GENETIC DIVERSITY OF MYCOTOXIGENIC MOLDS ASSOCIATED WITH CEREALS IN KARAMOJA SUB-REGION, UGANDA

BY

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A DISSERTATION REPORT SUBMITTED TO THE DIRECTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN MOLECULAR BIOLOGY AND BIOTECHNOLOGY OF GULU UNIVERSITY

AUGUST, 2026

DECLARATION

I, Odongo Solomon Otim, hereby declare that this research paper is my original work and that I have acknowledged all the sources that I have used in the paper. I have not copied, plagiarized, or fabricated any part of this paper from any other source. I have followed the ethical principles and guidelines of Gulu University in conducting this research. I also declare that this paper has not been submitted for any other degree or qualification at this or any other institution.

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APPROVAL

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ACRONYMS/ ABBREVIATION

AICc	Akaike Information Criterion, corrected
BLAST	Basic Local Alignment Search Tool
CTAB	Cetyltrimethylammonium Bromide
EF	Elongation Factor
FAO	Food and Agriculture Organization
ITS	Internal Transcribed Spacer
NDPIV	Fourth National Development Plan
PCR	Polymerase Chain Reaction
PDA	Potatoes Dextrose Agar
PDB	Potatoes Dextrose Broth
SQUEAC	Simplified Lqas Evaluation of Access and Coverage and Semi-Quantitative Evaluation of Access and Coverage
UNICEF	United Nation Children Emergency Fund
WFP	World Food Programme
WHO	World Health Organization

ABSTRACT

Mycotoxin contamination in cereals severely threatens food safety, community health, and economic security in sub-Saharan Africa, with the semi-arid Karamoja sub-region of Uganda being particularly vulnerable due to its climate and high rates of malnutrition. This research was mainly to determine the genetic diversity of mycotoxigenic fungal species in staple cereals maize, sorghum, and millet across Karamoja to understand the underlying drivers of contamination. A cross-sectional survey of 108 grain samples from six districts revealed an alarming contamination prevalence of 94%, with only 6% of samples being mold-free. Maize was the most susceptible crop (33% of contaminated samples), followed by sorghum (31%) and millet (28%). Contamination was pervasive across all districts, with Kotido and Karenga exhibiting 100% prevalence. Molecular identification, utilizing PCR amplification of the gene EF-1α and Sanger sequencing, identified *Aspergillus* species as the predominant genus, constituting over 70% of isolates. *A. flavus* and *A. niger* contributed the highest proportion of species, with *A. oryzae* also being prevalent. *Fusarium* species accounted for 22% of isolates, while *Penicillium* and *Alternaria* were minor constituents. Phylogenetic analysis demonstrated a great genetic similarity within the *A. flavus* and other isolates, indicating a homogenous population capable of colonizing all three cereal types regardless of the district of origin. The study concludes that cereals in Karamoja are extensively contaminated with genetically stable, potentially mycotoxigenic fungi, with maize bearing the highest burden. These findings underscore an urgent need for integrated pre and post-harvest management strategies, targeted farmer education, and enhanced food safety monitoring to mitigate mycotoxin exposure, improve food security, and safeguard the health of the Karamoja population.

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CHAPTER ONE

1.0 INTRODUCTION

1.1. Background

The high diversity of molds significantly influences the occurrence and contamination level of mycotoxins in crop produce. Genetic diversity refers to the variation in genetic composition among individuals within and between populations of the same species. In fungi, genetic diversity arises through mutation, recombination, gene flow, and adaptation to different ecological conditions, resulting in populations with varying biological characteristics and ecological fitness (McDonald and Linde, 2002; Taylor *et al*., 1999). Mycotoxigenic molds exhibit considerable adaptability, allowing them to successfully colonize various crops and persist in different environments (Moretti *et al*., 2013).

In mycotoxigenic fungi, genetic diversity influences ecological adaptation, host colonization, pathogenicity, and the ability to produce mycotoxins. Differences in genes involved in mycotoxin biosynthesis and regulation may result in variations in toxigenic potential among strains of the same species, leading to differences in contamination levels among crops and environments (Geiser *et al*., 2000; Ehrlich, 2014). Consequently, understanding the genetic diversity of mycotoxigenic fungi is important for tracking fungal populations, predicting contamination risks, and developing effective strategies for the management of mycotoxin contamination. Prominent mycotoxin-producing molds include *Aspergillus, Fusarium,* and *Penicillium*, which produce a variety of mycotoxins including aflatoxins, sterigmatocystin, fumonisins, ochratoxins, patulin, zearalenone, and citrinin (Reddy *et al*., 2008).

In Uganda, aflatoxins and fumonisins are the predominant mycotoxins contaminating food and feed commodities (DSM World Mycotoxin Survey, 2023). Wokorach *et al*. (2021) reported widespread contamination of staple grains, including sorghum, maize, groundnuts, and millet, across Northern Uganda, with only 7% of samples found to be free from mycotoxins. Sorghum was particularly susceptible to contamination compared to millet. Similar contamination patterns have been reported in the Karamoja sub-region, where cereals constitute a major component of household diets.

The presence of aflatoxins and fumonisins in staple foods is of concern because these toxins reduce food quality and may contribute to adverse health outcomes through chronic dietary exposure.

Karamoja continues to experience high levels of malnutrition, driven by a complex interplay of factors including food insecurity, recurrent droughts, poverty, infectious diseases, limited dietary diversity, and inadequate access to health services. During the first round of mass malnutrition screening conducted across the nine districts of Karamoja, 249,859 children aged 6–59 months were screened, of whom 3.2% (9,306) and 13.9% (40,919) were classified as severely and moderately malnourished, respectively (UNICEF Uganda Karamoja Response Report, 2022). Furthermore, the Uganda Demographic and Health Survey (UDHS) 2022 reported a stunting prevalence of 34.0% in the region, with Kotido District recording the highest prevalence at 44.2% (Onyango *et al*., 2018). Although mycotoxins are unlikely to be the sole cause of malnutrition, growing evidence suggests that chronic exposure to mycotoxin-contaminated foods may exacerbate growth impairment and nutritional deficiencies, particularly among children who rely heavily on cereal-based diets.

This study intended to identify the mycotoxigenic mould species, compare their occurrences and determine the diversity of these molds isolated from cereal crops in the Karamoja sub-region. By so doing this, the study helped to understand why mycotoxin contamination erupts in some host populations and not others and would guide the different approaches to reduce food contamination by mycotoxin hence improving food safety in the Karamoja sub-region.

1.2. Statement of problem

Mycotoxin contamination remains a major food safety challenge worldwide, particularly in sub-Saharan Africa where warm climatic conditions favour the growth of mycotoxigenic fungi such as *Aspergillus, Fusarium*, and *Penicillium*. In Uganda, aflatoxins and fumonisins are among the most frequently occurring mycotoxins and have been detected in staple food crops including maize, sorghum, millet, and groundnuts (Lukwago *et al*., 2019; Wokorach *et al*., 2021). Chronic consumption of contaminated foods has been associated with adverse health effects, reduced agricultural productivity, loss of market value, and compromised food security. These

concerns are particularly important in the Karamoja sub-region, where cereals constitute a major component of household diets and where food insecurity and malnutrition remain persistent challenges.

Despite growing evidence of mycotoxin contamination in Uganda, there is limited information on the diversity and genetic composition of the mycotoxigenic fungal species associated with cereals in the Karamoja sub-region. Most previous studies have focused on the occurrence and levels of mycotoxins in food commodities, with little attention given to the fungal species responsible for contamination and their genetic relationships. Knowledge of the diversity and genetic variability of mycotoxigenic fungi is important because differences among fungal species and strains can influence ecological adaptation, host colonization, distribution, and toxigenic potential. The lack of such information limits the development of targeted strategies for monitoring and controlling mycotoxin contamination in cereals. Therefore, this study sought to identify mycotoxigenic fungal species associated with cereals in Karamoja, compare their occurrence, and determine their genetic diversity using molecular approaches.

1.3. General objective

This study majorly aimed at determining the genetic diversity of mycotoxigenic fungal species associated with cereals in Karamoja, using molecular techniques.

1.4. Specific objectives

i. To determine mycotoxigenic fungal species in cereals from Karamoja using molecular techniques.

ii. To compare the occurrence of mycotoxigenic fungal species in different cereal grain types from Karamoja.

iii. To determine the genetic relationship among mycotoxigenic molds isolated from different cereal types.

1.5. Research questions

i. What were the mycotoxigenic fungal species occurring in cereal grain from Karamoja?

ii. Which cereal grain types were mostly contaminated with mycotoxigenic molds?

iii. What was the genetic relationship among mycotoxigenic molds isolated from different cereal crops?

1.6. Scope of study

Three cereal grains were collected from homesteads and commercial stores; maize, millet and sorghum which are of much importance in the Karamoja sub-region because they are a major food source, income, and resilience for the people of Karamoja (Karamoja Resilience Support Unit, 2018). Cereals such as maize, sorghum, and millet are staple crops that grow well in the semi-arid climate and variable rainfall of Karamoja (FAO, 2013).

According to the 2015 Karamoja SQUEAC final report, the Karamoja sub-region covers an area of 27,528 km² which is divided into nine districts: Napak, Nakapiripirit, Nabilatuk, Abim, Amudat, Kaabong, Kotido, Karenga, and Moroto. Karamoja shares a border with South Sudan to the north and Kenya to the east. Its southern boundary lies next to Teso and Sebei regions while the western boarder line is formed by the Acholi and Lango regions (Around Uganda, 2022).

The study was done in 12 months, starting August 2024 and ending August 2025. The study followed the ethical guidelines and procedures of Gulu University (GUREC-2024-864) and obtained the necessary approvals and consent from the relevant authorities and participants.

1.7. Justification of study

According to the Parliament of Uganda, the country loses approximately US$38 million annually due to aflatoxin contamination, mainly through rejection of agricultural exports and reduced market competitiveness. Contamination of maize, groundnuts, and other staple crops continues to undermine food safety, public health, and trade, prompting calls for increased public awareness and stricter food safety regulations (Parliament of Uganda, 2023).

Mycotoxins frequently contaminate staple cereals consumed in the Karamoja sub-region, particularly maize, sorghum, and millet (Barrantes & Caravani, n.d.). The region is characterized by recurrent drought, food insecurity, poverty, and malnutrition, factors that may increase vulnerability to mycotoxigenic fungal contamination and its associated health effects (ReliefWeb, 2021; ReliefWeb, 2022). Furthermore, Wokorach *et al*. (2021) reported high levels of aflatoxins and fumonisins in staple grains from northern Uganda, including Karamoja, and estimated substantial dietary exposure among the local population.

The nutritional situation in Karamoja remains critical, with more than 89,000 children under five years and 10,000 pregnant or lactating mothers requiring emergency nutritional support (Uganda Radio Network, 2023). Despite evidence of mycotoxin contamination and exposure, little is known about the occurrence, species composition, and genetic diversity of mycotoxigenic molds associated with cereal crops in the region. This knowledge gap limits the development of effective monitoring, prevention, and regulatory strategies. Therefore, the novelty of this study lies in its integration of conventional fungal identification with molecular characterization and genetic diversity analysis of mycotoxigenic molds associated with major cereal crops in Karamoja, thereby generating baseline data to support future food safety interventions and mycotoxin management programs.

1.8. Significance of study

This study contributes to knowledge on the occurrence, species composition, and genetic diversity of mycotoxigenic molds associated with cereal crops in the Karamoja sub-region. By comparing the occurrence of these molds across different cereals and locations, the study provides baseline information that may support future assessments of mycotoxin contamination risks and food safety challenges within the region.

Alignment with Uganda’s National Development Plan

The findings are relevant to Uganda's Fourth National Development Plan (NDP IV), which prioritizes food security, public health, and agricultural productivity (National Planning Authority [NPA], 2025). By providing evidence on fungal contamination of

cereals, the study may inform food safety interventions, pre- and post-harvest management, and agricultural policy.

Contribution to Food Security and Agricultural Development

This study provides baseline data on mycotoxigenic fungi associated with major cereal crops in Karamoja. The findings may support researchers, extension personnel, policymakers, and regulatory agencies in developing evidence-based surveillance and management strategies to improve grain quality, reduce post-harvest losses, and enhance market access.

Contribution to Public Health and Food Safety

Mycotoxigenic fungi produce toxins that pose risks to human and animal health. By identifying fungal species and assessing their genetic diversity, this study provides evidence that may support food safety assessments, risk management, and stakeholder awareness on fungal contamination of cereals.

Scientific Contribution

A key contribution of this study is the generation of baseline molecular data on the diversity and genetic relationships of mycotoxigenic molds in Karamoja cereals. These findings provide a foundation for future research on fungal ecology, mycotoxin contamination, and targeted control strategies.

1.9. Theoretical framework

The process begun with cereal sampling, fungal isolation, involving culturing, sub-culturing, and morphological identification to obtain pure fungal colonies. For accurate species identification, the workflow advanced to molecular techniques, including DNA extraction and PCR assays. DNA amplicons were subjected to Sanger sequencing, followed by bioinformatics analysis such as NCBI BLASTn for taxonomic assignment, phylogenetic tree construction, and evaluation of sequence similarity and identity indices. The molecular and morphological classification provided insight on predominance and occurrence of molds in cereals, all of which culminated in determining fungal genetic diversity, providing insights into the

composition, distribution, and evolutionary relationships of mold species colonizing staple grains of Karamoja as illustrated in figure 1 bellow.

