DIVERSITY AND BIO-CONTROL OF MYCOTOXIN FUNGI FROM MAIZE, GROUNDNUTS AND SOILS OF WESTERN KENYA

BY

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DECLARATION

DECLARATION BY THE STUDENT

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ABSTRACT

Mycotoxin adulteration in maize, groundnuts and agricultural lands have destabilized Kenya's food safety and healthcare status overtime. This study focused on assessing the diversity of mycotoxin fungi in maize, groundnut, and soils in Western Kenya, determining *in-vitro* inhibitory indices, and the efficacy of selected fungi against the mycotoxin-producing fungi under field conditions. This involved biased sampling of contaminated maize, groundnut, and soils from Homa Bay, Migori, Siaya, and Busia Counties. Respective farmers were assessed on their awareness on mycotoxin mitigation measures in grains. Pure fungal cultures were grown on PDA media and incubated at 25-27 ^{o}C in a Gallenkamp incubator. Fungal identification was done at X400 microscopic magnification and then confirmed using pathology reference books and journals. After identification, *in-vitro* inhibitory indices of toxigenic and non-toxigenic species were done to determine their degree of antagonism against mycotoxin-producing fungi using the dual culture and the modified techniques after a 10-day incubation period. The next stage comprised testing of nine fungal isolates for their inhibitory capacity against 6 mycotoxin fungi. Finally, field efficacy tests for successful antagonists against mycotoxin fungi under a split-plot layout in RCBD were done for two sites in two seasons. The arrangement comprised two maize varieties serving as the main plots and seven treatments as subplots. Fungal diversity findings were presented in plates and description tables, while *in-vitro* and field data were analysed using ANOVA and DMRT at 5% significance level applied as a mean separation post-hoc using Genstat software version 16.0. Additionally, farmers in Busia, Siaya, Homa Bay and Migori counties were unaware of field and off-field causes and mitigations of mycotoxins in maize and groundnuts. Pure cultures of thirty-five diverse fungal isolates were obtained where14 diverse pathotypes were *Aspergillus* species followed by 8 and 4 diverse isolates from *Penicillium* and *Fusarium* genera respectively. Busia County had the highest sum of diverse isolates and Siaya recorded the lowest. For isolation frequency per sample type, soil samples (30 isolates) and groundnuts (9 isolates) had the most and least number of diverse fungal isolates. Twenty-two isolates were specific to environments/counties while 13 were not specific to a single region. Over 80% inhibition levels were observed in *T. harzianum*, MCMT3, MCMT4b and *Monascus* species *in-vitro* against mycotoxin fungi. However, isolates MCMT3, MCMT4b and *Monascus* species had the best ZIs against mycotoxin fungal isolates. In field conditions, *Monascus* spp., *T. harzianum,* MCMT3, MCMT4b, and the mixed concoction MCMT3, MCMT4b, and *Monascus* spp significantly repressed mycotoxin fungi. In conclusion, Western Kenya farmers were unaware of mycotoxin causes and best pre- and post-harvest extenuation measures. Mycotoxin fungi dominated maize, groundnut, and soils of Busia, Siaya, Homa Bay, and Migori counties of Kenya. Isolates MCMT3, MCMT4b, *Monascus* spp., and *T. harzianum* best repressed mycotoxin-producing fungi but did not display synergism in field conditions. This study recommends adequate capacity building on mycotoxin management strategies among Western Kenya farmers and mass production of isolates MCMT3, MCMT4b, and *Monascus* species for the management of mycotoxin fungi in maize and susceptible economic valuable crops to mycotoxin fungi.

DEDICATION

I dedicate this work to the University of Eldoret, farmers, pathologists, colleagues, parents, friends, and all interested in plant protection.

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LIST OF ABBREVIATIONS

AEZ – Agro-Ecological Zones

Afs - Aflatoxins

ANOVA – Analysis of Variance

BCA – Biological Control Agents

CDCP - Centers for Disease Control and Prevention

CFU – Colony Forming Units

DMRT – Duncan’s Multiple Range Test

FAOSTAT - Food and Agriculture Organization Corporate Statistical Database

FDA - Food and Drug Administration

KALRO - Kenya Agricultural and Livestock Research Organization

NCPB – National Cereals and Produce Board

OTA – Ochratoxin A

PAT – Patulin

PCN – Penicillic Acid

PCPB – Pest Control Products Board

PDA – Potato Dextrose Agar

PPB – Parts per Billion

QGIS - Quantum Geographic Information System

RCBD – Randomized Complete Block Design

SSR – Simple Sequence Repeats

VCG - Vegetative Compatibility Groups

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Maize (*Zea mays*) is an economically significant crop for over a billion humans worldwide (Kaushal *et al.,* 2022). This crop is ranked third after two major cereals, that is, wheat (*Triticum aestivum*) and rice (*Oryza sativa*) (Hasan, 2008; Kainyu, 2015). It contains crucial nutritional components important for human health. In Kenya, maize contributes to 68% and 32% of daily intakes of cereal and protein per person (Schroeder, *et al*., 2013). In Kenya, small scale farmers produce 70- 80 % of the crop realizing yield of at least 1.5 tons in every hectare. Kenya Agricultural and Livestock Research Organization (KALRO) statistics show that countrywide land area under maize production currently stands at 1.5 million hectares (KALRO, 2022). Unfortunately, average yields in Kenya are 1.8 tons per hectare. According to Njeru et al. (2022), these yields are far beneath the estimated expected yield of 6 tons in every hectare. They signify deficient maize production levels against rising consumption demands countrywide (Gacheri *et al*., 2022).

Mycotoxins significantly aggravate maize losses by deteriorating grain quality (Nji *et al*., 2022). Mycotoxins that are widely reported in Kenyan to contaminate maize grain comprise aflatoxins (AFs), ochratoxin A (OTA), fumonisins, patulin (PAT), penicillic acid (PCN) and zearalenone (Kagot *et al.,* 2022; Wafula *et al.,* 2022). In Kenya, maize is grown in regions that experience environmental conditions that promote fungal annexation and mycotoxin buildup (Nelson *et al*., 2015). Over 70% of Kenyan maize farmers have inadequate resources to grow the crop under environments that can shield the crop from mycotoxin adulteration. The serial processes in maize value chain from production to consumption have no appropriate regulations that guarantee food safety (Kang'ethe, *et al*., 2017). Important causative factors of mycotoxin contamination within maize value chain are inadequately understood by majority of farmers in Kenya (Kagot et al., 2022). In the Western region of this country, maize is widely grown and is known to be susceptible to AF accumulation.

To achieve sustainability, biological control measures that prevent mycotoxin hypes in maize, have been identified in the past. For the case of AFs, among the best utilized strategies is the institution of non-AF strains in maize fields to prevent colonization of the crops by their relative toxigenic counterparts (Dorner, 2004; Mamo *et al*., 2022). This strategy enables the non-toxigenic strains to effectively compete and exclude AF-producing strains, reducing the likelihood of crop and environment contamination (Ortega-Beltran & Bandyopadhyay, 2021; Zhang *et al*., 2020). An important historical milestone was the formulation and approval of AflaSafe KE01^{TM} as a commercial biological product with a capacity to control AFs accumulation in maize grown in Eastern Kenya (Cotty & Mellon, 2006; Okun *et al*., 2015). Unfortunately, the product development processes were solely focused on Eastern Kenya. Nationwide performance data of the product lacks across all maize production zones. This gap is intensified as there are no registered bio-control products with capacity to mitigate other important mycotoxins in maize.

To develop effective mycotoxin mitigation strategies, assessing the prevalence of toxin-producing fungi is an essential procedure. There is a need to evaluate contamination levels and the factors associated with the accumulation of mycotoxins. Thus, this research aimed to assess the diversity of non-toxigenic and toxigenic fungi in groundnuts and maize fields. The two fungal groups were interacted in the laboratory. Finally, a formulation was made from the best-performing antagonists, and the efficacious biological concoction of successful antagonists was tested in field conditions.

1.2 Problem statement

Sufficient production and ingestion of mycotoxin free food and feed is a vital international challenge in tropical regions. It is practically a crucial challenge in most African countries to warrant production and distribution of quality food and feed. However, it is approaching a unprecedented tragic degrees increasing the possibilities of human fatality in Kenya (FAOSTAT, 2016). Exposure, where it is low at chronic levels, causes cancer among other illnesses resulting to death under acute acquaintances (Muthomi, 2018). Mycotoxin contamination is devastating across entire maize production value chain, affecting all stakeholders such as farmers and traders. Commercial production of food material is fairly regulated. More attention has been focused on the formal market and little attention is given to the informal markets which form the bulk of this entire market (Kagot *et al*., 2022). It is common to find between 30-40% of highly contaminated food material in Kenya.

The history of mycotoxins is long and dates back to time immemorial. For instance, AFs were first reported in Turkey in 1960, where over 100,000 turkeys and other birds such as ducklings died (Alakonya & Monda, 2013; Negash, 2018). In previous decades, Kenyan have suffered several fatal mycotoxin epidemics since 1981 where hundreds of people died by consuming spoiled food (Okoth, 2016). For instance, in 2004 over 100 Kenyan nationals died due to AF poisoning (Brown *et al*., 2013; Mahuku, *et al*., 2018). Fatality records indicate that Kenya is the only country globally that has sequentially experienced lethal aflatoxicosis outbreaks over the last 40 years (Julia, 2005; Muthomi *et al*., 2009; Probst *et al*., 2004).

Worldwide reports exist on mycotoxin accumulation and poisoning in maize. Reports indicate up to 2377.1 parts per billion (ppb) of mycotoxins in maize and groundnuts produced in Nyanza and Nairobi regions (Mutegi *et al*., 2013; Ndisio, 2015). Majority of Kenyan mycotoxin outbreaks have occurred in the Eastern Kenya when people consumed maize they produced for subsistence. According to Kachapulula, *et al*., (2017) and Okoth, *et al*., (2012), the Eastern region shelters a significant population of the S-strain morphotypes of *Aspergillus flavus*. Among the highest outbreaks that occurred between 2004 and 2006 in Kenya, several people succumbed to AF poisoning in diverse regions including the Eastern Province in areas like Mbeere and Kitui. Several thousands of humans were exposed to dangerous levels of AF quantities (CDCP, 2004).

Besides these fatal poisoning episodes, ingestion of trifling mycotoxin-adulterated maize products is a more significant health hazard especially in Western Kenya. It is common to find between 30-40% of highly adulterated food material circulation in Kenya (Ngure et al., 2021). Maize samples with 73.3 μg/kg AF levels were reported from Western Kenya (Kagot et al., 2022). Nine years prior, Mutegi *et al* (2013) also found that groundnuts of Western, and Nyanza regions had dangerously high AF levels. These statistics imply the possibility of significantly deficient mitigation measures against mycotoxin contamination in the region thus warranting urgent development of sustainable mitigation measures.

1.3 Justification of the study

Mycotoxins contribute to losses of up to 25% in the world's agricultural produce (Jalili, 2015). Financial losses due to AFs are encountered to a greater extent in developing countries (ICRISAT, 2016). Most mycotoxins in food are teratogenic and carcinogenic, causing death through illnesses like liver cancer in humans (Elsanhoty *et al.*, 2013). Approximately 5 billion individuals globally are recurrently exposed to these mycotoxins by consuming grains contaminated with mycotoxins. Reports indicate that AFs levels in maize has been reported as high as 2377.1 ppb in risky areas (Atela, *et al.*, 2016; Probst *et al.*, 2007; Salano *et al.*, 2015).

Efficacious approved biological products exist to manage mycotoxins at field conditions. A preliminary study was done in 2015 to formulate and develop the first fungal biocontrol product, Aflasafe KE01™, for application in Kenya (Migwi *et al.*, 2020). Currently, the product is already availed to farmers for purchase in some regions of Eastern Kenya. As expected, in certain events of biological control, the interactions in the field are entirely different from what is observed in the laboratory due to more complex and unpredictable interactions in natural environments. Even a successful case in another country may not be directly implemented by simply extrapolating results. In some interactions of organisms, the outcome has turned negative and even disastrous.

Before efficacy testing in Kenya, understanding the associations between toxigenic and non-toxigenic fungi is crucial. For proper management of mycotoxins, there is need to formulate products with diverse modes of action made from the broad diversity of locally existing fungi and test this alongside Aflasafe KE01™ (Wild, Miller, & Groopman, 2016). In addition to efficacy testing of Aflasafe KE01™ within Western Kenya's agricultural viable lands , this research aimed to formulate a new bio-control product comprising native non-toxigenic fungi. The target is to assist in extending early intervention techniques in maize production regions where intercropping is common. The initial step involved the isolation and morphological characterization of all fungi obtained from soils, maize and groundnut grains from Western regions of Kenya. *In-vitro* interaction studies followed this to identify and verify non-toxigenic fungi with capacity to repress the growth of mycotoxin fungi and ultimately limit potential levels of toxins produced. Best-performing antagonists were used to formulate an efficacious biological concoction applied in the field.

1.4 Objectives

1.4.1 Broad objective

To promote food and nutritional security through bio-control of mycotoxin fungi affecting optimal production of maize and groundnuts in Western Kenya.

1.4.2 Specific objectives

1. To evaluate the diversity of mycotoxin fungi in maize, groundnut, and soil samples in Western Kenya.
2. To determine i*n-vitro* inhibitory indices of selected fungi against the mycotoxin-producing fungal species from Western Kenya.
3. To assess the efficacy of selected bio-control agents *(in-vitro)* against mycotoxin-producing fungal isolates in maize in Western Kenya.

1.5 Research questions

1.5.1 Objective 1

1. Which fungal pathogens were present in samples of maize, groundnuts, and soils of Western Kenya?
2. Which major mycotoxin-producing fungi were among the fungal isolates?
3. How diverse were isolates of major genera of toxigenic species based on fungal morphology?

1.5.2 Objective 2

1. Which fungal antagonists recorded maximum and minimum inhibitions against mycotoxin fungi *in-vitro*?
2. Which of the selected fungi formed clear zones of inhibition against mycotoxin fungi species?
3. Which antagonists overgrew the test fungi *in-vitro*?

1.5.3 Objective 3

1. Which fungal concoction was most effective against AF fungi in the field?

2. Which biological concoction mixtures displayed synergism?

3. How did the test fungi perform compared to the standard product (Aflasafe KE01™)?

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview

This chapter reviews past literature on mycotoxins' occurrence, spread, and bio-control in food crops. The concept of mycotoxins is explored. Major mycotoxins in cereals in Kenya described include AFs, OTA, PAT, and PCN. The etiology of mycotoxin fungi is reviewed with a focus on host preference and environmental conditions suitable for their development. The biology and ecology of significant mycotoxin-producing fungi are described. This chapter also discusses regulatory measures employed internationally and in Kenya to prevent mycotoxin poisoning on consumers. Lastly, a review is done on *in-vitro* and field efficacy studies on mycotoxin-producing fungi.

2.2 Mycotoxins

The term mycotoxins is a general tag referring to more than 52 toxic compounds of fungal origin that occur naturally in nature without causing any known harm (Rossi, Gallo, & Bertuzzi, 2020). However, these compounds become poisonous only when ingested as they can result in serious health effects on livestock and humans. Some intermediate derivatives from aflatoxins include sterigmatocystin and aspergillic acid (Kubosaki *et al*., 2020). Attention is accorded to these compounds because of their direct effect on human well-being (Owiro, 2019; Yitbareka & Tamirb, 2013).

2.2.1 AFs

AFs refer to a collection of secondary metabolites yielded by certain members of *Aspergillus* fungi. The members of these fungi are essential because they are the primary producers of the poisons from which the name AF is derived. They include *Aspergillus flavus*, *Aspergillus nomius,* and *Aspergillus parasiticus* (Negash, 2018). Species and strains that produce these toxins have grown in diverse substrates. Examples of common food products where the fungi grow include maize, cassava and peanuts (Mahmood, *et al*., 2017). Therefore, virtually all food material requires post-harvest processing: drying and storage to prevent contamination by AFs (Pepple, Chukunda, & Ukoima, 2016).

