14,564	0	626
MS25	7,155	11,503	393	3,197
MS31	9,052	11,187	38	2,045
MS34	13,335	16,191	301	2,497
MS36	9,575	13,526	536	1,254
MS39	6,846	15,995	0	630
MS119	14,507	14,617	0	68
MS122	7,551	15,132	61	422
Mean	**9,696^{B}**	**14,129^{A}**	**149^{b}**	**1,408^{a}**

$^{A \text{ and } B}$ indicate significant difference (p=0.002) in the means of fungal reads between accept and reject streams
$^{a \text{ and } b}$ indicate significance difference (p= 0.002) in the means of bacterial reads between accept and reject streams

Table 7: Means of most abundant fungal genera and bacterial families in accepted and rejected streams

Fungal genera	Reject	Accept	*Bacterial family*	Reject	Accept
Aspergillus	595	1363	*Acetobacteraceae*	26	1
Candida	157	85	*Alcaligenaceae*	8	1
Clavispora	2	1	*Burkholderiaceae*	178	24
Fusarium	13249***	7914	*Enterobacteriaceae*	611**	74
Geotrichum	2	0	*Erwiniaceae*	134**	9
Gibberella	12	16	*Microbacteriaceae*	7	0
Kodamaea	45	128	*Moraxellaceae*	6**	0
Lasiodiplodia	40	32	*Paenibacillaceae*	38	0
Lectera	0	0	*Rhizobiaceae*	24	0
Penicillium	15	140	*Rhodanobacteraceae*	25	8
Phialemoniopsis	1	0	*Sphingobacteriaceae*	8	0
Pichia	8	11	*Streptococcaceae*	7	1
Saitozyma	1	0	*Streptomycetaceae*	22	4
Starmera	2	2	*Weeksellaceae*	9	0
Trichosporon	0	0	*Xanthomonadaceae*	44**	0
Wallemia	0	5	*Yersiniaceae*	250	26

*** indicates the total *Fusarium* in the accept and rejected stream differed significantly at p = 0.001
** indicates the total populations of Enterobacteriaceae, Erwiniaceae and Xanthomonadaceae in the accept and rejected streams differed significantly at $p < 0.05$

4.5 Discussion

4.5.1Efficacy of multi-spectral sorting in reduction of fumonisins

In mitigation of fumonisins in maize after harvesting the study found that multi-spectral sorting was highly efficacious in reduction of fumonisins. Similar findings were also observed by Stasiewicz *et al*. (2017). In addition to this, the study provides evidence on standardization uses of a country algorithm calibrated to visibly high-risk kernels since calibrations were derived from Ghanian maize and tested on Kenyan maize. Therefore, this work represents both an improvement of spectral sorting and suggests a practical, generalizable approach to developing sorting algorithms. The multi-spectral sorter can reject kernels that appear broken, moldy and insect infested which are features associated with mycotoxin contamination. In general, spectral sorting eliminates biasness in bulk testing which may condemn consignments of maize that contain a few pockets of mycotoxins thus increasing food and nutritional security.

4.5.2 Efficacy of multi-spectral sorting in reduction of toxigenic *Fusarium*

The population of toxigenic *Fusarium* that have the potential to produce and accumulate further fumonisins were significantly reduced after multi-spectral sorting. Other spectral methods like hyperspectral imaging has been reviewed to be an effective tool in management of *Fusarium* and DON (Femenias *et al*., 2020). Laser induced fluorescence spectroscopy has also been shown to be effective in management of *Aspergillus* and aflatoxin B1 in rice (Wang *et al*., 2022). Spectral techniques offer non-destructive methods compared to conventional methods in detection and reduction of fungi and mycotoxins. Despite this advantage, the lack of homogeneity in dispersal of mycotoxin in a sample, interference from impurities in the matrix, robustness of the models, limits of detection, efficiency of sorting, as well as development of instruments is still a limitation (Wu *et al*., 2018). The multi-spectral sorter employed in this study was able to simultaneously detect toxigenic *Fusarium* infected and fumonisin contaminated kernels using the physical traits of the kernel (broken, insect damaged and moldy). The adoption of this technology by the small-scale farmer will ensure maize produce quality across the value chain as the causative agents that deteriorate maize will have been eliminated. This will further prevent spoilage of maize and reduce marketable volumes.

4.5.3 Efficacy of multi-spectral sorting in reduction of other microbes

Apart from fungi observed as a contaminant of maize, the current study also documents co-occurrence of bacteria. *Fusarium* was the most abundant genus in the fungi while *Enterobacteriaceae* was the most abundant family of bacteria in the maize kernels. A recent study also indicated predominance of *Fusarium* species on maize residues (Cobo-Díaz *et al.*, 2022). Another study in Poland indicates, *Fusarium subglutinans, F. verticillioides,* and *F. temperatum* have been linked with maize ear rots (Czembor *et al.*, 2014). Furthermore, *F. proliferatum, F. vertillioides, F. subglutinans* and *F. oxysporum* have been isolated in harvested maize in Kenya (Maina *et al.*, 2017). In Kosovo, *Fusarium* ear rots incidences account for 0.7 to 40 % of diseased ears (Shala-Mayrhofer *et al.*, 2013). However, a study on maize in Cote de Ivore indicated *Aspergillus niger* to be most common contaminant of maize (Kakou *et al.*, 2020). The presence of toxigenic fungi causes a risk of mycotoxin production, deterioration of quality and spoilage in maize. On the other hand, *Enterobacteriaceae* has been observed in maize roots together *Yersiniaceae* family (Prischmann *et al.*, 2008). Enterobacteriaceae family includes most human pathogens like Salmonella, *Escherichia* and *Shigella* (Baylis, 2006). Other bacterial families associated with maize diseases include: *Erwiniaceae* bacteria specifically *Erwinia chrysanthemi* (Jackson-Ziems *et al.*, 2014); *Bacillaceae* specifically *Bacillus subtilis* that causes seed rot-seedling blight of maize and *Pseudomoniaceae* specifically *Pseudomonas syringae* that causes bacterial spot of maize (Butsenko *et al.*, 2018). The presence of bacteria on maize kernels that are eventually used as seeds may cause infection in the plant. Furthermore, bacteria may cause food spoilage and poisoning (Omodele *et al.*, 2020). Multi-spectral sorting reduced overall population of microbes in maize. Other methods to reduce microbes may include use of irradiation (Mohamed *et al.*, 2022), chemicals for example fungicides Reddy et al., 2021) or biocontrol techniques (Bacon *et al.*, 2001). The low- cost multi-spectral sorting is a promising post-harvest intervention in the maize value chain and efforts must be geared towards upscaling and adoption of the technology to farmers and millers.

4.6 Conclusion

Use of high-risk features to sort maize kernels reduces fumonisin, toxigenic *Fusarium* and other microbes in maize kernels. This technique is therefore a promising post-harvest technique in ensuring maize safety and quality. The study also shows using a generalizable approach in the

use of sorting algorithms, since the algorithm were developed on maize from Ghana to sort Kenyan maize. This technology can be employed by national strategic food reserves, government stores, millers, cereal aggregators to ensure all the kernels they receive, and process are of safe and of good quality.

CHAPTER FIVE

EFFICACY OF E-BEAM IRRADIATION IN REDUCTION OF *ASPERGILLUS* SECTION *FLAVI* AND *FUSARIUM* POPULATION AND RESULTING AFLATOXIN AND FUMONISIN IN MAIZE

5.1 Abstract

Aflatoxins and fumonisins produced by *Aspergillus* and *Fusarium* respectively have been a major source of concern especially in stored maize. Most post-harvest interventions arrest microbial growth by inactivating the fungi, the fungal spores lay dormant in food. Thus there is need for mitigation strategies that completely eliminate toxigenic fungi.E-beam irradiation has been used as a phytosanitary treatment in removal of harmful microbes though not much evidence has been published in naturally contaminated maize with mycotoxigenic fungi and mycotoxins. This study aimed at investigating the effectiveness of E-beam irradiation in reduction of *Aspergillus* section *Flavi* and *Fusarium* and the degradation of aflatoxins and fumonisins in maize samples in eastern Kenya that had high mycotoxin levels. Ninety-seven maize samples were tested for total aflatoxins and fumonisins using ELISA method. For E-beam efficacy, 24 samples that had greater than100 µg/kg of total aflatoxins and greater than 1000 µg/g of total fumonisins were selected. Before E-beam treatment at 5 kGy, 10 kGy and 20 kGy, the samples were mixed with sterile deionized water and sub sampled into four for the three doses and one control. The plating method was used to determine total fungal load while ELISA method was used for quantification of total aflatoxins and fumonisins. qPCR was used to identify and quantify *Aspergillus* and *Fusarium* nucleic acid present in the samples. The level of contamination with aflatoxins increased as the population of *Aspergillus* species increased ($r = 0.54$; $p = 0.007$). Similarly, the levels of fumonisins also increased as the population of *Fusarium* species increased ($r = 0.68$; $p < 0.001$). The population of viable fungi was 3.7 log CFU/g in the control samples after E-beam treatment at 5 kGy and greater dose, the fungal population was reduced to below limits of detection (> 1.9 log reduction). Aflatoxins were also reduced by an average 6.2 ng/g at 20 kGy but there was no reduction in fumonisins. This study confirms the elimination of fungi on food matrix by use of E-beam irradiation and radiosensitivity of pre-formed aflatoxins at 20 kGy. Furthermore, there is need to explore higher doses that may destroy fumonisins.

5.2 Introduction

The main maize delicacy in Kenya is *ugali* (stiffened porridge) made from ground maize. In the Kenyan maize value chain, mycotoxins specifically aflatoxins and fumonisins, are a major health concern. There have been repeated cases of aflatoxicosis in contaminated maize. In 2010 alone, the government of Kenya through the Ministry of Public Health, condemned and destroyed 2.3 million bags of maize because of aflatoxins contamination cause by toxic *Aspergillus* molds infection (Schmidt, 2013). Most poultry farmers feed chickens physically discolored and moldy maize grains that appear unappealing for human consumption. Once animals consume aflatoxin contaminated feeds they pass down the mycotoxins in their meat, eggs for poultry, and milk in the case of cattle (FDA, 2023).

Most mycotoxin mitigation strategies target the toxigenic fungi but a few tackles the already pre-formed mycotoxins. To achieve maximum reduction of mycotoxins in maize integration of both before and after harvest mitigations are necessary (Liu *et al*., 2022). One potential intervention in reduction of mycotoxins in red pepper has been the use of E-beam (Woldemariam *et al*., 2021). The efficacy of E-beam and gamma irradiation in reduction of *Aspergillus* and aflatoxin B1 have been demonstrated in Brazil nuts that were artificially inoculated with *Aspergillus* producing aflatoxin (Assunção *et al*., 2015).

E-beam irradiation can increase the shelf life of cereals and other food commodities by preventing post-harvest spoilage thus enhancing food safety and security. These potential benefits of E-beam as an ionizing technology is well illustrated on mycotoxins and fungi (Lung *et al*., 2015; Pillai & Shayanfar, 2017; Pillai & Shayanfar, 2018; Woldemariam *et al*., 2021). There are two mechanisms of action of ionizing irradiation: First, is directly where energetic photons or electrons act by breaking covalent and hydrogen bond, second is indirectly, where the radiolytic products of splitting water molecules that is hydroxide and hydrogen ions cause extensive covalent and hydrogen bond breaks (Khaneghah *et al*., 2020). E-beam irradiation is more effective compared to other methods of mitigating mycotoxin in that there is complete elimination of microbes after irradiation. Furthermore, at recommended doses of 10 kGy no physico-chemical changes occur to the food matrix.

5.3 Materials and Methods

5.3.1 Sample collection and processing

Ninety-seven ground maize samples were obtained from the Regional Mycotoxin Laboratory in KALRO Katumani being a portion of samples with aflatoxin levels > 10 µg/kg in another joint study by the International Institute of Tropical Agriculture (IITA), Innovation for Poverty Action (IPA)and International Food Policy Research Institute (IFPRI), whereas one kilogram of maize which was dry, shelled and stored in farmers' stores in kernel form were collected in Upper Eastern Kenya counties: Embu, Meru and Tharaka Nithi Counties. The samples were screened for aflatoxins using a commercial lateral flow assay ELISA kit (Accuscan Reveal Q+ Pro-reader, Neogen, USA). A hundred grams of the 97 samples were double packed in zip lock bags, transported to the University of Illinois for further analysis using E-beam irradiation. U.S Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) import permit to transport live plant pests, noxious weeds, and soil, P526P-21-00992_20210303 was used together with a phytosanitary certificate obtained from Kenya Plant Health Inspectorate Services (KEPHIS). Samples were packed in four containment levels to avoid leakage during transportation.

5.3.2 Determination of baseline aflatoxin and fumonisin analysis in maize flour

At the University of Illinois, the samples were re-tested for aflatoxin and fumonisin as described in Section 3.4.4. 24 out of 97 samples with aflatoxin levels greater than 100 µg/kg and fumonisin level greater than1000 µg/g were selected for E-beam irradiation efficacy studies.

5.3.3 Preparation of maize slurry and subsampling

A maize slurry was prepared by mixing ten grams of maize flour with 50 ml of sterile deionized water in each of the 24 samples. The presence of water in the slurry increases efficacy of E-beam irradiation in degradation of mycotoxins. Four subsamples were generated from each sample for treatment with three different doses and a control. For E-beam treatment done at Texas A & M University (TAMU), seven milliliters of slurry were transferred into sterile, plastic sample packaging bags (small Whirl Pak, VWR, USA), double packed, and heat-sealed into 95 kPa Transport Bags (Therapak, VWR, USA). The samples were stored in a cold room at 8 ^{o}C after packaging and later shipped to TAMU overnight with ice packs.

5.3.4 E-beam Irradiation on maize slurry

The E-beam treatment was done at the National Center for Electron Beam Research facility in TAMU using a 10 MeV, 15 kW S-band linear accelerator. The samples were treated in flat heat-sealed bags with doses of 5, 10, and 20 kGy as demonstrated in Table 8. Alanine dosimeters used in international standards were used to measure treatment doses. A Bruker e-Scan EPR spectrometer (Billerica, MA, USA) was used to read the alanine dosimeters. The product conveyor speed was used to calibrate the doses. The rate of dose of the 10 MeV, s-band 15 kW linac was approximately 3 kGy/s. Preliminary experiments were done to ascertain that doses were uniformly delivered to the samples. Dose uniformity ratios of 1 were measured between the above and below the surface of the bags to ascertain that E-beam penetration was not causing a variation in treatment of the samples. A set of control sample that was not irradiated was also included to compare the efficacy of E-beam between irradiated and non-irradiated samples and to assess whether transportation of the sample from Illinois to Texas affected the sample in any way.

Table 8: Dose distribution during slurry treatment in whirl-pak bags

Intended dose (kGy)	Measured dose (kGy)	Dose location	Dose uniformity ratio (Max/Min Dose)
5	4.709	Top	1.02
	4.821	Bottom	
10	9.496	Top	1.02
	9.713	Bottom	
20	20.407	Top	1.00
	20.378	Bottom	

Source: Mohamed *et al.* (2022)

5.3.5 Microbial analysis

5.3.5.1 Isolation of fungi in maize

Viable colonies of fungi were isolated after E-beam treatment, samples were shipped from Texas A&M University to University of Illinois for microbial isolation. Viable fungi were isolated from every control and E-beam irradiated maize slurry sample by doing serial dilution where 1 ml of

slurry was diluted in phosphate-buffered saline and 50 µl aliquots were pipetted and plated on Potato Dextrose Agar (PDA) as in Section 3.1.5.

5.3.5.2 Molecular identification of *Aspergillus* and *Fusarium* in maize

The presence of *Aspergillus* and *Fusarium* species DNA fragments was tested in all control samples and not in E-beam treated samples because from microbial isolation step, no viable fungi was detected and E-beam is known to degenerate DNA fragments (Bergen *et al*., 2005). DNA was extracted from four source: First source was from pure cultures of *Aspergillus flavus* S-strain which was isolated from aflatoxin contaminated maize sample; the second source was pure culture of *Fusarium proliferatum* isolated from fumonisin contaminated maize sample the third source was from pure uncontaminated maize and lastly from un-irradiated contaminated maize slurry samples (control samples).

Standard curves for *Aspergillus* (Mideros *et al*., 2009) and *Fusarium* DNA were developed. *A. flavus* S-strain and *F. proliferatum* were isolated from maize sample. One gram of ground contaminated maize sample was mixed with 9 ml of sterile deionized water and an aliquot of 50 µl was plated on PDA. The PDA plates were incubated for 5 days at 31 ^{o}C and later pure culture of each organism were sub-cultured in PDA. After that 5 ml of Glucose Yeast Extract Peptone (GYEP) broth was used to wash the colonies and then the colonies were sub-cultured into sterile petri dishes containing 8 ml of GYEP broth. After 48 hours of incubation the GYEP broth was centrifuged at room temperature (25 ^{o}C). Sterile deionized (DI) water was used to wash the cell pellets. Sterilized de-ionized water was added to cell pellets and 1 ml of cells was used to extract DNA using IBI kit protocol. Quantification of Extracted DNA was done using a Nano drop (2000C, Thermo Fisher Scientific, USA) and recorded in ng/µl. 260/280 ratio greater than 2.0 was used to determine purity of DNA. The *Aspergillus* and *Fusarium* DNA that had 260/280 ratio greater than 2.0 were later diluted in pure maize DNA and used for developing qPCR mixed standard curves.

Uncontaminated maize that did not have either fungal DNA or mycotoxins was also used in DNA extraction. This DNA extracted from uncontaminated maize was used to imitate the matrix background to carry the pure culture of *Aspergillus* and *Fusarium* DNA like it occurs in the

contaminated samples. Lastly, an IBI extraction kit protocol (IBI Scientific, Iowa, USA) was used to extract DNA from 100 mg of each maize slurry samples (control samples) using. Pathogen DNA was serially diluted where 0.001, 0.01, 0.1, 1 and 10 ng/µl was mixed with 1 ng/µl of uncontaminated maize DNA to make a mixed standard curve.

qPCR was performed in a Q-machine (model 95900-4C, Quantabio, MA, USA) for detection of total *Aspergillus* DNA in the slurry samples using the internal transcribed spacer 1 (ITS1) primers and Taqman probes were obtained from Life Technologies Corporation, USA: Forward primer 5-ATCATTACCGAGTGTAGGGTTCCT-3′; reverse primer: 5′-GCCGAAGCAACTAAGGTACAGTAAA-3′) and TaqMan probe was 5′FAM-CGAGCCCAACCTCCCACCCG-3′TAMRA, which produced a 73 bp amplicons. 12.5 µl of TaqMan Fast Advanced Master Mix (Life Technology Corporation, USA) were added to 1.9 µl of 10 µM of each forward and reverse primer, followed by 0.2 µl of 25 µM probe, 5.5 µl Nuclease free PCR water and 3 µl of template DNA extracted from each sample were mixed to make 25µl reaction mix (1X). A two-profile qPCR reaction was carried out with a pre-cycle phase of 50 °C for 2 min; then 95 °C for 10 min followed by 40 cycles of 95, 59, and 72 °C for 30 s each.

Method used by Bluhm *et al* (2004) was adopted and slightly modified for detection of *Fusarium* DNA. ITS primers and probes were obtained from Life Technologies Corporation, USA: ITS forward-
5'-AACTCCCAAACCCCTGTGAACATA-3'; ITS reverse- 5'-TTTAACGGCGTGGCCGC-3' and ITS Taqman probe 5'-CGCTCGAACAGGCATGCCCGCCAGAATAC-3'TAMRA which produced in a 431 bp amplicon product. 10 µl of TaqMan Fast Advanced Master Mix (Life Technology Corporation, USA) was added to 1 µl of 10 µM of each forward and reverse primer, followed by 0.1 µl of 20 µM probe and 2.9 µl Nuclease free PCR water and then 5 µl of template DNA extracted from each test sample were mixed to make a 20 µl reaction mix (1X). The reaction was carried out in a two-profile cycle: A pre-cycle phase of 50 °C for 2 min; then 95 °C for 10 min followed by 40 cycles of 95 ^{o}C for 15 sec and 60 ^{o}C for 2 min each.

