CORRELATION BETWEEN MAIZE LETHAL NECROSIS DISEASE AND MYCOTOXIN IN MAIZE IN BOMET, NAROK AND NAKURU COUNTIES, KENYA.

BY

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DECLARATION

I **Evans Natembeya Mwasame** declare that this thesis is my original work and it has not been presented for award of a degree in any other university or any other award.

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Signature.....Date.....4th November 2022

We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as university supervisors

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LIST OF ABBREVIATIONS

AGRA	Alliance for Green Revolution in Africa
CDC	Centre for Disease Control
CYMMIT	International Maize and Wheat Improvement Centre
DRC	Democratic Republic of Congo
ELISA	Enzyme Linked Immunosorbent Assay
FAOSTAT	Food and Agriculture Organization Corporate Statistics Database
HPLC	High Perfomance Liquid Chromatography
KARI	Kenya Agricultural Research Institute
MCMV	Maize Chlorotic Mottle Virus
MDMV	Maize Dwarf Mosaic Virus
MLND	Maize Lethal Necrosis Disease
MOA	Ministry of Agriculture
MT	Metric Tonnes
NA	Nutrient Agar
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar.
RNA	Ribonucleic Acid
SCMV	Sugarcane Mosaic Virus
SNA	Synthetic Nutrient Agar
USA	United States of America

ABSTRACT

Maize Lethal Necrosis Disease (MLND) is believed to increase concomitant toxic fungal infections in maize. The goal of this study was to discover a link between MLND and fungal infections. During the 2018/2019 maize growing seasons, a Randomized Complete Block Design experiment with three replicates was set up in farmer's fields in three experimental sites in Bomet, Narok, and Nakuru Counties. Six hybrid maize varieties classified as resistant (KATEH16-02 and DK777), tolerant (WE5135 and WE5140), and susceptible (DUMA 43 and PH30G-20) to MLND were used. A total of 54 maize grain samples were collected. Fungal colonies were isolated on Potato Dextrose Agar medium and identified using morphological and microscopic techniques. ELISA test was used to quantify aflatoxins. The samples yielded 4561 fungal isolates, with Fusarium being the most predominant genus (79.75%), followed by *Penicillium* (16.03%) and *Aspergillus* (1.933%). A positive significant correlation between the MLND bioassay score, *Penicilium* and *Aspergillus* infection was achieved, ($r = 0.429$, $p = 0.001$) and ($r = 0.275$, $P = 0.5$) respectively. Aflatoxin concentration levels of between 0.3 and 2.8 µg/kg were detected in 38.89 % of the samples. Aflatoxin and MLND were significantly correlated ($p = 0.001$, $r^2 = 0.547$). Although this study was only able to show Aflatoxins, there is a strong likelihood of other mycotoxins occurring

in maize grains in higher proportions. Therefore the public should be made aware of the mycotoxin risks associated with consumption of MLND infected maize.

CHAPTER ONE

1.1 BACKGROUND

Maize plays an important role in people's livelihoods in Kenya as it is the most valuable staple food for the country. Maize availability in Kenya is tied to food security, as it forms an important component of the feed and food system as well as a major income source for households in the rural setting (Tefera *et al*., 2011). The crop is cultivated by more than 38% of Kenya's food crop farmers, with Rift-Valley region being the main maize producer, accounting for 90% of annual maize production. Uasin-Gishu, Trans-Nzoia, and Nandi are the main producing counties in the Rift Valley (Nyoro *et al*., 2001). Bungoma, Kakamega, Busia, Nyanza, and parts of Eastern counties are also major maize producing counties.

Approximately three million smallholder farmers with an average of two hectares of land produce 70% of Kenya's total maize. Commercial farmers grow the remaining 30% of maize on average over 20 acres of land. The majority of maize produced by small-scale farmers (60%) is consumed by the farmers themselves, with the remainder sold to the local market. Large-scale farmers grow maize primarily for commercial purposes. Their maize is sold to millers and to the national cereal boards (Nyoro *et al*., 2001).

Despite the fact that maize is a primary staple food, production in most African countries remains low (FAOSTAT, 2014). Due to a variety of factors, including diseases such as Maize Lethal Necrosis Disease (MLND), pests such as stem borers and fall army worms and fungal infections. Fungal infections leads to production of mycotoxins, the most prominent of which is aflatoxin (Gnonlonfin *et al*., 2013).