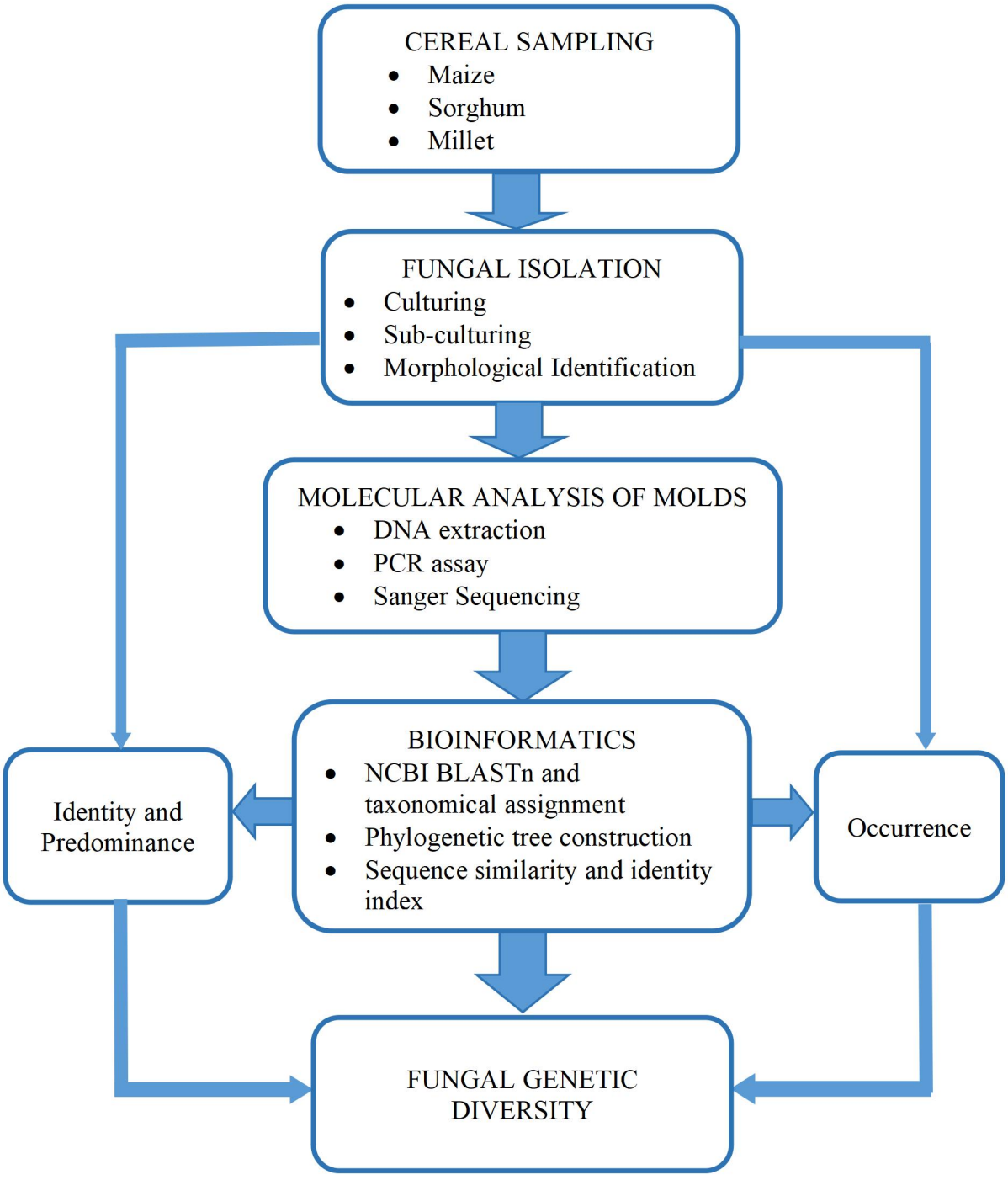


Figure 1: Theoretical framework of the study

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mycotoxigenic Fungi and Food Safety

Mycotoxigenic fungi are filamentous fungi capable of colonizing agricultural commodities and producing toxic secondary metabolites under favorable environmental conditions. The genera *Aspergillus*, *Fusarium*, and *Penicillium* are recognized as the most important mycotoxigenic fungi associated with cereal grains worldwide because of their frequent occurrence and ability to persist during pre-harvest, harvest, and storage stages (Pitt & Hocking, 2009; Magan *et al*., 2010). Their distribution is influenced by multiple factors, including temperature, moisture content, substrate composition, and agricultural practices, resulting in substantial variation in contamination patterns across geographical regions. While considerable knowledge exists regarding the occurrence of these fungi in major cereal-producing regions, their population structure and ecological adaptation remain poorly understood in many parts of sub-Saharan Africa, particularly in semi-arid environments.

The food safety significance of mycotoxigenic fungi extends beyond their mere presence in food commodities. Studies have demonstrated that fungal strains within the same species can differ markedly in their capacity to colonize hosts, compete with other microorganisms, and synthesize mycotoxins due to underlying genetic variation (Geiser *et al*., 2000; Ehrlich, 2014). Consequently, fungal identification at the genus or species level alone may not adequately predict contamination risk. Recent advances in molecular biology have highlighted the importance of characterizing fungal populations and their genetic diversity to better understand patterns of adaptation, dispersal, and toxigenic potential (de Vries *et al*., 2017). Such information is increasingly recognized as essential for developing effective surveillance and management strategies aimed at improving food safety.

In Uganda, studies have reported widespread contamination of staple cereals by mycotoxigenic fungi, particularly species of *Aspergillus* and *Fusarium* (Echodu *et al*., 2019; Wokorach *et al*., 2021). However, most investigations have focused on fungal occurrence and mycotoxin contamination levels, with limited attention given to the genetic diversity and phylogenetic relationships of the fungal populations involved.

This knowledge gap is especially evident in the Karamoja sub-region, where cereals constitute a major component of household diets and where environmental conditions may favor fungal proliferation. Therefore, understanding the diversity and genetic relationships of mycotoxigenic fungi associated with cereals is critical for identifying region-specific contamination risks and informing targeted food safety interventions.

2.1.1 Mycotoxigenic Fungi of Agricultural Importance

Several fungal genera are recognized as major contributors to food contamination and food safety risks due to their widespread occurrence in agricultural systems and their ability to colonize economically important crops. Among these, *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* are the most frequently reported mycotoxigenic fungi associated with cereals, legumes, nuts, fruits, and other food commodities worldwide (Pitt & Hocking, 2009; Eskola *et al*., 2020). Their prevalence is influenced by environmental conditions, agronomic practices, crop type, and storage conditions, resulting in considerable geographical variation in fungal communities. Understanding the ecology and diversity of these fungal groups is therefore fundamental for assessing food contamination risks and developing effective control strategies.

Species of *Aspergillus* are particularly important in tropical and subtropical regions where high temperatures and humidity favour fungal growth. Members of the genus, especially *A. flavus*, *A. parasiticus, A. niger, and A. ochraceus*, frequently colonize maize, groundnuts, sorghum, and millet both before and after harvest (Klich, 2007). In contrast, *Fusarium* species are commonly associated with field infections and are widely distributed in cereal production systems. Species such as F. verticillioides, F. proliferatum, F. graminearum, and F. oxysporum are important pathogens of maize, wheat, sorghum, and other cereals, with their occurrence often influenced by climatic conditions and host susceptibility (Leslie & Summerell, 2006). Studies have shown substantial genetic variability within both *Aspergillus* and *Fusarium* populations, suggesting that different strains may vary in ecological fitness, pathogenicity, and contamination potential (Acur *et al*., 2020; de Vries *et al*., 2017).

Although *Penicillium* and *Alternaria* have received comparatively less attention in cereal-based food safety studies, they remain important contaminants of agricultural commodities. *Penicillium* species are commonly associated with stored products and

can thrive under lower temperatures and moisture conditions than many other fungi, enabling them to persist during prolonged storage (Pitt & Hocking, 2009). Similarly, *Alternaria* species are ubiquitous plant-associated fungi that infect crops in the field and during storage, particularly under conditions of environmental stress (Lee *et al*., 2015). Despite evidence of their occurrence in cereal production systems, information regarding their genetic diversity, distribution, and phylogenetic relationships in Uganda remains limited. This knowledge gap highlights the need for molecular characterization studies that can provide region-specific information on the diversity and relatedness of mycotoxigenic fungi associated with staple cereals in Karamoja.

2.1.2 Relationship Between Fungal Contamination and Mycotoxin Production

The presence of mycotoxigenic fungi in food commodities does not necessarily indicate the presence of mycotoxins, as toxin production depends on a complex interaction between fungal genetics and environmental conditions. Studies have shown that not all isolates within a mycotoxigenic species possess the genetic capacity to synthesize toxins, and even toxigenic strains may fail to produce detectable toxin levels under unfavorable conditions (Geiser *et al*., 2000; Klich, 2007). For example, both toxigenic and non-toxigenic strains of *Aspergillus flavus* commonly occur within the same agroecosystem, often exhibiting considerable differences in secondary metabolite biosynthesis pathways. Consequently, reliance on morphological identification alone provides limited information regarding the actual contamination risk posed by fungal populations. Accurate species and strain identification using molecular approaches is therefore essential for distinguishing potentially toxigenic isolates from non-toxigenic counterparts and for improving food safety risk assessment (Ehrlich, 2014; Kizis *et al*., 2021).

Recent advances in molecular genetics have further demonstrated that variation in toxigenic potential is closely linked to genetic diversity within fungal populations. Differences in toxin biosynthesis genes, gene regulation mechanisms, and evolutionary adaptation can result in substantial variability in toxin-producing capabilities among closely related isolates (de Vries *et al*., 2017; Acur *et al*., 2020). As a result, characterization of fungal diversity and phylogenetic relationships has become increasingly important for understanding contamination dynamics and designing targeted management strategies. However, information on the genetic

diversity of mycotoxigenic fungi associated with staple cereals in Uganda, particularly in Karamoja, remains limited. This knowledge gap highlights the need to investigate the diversity and genetic relationships of fungal populations, since different fungal populations may differ significantly in their toxigenic potential and associated food safety risks.

2.2 Occurrence of Mycotoxigenic Fungi in Cereals

Cereal grains provide a favorable substrate for the growth of numerous fungal species during cultivation, harvesting, processing, and storage. Among these, mycotoxigenic fungi belonging to the genera *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* are the most frequently reported contaminants of staple cereals worldwide (Pitt & Hocking, 2009; Eskola *et al*., 2020). Their occurrence is influenced by environmental conditions, agronomic practices, storage systems, and crop characteristics, resulting in considerable variation in fungal communities among different cereal types and geographical regions. Understanding the occurrence of these fungi in major staple cereals is important for assessing contamination risks and identifying factors that may influence fungal diversity and persistence.

2.2.1 Mycotoxigenic Fungi Associated with Maize

Maize is one of the cereal crops most susceptible to colonization by mycotoxigenic fungi due to its high nutrient content and vulnerability to insect damage and environmental stress. Studies conducted across Africa, Asia, and Latin America consistently report frequent contamination of maize by *Aspergillus* and *Fusarium* species, particularly *A. flavus, A. niger*, *F. verticillioides,* and *F. proliferatum* (Wokorach *et al*., 2021; Leslie & Summerell, 2006). In Uganda, maize has repeatedly been identified as a major reservoir of mycotoxigenic fungi and associated mycotoxin contamination. However, most investigations have focused on fungal occurrence and toxin levels, with relatively limited attention given to the genetic diversity and population structure of the fungal isolates involved.

2.2.2 Mycotoxigenic Fungi Associated with Sorghum

Sorghum is an important staple crop in many semi-arid regions where it is valued for its drought tolerance and adaptability to marginal environments. Despite these advantages, sorghum is frequently colonized by mycotoxigenic fungi both in the field

and during storage. Species of *Fusarium*, *Aspergillus*, and *Alternaria* have commonly been isolated from sorghum grains, with their prevalence varying according to climatic conditions and post-harvest handling practices (Williams & McDonald, 1983; Wokorach *et al*., 2021). Although fungal contamination of sorghum has been documented in several African countries, information regarding the molecular diversity and evolutionary relationships of the associated fungal populations remains scarce.

2.2.3 Mycotoxigenic Fungi Associated with Millet

Millet is widely cultivated in dryland regions and serves as an important food security crop due to its resilience under harsh environmental conditions. Nevertheless, millet grains are not exempt from fungal contamination and have been reported to harbor species of *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* during production and storage (Echodu *et al*., 2019; Wokorach *et al*., 2021). While contamination levels in millet are often lower than those reported for maize, the crop can still serve as a reservoir for potentially toxigenic fungi. Existing studies have largely concentrated on fungal occurrence and mycotoxin contamination, leaving substantial gaps in knowledge regarding the genetic diversity and phylogenetic relationships of fungal isolates associated with millet.

2.2.4 Co-occurrence of Fungal Species in Cereals

Multiple mycotoxigenic fungal species frequently occur simultaneously within the same cereal grain or storage environment, creating complex fungal communities. Studies have shown that *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* species often coexist in cereal commodities, where they may compete, interact, or occupy different ecological niches depending on environmental conditions (Gruber-Dorninger *et al*., 2019; Eskola *et al*., 2020). Such co-occurrence can influence contamination dynamics and may complicate control measures. However, most available studies primarily describe the presence and prevalence of fungal contaminants, while comparatively few have investigated the diversity, genetic relationships, and population structure of co-occurring fungal species. This limitation highlights the need for molecular studies aimed at understanding the diversity of mycotoxigenic fungal communities associated with staple cereals in specific agro-ecological regions such as Karamoja.

2.3 Distribution of Mycotoxigenic Fungi in East Africa

Mycotoxigenic fungi are widely distributed throughout East Africa, where warm temperatures, seasonal droughts, and inadequate post-harvest management create favorable conditions for fungal colonization of cereals. Species belonging to the genera *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* have been reported in maize, sorghum, millet, and other staple crops across the region (Matumba *et al*., 2015; Wokorach *et al*., 2021). Their distribution varies among agro-ecological zones, reflecting differences in climate, crop production systems, and storage practices. Consequently, understanding regional patterns of fungal occurrence is important for identifying areas at heightened risk of contamination and for guiding targeted food safety interventions.

2.3.1 Evidence from Uganda

Studies conducted in Uganda have consistently reported the presence of mycotoxigenic fungi in major staple cereals, particularly maize, sorghum, and millet. Molecular and culture-based investigations have identified *Aspergillus flavus, A. niger, Fusarium* spp., and *Penicillium* spp. as common contaminants of stored grains (Echodu *et al*., 2019; Wokorach *et al*., 2021). Research has primarily focused on contamination prevalence and associated mycotoxins, revealing widespread food safety concerns in both rural and commercial production systems. However, relatively few studies have examined the genetic diversity and phylogenetic relationships of the fungal populations responsible for contamination.

2.3.2 Evidence from Kenya, Tanzania, and Ethiopia

Similar fungal communities have been reported across neighboring East African countries. In Kenya, recurrent contamination of maize by *Aspergillus flavus* has been linked to several aflatoxicosis outbreaks, particularly in drought-prone regions (Probst *et al*., 2007). Studies from Tanzania and Ethiopia have likewise documented the frequent occurrence of *Aspergillus* and *Fusarium* species in cereals and other food crops, with contamination patterns strongly influenced by environmental conditions and post-harvest handling practices (Mohamed *et al*., 2016; Chala *et al*., 2014). Despite increasing reports of contamination, molecular investigations of fungal population diversity remain limited across much of the region.

2.3.3 Environmental and Climatic Factors Influencing Fungal Occurrence

The occurrence and persistence of mycotoxigenic fungi are strongly influenced by environmental and management factors. Elevated temperatures and high humidity promote fungal growth and colonization of cereal grains, while prolonged drought stress can increase crop susceptibility to infection, particularly by *Aspergillus* species (Magan *et al*., 2010). Poor storage conditions, including inadequate drying, high grain moisture content, and insufficient aeration, further facilitate fungal proliferation after harvest. These factors often interact to determine both the abundance and composition of fungal communities within cereal production systems.

2.3.4 The Karamoja Context

Karamoja is a semi-arid region characterized by recurrent droughts, erratic rainfall patterns, and chronic food insecurity, conditions that can favor fungal colonization and persistence in stored cereals. The population relies heavily on maize, sorghum, and millet as staple foods, making cereal safety a critical public health concern (Kathlee Freeman and Nyasimi, 2017; WFP, 2024). Although studies have documented mycotoxin contamination in northern Uganda, information specifically describing the diversity and distribution of mycotoxigenic fungi in Karamoja remains scarce. Most available research has concentrated on toxin occurrence rather than the fungal populations responsible for contamination. Consequently, there is limited understanding of the diversity, genetic relationships, and ecological adaptation of mycotoxigenic fungi in this unique agro-ecological region.

2.4 Diversity of Mycotoxigenic Fungi

The diversity of mycotoxigenic fungi refers to the variety of fungal species and strains capable of colonizing agricultural commodities and potentially producing mycotoxins. Understanding fungal diversity is important because different species and strains vary in their ecological adaptation, competitiveness, pathogenicity, and toxigenic potential (Leslie & Summerell, 2006; Frisvad *et al*., 2019). Studies from various cereal-growing regions have shown that fungal communities are often composed of multiple species belonging to *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria*, with their composition varying according to environmental and agronomic conditions.

Consequently, assessing fungal diversity provides a more comprehensive understanding of contamination risks than merely documenting fungal occurrence.

2.4.1 Species Diversity

Species diversity among mycotoxigenic fungi is widely recognized in cereal production systems worldwide. Within a single genus, multiple species may coexist and occupy similar ecological niches while differing in their ability to colonize crops and produce toxins. For example, *Aspergillus flavus, A. niger*, and *A. ochraceus*, as well as several *Fusarium* species, have frequently been reported from the same cereal samples (Pitt & Hocking, 2009; Wokorach *et al*., 2021). Such diversity contributes to variation in contamination patterns and food safety risks across different production systems and geographical regions.