The amount of pathogen DNA was extrapolated from the mixed standard curves equations. The standard curve equation for *Aspergillus* was y= -3.4989x + 20.064 and the efficiency of the reaction was 93 %, R = 0.99. For *Fusarium* the mixed standard curve equation was y=-3.3778x+21.127 while the efficiency of the reaction was 98 %, R = 0.99. The infection coefficient (IC) was calculated as a proportion of fungal pathogen DNA over the total DNA in the sample and was used to estimate the pathogen in the sample.

5.3.6 Aflatoxin and fumonisin analysis

Aflatoxin and fumonisin in each E-beam-treated, and control sample was quantified by mixing 1 ml of slurry with 5 ml of 80% methanol. The procedure described in Section 3.4.4 was used to quantify the mycotoxins.

5.3.7 Data analysis

Open-source R software version 4.1.3 was used in data analysis. To determine the effectiveness of E-beam irradiation in reducing of *Aspergillus* section *Flavi* and *Fusarium* population and resulting aflatoxin and fumonisin in maize slurry, the mean difference of mycotoxin levels between the E-beam irradiated sample and non-irradiated sample was tested to be significantly different from zero using a Student t-test with False Discovery Rate correction. For each of the six conditions, that is aflatoxin and fumonisin at 5, 10 and 20 kGy p-values were adjusted in R using P. adjust function.

5.4 Results

5.4.1 Aflatoxin and fumonisin levels in high aflatoxin preselected samples

The mean aflatoxin level was 130 µg/kg and levels ranged from 10.2 to 1290 µg/kg. Out of the 97 maize samples tested 82 maize samples that were initially pre-selected for high aflatoxin level had high levels above the KEBS threshold (> 10 µg/kg). The other 15 samples had low levels of aflatoxins below KEBS threshold (< 10 µg/kg), the mean aflatoxin level was 5.7 µg/kg and levels varied between 1.3 to 9.7 µg/kg. Additionally, out of the 97 samples, 57 had fumonisin levels above the KEBS threshold (> 1000 µg/kg) with a mean of 3580 µg/kg and fumonisin level ranging between 1030 and 11600 µg/kg as shown in Figure. 4.

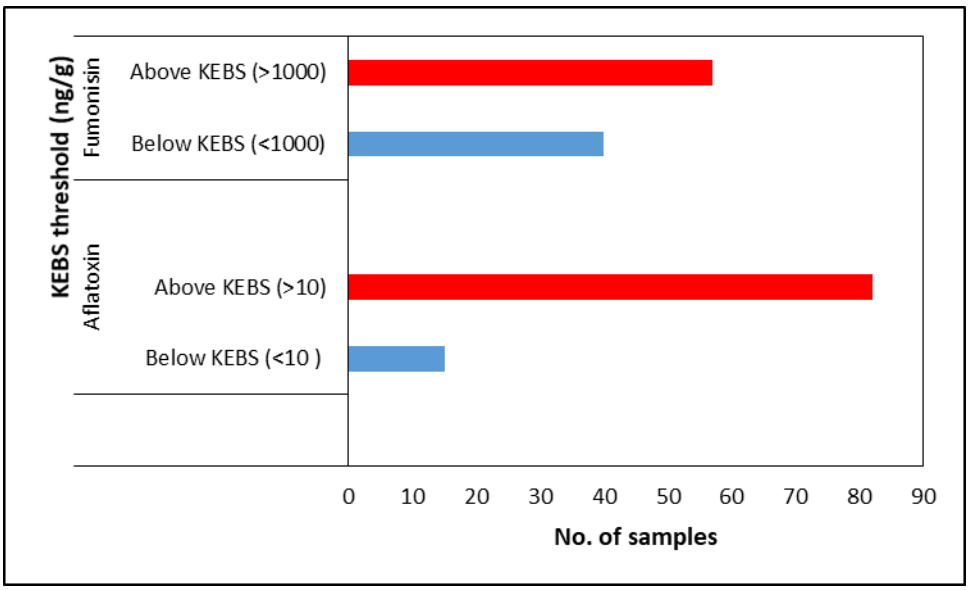

Figure 4: Baseline aflatoxin and fumonisin levels (KEBS threshold) in maize flour sample from Upper Eastern Kenya

5.4.2 Reduction in population of *Aspergillus* and *Fusarium* after E-beam irradiation

After E-beam Irradiation at 5, 10 and 20 kGy there was no viable fungal growth on PDA. All fungi were destroyed by E-beam resulting in a 3.7 log reduction as shown in Table 9. *Aspergillus* and *Fusarium* were detected in E-beam non-irradiated samples (control) by qPCR assays with efficiency of the reaction of 93 and 98 % for *Aspergillus* and *Fusarium* respectively. The mean of *Aspergillus* infection coefficient (IC) in maize was significantly lower ($p < 0.001$) than *Fusarium* as shown in Table 10. The *Aspergillus* and *Fusarium* co-occurred together and were positively correlated ($r = 0.5$, $p = 0.03$) between the IC of *Fusarium* and *Aspergillus*.

Table 9 Reduction of fungal population (log CFU/g) in maize after E-beam Irradiation

S/No	Sample code	Before E-beam	After E-beam	Log Reduction
1	IM105	3.9	<1.9	>2.0
2	IM119	3.3	<1.9	>1.4
3	IM120	4.2	<1.9	>2.3
4	IM123	4.5	<1.9	>2.6
5	IM145	3.8	<1.9	>1.9
6	IM162	2.8	<1.9	>0.9
7	IM166	4.2	<1.9	>2.3
8	IM168	<1.9	<1.9	>0.0
9	IM171	2.6	<1.9	>0.7
10	IM180	4.1	<1.9	>2.2
11	IM184	3.6	<1.9	>1.7
12	IM202	3.5	<1.9	>1.6
13	IM233	3.5	<1.9	>1.6
14	IM242	<1.9	<1.9	>0.0
15	IM251	3.1	<1.9	>1.2
16	IM252	3.8	<1.9	>1.9
17	IM253	2.9	<1.9	>1.0
18	IM255	4.6	<1.9	>2.7
19	IM266	4.1	<1.9	>2.2
20	IM273	3.9	<1.9	>2.0
21	IM292	3.9	<1.9	>2.0
22	IM299	3.8	<1.9	>1.9
23	IM305	4.1	<1.9	>2.2
24	IM328	4.0	<1.9	>2.1
Average		**3.7**	**<1.9**	**>1.8**

Table 10: Infection coefficients of *Aspergillus* and *Fusarium* DNA in maize (log ratio of pathogen in total DNA)

	Infection Coefficient (Log)	
Sample No.	***Aspergillus***	***Fusarium***
1	-2.86	-1.06
2	-3.62	-0.79
3	-3.08	-0.50
4	-2.09	0.36
5	-2.97	0.63
6	-3.34	-0.77
7	-2.17	-0.48
8	-2.87	0.46
9	-2.20	-0.50
10	-2.56	-0.10
11	-2.47	-1.07
12	-3.01	-0.11
13	-3.52	0.29
14	-4.07	-1.89
15	-2.61	-0.71
16	-3.73	-0.75
17	-2.59	-0.17
18	-3.28	-0.85
19	-2.16	0.09
20	-2.26	0.56
21	-2.78	0.75
22	-2.59	-0.05
23	-2.07	-0.28
Mean	-3.44	-0.81***

*** Significant difference in means of *Aspergillus* and *Fusarium* IC ($p < 0.001$, $t = 19.37$)
Infection coefficients expressed in log scale (pathogen/Total DNA ratio) of total *Aspergillus* (n=23) and *Fusarium* (n=23) DNA in maize DNA

5.4.3 Correlation between mycotoxins and fungal load

The levels of aflatoxins and IC of *Aspergillus* correlated positively. The levels of aflatoxins increased as the IC of *Aspergillus* increased. Furthermore, a highly significant positive correlation was also seen between fumonisins, and the IC of *Fusarium* as shown in Table 11. The *Aspergillus* IC varied between -4.08 to -2.09 log while the aflatoxins levels in the non-irradiated slurry sample varied between less than LOD of 0.0 log (µg/kg) to 2 log (µg/kg). Likewise, the

Fusarium IC varied between -1.89 - 0.75 log while the fumonisin levels ranged from 1.0 - 3.9 log(µg/kg).

Table 11: Correlation *Aspergillus*, aflatoxin, *Fusarium* and fumonisin

	Aspergillus	**Aflatoxin**	***Fusarium***	**Fumonisin**
Aspergillus	-			
Aflatoxin	+0.54*	-		
Fusarium	+0.46*	$+0.29^{ns}$	-	
Fumonisin	$+0.23^{ns}$	$+0.30^{ns}$	+0.68**	-

** Correlation coefficient significant at $p \leq 0.001$; * correlation coefficient significant at $p \leq 0.05$; ns - not significant; correlation coefficient significant at $p \leq 0.05$

5.4.4 Reduction in mycotoxins after E-beam irradiation

5.4.4.1 Reduction of aflatoxins after E-beam treatment

After E-beam treatment at 20 kGy, there was an average 6.4 µg/kg reduction in aflatoxins (False Discovery Rate (FDR) corrected p = 0.03). The mean of aflatoxin in irradiated samples at 20 kGy was significantly different from the non-irradiated sample. However, the aflatoxins levels did not differ significantly after E-beam dosing at 10 and 5 kGy dose treatment respectively as shown in Table 12. The aflatoxin levels before E-beam treatment ranged between < LOD (0.0 log µg/kg) to 2 log µg/kg. After E-beam treatment at 20 kGy there was significant aflatoxin reduction in 14/24 samples while at 5 and 10 kGy aflatoxin reduction occurred in only 10/24 samples.

5.4.4.2 Reduction of fumonisins after E-beam treatment

After E-beam treatment at 20, 10 and 5 kGy, no reduction seen in fumonisin (FDR corrected p > 0.05) as shown in Table 13. The fumonisin levels before E-beam treatment ranged between 1log µg/kg to 3.9 log µg/kg. Reduction in fumonisin level was seen only in 8/24 and 9/24 samples at 20 and 5 kGy respectively. No reduction in fumonisin was seen at 10 kGy.

Table 12: Aflatoxin levels (log ng/g) in control and E-Beam irradiation treated maize slurry samples

S/No.	0kGy (control)	20kGy	10kGy	5kGy
1	<0.0	<0.0	<0.0	<0.0
2	<0.0	0.26	<0.0	1.14
3	0.41	0.51	0.72	0.48
4	0.91	0.45	1.13	0.88
5	0.27	<0.0	0.08	0.16
6	0.92	0.09	0.39	0.46
7	1.60	0.19	0.65	0.49
8	1.03	0.11	1.77	0.64
9	1.18	0.59	0.92	1.11
10	2.04	2.16	1.32	1.55
11	0.56	0.60	0.33	0.74
12	0.41	0.61	0.71	0.44
13	1.00	0.63	1.07	0.85
14	0.16	0.53	0.17	0.29
15	1.19	1.88	1.47	1.18
16	0.41	0.47	0.53	0.84
17	0.47	0.07	0.38	0.61
18	1.00	1.15	1.37	1.83
19	1.54	1.08	1.53	1.27
20	1.26	0.80	1.29	1.73
21	0.51	0.31	0.84	0.59
22	0.77	0.33	0.87	0.81
23	0.94	0.68	0.82	2.47
24	1.03	0.24	0.65	1.04
Mean	0.83a	0.55b	0.82a	0.94a

Different letters on accompanying the means show significant difference at $p < 0.05$
<LOD = 0.0 µg/kg

Table 13: Fumonisin levels (log µg/kg) in control and eBeam irradiation treated maize slurry samples

S/No.	0kGy (control)	20kGy	10kGy	5kGy
1	1.0	2.65	<1.00	2.26
2	2.55	2.91	2.71	3.05
3	1.71	2.86	2.52	2.61
4	3.16	3.19	3.11	3.29
5	2.89	2.98	2.58	2.92
6	2.90	2.83	2.26	3.01
7	2.69	2.48	1.99	2.60
8	3.33	3.17	3.30	3.55
9	2.68	2.69	2.78	2.72
10	2.50	2.92	2.91	3.16
11	2.50	2.65	2.70	3.03
12	3.59	3.59	3.62	3.54
13	3.87	3.74	3.70	3.83
14	1.69	<1.00	1.57	2.14
15	2.04	1.92	2.65	2.67
16	2.28	0.61	2.68	2.07
17	3.46	3.05	3.29	3.32
18	3.14	3.27	3.33	3.37
19	2.95	3.11	3.27	3.03
20	3.55	3.55	3.77	3.68
21	3.13	3.20	3.17	3.11
22	2.73	2.37	2.47	2.47
23	3.01	2.89	3.04	2.83
24	2.48	3.07	2.65	2.25
Mean	2.74^{ns}	2.74^{ns}	2.75^{ns}	2.94^{ns}

ns- means of fumonisin levels did not differ significantly at $p < 0.05$
<LOD = 1.00 µg/kg

5.5 Discussion

5.5.1 Correlation among mycotoxin and mycotoxin producing fungi in high mycotoxin samples

This study shows the mycotoxin levels screened in the sample were directly related to the infection coefficient of fungi in the samples i.e., *Aspergillus* spp. and aflatoxins levels recorded and *Fusarium* spp. an fumonisins levels recorded in the slurry samples. Besides that, the biomass of *Aspergillus* and *Fusarium* spp. in the high mycotoxin contaminated samples increased concurrently. In another study involving field inoculated maize, a similar significant correlation (r=0.85) between *Aspergillus* biomass and aflatoxin levels was observed. While in inoculated

wheat a positive correlation (r=0.85) between *Fusarium* and fumonisin level has also been observed (Siou *et al.*, 2014). Mostly, traditional culture plating methods are used in studies to investigate prevalence of mycotoxigenic fungi in samples that are naturally contaminated, while this study used modern qPCR technique. Traditional plating method was used to isolate *Aspergillus flavus* S-strain (97.9 % of total *Aspergillus* isolated) that related positively with the aflatoxin level observed (0.27 to 4400 µg/kg) in maize in Kenya. Similarly, in another study done on Nigerian poultry feed samples by Ezekiel *et al.* (2014), 91% of *A. flavus* was recovered which positively correlated with aflatoxin B_1 levels produced in vivo (40 to 441,000 µg/kg). However, production of mycotoxin does not occur in all fungi but only certain members of the fungi that carry the genes responsible for toxin production (Moretti *et al.*, 2013). The toxigenicity of mycotoxigenic fungi differs among different strains in terms of the type and quantity of toxin produced. Aflatoxin production is caused by presence of functional gene of *Nor-1* in toxigenic *Aspergillus* (Iheanacho *et al.*, 2014). Atoxigenic members of *Aspergillus* section *Flavi* that are not able to produce aflatoxins have a gene deletion in the sequence used in aflatoxin biosynthesis (Probst *et al.*, 2014). Aflatoxin production is mostly pronounced in *A. flavus S*-strain and *A. parasiticus* (Probst *et al.*, 2007). Similarly, only a few *Fusarium* spp. that express the FUM gene have the ability to secrete fumonisins (Bluhm *et al.*, 2004).

Growth of *Aspergillus* and *Fusarium* species in a mixed culture *in vitro* showed the two fungi inhibited growth of each other (Chen *et al.*, 2021). However, in this study, the biomass of *Aspergillus* correlated positively with *Fusarium* biomass in the high aflatoxin and fumonisin samples that were naturally contaminated. This can be attributed to fluctuating environmental conditions that may favour optimal growth conditions for *Aspergillus* and *Fusarium.*

5.5.2 Efficacy of E-beam on *Aspergillus* section *Flavi* and *Fusarium*

In the current study, at least 4.6 logs of fungi in naturally contaminated maize slurry were reduced by low E-beam irradiation at 5 kGy. This finding is in concurrence with an earlier study that showed 5 kGy eBeam dose on waste water caused a reduction of coliphage, total coliforms and total flora by 3 logs (Farooq *et al.*, 1993). The E-beam dose of 5 kGy has also been shown in other studies to eliminate the total microbial load in turmeric powder (Esmaeili *et al.*, 2018) while a slightly lower dose of 4.8 kGy eliminated *Aspergillus* in maize (Nemţanu *et al.*, 2014).

Furthermore, E-beam dose of 4 kGy has also been shown in a recent study to be equally effective in reduction of yeast and other fungi in red pepper by 2 and 3.4 logs respectively (Woldemariam *et al.*, 2021). A slightly higher dose of 6 kGy of eBeam was effective in inactivating fungal spores (Etter *et al.*, 2018). Increasing the E-beam dose to 10 kGy has also been found to be effective in controlling microbes in soybean (Zhang et al., 2018). The stage of fungal infection present in the sample (hyphae or spores); water content of fungal infection stage or matrix of the sample; age of spores; sample pretreatment procedures i.e., freezing or heating determines the sensitivity of fungi to irradiation (Calado *et al.*, 2014). Sensitivity of different organisms to radiation also differs, *Penicillium* is more sensitive when exposed to E-beam irradiation compared to *Fusarium* and *Aspergillus* which is least sensitive. (Nemţanu *et al.*, 2014).

As a food safety measure, elimination of harmful fungi in flour may increase the shelf life of flour by preventing further fungal growth and inhibiting toxin production and accumulation. Moreover, E-Beam is effective in reducing harmful microbes in other food crops like spices (Gryczka *et al.*, 2020), meat and meat products and horticulture value chain for example the vegetables and fruits like mangoes (Khaneghah *et al.*, 2020). Irradiation of food has become more popular in recent time especially for phytosanitary measure, approval of irradiation application of food had been done by more than 60 countries worldwide (Eustice, 2017). The maximum irradiation doses applied on food differs in each product and in each country, as every country have their own set regulations (Freitas-Silva *et al.*, 2014). The dose recommended physio-chemical characteristics of food is 10 kGy. This study evaluated both sides of the recommended dose that is half (5 kGy) and double (20 kGy). However, in treatment of tropical fruits, pepper, persimmon, and tomatoes low doses of1 kGy have been used while for herbs and spices, high doses of 30 kGy have been permitted (Freitas-Silva *et al.*, 2014).

Food security is compromised when the maize samples are inappropriately stored in moist and warm conditions, toxigenic fungi present in the maize may increase toxin synthesis and contamination (Darwish *et al.*, 2014). For farmers and millers, if contaminated products are stored or processed together with other products, cross contamination may occur hence causing more economic losses. Furthermore, high mycotoxins contamination in the raw materials down

the value chain at the producer level may find its way into finished products produced by local food processing industries thus resulting into poor quality product , condemnation of exports (Mamo *et al.*, 2020) and diminished brand integrity (Chemuniqué, 2019).

5.5.3 Efficacy of E-beam in reduction of aflatoxins and fumonisins

Total aflatoxin reduction was observed at E-beam dose of 20 kGy in maize slurry that was naturally contaminated with mycotoxigenic fungi and mycotoxins while the reduction of aflatoxins at 10 kGy was not significant. This may have been caused by the high dosage of E-beam at 20 kGy that caused the breaking down the aflatoxin in the aqueous medium of the slurry. Maximum electron adsorption causing degradation is enhanced when the concentration of mycotoxin increases (Liu *et al.*, 2016) thus the direct effect of E-beam irradiation. Efficacy of E-beam in degradation of mycotoxins is also favoured by the high water activity of the sample, the higher the degradation when moisture content is high (Liu *et al.*, 2016). Thus the indirect effect of E-beam occurs when the bonds in the mycotoxins are broken by the products of water hydrolysis: hydroxides and hydrogen ions (D'Ovidio *et al.*, 2007). Furthermore the mutagenicity and cytotoxicitiy effect of E-beam degraded products of mycotoxins is less compared to the original mycotoxin (Liu *et al.*, 2016). In a dry matrix for example chili pepper powder, doses of up to 30 kGy of E-beam have been found not to be effective in reducing aflatoxins but in dry chilli pepper, they significantly reduced ochratoxin A by 25 % (Woldemariam *et al.*, 2021). Additionally, other irradiation techniques like gamma are also affected by moisture content in degradation of mycotoxin (Wang *et al.*, 2020). Gamma irradiation at 30 kGy has been shown by Jalili *et al.* (2012) to reduce ochratoxin A, AF B_1, AF B_2, AF G_1 and AF G_2, by 55.2 %, 50.6 %, 39.2 %, 47.7 % and 42.9 % reduction on pepper with 18% moisture content. Therefore, for complete degradation of mycotoxins, higher doses of up to 50-100 kGy have been recommended (Temcharoen & Thilly, 1982). However, other studies have shown effective reduction of aflatoxin B_1 in Brazilian nut (Assuncao *et al.*, 2015) and beans (Supriya *et al.*, 2014) at E-beam at a lower dose of 10 and 15 kGy. .