AFs were discovered in 1960 in Turkey when hundreds of thousands of poultry died from consuming *A. flavus*-infested groundnut meal (Joanne, Linda, & Christopher, 2008). Key habitat areas of *Aspergillus* include soil, decomposing vegetation, and deteriorating grain. Fungal species of this genus thrive in all forms of organic substrates under favorable environmental conditions. Human foods frequently affected include commonly consumed cereals like maize and sorghum. Other favourable substrates for the fungus include oilseeds like groundnuts and soya bean (Patel *et al.*, 2015). There are 18 diverse AFs, with dominant types including AFs B1, B2, G1, and G2. AF B1 is mainly found in hefty levels in preferred substrates and is regarded as the most lethal (Marchese, *et al*., 2018).

Different species of *Aspergillus* may produce specific AFs. For example, *A. parasiticus* produces AFs B1, B2, G1, and G2, whereas *A. flavus* produces only B1 and B2. AFs M1 and M2 are metabolic products of AF B1 and B2 when ingested by animals (Wacoo *et al*., 2014). AF B2A and G2A have been isolated from *A. flavus* and *A. parasiticus* but in minute amounts (Benkerroum, 2020). While these toxins are not utilized at any metabolic process of the fungi during their growth or development, they offer protection to the fungus by weakening the receiving host and improve the infected environment to favour further fungal proliferation (Fox & Howlett, 2008). Aflatoxicol is a product of reductive metabolism of AF B1 and is usually exuded in products like milk and eggs and in excreted wastes of dairy and poultry that have ingested AF B1. AF GM1 and parasiticol are examples of other toxins produced by *A. flavus* (Díaz-zaragoza, *et al*., 2014; Filazi & Sireli, 2013).

2.2.2 OTA

OTA is a typical cereal contaminant in Kenya produced by fungi from *Penicillium* and *Aspergillus* genera (Tao *et al*., 2018). The most common *Aspergillus* species known for producing this toxin are *A. ochraceous, A. carbonarious,* and *A. niger.* It is the most toxic form of the ochratoxins group of metabolites and has fatal implications on human health, such as liver inflammation (Wang *et al*., 2019). This toxin is cancerous and can be stable even under heat. This stability makes it difficult to treat foods once produced because high heat levels are likely to destroy the foods by denaturing their form and crucial nutritional compounds (Tao *et al*., 2018). These toxins have been detected in various human and animal foods such as cereals, fruits, and hay. Its potential to cause fatalities led to the establishment of threshold levels by the European Commission on all exported and imported products (Schrenk *et al*., 2020). For example, the highest allowable threshold for raw cereals is 5.0 ppb.

Kibebe (2018) detected OTA from wheat and sorghum samples obtained in Nairobi, Kenya. Although they found that the levels were lower than the maximum thresholds, they concluded that high OTA frequencies in cereals in Nairobi County are a potential human health factor in the region. Mutiga *et al*. (2021) reported that Kenya does not have an extensive regulatory framework against mycotoxins. Toxins such as OTA have not been labelled maximum allowable limits by the regulatory authorities. These toxins have also been detected in rice sourced from the Mwea irrigation scheme in the 2018-2019 growing seasons.

2.2.3 PAT and PCN

PAT is a critical mycotoxin found in various food staffs consumed by humans (Brito *et al*., 2022). It is produced by several fungal species of *Aspergillus* and *Penicillium* genera growing in rotting foods. This toxin damages human organs such as kidneys and the liver. PAT is genotoxic, but its cancer-inducing properties have not been widely explored (Carvajal-Moreno, 2021). The diversity and prevalence of PAT-producing fungi in Kenya are limited, but mycotoxin studies in Africa have detected its presence (Wafula *et al*., 2022). Conversely, PCN producing fungi have been detected in grains in Kenya. It is also produced by several fungi from *Aspergillus* and *Penicillium* genera. The large number of strains that produce PCN indicate these mycotoxins' ubiquity (Ismaiel & Papenbrock, 2015). This toxin also increases at low temperatures, posing a great danger to refrigerated foods.

2.3 Ecology and biology of major toxigenic species

Aspergillus and *Penicillium* species are ubiquitous in the environment grow in diverse habitats such as soils and plants. They are found in open and closed spaces where air, water and food items are contained. *A. flavus* and *A. parasiticus* share several similarities. For example, they occur as saprophytic organisms on decaying plants and in soils. These species, although have a global distribution, they are mostly detected in tropical environments with wide ranges of weather conditions like temperature and humidity (Yoshimi, Miyazawa, & Abe, 2016).

Aspergillus species produce non-septate conidiophores and have enflamed tips where phialides are found. These distinct reproductive cells are either uniseriate or petite progressions (Yoshimi *et al*., 2016). When cultured on Czapek's agar the colonies of *A. flavus* appear greenish and yellowish. They sterigmata are usually biseriate and have sclerotia with red to brown colouration. The spores are excellently coarsened and exhibit shape variations from ovate to globular (Thathana *et al*., 2017). The mycelia surface of *A. parasiticus* appears dark green on Czapek’s media but maintain the green colour with age, and their sterigmata have a uniseriate pattern. The fungus lacks sclerotia, and the spores are roughened. *A. parasiticus* colonies depict uniformity in size and shape (Iheanacho, *et al*., 2014).

2.3.1 Environmental conditions promoting mycotoxin adulteration in agricultural products

Environmental conditions are vital dependable influences for mould growth and toxin production, leading to mycotoxin adulteration in foods and feeds. Adulteration of harvested and processed cultivated foodstuffs can occur on- and off-field stages (Omara *et al*., 2020). While *A. parasiticus* thrives in soils and peanuts, *A. flavus* is mostly detected in the aerial parts of a plant like flowers and mostly infects corn. Mycotoxin accumulation in maize and oilseeds are favoured by high moisture (over 80%) and high temperature levels (10-40 ^{o}C) (Agriopoulou, Stamatelopoulou, & Varzakas, 2020; Mahukua, *et al*., 2019).

Drought conditions favour spore production by *Aspergillus* (Sibakwe,*et al*., 2017). Low levels of major soil minerals like nitrogen and other stressors that limit pollination favour Ochratoxin A production (Marin & Taranu, 2012; Atumo, 2020). Both dry and undried maize grain remaining in the field is susceptible to infection by mycotoxin producing fungal species. Infections are also favoured by poor storage conditions of feeds and grains (Lunyasunya *et al*., 2005).

The harvest time has also been shown to influence build-up of mycotoxins such as AFs. It is because *Aspergillus* does not effectively compete with other fungi at moisture levels of less than 20%. Therefore, a key management tip is to conduct harvesting at moisture levels of 20% and then drying the grains to attain moisture levels to below 15% (Muga, Marenya, & Workneh, 2019). Low oxygen content diminishes mycotoxin formation (Marin & Taranu, 2012). Insect infestation in grains cause physical injuries on grain allowing entry by *Aspergillus* and *Penicillium* species leading to adulteration (Bowen *et al.,* 2014; Dowd, 2003). Inappropriate storage of protein enhancements like cottonseed cakes habour mould often detected in homemade feed concentrates of smallholders (Alvarado *et al*., 2017). Traditional maize storage systems such as granaries may favour growth of toxigenic fungi on wet kernels when their environment is warm and floors are dusty (Eduardo *et al*., 2005; Mutiga, Mushongi, & Kangéthe, 2019).

When these storage places are elevated and are kept clean, they assist in isolating the maize from fungal spores and insect infestations. Although drying is a recommended preventative management strategy, most mycotoxin fungal spores remain attached to favourable agricultural products after drying and grow under light conditions (Dagnas & Membre, 2013). Unfortunately, most stakeholders especially traders in this value chain do not employ appropriate protective measures against insect pest infestation and fungal colonization in grain. Elimination of possible moisture sources in foods and feed at handling and storage phases is vital to prevent prolific growth and reproduction of mycotoxin fungi.

2.4 Prevalence of non-toxigenic strains in Kenya

In Kenya, the laboratory production of non-toxigenic strains has been focused mostly on *Aspergillus flavus*. It has been established that some strains cannot produce toxins (Bandyopadhyay *et al*., 2016). In some cases non-toxic producers are applied as biocontrol of the toxigenic strains. They are selected as biocontrol based on the inability to mate with toxin-producing forms, as shown through genetic profiling using simple sequence repeats (SSR) and Vegetative Compatibility Grouping (VCG) (Moral *et al*., 2020; Shenge *et al*., 2019). Non-toxigenic strains are ecologically competitive. This method applies competitive exclusion necessitating physical obstruction of growth or entrance of the mycotoxin producing pathotypes to the seed. In the case of *A. flavus*, based on functional, genetic, and morphology, this pathogen can be clustered into L- and S- strains characterized by production of low quantities of large sclerotia and several petite sclerotia (<400 µm) respectively (Ndisio, 2015).

For almost two decades, biological control strategies have been pursued for utilizing non-AF (non-toxigenic) pathotypes to curb AF adulteration on crops (Dorner, 2004). In this case, the non-toxigenic strains are given a competitive gain to deny AF-producing fungi growth opportunities hence decreasing their contamination impact. Effective approaches have been realized by utilizing native non-toxigenic fungal strains (Cotty & Mellon, 2006; Okun *et al*., 2015).

Studies by Okun *et al*., 2015 indicate high variations of forms of non-toxigenic species in Kenya. Reports show that non-toxigenic strains or L-morphotypes of both *A. flavus* and

A. parasiticus occur naturally, but this depends on the ecological niche. *Aspergillus* species were positively detected in all soil samples obtained from Kenyan regions. The L-strain morphotypes were the most common occurring in over 50% of the samples and then followed closely by the S-strains. Among *Aspergillus* species, *A. flavus* and *A. parasiticus* recorded the highest detection frequencies. There were variations in toxin to non-toxigenic strain ratio of *Aspergillus* across ecological regions. For example, while Msambweni region had the lowest strain ratio Makindu had the highest ratio (Okun *et al*., 2015). The outcomes of this research signal that Eastern Kenya contains potentially a high diversity of non-toxigenic fungal species that may be utilized to develop biological controls against mycotoxin fungi in that region.

Another study that identified non-toxigenic strains in Kenyan agroecological zones was by Pobst *et al*. (2011). This study sampled 12 districts of Kenyan regions formerly known as provinces, including Eastern, Coast, Rift Valley and Nairobi. While there were 96 strains of mycotoxin non-producing *A. flavus,* 12 of these isolates significantly reduced AF levels by over 80%. This study found that non-toxigenic strains in these regions have a high potential of mitigating AF contamination to significant levels hence reducing mycotoxin outbreaks in the region.

2.5 Regulations of mycotoxins

2.5.1 International regulations

In the United States, the Food and Drug Administration (FDA) termed mycotoxins, such as AFs, as unavoidable food poisons and enforced the highest acceptable limit in agricultural products. However, the maximum residual limits vary from 10ppb for food (human beings) to 20 ppb for feed (domestic animals: such as ruminants) (Liu & Wu, 2010; Williams *et al*., 2004). Thus, if AFs are detected beyond human safety levels (10ppb), the material is converted to feed through further downstream processing. While this (10ppb) level is tolerated among certain countries, there seem to be differences in the enforcement as countries are at liberty to impose more stringent requirements on trading partners. The European Commission has placed various regulations to manage mycotoxins. Major toxins such as AFs and ochratoxin A have been prioritized, and the commission has signed several agreements with neighboring states to address these toxins. By 2018, mycotoxins were the primary reason for border rejection of products in Europe, with more than 500 notifications (Daou *et al*., 2021).

2.5.2 Local regulation of mycotoxins

The practice of stringent regulation works well in Europe and Asia, but the same does not apply in Africa. By contrast, most of the populance in Africa depend on on cereals for their everyday nourishment, and it is nearly impossible to enforce these regulations in practice (Probst, *et al*., 2011). In Kenya, for example, food declared unfit for human consumption by the NCPB is often sold in an unregulated market dominated by smallholder farmers and producers. This is a significant cause of non-adherence; hence a large part of the population, as in the rest of Africa, keep on consuming food with mycotoxins exceeding permissible threshold (Atela, *et al*., 2016; Salano *et al*., 2015). However, the scenario is more severe in Kenya and Tanzania, where human fatalities have occurred in the past (Wild, *et al*., 2016). The difficulty in implementing the stringent requirements for AF regulations strengthens the view that AFs are a silent killer and a disaster waiting to happen. This study sought to tackle mycotoxin dilemma in maize to lower the toxin levels circulating in the food chain enabling more access to trading blocs among states in agreement.

2.6 Mycotoxin management approaches

Mycotoxins have received considerable research attention to understand the biology, epidemiology, and manifestation of associated fungi. Several efforts have been underway to improve some practices that predispose crops to contamination and apply them on large scale (Obonyo & Salano, 2018). Matumba *et al*. (2021) outlined 5 solutions to preventing and managing mycotoxins in cereals. These strategies are being employed in various degrees throughout the world. The first strategy is to maintain plant vigour through timely planting well-performing varieties using healthy seeds and routine management and fertilization practices. The second key entails reducing toxin-producing fungi at pre-harvest and in storage by crop rotation, optimal fungicide application, reduction of crop-soil contact, field sanitation, and disinfection of storage bags and facilities. The third technique is by protecting husks and the pericarp to maintain the integrity of the grains. It minimises mechanical damage to the grains by using chemicals such as rodenticides. The last two strategies include rapid reduction of grain moisture and cleaning mycotoxin fungi preferred spots (Ayeni et al., 2021). While these strategies may be promising, in Kenya, there hasn't been much attention to reducing and possibly eliminating the toxins from food material through BCAs (Wild *et al*., 2016).

2.6.1 Pre-harvest and post-harvest handling techniques

Crop mycotoxin adulteration is a multifaceted procedure that commences in the farm, ensuing from ecological and biological elements like host vulnerability, high-temperatures, insect infestation, and mycotoxin-producing capacities of fungi within specific habitats (Ojiambo *et al*., 2018). The contamination process in the field begins with the causative fungi colonizing the crop and "waiting" for the right conditions to grow to maturity, where they produce toxins (Salano *et al*., 2015). This necessitates both field and off-field interventions. Crop exposure to mycotoxin ultimately leads to adulteration by mycotoxins. During processing and storage, this may either happen at on-field or post-harvest (Mahukua *et al*., 2019). A simple drying device has been designed for use at subsistence levels. This device is still at patenting stage, but the trial phase showed that the device could significantly lower AF levels in Maize crops (Walker & Davies, 2013). It is currently being tested in other crop models. There is a need to examine other pre- and post-harvest strategies and their effect on reducing toxins in agricultural products.

2.6.2 Biological management of mycotoxin producers

Biological control is anchored on the premise that it is possible to use live organisms to control another often-harmful target organism, bringing it under control and reducing the harm caused by the other harmful organisms (Unnevehr & Grace, 2013). Most mycotoxin producing fungal species closely link with diverse crops steering contamination in storage and handling spaces. Maize and groundnut are valuable staples for millions of people across Africa, being among the key crops that are highly contaminated and also consumed. It follows that they should be ranked highly among crops to control AFs (Wild *et al*., 2016). Within the *Aspergillus* genus, several members cannot produce AFs (non-toxigenic) (Alakonya & Monda, 2016). It is also believed that careful selection of non-toxigenic genotypes in bio-control products can competently reduce mycotoxin adulteration of crops if introduced before flower formation and pollination (Bandyopadhyay *et al.*, 2016; Moral *et al.*, 2020; Shenge *et al.*, 2019).

Previous reviews revealed that no local groundnut variety or maize is resistant to the accumulation of mycotoxins such as AF (Ndisio, 2015). Were *et al*. (2015) isolated mycotoxin-producing fungi in groundnuts from Bungoma County but did not examine their capacity to produce toxins. Mutegi *et al*. (2013) studied the incidence of *Aspergillus* section Flavi in Homa Bay County but did not provide sufficient information on the specific regions sampled. Therefore, little has been done to document the diversity of *Aspergillus* and *Penicillium* strains and possible mycotoxins produced in Western Kenya.