2.4.2 Factors Influencing Fungal Diversity

Fungal diversity is shaped by a combination of biological and environmental factors. Host crop characteristics influence fungal colonization by providing different nutritional and ecological conditions, resulting in variation in fungal communities among maize, sorghum, and millet (Leslie & Summerell, 2006). Geography and climate further affect fungal distribution, with temperature, rainfall patterns, and humidity determining the suitability of environments for specific fungal species. In addition, storage conditions such as grain moisture content, storage duration, and aeration influence the survival and proliferation of fungal populations after harvest (Magan *et al*., 2010). These interacting factors contribute to considerable differences in fungal diversity between regions and cereal types.

2.4.3 Significance of Fungal Diversity in Food Safety

Fungal diversity has important implications for food safety because different fungal species and strains exhibit varying capacities to produce mycotoxins and adapt to environmental conditions. Diverse fungal communities may increase the likelihood of contamination by multiple toxigenic species and complicate mitigation strategies (Frisvad *et al*., 2019). Furthermore, closely related fungal isolates may differ substantially in their toxigenic potential due to genetic variation, making diversity assessment essential for understanding contamination risks. Despite this importance, many studies continue to rely primarily on morphological identification methods,

which may fail to accurately distinguish closely related species and cryptic taxa. Consequently, morphological approaches alone are insufficient for comprehensive assessment of fungal diversity, highlighting the need for molecular tools capable of providing higher taxonomic resolution.

2.5 Isolation and Culturing of Mycotoxigenic Fungi

Isolation and culturing remain fundamental procedures in mycological studies because they enable the recovery, purification, and preliminary characterization of fungal contaminants from food commodities. These methods provide important information on fungal occurrence, abundance, colony characteristics, and ecological distribution within different substrates (Pitt & Hocking, 2009). Although advances in molecular biology have expanded the tools available for fungal identification, conventional isolation techniques continue to play a critical role in obtaining pure cultures for downstream analyses, including molecular characterization and phylogenetic studies.

2.5.1 Sampling Approaches

The accuracy of fungal detection is highly dependent on the sampling strategy employed. Representative sampling is essential because fungal contamination is often unevenly distributed within grain lots due to variations in moisture content, storage conditions, and insect activity (Whitaker, 2006). Consequently, studies commonly collect multiple subsamples from different locations within storage facilities or grain batches and combine them into composite samples to minimize sampling bias and improve the reliability of contamination assessments.

2.5.2 Isolation Methods

Isolation of mycotoxigenic fungi from cereals is commonly achieved using direct plating or dilution-based techniques on selective culture media. Media such as Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and Dichloran Rose Bengal Chloramphenicol Agar (DRBC) are widely used because they support fungal growth while suppressing bacterial contamination (Samson *et al.*, 2010). Following incubation, distinct fungal colonies are sub-cultured repeatedly to obtain pure isolates suitable for subsequent identification and characterization.

2.5.3 Morphological Identification

Morphological identification relies on the observation of colony appearance and microscopic structures such as conidia, conidiophores, hyphae, and spore-bearing structures. This approach has historically formed the basis of fungal taxonomy and remains widely used due to its simplicity and relatively low cost (Klich, 2002). Using established taxonomic keys, researchers can often identify fungal isolates to the genus level and, in some cases, to the species level. Morphological characterization therefore provides an important preliminary assessment of fungal diversity within cereal samples.

2.5.4 Strengths and Limitations of Conventional Methods

Conventional culture-based and morphological methods offer several advantages, including low operational costs, direct observation of fungal characteristics, and the ability to obtain viable isolates for further study (Samson *et al*., 2010). However, these methods also have notable limitations. Morphologically similar species may be difficult to distinguish accurately, while environmental conditions and culture media can influence fungal appearance, leading to misidentification. In addition, cryptic species and genetically distinct strains often cannot be differentiated using morphology alone (Frisvad *et al*., 2019). As a result, conventional approaches may underestimate fungal diversity and provide insufficient resolution for studying population structure and genetic relationships. These limitations have driven the increasing adoption of molecular techniques for more accurate identification and diversity assessment of mycotoxigenic fungi.

2.6 Molecular Identification of Mycotoxigenic Fungi

Molecular identification has become an essential tool in fungal taxonomy due to the limitations of conventional morphological methods in distinguishing closely related species. DNA-based approaches utilize genetic markers to identify fungi based on sequence variation, providing greater accuracy, reproducibility, and taxonomic resolution than phenotype-based methods (Nilsson *et al*., 2019). These techniques have significantly improved the characterization of mycotoxigenic fungi associated with agricultural commodities and have facilitated studies of fungal diversity, phylogeny, and population structure across different environments.

2.6.1 DNA-Based Fungal Identification

DNA-based fungal identification involves the extraction, amplification, and sequencing of specific genomic regions followed by comparison with reference sequences in public databases. The approach enables accurate identification even when morphological features are ambiguous, absent, or influenced by environmental conditions (Schoch *et al*., 2012). Consequently, molecular methods have become widely adopted in studies investigating cereal-associated fungal communities, where precise species identification is necessary for understanding contamination risks and ecological relationships.

DNA-based identification of fungi commonly utilizes either universal or species-specific primers depending on the objective of the study. Universal primers target conserved genomic regions shared across a broad range of fungal taxa, enabling the detection and identification of diverse fungal communities from environmental or food samples. In contrast, species-specific primers are designed to amplify DNA sequences unique to a particular fungal species or group, allowing rapid and highly sensitive detection of target organisms (Lievens & Thomma, 2005). The choice of primer type depends on whether the aim is broad-spectrum fungal identification, biodiversity assessment, or targeted detection of known mycotoxigenic species. PCR-based detection systems generally involve DNA extraction, amplification using appropriate primers, visualization of amplified products, and sequence analysis for species confirmation. A general overview of PCR-based detection technologies and the mechanism by which mycotoxigenic fungi are detected is presented in Figure 2.

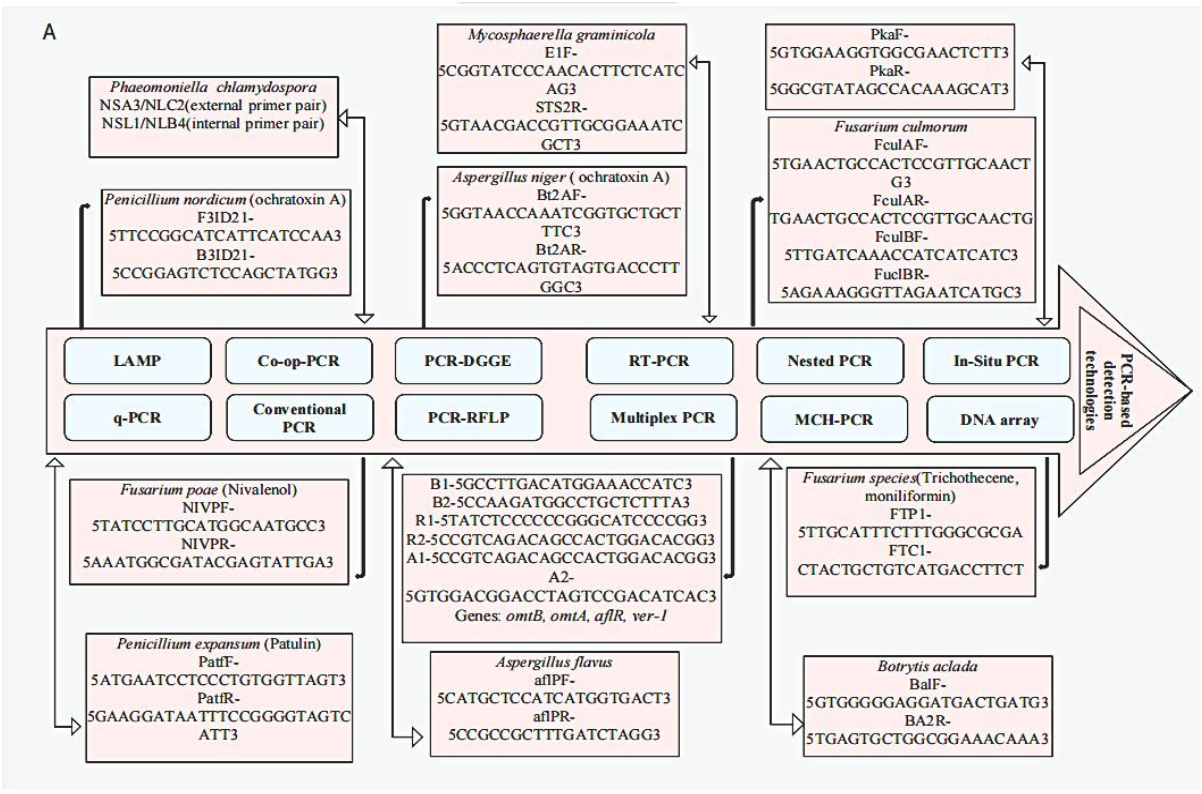


Figure 2: (A) Flow chart showing PCR detection methods, including the specific target genes sequence sources. (B) An overview of the standard detection protocol. Source: doi/pdf/10.3920/WMJ2019.2455

2.6.2 Molecular Markers Used in Fungal Taxonomy

Several genetic markers have been employed for fungal identification and phylogenetic studies. The internal transcribed spacer (ITS) region is the most widely used universal fungal barcode because of its high amplification success and extensive representation in sequence databases (Schoch *et al*., 2012). However, ITS often lacks sufficient resolution to distinguish closely related species within important genera such as *Aspergillus* and *Fusarium*. To overcome this limitation, protein-coding genes such as β-tubulin (BenA) and translation elongation factor 1-alpha (TEF1-α) are frequently used. These markers generally exhibit higher sequence variability and therefore provide improved discrimination among closely related taxa (Samson *et al*., 2014; O'Donnell *et al*., 2015).

2.6.3 Advantages of TEF1-α in Species Discrimination

Among the commonly used molecular markers, TEF1-α has proven particularly valuable for species-level identification of mycotoxigenic fungi. The marker contains conserved regions suitable for primer binding as well as variable regions that allow discrimination among closely related species and strains (O'Donnell *et al*., 2015). Several studies have demonstrated that TEF1-α provides greater taxonomic resolution than ITS for members of the genera *Fusarium* and *Aspergillus*, making it highly useful for phylogenetic analyses and diversity studies (Carbone & Kohn, 1999; Geiser *et al*., 2007). Its ability to reveal subtle genetic differences has made it an important marker for investigating fungal population diversity and evolutionary relationships.

2.6.4 Applications in Cereal-Associated Fungi

Molecular markers have been extensively applied in the identification and characterization of fungi associated with cereal crops. Sequence-based approaches have enabled the detection of species complexes, clarification of taxonomic relationships, and assessment of genetic diversity among fungal isolates recovered from maize, sorghum, millet, and other cereals (Wokorach *et al*., 2021; O'Donnell *et al*., 2015). Despite these advances, many studies in East Africa have primarily focused on identifying fungal contaminants or quantifying mycotoxin occurrence, with limited emphasis on fungal population diversity. Consequently, molecular characterization using high-resolution markers such as TEF1-α remains important for

improving understanding of the diversity and genetic relationships of mycotoxigenic fungi associated with cereals in under-studied regions such as Karamoja.

2.7 Genetic Diversity of Mycotoxigenic Fungi

Genetic diversity refers to the variation in genetic composition among individuals, populations, or species and is a fundamental component of fungal ecology and evolution. In mycotoxigenic fungi, genetic diversity influences physiological traits such as growth rate, environmental adaptation, pathogenicity, and toxin production (McDonald & Linde, 2002). Understanding the genetic diversity of fungal populations is therefore important for elucidating contamination dynamics, evolutionary relationships, and potential food safety risks associated with cereal production systems.

2.7.1 Concept of Genetic Diversity in Fungi

Genetic diversity in fungi arises through mutation, recombination, gene flow, and evolutionary adaptation to different ecological niches. This diversity may occur both between species and among strains within the same species, resulting in substantial variation in biological characteristics (Taylor *et al*., 2006). In agricultural environments, genetically diverse fungal populations are often better able to survive environmental stress, exploit different hosts, and adapt to changing climatic conditions, making diversity studies important for understanding fungal persistence and spread.

2.7.2 Genetic Diversity Within *Aspergillus* Species

Considerable genetic diversity has been reported within species of the genus *Aspergillus*, particularly *A. flavus*, one of the most important fungal contaminants of cereals in tropical regions. Molecular studies have revealed the existence of genetically distinct populations differing in ecological adaptation, reproductive biology, and toxin-producing capability (Geiser *et al*., 2000; Ehrlich, 2014). Such variation can influence contamination patterns and may explain differences in fungal prevalence and toxigenic potential observed among geographical regions and crop types.

2.7.3 Genetic Diversity Within *Fusarium* Species

Species of *Fusarium* also exhibit extensive genetic diversity, often existing as species complexes composed of closely related but genetically distinct lineages. Molecular analyses have demonstrated substantial variation among *Fusarium* populations with respect to host preference, geographic distribution, pathogenicity, and toxin production (Leslie & Summerell, 2006; O'Donnell *et al*., 2015). This diversity has important implications for understanding the ecology and epidemiology of cereal-associated fungal contaminants and for developing effective management strategies.

2.7.4 Implications of Genetic Diversity

Genetic diversity contributes significantly to the adaptive capacity of fungal populations. Diverse populations are more likely to survive environmental fluctuations, colonize a broader range of hosts, and evolve resistance to management interventions (McDonald & Linde, 2002). In mycotoxigenic fungi, genetic variation is also associated with differences in toxigenic potential, meaning that isolates belonging to the same species may differ considerably in their ability to produce toxins (Ehrlich, 2014). Consequently, knowledge of fungal genetic diversity is important for predicting contamination risks, understanding host-pathogen interactions, and designing effective disease and food safety management programs.

2.7.5 Molecular Approaches for Diversity Assessment

Advances in molecular biology have provided powerful tools for investigating fungal diversity and genetic relationships. DNA sequencing allows the characterization of genetic variation within and among fungal populations, while BLAST analysis facilitates species identification through comparison with reference sequences in public databases (Johnson *et al*., 2008). Phylogenetic analysis further enables the reconstruction of evolutionary relationships and assessment of genetic relatedness among isolates (Tamura *et al*., 2021). Together, these approaches provide a robust framework for studying fungal diversity beyond what can be achieved using conventional morphological methods alone.

2.7.6 Knowledge Gaps in East Africa and Karamoja

Although numerous studies in East Africa have documented the occurrence of mycotoxigenic fungi and the prevalence of mycotoxins in cereal crops, relatively few have investigated the genetic diversity of the fungal populations involved (Echodu *et*

al., 2019; Wokorach *et al*., 2021). Most available studies focus on contamination levels and species occurrence, providing limited insight into the evolutionary relationships and population structure of fungal isolates. This gap is particularly evident in the Karamoja sub-region, where environmental conditions, dependence on cereals, and recurrent food insecurity may influence fungal diversity and distribution. Although several studies have documented mycotoxin contamination in cereals in East Africa, limited information exists on the diversity and genetic relationships of mycotoxigenic fungi associated with cereals in the Karamoja sub-region of Uganda. This knowledge gap justifies the present study.

2.8 Control and Management of Mycotoxigenic Fungi and Mycotoxins

The control of mycotoxigenic fungi and their associated toxins requires an integrated approach that addresses contamination throughout the crop production and storage chain. Because fungal infection can occur before harvest and continue during storage, effective management strategies must combine agronomic, biological, post-harvest, and monitoring interventions (Magan & Aldred, 2007). Such approaches aim to reduce fungal colonization, limit mycotoxin production, and improve food safety, particularly in regions where cereals constitute staple foods.