5.6 Conclusion

E-beam irradiation was effective in reduction of *Aspergillus* section *flavi* and *Fusarium* species. Aflatoxins in the maize slurry were also reduced but not fumonisins, this study indicates E-beam

as a promising intervention. E-beam was able to eliminate all microbes, especially *Aspergillus* and *Fusarium* which positively correlated to aflatoxin and fumonisin in the maize respectively. E-beam can be used as last line of defense against mycotoxins, thus increasing shelf life and to remediate condemned maize flour.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1 GENERAL DISCUSSION

Post harvest interventions that are used to mitigate mycotoxins act like the last line of defense mechanism against mycotoxin contamination in food. When the microbes escape pre-harvest and during harvest interventions, post-harvest interventions become the only option in reduction of the mycotoxigenic fungi and mycotoxins. However, effective management of mycotoxins employs both pre- and post-harvest interventions (Logrieco *et al.*, 2021). When the toxigenic fungi escape pre-harvest intervention, spectral sorting can be used to manage mycotoxins after harvest. This study also demonstrated the efficacy of multi-spectral sorting in reduction of fumonisins, which was the most common mycotoxin in the standing maize crop. Unlike maize from a standing crop that is contaminated by *Fusarium* mostly, maize that has been stored in farmers stores are mostly contaminated with both the storage fungi *Aspergillus* (Maina et al., 2016) and the field fungi *Fusarium* (Maina et al., 2017). Post- harvest contamination of aflatoxins is most likely to occur during storage (Kumar *et al.*, 2021) and its production is highly favored by darkness and inhibited by sunlight (Rushing & Selim, 2019). Positive correlation between *Aspergillus* and aflatoxin has also been reported in stored rice (Fatemeh *et al.*, 2011) and red pepper (Woldemariam *et al.*, 2021). Similarly, *Fusarium* has been positively correlated to fumonisin levels in corn (Ono *et al.*, 2006). E-beam irradiation has been found efficacious in elimination of fungi (Nemţanu *et al.*, 2014) as well as mycotoxins like ochratoxin A (Woldemariam *et al.*, 2021); aflatoxins (Assuncao *et al.*, 2015) and deoxynivalenol (Stepanik *et al.*, 2007). This technology serves as a phytosanitary intervention in arresting growth of harmful fungi thus reducing spoilage and further toxin production during storage. Elimination of both the mycotoxigenic fungi and mycotoxins is key thus methods like E-beam become handy in reduction of *Aspergillus, Fusarium* and aflatoxin but not fumonisin which was effectively reduced by multi-spectral sorting. Despite post-harvest interventions being key in managing mycotoxins, pre-harvest good agronomic practices play a key role in managing the contamination at field level which serves as the primary source of infection to the crop while poor agricultural practices like the use of mixed varieties (both local and improved) increases fumonisins and the use of tractors for tillage increases aflatoxins in freshly harvested maize. The high fumonisin levels in mixed variety cropping are because most local varieties are highly

susceptible to infection and farmers mostly recycle them (Wambugu *et al.*, 2012) thus, may act as a source of inoculum to contaminate the other varieties in the field when mixed. Farmers also tend to share/ hire tractors and when not properly sanitized, the tractor can carry contaminants from one farm to another. Furthermore, machine tillage may cause damage to the crop residues hence exposing them for fungal contamination. Despite the poor practices that expose the crop to mycotoxin contamination, The spectral sorter was able to reject < 2% of the maize which is in concurrence with a recent study where aflatoxin in highly contaminated and low contaminated maize was reduced by 93% and 40.7% with a low reject rate of 1.02% and 1.34% respectively (Yao *et al.*, 2023). Multi spectral sorting has been shown to be effective in reducing both aflatoxins and fumonisins (Chavez *et al.*, 2023). Studies on use of multi-spectral sorter have majorly focused on mycotoxins reduction for example aflatoxins and fumonisins. Not much has been published on the use of multi-spectral sorting in reduction of toxigenic microbes. The reduction of toxigenic *Fusarium* is thus novel to this study. In addition to this, stored farmers' maize which has high levels of *Aspergillus* and *Fusarium* also has high levels of aflatoxins and fumonisins can be terminally sterilized by E-beam irradiation thus avoiding further spoilage and toxin production.

*Fusarium*l. *Fusarium*

FusariumFusariumFusarium.

6.2 CONCLUSION

Farmers' practices before harvest contribute to introduction, proliferation, growth and survival of the fungi that produce mycotoxins at pre-harvest stages. These pre-formed mycotoxins in the field are mostly fumonisins which together with the associating mycotoxigenic *Fusarium* can be effectively reduced by multi-spectral sorting. Furthermore, storge samples which have aflatoxins and *Aspergillus* in addition to fumonisins and *Fusarium* can also be decontaminated by E-beam irradiation. In Eastern Kenya, the use of tractors, planting of mixed local and improved varieties increased mycotoxin contamination while agronomic practices did not affect the fungal population. This shows that farmer practices affect the toxin production and consequently

mycotoxins contamination in maize conditions and not necessarily the population of toxin producing fungal pathogen. The multi-spectral sorter is therefore a promising technology for millers who buy maize from farmers and traders instead of testing the maize using bulk analysis which may be biased. Moreover, E-beam irradiation treatment could be used to remediate already contaminated products instead of condemnation for destruction, especially when food insecurity makes it a possible choice other than to destroy contaminated maize.

6.3 RECOMMENDATIONS

The following recommendations are drawn based on the findings of this study: There is need to raise awareness on sharing of farm tools that may be contaminated among farmers, and to increase awareness on use of certified seed varieties suitable for a certain region.

1. More research to be done to evaluate the agronomic practices that influence the prevalence of fungi in the soil.
2. There is a need to develop policies that ensure mycotoxin surveillance at farmer level not just at market level.
3. Upscaling the multi-spectral sorting technology for large scale users like strategic government food reserves and stores.
4. There is a need to develop more algorithms for other crops that are contaminated by mycotoxins as well such as sorghum, millet, barley, peanuts and beans.
5. Since fumonisin levels in maize slurry were not reduced by up to 20 kGy of E-beam, evaluation of higher doses may be required.
6. There is need to develop policies on adoption of new technologies that can assist farmers, millers in meeting phytosanitary requirements.
7. There is need to create awareness and adoption of new technologies like E-beam technology in Kenya especially to stakeholders like farmers, millers, retailers and policy makers.

REFERENCES

Abbas, H. K., Weaver, M. A., Zablotowicz, R. M., Horn, B. W., & Shier, W. T. (2005). Relationships between aflatoxin production and sclerotia formation among isolates of Aspergillus section Flavi from the Mississippi Delta. *Netherlands Journal of Plant Pathology*, *112*(3), 283–287. https://doi.org/10.1007/s10658-004-4888-8

Adekunle, A. A., & Bassir, O. (1973). The effects of aflatoxin B1 and palmotoxins B0 and G0 on the germination and leaf colour of the cowpea (vigna sinensis). *Mycopathologia et Mycologia Applicata*, *51*(4), 299–305. https://doi.org/10.1007/BF02057800

Adhikari, B. N., Bandyopadhyay, R., & Cotty, P. J. (2016). Degeneration of aflatoxin gene clusters in *Aspergillus flavus* from Africa and North America. *AMB Express*, *6*(1), 62. https://doi.org/10.1186/s13568-016-0228-6

Agriopoulou, S., Stamatelopoulou, E., & Varzakas, T. (2020). Advances in Analysis and Detection of Major Mycotoxins in Foods. *Foods*, *9*(4), Article 4. https://doi.org/10.3390/foods9040518

Ahmadsah, L. S. F., Kim, E., Jung, Y.-S., & Kim, H.-Y. (2018). *Identification of LAB and Fungi in Laru, a Fermentation Starter, by PCR-DGGE, SDS-PAGE, and MALDI-TOF MS*. *28*(1), 32–39. https://doi.org/10.4014/jmb.1705.05044

Akhila, P. P., Sunooj, K. V., Aaliya, B., Navaf, M., Sudheesh, C., Sabu, S., Sasidharan, A., Mir, S. A., George, J., & Mousavi Khaneghah, A. (2021). Application of electromagnetic radiations for decontamination of fungi and mycotoxins in food products: A comprehensive review. *Trends in Food Science & Technology*, *114*, 399–409. https://doi.org/10.1016/j.tifs.2021.06.013

Akkina, R. C., Payala, V., Maganti, S. S., Akkina, R. C., Payala, V., & Maganti, S. S. (2022). Tools for Rapid Detection and Control of Foodborne Microbial Pathogens. In *Foodborne*

Pathogens—Recent Advances in Control and Detection. IntechOpen. https://doi.org/10.5772/intechopen.103938

Aladhadh, M. (2023). A Review of Modern Methods for the Detection of Foodborne Pathogens. *Microorganisms*, *11*(5), Article 5. https://doi.org/10.3390/microorganisms11051111

Al-Hmoud, N., Ibrahim, M. A., Al-Rousan, H., & Alseyah, A. (2012). The Prevalence of Aflatoxinogenic *Aspergillus parasiticus* in Jordan. *International Journal of Microbiology*, *2012*, 675361. https://doi.org/10.1155/2012/675361

Almekinders, C., Hebinck, P., Marinus, W., Kiaka, R., & Waswa, W. (2021). Why farmers use so many different maize varieties in West Kenya. *Outlook on Agriculture*, *50*, 406–417. https://doi.org/10.1177/00307270211054211

Anankware, P. J., Fatunbi, A. O., Afreh-Nuamah, K, Obeng-Ofori, D, & Ansah, A.F. (2012). *Efficacy of the Multiple-Layer Hermetic Storage Bag for Biorational Management Of primary Beetle Pests of Stored Maize*. *5*(1), 47–53.

Ankwasa, E. M., Imade, F., & Tanvir, A. (2021). Update on mycotoxin contamination of maize and peanuts in East African Community countries. *Journal of Food Science and Nutrition Therapy*, 001–010. https://doi.org/10.17352/jfsnt.000026

Aoun, M., Stafstrom, W., Priest, P., Fuchs, J., Windham, G. L., Williams, W. P., & Nelson, R. J. (2020). Low-cost grain sorting technologies to reduce mycotoxin contamination in maize and groundnut. *Food Control*, *118*, 107363. https://doi.org/10.1016/j.foodcont.2020.107363

Ariño, A., Herrera, M., Juan, T., Estopañan, G., Carramiñana, J. J., Rota, C., & Herrera, A. (2009). Influence of agricultural practices on the contamination of maize by fumonisin

mycotoxins. *Journal of Food Protection*, *72*(4), 898–902. https://doi.org/10.4315/0362-028X-72.4.898

Armstrong, A. E., Rossoff, J., Hollemon, D., Hong, D. K., Muller, W. J., & Chaudhury, S. (2019). Cell-free DNA next-generation sequencing successfully detects infectious pathogens in pediatric oncology and hematopoietic stem cell transplant patients at risk for invasive fungal disease. *Pediatric Blood & Cancer*, *66*(7), e27734. https://doi.org/10.1002/pbc.27734

Arnarson, A. (2019, May 16). *Corn 101: Nutrition Facts and Health Benefits*. Healthline. https://www.healthline.com/nutrition/foods/corn

Assuncao, E., Reis, T., Baquiao, A., & Correa, B. (2015). Effects of Gamma and Electron Beam Radiation on Brazil Nuts Artificially Inoculated with *Aspergillus flavus*. *Journal of Food Protection*, *78*(7), 1397–1401. https://doi.org/10.4315/0362-028X.JFP-14-595

Atehnkeng, J., Ojiambo, P. S., Donner, M., Ikotun, T., Sikora, R. A., Cotty, P. J., & Bandyopadhyay, R. (2008). Distribution and toxigenicity of Aspergillus species isolated from maize kernels from three agro-ecological zones in Nigeria. *International Journal of Food Microbiology*, *122*(1–2), 74–84. https://doi.org/10.1016/j.ijfoodmicro.2007.11.062

Aziz, N., & Moussa, L. (2002). Influence of γ-radiation on mycotoxin producing moulds and mycotoxins in fruits. *Food Control*, *13*, 281–288. https://doi.org/10.1016/S0956-7135(02)00028-2

Bacon, C. W., Yates, I. E., Hinton, D. M., & Meredith, F. (2001). Biological control of *FusariumFusarium moniliforme* in maize. *Environmental Health Perspectives*, *109 Suppl 2*(Suppl 2), 325–332. https://doi.org/10.1289/ehp.01109s2325

Bader, O. (2017). Fungal Species Identification by MALDI-ToF Mass Spectrometry. *Methods in Molecular Biology (Clifton, N.J.), 1508*, 323–337. https://doi.org/10.1007/978-1-4939-6515-1_19

Bakri,A, Mehta, K, & Lance, D. R. (2016, April 13). *Sterile insect technique* [Text]. IAEA. https://www.iaea.org/topics/sterile-insect-technique

Batchelor, W. D., Suresh, L. M., Zhen, X., Beyene, Y., Wilson, M., Kruseman, G., & Prasanna, B. (2020). Simulation of Maize Lethal Necrosis (MLN) Damage Using the CERES-Maize Model. *Agronomy*, *10*(5), Article 5. https://doi.org/10.3390/agronomy10050710

Battilani, P., Rossi, V., Giorni, P., Pietri, A., Gualla, A., van der Fels-Klerx, H. j., Booij, C. j. h., Moretti, A., Logrieco, A., Miglietta, F., Toscano, P., Miraglia, M., De Santis, B., & Brera, C. (2012). Modelling, predicting and mapping the emergence of aflatoxins in cereals in the EU due to climate change. *EFSA Supporting Publications*, *9*(1), 223E. https://doi.org/10.2903/sp.efsa.2012.EN-223

Baylis, C. L. (2006). Enterobacteriaceae. *Food Spoilage Microorganisms*, 624–667. https://doi.org/10.1533/9781845691417.5.624

Beley, M. A. J., & Teves, F. G. (2013). *Isolation of Fungal Species and Detection of Aflatoxin from Soy Milk Products using ELISA method*. *2*.

Bergen, A. W., Qi, Y., Haque, K. A., Welch, R. A., Garcia-Closas, M., Chanock, S. J., Vaught, J., & Castle, P. E. (2005). Effects of electron-beam irradiation on whole genome amplification. *Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology*, *14*(4), 1016–1019. https://doi.org/10.1158/1055-9965.EPI-04-0686

Bhat, R., & Sridhar, K. R. (2008). Nutritional quality evaluation of electron beam-irradiated lotus (Nelumbo nucifera) seeds. *Food Chemistry*, *107*(1), 174–184. https://doi.org/10.1016/j.foodchem.2007.08.002

Bhat, R., Sridhar, K. R., & Karim, A. A. (2010). Microbial quality evaluation and effective decontamination of nutraceutically valued lotus seeds by electron beams and gamma irradiation. *Radiation Physics and Chemistry*, *79*(9), 976–981. https://doi.org/10.1016/j.radphyschem.2010.04.002

Bhat, R., Sridhar, K. R., & Tomita-Yokotani, K. (2007). Effect of ionizing radiation on antinutritional features of velvet bean seeds (Mucuna pruriens). *Food Chemistry*, *103*(3), 860–866. https://doi.org/10.1016/j.foodchem.2006.09.037

Bisht, B., Bhatnagar, P., Gururani, P., Kumar, V., Tomar, M. S., Sinhmar, R., Rathi, N., & Kumar, S. (2021). Food irradiation: Effect of ionizing and non-ionizing radiations on preservation of fruits and vegetables– a review. *Trends in Food Science & Technology*, *114*, 372–385. https://doi.org/10.1016/j.tifs.2021.06.002

Blount, W. (1961). Turkey "X" disease. *J Br Turkey Fed*, *9*, 52–77.

Bluhm, B. H., Cousin, M. A., & Woloshuk, C. P. (2004). Multiplex real-time PCR detection of fumonisin-producing and trichothecene-producing groups of *Fusarium* species. *Journal of Food Protection*, *67*(3), 536–543. https://doi.org/10.4315/0362-028X-67.3.536

Bottalico, A. (1998). *Fusarium* Diseases of Cereals: Species Complex and Related Mycotoxin Profiles, in Europe. *Journal of Plant Pathology*, *80*(2), 85–103.

Braun, M. S., & Wink, M. (2018). Exposure, occurrence, and chemistry of fumonisins and their cryptic derivatives. *Comprehensive Reviews in Food Science and Food Safety*, *17*(3), 769–791. https://doi.org/10.1111/1541-4337.12334

Brodal, G., Aamot, H. U., Almvik, M., & Hofgaard, I. S. (2020). Removal of small kernels reduces the content of *Fusarium* mycotoxins in oat grain. *Toxins*, *12*(5), 346. https://doi.org/10.3390/toxins12050346

Brzonkalik, K., Hümmer, D., Syldatk, C., & Neumann, A. (2012). Influence of pH and carbon to nitrogen ratio on mycotoxin production by *Alternaria alternata* in submerged cultivation. *AMB Express*, *2*(1), 28. https://doi.org/10.1186/2191-0855-2-28

Burmistrova, N. A., Rusanova, T. Y., Yurasov, N. A., Goryacheva, I. Y., & De Saeger, S. (2014). Multi-detection of mycotoxins by membrane based flow-through immunoassay. *Food Control*, *46*, 462–469. https://doi.org/10.1016/j.foodcont.2014.05.036

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T. (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry*, *55*(4), 611–622. https://doi.org/10.1373/clinchem.2008.112797

Butsenko, L., Kalinichenko, A., Pasichnyk, L., Nepran, I., & Patyka, V. (2018). IDENTIFICATION OF PHYTOPATHOGENIC BACTERIA IN MAIZE SEEDS IN UKRAINE. *The Journal "Agriculture and Forestry"*, *64*(4). https://www.academia.edu/58962521/Identification_of_Phytopathogenic_Bacteria_in_Maize_Seeds_in_Ukraine

Calado, T., Venâncio, A., & Abrunhosa, L. (2014). Irradiation for Mold and Mycotoxin Control: A Review. *Comprehensive Reviews in Food Science and Food Safety*, *13*(5), 1049–1061. https://doi.org/10.1111/1541-4337.12095

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal, 11*(12), Article 12. https://doi.org/10.1038/ismej.2017.119

Capcarova, M., Zbynovska, K., Kalafova, A., Bulla, J., & Bielik, P. (2016). Environment contamination by mycotoxins and their occurrence in food and feed: Physiological aspects and economical approach. *Journal of Environmental Science and Health, Part B, 51*(4), 236–244. https://doi.org/10.1080/03601234.2015.1120617

Capraru, O., Albota, F, Virgolici, M, Negut, D, & Moise, V. (2023). *E-beam simulation for cultural heritage cellulosic material preservation at Irasm* (75; Romanian Reports in Physics, p. 801). https://rrp.nipne.ro/2023/AN75801.pdf

Centre for Food Safety. (2023). *Effects on Farmers & the Environment | | Effects on Farmers & the Environment*. Center for Food Safety. https://www.centerforfoodsafety.org/issues/303/seeds/effects-on-farmers-and-the-environment

CGIAR, Perfomance report. (2019). Aflasafe: Safeguarding health and livelihoods with biocontrol technology in Africa. *CGIAR*. https://www.cgiar.org/annual-report/performance-report-2019/aflasafe/

Chavez, R. A., Cheng, X., & Stasiewicz, M. J. (2020). A review of the methodology of analyzing aflatoxin and fumonisin in single corn kernels and the potential impacts of these methods on food Security. *Foods, 9*(3), Article 3. https://doi.org/10.3390/foods9030297