Maize Lethal Necrosi Disease is a serious disease that was discovered in Kenya in 2011 (Wangai *et al*., 2012). Since its discovery, the disease has spread to other East African countries such as Rwanda, Tanzania, Uganda, Ethiopia, and the Democratic Republic of the Congo. Kenyan counties most affected by MLND include Nakuru, Bomet, Narok, Baringo, Uasin Gishu, Kisii, Trans-Nzoia, Bungoma, and Elgeyo-Marakwet, which produce a lot of maize and are known as the country's bread basket.

In addition to increasing food insecurity and extreme poverty, Maize Lethal Necrosis infection is believed to predispose maize to mycotoxin contamination (Wangai *et al*., 2012). Maize Lethal Necrosi Disease provide favourable conditions for proliferation and accumulation of mycotoxigenic fungi. Such conditions

include rotting of maize kernels, breakages of maize grains and high moisture retention (Mutiga *et al.*, 2015).

Mycotoxins are toxic substances produced as secondary metabolites on a variety of food items by fungal species such as *Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp. and have been linked to massive agricultural losses around the world, particularly in maize and peanuts (Cardona and Johnson, 2014). Kenya has experienced fatal aflatoxicosis epidemics in 1982, 2001, 2004, and 2005 over the last three decades. The most recent outbreak occurred in 2004 in Eastern Province (Machakos, Makueni, Kitui, and Embu) and resulted in 150 deaths (CDC, 2004 and Chemining'wa *et al*., 2009).

While the majority of serious cases occur in Kenya's lower Eastern region, aflatoxin contamination has been discovered in other parts of the country's maize-growing regions (Ongoma, 2013). According to Sirma *et al*. (2015), aflatoxin contamination levels ranging from 0.17 to 5.3 g/kg were found in 67% of maize samples from Kenya's Rift Valley region, which produces the majority of the country's maize. According to Nduti *et al*. (2017), a large amount of aflatoxin was also found in samples collected from Western, Eastern, and Nairobi in Kenya.

The recurring shortage of maize and the resulting increase in food prices have recently been established as the causative effects of stagnating and continuing declines in maize production and yield per hectare (Achon *et al*., 2017). According to FAOSTAT (2014), the decrease in production is caused by below-average rainfall as well as delayed and insufficient farm input supplies. The spread of MLND, *Striga* infestation, and declining soil fertility have all contributed to decreased maize production (FAOSTAT, 2014). Increased post-harvest losses due to insufficient drying and storage practices, as well as early sales of green maize, have exacerbated shortages, with the former contributing to high levels of aflatoxin contamination (Kariuki, 2017).

According to Olwande (2012), inadequate pre and post-harvest pest and disease management practices worsen the declining yield of maize, accounting for 12–20 per cent of post-harvest losses. Poor harvest and grain handling by relevant stakeholders and farmers in the value chain results in pathogen contamination and other losses, lowering the quality of produce (Mutungi and Affognon, 2013; Abdullahi *et al*., 2015). Predisposing factors for Maize Lethal Necrosi Disease, combined with poor grain handling, definitely predispose maize to aflatoxin

contamination, resulting in serious animal and human health problems (Sirma *et al.*, 2015).

1.2 Statement of Research Problem

Maize Lethal Necrosis is caused by the co-infection of Maize Chlorotic Mottle Virus (MCMV) and Sugarcane Mosaic Virus (SCMV). Though the presence of a single virus (MCMV) infection can result in significant reductions in both quality and yields of maize (Wangai *et al.*, 2012; De Groote *et al.*, 2013). Maize that has been infected with MLND is more susceptible to other diseases and pests. Given the importance of maize to Kenya's food security and agricultural productivity, maize production threats such as Maize Lethal Necrosis disease and maize mycotoxin pose a significant threat to the country's food security (De Groote *et al*., 2013).

Many studies have found a direct link between MLND and declining maize production (Wangai *et al*., 2012). Other studies have looked into the relationship between aflatoxin, food security, and public health (Ali and Yan, 2012, Bhutta *et al*., 2013). However, research on the extent to which MLND-infected maize's physiological and pathological conditions make it susceptible to Mycotoxin contamination is lacking. This study was particularly interested in determining the

relationship between Maize Lethal Necrosis Disease and mycotoxin, with a special focus on maize fungal and aflatoxin in maize, which had not previously been investigated.

1.3 Justification

With the emergence of MLND and the massive damage it causes on maize in farmer's fields, the disease is likely to predispose maize to fungal infection. The presence of fungi and mycotoxin levels in maize from MLND hotspots should be assessed as a next step in the prevention and management of aflatoxicoses. The incidences of mycotoxin infection and their link to aflatoxin contamination have not received adequate attention in MLND-prone areas. Mycological and aflatoxin safety is a major public health concern in Kenya due to the high consumption of maize.