2.8.1 Pre-harvest Management

Pre-harvest management focuses on reducing fungal infection in the field through good agricultural practices. Strategies such as the use of resistant crop varieties, timely planting, crop rotation, proper irrigation, insect pest control, and minimization of plant stress have been shown to reduce susceptibility to infection by mycotoxigenic fungi (Munkvold, 2017). Since drought stress and insect damage often predispose cereals to fungal colonization, improving field management practices can significantly reduce contamination risks before harvest.

2.8.2 Post-harvest Management

Post-harvest interventions are critical because fungal growth and mycotoxin accumulation frequently continue during storage. Effective measures include rapid drying of grains to safe moisture levels, proper cleaning and sorting, use of well-ventilated storage facilities, and protection from insect infestation and water ingress (Magan *et al*., 2010). Studies have shown that maintaining low grain moisture content

and favorable storage conditions can substantially limit fungal proliferation and reduce the likelihood of mycotoxin accumulation in stored cereals.

2.8.3 Biological Control Approaches

Biological control has emerged as a promising strategy for reducing contamination by mycotoxigenic fungi. One of the most successful approaches involves the application of non-toxigenic strains of *Aspergillus flavus* that competitively exclude toxin-producing strains from colonizing crops (Ehrlich, 2014; Bandyopadhyay *et al*., 2016). Other biological control agents, including beneficial bacteria and fungi, have also shown potential in suppressing fungal growth and reducing toxin production. These environmentally friendly approaches offer sustainable alternatives to chemical control methods and are increasingly being incorporated into integrated management programs.

2.8.4 Molecular Surveillance and Future Directions

Recent advances in molecular biology have enhanced the ability to detect, identify, and monitor mycotoxigenic fungi with greater accuracy and sensitivity. DNA-based tools enable early detection of fungal contaminants, characterization of population diversity, and identification of potentially toxigenic strains before significant contamination occurs (Kizis *et al*., 2021). Molecular surveillance can therefore support targeted intervention strategies and improve risk assessment programs. As fungal populations continue to evolve under changing environmental conditions, integrating molecular monitoring with conventional management approaches will be essential for strengthening food safety systems and developing effective long-term control strategies.

The reviewed literature indicates that mycotoxigenic fungi are widely associated with cereal crops and remain a significant food safety concern, particularly in East Africa where favourable climatic conditions promote fungal growth and mycotoxin contamination. Although several studies have documented the occurrence and health implications of mycotoxins in cereals, relatively few have investigated the diversity, distribution, and genetic relationships of the fungal species responsible for contamination. In Uganda, especially the Karamoja sub-region, molecular information on mycotoxigenic fungi remains limited despite evidence of widespread

contamination of staple cereals. This knowledge gap hinders the development of targeted surveillance and effective management strategies. Therefore, this study was undertaken to identify the mycotoxigenic molds associated with major cereal crops in Karamoja, compare their occurrence across cereal types and districts, and determine their genetic diversity using molecular approaches, thereby generating baseline information to support future food safety interventions and research.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Study area

This research was conducted in Uganda's Karamoja sub-region, occupying approximately 27,528 km² and consisting of nine administrative districts: Abim, Amudat, Kaabong, Kotido, Karenga, Moroto, Nakapiripirit, Nabilatuk, and Napak (Karamoja SQUEAC Final Report, 2015) as shown in figure 3. The area features a varied landscape that includes mountain ranges, savannah vegetation, and broad semi-arid plains. Mount Moroto, rising to about 3,000 metres above sea level, forms a prominent landmark within the sub-region. According to the Uganda Bureau of Statistics (2022), Karamoja's population was estimated at roughly 1.4 million people. The sub-region is primarily inhabited by the Karamojong community, whose livelihoods are largely based on pastoralism, with cattle keeping and semi-nomadic movements as defining cultural practices.

The Karamoja sub-region has experienced increasing climatic variability over the past three decades, characterized by erratic rainfall patterns, prolonged dry spells, and rising temperatures (Egeru *et al*., 2014; Kathlee Freeman and Mary Nyasimi, 2017; WFP, 2024). Although the rainy season has extended from approximately March to December, rainfall remains highly variable in both timing and amount, making crop production unpredictable (WFP, 2024). These climatic conditions adversely affect crop productivity, post-harvest grain handling, water availability, and livestock production, thereby exacerbating food insecurity and livelihood challenges in the region (Kathleen Freeman and Mary Nyasimi, 2017; WFP, 2024). From a mycological perspective, fluctuations in temperature and moisture create selective environmental pressures that influence the distribution, survival, and adaptation of mycotoxigenic fungi. Such conditions may favour the persistence of particular fungal species with greater ecological fitness, potentially contributing to differences in fungal population structure and genetic diversity across cereal-growing environments.

Economically, the region engages in cattle keeping, mining, and trading in agricultural produce with neighboring districts. While Karamoja's infrastructural development has historically lagged behind the rest of Uganda, concerted efforts by

the government and international organizations have resulted in significant improvements in recent years (Around Uganda, 2022).

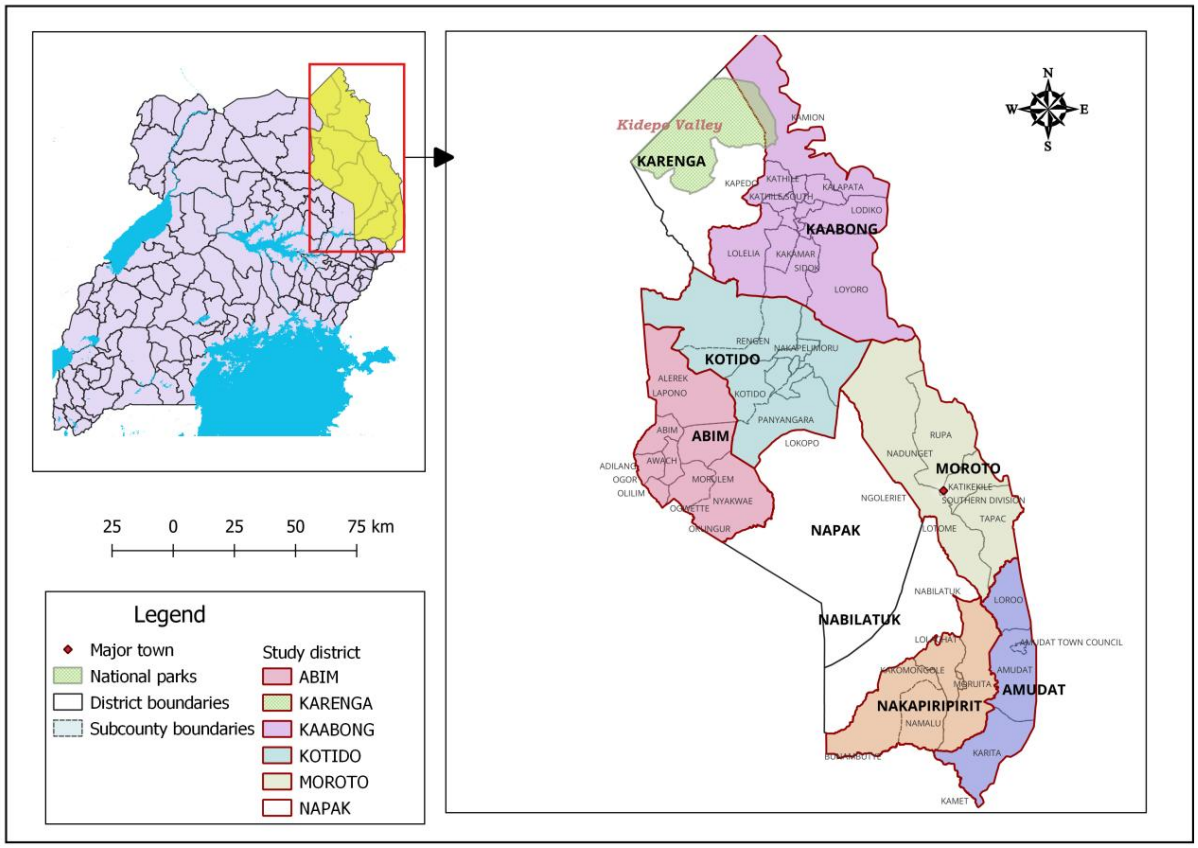


Figure 3: A map of Karamoja sub-region showing the sample area. Source: Mapchart.net

3.2. Study design

This study employed a cross-sectional quantitative research design. Samples of the three major staple cereals (maize, sorghum, and millet) were collected from three sub-counties within each selected district using stratified random sampling. Primary data were generated through laboratory analysis, where fungal isolates were identified and characterized using PCR-based molecular techniques. In addition, household surveys were conducted using structured questionnaires to collect information on cereal harvesting, drying, storage, and consumption practices. Survey data were summarized using descriptive statistics and used to provide contextual information for interpreting the occurrence and distribution of mycotoxigenic fungi identified through molecular analyses.

3.3. Sample size determination

The sample size for this study was determined using Cochran's formula for estimating proportions in large populations (Israel, 1992). An initial sample size ((n_0)) was first calculated and then adjusted using the finite population correction (FPC) because of the estimated number of sampling frames.

The initial sample size was calculated using Cochran's formula:

$$n_0=\frac{Z^2P(1-P)}{e^2}$$

Where:

(n_0) = initial sample size for an infinite population;

(Z) = standard normal deviate corresponding to the desired confidence level (1.96 for a 95% confidence level);

(P) = estimated proportion of the attribute of interest in the population. In the absence of previous estimates for the study area, a value of 0.5 was adopted because it provides the maximum sample size and ensures adequate statistical precision;

(e) = margin of error (0.05).

Substitution

$$n_0=\frac{(1.96)^2\times0.5(1-0.5)}{(0.05)^2} \qquad n_0=\frac{3.8416\times0.25}{0.0025}$$

$$n_0=384.16\approx385$$

Because the total estimated sampling frame was 150, the sample size was adjusted using Cochran's finite population correction formula:

$$n=\frac{n_0}{1+\left(\frac{n_0-1}{N}\right)}$$

(n) = adjusted sample size for a finite population;

(n_0) = initial sample size (385);

(N) = total number of eligible cereal samples in the sampling frame (150).

Substitution

$$n=\frac{385}{1+\left(\frac{385-1}{150}\right)} \qquad n=\frac{385}{1+\frac{384}{150}} \qquad n=108.15\approx108$$

$$n=\frac{385}{3.56}$$

3.4. Sampling procedures

A stratified random sampling approach was employed to obtain a 500g representative cereal grain samples (maize, sorghum, and millet) from different households across six districts. These districts were selected to provide broad geographical coverage of the region while capturing variations in cereal production, climatic conditions, and farming practices. Together, they represent the major cereal-growing areas of Karamoja and share similar agroecological characteristics. The sampling procedure involved a simple random collection of maize, sorghum, and millet samples across six districts. From each district, three homesteads were selected, and at every homestead, two samples of each cereal type were obtained. This yielded a total of 36 samples per cereal crop (6 districts × 3 homesteads × 2 samples), and 108 samples overall for the three crops combined, as shown in Table 1. All samples were collected under sterile conditions using polythene bags, properly labeled, and subsequently transported to Gulu University's multifunctional laboratory for further analysis.

Table 1:

Number of samples and their sources

Samples	No. of districts	No. of homesteads per district	No. of samples per homestead	Sub-total
Maize	6	3	2	36
Sorghum			2	36
Millet			2	36
Total	6	3	6	**108**

3.5. Isolation of molds

The seeds were cultured on Potato Dextrose Agar (PDA) and incubated at 30 °C for about three days to promote mixed fungal colony development (Farson, 2021). Each 250ml of PDA was added 250mg of penicillin tablet to suppress bacterial contamination (Jayaram and Nagao, 2018). After visible growth appeared, a small fragment from each fungal colony was sub-cultured on a fresh PDA plate to obtain pure isolates. The resulting colonies were

examined microscopically and identified according to their morphological features (Samson et al., 2014). Each pure isolate was then transferred into Potato Dextrose Broth (PDB) and maintained at 30 °C for five to seven days under continuous shaking or stirring (Todorović *et al*., 2023). The fungal spores obtained from these cultures were harvested through centrifugation and stored at 4 °C for subsequent analyses (Todorović *et al*., 2023).

Morphological identification based on colony characteristics and microscopic features provided the preliminary classification of fungal isolates. These identifications were subsequently validated by PCR amplification and sequencing of the TEF1-α gene, followed by BLASTn comparison with reference sequences in the NCBI database and phylogenetic analysis. Agreement between the morphological and molecular results was used to confirm the identity of the fungal isolates.

3.5.1. Molecular identification of molds

DNA extraction was conducted using the reliable and cost effective CTAB protocol as outlined by Carr (2022). The PDB culture containing fungal spores was centrifuged at maximum speed for about five minutes to collect the spores as a pellet. The liquid supernatant was discarded, leaving the fungal residue at the base of the tube. The resulting spore pellet was moved into a clean mortar, mixed with 500 microliters of CTAB buffer, and ground gently with a pestle until a uniform, cloudy mixture was obtained. The homogenized sample was then transferred back into a microcentrifuge tube, and 10 microliters of proteinase K added. The mixture was thoroughly vortexed and incubated in a water bath at 65 °C for 30 minutes.

Next, 500 microliters of a chloroform : isoamyl alcohol solution (24:1, v/v) was added, followed by vortexing until well mixed. The mixture was centrifuged at 13,000 rpm for 15 minutes. The upper aqueous layer (approximately 300 microliters) was carefully transferred into a fresh tube, to which 300 microliters of cold isopropanol (kept at −20 °C) was added. The mixture was again vortexed and centrifuged at 13,000 rpm for 15 minutes to form a DNA pellet. The supernatant was discarded, and the DNA precipitate at the tube's bottom was retained.

The pellet was washed with 150 microliters of ethanol (70%) and centrifuged again at 13,000 rpm for 2 minutes. The ethanol was poured off, leaving the DNA pellet in the tube. The tube was left open briefly to allow evaporation of any remaining ethanol. Finally, the pellet was

dissolved in 50 microliters of nuclease free water and stored for later use. The quality and integrity of the extracted genomic DNA were assessed by agarose gel electrophoresis. DNA samples showing distinct, intact bands with minimal smearing were considered of sufficient quality for PCR amplification, while poor-quality samples were excluded or re-extracted before downstream molecular analyses.

3.5.2. PCR Assay

PCR assay was run using ITS and E.F primers, whose respective sequences used are summarized in Table 2. The PCR process began with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles that included denaturation at 95°C for 30 seconds, annealing at 52°C for 30 seconds (which varied depending on the primer used), and extension at 72°C for 1 minute. A final extension step was carried out at 72°C for 10 minutes, after which the reaction was maintained at 4°C. The amplicons were then checked on an agarose gel to verify the quality and amount of amplified DNA (Farooq *et al*., 2020).

Table 2:

List of primers and their corresponding sequences used for amplification of ribosomal RNA (ITS region) and elongation factor 1-alpha (TEF1-α) gene in fungal isolates (Gardes & Bruns, 1993; Carbone & Kohn, 1999).

Target gene	Primer name	Primer sequence
Ribosomal RNA	ITS1-F	5’-CTTGGTCATTTAGAGGAAGTAA-3’
	ITS4-B	5’-TCCTCCGCTTATTGATATGC-3’
TEF 1-alpha	EF1-728F	5’-CATCGAGAAGTTCGAGAAGG-3’
	EF2	5’-GGARGTACCAGTSATCATG-3’

To ensure the reliability of the PCR assay, both positive and negative controls were included in each amplification run. The positive control consisted of DNA from a previously confirmed fungal isolate known to amplify successfully with the selected primer set, while the negative control contained all PCR reagents except template DNA, with nuclease-free water added instead. The controls were used to verify successful amplification and to detect possible contamination of PCR reagents or reaction mixtures.