Chavez, R. A., Opit, G., Opoku, B., & Stasiewicz, M. J. (2023). Spectral kernel sorting based on high-risk visual features associated with mycotoxin contamination reduces aflatoxin and

fumonisin contamination in maize from Ghana. *Food Control, 151*(109788). https://doi.org/10.1016/j.foodcont.2023.109788

Chemuniqué. (2019, November 1). Mycotoxin contamination: The socio-economic impact. *Chemuniqué*. https://www.chemunique.co.za/ruminant/dairy/mycotoxin-contamination-the-socio-economic-impact/

Chen, C., Mitchell, N. J., Gratz, J., Houpt, E. R., Gong, Y., Egner, P. A., Groopman, J. D., Riley, R. T., Showker, J. L., Svensen, E., Mduma, E. R., Patil, C. L., & Wu, F. (2018). Exposure to aflatoxin and fumonisin in children at risk for growth impairment in rural Tanzania. *Environment International, 115*, 29–37. https://doi.org/10.1016/j.envint.2018.03.001

Chen, X., Landschoot, S., Detavernier, C., De Saeger, S., Rajkovic, A., & Audenaert, K. (2021). Cross-talk between *Fusarium verticillioides* and *Aspergillus flavus* in vitro and in planta. *Mycotoxin Research, 37*(3), 229–240. https://doi.org/10.1007/s12550-021-00435-x

Chen, X., Qiu, Y., Zhang, J., Guo, Y., Ding, Y., & Lyu, F. (2022). Degradation efficiency and products of deoxynivalenol treated by cold plasma and its application in wheat. *Food Control, 136*, 108874. https://doi.org/10.1016/j.foodcont.2022.108874

Chmielewska-Śmietanko, D., Gryczka, U., Migdał, W., & Kopeć, K. (2018). Electron beam for preservation of biodeteriorated cultural heritage paper-based objects. *Radiation Physics and Chemistry, 143*, 89–93. https://doi.org/10.1016/j.radphyschem.2017.07.008

Chu, X., Wang, W., Yoon, S.-C., Ni, X., & Heitschmidt, G. W. (2017). Detection of aflatoxin B1 (AFB1) in individual maize kernels using short wave infrared (SWIR) hyperspectral imaging. *Biosystems Engineering, 157*, 13–23. https://doi.org/10.1016/j.biosystemseng.2017.02.005

CIMMYT. (2017, June 30). Farmers at center of sustainable agriculture in Kenya. *CIMMYT*. https://www.cimmyt.org/news/farmers-at-center-of-sustainable-agriculture-in-kenya/

Claeys, L., Romano, C., De Ruyck, K., Wilson, H., Fervers, B., Korenjak, M., Zavadil, J., Gunter, M. J., De Saeger, S., De Boevre, M., & Huybrechts, I. (2020). Mycotoxin exposure and human cancer risk: A systematic review of epidemiological studies. *Comprehensive Reviews in Food Science and Food Safety*, *19*(4), 1449–1464. https://doi.org/10.1111/1541-4337.12567

Cobo-Díaz, J. F., Legrand, F., Le Floch, G., & Picot, A. (2022). Influence of Maize Residues in Shaping Soil Microbiota and *Fusarium* spp. Communities. *Microbial Ecology*, *83*(3), 702–713. https://doi.org/10.1007/s00248-021-01797-6

Codex Alimentarius. (1997). *General Standard for Contaminants and Toxins in Food and Feed* [Codex standard]. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B193-1995%252FCXS_193e.pdf

Costa, S. J. (2014). *An 'Action Research' evaluation trial from Uganda and Burkina Faso.* https://documents.wfp.org/stellent/groups/public/documents/special_initiatives/WFP265205.pdf

Crisan, E. (1973). *Effects of Aflatoxin on Seeding Growth and Ultrastructure in Plants | Applied Microbiology*. https://journals.asm.org/doi/10.1128/am.26.6.991-1000.1973

Cruz, D. R., Leandro, L. F. S., & Munkvold, G. P. (2019). Effects of Temperature and pH on *Fusarium oxysporum* and Soybean Seedling Disease. *Plant Disease*, *103*(12), 3234–3243. https://doi.org/10.1094/PDIS-11-18-1952-RE

Czembor, E., Stępień, Ł., & Waśkiewicz, A. (2014). *Fusarium* temperatum as a New Species Causing Ear Rot on Maize in Poland. *Plant Disease*, *98*(7), 1001. https://doi.org/10.1094/PDIS-11-13-1184-PDN

Da Costa, A. (2013). Production of tannase and gallic acid by *Aspergillus tamarii* in submerged and solid state cultures. *African Journal of Biochemistry Research*, *7*(10), 197–202. https://doi.org/10.5897/AJBR12.073

Da Silva, N., Taniwaki, M. H., Junqueira, C., Silveira, N., Okazaki, M., & Gomes, R. (2018). *Microbiological Examination Methods of Food and Water: A Laboratory Manual ... - Neusely da Silva, Marta H. Taniwaki, Valéria C.A. Junqueira, Neliane Silveira, Margarete Midori Okazaki, Renato Abeilar Romeiro Gomes—Google Books*. https://books.google.co.ke/books/about/Microbiological_Examination_Methods_of_F.html?id=i7J5DwAAQBAJ&redir_esc=y

Daou, R., Joubrane, K., Maroun, R. G., Khabbaz, L. R., Ismail, A., Khoury, A. E., Daou, R., Joubrane, K., Maroun, R. G., Khabbaz, L. R., Ismail, A., & Khoury, A. E. (2021). Mycotoxins: Factors influencing production and control strategies. *AIMS Agriculture and Food*, *6*(1), Article agrfood-06-01-025. https://doi.org/10.3934/agrfood.2021025

Darwish, W. S., Ikenaka, Y., Nakayama, S. M. M., & Ishizuka, M. (2014). An Overview on Mycotoxin Contamination of Foods in Africa. *The Journal of Veterinary Medical Science*, *76*(6), 789–797. https://doi.org/10.1292/jvms.13-0563

Dasan, B. G., Mutlu, M., & Boyaci, I. H. (2016). Decontamination of *Aspergillus flavus* and *Aspergillus parasiticus* spores on hazelnuts via atmospheric pressure fluidized bed plasma reactor. *International Journal of Food Microbiology*, *216*, 50–59. https://doi.org/10.1016/j.ijfoodmicro.2015.09.006

De Arriola, M. del C., De Porres, E., De Cabrera, S., De Zepeda, M., & Rolz, C. (1988). Aflatoxin fate during alkaline cooking of corn for tortilla preparation. *Journal of Agricultural and Food Chemistry*, *36*(3), 530–533. https://doi.org/10.1021/jf00081a030

Deepa, N., Achar, P. N., & Sreenivasa, M. Y. (2021). Current perspectives of biocontrol agents for management of *Fusarium verticillioides* and Its fumonisin in cereals-A review. *Journal of Fungi (Basel, Switzerland)*, *7*(9), 776. https://doi.org/10.3390/jof7090776

Degraeve, S., Madege, R. R., Audenaert, K., Kamala, A., Ortiz, J., Kimanya, M., Tiisekwa, B., De Meulenaer, B., & Haesaert, G. (2016). Impact of local pre-harvest management practices in maize on the occurrence of *Fusarium* species and associated mycotoxins in two agro-ecosystems in Tanzania. *Food Control*, *59*, 225–233. https://doi.org/10.1016/j.foodcont.2015.05.028

Di Febo, T., Schirone, M., Visciano, P., Portanti, O., Armillotta, G., Persiani, T., Di Giannatale, E., Tittarelli, M., & Luciani, M. (2019). Development of a Capture ELISA for Rapid Detection of Salmonella enterica in Food Samples. *Food Analytical Methods*, *12*(2), 322–330. https://doi.org/10.1007/s12161-018-1363-2

Dix, N. J., & Webster, J. (1995). Fungi of Extreme Environments. In N. J. Dix & J. Webster (Eds.), *Fungal Ecology* (pp. 322–340). Springer Netherlands. https://doi.org/10.1007/978-94-011-0693-1_12

D-map.com. (2023). *Kenya free map, free blank map, free outline map, free base map outline, counties, names, color*. https://d-maps.com/carte.php?num_car=35032&lang=en

Domenico, A., Christ, D., Hashimoto, E. H., Busso, C., & Coelho, S. R. M. (2015). Evaluation of quality attributes and the incidence of *Fusarium* sp. And *Aspergillus* sp. In different types

of maize storage. *Journal of Stored Products Research, 61*, 59–64. https://doi.org/10.1016/j.jspr.2014.12.001

Doohan, F. M., Brennan, J., & Cooke, B. M. (2003). Influence of Climatic Factors on *Fusarium* Species Pathogenic to Cereals. *European Journal of Plant Pathology*, *109*(7), 755–768. https://doi.org/10.1023/A:1026090626994

D'Ovidio, K. L., Trucksess, M. W., Devries, J. W., & Bean, G. (2007). Effects of irradiation on fungi and fumonisin B1 in corn, and of microwave-popping on fumonisins in popcorn. *Food Additives & Contaminants*, *24*(7), 735–743. https://doi.org/10.1080/02652030701216453

Dowell, F. E., Pearson, T. C., Maghirang, E. B., Xie, F., & Wicklow, D. T. (2002). Reflectance and Transmittance Spectroscopy Applied to Detecting Fumonisin in Single Corn Kernels Infected with *Fusarium verticillioides*. *Cereal Chemistry*, *79*(2), 222–226. https://doi.org/10.1094/CCHEM.2002.79.2.222

Dura, S. (2022, May 6). *Toxigenic Fungi And Fungal Mycotoxins In Food- An Overview*. Microbe Notes. https://microbenotes.com/fungal-mycotoxins-in-food/

Duran, R., Cary, J. W., & Calvo, A. M. (2010). Role of the Osmotic Stress Regulatory Pathway in Morphogenesis and Secondary Metabolism in Filamentous Fungi. *Toxins*, *2*(4), 367–381. https://doi.org/10.3390/toxins2040367

Edgar, R. C. (2017). Accuracy of microbial community diversity estimated by closed- and open-reference OTUs. *PeerJ*, *5*, e3889. https://doi.org/10.7717/peerj.3889

Egrutz. (2018, March 28). Horse Labor Instead of Tractors. *Small Farmer's Journal*. https://smallfarmersjournal.com/horse-labor-instead-of-tractors/

Ehrlich, K. C. (2014). Non-aflatoxigenic *Aspergillus flavus* to prevent aflatoxin contamination in crops: Advantages and limitations. *Frontiers in Microbiology*, *5*, 50. https://doi.org/10.3389/fmicb.2014.00050

Ehrlich, K. C., Chang, P.-K., Yu, J., & Cotty, P. J. (2004). Aflatoxin Biosynthesis Cluster Gene *cypA* Is Required for G Aflatoxin Formation. *Applied and Environmental Microbiology*, *70*(11), 6518–6524. https://doi.org/10.1128/AEM.70.11.6518-6524.2004

Ekwomadu, T. I., Akinola, S. A., & Mwanza, M. (2021). *Fusarium* Mycotoxins, Their Metabolites (Free, Emerging, and Masked), Food Safety Concerns, and Health Impacts. *International Journal of Environmental Research and Public Health*, *18*(22), 11741. https://doi.org/10.3390/ijerph182211741

Ekwomadu, T. I., Dada, T. A., Nleya, N., Gopane, R., Sulyok, M., & Mwanza, M. (2020). Variation of *Fusarium* Free, Masked, and Emerging Mycotoxin Metabolites in Maize from Agriculture Regions of South Africa. *Toxins*, *12*(3), Article 3. https://doi.org/10.3390/toxins12030149

Elbehiry, A., Marzouk, E., Hamada, M., Al-Dubaib, M., Alyamani, E., Moussa, I. M., AlRowaidhan, A., & Hemeg, H. A. (2017). Application of MALDI-TOF MS fingerprinting as a quick tool for identification and clustering of foodborne pathogens isolated from food products. *The New Microbiologica*, *40*(4), 269–278.

Elfil, M., & Negida, A. (2017). Sampling methods in Clinical Research; an Educational Review. *Emergency*, *5*(1), e52.

EL-Saeid, M. H., Alotaibi, M. O., Alshabanat, M., Alharbi, K., Altowyan, A. S., & Al-Anazy, M. (2021). Photo-Catalytic Remediation of Pesticides in Wastewater Using UV/TiO2. *Water*, *13*(21), Article 21. https://doi.org/10.3390/w13213080

Esmaeili, S., Barzegar, M., Sahari, M. A., & Berengi-Ardestani, S. (2018). Effect of gamma irradiation under various atmospheres of packaging on the microbial and physicochemical properties of turmeric powder. *Radiation Physics and Chemistry*, *148*, 60–67. https://doi.org/10.1016/j.radphyschem.2018.02.028

Esua, O. J., Cheng, J.-H., & Sun, D.-W. (2020). Antimicrobial activities of plasma-functionalized liquids against foodborne pathogens on grass carp (Ctenopharyngodon Idella). *Applied Microbiology and Biotechnology*, *104*(22), 9581–9594. https://doi.org/10.1007/s00253-020-10926-z

Etter, D., Rupp, A., Prange, A., & Drissner, D. (2018). *Inactivation of mould spores in a model system and on raisins by low-energy electron beam | Elsevier Enhanced Reader*. https://doi.org/10.1016/j.foodcont.2018.05.006

Eustice, R. F. (2017). *Global Status and Commercial Applications of Food Irradiation*. https://doi.org/10.1039/9781788010252-00397

Ezekiel, C. N., Atehnkeng, J., Odebode, A. C., & Bandyopadhyay, R. (2014). Distribution of aflatoxigenic *Aspergillus* section *Flavi* in commercial poultry feed in Nigeria. *International Journal of Food Microbiology*, *189*, 18–25. https://doi.org/10.1016/j.ijfoodmicro.2014.07.026

Ezekiel, C. N., Ortega-Beltran, A., & Bandyopadhyay, R. (n.d). *The Need for Integrated Approaches to Address Food Safety Risk: The Case of Mycotoxins in Africa*.

Fan, X., & Niemira, B. A. (2020). Gamma Ray, Electron Beam, and X-ray Irradiation. In A. Demirci, H. Feng, & K. Krishnamurthy (Eds.), *Food Safety Engineering* (pp. 471–492). Springer International Publishing. https://doi.org/10.1007/978-3-030-42660-6_18

Fandohan, P., Gnonlonfin, B., Hell, K., Marasas, W. F. O., & Wingfield, M. J. (2005). Natural occurrence of *Fusarium* and subsequent fumonisin contamination in preharvest and stored maize in Benin, West Africa. *International Journal of Food Microbiology*, *99*(2), 173–183. https://doi.org/10.1016/j.ijfoodmicro.2004.08.012

Faraji, H., Yazdi, F. T., & Razmi, N. (2022). The influence of ultraviolet radiation on aflatoxin producing Aspergillus species' isolated from Iranian rice. *Toxicology Reports*, *9*, 1528–1536. https://doi.org/10.1016/j.toxrep.2022.07.007

FarmLINK Kenya. (2017). *Maize Production in Kenya – FarmLINK Kenya*. http://www.farmlinkkenya.com/maize-production/

Farooq, S., Kurucz, C. N., Waite, T. D., & Cooper, W. J. (1993). Disinfection of wastewaters: High-energy electron vs gamma irradiation. *Water Research*, *27*(7), 1177–1184. https://doi.org/10.1016/0043-1354(93)90009-7

Fatemeh, Z., Alireza, K., & Hojjatollah, S. (2011). Evaluation of the correlation between Aflatoxin B1 production and Aspergillus contamination in rice bran from northern Iran. *African Journal of Microbiology Research*, *5*(11), 1306–1310. https://doi.org/10.5897/AJMR11.132

FDA, C. for V. (2023). Chemical Hazards. *FDA*. https://www.fda.gov/animal-veterinary/biological-chemical-and-physical-contaminants-animal-food/chemical-hazards

Femenias, A., Gatius, F., Ramos, A. J., Sanchis, V., & Marín, S. (2020). Use of hyperspectral imaging as a tool for *Fusarium* and deoxynivalenol risk management in cereals: A review. *Food Control*, *108*, 106819. https://doi.org/10.1016/j.foodcont.2019.106819

Ferrara, M., Perrone, G., Gallo, A., Epifani, F., Visconti, A., & Susca, A. (2015). Development of loop-mediated isothermal amplification (LAMP) assay for the rapid detection of

Penicillium nordicum in dry-cured meat products. *International Journal of Food Microbiology, 202*, 42–47. https://doi.org/10.1016/j.ijfoodmicro.2015.02.021

Ferrigo, D., Raiola, A., & Causin, R. (2014). Plant stress and mycotoxin accumulation in maize. *Agrochimica -Pisa-, LVII*, 116–127.

FEWS NET. (2021, October 27). *East Africa Regional Maize Supply and Market Outlook, October 2021—Kenya | ReliefWeb*. https://reliefweb.int/report/kenya/east-africa-regional-maize-supply-and-market-outlook-october-2021

Fox, G., & Manley, M. (2014). Applications of single kernel conventional and hyperspectral imaging near infrared spectroscopy in cereals. *Journal of the Science of Food and Agriculture, 94*(2), 174–179. https://doi.org/10.1002/jsfa.6367

Freitas-Silva, O., Oliveira, P., & Junior, M. (2014). Potential of Electron Beams to Control Mycotoxigenic Fungi in Food. *Food Engineering Reviews, 7*. https://doi.org/10.1007/s12393-014-9093-8

Freitas-Silva, O., & Venâncio, A. (2010). Ozone applications to prevent and degrade mycotoxins: A review. *Drug Metabolism Reviews, 42*(4), 612–620. https://doi.org/10.3109/03602532.2010.484461

Furtas, R. (2018). *Overview of certified seed and farmer saved seed*. Economic and Competitiveness Branch, Alberta Government. https://www1.agric.gov.ab.ca/$Department/deptdocs.nsf/all/econ15976/$FILE/Overview%20of%20Certified%20Seed%20and%20Farmer%20Saved%20Seed%20II.pdf#:~:text=The%20top%20reasons%20to%20use%20certified%20seed%20according,towards%20certified%20seed%20were%20dependent%20on%20producer%20demographics.