2.6.3 *In-vitro* screening of bio-control agents

In-vitro assessment to test the capacity of BCAs (BCAs) to antagonize pathogenic fungi has been widely employed for grouping and explicating the mechanisms used. *Trichoderma* species and *Pseudomonas* species are among the most utilized BCAs worldwide (Begum *et al*., 2008). *In vitro* studies to evaluate the ability of BCAs to detoxify AFs have been done in past studies (Elsanhoty *et al.*, 2013). Alternatively, the capability of AF non-producing strains to competitively exclude aflnon-toxigenic fungi has been investigated *in-vitro* to ultimately reduce the potential total amount of AFs to be produced. This has been achieved using the dual culture technique either through co-inoculation of grains with non-toxigenic and toxigenic agents on grains or growing them side by side on culture media (Calistru, McLean, & Berjak, 1997; Hruska *et al*., 2014). It has also been used to study gene-coding repression for AFs production by non-toxigenic strains (Hua *et al*., 2019).

2.6.4 Field efficacy assessments

Inoculation of non-toxigenic strains has been tested and found safe and environmentally friendly in the USA (Nesic et al., 2021). Later the concept and practice were scaled up to over a million acres where susceptible hosts were treated (Cotty, Probst, & Jaime-Garcia, 2008). The technology is currently being improved for use in sub-Saharan Africa with the intention to develop bio-control products, under the Aflasafe tradename, for 11 countries in Africa, including Kenya. In 2015, a preliminary study was formulated and set Kenya's first mycotoxin fungal bio-control product, Aflasafe KE01™. It was later registered that year by PCPB as a bio-pesticide. A factory to manufacture the inoculum was set up, and initial laboratory experiments on efficacy were carried out in Kibwezi, indicating chances of success. After the seemingly successful event, the Kenyan Government purchased 270 tons of Aflasafe KE01™ and applied it in AF hotspots in Bura District (Bandyopadhyay, *et al*., 2016). Findings of field trials in Wote District point to the need for a more thorough and possibly independent re-assessment of the safety and efficacy of Aflasafe KE01™ since the levels of toxins in both test and control farmer groups are ostensibly higher than expected (Bakari, 2016).

The field has fewer chances of recombination between toxin producers and non-producers since the two sets of fungi are selected from different vegetative compatibility groups (VCGs). At this point, the chances of genetic transmissions and fusion of the hyphae are minimal (Kagot, Okoth, Boevre, & Saeger, 2019; Moral *et al*., 2020). The application of non-toxigenic forms relies on the principle of competitive exclusion/out competition, where before colonization of kernels by aflnon-toxigenic fungi, non-toxigenic strains are introduced.

Testing other fungi outside the genus *Aspergillus* is of a great boost as it helps broaden the scope of options for biological control. Generally, fungi used as bio-control agents employ varied mechanisms for antibiosis. Strains of gram-positive rhizobacteria such as *Paenibacillus polymixa* and rhizobia fungi as *Trichoderma* species produce lytic enzymes and have been explored and utilized further to manage phytopathogenic fungi (Raza, Yang, & Shen, 2008; Smitha *et al*., 2014). Several bio-fungicides, such as Protect, have been formulated and utilize these proteins to manage infectious fungi (Hamid, *et al*., 2013; Jadhav & Sayyed, 2016).

2.7 Way forward

This study focused on distinguishing the normal manifestation of diverse strains of *Aspergillus* and *Penicillium* species and the ecological occurrence of both toxigenic and non-toxigenic species. It was a critical step toward identifying the proportion of non-toxigenic species and other antagonistic fungi in natural environments. It is an essential step toward approximating their pertinency in the biological management of the more pathologically and economically critical mycotoxin producing strains in Kenya. Field tests of suitable antagonists helped evaluate the possible utilization of the fungi and their compounds as bio-control agents.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Overview

This section describes the protocol and the materials used to collect credible data that sufficiently answers the research questions. Busia, Siaya, Homa Bay, and Migori regions of Western Kenya were chosen for grain and sample collection based on the intensity of farming practices and environmental conditions that favour the occurrence and proliferation of mycotoxin fungi. It also explains the fungal isolation and incubation procedures using a laboratory-made Potato Dextrose Agar (PDA) media to grow pure cultures for morphological diversity assessment from groundnut, maize grain and soil samples. The third section describes the *in-vitro* assays to test for antagonistic properties of newly identified species from the successfully isolated fungi. The last section of this chapter describes procedures used in laying field experiments to identify the most efficacious BCAs against mycotoxin-producing fungal isolates affecting maize production in Western Kenya.

3.2 Study sites

Surveys were conducted during June and July 2019 in three sub-counties of Busia, Siaya, Homa Bay, and Migori counties (Table 1). Homa Bay and Bungoma have been associated with AF in Kenya. Little has been studied in Busia, Siaya, and Migori counties (Njoki et al., 2023). The four study regions were chosen based on the production of groundnuts and maize and their diverse agroecological conditions. Fungal isolation and *in-vitro* assessment studies were performed at the University of Eldoret, while field experiments were laid in Busia and Kisumu counties. The regional survey involved sampling maize grains, groundnut grains, soil from intercrop, and pure stands of the two crops. Global Positioning System (GPS)-sensing applications and administrative block were used for site identification.

Table 1: Counties and Sub Counties of sampling sites

County	Sub Counties
Homa Bay	Homa Bay Town, Rachuonyo North, and Rangwe
Busia	Butula and Matayos
Migori	Suna East, Suna West and Awendo
Siaya	Ugenya

3.3 Fungal diversity in maize, groundnut and soils of Western Kenya

3.3.1 Study location and environmental characteristics

Surveys were conducted during June and July 2019 in three sub-counties of Busia, Siaya, Homa Bay, and Migori counties (Table 1 and Figure 1). The four study regions were chosen based on the production of groundnuts and maize, and diversity in agro-ecological conditions. The study regions were clustered based on administrative boundaries (Taherdoost, 2016). Biased sampling was used where farmers with maize and groundnuts intercrops from the previous season were targeted. This strategy was used ensure precise collection of adulterated maize. Groundnut and soil samples.

Homa Bay County receives annual temperatures of 21.7^{o}C, 1330.9mm of rainfall, and 64.8% relative humidity. The highest temperatures (22.5^{o}C) are recorded in February and March, while the highest relative humidity rates (71.5%) are recorded in May

(Weatherbase, 2022). Siaya County records annual temperatures of 21.4 ^{o}C, yearly rainfall of 2143.8mm, and annual average humidity of 77.25%. The highest relative humidity (82.74%) is recorded in April, and the highest temperatures (22.7°C) are recorded in February (Climate Data, 2022a). The average annual temperature level recorded in Migori County is 21.0°C, and precipitation levels of 1521.5mm per year. The highest temperatures (21.9°C) are recorded in February, while April is the month that records the highest relative humidity (76.88%) (Climate Data, 2022b). Busia County records annual average temperatures of 21.8°C and precipitation levels of 2291.1mm. May records the highest relative humidity levels (82.59%), while the most elevated temperatures are recorded in February (29.3°C) (Climate Data, 2022c).

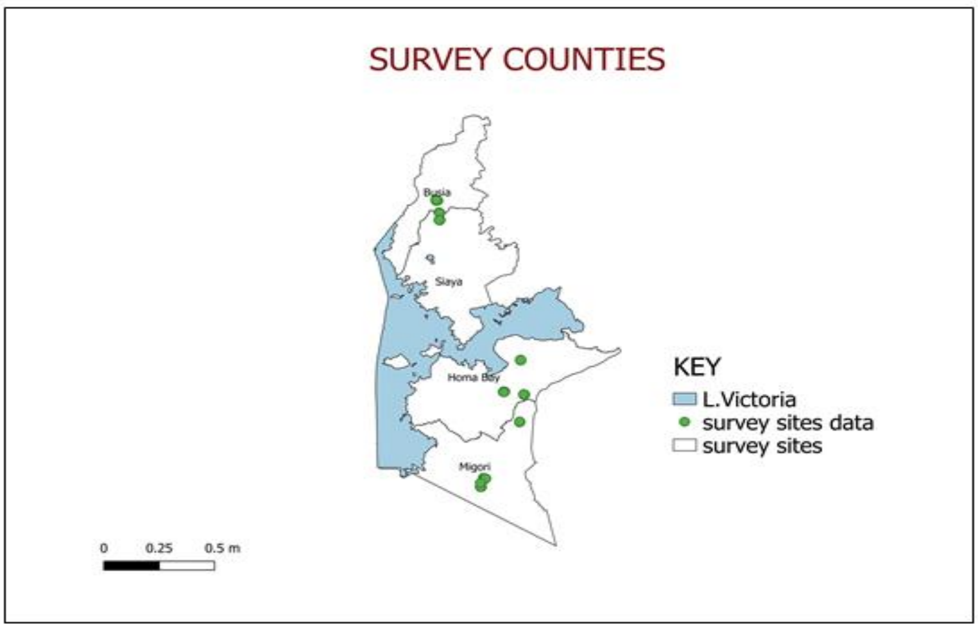


Figure 1: Geographical mapping of the survey area using QGIS location mapping

3.3.2 Sampling strategy

Grain samples of maize and groundnuts, and soils weighing 250g each were obtained from the selected farmers. Soil samples were obtained from the fields where the grains were harvested in the previous season. Samples were aseptically transported to the laboratories to isolate fungi (Salano *et al*., 2015). This procedure assisted in determination of the incidence and prevalence of AFs in diverse farming practices and environmental conditions. Forty-six maize and groundnut farmers were sampled and interviewed on their awareness and mitigation measures against mycotoxin fungi occurrence using semi-structured questionnaires (Appendix I).

3.3.3 Fungal isolation and purification from grains and soils

Commercial TM Media PDA at 39g/l concentration in sterile distilled water was heated to ensure a uniform mixture. The media was sterilised at 121 °C for 15 minutes. Contaminating bacteria were controlled by adding streptomycin antibiotic at 1g/litre of PDA (Pardley & Sharma, 2010). The grains were surface pasteurised in 1% sodium hypochlorite (NaOCl) for 60 seconds, rinsed three times in antiseptic water, and then placed on solidified sterilised PDA media. The gains were subjected to 27^{o}C Gallen Kamp incubator conditions in alternating light and dark at the rate of five grains per plate (Salano *et al*., 2016). After five days, culture purification was done where a representative mycelium was inoculated on new PDA plate and incubated at similar light and temperature conditions. The emerging colonies were sub-cultured and pure cultures were obtained subsequently for use in succeeding studies (Were *et al*., 2015).

The soils were put through a sieve using a 2-millimetre mesh to get rid of crop remains. One gram of the sieved soil was mixed with 9 ml antispetic water. Serial dilutions to 10^{-3}

of the suspension were done and a 200 microlitres aliquot was evenly dispersed on PDA media in two replicates (Muthomi *et al*., 2009). Incubation procedures lasted for five days and fungal colonies counted. The Colony Forming Units per gram (CFU g^{-1}) of soil was calculated according to Odhiambo, Murage, and Wagara (2013);

CFU g^{-1} Soil = *Number of colonies / (Amount plated x dilution factor)*

3.3.4 Fungal identification and diversity assessment

Fungal isolates across the study areas were analysed using morphologically descriptive assays. Further classification was completed through pathology reference books and journals such as Agrios (2005), Lucas (2009), Tronsmo *et al.*, (2020), and Khokhar and Bajwa, (2015). By use of a light microscope at X400 magnification the fungi were identified and clustered according to their spore and mycelial morphologies such as shape, colour and texture. The fungal isolates were also categorised according to AEZs (Counties) of isolation to determine whether they occurred within a specific region or in more than one geographical space.

3.4 *In vitro* inhibition of selected fungi against mycotoxin fungi

This research was done at the University of Eldoret Crop Protection Laboratory 1. A sum of six fungal isolates known to produce mycotoxins and nine fungal isolates without clear history of mycotoxin production were obtained from the experiment in section 3.3 of this study.

3.4.1 *In-vitro* assays against mycotoxin fungi

Inhibition characteristics of selected fungal isolates was tested against against mycotoxin-producing fungi. The assays were done by growing the two groups of fungi on PDA media using two different placement methods within a petri-dish (Maurya, Singh, & Tomer, 2014; Kumar *et al.*, 2020). Antagonistic fungal isolates selected included *Biatriospora* species, *Coniothyrium species, Epichloe* species, MCHB2 (unidentified), *Phialemoniopsis* species, *Trichoderma species*, MCMT4 (unclassified) and *Monascus* species. These nine isolates were assesssed against three AF-producing fungal species (*Aspergillus flavus, Aspergillus parasiticus,* and *Aspergillus nomius*); two PAT and PCN (*Penicillium corrylophillum* and *Penicillium auratiogriseum*); and one OTA-producing fungal isolate (*Aspergillus niger*).

Among the two methods used, the first was a dual culture while the second was the author's modified technique. For the dual culture technique, the test fungal isolates were plated at opposite and equidistant points against the antagonist from the periphery of a PDA enriched petri-dish. In comparison, for the modified technique, the antagonistic fungal isolate was cultured at four equidistant positions while the test/mycotoxin fungi placed at the middle (Figure 2). Each arrangement was replicated three times for every test.

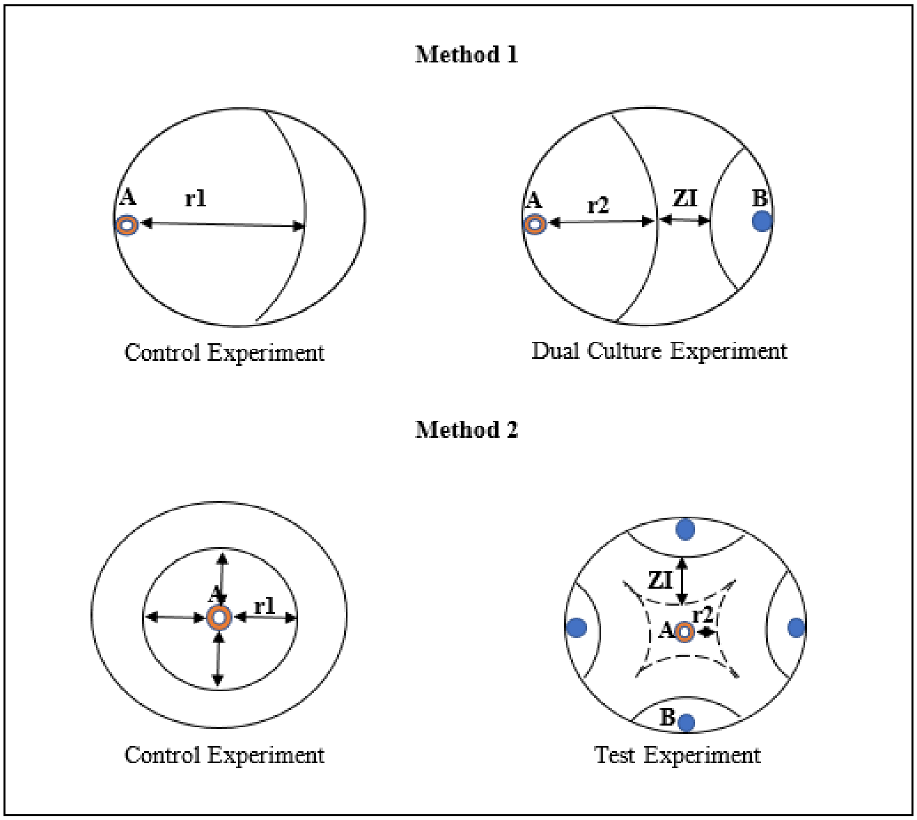


Figure 2: Isolate placement illustration myctoxin/test (A) and antagonist (B) fungi using the dual culture (method 1) and the modified method (method 2)

3.4.2 Data collection

After a 7-day incubation period, the width of inhibition zones (IZ) in millimeters was measured. Percentage inhibition levels were calculated according to Maurya *et al.*, (2014).

$$\mathbf{I}(\%) = \frac{r1 - r2}{r1} x\ 100$$

Where: I = Percentage inhibition, r_1 = length of longest radius in millimeters of the test/toxigenic fungi grown in the control experiment and r_2 = the length of radius in millimeters of mycotoxin fungi in the test experiment.