3.6. Sanger sequencing

The DNA amplicons were sequenced using Sanger sequencing technology from Inqababiotec, located in Pretoria, South Africa. The method provides highly accurate sequence data for single-gene targets, is cost-effective for a relatively small number of samples, and is widely accepted for fungal taxonomic studies. During Sanger sequencing, each double-stranded DNA was split into two single strands, and a primer complementary to one end of the template strand was attached. The sequencing process involved four polymerase reactions containing the standard nucleotides, after which one type of chain-terminating nucleotide was added. The fluorescently labeled chain-terminating nucleotides stop DNA synthesis once incorporated, allowing the resulting fragments to be analyzed for sequence determination. The resulting fragments were separated by capillary electrophoresis according to their size and detected by a laser scanner.

3.6.1. Sequence quality check

The raw nucleotide sequences generated by Sanger sequencing were imported into MEGA version 11 (MEGA Software Suite, 2021) for quality assessment and subsequent analyses. Sequence quality was evaluated by visual inspection of the electropherogram peaks, where clear, well-resolved peaks indicated reliable base calls, while ambiguous or overlapping peaks were considered low-quality regions. Low-quality nucleotides, particularly those occurring at the 5′ and 3′ ends of the sequences (Figure 4), were trimmed to improve sequence accuracy and minimize errors in downstream analyses. The resulting high-quality trimmed sequences were then exported in FASTA format for subsequent BLASTn searches, multiple sequence alignment, and phylogenetic analysis.

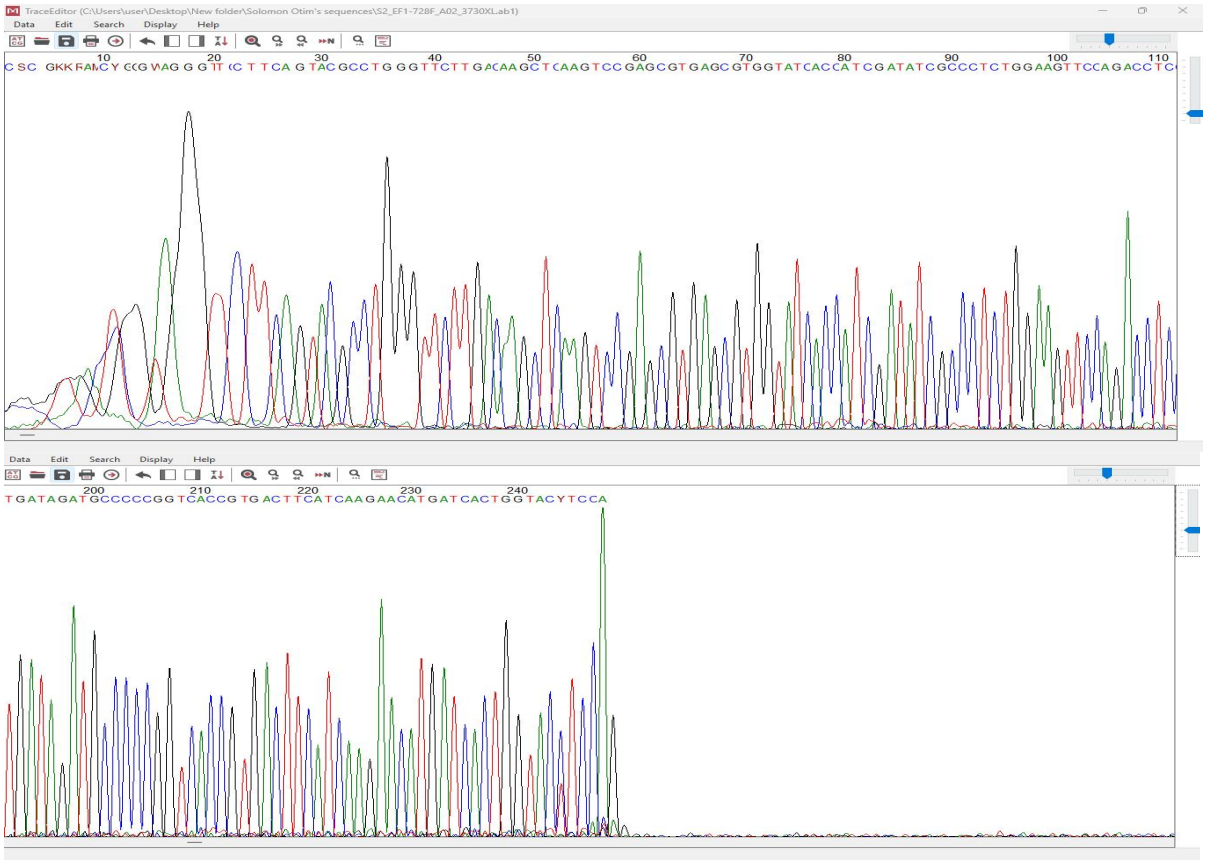


Figure 4: Electropherogram of a sample (S2_EF1-728F_A02_3730XL) showing low quality regions at the start and at the end.

3.6.2. Taxonomy assignment

The clean FASTA sequences were BLAST searched in the NCBI database using BLASTn tool to identify the closest species based on the best hit produced by the BLAST. The BLASTn results were summarized in Table 1 in the appendix.

3.6.3. Phylogenetic tree construction

Multiple sequence alignment was performed using the MUSCLE algorithm implemented in MEGA 11. The algorithm provides reliable alignment of homologous nucleotide sequences and is widely used for phylogenetic analyses of fungal gene regions (Tamura, Stecher, and Kumar, 2021). The alignment was configured with specific parameter settings to optimize accuracy and consistency. Gap penalties were set such that the gap opening cost was –400.00, while the gap extension penalty maintained at 0.00, ensuring that new gaps were strongly discouraged but extensions of existing gaps were permitted with minimal penalty. For computational efficiency, the maximum memory allocation was 2048 MB, and the algorithm

was allowed to run for up to 16 iterations. Clustering during the first and second iterations, as well as in subsequent iterations, was done using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method. Additionally, the minimum diagonal length (Lambda) was set at 24, which controls the length of diagonals retained in the alignment process. These settings ensured a balance between computational speed and the biological accuracy of the final multiple sequence alignment shown in Figure 5.

A nucleotide substitution model (Table 3) with the lowest Akaike Information Criterion, corrected (AICc), was selected to construct a maximum likelihood phylogenetic tree with 1000 bootstrap replication values to show the relatedness between the organisms and how each organism has evolved.

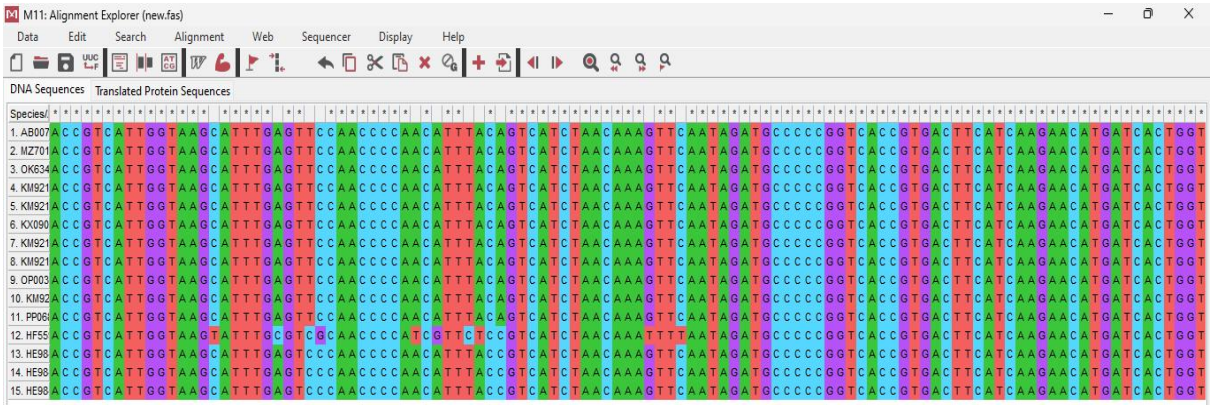


Figure 5: Multiple sequence alignment performed in MEGA 11.

Table 3:

Best nucleotide substitution models with the lowest AICc, used to construct maximum likelihood phylogenetic trees

S/No	Organism	Total samples	Nucleotide substitution model
01	*A. flavus*	07	Jukes Cantor (JC)
02	*A. oryzae*	15	JC
03	*A. niger*	05	Kimura 2 parameter (K2)
04	*F. chlamydosporum*	01	K2
05	*F. oxysporum*	01	JC

3.6.4. Sequencing similarity and identity index

Similar species isolated from different food types were compared to highlight the genetic relationship of fungal isolates across different food types. The sequence similarity and identity index among the organisms were recorded.

3.7. Data storage and quality management

The research findings were kept in safe folders encrypted by a password, only accessible by the researcher. A copy of the research findings was uploaded to a password-protected cloud-based storage like Google Drive. Quality management was crucial to ensure the reliability, reproducibility, and overall validity of scientific work. It was an ongoing process throughout the research cycle aimed at enhancing the quality of the findings. This was being done in the following ways: Data collection was accurate and consistent, standardized protocols were used, and data entry was validated for errors. Appropriate statistical methods were used; software tools and scripts used for analysis were also validated before analysis. Colleagues and experts in the field were consulted for feedback, and any identified issues or discrepancies were addressed.

3.7.1. Data dissemination plan

The data from the findings will be published in a well-known science journal. The data will also be disseminated to the community stakeholders in a community gathering. Posters and fliers will also be developed and used to disseminate the information.

3.7.2. Community engagement plan

Consultations were done with the relevant stakeholders, e.g., the LC1, store owners, and the local guide (translator). Questionnaires were also used to collect some information pertaining to the agronomic practices of the locals.

3.7.3. Biosafety considerations

Decontamination was achieved in the following ways: The used petri dishes were sterilized by autoclaving and incinerating. Fungal isolates were kept at -20 °C freezers with restricted access. After completion of the study, the molds were autoclaved and incinerated to avoid them getting out of the Lab.

3.8. Data analysis

Data obtained from laboratory analyses were entered into Microsoft Excel 2019 for coding, cleaning, and validation before being exported to IBM Statistical Package for Social Sciences (SPSS) version 25.0 for statistical analysis. Sequence-based analyses were performed using Molecular Evolutionary Genetics Analysis (MEGA) version 11 and the Basic Local Alignment Search Tool for nucleotides (BLASTn) available at the National Center for Biotechnology Information (NCBI). The analytical approaches used for each study objective are described below.

Objective 1: To identify the mycotoxigenic moulds associated with cereals in Karamoja

Fungal isolates were initially identified based on colony morphology and microscopic characteristics following culturing and sub-culturing procedures. Molecular identification was subsequently performed to confirm species identity. Raw sequence chromatograms generated through Sanger sequencing were imported into MEGA version 11 (Tamura *et al*., 2021), where sequence quality was assessed by examining electropherogram peaks. Low-quality nucleotide regions at the beginning and end of sequences were trimmed to obtain high-quality consensus sequences.

The cleaned sequences were exported in FASTA format and subjected to BLASTn searches against the NCBI GenBank database to identify fungal isolates based on sequence similarity with reference sequences (Johnson *et al.*, 2008). The fungal species identified were summarized using descriptive statistics, including frequencies and percentages. The distribution of mould species across cereal types was presented using tables and graphical illustrations.

Objective 2: To compare the occurrence of mycotoxigenic moulds associated with cereals in Karamoja

Descriptive statistical analyses were performed using SPSS version 25.0 to determine the occurrence of mycotoxigenic mould species in maize, sorghum, and millet samples. Frequencies and percentages were calculated to establish the prevalence of each fungal species within the different cereal types.

Cross-tabulation analysis was employed to compare the occurrence of mycotoxigenic moulds across the cereal types. The results were presented in tables and bar charts to

facilitate interpretation of occurrence patterns and contamination trends among the cereals (Field, 2018).

Objective 3: To determine the genetic relationship among the mycotoxigenic moulds associated with cereals in Karamoja

High-quality nucleotide sequences were aligned using the MUSCLE algorithm implemented in MEGA version 11 (Tamura *et al.,* 2021). Multiple sequence alignment was conducted to identify homologous nucleotide positions among the fungal isolates.

The most suitable nucleotide substitution model for each fungal species was selected based on the corrected Akaike Information Criterion (AICc), with the model having the lowest AICc value considered the best fit for phylogenetic analysis (Posada & Buckley, 2004). Maximum likelihood phylogenetic trees were constructed using 1,000 bootstrap replicates to assess the robustness of the inferred phylogenetic relationships (Felsenstein, 1981). Reference sequences retrieved from GenBank and appropriate outgroup sequences were incorporated to facilitate accurate rooting and interpretation of the phylogenetic trees.

Sequence similarity and identity indices among isolates originating from different cereal types were determined to assess genetic relatedness. The clustering patterns observed in the phylogenetic trees were used to infer evolutionary relationships, genetic similarity, and genetic divergence among the mycotoxigenic mould isolates. Bootstrap values greater than 70% were considered indicative of strong support for phylogenetic groupings (Hillis & Bull, 1993).

3.9. Ethical considerations

The research was done following the guidelines from Gulu University Research and Ethical Committee “GUREC-2024-864”. Permission to carry out field activities was sought from relevant authorities, like the area councillor. Consent forms were used to seek the consent of other participants who got involved in the study. The participants who were engaged for a relatively long time were compensated for the time offered. Participants' confidentiality was protected by assigning unique identification codes to questionnaires instead of recording personal identifiers such as names. Information collected was used solely for research purposes and was accessible only to the researcher and authorized supervisors. All data were securely stored, and the findings are presented in aggregate form to ensure that individual participants cannot be identified.

This chapter described the materials and methods used to achieve the study objectives. It outlined the study area, sample collection, laboratory isolation and identification of mycotoxigenic molds from cereal samples, molecular characterization using the TEF1-α gene region, and phylogenetic analyses employed to determine genetic diversity. The methods adopted provided a systematic approach for identifying the fungal species present, comparing their occurrence across cereal types and districts, and assessing their genetic relationships. The findings generated using these procedures are presented in the following chapter.

3.10. Limitations and Delimitations of the Study

This study was limited to maize, sorghum, and millet collected from six districts of the Karamoja sub-region, and therefore the findings may not fully represent the occurrence and genetic diversity of mycotoxigenic moulds in other cereal crops or districts within Uganda. In addition, molecular characterization was based on sequencing of the TEF1-α gene, which provides reliable species identification but may not capture the full extent of genetic variation that could be resolved using multilocus or whole-genome sequencing approaches.

Furthermore, only culturable fungal isolates recovered under the selected laboratory conditions were included in the molecular analyses, potentially excluding non-culturable or slow-growing fungal species. Nevertheless, the use of standardized sampling procedures, morphological characterization, PCR amplification, Sanger sequencing, and phylogenetic analysis provided robust and reliable data that adequately addressed the study objectives on the occurrence, predominance, and genetic diversity of mycotoxigenic moulds associated with cereals in the Karamoja sub-region.

CHAPTER FOUR

4.0. RESULTS

4.1. Occurrence of moulds in cereals

A total sum of 108 cereal samples were picked mainly from domestic homesteads (80 samples) and sometimes from commercial stores (28 samples) across each districts across Karamoja to assess the occurrence of mould contamination. Results (Figure 6) revealed that only 6% of the samples were free from mould contamination, while the majority (94%) were contaminated.