Garber, R. K., & Cotty, P. J. (1997). Formation of Sclerotia and Aflatoxins in Developing Cotton Bolls Infected by the S Strain of *Aspergillus flavus* and Potential for Biocontrol with an Atoxigenic Strain. *Phytopathology®*, *87*(9), 940–945. https://doi.org/10.1094/PHYTO.1997.87.9.940

Garrido-Bazan, V., Mahuku, G., Bibbins-Martinez, M., Arroyo-Bacerra, A., & Villalobos-López, M. Á. (2018). Dissection of mechanisms of resistance to *Aspergillus flavus* and aflatoxin using tropical maize germplasm. *World Mycotoxin Journal*, *11*(2), 215–224. https://doi.org/10.3920/WMJ2017.2219

Gautam, R. K., & Venugopal, V. (2021). Electron beam irradiation to control biohazards in seafood. *Food Control*, *130*, 108320. https://doi.org/10.1016/j.foodcont.2021.108320

Gelineau-van Waes, J., Voss, K. A., Stevens, V. L., Speer, M. C., & Riley, R. T. (2009). Maternal fumonisin exposure as a risk factor for neural tube defects. *Advances in Food and Nutrition Research*, *56*, 145–181. https://doi.org/10.1016/S1043-4526(08)00605-0

Ghanem, E., Orfi, M., & Shamma, M. (2008). Effect of gamma radiation on the inactivation of aflatoxin B1 in food and feed crops. *Brazilian Journal of Microbiology : [Publication of the Brazilian Society for Microbiology]*, *39*, 787–791. https://doi.org/10.1590/S1517-838220080004000035

Giorni, P., Magan, N., Pietri, A., Bertuzzi, T., & Battilani, P. (2007). Studies on Aspergillus section Flavi isolated from maize in northern Italy. *International Journal of Food Microbiology*, *113*(3), 330–338. https://doi.org/10.1016/j.ijfoodmicro.2006.09.007

Glenn, A. E., Zitomer, N. C., Zimeri, A. M., Williams, L. D., Riley, R. T., & Proctor, R. H. (2008). Transformation-mediated complementation of a FUM gene cluster deletion in *Fusarium verticillioides* restores both fumonisin production and pathogenicity on maize

seedlings. *Molecular Plant-Microbe Interactions: MPMI*, *21*(1), 87–97. https://doi.org/10.1094/MPMI-21-1-0087

Gong, Y., Hounsa, A., Egal, S., Turner, P. C., Sutcliffe, A. E., Hall, A. J., Cardwell, K., & Wild, C. P. (2004). Postweaning Exposure to Aflatoxin Results in Impaired Child Growth: A Longitudinal Study in Benin, West Africa. *Environmental Health Perspectives*, *112*(13), 1334–1338. https://doi.org/10.1289/ehp.6954

Goryacheva, I. Y., De Saeger, S., Eremin, S. A., & Van Peteghem, C. (2007). Immunochemical methods for rapid mycotoxin detection: Evolution from single to multiple analyte screening: a review. *Food Additives and Contaminants*, *24*(10), 1169–1183. https://doi.org/10.1080/02652030701557179

Gouda, M. K., & Abdel-Naby, M. A. (2002). Catalytic properties of the immobilized Aspergillus tamarii xylanase. *Microbiological Research*, *157*(4), 275–281. https://doi.org/10.1078/0944-5013-00165

Gowthami, R., Vanniarajan, C., Souframanien, J., Veni, K., & Renganathan, V. G. (2021). Efficiency of electron beam over gamma rays to induce desirable grain-type mutation in rice (Oryza sativa L.). *International Journal of Radiation Biology*, *97*(5), 727–736. https://doi.org/10.1080/09553002.2021.1889702

Greeff-Laubscher, M. R., Beukes, I., Marais, G. J., & Jacobs, K. (2019). Mycotoxin production by three different toxigenic fungi genera on formulated abalone feed and the effect of an aquatic environment on fumonisins. *Mycology*, *11*(2), 105–117. https://doi.org/10.1080/21501203.2019.1604575

Gryczka, U., Kameya, H., Kimura, K., Todoriki, S., Migdał, W., & Bułka, S. (2020). Efficacy of low energy electron beam on microbial decontamination of spices. *Radiation Physics and Chemistry*, *170*, 108662. https://doi.org/10.1016/j.radphyschem.2019.108662

Guera, O. G. M., Castrejón-Ayala, F., Robledo, N., Jiménez-Pérez, A., Sánchez-Rivera, G., Salazar-Marcial, L., & Flores Moctezuma, H. E. (2021). Effectiveness of Push–Pull Systems to Fall Armyworm (*Spodoptera frugiperda*) Management in Maize Crops in Morelos, Mexico. *Insects*, *12*(4), 298. https://doi.org/10.3390/insects12040298

Haarhoff, S. J., & Swanepoel, P. A. (2018). Plant Population and Maize Grain Yield: A Global Systematic Review of Rainfed Trials. *Crop Science*, *58*(5), 1819–1829. https://doi.org/10.2135/cropsci2018.01.0003

Haff, R. P., & Pearson, T. C. (2006). Spectral Band Selection for Optical Sorting of Pistachio Nut Defects. *Transactions of the ASABE*, *49*. https://doi.org/10.13031/2013.21716

Hallman, G. J. (2013). Control of stored product pests by ionizing radiation. *Journal of Stored Products Research*, *52*, 36–41. https://doi.org/10.1016/j.jspr.2012.10.001

Hamad, G. M., Mehany, T., Simal-Gandara, J., Abou-Alella, S., Esua, O. J., Abdel-Wahhab, M. A., & Hafez, E. E. (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

Han, D., Yao, H., Hruska, Z., Kincaid, R., Ramezanpour, C., Rajasekaran, K., & Bhatnagar, D. (2018). Development of high speed dual-camera system for batch screening of aflatoxin contamination of corn using multispectral fluorescence imaging. *Sensing for Agriculture and Food Quality and Safety X*, *10665*, 63–71. https://doi.org/10.1117/12.2305148

Harrell, C. R., Djonov, V., Fellabaum, C., & Volarevic, V. (2018). Risks of Using Sterilization by Gamma Radiation: The Other Side of the Coin. *International Journal of Medical Sciences*, *15*(3), 274–279. https://doi.org/10.7150/ijms.22644

Harrison, L. R., Colvin, B. M., Greene, J. T., Newman, L. E., & Cole, J. R. (1990). Pulmonary edema and hydrothorax in swine produced by fumonisin B1, a toxic metabolite of Fusarium *moniliforme*. *Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc*, *2*(3), 217–221. https://doi.org/10.1177/104063879000200312

Hell, K., & Mutegi, C. (2011). Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa. *African Journal of Microbiology Research*, *5*, 459–466.

Hojnik, N., Modic, M., Walsh, J. L., Žigon, D., Javornik, U., Plavec, J., Žegura, B., Filipič, M., & Cvelbar, U. (2021). Unravelling the pathways of air plasma induced aflatoxin B1 degradation and detoxification. *Journal of Hazardous Materials*, *403*, 123593. https://doi.org/10.1016/j.jhazmat.2020.123593

Homa, M., Manikandan, P., Szekeres, A., Kiss, N., Kocsubé, S., Kredics, L., Alshehri, B., Dukhyil, A. A. B., Revathi, R., Narendran, V., Vágvölgyi, C., Shobana, C. S., & Papp, T. (2019). Characterization of *Aspergillus tamarii* Strains From Human Keratomycoses: Molecular Identification, Antifungal Susceptibility Patterns and Cyclopiazonic Acid Producing Abilities. *Frontiers in Microbiology*, *10*. https://www.frontiersin.org/articles/10.3389/fmicb.2019.02249

Hooshmand, H., & Klopfenstein, C. F. (1995). Effects of gamma irradiation on mycotoxin disappearance and amino acid contents of corn, wheat, and soybeans with different

moisture contents. *Plant Foods for Human Nutrition*, *47*(3), 227–238. https://doi.org/10.1007/BF01088331

Houbraken, J., Kocsubé, S., Visagie, C. M., Yilmaz, N., Wang, X.-C., Meijer, M., Kraak, B., Hubka, V., Bensch, K., Samson, R. A., & Frisvad, J. C. (2020). Classification of *Aspergillus, Penicillium, Talaromyces* and related genera (*Eurotiales*): An overview of families, genera, subgenera, sections, series and species. *Studies in Mycology*, *95*, 5–169. https://doi.org/10.1016/j.simyco.2020.05.002

IARC. (2002). *Aflatoxins. IARC Monograph on the Evaluation of Carcinogenic Risks to Humans*. *82*, 171.

ICRISAT. (2020, March 29). *Farmers in Eastern Kenya reap big profits from improved varieties of drought-tolerant crops—Kenya | ReliefWeb*. https://reliefweb.int/report/kenya/farmers-eastern-kenya-reap-big-profits-improved-varieties-drought-tolerant-crops

Iheanacho, H. E., Dutton, M. F., Steenkamp, P. A., Steenkamp, L., Makun, H. A., Swart, A., & Mthombeni, J. Q. (2014). Real time PCR of Nor~1 (aflD) gene of aflatoxin producing fungi and its correlative quantization to aflatoxin levels in South African compound feeds. *Journal of Microbiological Methods*, *97*, 63–67. https://doi.org/10.1016/j.mimet.2013.12.014

International Agency for Research on Cancer (IARC). (1993). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins* (Vol. 56). IARC Working Group, WHO, Lyon.

Iqdiam, B. M., Abuagela, M. O., Boz, Z., Marshall, S. M., Goodrich-Schneider, R., Sims, C. A., Marshall, M. R., MacIntosh, A. J., & Welt, B. A. (2020). Effects of atmospheric pressure

plasma jet treatment on aflatoxin level, physiochemical quality, and sensory attributes of peanuts. *Journal of Food Processing and Preservation*, *44*(1), e14305. https://doi.org/10.1111/jfpp.14305

Iqdiam, B. M., Feizollahi, E., Arif, M. F., Jeganathan, B., Vasanthan, T., Thilakarathna, M. S., & Roopesh, M. S. (2021). Reduction of T-2 and HT-2 mycotoxins by atmospheric cold plasma and its impact on quality changes and germination of wheat grains. *Journal of Food Science*, *86*(4), 1354–1371. https://doi.org/10.1111/1750-3841.15658

Ismaiel, A., & Papenbrock, J. (2015). Mycotoxins: Producing Fungi and Mechanisms of Phytotoxicity. *Agriculture*, *2015*, 492–537. https://doi.org/10.3390/agriculture5030492

Ita, B. N., Ariga, E. S., Michieka, R. W., & Muiru, W. M. (2014, August 19). *Comparative Efficiency of Tillage Practices in Maize – Current Agriculture Research Journal*. https://www.agriculturejournal.org/volume2number2/comparative-efficiency-of-tillage-practices-in-maize/

Ito, Y., Peterson, S. W., & Goto, T. (1998). Properties of *Aspergillus tamarii, A. caelatus* and related species from acidic tea field soils in Japan. *Mycopathologia*, *144*(3), 169–175. https://doi.org/10.1023/a:1007021527106

Jackson-Ziems, T. A., Rees, J. M., & Harveson, R. M. (2014). *Common Stalk Rot Diseases of Corn*. https://extensionpublications.unl.edu/assets/pdf/ec1898.pdf

Jagadeesan, B., Gerner-Smidt, P., Allard, M. W., Leuillet, S., Winkler, A., Xiao, Y., Chaffron, S., Van Der Vossen, J., Tang, S., Katase, M., McClure, P., Kimura, B., Ching Chai, L., Chapman, J., & Grant, K. (2019). The use of next generation sequencing for improving food safety: Translation into practice. *Food Microbiology*, *79*, 96–115. https://doi.org/10.1016/j.fm.2018.11.005

Jalili, M., Jinap, S., & Noranizan, A. (2010). Effect of gamma radiation on reduction of mycotoxins in black pepper. *Food Control*, *21*(10), 1388–1393. https://doi.org/10.1016/j.foodcont.2010.04.012

Janik, E., Niemcewicz, M., Podogrocki, M., Ceremuga, M., Gorniak, L., Stela, M., & Bijak, M. (2021). The Existing Methods and Novel Approaches in Mycotoxins' Detection. *Molecules*, *26*(13), 3981. https://doi.org/10.3390/molecules26133981

Jasson, V., Jacxsens, L., Luning, P., Rajkovic, A., & Uyttendaele, M. (2010). Alternative microbial methods: An overview and selection criteria. *Food Microbiology*, *27*(6), 710–730. https://doi.org/10.1016/j.fm.2010.04.008

Jena, P. R. (2022). Extension services as key determining factor for adoption of minimum tillage practice in Kenya: A plot level analysis. *Journal of Public Affairs*, *22*(S1), e2724. https://doi.org/10.1002/pa.2724

Jiang, S., Chen, Y., Han, S., Lv, L., & Li, L. (2022). Next-Generation Sequencing Applications for the Study of Fungal Pathogens. *Microorganisms*, *10*(10), 1882. https://doi.org/10.3390/microorganisms10101882

Joint FAO/IAEA/WHO Expert Committee. (1981). *Wholesomeness of irradiated food: Report of a Joint FAO/IAEA/WHO Expert Committee [meeting held in Geneva from 27 October to 3 November 1980]*. World Health Organization. https://apps.who.int/iris/handle/10665/41508

Joint FAO/WHO Expert Committee on Food Additives. (2001). *Safety evaluation of certain mycotoxins in food*. FAO. https://apps.who.int/iris/handle/10665/42467

Joint FAO/WHO Expert Committee on Food Additives. (2002). *Evaluation of certain mycotoxins in food: Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives*. https://apps.who.int/iris/handle/10665/42448?show=full

Juraschek, L. M., Kappenberg, A., & Amelung, W. (2022). Mycotoxins in soil and environment. *Science of The Total Environment*, *814*, 152425. https://doi.org/10.1016/j.scitotenv.2021.152425

Kakou, M. A., Adja, N. A., Johnson, F., Kouadia, A. M. A., Tienebo, E.-O., T. Kouadio, K., Danho, M., & Abo, K. (2020). Identification of Maize Insects and Fungi Affecting Sanitary and Physiological Quality of Stored maize Grains in Central Cote d'Ivoire. *Asian Journal of Crop Science*, *13*(1), 36–45. https://doi.org/10.3923/ajcs.2021.36.45

Kamano, H. M., Okoth, M., Makau, W.-K., & Patrick, P. (2021). Use and efficacy of low temperature plasma in foods: Promising intervention on aflatoxin control in maize in Kenya – A review. *African Journal of Food, Agriculture, Nutrition and Development*, *21*(9), 18662–18675. https://doi.org/10.18697/ajfand.104.20950

Kamil, O., Lupuliasa, D., Drăgănescu, D., Vlaia, L., Davila, C., & Vuia, T. (2011). *Interrelations of Drying Heat and Survival of Different Fungal Spores Within the Tablets Formulation*. https://www.semanticscholar.org/paper/INTERRELATIONS-OF-DRYING-HEAT-AND-SURVIVAL-OF-THE-Kamil-Lupuliasa/97b5d470b7c6dad4359cdda4f5aa56287d45fbe5

Kamle, M., Mahato, D. K., Devi, S., Lee, K. E., Kang, S. G., & Kumar, P. (2019). Fumonisins: Impact on agriculture, food, and human health and their management Strategies. *Toxins*, *11*(6), 328. https://doi.org/10.3390/toxins11060328

Kang'ethe, E. (2011). *Situation Analysis: Improving Food Safety in The Maize Value Chain in Kenya* (p. 11). University of Nairobi. https://www.academia.edu/es/24841078/SITUATION_ANALYSIS_IMPROVING_FOOD_SAFETY_IN_THE_MAIZE_VALUE_CHAIN_IN_KENYA_Report_prepared_for_FAO_by

Kang'ethe, E., Gatwiri, M., Sirma, A., Ouko, O., Mburugu, K., Kitala, P., Gitahi, N., Nderitu, J., Mung'atu, J., Hietaniemi, V., Joutsjoki, V., & Korhonen, H. (2017). Exposure of Kenyan population to aflatoxins in foods with special reference to Nandi and Makueni counties. *Food Quality and Safety*, *1*. https://doi.org/10.1093/fqsafe/fyx011

Kariuki, G., Njaramba, J., & Ombuki, C. (2020). Maize Output Supply Response to Climate Change in Kenya: An Econometric Analysis. *European Scientific Journal*, *Vol.16*, 63–83. https://doi.org/10.19044/esj.2020.v16n3p63

Karlovsky, P., Suman, M., Berthiller, F., De Meester, J., Eisenbrand, G., Perrin, I., Oswald, I. P., Speijers, G., Chiodini, A., Recker, T., & Dussort, P. (2016). Impact of food processing and detoxification treatments on mycotoxin contamination. *Mycotoxin Research*, *32*(4), 179–205. https://doi.org/10.1007/s12550-016-0257-7

Khamila, S., Ndaka, D., Makokha, A., Kyallo, F., Kinyanjui, P. K., Kanensi, O. J., & Mwai, J. (2019). Status of commercial maize milling industry and flour fortification in Kenya. *African Journal of Food Science*, *13*(3), 65–82. https://doi.org/10.5897/AJFS2018.1782

Khan, R., Ghazali, F. M., Mahyudin, N. A., & Samsudin, N. I. P. (2021). Biocontrol of Aflatoxins Using Non-Aflatoxigenic *Aspergillus flavus*: A Literature Review. *Journal of Fungi (Basel, Switzerland)*, *7*(5), 381. https://doi.org/10.3390/jof7050381

Khan, Z., Midega, C., Pittchar, J., Pickett, J., & Bruce, T. (2011). Push—pull technology: A conservation agriculture approach for integrated management of insect pests, weeds and soil health in Africa: UK government's Foresight Food and Farming Futures project. *International Journal of Agricultural Sustainability*, *9*(1), 162–170. https://doi.org/10.3763/ijas.2010.0558

Khan, Z., & Pickett, J. (2004). The "push-pull" strategy for stem borer management: A case study in exploiting biodiversity and chemical ecology. *The "Push-Pull" Strategy for Stemborer Management: A Case Study in Exploiting Biodiversity and Chemical Ecology*, 155–164.

Khan, Z. R., Midega, C. A. O., Njuguna, E. M., Amudavi, D. M., Wanyama, J. M., & Pickett, J. A. (2008). Economic performance of the 'push–pull' technology for stemborer and Striga control in smallholder farming systems in western Kenya. *Crop Protection*, *27*(7), 1084–1097. https://doi.org/10.1016/j.cropro.2008.01.005

Khaneghah, A., Moosavi, M. H., Oliveira, CarlosA. F., Vanin, F., & Sant'Ana, A. (2020). *Electron beam irradiation to reduce the mycotoxin and microbial contaminations of cereal-based products: An overview—PubMed.* https://pubmed.ncbi.nlm.nih.gov/32640360/

Kikuchi, O. k., Todoriki, S., Saito, M., & Hayashi, T. (2003). Efficacy of Soft-electron (Low-energy Electron Beam) for Soybean Decontamination in Comparison with Gamma-rays. *Journal of Food Science*, *68*(2), 649–652. https://doi.org/10.1111/j.1365-2621.2003.tb05725.x

Kim, D. M., Chung, S. H., & Chun, H. S. (2011). Multiplex PCR assay for the detection of aflatoxigenic and non-aflatoxigenic fungi in meju, a Korean fermented soybean food starter. *Food Microbiology*, *28*(7), 1402–1408. https://doi.org/10.1016/j.fm.2011.06.017

Kimanya, M. E., Routledge, M. N., Mpolya, E., Ezekiel, C. N., Shirima, C. P., & Gong, Y. Y. (2021). Estimating the risk of aflatoxin-induced liver cancer in Tanzania based on biomarker data. *PLOS ONE*, *16*(3), e0247281. https://doi.org/10.1371/journal.pone.0247281

Kimeli, C. M. (2013). *Determining Factors That Affect Maize Production In Turbo Constituency, Kenya*. http://erepository.uonbi.ac.ke/bitstream/handle/11295/56008/Chumo_Determining%20Factors%20That%20Affect%20Maize%20Production%20In%20Turbo%20Constituency.pdf?sequence=3

Kiš, M., Milošević, S., Vulić, A., Herceg, Z., Vukušić, T., & Pleadin, J. (2020). Efficacy of low pressure DBD plasma in the reduction of T-2 and HT-2 toxin in oat flour. *Food Chemistry*, *316*, 126372. https://doi.org/10.1016/j.foodchem.2020.126372

Kochiieru, Y., Mankevičienė, A., Cesevičienė, J., Semaškienė, R., Dabkevičius, Z., & Janavičienė, S. (2020). The influence of harvesting time and meteorological conditions on the occurrence of *Fusarium* species and mycotoxin contamination of spring cereals. *Journal of the Science of Food and Agriculture*, *100*(7), 2999–3006. https://doi.org/10.1002/jsfa.10330

Kokkonen, M., Jestoi, M., & Rizzo, A. (2005). The effect of substrate on mycotoxin production of selected *Penicillium* strains. *International Journal of Food Microbiology*, *99*(2), 207–214. https://doi.org/10.1016/j.ijfoodmicro.2004.08.014

Komen, J., Mutoko, M., Wanyama, J., C, R., & Mose, L. (2010). *Economics of Post-Harvest Maize Grain Losses in Trans Nzoia and Uasin Gishu Districts of Northwest Kenya*. https://www.researchgate.net/profile/Morgan-Mutoko/publication/262157107_ECONOMICS_OF_POST-HARVEST_MAIZE_GRAIN_LOSSES_IN_TRANS_NZOIA_AND_UASIN_GISHU_DISTRICTS_OF_NORTHWEST_KENYA/links/0a85e536ca6666f05d000000/ECONOMICS-OF-POST-HARVEST-MAIZE-GRAIN-LOSSES-IN-TRANS-NZOIA-AND-UASIN-GISHU-DISTRICTS-OF-NORTHWEST-KENYA.pdf