A visual scale of 1-5 was also used to measure the degree of antagonism. Where: 1 = the antagonist completely covered the whole petri-dish and grew over the mycotoxin fungi, 2 = the antagonistic fungi grew and occupied at least 2/3 of the petri-dish, 3 = the test and antagonistic fungal isolates shared equal portions of the surface of the plated media, 4 = the test fungal isolate occupied to more than 2/3 of the surface area and 5 = the test fungi occupied the whole plate and grew over the antagonistic fungal isolate (Kucuk & Kyvanc, 2011).

3.5 Efficacy of biocontrol agents against mycotoxin fungi in maize under field conditions

3.5.1 Site characteristics

The field research was done for two seasons in Kibos and Sega regions of Kisumu and Siaya counties. The study sites were selected based on their rich history in maize production, occurrence in different AEZs and suitable conditions for mycotoxin fungi infection and growth. Kibos site receives 1464 mm annual rainfall and 23^{o}C average daily temperatures. The site's altitude is 1184m above sea level at -0.06994N and 34.81688 E. The soils pH ranges from 5 to 6 (Juma *et al*, 2018). Alternatively, Sega site receives average rainfall amounts of up to 1450mm annually while temperature vary from 15-30^{o}C. The site's altitude stands at 1120m asl and is located at 0.250425995N and 34.20243912E. Ferralsols are among the most dominant soil types of the area (Okaron, 2017; Owino *et al*., 2015).

3.5.2 Sowing and study design

The field research was planted in a split-plot layout using Randomized Complete Block Design (RCBD) in two sites with treatments replicated four times. The main plots of the split plot comprised the Duma and Punda milia maize varieties known to be susceptible hosts of AF producing fungi. The sub plots comprised the seven fungal concoctions including *Trichoderma harzianum*, *Monascus* species, isolates MCMT3, MCBT4b, a mixture of *Monascus* sp, MCMT3, and MCBT4B, AflaSafe KE01^{TM} standard check and the untreated negative control. The plots measured 18.75m^2 while inter- and intra-row spaces were maintained as 75cm by 25cm respectively. During planting, Di-Ammonium Phosphate fertiliser of 18% N, 46% P and 2.5% S was added. The equivalents of nitrogen and phosphorus were 22.5kg/Ha and 25.13kg/Ha, and 0.042 kg/plot and 0.047kg/plot respectively. Weeding was completed at 21 and 56 days after planting. Calcium ammonium nitrate was applied as a top dress at 65g of N per plot at 21 days after crop emergence after the first weeding. The experiment was planted for two seasons including the long rains and short rains of 2020 in Western Kenya.

3.5.3 Concoction formulation and application

Fungal spores were washed from the petri-dishes by adding 10 milliliters of distilled water to the mycelial surface. A sterilized glass rod was used to gently scrub the surface to detach conidia from the colonies and suspend them in water. The spore suspension was transferred into sterile conical flasks where a concoction of 4 suspensions/litre was attained (Atehnkeng, *et al*., 2008). The mixed concoction treatment consisted of mixing the individual suspensions at equal concentrations. For proper storage, the fungal concoctions were refrigerated at 4^{o}C (Atehnkeng, *et al*., 2008).

When the field maize attained 50-70% silking, inoculation of the concoctions was done on the ears at 4ml per ear using clean syringes. For the standard treatment AflaSafe

KE01^{TM}, applications were done at 14 days prior to flowering which at 40 Kg/ha (Atehnkeng, *et al*., 2008).

3.5.4 Fungal diversity in harvested kernels

At maturity 20 samples of ears per treatment/subplots were obtained and were sundried until grain moisture content reached 13%. Symptomatic grains were sampled and were surface pasteurized using 1% of NaOCl for two minutes and then washed thrice using disinfected water. PDA was prepared and sterilized using procedures outlined in section 3.3.3. Afterwards, streptomycin was applied as an antibiotic to the media to repress growing bacteria on the media (Gulbis *et al*., 2016). Five grains were placed into the media dispensed on petri-dishes in triplicates and then incubated in sterile conditions at 25-27^{o}C in darkness. After 5 days, fungi were observed to grow from the cultured seeds and identified according to their morphological features of the mycelia, spores and spore-bearing structures (Gulbis *et al*., 2016). Percentage incidences of mycotoxin fungi propagating from the cultured seeds were recorded. Key mycotoxin fungi targeted in the assessments include *A. flavus, A. parasiticus*, *A. nomius*, *P. corrylophilum*, *P. auratiogriseum* and *A. niger*. These fungi were grouped into three categories including AF, PAT, PCN and OTA producing fungi. Fungal diversity assessments were also made by recording the total number of fungal species per petri-dish.

3.5.5 Experimental Layout and statistical model

The layout for the field experiment is shown in figure 3 below.

Block/Rep	Main Plots													
	Subplot A (Duma variety)							Subplot B (Punda variety)						
1	T5	T1	T3	T2	T6	T7	T4	T2	T4	T5	T7	T1	T6	T3
2	T1	T3	T4	T6	T5	T2	T7	T3	T2	T1	T6	T4	T7	T5
3	T5	T1	T6	T4	T2	T7	T3	T4	T5	T1	T3	T7	T6	T2
4	T7	T6	T4	T1	T3	T5	T2	T3	T2	T7	T5	T6	T1	T4

Key

Treatment 1 (T1) = Co-inoculation of isolates MCMT3, MCMT4b and *Monascus* species.
Treatment 2 (T2) = *Monascus* species
Treatment 3 (T3) = Isolate MCMT3
Treatment 4 (T4) = *Trichoderma harzianum*
Treatment 5 (T5) = Isolate MCMT4b
Treatment 6 (T6) = Aflasafe KE01^{TM} (Positive check)
Treatment 7 (T7) = Control/Untreated experiment (Negative check)

Figure 3: Study layout of a split-plot arrangement in RCBD.

Experimental Model

$Y_{ijkl} = \mu + \beta_i + \alpha_j + \sum_{(a)ij} + \lambda_k + \alpha\lambda_{jk} + \sum_{(b)ijkl}$

Where: Y_{ijkl} = total observation, μ = overall mean, β_i = i^{th} effect of block, α_j = j^{th} effect of the main plot, $\sum_{(a)ij}$ = error due to the i^{th} and j^{th} effects, λ_k = k^{th} effect of the subplot, $\alpha\lambda_{jk}$ = Interaction between the j^{th} level of the main plot and the k^{th} level of the subplot and $\sum_{(b)ijkl}$ = residual effect.

3.6 Statistical analysis

Data obtained from farmer practices and their knowledge status against mycotoxin contamination were analysed using descriptive statistics using bar graphs with standard error bars. The data on inhibitory indices was analyzed for ANOVA using Genstat software version 16.0. Pictorials were organized in figures to visually illustrate the antagonism effects of tested siolates. Similarly, for the field studies, incidence data depicting performance of the applied treatments and fungal diversity were subjected to descriptive statistics using Excel software of Microsoft 365. Seasonal and varietal performance trends of the treatments were depicted in line and bar graphs with custom error bars. Numerical data was subjected to ANOVA and DMRT used as a post hoc at 95% confidence interval for all mean separations in this study.

CHAPTER FOUR

RESULTS

4.1 Overview

This section entails the findings obtained in this research. The first section provides detailed information on the current status on farmer practices, awareness levels and mycotoxin mitigation measures. The second section outlines vital findings on fungal morphological diversity and environmental specificity of isolated fungi from the sourced samples. The third section contains findings on *in-vitro* assays between identified antagonists and major mycotoxin-producing fungi. Finally, the performance of successful antagonists from *in-vitro* studies is revealed in the last part of this section.

4.2 Farmers' cognizance on mycotoxin mitigation

Regarding farmer awareness of causes and mitigation measures of mycotoxins, Migori County registered the highest frequency while Siaya had the lowest in both aspects. More than 10% of respondents in Busia, Siaya, Migori, and Homa Bay counties were aware that improper drying, rainfall at harvesting, dampness, and high moisture content are causative factors of AF accumulation in maize and groundnuts. However, less than 20% of respondents from all sampled regions were aware that improper storage, soil infection, pest damage, maize rotting, bad seeds, chemical sprays on wet cereals, delayed harvesting, and untreated cereals were causative factors of mycotoxin accumulation (Figure 4). Further, farmer practice assessments revealed that over 60% of the interviewees stored their harvested groundnuts and maize in gunny bags while less than 40% stored their grains in granaries (Figure 4).

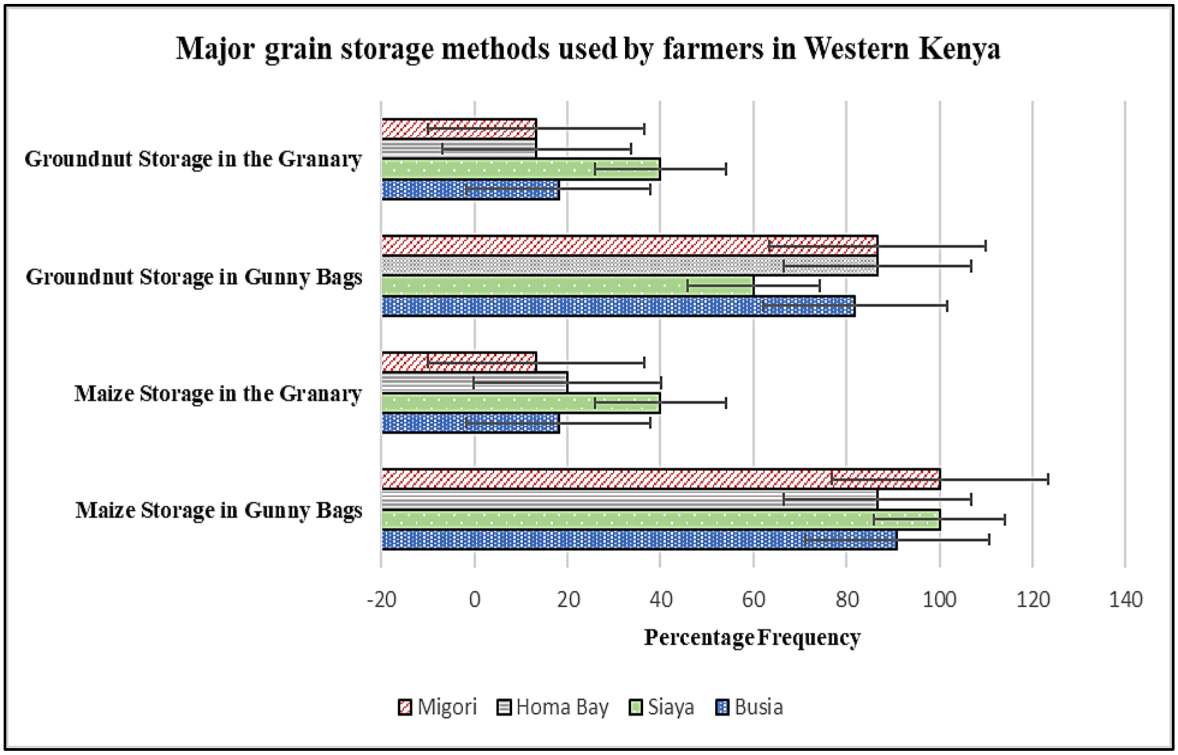


Figure 4: Storage techniques used by respondents of harvested grain (groundnut and maize)

Farmer evaluations on mycotoxin mitigation uncovered that respondents were least aware of strategies used to mitigate their accumulation (Figure 5). Appropriate drying and grain storage techniques emerged as the only key techniques known by at least 40% of the respondents across the study regions. Conversely, other alleviation strategies such as growing resistant genotypes, chemical use and crop rotation recorded less than 20% among all the study regions (Figure 5).

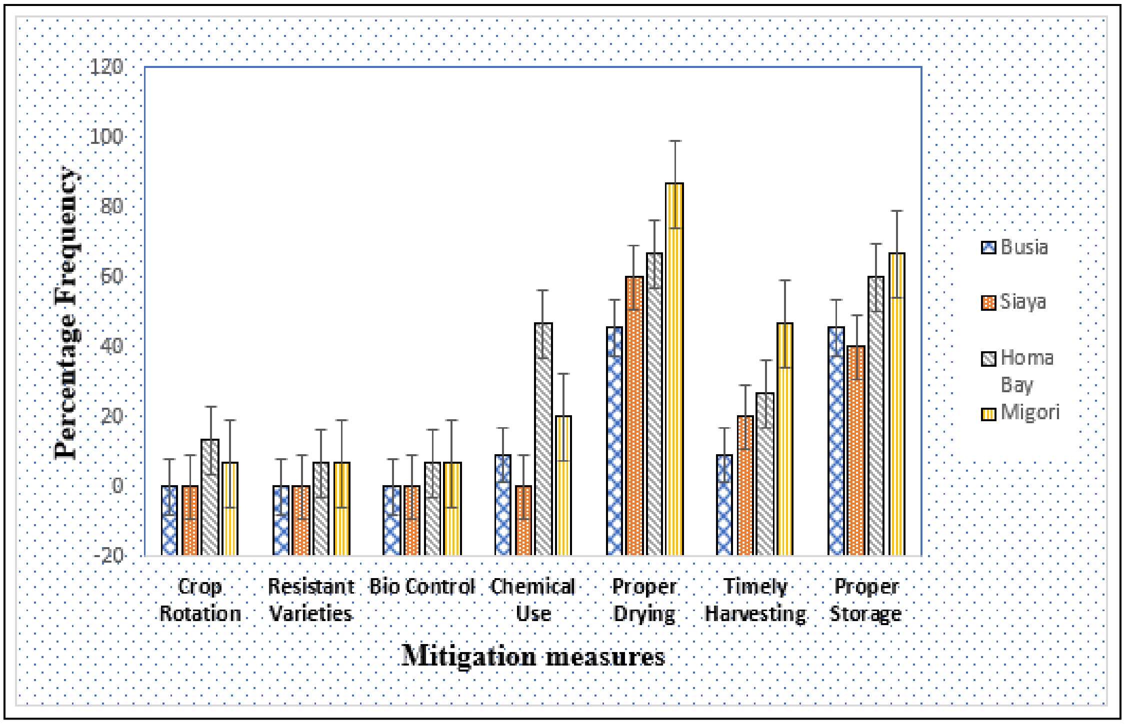


Figure 5: Farmer's response on mycotoxin mitigation in groundnut and maize

4.3 Fungal diversity in grains and soils of Western Kenya

Fungal diversity and identification assessments recorded 35 different/diverse fungal isolates across the four study regions of Western Kenya and across the grain and soil samples. Furthermore, several morphotypes were observed within a single species were noted phenotypically in plates at a microscopic level based on mycelial and spore features. Among the 35 diverse isolates, *Aspergillus* species were the most frequently isolated with 14 diverse pathotypes (Table 2 and Table 3). The second and third most frequently obtained isolates belonged to *Penicillium* (8 isolates) and *Fusarium* (4 isolates), respectively (Table 2 and Table 3). Only 4 isolates remained unidentified whereas 34 diverse isolates were identified to the genus level.

4.4 Environmental distribution of fungal isolates from Western Kenya

In general, most fungal isolates from the maize, groundnut and soil sample sets were environment specific. Across the sample types, most fungal isolates were traced to Busia County samples while Siaya County recording the least. Numerically, 13 fungal isolates were not specific to a single environment (Figure 6). In terms of sample types, maize and groundnuts of Busia County had the most frequently isolated environmentally non-specific fungal isolates (Table 2).