Aflatoxigenic fungi, particularly *Aspergillus flavus*, are among the fungi that have had epidemiological significance in Kenya's public health. Aflatoxin-contaminated foods cause acute and chronic pathological human illnesses known collectively as aflatoxicoses. The study's data sheds light on MLND's role as a precursor to mycotoxin outbreaks in these areas. It also provides useful information on aflatoxin levels in maize samples.

1.4 General Objective

To determine whether maize lethal necrosis disease is a precursor to mycotoxin contamination in maize.

1.4.1 Specific Objectives:

i) To identify and characterize mycotoxin causing fungi from Maize Lethal Necrosis Disease infected maize.

ii) To profile and quantify aflatoxin in Maize Lethal Necrosis Disease-infected maize.

iii) To correlate aflatoxin contamination levels with Maize Lethal Necrosis Disease infection in infected maize.

1.5 Hypotheses

The study tested the following hypotheses:

i) Maize Lethal Necrosis Disease infected maize is infected by several mycotoxins causing fungi.

ii) Maize Lethal Necrosis Disease infected maize has significantly high levels of aflatoxin.

iii) There is a correlation between Maize Lethal Necrosis Disease infection and contamination of maize by mycotoxins in Kenya.

1.4 Conceptual framework

Numerous factors, both biotic and abiotic, are blamed for the decline in maize yield. Abiotic factors like temperature change, unpredictable rainfall, drought, and climate change have a significant impact on the nation's maize production. High rainfall causes rotting in maize and thus fungal infection. Climate change is causing the emergence of new diseases, such as maize lethal necrosis. With high disease incidences such as Maize Lethal Necrosis Disease, the quality and quantity of maize is compromised due to grain deformities, wrinkling, rotting, and maize becomes susceptible to breakages during harvesting. These Maize Lethal Necrosis Disease induced factors make maize susceptible to fungal infection and, as a result, contamination with myctoxins. The study hypothesized that Maize Lethal Necrosis Disease affected grains, create favorable conditions for aflatoxin contamination, as illustrated (Figure 1.1).