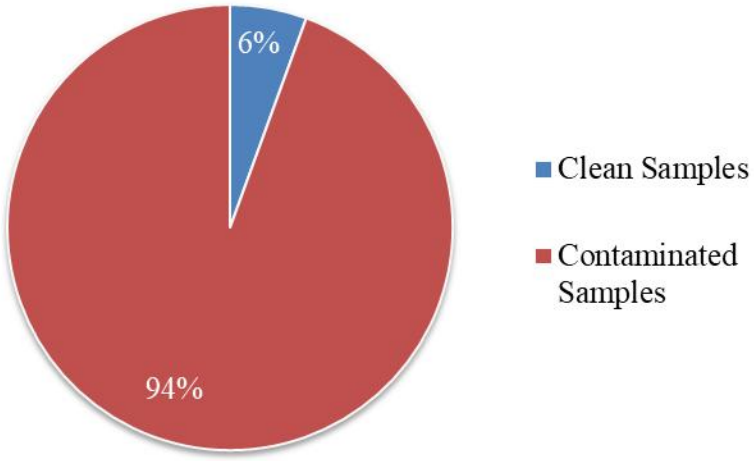


Figure 6: Percentage contamination of cereal samples collected from Karamoja sub region

4.1.1. Co-contamination

During culturing, 38 cereal samples exhibited evidence of co-contamination, where multiple fungal genera were present simultaneously. Figure 7 reveals overlapping growth of potential mycotoxigenic species (points 1,2 and 3) in one of the samples.

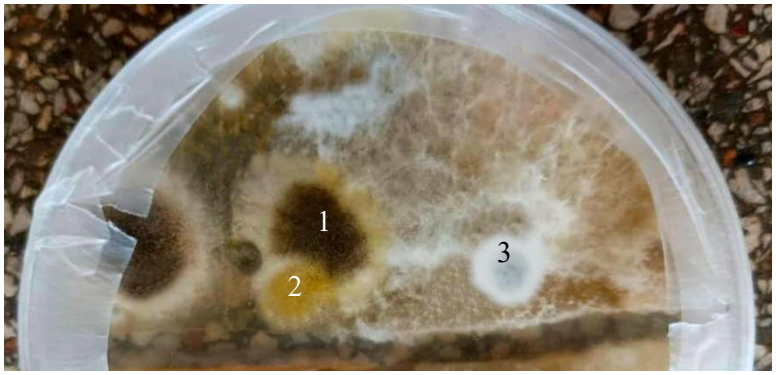


Figure 7: Co-contaminated fungal cultures, showing simultaneous growth of different fungal genera creates the perfect conditions for potentially toxic mycotoxigenic species to thrive (labeled 1, 2, and 3) within a single sample during culturing.

4.1.2. Contamination level in different cereal types

Out of the 94% contaminated samples, maize represented the highest proportion at 33%, followed closely by sorghum at 31%, while millet showed the lowest contamination level at 28% as shown in Figure 8.

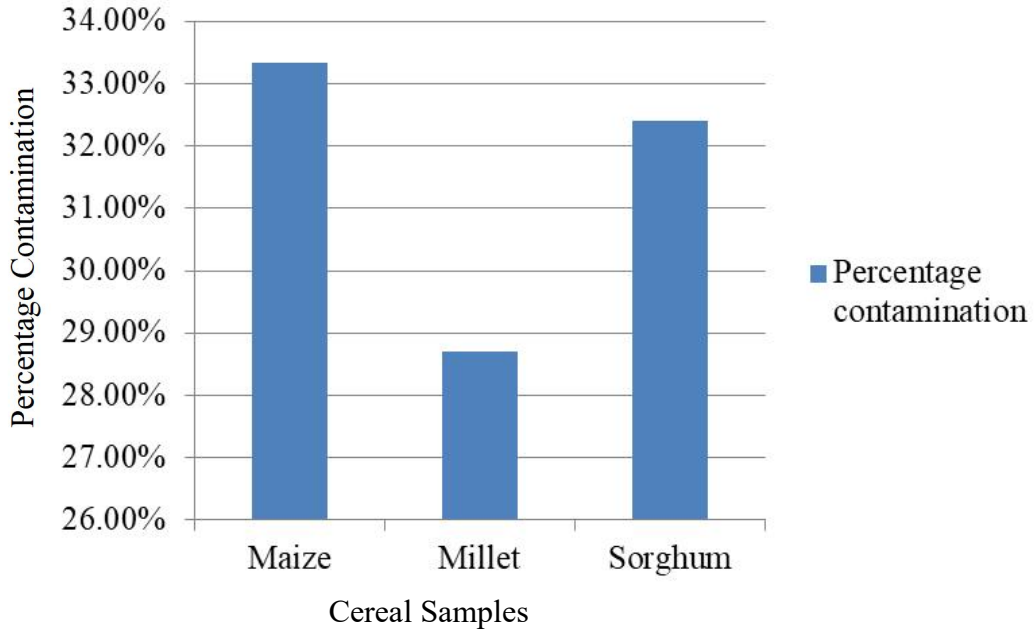


Figure 8: Percentage contamination levels of the three cereal types; maize, millet, and sorghum.

4.1.3. Contamination level in different districts

Different districts had varying grain contamination levels ranging from the highest contamination of 100% in the districts of Kotido and Karenga, followed by 94.4% in the districts of Abim and Moroto, and the least was 88.8% in the districts of Kaabong and Napak, as shown in Figure 9.

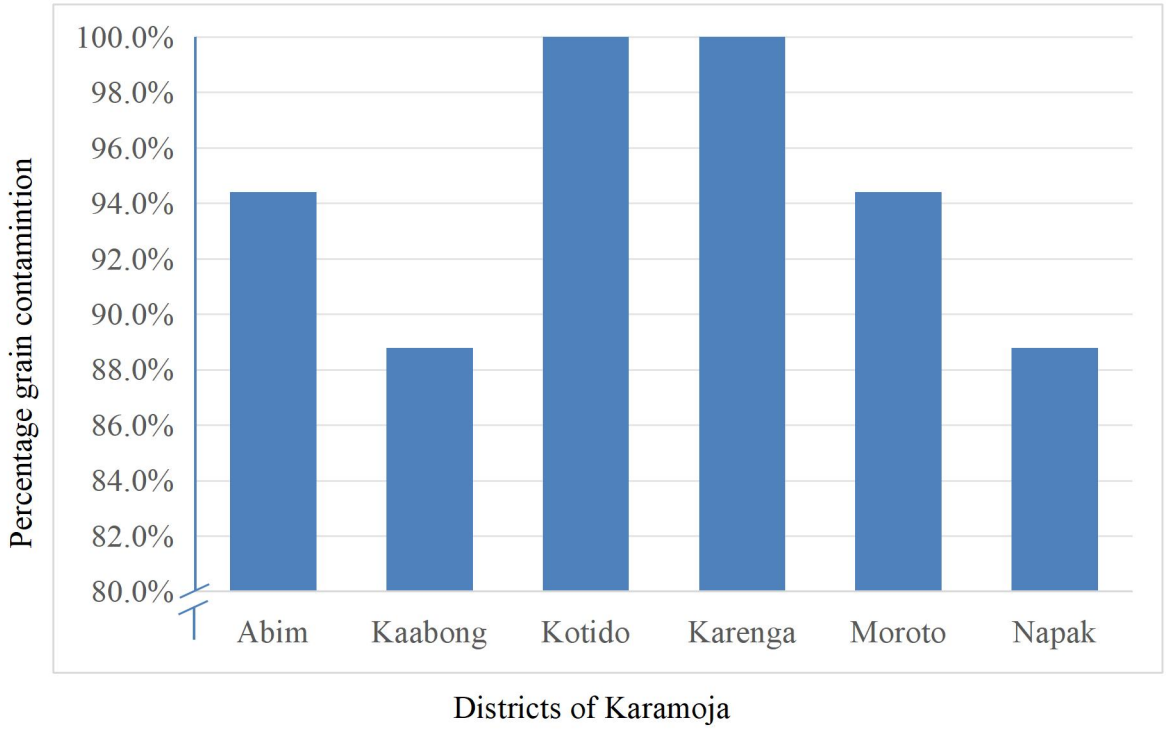


Figure 9: Percentage grain contamination in the districts of Karamoja.

4.1.4. Contamination level with respect to sample sources

79 out of the 80 samples sourced from domestic homesteads were contaminated while 23 out of the 28 samples picked from commercial stores were contaminated as shown in Figures 10(a) and 10(b).

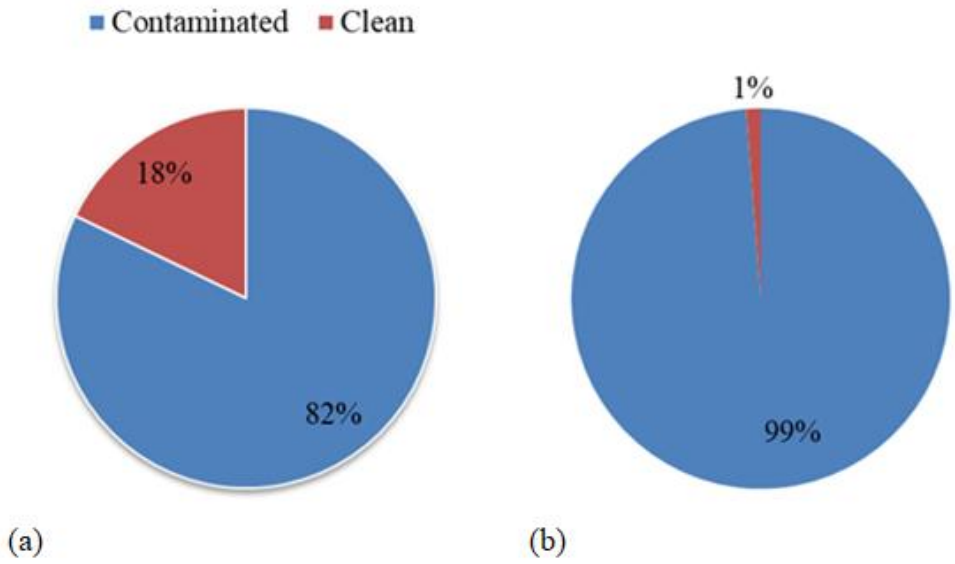


Figure 10: Percentage of clean and contaminated samples in *(a)* commercial grains picked from stores and *(b)* domestic grains picked from homesteads.

4.2. Predominance of molds in cereals

Analysis of the different fungal isolates revealed variation in their relative abundance across the cereal samples, as shown in Figure 11. *Aspergillus* spp. represented more than 70% of the isolates, followed by *Fusarium* spp, which represented 22% of the isolates. *Penicillium* spp and *Alternaria* spp. were present at relatively lower proportions, representing 6% and 2% respectively. This distribution pattern indicates that *Aspergillus* species dominate the mycobiota of the cereals, while *Alternaria* spp. occur only as minor contaminants.

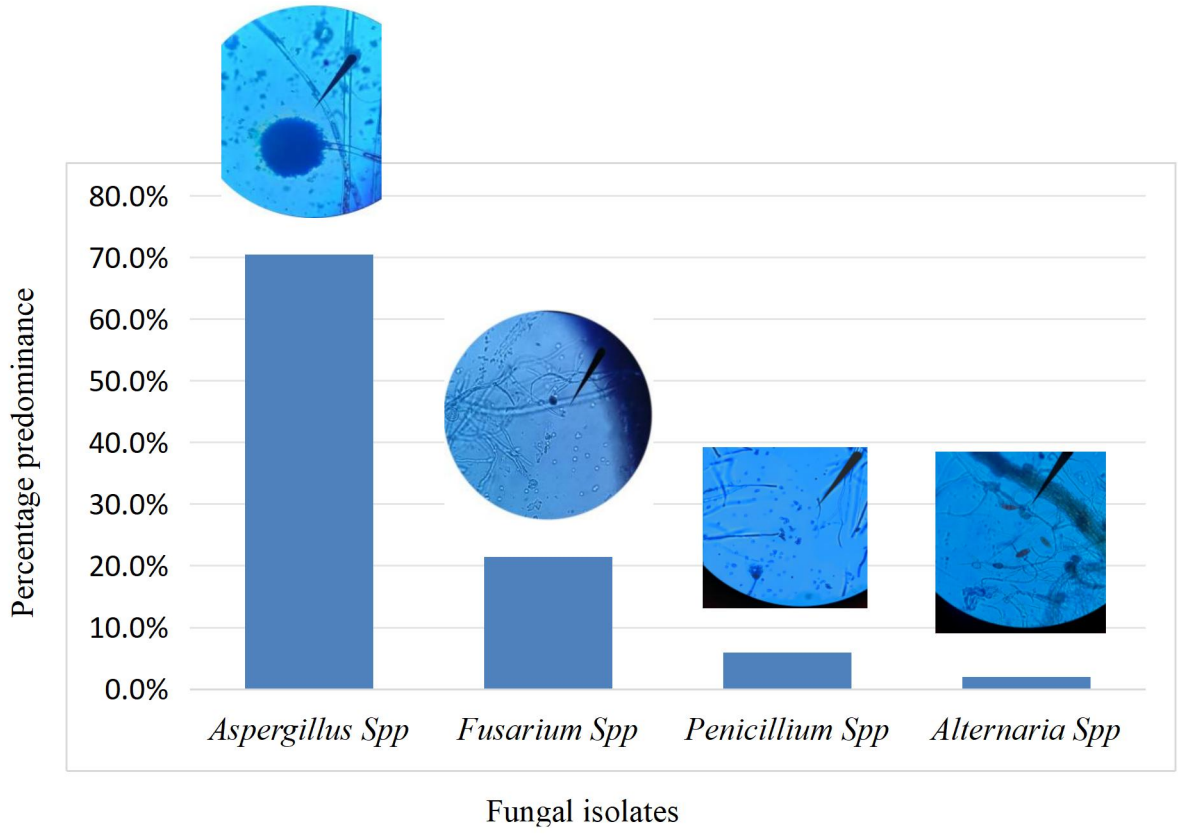


***Figure 11*:** Percentage predominance of fungal species isolated from cereal samples from Karamoja sub region.

4.3. Genetic diversity of mycotoxin producing molds

4.3.1. PCR Amplification

Genomic DNA was successfully extracted from fungal spores using the CTAB extraction method as described by Carr *et. al.* (2022). PCR amplification was performed using both ITS and EF-1α primers. EF-1α primers produced the most consistent and reliable amplification results hence preferred (gel image in Figure 12).

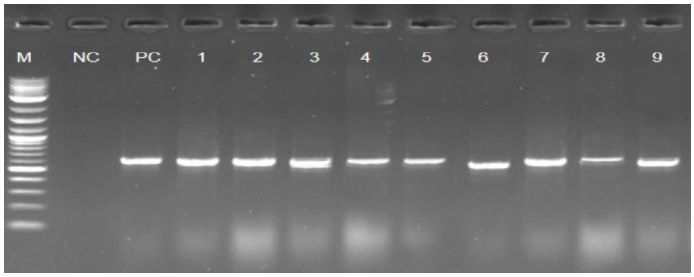


Figure 12: Agarose gel electrophoresis of TEF1-α PCR amplicons from representative fungal isolates. M = 100 bp DNA ladder; NC = negative control; PC = positive control; lanes 1–9 = fungal isolates. The amplified fragments were approximately 300 bp in size.

4.3.2. The BLASTn

Following successful sequencing of the EF1-α PCR amplicons, the sequences were subjected to BLASTn searches against the NCBI nucleotide database for species identification. The best matches for each isolate are presented in Appendix Table 1. Analysis of the 29 isolates identified two fungal genera, *Aspergillus* and *Fusarium*. *Aspergillus* predominated, with *A. oryzae* (15 isolates), *A. flavus* (7), and *A. niger* (5), while *Fusarium chlamydosporum* and *F. oxysporum* were each represented by one isolate (Figure 13). Contamination levels of the major mycotoxigenic species across the three cereal types are presented in Figure 14.