In maize samples, *A. flavus* (MUG5) was the most frequently isolated fungus detected across all the study areas but absent in all groundnut samples. Similarly, *Fusarium oxysporium* (MGW5) and *A. niger* (MBT2) were traced in three Counties out of the total four. Interestingly, *A. flavus* (GMT3) record the highest detection frequencies in groundnuts except for Migori County where it was not detected. In comparison, *A. niger* (MBT2) was detected in all sampling regions hence emerging as the most occurring isolate in the assessed soil samples (Table 2).

In addition to regional non-specificity, some of the isolates were specific to sample types. For instance, the detection of *A. flavus* (MUG5) occurred in maize samples from all the counties and from soils of Siaya County. Also, while *Aspergillus flavus* (GMT3) was only specific to groundnut samples, *Aspergillus nomius*, MCMT4b, and *Biatriospora* species were specific soil samples.

Besides regional non-specificity, some of the isolates were specific to sample types. *Aspergillus flavus* (MUG5) was found in maize samples alone in all counties and in soils of Siaya County. *Aspergillus flavus* (GMT3) was only specific to groundnut samples. Isolates that were specific to soil samples include *Aspergillus nomius*, MCMT4b, and *Biatriospora* species.

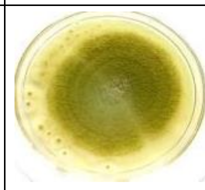
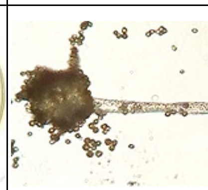
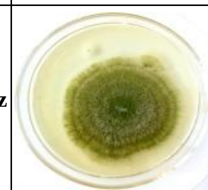
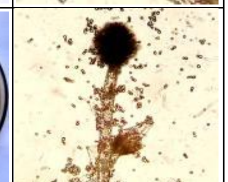
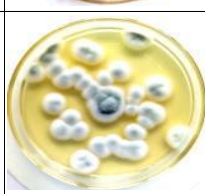
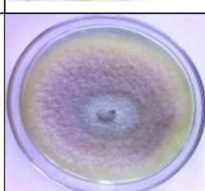
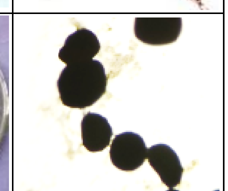
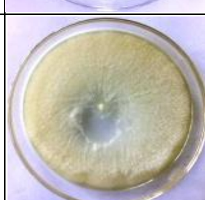
No	Identification	Mycelial morphology & colour	Spore morphology (X400 magnification)	No	Identification	Mycelial morphology & colour	Spore morphology (X400 magnification)
1	***Aspergillus flavus*** **(MUG5) (Makhlouf *et al.*, 2019)**			8	***Aspergillus nomius*** **(Azeez *et al* ., 2016)**		
2	***Aspergillus niger*** **(Moslem *et al.* , 2010)**			9	***Aspergillus flavus*** **(GMT3) Makhlouf *et al* ., 2019)**		
3	***Penicillium auratiogriseum*** **(Khokhar & Bajwa, 2015)**			10	***Fusarium*** **species (MCBT1) (Ignjatov *et al* ., 2018)**		
4	***Penicillium*** **species (MMT2) (Peterson *et al* ., 2015)**			11	***Penicillium chrysogenum*** **(Xia *et al* ., 2018)**		
5	***Fusarium oxysporium*** **(MGW5) (Teixeira *et al* ., 2017)**			12	**MCMT4b (Unidentified)**		
6	***Fusarium proliferatum*** **(Husain *et al* ., 2017)**			13	***Trichoderma harzianum*** **(Rachniyom, & Jaenaksorn, 2008)**		
7	***Biatriospora*** **species (Kolarik *et al* ., 2017)**						

Figure 6: Environmental non-specific diverse fungal isolates

Table 2: Detection frequencies of environmental or region non-specific isolates

Isolate Identity	WESTERN KENYA COUNTIES: Busia			Siaya			Homa Bay			Migori			Isolation Frequency
	M	G	S	M	G	S	M	G	S	M	G	S	
A. flavus (MUG5)	+	-	-	+	-	+	+	-	-	+	-	-	5
A. niger	+	+	+	+	-	+	-	-	+	+	-	+	8
Penicillium auratiogriseum	+	+	-	-	+	-	-	+	+	-	+	-	6
Penicillium species (MMT2)	+	+	+	-	-	-	-	-	-	-	+	-	4
Fusarium oxysporum (MGW5)	+	-	+	+	+	-	+	-	-	-	-	-	5
Fusarium proliferatum	+	-	-	-	-	+	-	+	-	-	-	-	3
Aspergillus nomius	-	-	+	-	-	-	-	-	+	-	-	-	2
A. flavus (GMT3)	-	+	-	-	+	-	-	+	-	-	-	-	3
Fusarium species (MCBT1)	-	+	+	-	-	+	-	-	+	-	-	+	5
Penicillium chrysogenum	-	-	+	-	-	-	-	+	-	-	-	+	3
MCMT4b (Unidentified)	-	-	-	-	-	+	-	-	+	-	-	-	2
T. harzianum	-	-	-	-	-	-	+	-	-	-	-	+	2
Biatriospora species	-	-	-	-	-	-	-	-	+	-	-	+	2
Number of diverse fungi per sample	6	5	6	3	3	5	3	4	6	2	2	5	50
Number of divserse fungi per region/County	17			11			13			9			

Key

M - Maize
G - Groundnut
S - Soil

In addition to environment/regional-specific fungal isolates in Table 2, 22 of the total isolates were region/County-specific (Figure 7 and 8). Taxonomically, 10 isolates were from *Aspergillus* genus, while 1 isolated was *Penicillium* species. When classified into regions, 8 isolates were from Busia County (7 from soil samples and 1 from maize) with 3 from Siaya County soil samples (Table 3).

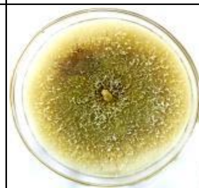
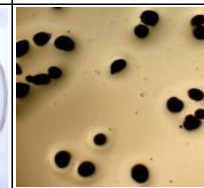
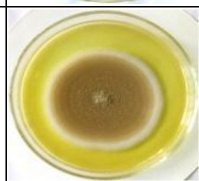
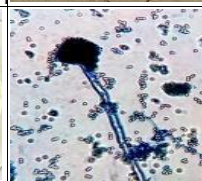
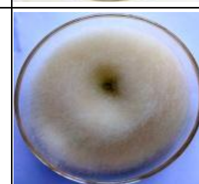
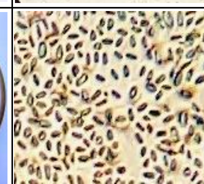
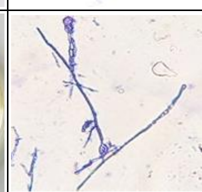
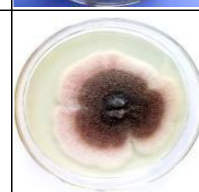
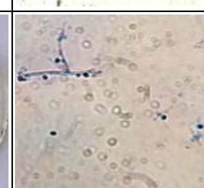
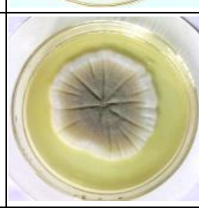
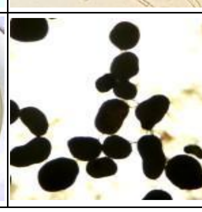
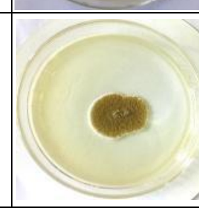
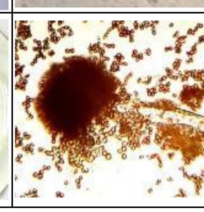
No	Identification	Mycelial morphology & colour	Spore morphology (X400 magnification)	No	Identification	Mycelial morphology & colour	Spore morphology (X400 magnification)
1	MMT3 (Unidentified)			7	MCMT3 (Unidentified)		
2	*Aspergillus terreus* (MCBT6) (Upendra *et al* ., 2013)			8	MCMBT3 (Unidentified)		
3	*Aspergillus tubingensis* (Guerrero *et al* ., 2021)			9	*Athrinium sacchari* (Wang *et al* ., 2018)		
4	*Aspergillus Candidus* (Ulloa *et al* ., 2006)			10	*Coniothyrium olivaceum* (Abdollahi Aghdam & Fotouhifar, 2016)		
5	*Monascus* species (Virk *et al* ., 2020*)*			11	*Epichloe* species		
6	MCMT4a (Unidentified)			12	*Aspergillus terreus* (MGW1) (Upendra *et al* ., 2013)		

Figure 7: Environment/region-specific diverse fungal isolates

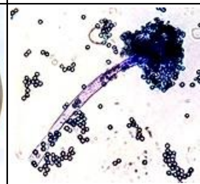
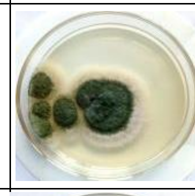
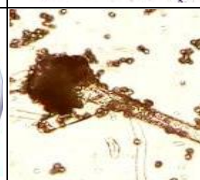
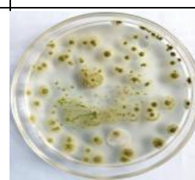
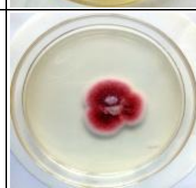
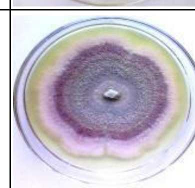
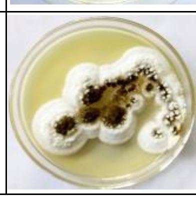
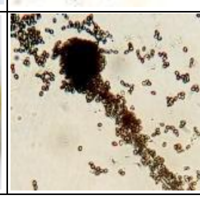
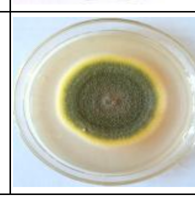
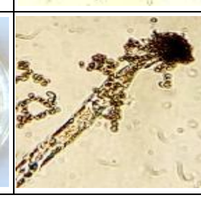
No	Identification	Mycelial morphology & colour	Spore morphology (X400 magnification)	No	Identification	Mycelial morphology & colour	Spore morphology (X400 magnification)
13	***Aspergillus flavipes*** ***(*****Kebeish & El-Sayed, 2012*****)***			18	***Aspergillus parasiticus*** **(Al-Hmoud *et al*., 2012)**		
14	***Aspergillus tamarii*** **(Jayshree & Seema, 2018)**			19	***Aspergillus oryzae*** **(Mahmoud & Zohri, 2021*****)***		
15	**MCHB2 (Unidentified)**			20	***Penicillium*** **species (MCSW3) (Suhaib *et al*., 2011)**		
16	**MCRN1 (Unidentified)**			21	***Phialemoniopsis endophytica*** **(Su *et al*., 2016)**		
17	***Aspergillus species*** **(MSE2) (Asan, 2003)**			22	***Aspergillus*** **species (MCSW1) (Pitt & Hocking, 2022)**		

Figure 8: Environmental specific diverse fungal isolates (Cont')

Table 3: Detection frequency of region-specific isolates

Fungal isolates' identification	WESTERN KENYA COUNTIES												Isolation/Detection Frequency
	Busia			Siaya			Homa Bay			Migori			
	M	G	S	M	G	S	M	G	S	M	G	S	
MMT3 (Unidentified)	+	-	-	-	-	-	-	-	-	-	-	-	1
Aspergillus terreus (MCBT6)	-	-	+	-	-	-	-	-	-	-	-	-	1
Aspergillus tubingensis	-	-	+	-	-	-	-	-	-	-	-	-	1
Aspergillus Candidus	-	-	+	-	-	-	-	-	-	-	-	-	1
Monascus species	-	-	+	-	-	-	-	-	-	-	-	-	1
MCMT4a (Unidentified)	-	-	+	-	-	-	-	-	-	-	-	-	1
MCMT3 (Unidentified)	-	-	+	-	-	-	-	-	-	-	-	-	1
MCMBT3 (Unidentified)	-	-	+	-	-	-	-	-	-	-	-	-	1
Athrinium sacchari	-	-	-	-	-	+	-	-	-	-	-	-	1
Coniothyrium olivaceum	-	-	-	-	-	+	-	-	-	-	-	-	1
Epichloe species	-	-	-	-	-	+	-	-	-	-	-	-	1
Aspergillus terreus (MGW1)	-	-	-	-	-	-	+	-	-	-	-	-	1
Aspergillus flavipes	-	-	-	-	-	-	+	-	-	-	-	-	1
Aspergillus tamarii	-	-	-	-	-	-	-	+	-	-	-	-	1
MCHB2 (Unidentified)	-	-	-	-	-	-	-	-	+	-	-	-	1
MCRN1 (Unidentified)	-	-	-	-	-	-	-	-	+	-	-	-	1
Aspergillus species (MSE2)	-	-	-	-	-	-	-	-	-	+	+	+	3
Aspergillus parasiticus	-	-	-	-	-	-	-	-	-	+	-	-	1
Aspergillus oryzae	-	-	-	-	-	-	-	-	-	-	-	+	1
Penicillium species (MCSW3)	-	-	-	-	-	-	-	-	-	-	-	+	1
Phialemoniopsis endophytica	-	-	-	-	-	-	-	-	-	-	-	+	1
Aspergillus species (MCSW1)	-	-	-	-	-	-	-	-	-	-	-	+	1
Number of fungi per sample	**1**	**0**	**7**	**0**	**0**	**3**	**2**	**1**	**2**	**2**	**1**	**5**	
Number of fungi per region (County)	8			3			5			8			**24**

Key

M = Maize grain
G = Groundnut grain
S = Soil sample

4.5 Colony Forming Units (CFU) of soil fungi in Western Kenya

Fungal colony assessments revealed non-significant differences of CFUg^{-1} among the four study regions (Busia, Siaya, Homa Bay and Migori) (Figure 9). However, variations were observed between CFUs where Homa Bay recorded the highest differences. The highest variations occurred in Siaya which had a mean of over 17 CFUg-^{1}. Also, for sample spreading (whiskers), Busia County had the highest tally of CFUg^{-1}, that is, over 30 CFUg^{-1}and Homa Bay recording the least (<5 CFUg^{-1}) (Figure 9).

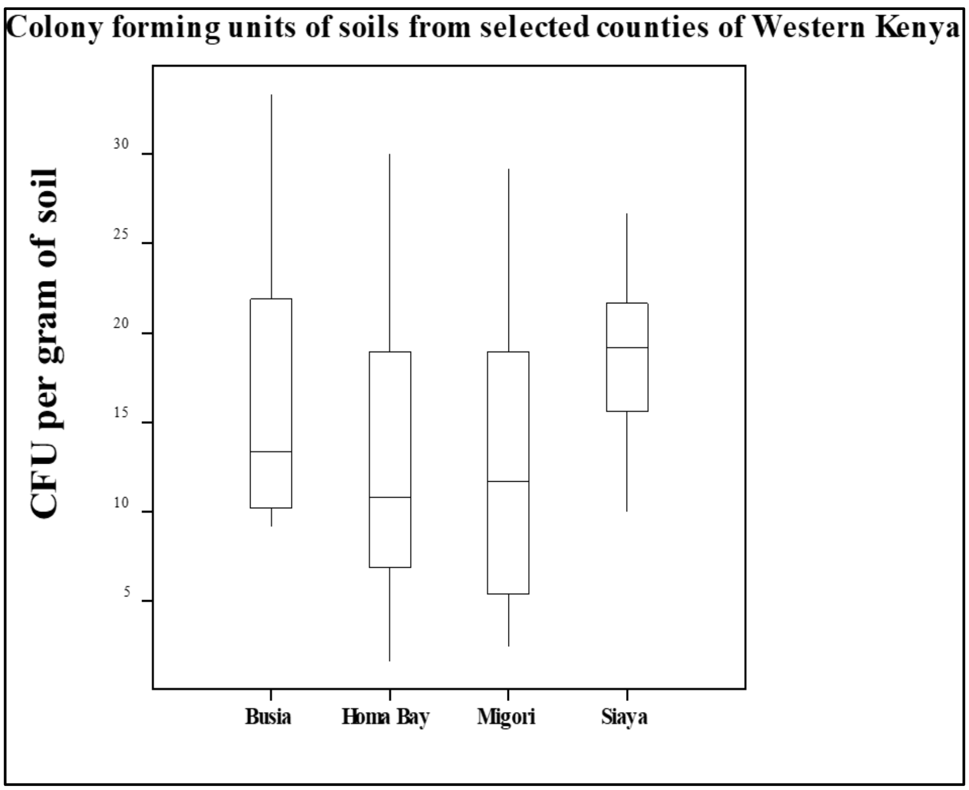


Figure 9: CFUg^{-1}of soil inhabiting fungal isolates

4.6 *In-Vitro* Inhibitory Indices against Mycotoxins from Western Kenya

4.6.1 Percentage inhibition of mycotoxin-producing fungi

The nine antagonistic fungal isolates performed uniquely against the major AF-producing fungal species. *T. harzianum* exhibited the best inhibition levels (> 60%) *in-vitro* using the dual culture method against *A. flavus, A. parasiticus, A. niger, P. corrylophylum* and *P. auratiogriseum*. Uind the same technique *A. nomius* exhibited less inhibition when grown against *T. harzianum* but was best supressed by MCMT4b. In addition to *A. nomius*, isolate MCMT4b expressed parallel inhibition levels (>40%) against *P. auratiogriseum*. Like *T. harzianum* and MCMT4b, *Monascus* species and isolate MCMT3 significantly repressed all the mycotoxin fungi assessed (Table 4).