Kotani, A. (2012). [Development of HPLC with high-sensitive and precise electrochemical detection enabling dynamic analysis of compounds of biological importance]. *Yakugaku Zasshi: Journal of the Pharmaceutical Society of Japan*, *132*(9), 985–991. https://doi.org/10.1248/yakushi.132.985

Kottapalli, B., Wolf-Hall, C. E., & Schwarz, P. (2006). Effect of electron-beam irradiation on the safety and quality of *Fusarium*-infected malting barley. *International Journal of Food Microbiology*, *110*(3), 224–231. https://doi.org/10.1016/j.ijfoodmicro.2006.04.007

Kumar, A., Pathak, H., Bhadauria, S., & Sudan, J. (2021). Aflatoxin contamination in food crops: Causes, detection, and management: a review. *Food Production, Processing and Nutrition*, *3*(1), 17. https://doi.org/10.1186/s43014-021-00064-y

Kumari, R. (2021, July 19). ELISA- Principle, Types, Uses, Advantages and Disadvantages. *Microbiology Notes*. https://microbiologynotes.org/elisa-principle-types-uses-advantages-and-disadvantages/

Kumeda, Y., & Asao, T. (1996). Single-strand conformation polymorphism analysis of PCR-amplified ribosomal DNA internal transcribed spacers to differentiate species of

Aspergillus section *Flavi*. *Applied and Environmental Microbiology*, *62*(8), 2947–2952. https://doi.org/10.1128/aem.62.8.2947-2952.1996

Kwon, S., Kwon, H. J., Ryu, J. H., & Kim, Y. R. (2020). Dose Effect of Phytosanitary Irradiation on the Postharvest Quality of Cut Flowers. *Journal of People, Plants, and Environment*, *23*(2), 171–178. https://doi.org/10.11628/ksppe.2020.23.2.171

Kyung, H.-K., Ramakrishnan, S. R., & Kwon, J.-H. (2019). Dose rates of electron beam and gamma ray irradiation affect microbial decontamination and quality changes in dried red pepper (Capsicum annuum L.) powder. *Journal of the Science of Food and Agriculture*, *99*(2), 632–638. https://doi.org/10.1002/jsfa.9225

Laforgue, R., Guérin, L., Pernelle, J. J., Monnet, C., Dupont, J., & Bouix, M. (2009). Evaluation of PCR-DGGE methodology to monitor fungal communities on grapes. *Journal of Applied Microbiology*, *107*(4), 1208–1218. https://doi.org/10.1111/j.1365-2672.2009.04309.x

Lanza, C. M., Mazzaglia, A., Paladino, R., Auditore, L., Barnà, R. C., Loria, D., Trifirò, A., Trimarchi, M., & Bellia, G. (2013). Characterization of peeled and unpeeled almond (*Prunus amygdalus*) flour after electron beam processing. *Radiation Physics and Chemistry*, *86*, 140–144. https://doi.org/10.1016/j.radphyschem.2013.01.010

Leslie, J. F., & Summerell, B. A. (Eds.). (2006). *The Fusarium Laboratory Manual* (1st ed.). Wiley. https://doi.org/10.1002/9780470278376

Levasseur-Garcia, C. (2018). Updated Overview of Infrared Spectroscopy Methods for Detecting Mycotoxins on Cereals (Corn, Wheat, and Barley). *Toxins*, *10*(1), Article 1. https://doi.org/10.3390/toxins10010038

Li, R., Yang, S., Wang, D., Liang, J., Huang, T., Zhang, L., & Luo, A. (2021). Electron-beam irradiation delayed the postharvest senescence of kiwifruit during cold storage through regulating the reactive oxygen species metabolism. *Radiation Physics and Chemistry*, *189*, 109717. https://doi.org/10.1016/j.radphyschem.2021.109717

li, X., Chen, S., Aluoch, S. O., Mosongo, P., Cao, J., & Hu, C. (2018). Maize production status and yield limiting factors of Kenya. *Chinese Journal of Eco-Agriculture*, *26*, 567–573. https://doi.org/10.13930/j.cnki.cjea.170632

Liu, C., & Van der Fels-Klerx, H. J. (2021). *Toxins | Free Full-Text | Quantitative Modeling of Climate Change Impacts on Mycotoxins in Cereals: A Review*. https://www.mdpi.com/2072-6651/13/4/276

Liu, M., Zhao, L., Gong, G., Zhang, L., Shi, L., Dai, J., Han, Y., Wu, Y., Khalil, M. M., & Sun, L. (2022). Invited review: Remediation strategies for mycotoxin control in feed. *Journal of Animal Science and Biotechnology*, *13*, 19. https://doi.org/10.1186/s40104-021-00661-4

Liu, R., Wang, R., Lu, J., Chang, M., Jin, Q., Du, Z., Wang, S., Li, Q., & Wang, X. (2016). Degradation of AFB1 in aqueous medium by electron beam irradiation: Kinetics, pathway and toxicology. *Food Control*, *66*, 151–157. https://doi.org/10.1016/j.foodcont.2016.02.002

Liverpool-Tasie, L. S. O., Turna, N. S., Ademola, O., Obadina, A., & Wu, F. (2019). The occurrence and co-occurrence of aflatoxin and fumonisin along the maize value chain in southwest Nigeria. *Food and Chemical Toxicology*, *129*, 458–465. https://doi.org/10.1016/j.fct.2019.05.008

Logrieco, A., Battilani, P., Leggieri, M. C., Jiang, Y., Haesaert, G., Lanubile, A., Mahuku, G., Mesterházy, A., Ortega-Beltran, A., Pasti, M., Smeu, I., Torres, A., Xu, J., & Munkvold, G. (2021). Perspectives on Global Mycotoxin Issues and Management From the MycoKey Maize Working Group. *Plant Disease*, *105*(3), 525–537. https://doi.org/10.1094/PDIS-06-20-1322-FE

Lung, H. M., Cheng, Y. C., Chang, Y. H., Huang, H. W., Yang, B. B., & Wang, C. Y. (2015). Microbial decontamination of food by electron beam irradiation. *Trends in Food Science &Technology*, *44*(1), 66–78. https://doi.org/10.1016/j.tifs.2015.03.005

Madala, N., Saeger, S., Boevre, M., Olotu, I., Njobeh, P., Adebo, O., & Gbashi, S. (2018). *The Socio-Economic Impact of Mycotoxin Contamination in Africa*. https://doi.org/10.5772/intechopen.79328

Magan, N. (2004). Mycotoxins in Food: Detection and Control. In *International Journal of Food Science and Technology—INT J FOOD SCI TECHNOL* (Vol. 40). https://doi.org/10.1201/9781439823361

Magnoli, A. P., Poloni, V. L., & Cavaglieri, L. (2019). Impact of mycotoxin contamination in the animal feed industry. *Current Opinion in Food Science*, *29*, 99–108. https://doi.org/10.1016/j.cofs.2019.08.009

Maina, A. W., Wagacha, J. M., Mwaura, F. B., Muthomi, J. W., & Woloshuk, C. P. (2017). Assessment of Farmers Maize Production Practices and Effect of Triple-Layer Hermetic Storage on the Population of *Fusarium* Spp. And Fumonisin Contamination. *World Journal of Agricultural Research*. https://doi.org/10.12691/wjar-5-1-4

Maina, A., Wagacha, M., Mwaura, F., Muthomi, J. W., & Woloshuk, C. (2016). Postharvest Practices of Maize Farmers in Kaiti District, Kenya and the Impact of Hermetic Storage

on Populations of *Aspergillus* Spp. And Aflatoxin Contamination. *Journal of Food Research*, *5*, 53. https://doi.org/10.5539/jfr.v5n6p53

Mamo, F. T., Abate, B. A., Tesfaye, K., Nie, C., Wang, G., & Liu, Y. (2020). Mycotoxins in Ethiopia: A Review on Prevalence, Economic and Health Impacts. *Toxins*, *12*(10), Article 10. https://doi.org/10.3390/toxins12100648

Mannaa, M., & Kim, K. D. (2017). Influence of Temperature and Water Activity on Deleterious Fungi and Mycotoxin Production during Grain Storage. *Mycobiology*, *45*(4), 240–254. https://doi.org/10.5941/MYCO.2017.45.4.240

Mansfield, M. A., De Wolf, E. D., & Kuldau, G. A. (2005). Relationships between weather conditions, agronomic practices, and fermentation characteristics with deoxynivalenol content in fresh and ensiled maize. *Plant Disease*, *89*(11), 1151–1157. https://doi.org/10.1094/PD-89-1151

Marasas, W. F., Kellerman, T. S., Gelderblom, W. C., Coetzer, J. A., Thiel, P. G., & van der Lugt, J. J. (1988). Leukoencephalomalacia in a horse induced by fumonisin B1 isolated from *Fusarium moniliforme*. *The Onderstepoort Journal of Veterinary Research*, *55*(4), 197–203.

Marasas, W. F. O. (1995). Fumonisins: Their implications for human and animal health. *Natural Toxins*, *3*(4), 193–198. https://doi.org/10.1002/nt.2620030405

Marasas, W. F. O. (2001). Discovery and Occurrence of the Fumonisins: A Historical Perspective. *Environmental Health Perspectives*, *109*, 5.

Marin, S., Ramos, A. J., Cano-Sancho, G., & Sanchis, V. (2013). Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food and Chemical Toxicology: An International*

Journal Published for the British Industrial Biological Research Association, *60*, 218–237. https://doi.org/10.1016/j.fct.2013.07.047

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), Article 1. https://doi.org/10.14806/ej.17.1.200

Masese, A., N., Mbithi, L. M., Joshi, N., P., & Machuka, S., M. (2022). Determinants of Maize Production and Its Supply Response in Kenya. *International Journal of Agronomy*, *2022*, e2597283. https://doi.org/10.1155/2022/2597283

Matumba, L., Van Poucke, C., Njumbe Ediage, E., Jacobs, B., & De Saeger, S. (2015). Effectiveness of hand sorting, flotation/washing, dehulling and combinations thereof on the decontamination of mycotoxin-contaminated white maize. *Food Additives & Contaminants: Part A*, *32*(6), 960–969. https://doi.org/10.1080/19440049.2015.1029535

Mbithi, M. (2000). *A comparison of maize production in Kenya before and during liberalisation period*. https://profiles.uonbi.ac.ke/lmbithi/publications/comparison-maize-production-kenya-and-during-liberalisation-period

Medina, A., Akbar, A., Baazeem, A., Rodriguez, A., & Magan, N. (2017). Climate change, food security and mycotoxins: Do we know enough? *Fungal Biology Reviews*, *31*(3), 143–154. https://doi.org/10.1016/j.fbr.2017.04.002

Medina, Á., Rodríguez, A., & Magan, N. (2015). Climate change and mycotoxigenic fungi: Impacts on mycotoxin production. *Current Opinion in Food Science*, *5*, 99–104. https://doi.org/10.1016/j.cofs.2015.11.002

Mehan, V. K., Mcdonald, D., Haravu, L. J., & Jayanthi, S. (1991, November 1). *Groundnut Aflatoxin Problem: Review and Literature Database*.

https://www.semanticscholar.org/paper/Groundnut-Aflatoxin-Problem%3A-Review-and-Literature-Mehan-Mcdonald/db24ace1c5ed6a7b8b2491782dcf51490b9c520e

Meseka, S., Williams, W. P., Warburton, M. L., Brown, R. L., Augusto, J., Ortega-Beltran, A., Bandyopadhyay, R., & Menkir, A. (2018). Heterotic affinity and combining ability of exotic maize inbred lines for resistance to aflatoxin accumulation. *Euphytica*, *214*(10), 184. https://doi.org/10.1007/s10681-018-2254-8

Midega, C., Pittchar, J., Pickett, J., Hailu, G., & Khan, Z. (2018). *[PDF] A climate-adapted push-pull system effectively controls fall armyworm, Spodoptera frugiperda (J E Smith), in maize in East Africa | Semantic Scholar*. https://www.semanticscholar.org/paper/A-climate-adapted-push-pull-system-effectively-fall-Midega-Pittchar/1e225211965c832e9d6500b6d435cb1c358508cf

Mideros, S. X., Windham, G. L., Williams, W. P., & Nelson, R. J. (2009). Aspergillus flavus Biomass in Maize Estimated by Quantitative Real-Time Polymerase Chain Reaction Is Strongly Correlated with Aflatoxin Concentration. *Plant Disease*, *93*(11), 1163–1170. https://doi.org/10.1094/PDIS-93-11-1163

Ministry of Agriculture, Livestock, Fisheries and Cooperatives. (2022, September 20). Fertilizer Subsidy 2022. *Ministry of Agriculture, Livestock, Fisheries and Co-Operatives*. https://kilimo.go.ke/fertilizer-subsidy-2022/

Mitchell, N. J., Bowers, E., Hurburgh, C., & Wu, F. (2016). Potential economic losses to the US corn industry from aflatoxin contamination. *Food Additives & Contaminants: Part A*, *33*(3), 540–550. https://doi.org/10.1080/19440049.2016.1138545

Mogeni, E. (2019). Institutional and Social Economic Factors Affecting Productivity of Maize in Kenya: A Case of Transzoia and Machakos Counties. *International Journal of Scientific*

and Research Publications (IJSRP), IJSRP Volume 9, ,. https://doi.org/10.29322/IJSRP.9.02.2019.p8632

Mohamed, A. B. (2016). *Population of Aspergillus section Flavi and aflatoxin contamination in maize from fields treated with atoxigenic Aspergillus flavus (aflasafe ke01) in lower eastern Kenya*. http://erepository.uonbi.ac.ke/bitstream/handle/11295/97375/Mohamed_Population%20of%20aspergillus%20section%20flavi%20and%20aflatoxin.pdf?sequence=1&isAllowed=y

Mohamed, A. B., Chavez, R. A., Wagacha, M. J., Mutegi, C. K., Muthomi, J. W., Pillai, S. D., & Stasiewicz, M. J. (2022). Efficacy of electron beam irradiation in reduction of mycotoxin-producing fungi, aflatoxin, and fumonisin, in naturally contaminated maize slurry. *Toxicon: X*, *16*, 100141. https://doi.org/10.1016/j.toxcx.2022.100141

Moral, J., Garcia-Lopez, M. T., Camiletti, B. X., Jaime, R., Michailides, T. J., Bandyopadhyay, R., & Ortega-Beltran, A. (2020). *Present Status and Perspective on the Future Use of Aflatoxin Biocontrol Products*. https://dissem.in/p/117869171/present-status-and-perspective-on-the-future-use-of-aflatoxin-biocontrol-products/

Morales, L., Marino, T. P., Wenndt, A. J., Fouts, J. Q., Holland, J. B., & Nelson, R. J. (2018). Dissecting Symptomatology and Fumonisin Contamination Produced by *Fusarium verticillioides* in Maize Ears. *Phytopathology*, *108*(12), 1475–1485. https://doi.org/10.1094/PHYTO-05-18-0167-R

Moreno-Pedraza, A., Valdés-Santiago, L., Hernández-Valadez, L. J., Higuera, A. R.-S., Winkler, R., & Peña, D. L. G. (2015). Reduction of aflatoxin B1 during tortilla production and identification of degradation by-products by direct-injection electrospray mass

spectrometry. *Salud Pública de México*, *57*(1), Article 1. https://doi.org/10.21149/spm.v57i1.7402

Moretti, A., Susca, A., Mulé, G., Logrieco, A. F., & Proctor, R. H. (2013). Molecular biodiversity of mycotoxigenic fungi that threaten food safety. *International Journal of Food Microbiology*, *167*(1), 57–66. https://doi.org/10.1016/j.ijfoodmicro.2013.06.033

Moses, J. A., Jayas, D. S., & Alagusundaram, K. (2015). Climate Change and its Implications on Stored Food Grains. *Agricultural Research*, *4*(1), 21–30. https://doi.org/10.1007/s40003-015-0152-z

Mossel, D. A. (1979). *Decontamination of Animal Feeds by Irradiation: Proceedings of an Advisory Group Meeting on Radiation Treatment of Animal Feeds*. International Atomic Energy Agency. https://books.google.co.ke/books?id=_Svrv65Qt-wC

Mostafavi, H., A., Mirmajlessi, S., M., & Fathollahi, H. (2012). *Potential of food irradiation: Benefits and limitations In: Eissa AH (ed) Trends in Vital Food and Control Engineering*. https://www.intechopen.com/books/1936

Mostrom, M. S. (2021). *Fumonisin Toxicosis in Animals—Toxicology*. Merck Veterinary Manual. https://www.merckvetmanual.com/toxicology/mycotoxicoses/fumonisin-toxicosis-in-animals

Mucheru-Muna, M., Mugendi, D., Pypers, P., Mugwe, J., Vanlauwe, B., Merckx, R., & Kungu, J. (2011). *Increasing Productivity Through Maize–Legume Intercropping in Central Kenya* (pp. 843–858). https://doi.org/10.1007/978-90-481-2543-2_84

Mugwe, J., Mugendi, D., Kungu, J., & Mucheru-Muna, M. (2010). Effect of Plant Biomass, Manure and Inorganic Fertiliser on Maize Yield in the Central Highlands Of Kenya.