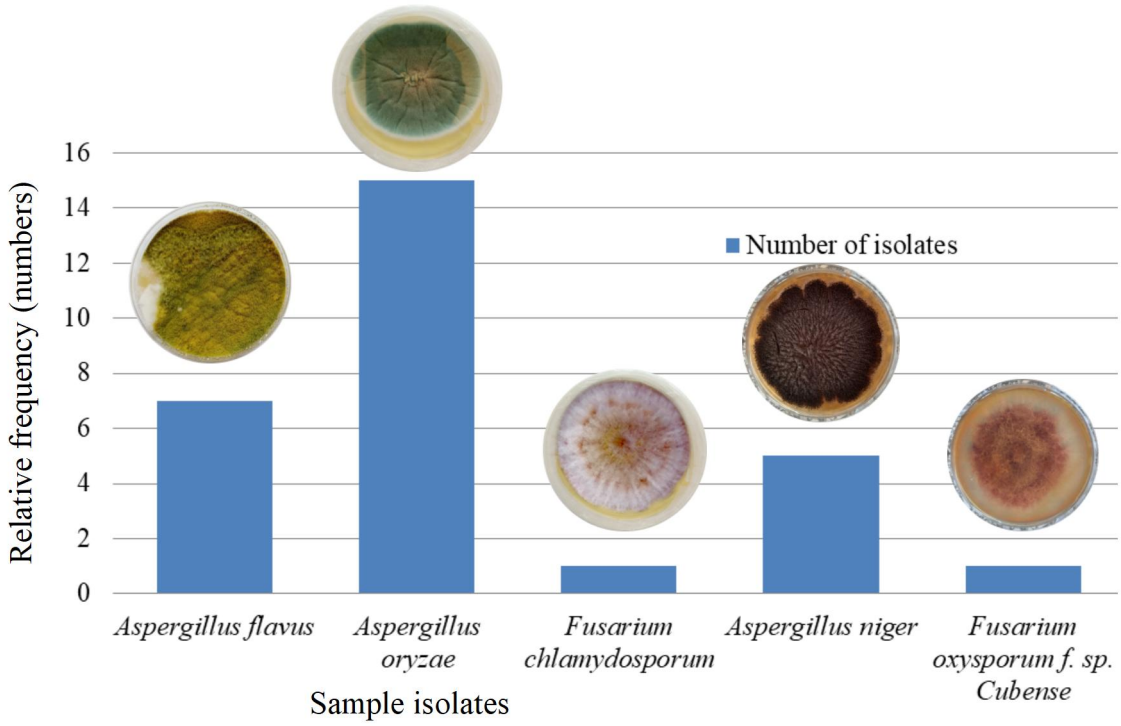


Figure 13: Relative frequency (numbers) of each sample isolates

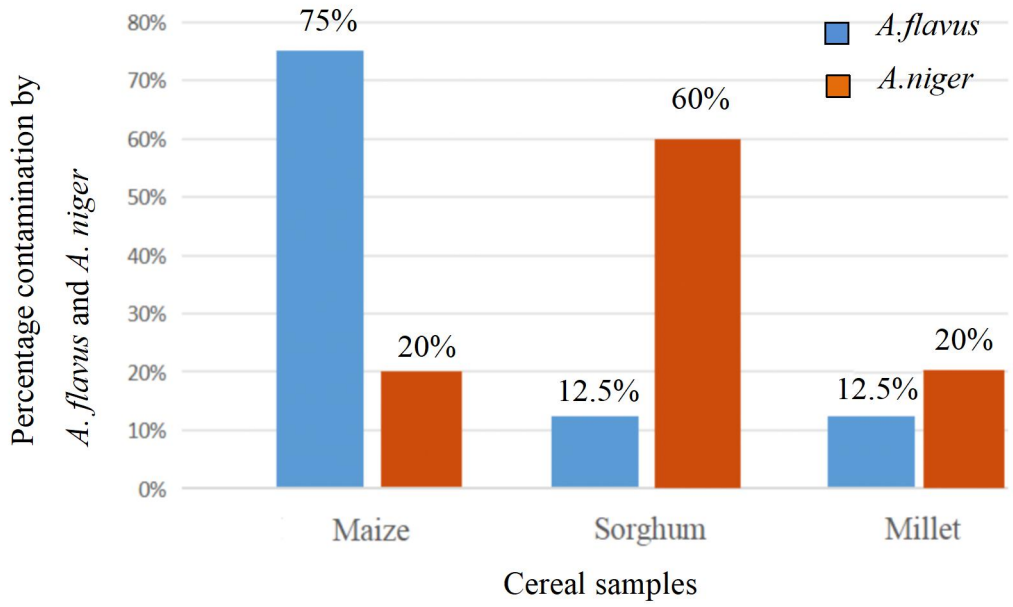


Figure 14: Percentage contamination by *A. flavus* and *A. niger* in different cereal samples

4.3.3. Phylogenetic tree

Phylogenetic Analysis of *Aspergillus flavus* Isolates

A Maximum Likelihood phylogenetic tree was built following the Jukes–Cantor substitution model with 1000 bootstrap replicates to examine the evolutionary relationships of *Aspergillus flavus* isolates (Figure 15). The tree was rooted with an outgroup sequence (red square), and reference sequences from GenBank are shown as green open rings.

All study isolates (green filled circles) grouped within the *A. flavus* clade with the reference sequences, which confirms their taxonomic placement as *A. flavus*. High bootstrap values across the major branches (>70%) indicate good phylogenetic support.

The isolates from different cereal grains and districts (S1: millet, Moroto; S4: maize, Abim; S6: sorghum, Kaabong; S31: maize, Moroto; S32: maize, Nakapiripirit; S38: maize, Kaabong; S40: maize, Abim) did not form separate sub-clusters. Instead, they were distributed within a single clade with the reference strains, which shows limited sequence difference among the isolates.

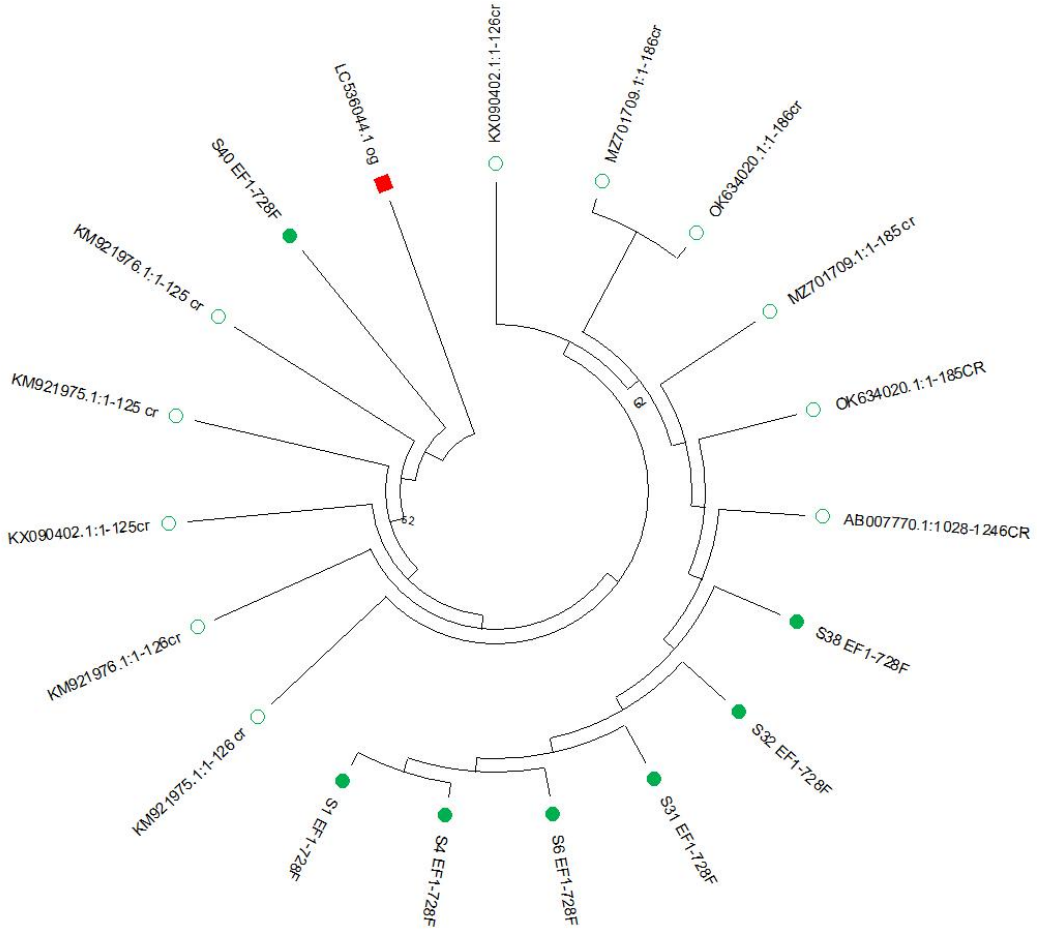


Figure 15: Phylogenetic tree of *Aspergillus flavus* isolates. The green shaded circles are query samples, the green rings are the reference organisms and the red box is the out group organism.

Phylogenetic analysis of *Aspergillus niger*

The *Aspergillus niger* phylogenetic tree was made using the Kimura 2-parameter model. The sequences clustered closely with reference sequences from the NCBI database, which shows similarity among isolates (S25, S13, S34, S42, and S12). Strong bootstrap values (e.g., 85 and 87) support the branching reliability. The reference sequence LC468066.1 was used as the outgroup (red), which clearly separates the *A. niger* isolates from other related species, as shown in Figure 16.

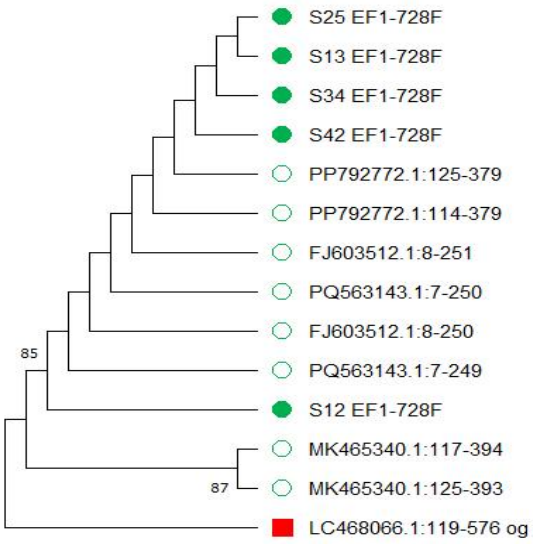


Figure 16: Phylogenetic tree of *Aspergillus niger* in the isolates. The green shaded circles are query samples, the green rings are the reference organisms and the red box is the out group organism.

Phylogenetic analysis of *Penicillium citrinum*

The *Penicillium citrinum* phylogenetic tree was made using the Jukes-Cantor model. The isolate (S36 EF1-728F) grouped closely with reference sequences like OP066898.1 and OP066911.1, forming a supported clade. Bootstrap values (e.g., 66, and 76) give reasonable support for the evolutionary relationships. The sequence LC468066.1 was included as an out-group (red), clearly distinguished from *P. citrinum* related species, as shown in Figure 17.

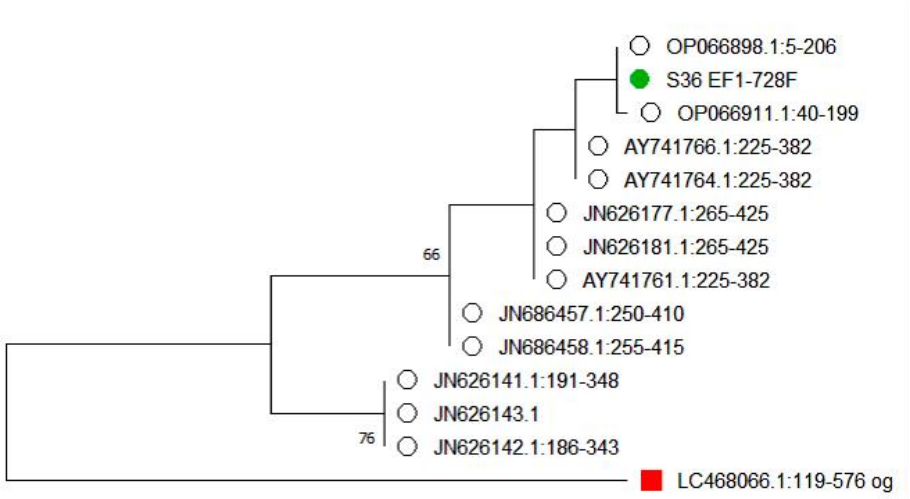


Figure 17: Phylogenetic tree of *Penicillium citrinum* in the samples. The green shaded circles are query samples, the green rings are the reference organisms and the red box is the out group organism.

4.3.4. The sequence similarity and identity index among the organisms

BLAST Sequence Identity

The percentage identity values from BLASTn searches of all isolates against GenBank reference sequences showed similarity to their closest database matches, with identity values ranging from 95.2% to 100%. Most isolates showed ≥ 98% identity, and this indicates a strong species-level matches, while a few had slightly lower values (95 – 96%), suggesting minor differences from reference sequences. Overall, our findings showed that the isolates were highly conserved and reliably identified through BLASTn analysis.

This chapter presented the findings on the occurrence and genetic diversity of mycotoxigenic molds associated with cereal crops in the Karamoja sub-region. The results revealed a high prevalence of fungal contamination across the sampled cereals, with *Aspergillus* spp. being the predominant genus, followed by *Fusarium, Penicillium*, and *Alternaria*. Maize recorded the highest level of contamination, while Kotido and Karenga districts exhibited the greatest contamination prevalence. Molecular characterization using the TEF1-α gene successfully identified several mycotoxigenic fungal species, including *Aspergillus flavus, A. niger, Fusarium* spp., and *Penicillium citrinum*. Phylogenetic analyses further confirmed the species identities and demonstrated close genetic relationships among isolates from different cereal types and districts. Collectively, these findings address the study objectives and provide the basis for the interpretation and comparison with existing literature presented in the subsequent discussion chapter.

CHAPTER FIVE

5.0: DISCUSSION OF RESULTS

5.1 Occurrence

In this study, we assessed the occurrence and predominance of moulds in cereal samples from Karamoja to determine the extent of fungal contamination and the key species involved. Our findings revealed a high prevalence, with 94% of the samples contaminated and only 6% remaining free of moulds. This agrees with a study linking fungal occurrence to warm, humid conditions and poor handling (Mwadzingeni *et al*., 2024) and with another study from Northern Uganda where just 7% of grains were toxin-free (Wokorach *et al*., 2021). The high prevalence suggests cereals in Karamoja are highly prone to fungal colonization, compromising safety and quality.

5.1.1. Co-contamination

Co-contamination was observed in some cereal samples, where more than one mycotoxigenic fungal genus occurred within the same sample. The simultaneous presence of genera such as *Aspergillus, Fusarium, Penicillium,* and *Alternaria* is of particular concern because different fungal species may produce different classes of mycotoxins, thereby increasing the potential risk to food safety. Mixed fungal populations may also interact during colonization, creating conditions that favour fungal persistence and complicating efforts aimed at preventing contamination through conventional storage and handling practices.

The occurrence of co-contamination in this study agrees with findings from other cereal-producing regions, where multiple mycotoxigenic fungi commonly coexist in the same grain samples (Goda et al., 2025). Such co-contamination increases the risk of simultaneous exposure to multiple mycotoxins with additive or synergistic health effects. These findings underscore the need for integrated fungal surveillance and improved pre- and post-harvest management practices in Karamoja's cereal value chain.