[1]Adjustment of the dual culture method surfaced distinct and increased supression for *T. harzianum* against the tested mycotoxin fungi to over 80% inhibitory levels (Table 5). In contrast to the dual culture method the rest antagonistic fungi lower (< 40%) inhibition indices against mycotoxin fungi *vitro* conditions.[2]

Table 4: Percentage inhibition of toxigenic fungi *in-vitro* using dual (1:1) culture

Antagonist Fungi	*A. flavus*	*A. Parasiticus*	*A. nomius*	*A. niger*	*P. corrylophilum*	*P. auratiogriseum*
Isolate MCMT3	5.34a	49.49c	20.08a	23.47b	12.03a	3.15a
Isolate MCMT4b	4.27a	30.87b	53.56c	6.34a	31.28b	54.33c
Monascus sp.	15.26b	14.86a	34.81b	19.89b	8.21a	14.99b
T. harzianum	58.31c	88.55d	31.17b	79.01c	87.21c	66.94d
MEAN	20.8	45.94	34.91	32.18	34.68	34.85
Probability	<.001	<.001	<.001	<.001	<.001	<.001
S.E	2.023	2.872	3.171	1.987	3.43	1.841
S.E.D	1.652	2.345	2.589	1.622	2.8	1.503
% CV	9.7	6.3	9.1	6.2	9.9	5.3

[1] Percentage inhibitions with different letters are statistically different at 5% significance level
[2] Percentage inhibitions with different letters statistically differ at 95% confidence interval

Table 5: Percentage inhibition of toxigenic fungi *in-vitro* using the modified (4:1) technique

Antagonist Fungi	*A. flavus*	*A. Parasiticus*	*A. nomius*	*A. niger*	*P. corrylophilum*	*P. auratiogriseum*
Isolate MCMT3	23.49b	23.12b	11.45a	28.76a	14.74a	5.02a
Isolate MCMT4b	6.77a	4.91a	16.95b	31.64b	21.63b	47.94c
Monascus sp.	38.32c	25.32b	33.76c	47.93c	41.22c	11.20b
T. harzianum	80.42d	88.55c	87.19d	87.24d	87.21d	87.42d
MEAN	37.25	35.48	37.34	48.89	41.2	37.9
Probability	<.001	<.001	<.001	<.001	<.001	<.001
S.E	1.374	1.375	1.482	1.408	2.997	1.975
S.E.D	1.122	1.123	1.21	1.15	2.447	1.613
% CV	3.7	3.9	4	2.9	7.3	5.2

4.6.2 Inhibition of mycotoxin-producing fungi in-vitro using the 1-5 rating scale

According to the inhibition scale the best repression levels against mycotoxin fungi were observed in *T. harzianum,* MCMT3, and *Monascus* species had. In particular, *T. harzianum* best supressed mycotxin fungi to < 2.5. In contrast, notable differences were not observed among *T. harzianum, Monascus* species and MCMT3 against *P. auratiogriseum*. Additionally, isolate MCMT4b expressed significant positive antagonistic levels against *A. Parasiticus, P. corrylophylum,* and *P. auratiogriseum*. All mycotoxin fungi were not positively supressed by *Biastrospora* species, *C. olivaceum, Epichloe* species, MCHB2, and *P. endophytica* (Table 6).

Table 6: Mycotoxin fungi suppression using the inhibition scale (1-5) in dual culture

Antagonist Fungi	*A. flavus*	*A. Parasiticus*	*A. nomius*	*A. niger*	*P. corrylophilum*	*P. auratiogriseum*
***Biatriospora* sp.**	4.133de	5e	4.6d	5f	4.333e	5e
C. olivaceum	3.7d	4.3d	4.367d	3.867d	3.9d	4.433d
***Epichloe* sp.**	4.333e	4.767e	4.733d	4.8ef	4.6ef	3.933c
Isolate MCMT3	2.5b	2.667b	3.267b	2.667b	3.133c	2.367a
Isolate MCMT4b	3.8d	2.9b	3.367bc	3.433c	2.5b	2.867b
Isolate MCHB2	4.967f	3.967d	4.433d	5f	4.867f	4.633de
***Monascus* sp.**	3.133c	3.433c	3.267b	3.867d	3.133c	2.367a
P. endophytica	3.767d	4.7e	4.067cd	4.6e	4.767f	4.6de
T. harzianum	1.367a	1.7a	1.767a	2.133a	1.733a	2.1a
MEAN	3.522	3.715	3.763	3.93	3.663	3.589
Probability	<.001	<.001	<.001	<.001	<.001	<.001
S.E	0.1764	0.0339	0.1302	0.0714	0.14	0.1567
S.E.D	0.238	0.1855	0.3408	0.1356	0.0525	0.1868
% CV	5	0.9	3.5	1.8	1.4	4.4

[3]

4.6.3 Inhibition of mycotoxin-producing fungi-based on zones of inhibition

Positive or successful inhibitions against the examined mycotoxin fungi were exhibited by isolates MCMT3, MCMT4b, *T. harzianum*, *Monascus* species and *P. endophytica* in the dual culture technique (Table 7 and Figure 10). Comparatively, isolates MCMT3, MCMT4b and *Monascus* species best formed ZIs (>1mm) (Table 8). The rest of the antagonistic fungal isolates tested did not exhibit capacity to form inhibition zones .

[3] Percentage inhibitions with different letters statistically differ at 95% confidence interval

Table 7: Inhibition of mycotoxin-producing fungi by ZI width in millimetres using the dual culture

Antagonist Fungi	*A. flavus*	*A. Parasiticus*	*A. nomius*	*A. niger*	*P. corrylophilum*	*P. auratiogriseum*
Isolate MCMT3	3.0a	2.7ab	7.0c	4.0b	3.7b	5.0a
Isolate MCMT4b	29.0c	1.7a	7.0c	6.0c	6.0c	5.7a
***Monascus* sp.**	2.7a	2.3a	2.7a	2.0a	4.3bc	4.7a
T. harzianum	20.0b	6.3c	2.3a	4.7b	1.3a	22.7b
P. endophytica	4.0a	4.0b	5.3b	6.0c	3.3b	3.3a
MEAN	11.73	3.4	4.87	4.53	3.73	8.27
Probability	<.001	<.001	<.001	<.001	0.002	<.001
S.E	1.366	0.816	0.894	0.516	0.931	1.693
S.E.D	1.116	0.667	0.73	0.422	0.76	1.382
% CV	11.6	24	18.4	11.4	24.9	20.5

Table 8: Inhibition of mycotoxin-producing fungi based on the ZI width in millimetres using the modified method

Antagonist Fungi	*A. flavus*	*A. Parasiticus*	*A. nomius*	*A. niger*	*P. corrylophilum*	*P. auratiogriseum*
Isolate MCMT3	2.7a	1.7a	2.7a	2.0a	4.3a	3.7a
Isolate MCMT4b	3.0a	1.7a	6.3b	4.0b	3.0a	4.0a
***Monascus* sp.**	6.0b	3.3b	4.3ab	5.0b	4.0a	11.7b
MEAN	3.89	0.019	4.44	3.67	3.78	6.44
Probability	0.027	2.22	0.023	0.002	0.236	<.001
S.E	1.202	0.577	1.155	0.577	0.882	1.374
S.E.D	0.981	0.471	0.943	0.471	0.72	1.122
% CV	30.9	26	26	15.7	23.3	21.3

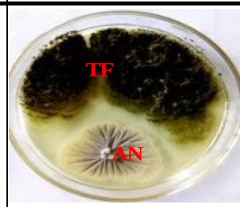
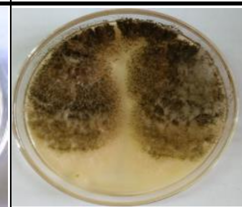
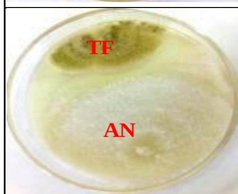
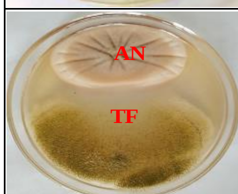
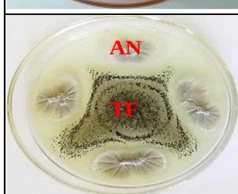
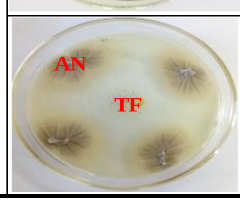
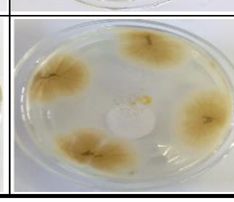
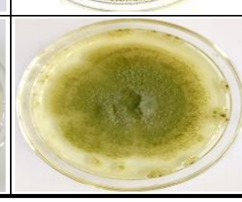
Culture Method	Observe (Mycelial) Surface	Reverse (substrate) surface	Control Experiment (Mycotpxin fungi)	Antagonist (AN) and Test Fungi (TF)
Dual Culture Method				**AN=Isolate MCMT3** **TF = *A. parasiticus***
				**AN=*T. harzianum*** **TF = *A. flavus***
				**AN = Isolate MCMT4b** **TF = *A. flavus***
Modified Culture Method				**AN = *Monascus* species** **TF = *A. parasiticus***
				**AN = Isolate MCMT3** **TF = *A. niger***
				**AN = Isolate MCMT3** **TF = *A. flavus***

Figure 10: An illustration of best performing antagonists *in-vitro using* dual culture and the modified method

4.7 The effect of efficacious biological concoctions on incidences of toxigenic fungi and fungal diversity at post-harvest

4.7.1 Performance of applied bio-controls against mycotoxin fungi

In terms of percentage incidences, there were no notable differences observed in grain inoculated with *Monascus* species, MCMT3, MCMT4b, *T. harzianum* and mixture treatment AF, PAT, PCN, and OTA fungi in Sega and Kibos sites. Lowest incidences (<15%) of mycotoxin fungi were recorded in grains inovulated with *Monascus* species, MCMT3, MCMT4b and mixture treatment. These incidences differed significantly with those of the untreated control in Sega and Kibos sites except incidences recorded in the mixed concoction treatment against PAT & PCN fungi in Sega site (Figure 11).

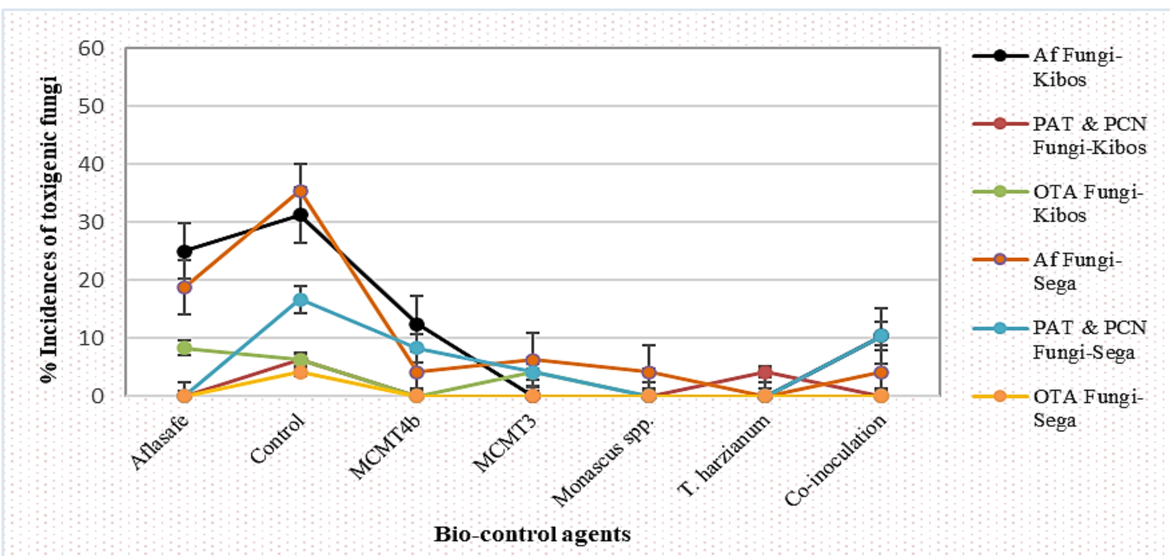


Figure 11: Performance of applied biological concoctions against toxigenic fungi during the long rains season

On the other hand, all treatments namely *Monascus* species, MCMT3, MCMT4b, *T. harzianum* and the mixed concoction exhibited no significant differences against AF, OTA, PAT and PCN-fungi percentage incidences in Sega site. Their performances replicated in Kibos with an esception of MCMT4b and MCMT3 concoctions that did not

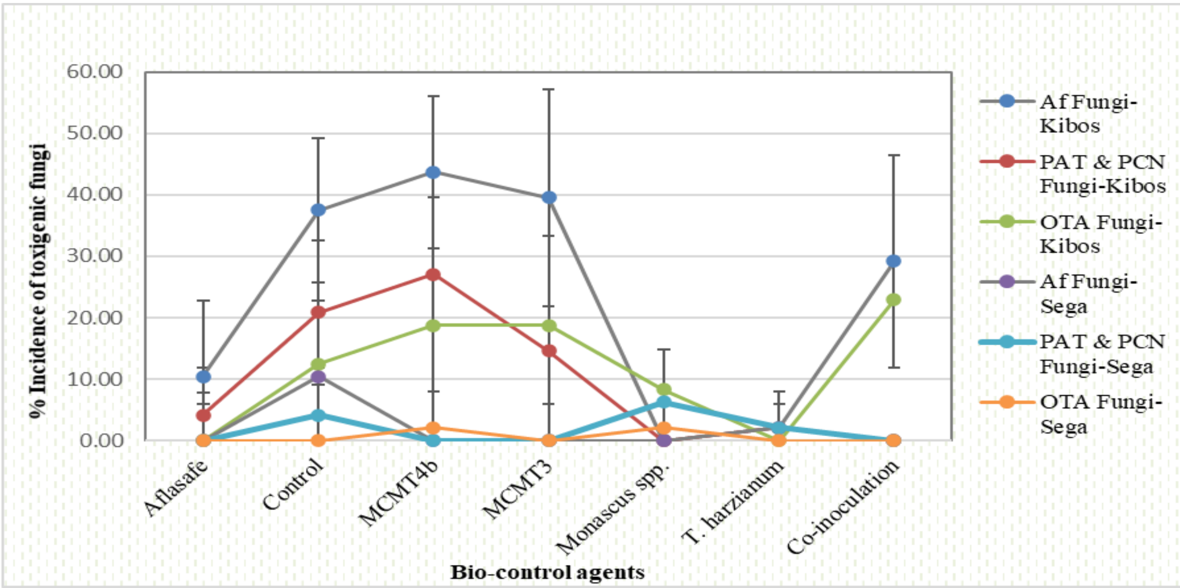


Figure 12: Performance of bio-controls against toxigenic fungal incidences during short rains

exhibit notable differences with AflaSafe KE01™ against all mycotoxin fungi except for OTA fungi (Figure 12). Tellingly, in Kibos, MCTM3 did not outperform the control experiment in repressing percentage incidences of all tested toxigenic fungal isolates (Figure 12).