African Crop Science Journal (ISSN: 1021-9730) Vol 15 Num 3, 15. https://doi.org/10.4314/acsj.v15i3.54424

Muhammad, L., Njoroge, K., Bett, C., Mwangi, W., Verkuijl, H., De Groote, H., Muhammad, L., Njoroge, K., Bett, C., Mwangi, W., Verkuijl, H., & De Groote, H. (2003). *The Seed Industry for Dryland Crops in Eastern Kenya*. https://doi.org/10.22004/AG.ECON.56108

Mukanga, M., Derera, J., Tongoona, P., & Laing, M. D. (2010). A survey of pre-harvest ear rot diseases of maize and associated mycotoxins in south and central Zambia. *International Journal of Food Microbiology*, *141*(3), 213–221. https://doi.org/10.1016/j.ijfoodmicro.2010.05.011

Munkvold, G. P. (2017). *Fusarium* species and their associated mycotoxins. In A. Moretti & A. Susca (Eds.), *Mycotoxigenic Fungi: Methods and Protocols* (pp. 51–106). Springer. https://doi.org/10.1007/978-1-4939-6707-0_4

Murithi, M. E. (2014). *Prevallence of Fusarium and Aspergillus in maize grain from Kitui, Machakos and Meru and the use of Near Infrared light sorting to remove fumonisins and aflatoxin contaminated grains in Kenya*. University of Nairobi. http://erepository.uonbi.ac.ke/bitstream/handle/11295/76919/Murithi_Prevalence%20Of%20*Fusarium*%20And%20Aspergillus%20Species%20In%20Maize%20Grain%20From%20Kitui%2C%20Machakos%20And%20Meru%20And%20Use%20Of%20Near%20Infra-Red%20Light%20Sorting%20.....pdf?sequence=3

Mutambuki, K., & Likhayo, P. (2021). Efficacy of different hermetic bag storage technologies against insect pests and aflatoxin incidence in stored maize grain. *Bulletin of Entomological Research*, *111*(4), 499–510. https://doi.org/10.1017/S0007485321000213

Mutegi, C. K., Cotty, P. J., & Bandyopadhyay, R. (2018). Prevalence and mitigation of aflatoxins in Kenya (1960-to date). *World Mycotoxin Journal*, *11*(3), 341–357. https://doi.org/10.3920/WMJ2018.2362

Mutegi, C. K., Ngugi, H. K., Hendriks, S. L., & Jones, R. B. (2012). Factors associated with the incidence of *Aspergillus* section *Flavi* and aflatoxin contamination of peanuts in the Busia and Homa bay districts of western Kenya. *Plant Pathology*, *61*(6), 1143–1153. https://doi.org/10.1111/j.1365-3059.2012.02597.x

Mutiga, S. K., Hoffmann, V., Harvey, J. W., Milgroom, M. G., & Nelson, R. J. (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

Nada, S., Nikola, T., Bozidar, U., Ilija, D., & Andreja, R. (2022). Prevention and practical strategies to control mycotoxins in the wheat and maize chain. *Food Control*, *136*, 108855. https://doi.org/10.1016/j.foodcont.2022.108855

Nakuwa, M. S., Mongi, R., & Ngoma, S. (2023). Food handling practices, the prevalence of aflatoxin dietary exposure and its associated factors among children aged 6–23 months in Bukombe District, Tanzania. *NFS Journal*, *31*, 162–170. https://doi.org/10.1016/j.nfs.2023.05.004

Neme, K., & Mohammed, A. (2017). Mycotoxin occurrence in grains and the role of postharvest management as a mitigation strategies. A review. *Food Control*, *78*, 412–425. https://doi.org/10.1016/j.foodcont.2017.03.012

Nemţanu, M. R., Braşoveanu, M., Karaca, G., & Erper, İ. (2014). Inactivation effect of electron beam irradiation on fungal load of naturally contaminated maize seeds. *Journal of the Science of Food and Agriculture*, *94*(13), 2668–2673. https://doi.org/10.1002/jsfa.6607

Nesic, K., Pisinov, B., Jakšić, S., Tasic, A., Božidar, S., & Pavlovic, N. (2017). Comparison of elisa and HPLC methods for the detection of mycotoxins by analysing proficiency test results. *Zbornik Matice Srpske Za Prirodne Nauke*, *2017*, 79–93. https://doi.org/10.2298/ZMSPN1733079N

Ngindu, A., Johnson, B. K., Kenya, P. R., Ngira, J. A., Ocheng, D. M., Nandwa, H., Omondi, T. N., Jansen, A. J., Ngare, W., Kaviti, J. N., Gatei, D., & Siongok, T. A. (1982). Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. *Lancet (London, England)*, *1*(8285), 1346–1348. https://doi.org/10.1016/s0140-6736(82)92411-4

Nguyen, T., Palmer, J., Phan, N., Shi, H., Keener, K., & Flint, S. (2022). Control of aflatoxin M1 in skim milk by high voltage atmospheric cold plasma. *Food Chemistry*, *386*, 132814. https://doi.org/10.1016/j.foodchem.2022.132814

Nicholson, P. (2004). Rapid detection of mycotoxigenic fungi in plants. In *Mycotoxins in Food* (pp. 111–136). https://doi.org/10.1533/9781855739086.1.111

Nikolić, M., Savić, I., Nikolić, A., Jauković, M., Kandić, V., Stevanović, M., & Stanković, S. (2021). Toxigenic Species *Aspergillus parasiticus* Originating from Maize Kernels Grown in Serbia. *Toxins*, *13*(12), 847. https://doi.org/10.3390/toxins13120847

Njeru, F., Mwaura, S., Kusolwa, P. M., & Misinzo, G. (2022). Maize production systems, farmers' perception and current status of maize lethal necrosis in selected counties in Kenya. *All Life*, *15*(1), 692–705. https://doi.org/10.1080/26895293.2022.2085815

Njeru, N. K., Midega, C. A. O., Muthomi, J. W., Wagacha, J. M., & Khan, Z. R. (2020). Impact of push–pull cropping system on pest management and occurrence of ear rots and mycotoxin contamination of maize in western Kenya. *Plant Pathology*, *69*(9), 1644–1654. https://doi.org/10.1111/ppa.13259

Njeru, N., Midega, C., Muthomi, J. W., Wagacha, M., & Khan, Z. (2019). Influence of socio-economic and agronomic factors on aflatoxin and fumonisin contamination of maize in western Kenya. *Food Science & Nutrition*, *7*. https://doi.org/10.1002/fsn3.1070

Njeru, T. N. (2019, July 19). *Understanding the political economy of maize in Kenya*. The Conversation. http://theconversation.com/understanding-the-political-economy-of-maize-in-kenya-120336

Njeru, T. N. (2022). *Kenya's maize price has doubled in a year: Six ways to avoid a staple food shortage*. *The conversation*. https://phys.org/news/2022-09-kenya-maize-price-year-ways.html

Njoroge, A., Ibrahim, B., & Baributsa, D. (2019). Postharvest Management Practices of Grains in the Eastern Region of Kenya. *Journal of Agricultural Science*, *11*. https://doi.org/10.5539/jas.v11n3p33

Nyirahakizimana, H., Mwamburi, L., Wakhisi, J., Mutegi, C. K., Christie, M. E., & Wagacha, J. M. (2013). Occurrence of *Aspergillus* Species and Aflatoxin Contamination in Raw and Roasted Peanuts from Formal and Informal Markets in Eldoret and Kericho Towns, Kenya. *Advances in Microbiology*, *03*(04), 333–342. https://doi.org/10.4236/aim.2013.34047

Nyoro, J. K., Kirimi, L., & Jayne, T. S. (2004). *Competitiveness of Kenyan and Ugandan Maize Production: Challenges for the Future*. https://doi.org/10.22004/AG.ECON.55158

Odjo, S., Alakonya, A. E., Rosales-Nolasco, A., Molina, A. L., Muñoz, C., & Palacios-Rojas, N. (2022). Occurrence and postharvest strategies to help mitigate aflatoxins and fumonisins in maize and their co-exposure to consumers in Mexico and Central America. *Food Control*, *138*, 108968. https://doi.org/10.1016/j.foodcont.2022.108968

Oerke, E.-C., & Dehne, H.-W. (2004). Safeguarding production—Losses in major crops and the role of crop protection. *Crop Protection*, *23*(4), 275–285. https://doi.org/10.1016/j.cropro.2003.10.001

Ogunleye, A., & Olaiya, G. (2015). Isolation, identification and mycotoxin production of some mycoflora of dried stockfish (*Gadus morhua*). *Academic Journal of Science*, *4*, 345–363.

Omodele, A. D., Mudenda, H. B., Muma, J. B., & Munyeme, M. (2020). Species Identification of Mealie Meal Spoilage Organisms and Pathogenic Bacteria from Selected Food Stores in Lusaka District of Zambia. *Open Journal of Preventive Medicine*, *10*(8), Article 8. https://doi.org/10.4236/ojpm.2020.108016

Omori, A. M., Ono, E. Y. S., Bordini, J. G., Hirozawa, M. T., Fungaro, M. H. P., & Ono, M. A. (2018). Detection of *Fusarium verticillioides* by PCR-ELISA based on FUM21 gene. *Food Microbiology*, *73*, 160–167. https://doi.org/10.1016/j.fm.2018.01.020

Ono, E., Biazon, L., Silva, M., Vizoni, É., Sugiura, Y., Ueno, Y., & Hirooka, E. (2006). Fumonisins in Corn: Correlation with *Fusarium* sp. count, damaged kernels, protein and lipid content. *Brazilian Archives of Biology and Technology - BRAZ ARCH BIOL TECHNOL*, *49*. https://doi.org/10.1590/S1516-89132006000100008

Otieno, H., Zingore, S., Chemining'wa, G., & Gachene, C. (2020). Maize (Zea mays L.) Growth and Yield Response to Tillage Methods and Fertilizer Combinations in the Midland Agro-ecological Zones of Kenya. *Turkish Journal of Agriculture - Food Science and Technology*, *8*, 616. https://doi.org/10.24925/turjaf.v8i3.616-624.3097

Otsuki, T., Wilson, J. S., & Sewadeh, M. (2001). Saving two in a billion: *Food Policy*, *26*(5), 495–514. https://doi.org/10.1016/S0306-9192(01)00018-5

Ouma, J., Bett, E., & Mbataru, P. (2014). *Does Adoption of Improved Maize Varieties Enhance Household Food Security in Maize growing Zones of Eastern Kenya?* https://ir-library.ku.ac.ke/bitstream/handle/123456789/12359/Does%20Adoption%20of%20Improved%20Maize%20Varieties%20Enhance%20Household.....pdf;sequence=1

Ouma, J. O., & De Groote, H. (2011). Maize varieties and production constraints: Capturing farmers' perceptions through participatory rural appraisals (PRAs) in Eastern Kenya. *Journal of Development and Agricultural Economics, 3*(15). https://doi.org/10.5897/JDAE10.096

Özcelik, S., & Özcelik, N. (1990). Interacting effects of time, temperature, pH and simple sugars on biomass and toxic metabolite production by three *Alternaria* spp. *Mycopathologia*, *109*(3), 171–175. https://doi.org/10.1007/BF00436806

Parsons, M. W., & Munkvold, G. P. (2010). Associations of planting date, drought stress, and insects with *Fusarium* ear rot and fumonisin B1 contamination in California maize. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, *27*(5), 591–607. https://doi.org/10.1080/19440040903456337

Pascual, C., Barcos, A., Mandap, J. A., & Ocampo, E. (2016). Fumonisin-Producing *Fusarium* Species Causing Ear Rot of Corn in the Philippines. *Philippine Journal of Crop Science*, *41*, 12–21.

Pearson, T. C., Wicklow, D. T., & Brabec, D. L. (2009). Technical Note: Characteristics and Sorting of White Food Corn Contaminated with Mycotoxins. *Applied Engineering in Agriculture, 26*(1), 109–113. https://doi.org/10.13031/2013.29463

Pearson, T. C., Wicklow, D. T., Maghirang, E., Xie, F., & Dowell, F. (2001). Detecting aflatoxin in single corn kernels by transmittance and reflectance spectroscopy. *Transactions of the American Society of Agricultural Engineers, 44*. https://doi.org/10.13031/2013.6418

Perdoncini, M., Sereia, M., Poliseli-Scopel, F., Formigoni, M., Rigobello, E., Benetti, S., Cardoso, F., Marchi, L., Pedroso, C., Fernandes, P., Souza, T. H., Wielewski, P., Lima, E., Gregório, A., Zorzenon, M., Castro, J., Bel, V., Pozza, M., & Marques, L. (2019). *Growth of Fungal Cells and the Production of Mycotoxins*. https://doi.org/10.5772/intechopen.86533

Pfordt, A., Ramos Romero, L., Schiwek, S., Karlovsky, P., & von Tiedemann, A. (2020). Impact of environmental conditions and agronomic practices on the prevalence of *Fusarium* species associated with ear- and stalk rot in maize. *Pathogens, 9*(3), 236. https://doi.org/10.3390/pathogens9030236

Phan, L., Trần, T., Audenaert, K., Jacxsens, L., & Eeckhout, M. (2021). Contamination of *Fusarium proliferatum* and *Aspergillus flavus* in the Rice Chain Linked to Crop Seasons, Cultivation Regions, and Traditional Agricultural Practices in Mekong Delta, Vietnam. *Foods, 10*, 2064. https://doi.org/10.3390/foods10092064

Pildain, M. B., Frisvad, J. C., Vaamonde, G., Cabral, D., Varga, J., & Samson, R. A. (2008). Two novel aflatoxin-producing *Aspergillus* species from Argentinean peanuts. *International Journal of Systematic and Evolutionary Microbiology, 48*, 725–735. https://doi.org/10.1099/ijs.0.65123-0

Pillai, S. D., & Shayanfar, S. (2017). Electron beam technology and other irradiation technology applications in the food industry. *Applications of Radiation Chemistry in the Fields of*

Industry, Biotechnology and Environment, 249–268. https://doi.org/10.1007/s41061-016-0093-4

Pillai, S. D., & Shayanfar, S. (2018). Electron beam processing of fresh produce–A critical review. *Radiation Physics and Chemistry*, *143*, 85–88. https://doi.org/10.1016/j.radphyschem.2017.09.008

Porto, Y. D., Trombete, F. M., Freitas-Silva, O., de Castro, I. M., Direito, G. M., & Ascheri, J. L. R. (2019). Gaseous Ozonation to Reduce Aflatoxins Levels and Microbial Contamination in Corn Grits. *Microorganisms*, *7*(8), Article 8. https://doi.org/10.3390/microorganisms7080220

Prabhavathi, S., Sridhar, K., & Sanjeev, G. (2014). Fungal decontamination and enhancement of shelf life of edible split beans of wild legume *Canavalia maritima* by the electron beam irradiation. *Radiation Physics and Chemistry*, *96*, 5–11. https://doi.org/10.1016/j.radphyschem.2013.08.007

Prischmann, D., Lehman, R., Christie, A., & Dashiell, K. (2008). Characterization of bacteria isolated from maize roots: Emphasis on *Serratia* and infestation with corn rootworms (*Chrysomelidae: Diabrotica*). *Applied Soil Ecology*, *40*, 417–431. https://doi.org/10.1016/j.apsoil.2008.06.012

Probst, C., Bandyopadhyay, R., & Cotty, P. (2014). *Diversity of aflatoxin-producing fungi and their impact on food safety in sub-Saharan Africa*. *174*, 113–122. https://doi.org/10.1016/j.ijfoodmicro.2013.12.010

Probst, C., Bandyopadhyay, R., Price, L. E., & Cotty, P. J. (2011). Identification of Atoxigenic *Aspergillus flavus* Isolates to Reduce Aflatoxin Contamination of Maize in Kenya. *Plant Disease*, *95*(2), 212–218. https://doi.org/10.1094/PDIS-06-10-0438

Probst, C., Njapau, H., & Cotty, P. (2007). *Outbreak of an Acute Aflatoxicosis in Kenya in 2004: Identification of the Causal Agent*. *73*, 2762–2764. https://doi.org/doi:10.1128/AEM.02370-06

Puhova, I., Rubtsov, K., Kurzina, I., Kazakov, A., & Medovnik, A. (2015). Modification of Polymer Materials by Electron Beam Treatment. *Key Engineering Materials*, *670*, 118–125. https://doi.org/10.4028/www.scientific.net/KEM.670.118

Quéro, L., Girard, V., Pawtowski, A., Tréguer, S., Weill, A., Arend, S., Cellière, B., Polsinelli, S., Monnin, V., van Belkum, A., Vasseur, V., Nodet, P., & Mounier, J. (2019). Development and application of MALDI-TOF MS for identification of food spoilage fungi. *Food Microbiology*, *81*, 76–88. https://doi.org/10.1016/j.fm.2018.05.001

Quintilla, R., Kolecka, A., Casaregola, S., Daniel, H. M., Houbraken, J., Kostrzewa, M., Boekhout, T., & Groenewald, M. (2018). MALDI-TOF MS as a tool to identify foodborne yeasts and yeast-like fungi. *International Journal of Food Microbiology*, *266*, 109–118. https://doi.org/10.1016/j.ijfoodmicro.2017.11.016

Rahman, H. U., Yue, X., Ren, X., Zhang, W., Zhang, Q., & Li, P. (2020). Multiplex PCR assay to detect Aspergillus, Penicillium and *Fusarium* species simultaneously. *Food Additives & Contaminants: Part A*, *37*(11), 1939–1950. https://doi.org/10.1080/19440049.2020.1810860

Rahman, H. U., Yue, X., Yu, Q., Zhang, W., Zhang, Q., & Li, P. (2020). Current PCR-based methods for the detection of mycotoxigenic fungi in complex food and feed matrices. *World Mycotoxin Journal*, *13*(2), 139–150. https://doi.org/10.3920/WMJ2019.2455

Rahmani, A., Jinap, S., & Soleimany, F. (2009). Qualitative and Quantitative Analysis of Mycotoxins. *Comprehensive Reviews in Food Science and Food Safety*, *8*(3), 202–251. https://doi.org/10.1111/j.1541-4337.2009.00079.x

Rankin, M., & Grau, C. (2002). *Agronomic Considerations for Molds and Mycotoxins in Corn Silage*. *4*(1). https://fyi.extension.wisc.edu/forage/files/2014/01/Mycotoxins.pdf

Recha, J., James, Kinyangi, & Hellen Omondi. (2013). *Climate Related Risks and Opportunities for Agricultural Adaptation in Semi-Arid Eastern Kenya*. https://doi.org/10.13140/RG.2.1.2172.8728

Reddy, K. R. N., Salleh, B., Saad, B., Abbas, H. K., Abel, C. A., & Shier, W. T. (2010). An overview of mycotoxin contamination in foods and its implications for human health. *Journal of Toxicology - Toxin Reviews*, *29*(1), 3–26. https://doi.org/10.3109/15569541003598553

Reddy, R., Reddy, P., Reddy, R., & Sokka Reddy, S. (2021). *Management of Turcicum Leaf Blight of Maize Caused by Exserohilum Turcicum in Maize*. https://www.researchgate.net/profile/Talakanti-Rajeshwar-Redy/publication/349588685_Management_of_Turcicum_Leaf_Blight_of_Maize_Caused_by_Exserohilum_Turcicum_in_Maize/links/60374b92299bf1cc26ec0be9/Management-of-Turcicum-Leaf-Blight-of-Maize-Caused-by-Exserohilum-Turcicum-in-Maize.pdf

Reverberi, M., Ricelli, A., Zjalic, S., Fabbri, A. A., & Fanelli, C. (2010). Natural functions of mycotoxins and control of their biosynthesis in fungi. *Applied Microbiology and Biotechnology*, *87*(3), 899–911. https://doi.org/10.1007/s00253-010-2657-5

Richard, J., Payne, G., Desjardins, A., Maragos, C., Norred, W., & Pestka, J. (2003). Mycotoxins: Risks in plant, animal and human systems. *CAST Task Force Report*, *139*, 101–103.

Rodrigues, P., Venâncio, A., Kozakiewicz, Z., & Lima, N. (2009). A polyphasic approach to the identification of aflatoxigenic and non-aflatoxigenic strains of *Aspergillus* Section *Flavi* isolated from Portuguese almonds. *International Journal of Food Microbiology*, *129*(2), 187–193. https://doi.org/10.1016/j.ijfoodmicro.2008.11.023

Rodríguez-Aguilar, M., Solis, J., Flores-Ramírez, R., Díaz-Barriga, F., BA, Z.-O., & Cilia, G. (2020). Aflatoxins and the traditional process of nixtamalization in indigenous communities from the Huasteca Potosina region. *World Mycotoxin Journal*. https://doi.org/10.3920/wmj2019.2538

Rogovschi, V. D., Aquino, S., Nunes, T. C. F., Gonçalez, E., & Corrêa, B. (2009). *Use of electron beam on aflatoxins degradation in coconut agar*. https://www.ipen.br/biblioteca/2009/inac/15144.pdf

Rosa Junior, O. F., Dalcin, M. S., Nascimento, V. L., Haesbaert, F. M., Ferreira, T. P. de S., Fidelis, R. R., Sarmento, R. de A., Aguiar, R. W. de S., Oliveira, E. E. de, & Santos, G. R. D. (2019). Fumonisin production by *Fusarium verticillioides* in maize genotypes cultivated in different environments. *Toxins*, *11*(4), 215. https://doi.org/10.3390/toxins11040215

Rose, L. J., Okoth, S., Beukes, I., Ouko, A., Mouton, M., Flett, B. C., Makumbi, D., & Viljoen, A. (2017). Determining resistance to *Fusarium verticillioides* and fumonisin accumulation in African maize inbred lines resistant to *Aspergillus flavus* and aflatoxins. *Euphytica*, *213*(4), 93. https://doi.org/10.1007/s10681-017-1883-7

Rushing, B. R., & Selim, M. I. (2019). Aflatoxin B1: A review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. *Food and Chemical Toxicology*, *124*, 81–100. https://doi.org/10.1016/j.fct.2018.11.047

Sadler, T. W., Merrill, A. H., Stevens, V. L., Sullards, M. C., Wang, E., & Wang, P. (2002). Prevention of fumonisin B1-induced neural tube defects by folic acid. *Teratology*, *66*(4), 169–176. https://doi.org/10.1002/tera.10089

Sánchez-Bel, P., Martínez-Madrid, M. C., Egea, I., & Romojaro, F. (2005). Oil quality and sensory evaluation of almond (Prunus amygdalus) stored after electron beam processing. *Journal of Agricultural and Food Chemistry*, *53*(7), 2567–2573. https://doi.org/10.1021/jf040184r

Sapsford, K. E., Taitt, C. R., Fertig, S., Moore, M. H., Lassman, M. E., Maragos, C. M., & Shriver-Lake, L. C. (2006). Indirect competitive immunoassay for detection of aflatoxin B1 in corn and nut products using the array biosensor. *Biosensors and Bioelectronics*, *21*(12), 2298–2305. https://doi.org/10.1016/j.bios.2005.10.021