5.1.2. Contamination level in different cereal types

Among the contaminated cereal samples, maize exhibited the highest level of fungal contamination (33%), followed by sorghum (31%), while millet recorded the lowest contamination level (28%). These findings suggest that maize is more susceptible to

colonization by mycotoxigenic fungi than sorghum and millet. The relatively high contamination observed in maize may be attributed to its higher carbohydrate content, larger grain size, and greater moisture retention capacity, which provide favourable conditions for fungal growth during storage. In contrast, millet has been reported to possess structural and biochemical characteristics, including high levels of phenolic compounds and tannins, which may inhibit fungal growth and reduce its susceptibility to contamination (Adebiyi *et al.*, 2022).

The observed trend is consistent with findings from other studies that have reported maize as one of the cereal crops most vulnerable to fungal contamination and mycotoxin accumulation during storage. For instance, Chala *et al.* (2014) found higher frequencies of fungal contamination in maize compared to other cereals in Eastern Africa, attributing this to poor post-harvest handling practices and favourable environmental conditions for fungal proliferation. Although the differences between maize and sorghum in the present study were relatively small, both cereals demonstrated a greater susceptibility to contamination than millet. Overall, these results indicate that fungal contamination is widespread across all cereal types in Karamoja, with maize bearing the greatest contamination burden and therefore requiring particular attention in fungal pre and post-harvest management programmes.

5.1.3. Contamination level in different districts

The findings reveal a widespread occurrence of fungal contamination across all surveyed districts, with Kotido and Karenga recording the highest contamination levels (100%), followed by Abim and Moroto (94.4%), while Kaabong and Napak had the lowest contamination levels (88.8%). The consistently high contamination rates across the districts suggest that fungal contamination of cereals is a regional challenge rather than an isolated problem. These findings may reflect similarities in climatic conditions, cereal handling practices, storage systems, and limited implementation of effective post-harvest management strategies throughout the Karamoja sub-region.

The contamination levels observed in this study are considerably higher than those reported in other parts of East Africa. For example, Kaaya and Kyamuhangire (2006) reported fungal contamination in stored maize from different regions of Uganda, but at lower frequencies than those observed in the present study. The exceptionally high contamination levels recorded in Karamoja may be attributed to prolonged storage periods, inadequate drying of

grains before storage, and the warm environmental conditions that favour fungal growth and persistence. The narrow variation in contamination levels among districts further indicates that the factors driving contamination are widespread across the region. Consequently, the high prevalence of fungal contamination observed in this study raises concerns regarding food safety, food security, and potential exposure of local communities to mycotoxins, highlighting the need for strengthened fungal surveillance and improved post-harvest management practices throughout Karamoja.

5.1.4. Contamination level in samples from different sources

The contamination data reveals a significant disparity between grain samples sourced from domestic homesteads and those obtained from commercial stores. Of the 80 samples collected from households, an overwhelming 98.75% (79 samples) were contaminated, indicating that grains stored or handled domestically are highly vulnerable to contamination. In contrast, commercial stores showed a lower contamination rate of 82.14%, with 23 out of 28 samples affected. This difference suggests that commercial grain handling may involve better storage practices, more frequent quality checks that reduce exposure to contaminants. The higher contamination in domestic settings may be attributed to inadequate infrastructure, lack of awareness about safe storage, and or limited access to preservation technologies. However, the fact that contamination is still prevalent in both settings points to systemic issues in grain production and distribution.

5.2. Predominance

The present study revealed that *Aspergillus spp*. constituted more than 70% of all fungal isolates recovered from cereal grains in the Karamoja sub-region, making it the predominant fungal genus. This finding suggests that environmental conditions within the region strongly favour the growth and persistence of *Aspergillus species*. The warm temperatures, periodic drought conditions, and suboptimal storage practices in Karamoja may provide favourable conditions for the proliferation of *Aspergillus spp*., many of which are known producers of aflatoxins. The predominance of this genus is therefore of considerable concern due to its implications for food safety and public health.

The dominance of *Aspergillus spp*. observed in this study is consistent with findings reported elsewhere in East Africa. Chala *et al*. (2014) reported that *Aspergillus species* were among the most frequently isolated fungi from cereal grains in Ethiopia, particularly under

conditions characterized by inadequate drying and storage. Similarly, the relatively lower occurrence of *Fusarium spp*. (22%) in the present study may reflect the predominantly dry conditions of Karamoja, as *Fusarium species* are often associated with field infections and tend to thrive under relatively higher moisture conditions. *Penicillium spp*. (6%) and *Alternaria* spp. (2%) were recovered at much lower frequencies, suggesting a limited contribution to the overall fungal community. Nevertheless, their presence remains important because several species within these genera are capable of producing mycotoxins and contributing to grain spoilage. Overall, the fungal distribution pattern observed in this study indicates that environmental conditions and pre and post-harvest handling practices in Karamoja strongly favour *Aspergillus* colonization, particularly in maize, thereby increasing the potential risk of aflatoxin contamination.

5.3 Genetic diversity of fungal isolates

The molecular characterization and phylogenetic analyses demonstrated that the fungal isolates recovered from cereals in Karamoja comprised several mycotoxigenic species, including *A. flavus, A. niger, Fusarium chlamydosporum, Fusarium proliferatum,* and *Penicillium citrinum*. BLASTn analysis showed high sequence similarity between the study isolates and reference sequences in GenBank, with percentage identity values ranging from 95.2% to 100%. The high sequence identity values, together with the clustering patterns observed in the phylogenetic trees, confirmed the taxonomic identities of the isolates and demonstrated the reliability of EF1-α sequencing for fungal identification.

Phylogenetic analyses further revealed that isolates of *A. flavus, A. niger*, and *P. citrinum* clustered closely with their respective reference strains and were supported by moderate to high bootstrap values. Isolates originating from different cereal types and districts did not form distinct host-specific or location-specific clades but instead grouped within the same species lineages. This suggests limited genetic diversity among the fungal populations circulating in the Karamoja sub-region. Similar findings were reported by Atehnkeng *et al*. (2008), who observed widespread distribution of closely related *A. flavus* strains across different agroecological zones in Africa. Likewise, Probst *et al.* (2012) reported that *A. flavus* populations associated with staple crops often exhibit limited genetic differentiation despite occurring across geographically separated locations.

Although the EF1-α gene region revealed relatively low sequence variation among the isolates, the occurrence of multiple fungal species demonstrates considerable species diversity within cereal grains in Karamoja. The presence of *Aspergillus, Fusarium,* and *Penicillium species* is particularly important because members of these genera are associated with the production of aflatoxins, fumonisins, and other mycotoxins of public health significance. Therefore, the combined use of BLASTn analysis and phylogenetic inference not only confirmed species identity but also highlighted the widespread distribution of genetically related mycotoxigenic fungi across cereal types and districts within the Karamoja sub-region.

This chapter discussed the study findings in relation to existing literature and the study objectives. The discussion confirmed the widespread occurrence of mycotoxigenic molds in cereal crops from the Karamoja sub-region, with variations observed across cereal types and districts. It further demonstrated the predominance of *Aspergillus* species and highlighted the occurrence of other important mycotoxigenic genera, including *Fusarium, Penicillium*, and *Alternaria*. Molecular and phylogenetic analyses confirmed the identities of the fungal isolates and revealed close genetic relationships among isolates from different cereals and geographical locations. Collectively, these findings address the study objectives by identifying the mycotoxigenic molds present, comparing their occurrence, and characterizing their genetic diversity. The implications of these findings are summarized in the following chapter.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

6.1.1. Occurrence of Mycotoxigenic Moulds in Karamoja Cereals

This study confirms that fungal contamination in cereals across Karamoja is both pervasive and dominated by mycotoxigenic species. Virtually all sampled grains; maize, sorghum, and millet showed evidence of fungal infestation. The high prevalence of *A. flavus* and *A. niger*, both known for their aflatoxin production and other secondary metabolites, signals a serious food safety threat. The detection of *Fusarium* species, though less frequent, further compounds the risk due to their fumonisin production abilities.

The widespread nature of contamination suggests that the cereal handling conditions in Karamoja such as high humidity, poor drying practices, and inadequate storage infrastructure create a conducive environment for fungal proliferation. These findings align with global patterns observed in regions of tropics and subtropics, known for their disproportionately high post-harvest losses and mycotoxin exposure (Pandey *et al*., 2023).

6.1.2. Predominant Mycotoxigenic Moulds and Cereal Susceptibility

Among the isolates, *Aspergilus flavus* and *Aspergillus niger* were consistently dominant across all districts and cereal types. Maize emerged as the most vulnerable crop, likely due to its higher moisture retention and larger kernel surface area, which facilitate fungal colonization. Sorghum showed intermediate susceptibility, while millet demonstrated relative resilience possibly due to its smaller grain size and tighter husk structure.

The co-occurrence of multiple fungal genera within single samples presents a multifaceted challenge. Mixed contamination can lead to synergistic toxin production, complicating detection and mitigation efforts. This underscores the need for integrated mycotoxin management strategies that address both fungal diversity and cereal-specific vulnerabilities.

6.1.3. Genetic Relationship and Molecular Characterization

Molecular analysis using PCR amplification of EF1 alpha regions followed by Sanger sequencing provided robust identification of fungal isolates. BLASTn results confirmed very

high sequence similarity to reference strains, validating the classification of isolates as *A. flavus* and *A. niger*. Phylogenetic trees constructed using Maximum Likelihood methods (Jukes–Cantor for *A. flavus*, Kimura 2-parameter for *A. niger*) revealed strong bootstrap support for clustering with GenBank reference sequences.

Notably, isolates from different cereals and districts did not form distinct clades, indicating genetic homogeneity across commodities and geography. This suggests that *A. flavus* and *A. niger* populations in Karamoja are ecologically stable and well-adapted to local conditions. The genetic preservation of *A. flavus* across diverse substrates and environments highlights its evolutionary fitness and reinforces its role as a persistent contaminant.

Given *A. flavus*'s known aflatoxin biosynthesis pathways and its dominance in the region, the findings point to a chronic and systemic food safety risk. The genetic stability of these strains may also limit the success level of conventional mitigation strategies, necessitating innovative approaches like bio-control and genomic surveillance.

6.2 Recommendations

6.2.1 Strengthen Food Safety Monitoring and Infrastructure

The **Ministry of Agriculture, Animal Industry and Fisheries (MAAIF)**, in collaboration with the **Uganda National Bureau of Standards (UNBS)** and district local governments, should establish decentralized aflatoxin testing centres to facilitate timely detection and monitoring of cereal contamination. MAAIF and agricultural extension services should also promote the adoption of hermetic storage technologies such as Purdue Improved Crop Storage (PICS) bags and develop mobile-based advisory systems to disseminate information on contamination risks and appropriate post-harvest management practices.

6.2.2 Advance Genetic and Functional Characterization

Universities, the National Agricultural Research Organisation (NARO), and other research institutions should expand molecular studies by employing high-resolution markers such as microsatellites, single nucleotide polymorphisms (SNPs), and whole-genome sequencing to improve strain discrimination and understand fungal virulence and toxigenic potential. These institutions should also investigate the expression of aflatoxin biosynthesis genes under different environmental conditions and collaborate with regional and

international research partners to establish a fungal sequence database for surveillance and comparative studies.

6.2.3 Enhance Farmer Awareness and Capacity Building

District production departments, agricultural extension workers, non-governmental organizations (NGOs), and farmer cooperatives should implement continuous training programmes on Good Agricultural Practices (GAP) and Good Storage Practices (GSP). These stakeholders should also disseminate educational materials on safe drying, storage, and recognition of fungal spoilage, while supporting community-based drying platforms and shared storage facilities to minimize post-harvest fungal contamination.

6.2.4 Strengthen Policy and Regulatory Frameworks

The **Government of Uganda**, through **MAAIF**, **UNBS**, and relevant regulatory agencies, should strengthen enforcement of national aflatoxin standards for cereals marketed locally and internationally. Government should further support the registration and adoption of proven biological control technologies such as atoxigenic *Aspergillus flavus* strains and promote public-private partnerships to improve access to affordable aflatoxin testing services and safe grain storage technologies.

6.2.5 Integrate Mycotoxin Risk into Public Health Programmes

The **Ministry of Health (MoH)**, in collaboration with district health offices and development partners, should integrate mycotoxin risk communication into existing nutrition and food safety programmes. Health workers should be trained to recognize the health effects associated with chronic mycotoxin exposure and provide appropriate nutrition and food safety education, particularly to vulnerable populations such as children and pregnant women. Public health awareness campaigns should also be strengthened to improve community understanding of the long-term health risks associated with mycotoxin exposure.

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APPENDIX 1

Table 1: The summary of BLASTn results

Sample Identity	Scientific Name	Max Score	Total Score	Query Cover	E Value	Per. Ident	Acc. Len	Accession
S1	*Aspergillus flavus*	339	339	83%	1e-88	98.93%	835	OK634020
S2	*Aspergillus oryzae*	401	401	100%	2e-107	98.25%	3181	AB007770
S3	*Fusarium chlamydosporum*	832	832	100%	0.0	99.34%	577	LC468066
S4	*Aspergillus flavus*	335	335	84%	2e-87	98.92%	835	OK634020
S5	*Aspergillus oryzae*	291	291	99%	4e-74	91.55%	3181	AB007770
S6	*Aspergillus flavus*	329	329	83%	8e-86	99.44%	835	OK634020
S7	*Aspergillus oryzae*	364	364	100%	2e-96	94.98%	3181	AB007770
S9	*Aspergillus oryzae*	401	401	100%	2e-107	98.67%	3181	AB007770
S10	*Aspergillus oryzae*	407	407	100%	4e-109	99.12%	3181	AB007770
S12	*Aspergillus niger*	466	466	95%	8e-127	99.61%	379	PP792772
S13	*Aspergillus niger*	448	448	93%	3e-121	96.99%	379	PP792772
S14	*Aspergillus oryzae*	412	412	100%	9e-111	99.13%	3181	AB007770
S15	*Aspergillus oryzae*	305	305	99%	1e-78	91.89%	3181	AB007770
S16	*Aspergillus oryzae*	394	394	99%	3e-105	98.22%	3181	AB007770
S21	*Aspergillus oryzae*	390	390	99%	4e-104	98.19%	3181	AB007770
S25	*Aspergillus niger*	451	451	100%	2e-122	96.67%	121589	AM270408
S26	*Aspergillus oryzae*	394	394	99%	3e-105	98.63%	3181	AB007770
S27	*Aspergillus oryzae*	405	405	99%	1e-108	99.55%	3181	AB007770
S28	*Aspergillus oryzae*	374	374	66%	6e-99	95.91%	3181	AB007770
S29	*Aspergillus oryzae*	405	405	100%	1e-108	99.55%	3181	AB007770
S30	*Aspergillus oryzae*	361	361	100%	3e-95	94.52%	3181	AB007770
S31	*Aspergillus flavus*	305	305	85%	1e-78	94.62%	835	OK634020
S32	*Aspergillus flavus*	337	337	85%	5e-88	98.92%	835	OK634020
S34	*Aspergillus niger*	270	270	94%	6e-68	83.06%	379	PP792772
S38	*Aspergillus flavus*	340	340	83%	4e-89	99.46%	835	OK634020
S39	*Fusarium oxysporum f. sp. Cubense*	95.2	95.2	74%	4e-15	75.47%	710	MT809757
S40	*Aspergillus flavus*	318	318	84%	2e-82	96.24%	835	OK634020
S41	*Aspergillus oryzae*	398	398	100%	2e-106	98.64%	3181	AB007770
S42	*Aspergillus niger*	468	468	100%	2e-127	97.08%	121589	AM270408