4.7.2 Effect applied fungal concoctions on diversity of other fungi

In Kibos, there were no significant variations in the effect of *Monascus* species, MCMT3, MCMT4b, *T. harzianum* and the mixed concoction on fungal diversity in both seasons. Similar effects were recorded in Sega with an exception of *Monascus* spp. and *T. harzianum.* These fungal concoctions had the lowest fungal diversity levels. However, they differed notably with MCMT3 and the mixed concoction in the long rains (Figure 13).

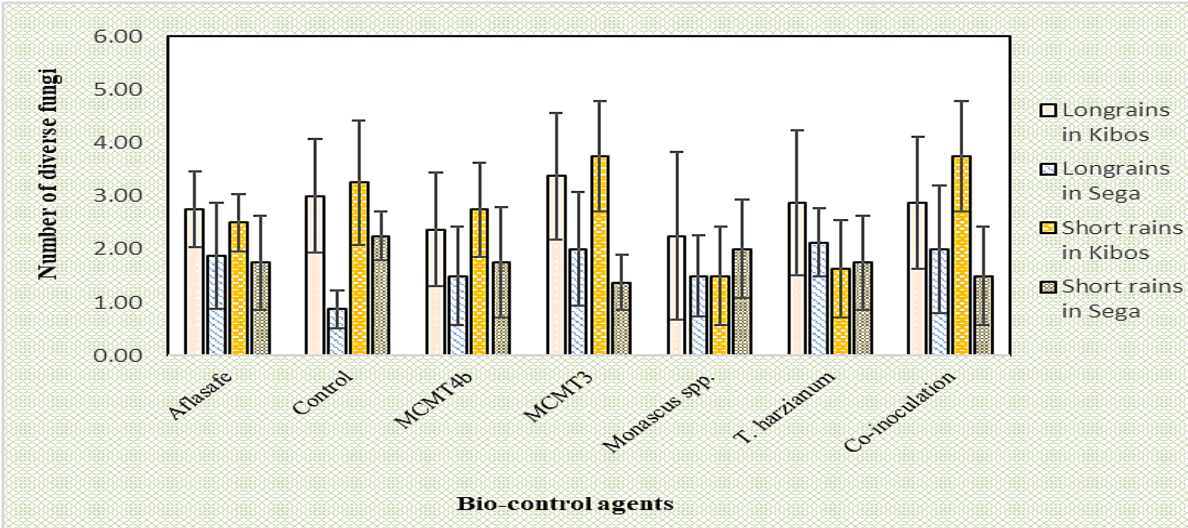


Figure 13: Effect applied fungal concoctions on diversity of other fungi during long and short rains of Western Kenya

4.7.3 Efficacy of the tested BCA's as influenced by maize variety

Maize variety had no notable effect on the effects of the assessed biological concoctions. Their performances had a similar effect in reducing incidences of mycotoxin fungi across the two varieties. In addition to the similar performances, higher incidences of AF fungi in Punda milia than Duma variety were observed (Figure 14). For fungal diversity assessments, the results revealed non-significant variety effects on biological concoctions, performance against fungal diversity (Figure 15).

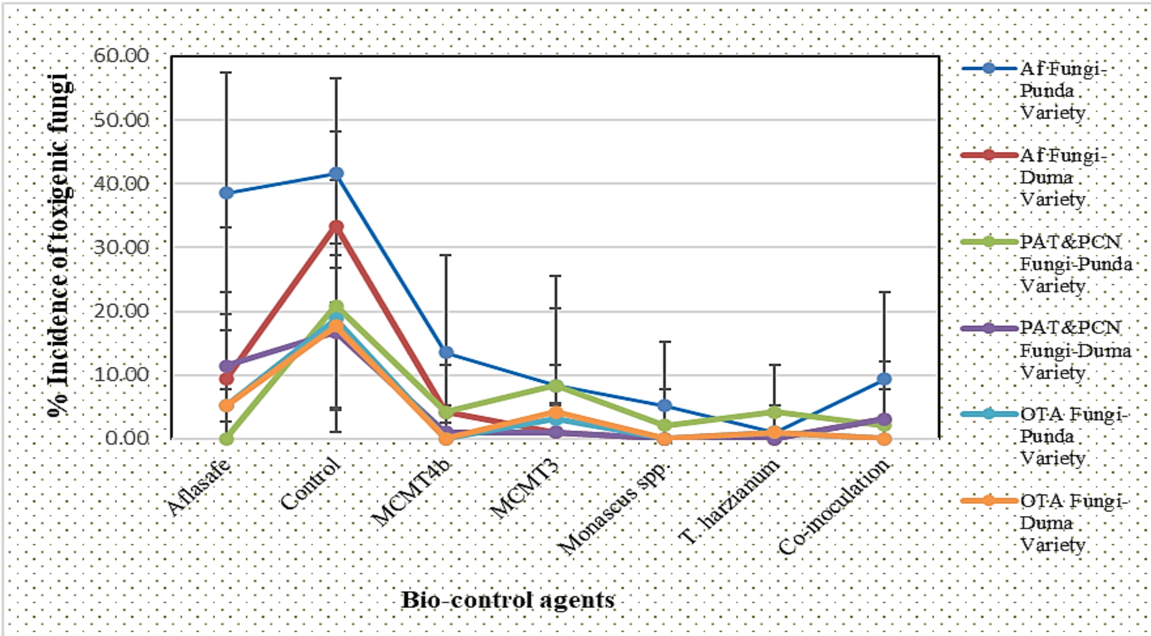


Figure 14: Performance of applied fungal concoctions against toxigenic fungal incidences as affected by maize variety

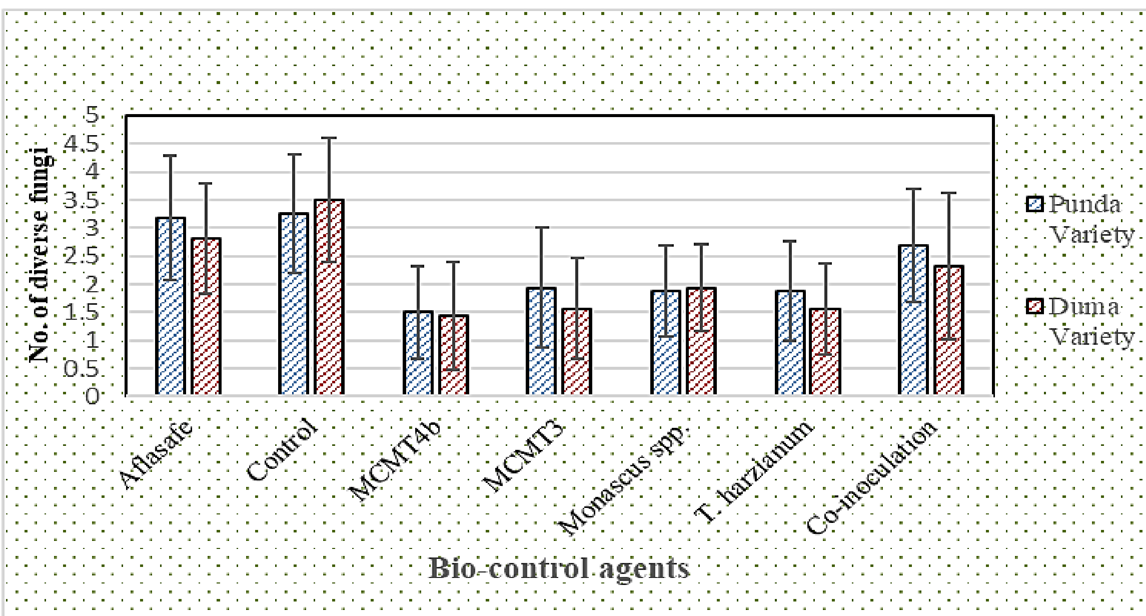


Figure 15: Performance of applied BCAs against fungal diversity across maize varieties

4.7.4 Effects of applied biological concoctions the quality of maize grain

Grain quality in terms of colour also revealed that the applied biological concoctions differed in their performances. The best grain quality was obtained in ear inoculations using *Monascus* spp., MCMT3, MCMT4b, *T. harzianum* and the mixed concoction (Figure 16). Grains harvested from untreated and AflaSafe KE01™ - applied grains/subplots were decoloured and had a yellowish-green appearance with visible toxigenic moulds growing along the longitudinal fissures.

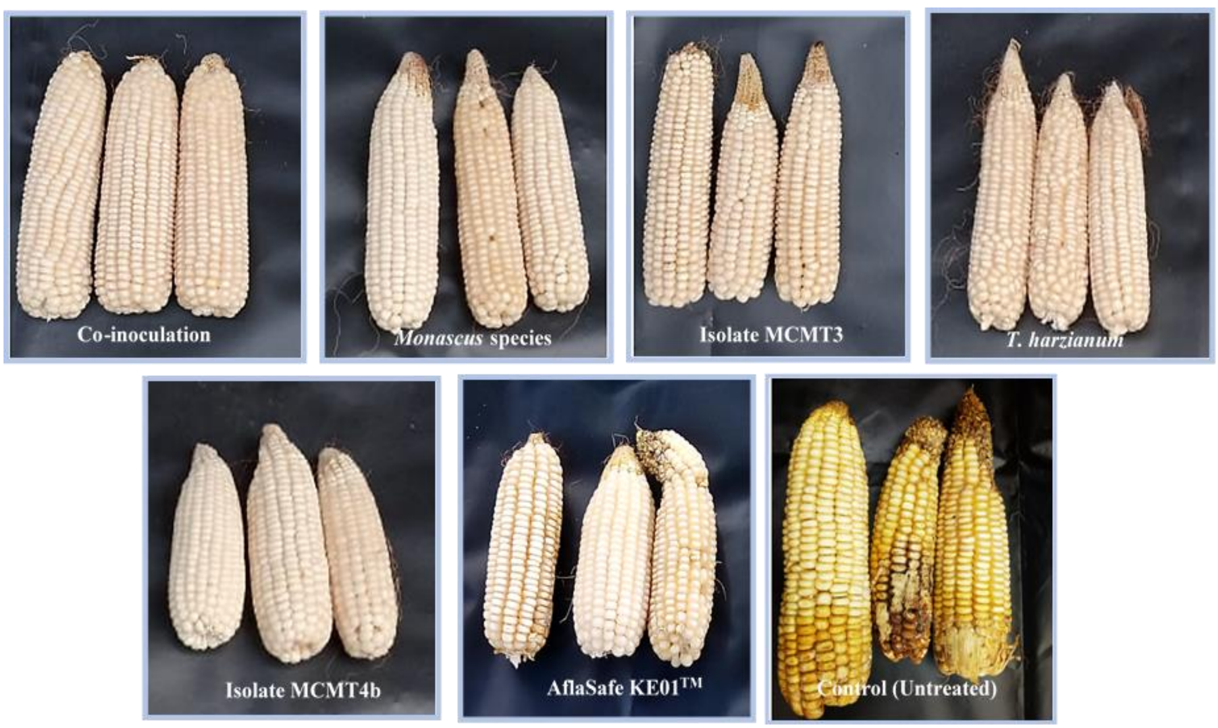


Figure 16: Effects of applied concoctions on grain quality

CHAPTER FIVE

DISCUSSION

5.1 Farmer cognizance and fungal diversity

Respondents from all the study regions expressed insufficient knowledge of the causes and palliation of AFs. This study adds tremendously to mycotoxin literature because it provides a current overview of AF awareness levels in Western Kenya which is undocumented in numerous studies on the topic in Kenya and Africa (Bauchet *et al*., 2021; Kaale *et al*., 2021; Meijer *et al*., 2021; Stepman, 2018). This knowledge is consequential since farmers within the study region are more likely to deliberately apply crop management practices favour mycotoxin accumulation. For example, gunny bags lead to humidity build-up favouring fungal proliferation when put on floors (Bbosa *et al*., 2013; Costa *et al*., 2019; Singh *et al*., 2022). Such practices expose the grains to mechanical damages and pest infestations, favour penetration and multiplication of mycotoxin-producing fungi (Birgen *et al*., 2020; Matumba *et al*., 2021; Niyibituronsa *et al*., 2020). Likewise, farmers may neglect useful techniques like crop rotation that break disease and pest cycles (Fouche, Claassens, & Maboeta, 2020). These consequences imply the central role of knowledge by farmers useful to repress mycotoxin contamination (Mutegi *et al*., 2018; Patel *et al*., 2015). Although useful knowledge is acquired in important platforms such as farmer discussion forums, there is limited participation within the region.

5.2 Fungal diversity and environmental specificity

Key evaluations on fungal diversity exhibited a wide-ranging genetic bank of fungal species inhabiting soils and grains of Western Kenya. This study confirms that mycotoxin accumulation is still a major threat to Kenya's food security. It is because the most frequently isolated fungi were species of major genera that generate mycotoxins, that is, *Aspergillus*, *Penicillium*, and *Fusarium* (Martínez-Culebras *et al*., 2021).

The vast diversity revealed in mycotoxin and virulent species depicts their profusion and genetic potential to have widespread distribution by evolving through the swapping of genes (Ropars *et al*., 2014; Were *et al*., 2015). For instance, *A. flavus* was highest ranked fungal isolate in frequency but was also recorded in two diverse pathotypes suggesting its evolutionary potential. This occurrence which confirms its regional universality shows that mycotoxin adulteration will persist in Western Kenya for several years if left unattended (Gupta *et al*., 2022; Brauer *et al*., 2020; Njoroge, 2018).

While elevated diversity levels of mycotoxin fungi could be a looming management risk, they also provide a gateway to the innovation of efficacious biological control technologies such as the use of non-toxigenic strains. Besides variations within a single genus, differences between genera were the highest hence a high likelihood identifying several other BCAs to manage mycotoxin occurrence in grains and soils. As an illustration, since *A. flavus* (MUG5) was isolated from both maize and soils it indicates that this fungus a seed borne and a soil pathogen. Therefore, the best management practice for this pathogen is that which would manage its buildup in the soil atmosphere and on grain.

Additional analysis on diversity discloses that most of fungi were derived from the soils (Table 2 & Table 3). By assessing the study counties, the diversity extent of the species among soil samples correlated with the diversity degrees recorded among maize and groundnut samples (Table 1). For instance, most fungal isolates were detected in Busia County samples with mycotoxin fungi registering the highest frequencies. The biology and reproduction of these species could be an important link in understanding this occurrence. As an illustration, *Aspergillus* and *Penicillium* species generate several air-borne spores thus spread directly from soils to seeds within production fields (Houbraken *et al*., 2020; Houbraken, de Vries & Samson, 2014).

In addition to soils, more fungal isolates were isolated from maize grains than in groundnuts. These findings could be associated to their specificity to pathogen and/or race since the division *Forma specialis* of these toxigenic fungal species into diverse races justifies the occurrence where fungi with similar morphologies may have different virulence levels on hosts (Jayawardena *et al*., 2021). Nonetheless, this research cannot entirely discover this occurrence since molecular idnetifcation of the fungal isolates lacked. Additionally, the grains examined were sampled from the research area; thus data generalization is restrained by sampling limitations.