Schaarschmidt, S., & Fauhl-Hassek, C. (2019). Mycotoxins during the Processes of Nixtamalization and Tortilla Production. *Toxins*, *11*(4), Article 4. https://doi.org/10.3390/toxins11040227

Scheidegger, K., & Payne, G. (2003). Unlocking the Secrets Behind Secondary Metabolism: A Review of *Aspergillus flavus* from Pathogenicity to Functional Genomics. *Toxin Reviews*, *22*, 423–459. https://doi.org/10.1081/TXR-120024100

Schmidt, C. W. (2013). Breaking the Mold: New Strategies for Fighting Aflatoxins. *Environmental Health Perspectives*, *121*(9), A270–A275. https://doi.org/10.1289/ehp.121-a270

Serna-Saldivar, S. O. (2015). Chapter 1—History of Corn and Wheat Tortillas. In L. W. Rooney & S. O. Serna-Saldivar (Eds.), *Tortillas* (pp. 1–28). AACC International Press. https://doi.org/10.1016/B978-1-891127-88-5.50001-3

Sexton, D. J., Kordalewska, M., Bentz, M. L., Welsh, R. M., Perlin, D. S., & Litvintseva, A. P. (2018). Direct Detection of Emergent Fungal Pathogen Candida auris in Clinical Skin Swabs by SYBR Green-Based Quantitative PCR Assay. *Journal of Clinical Microbiology*, *56*(12), 10.1128/jcm.01337-18. https://doi.org/10.1128/jcm.01337-18

Shala-Mayrhofer, V., Varga, E., Marjakaj, R., Berthiller, F., Musolli, A., Berisha, D., Kelmendi, B., & Lemmens, M. (2013). Investigations on *Fusarium* spp. And their mycotoxins causing *Fusarium* ear rot of maize in Kosovo. *Food Additives & Contaminants. Part B, Surveillance*, *6*(4), 237–243. https://doi.org/10.1080/19393210.2013.804885

Shawrang, P., Sadeghi, A. A., Behgar, M., Zareshahi, H., & Shahhoseini, G. (2011). Study of chemical compositions, anti-nutritional contents and digestibility of electron beam irradiated sorghum grains. *Food Chemistry*, *125*(2), 376–379. https://doi.org/10.1016/j.foodchem.2010.09.010

Shephard, G. S. (2016). Current Status of Mycotoxin Analysis: A Critical Review. *Journal of AOAC International*, *99*(4), 842–848. https://doi.org/10.5740/jaoacint.16-0111

Shi, H., Ileleji, K., Stroshine, R. L., Keener, K., & Jensen, J. L. (2017). Reduction of Aflatoxin in Corn by High Voltage Atmospheric Cold Plasma. *Food and Bioprocess Technology*, *10*(6), 1042–1052. https://doi.org/10.1007/s11947-017-1873-8

Shotwell, O. L., & Hesseltine, C. (1981). *Cereal Chem 1981 | Use of Bright Greenish Yellow Fluorescence as a Presumptive Test for Aflatoxin in Corn*.

https://www.cerealsgrains.org/publications/cc/backissues/1981/Documents/CC1981a32.html

Sibiya, J., Tongoona, P., Derera, J., & Makanda, I. (2013). Smallholder farmers' perceptions of maize diseases, pests, and other production constraints, their implications for maize breeding and evaluation of local maize cultivars in KwaZulu-Natal, South Africa. *African Journal of Agricultural Research*, *8*, 1790–1798. https://doi.org/10.5897/AJAR12.1906

Simiyu, S. W. (2014). *Factors influencing maize production among small scale Farmers in Kenya, A caseof Bungoma central sub county*. https://www.semanticscholar.org/paper/Factors-influencing-maize-production-among-small-in-Simiyu/cf029979b5e55391eb98684d81a8c113be8aefdc

Singh, S., Kumar, V., Dhanjal, D. S., Datta, S., Prasad, R., & Singh, J. (2020). Biological Biosensors for Monitoring and Diagnosis. *Microbial Biotechnology: Basic Research and Applications*, 317–335. https://doi.org/10.1007/978-981-15-2817-0_14

Sinha, K. K., & Kumari, P. (1990). Some physiological abnormalities induced by aflatoxin B1 in mung seeds (Vigna radiata variety Pusa Baishakhi). *Mycopathologia*, *110*(2), 77–79. https://doi.org/10.1007/BF00446994

Sinha, K. K., & Sinha, A. K. (1993). Effect of aflatoxin B1 on germination index and seedling growth in wheat varieties. *Mycopathologia*, *123*(3), 165–169. https://doi.org/10.1007/BF01111268

Siou, D., Gélisse, S., Laval, V., Repinçay, C., Canalès, R., Suffert, F., & Lannou, C. (2014). Effect of wheat spike infection timing on *Fusarium* head blight development and mycotoxin accumulation. *Plant Pathology*, *63*(2), 390–399. https://doi.org/10.1111/ppa.12106

Sipos, P., Peles, F., Brassó, D. L., Béri, B., Pusztahelyi, T., Pócsi, I., & Győri, Z. (2021). Physical and chemical methods for reduction in aflatoxin content of feed and food. *Toxins*, *13*(3), 204. https://doi.org/10.3390/toxins13030204

Small, I. M., Flett, B. C., Marasas, W. F. O., McLeod, A., Stander, M. A., & Viljoen, A. (2012). Resistance in Maize Inbred Lines to *Fusarium verticillioides* and Fumonisin Accumulation in South Africa. *Plant Disease*, *96*(6), 881–888. https://doi.org/10.1094/PDIS-08-11-0695

Smith, M.-C., Madec, S., Coton, E., & Hymery, N. (2016). Natural Co-Occurrence of Mycotoxins in Foods and Feeds and Their in vitro Combined Toxicological Effects. *Toxins*, *8*(4), Article 4. https://doi.org/10.3390/toxins8040094

Sserumaga, J. P., Ortega-Beltran, A., Wagacha, J. M., Mutegi, C. K., & Bandyopadhyay, R. (2020). Aflatoxin-producing fungi associated with pre-harvest maize contamination in Uganda. *International Journal of Food Microbiology*, *313*, 108376. https://doi.org/10.1016/j.ijfoodmicro.2019.108376

Stafstrom, W., Wushensky, J., Fuchs, J., Xu, W., Ezera, N., & Nelson, R. J. (2021a). Validation and Application of a Low-Cost Sorting Device for Fumonisin Reduction in Maize. *Toxins*, *13*(9), Article 9. https://doi.org/10.3390/toxins13090652

Stafstrom, W., Wushensky, J., Fuchs, J., Xu, W., Ezera, N., & Nelson, R. J. (2021b). Validation and Application of a Low-Cost Sorting Device for Fumonisin Reduction in Maize. *Toxins*, *13*(9), Article 9. https://doi.org/10.3390/toxins13090652

Stasiewicz, M. J., Falade, T. D. O., Mutuma, M., Mutiga, S. K., Harvey, J. J. W., Fox, G., Pearson, T. C., Muthomi, J. W., & Nelson, R. J. (2017). Multi-spectral kernel sorting to

reduce aflatoxins and fumonisins in Kenyan maize. *Food Control*, *78*, 203–214. https://doi.org/10.1016/j.foodcont.2017.02.038

Stepanik, T., Kost, D., Nowicki†, T., & Gaba†, D. (2007). Effects of electron beam irradiation on deoxynivalenol levels in distillers dried grain and solubles and in production intermediates. *Food Additives & Contaminants*, *24*(9), 1001–1006. https://doi.org/10.1080/02652030701329629

Supriya, P., Sridhar, K. R., & Ganesh, S. (2014). Fungal decontamination and enhancement of shelf life of edible split beans of wild legume *Canavalia maritima* by the electron beam irradiation. *Radiation Physics and Chemistry*, *96*, 5–11. https://doi.org/10.1016/j.radphyschem.2013.08.007

Suwarno, W. B., Hannok, P., Palacios-Rojas, N., Windham, G., Crossa, J., & Pixley, K. V. (2019). Provitamin A Carotenoids in Grain Reduce Aflatoxin Contamination of Maize While Combating Vitamin A Deficiency. *Frontiers in Plant Science*, *10*, 30. https://doi.org/10.3389/fpls.2019.00030

Tannous, J., Atoui, A., El Khoury, A., Kantar, S., Chdid, N., Oswald, I. P., Puel, O., & Lteif, R. (2015). Development of a real-time PCR assay for *Penicillium expansum* quantification and patulin estimation in apples. *Food Microbiology*, *50*, 28–37. https://doi.org/10.1016/j.fm.2015.03.001

Temcharoen, P., & Thilly, W. G. (1982). Removal of Aflatoxin B1 Toxicity but Not Mutagenicity by 1 Megarad Gamma Radiation of Peanut Meal. *Journal of Food Safety*, *4*(4), 199–205. https://doi.org/10.1111/j.1745-4565.1982.tb00445.x

Thanushree, M. P., Sailendri, D., Yoha, K. S., Moses, J. A., & Anandharamakrishnan, C. (2019). Mycotoxin contamination in food: An exposition on spices. *Trends in Food Science & Technology*, *93*, 69–80. https://doi.org/10.1016/j.tifs.2019.08.010

The East African. (2021, March 10). *Uganda ministers to meet over Kenya's ban on its maize*. The East African. https://www.theeastafrican.co.ke/tea/business/uganda-meeting-kenya-maize-ban-3317968

The Standard. (2020). *17 maize flour brands banned over high aflatoxin levels*. The Standard. https://www.standardmedia.co.ke/ureport/article/2001358551/17-maize-flour-brands-banned-over-high-aflatoxin-levels

Ulloa-sosa, M., & Schroeder, H. W. (1969). Note on aflatoxin decomposition in the process of making tortillas from corn. *Cereal Chemistry*, *46*, 397–400.

Uppala, S. S., Bowen, K. L., & Woods, F. M. (2013). Pre-harvest aflatoxin contamination and soluble sugars of peanut. *Peanut Science*, *40*(1), 40–51. https://doi.org/10.3146/PS12-9.1

van der Westhuizen, L., Shephard, G. S., Rheeder, J. P., Burger, H.-M., Gelderblom, W. C. A., Wild, C. P., & Gong, Y. Y. (2010). Simple intervention method to reduce fumonisin exposure in a subsistence maize-farming community in South Africa. *Food Additives & Contaminants: Part A*, *27*(11), 1582–1588. https://doi.org/10.1080/19440049.2010.508050

Varga, J., Frisvad, J. C., & Samson, R. A. (2011). Two new aflatoxin producing species, and an overview of *Aspergillus* section *Flavi*. *Studies in Mycology*, *69*, 57–80. https://doi.org/10.3114/sim.2011.69.05

Villavicencio, A. L. C. H., Fanaro, G. B., Araújo, M. M., Aquino, S., & Silva, P. V. da. (2007). Detection of *Phakopsora pachyrhizi* by polymerase chain reaction (PCR) and use of

germination test and DNA comet assay after e-bearn processing in soybean. *Radiation Physics and Chemistry*, *76*(11–12), 1878–1881.

Vylkova, S. (2017). *Environmental pH modulation by pathogenic fungi as a strategy to conquer the host | PLOS Pathogens*. https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1006149

Wagacha, J., & Muthomi, J. W. (2008). Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. *International Journal of Food Microbiology*, *124*, 1–12.

Wagner, K., Springer, B., Pires, V. P., & Keller, P. M. (2018). Molecular detection of fungal pathogens in clinical specimens by 18S rDNA high-throughput screening in comparison to ITS PCR and culture. *Scientific Reports*, *8*(1), Article 1. https://doi.org/10.1038/s41598-018-25129-w

Wambugu, P. W., Mathenge, P. W., Auma, E. O., & vanRheenen, H. A. (2012). Constraints to On-Farm Maize (*Zea mays* L.) Seed Production in Western Kenya: Plant Growth and Yield. *International Scholarly Research Notices*, *2012*, e153412. https://doi.org/10.5402/2012/153412

Wang, B., Shen, F., He, X., Fang, Y., Hu, Q., & Liu, X. (2022). Simultaneous detection of *Aspergillus* moulds and aflatoxin B1 contamination in rice by laser induced fluorescence spectroscopy. *Food Control*, *145*, 109485. https://doi.org/10.1016/j.foodcont.2022.109485

Wang, F., Xie, F., Xue, X., Wang, Z., Fan, B., & Ha, Y. (2011). Structure elucidation and toxicity analyses of the radiolytic products of aflatoxin B1 in methanol-water solution.

Journal of Hazardous Materials, *192*(3), 1192–1202. https://doi.org/10.1016/j.jhazmat.2011.06.027

Wang, P.-C., Yang, N., Liu, D., Qin, Z.-M., An, Y., & Chen, H.-B. (2020). Coupling effects of gamma irradiation and absorbed moisture on silicone foam. *Materials & Design*, *195*, 108998. https://doi.org/10.1016/j.matdes.2020.108998

Wang, X., Yan, J., Zhang, X., Zhang, S., & Chen, Y. (2020). Organic manure input improves soil water and nutrients use for sustainable maize (Zea mays. L) productivity on the Loess Plateau. *PLOS ONE*, *15*(8), e0238042. https://doi.org/10.1371/journal.pone.0238042

Wang, Y., Zhang, W., & Fu, L. (2017). *Food Spoilage Microorganisms | Ecology and Control*. https://www.taylorfrancis.com/books/mono/10.4324/9781315368887/food-spoilage-microorganisms-yanbo-wang-wangang-zhang-linglin-fu

Warth, B., Parich, A., Atehnkeng, J., Bandyopadhyay, R., Schuhmacher, R., Sulyok, M., & Krska, R. (2012). Quantitation of mycotoxins in food and feed from Burkina Faso and Mozambique using a modern LC-MS/MS multitoxin method. *Journal of Agricultural and Food Chemistry*, *60*(36), 9352–9363. https://doi.org/10.1021/jf302003n

Wegulo, S. N. (2012). Factors influencing deoxynivalenol accumulation in small grain cereals. *Toxins*, *4*(11), 1157–1180. https://doi.org/10.3390/toxins4111157

WHO. (2018). *Mycotoxins*. https://www.who.int/news-room/fact-sheets/detail/mycotoxins

Woldemariam, H. W., Kießling, M., Emire, S. A., Teshome, P. G., Töpfl, S., & Aganovic, K. (2021). Influence of electron beam treatment on naturally contaminated red pepper (Capsicum annuum L.) powder: Kinetics of microbial inactivation and physicochemical

quality changes. *Innovative Food Science & Emerging Technologies*, *67*, 102588. https://doi.org/10.1016/j.ifset.2020.102588

Wu, F. (2021). Chapter 27—Foodborne mycotoxins. In J. G. Morris & D. J. Vugia (Eds.), *Foodborne Infections and Intoxications (Fifth Edition)* (pp. 439–454). Academic Press. https://doi.org/10.1016/B978-0-12-819519-2.00027-X

Wu, Q., Xie, L., & Xu, H. (2018). Determination of toxigenic fungi and aflatoxins in nuts and dried fruits using imaging and spectroscopic techniques. *Food Chemistry*, *252*, 228–242. https://doi.org/10.1016/j.foodchem.2018.01.076

Yang, K., Li, K., Pan, L., Luo, X., Xing, J., Wang, J., Wang, L., Wang, R., Zhai, Y., & Chen, Z. (2020). Effect of Ozone and Electron Beam Irradiation on Degradation of Zearalenone and Ochratoxin A. *Toxins*, *12*(2), Article 2. https://doi.org/10.3390/toxins12020138

Yao, H., Hruska, Z., & Di Mavungu,D. (2015). Developments in detection and determination of aflatoxins. *World Mycotoxin Journal*, *8*(2), 181–191. https://doi.org/10.3920/WMJ2014.1797

Yao, H., Hruska, Z., Kincaid, R., Brown, R., Cleveland, T., & Bhatnagar, D. (2010). Correlation and classification of single kernel fluorescence hyperspectral data with aflatoxin concentration in corn kernels inoculated with *Aspergillus flavus* spores. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, *27*(5), 701–709. https://doi.org/10.1080/19440040903527368

Yao, H., Zhu, F., Kincaid, R., Hruska, Z., & Rajasekaran, K. (2023). A Low-Cost, Portable Device for Detecting and Sorting Aflatoxin-Contaminated Maize Kernels. *Toxins*, *15*(3), Article 3. https://doi.org/10.3390/toxins15030197

Yemmireddy, V., Adhikari, A., & Moreira, J. (2022). Effect of ultraviolet light treatment on microbiological safety and quality of fresh produce: An overview. *Frontiers in Nutrition*, *9*, 871243. https://doi.org/10.3389/fnut.2022.871243

Yu, S., Jia, B., Liu, N., Yu, D., & Wu, A. (2020). Evaluation of the Individual and Combined Toxicity of Fumonisin Mycotoxins in Human Gastric Epithelial Cells. *International Journal of Molecular Sciences*, *21*(16), Article 16. https://doi.org/10.3390/ijms21165917

Yu, S., Jia, B., Liu, N., Yu, D., Zhang, S., & Wu, A. (2021). Fumonisin B1 triggers carcinogenesis via HDAC/PI3K/Akt signalling pathway in human esophageal epithelial cells. *Science of The Total Environment*, *787*, 147405. https://doi.org/10.1016/j.scitotenv.2021.147405

Yun, S.-H., Lee, S.-W., Koo, H.-N., & Kim, G.-H. (2014). Assessment of electron beam-induced abnormal development and DNA damage in *Spodoptera litura* (F.) (*Lepidoptera: Noctuidae*). *Radiation Physics and Chemistry*, *96*, 44–49. https://doi.org/10.1016/j.radphyschem.2013.08.008

Zhai, Y., Pan, L., Luo, X., Zhang, Y., Wang, R., & Chen, Z. (2022). Effect of electron beam irradiation on storage, moisture and eating properties of high-moisture rice during storage. *Journal of Cereal Science*, *103*, 103407. https://doi.org/10.1016/j.jcs.2021.103407

Zhang, Z.-S., Xie, Q.-F., & Che, L.-M. (2018). Effects of gamma irradiation on aflatoxin B1 levels in soybean and on the properties of soybean and soybean oil. *Applied Radiation and Isotopes: Including Data, Instrumentation and Methods for Use in Agriculture, Industry and Medicine*, *139*, 224–230. https://doi.org/10.1016/j.apradiso.2018.05.003

Zheng, M. Z., Richard, J. L., & Binder, J. (2006). A Review of Rapid Methods for the Analysis of Mycotoxins. *Mycopathologia*, *161*(5), 261–273. https://doi.org/10.1007/s11046-006-0215-6

Zhu, F., Yao, H., Hruska, Z., Kincaid, R., Brown, R. L., Bhatnagar, D., & Cleveland, T. (2016). Integration of Fluorescence and Reflectance Visible Near-Infrared (VNIR) Hyperspectral Images for Detection of Aflatoxins in Corn Kernels. *Transactions of the ASABE*, *59*(3), 785–794. https://doi.org/10.13031/trans.59.11365

APPENDIX 1: QUESTIONNAIRE

Name of the farmer……

Tel No……

County……Sub-county……Village…….....

GPS co-ordinates (decimal degrees): ……

Note to data collector: This survey must be administered to the individual in the household primarily responsible for maize cultivation. DO NOT read out the possible responses to the respondent; select the option(s) that best reflect the respondent's answer.

1. When did you plant the maize?

a) Month _____

b) Week ( ) 1st ( ) 2nd ( ) 3rd ( ) 4th

2. How many acres have you planted with maize? _____

3. Is the maize intercropped? Yes ( ) □ Q5 No ( ) . *If No skip question 4*

4. With what is the maize intercropped? (Select all responses mentioned)

vegetables ( ) maize ( ) groundnuts ( ) beans ( ) sorghum ( ) other ( )……

5. Which variety of maize have you planted this season? ....

6. Did you use fertilizer on your maize? Yes ( ) No ( )

If yes, which fertilizers? …….

7. How was tillage done? …….

8. How was weeding done? .....

9. When was the maize harvested?.....

10. Was the maize dried prior to storage? Yes ( ) No ( ). If yes how was drying done?..... condition……..

11. Where will/is the maize stored? …….. packaging……. Condition……