5.3 Implications in performances of beneficial fungi in-vitro

The findings of *in-vitro* assays against toxigenic fungi disclosed that Isolate MCMT3, Isolate MCMT4b, and *Monascus* species significantly suppressed growth of all the examined mycotoxin fungi. The formation of notable ZIs against the test/mycotoxin fungal isolates, these antagonists revealed their capabilities to produce repressive chemicals against fungal proliferation (Lass-Flori, Perkhofer & Mayr, 2010). Suppression levels varied as evidenced by differences in ZIs which could be subject to differences in forms and quantities of antifungal compounds generated. Also, it could be caused by differences in their main suppression criteria such as antibiosis, competitive exclusion, mycoparasitism and hyperparasitism (Abdallah *et al*., 2018). Therefore, additional research in confirming action modes by each fungal isolate from the study region is critical.

Monascus species portrayed unique antagonism mechanism where the isolate produced red pigments when tested against *in-vitro*. These occurrences on pigment production were parallel Liu *et al*., (2018) study findings. Other researchers have documented the generation of red and orange stains generated and have documented their application in food processing using these pigments. For instance, Kim *et al*., (2006) uncovered that *Monascus* species colorants with L-and D- forms of amino acids had antimicrobial effects against *A. niger* and *Penicillium citrinum*. Also, these species are extensively utilized in the pharmaceutical field (Agboyibor *et al*., 2018). They are applied as detoxifying agents against AFs, PAT, PCN and OTA. However, they are underutilized despite their high potential as observed in *in-vitro* studies.

In contrast, fungal isolates MCMT3 and MCMT4 portrayed repressive effects against mycotoxin fungal isolates. Although, their conclusive identification lacked, they exhibited leutiomycete features. For example, when grown on PDA they had undulate edges, umbonate elevations with irregular surfaces. The two fungal isolates had irregular fissures on surfaces on media; their mycelial colour ranged from pewter to tan and was bordered by white borders on the observe surface. The spores were enclosed in whole bitunicate asci, a shared characteristic by leutiomycetes of ascomycota (Ekanayaka *et al*., 2019; Schoch *et al.*, 2009). These fungi established clear ZIs against all tested mycotoxin fungi hence capable of metabolizing lytic and secondary antifungal substances.

5.4 Inferences on performances of non-toxigenic fungi in the field

Variations in percentage incidences of mycotoxin fungi at post-harvest imply that *Monascus* spp, MCMT3, MCMT4b, *T. harzianum* and the mixed concoction suppressed proliferation of mycotoxin fungi in the field. The results on deterioration of grain quality verify these variations. Inter-seasonal performances incidence data of toxigenic fungi revealed that the short rains had more mycotoxin fungi than the long rains period except for AF producing where AflaSafe KE01^{TM} was applied in Kibos. These seasonal variations are a function of weather fluctuations. According to Krnjaja *et al*., (2019) crop stand and weather variations influence incidences of *Aspergillus* and *Penicillium* species in maize production zones. There are high possibilities that the weather and plant density variations beyond the study area might have influenced such variations. For incidence data obtained in AflaSafe KE01^{TM} maize cannot be fully ascertained because non-toxigenic *Aspergillus* strains present in the product were not distinguished from their toxigenic relatives. This signals an important research gap where future research must target genetic characterization of isolated fungi to achieve accuracy.

There no distinct varietal effects on the performance of applied bio-controls against mycotoxin fungi. However, incidence data revealed Punda milia was more susceptible variety than Duma. Similar trends were observed by Krnjaja *et al*., (2019) where varietal susceptibility to mycotoxin fungi were faintly displayed. According to Blandino *et al*. (2017), maize varieties are variably susceptible to ear rot. These differences translate to variations in varietal variations in their susceptibility to mycotoxin fungal infections. The 14-kDa maize trypsin inhibitor determines the susceptibility or resistance levels by the maize host plant (Soni *et al*., 2020). The protein obstructs the typical growth patterns of

A. flavus through instigation of conidial disnitegration and abnormal expansion of the hyphal structures (Pechanova & Pechan, 2015; Chen *et al*., 2016).

The PR-10 protein also assists in crop resistance development. It is because when the *pr*-10 genes are silenced, maize grains become more sensitive to heat stress which is an important gateway to fungal infections (Dhakal *et al*., 2017). The rachis also determines maize susceptibility to mycotoxin fungal infections because *A. flavus* uses it as a pathway to grow and reach the ears (Pechanova *et al*., 2010). Parallel to these findings, Jeremy and Tibor (2021) affirmed repression of *A. flavus* multiplication by the rachis. Therefore, rachis formation and developmental variations in maize varieties are critical determinants of their resistance to mycotoxin buildup in the field.

Performances of the mixed concoction in repressing mycotoxin fungal incidences revealed no synergistic effects of the combined fungi. The combination of isolates MCMT3, MCMT4b and *Monascus* spp. did not outperform the individual isolates in suppressing mycotoxin fungal incidences. These results infer possible presence of antagonistic forces between the combined isolates. Xu *et al*. (2011) recorded comparable observations and asserted that antagonism is a more likely phenomenon than synergism when bio-control agents are applied simultaneously. For this research, the lives organisms were used posing a high likelihood of competitive elimination and antibiosis processes. The co-inoculation of these fungal isolates to suppress mycotoxin fungi is an important research gap. While the evaluation of fungal diversity in maize disclosed trivial variations, more accurate strategies are needed. According to Thambugala *et al*., (2020), molecular methods are vital in determining the unprecedented effects of fungal bio-controls and in tracking their pathways. These techniques will assist in determining appropriate approaches of leveraging BCA's antifungal properties against mycotoxins.

CHAPTER SIX

CONCLUSIONS, RECOMMENDATIONS, AND WAY FORWARD

6.1 Conclusions

1. Farmers in Western Kenya were unaware of AF causes and best pre and post-harvest mitigation practices. Busia County had more diverse fungal isolates than Siaya, Homa Bay, and Migori Counties. Mycotoxin-producing *Aspergillus*, *Penicillium*, and *Fusarium* species dominated maize, groundnut, and soils of Busia, Siaya, Homa Bay, and Migori counties of Western Kenya.
2. Isolate MCMT3, Isolate MCMT4b, *Monascus* species, and *T. harzianum* significantly supressed *A. flavus, A. parasiticus, A. niger, P. corrylophylum,* and *P. auratiogriseum in-vitro.* Also, isolates MCMT3, MCMT4b, and *Monascus* species formed clear inhibition zones in *in-vitro* assays against *A. flavus*, *A. parasiticus, A. niger, P. corrylophylum,* and *P. auratiogriseum*.
3. *Monascus* species, MCMT3, MCMT4b, *T. harzianum* and the mixed concoction of *Monascus* species, MCMT3 and MCMT4b, significantly supressed incidences *A. flavus, A. parasiticus, A. nomius, A. niger, P. corrylophilum,* and *P. auratiogriseum* in field conditions. Individual and mixed combinations of MCMT3, MCMT4b, and *Monascus* species did not significant vary in repressing *A. flavus*, *A. nomius, A. parasiticus*, *A. niger, P. corrylophilum,* and *P. auratiogriseum* incidences in the field. Punda milia and Duma maize varieties did not significantly affect the performance of the assessed fungal concoctions.

6.2 Recommendations

1. There is a need for capacity building on efficient pre-harvest and post-harvest mycotoxin management strategies in Western Kenya.
2. Further studies and mass production of isolates MCMT3, MCMT4b and *Monascus* species, and their antifungal metabolites should be prioritized.
3. Isolates MCMT3, MCMT4b and *Monascus* species should be used as effective bio-controls against mycotoxins in maize and other susceptible hosts.

6.3 Way Forward

Extensive evaluation of grains and soils in Western Kenya should be the primary goal of future research. This study has revealed a high likelihood of finding some of the best BCAs against mycotoxin fungi in this region. Scientific efforts should be focused on formulating effective biological control products to manage mycotoxins in Western Kenya either as complimentaries or alternatives to Aflasafe KE01^{TM}.

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APPENDICES

APPENDIX I: Farmer questionnaire on maize and ground nut farming and mycotoxin knowledge during the 2018-2019 crop seasons.

SECTION A: FARMER IDENTIFICATION

Name of the farmer	
County	
Sub- County	
Altitude	
Longitude	
Maize sample no.	
Groundnut sample no.	
Monocrop soil sample no.	
Mixed cropping soil sample no.	

SECTION B: DEMOGRAPHIC INFORMATION (Mark (√)Appropriate Box)

1. Age in completed years……………………………………….. Sex: Male ☐ Female ☐
2. Education level: None ☐ Primary ☐ Secondary ☐ Above secondary ☐
3. Main source of income: Farming ☐ Salaried ☐ Self- employed ☐
   others (specify)………………………………………………………………
4. Do you have any forum for discussing Maize/ Groundnut/farming issues? Yes
   No
5. If Yes, name it……………………………………………………………………………

SECTION C: AGRONOMIC INFORMATION

1. How long have you been growing Maize/ Groundnut?

   Maize…….(yrs) Groundnut………(yrs)

2. How many acres of land do you own…………………………………………………………..
3. Name other crops grown on your farm………………………………………………………….

4. Rank the crops according to their profitability to you starting with the most profitable

Crop	Rank (with 1 most profitable)	Area planted this season
I		
II		
III		
IV		

5. Do you intercrop maize and groundnut?...............[Yes=1; No=2]
6. Which crops were cultivated previous season on the current land area groundnut?
   i. ………………………………
   ii. ………………………………
   iii. ………………………………
7. Where do you source your planting seeds?

   [Own=1; Neighbor=2; Local market=3; Commercial dealer=4;

   others (specify)……………………………………………………………..…………]

SECTION D: <u>FARMERS' KNOWLEDGE ON CAUSES OF MYCOTOXINS</u>

Cause	Aware [√]	Not Aware [√]
Improper storage		
Improper drying		
Rains at harvesting		
Store dampness		
Elevated air moisture levels		
Infection from soil		
Damage by pests		
Rotting of maize		
Bad seeds		
Applying pesticide on wet cereals		
Delayed harvesting	-	
Untreated cereals		
Do not know		

SECTION E: <u>FARMERS AWARENESS ON MANAGEMENT TECHNIQUES OF MYCOTOXINS</u>

Technique	Aware [√]	Not Aware [√]	Successful [√]	Unsuccessful [√]
1.Crop rotation				
2. Use of resistant varieties				
3. Bio control				
4. Pest control in the farm				
5. Proper drying of grains				
6. Timely harvesting				
7. Proper storage				
8. Others				
9. Not aware of any management				

APPENDIX II: ANOVA summaries for percentage inhibition of mycotoxin fungi using dual culture technique

Analysis of variance **Variate: *Aspergillus flavus***					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	5851.507	1950.502	476.45	<.001
Residual	8	32.750	4.094		
Total	11	5884.258			
Variate: *A. parasiticus*					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	9062.973	3020.991	366.27	<.001
Residual	8	65.984	8.248		
Total	11	9128.957			
Variate: *A. nomius*					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	1745.47	581.82	57.86	<.001
Residual	8	80.45	10.06		
Total	11	1825.92			
Variate: *A. niger*					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	9264.933	3088.311	782.10	<.001
Residual	8	31.590	3.949		
Total	11	9296.523			
Variate: *P. corrylophilum*					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	11956.29	3985.43	338.83	<.001
Residual	8	94.10	11.76		
Total	11	12050.39			
Variate: *P. auratiogriseum*					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	8426.404	2808.801	828.61	<.001
Residual	8	27.118	3.390		
Total	11	8453.523			

APPENDIX III: ANOVA summaries on percentage inhibition in-vitro of test/mycotoxin fungal isolates by applying the modified culture technique

Analysis of variance **Variate: *Aspergillus flavus***					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	8949.555	2983.185	1579.47	<.001
Residual	8	15.110	1.889		
Total	11	8964.665			
Variate: *A. parasiticus*					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	12019.757	4006.586	2117.75	<.001
Residual	8	15.135	1.892		
Total	11	12034.892			
Variate: *A. nomius*					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	10749.950	3583.317	1631.28	<.001
Residual	8	17.573	2.197		
Total	11	10767.523			
Variate: *A. niger*					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	6524.078	2174.693	1096.67	<.001
Residual	8	15.864	1.983		
Total	11	6539.942			
Variate: *P. corrylophilum*					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	9601.598	3200.533	356.39	<.001
Residual	8	71.843	8.980		
Total	11	9673.441			
Variate: *P. auratiogriseum*					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	13040.499	4346.833	1114.50	<.001
Residual	8	31.202	3.900		
Total	11	13071.701			

APPENDIX IV: ANOVA summaries on mycotoxin fungal repression using a 1-5 scale

Analysis of variance **Variate: *Aspergillus flavus***					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	27.38667	3.42333	40.27	<.001
Residual	8	1.36	0.085		
Total	11	29.30667			
Variate: *A. parasiticus*					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	30.10741	3.76343	72.91	<.001
Residual	8	0.82593	0.05162		
Total	11	30.95407			
Variate: *A. nomius*					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	21.5496	2.6937	15.46	<.001
Residual	8	2.7881	0.1743		
Total	11	24.643			
Variate: *A. niger*					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	25.72296	3.21537	116.53	<.001
Residual	8	0.44148	0.02759		
Total	11	26.2563			
Variate: *P. corrylophilum*					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	29.06296	3.63287	123.57	<.001
Residual	8	0.47037	0.0294		
Total	11	29.58296			
Variate: *P. auratiogriseum*					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	3	31.98667	3.99833	76.36	<.001
Residual	8	0.83778	0.05236		
Total	11	33.26667			

APPENDIX V: ANOVA tables for Inhibition of mycotoxin fungi based on zones of inhibitions in millimeters using the dual culture method

Analysis of variance **Variate:** ***Aspergillus flavus***					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	4	1754.267	438.567	234.95	<.001
Residual	10	18.667	1.867		
Total	14	1772.933			
Variate: ***A. parasiticus***					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	4	40.9333	10.2333	15.35	<.001
Residual	10	6.6667	0.6667		
Total	14	47.6000			
Variate: ***A. nomius***					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	4	61.7333	15.4333	19.29	<.001
Residual	10	8.0000	0.8000		
Total	14	69.7333			
Variate: ***A. niger***					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	4	33.0667	8.2667	31.00	<.001
Residual	10	2.6667	0.2667		
Total	14	35.7333			
Variate: ***P. corrylophilum***					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	4	34.2667	8.5667	9.88	0.002
Residual	10	8.6667	0.8667		
Total	14	42.9333			
Variate: ***P. auratiogriseum***					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	4	786.267	196.567	68.57	<.001
Residual	10	28.667	2.867		
Total	14	814.933			

APPENDIX VI: ANOVA tables for Inhibition of mycotoxin fungi based on zones of inhibitions in millimeters using the modified method

Analysis of variance **Variate:** ***Aspergillus flavus***					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	2	20.222	10.111	7.00	0.027
Residual	6	8.667	1.444		
Total	8	28.889			
Variate: ***A. parasiticus***					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	2	5.5556	2.7778	8.33	0.019
Residual	6	2.0000	0.3333		
Total	8	7.5556			
Variate: ***A. nomius***					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	2	20.222	10.111	7.58	0.023
Residual	6	8.0000	1.333		
Total	8	28.222			
Variate: ***A. niger***					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	2	14.0000	7.0000	21.00	0.002
Residual	6	2.0000	0.3333		
Total	8	16.0000			
Variate: ***P. corrylophilum***					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	2	2.8889	1.4444	1.86	0.236
Residual	6	4.6667	0.7778		
Total	8	7.5556			
Variate: ***P. auratiogriseum***					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antagonist	2	122.889	61.444	2.53	<.001
Residual	6	11.333	1.889		
Total	8	134.222			

APPENDIX VII: Similarity Report

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Certificate of Plagiarism Check for Thesis

Author Name	EDWARD MWAKICHWA MWATABU SAGR/SCH/ M/005/19
Course of Study	Type here...
Name of Guide	Type here...
Department	Type here...
Acceptable Maximum Limit	Type here...
Submitted By	titustoo@uoeld.ac.ke
Paper Title	DIVERSITY AND BIO-CONTROL OF MYCOTOXIN FUNGI FROM MAIZE, GROUNDNUTS AND SOILS OF WESTERN KENYA
Similarity	9%
Paper ID	2093677
Submission Date	2024-07-09 10:51:00

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