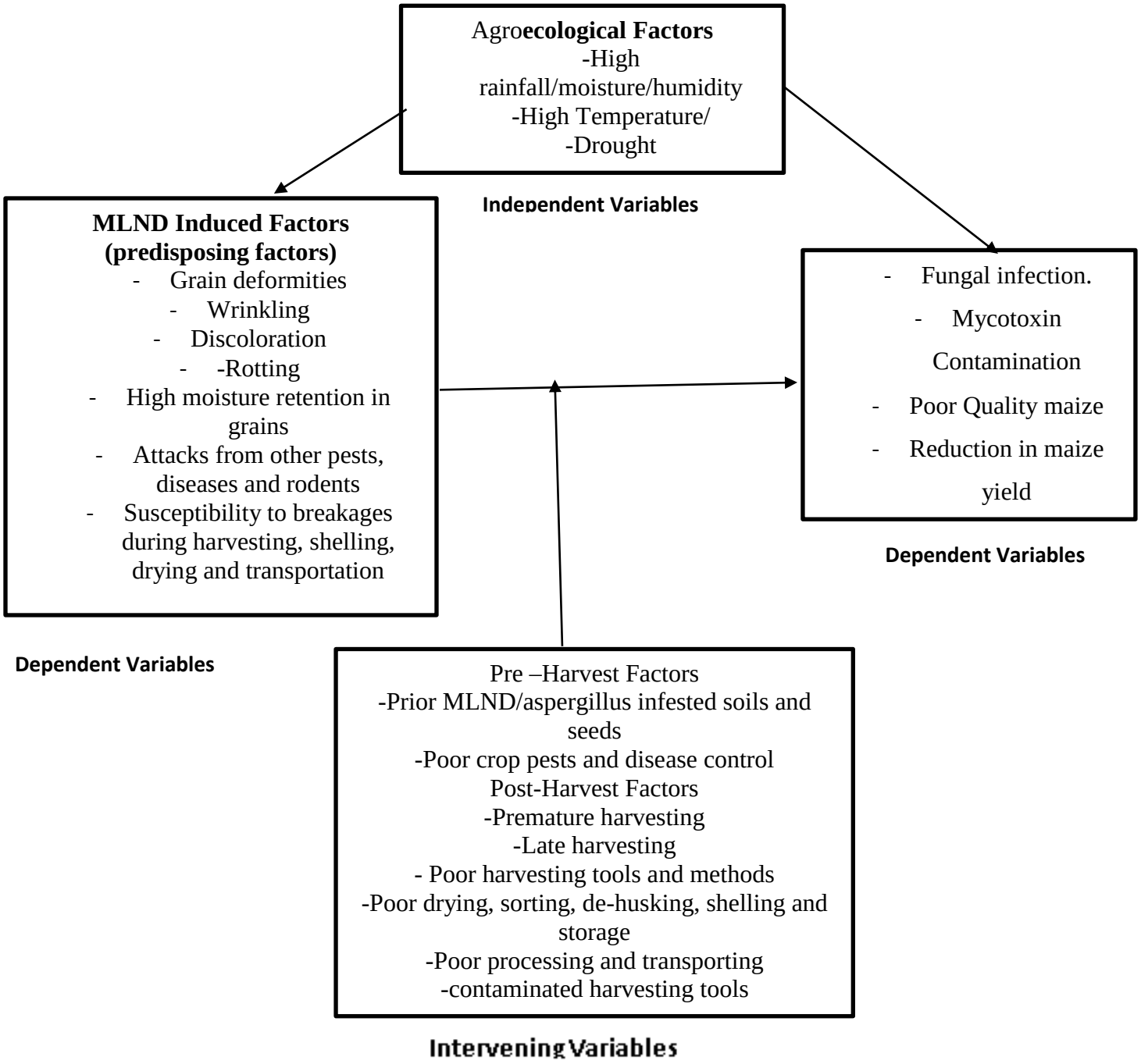


Figure1. 1: Conceptual framework for the study

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Maize production

Maize origin is traced from the Mexican highlands, it is one of the most widely cultivated cereal crops globally. The crop belongs to the family *poacea* which is one of the largest grass family members after rice and wheat (Bari *et al*., 2018). Maize is a popular crop because of its ability to grow and adapt in several range of environmental conditions. The crop thrives in soils that are nutrient-rich, well-drained, pH range from 5.5 to 7.0 and temperature from 14^0C to 16^0C. It needs between 250mm and 5000mm of rainfall (Maiti and Singh, 2017).

Globally, the United States of America is the leading producer of maize with an estimated 35% total maize production. It is the economic engine of the US economy, referred to as mother grain. In 2012 the total world maize production was 875,226,630 with United States of America accounting for 31%, China 24% and Brazil 8% (Ranum *et al.*, 2014).

2.2 Maize production in Kenya

The most important cereal crop in Kenya is maize, which is also a major source of income for rural households and plays an important role in the feed and

food chain. It provides nearly a third of the country's caloric needs and 80% of the feed rations. Maize production in Kenya has been a source of nutrition to many households providing carbohydrates which is a vital ingredient to human health. Maize production involves so many activities and this has been source of employment among the women and youth within the society. Farmers earn income through reaping of the output and this uplifts their living standards especially to the rural areas. Maize production is also essential as a source of food to the livestock like dairy cattle, pigs, and poultry among others (Mohajan, 2014).

Maize occupies approximately 56% of Kenya's arable land in terms of agricultural land use. About three million smallholder farmers with an average of 2 hectares of land produce 70% of Kenya's total maize. Commercial farmers grow the remaining 30% of maize on more than 20 acres of land on average. Annual production has ranged from 26 to 37 million bags in an average 1.8 million ha per year over the years, depending on climatic conditions (FAOSTAT, 2012). In Kenya, 75% of maize is grown between 1100 and 2900 meters above sea level in the mid and highlands.

The annual maize consumption in Kenya is estimated to be around 37 million bags (2.9 million MT) (Shiferaw *et al*., 2011; Mohajan, 2014). For

decades, the country has had a systemic deficit, forcing it to rely on imports. Imports from Uganda, Tanzania, and the global market are used to supplement production. The shortfalls are attributed to a variety of factors, including the country's heavy reliance on rain-fed agriculture to meet its food needs. Farmers are unable to produce at their best owing to unpredictable weather because of over reliance on rain-fed agriculture.

In addition to erratic weather, uncontrolled crop pests and diseases usually result in significant pre and postharvest losses, lower yields, and income. The most common crop pests in Kenya and other countries are armyworms, thrips, aphids, stem borers, mealy bugs, and nematodes (Wambugu, 2012, Wokabi, 2013). A variety of fungal infections (rust, spot, blight, smut, *Aspergillus*, and *Fusarium*) and viral diseases (Maize Streak Virus, Maize Lethal Necrosis Disease) also play a significant role in maize yield reduction.

Currently, the most serious disease is Maize Lethal Necrosis Disease (MLND), which has been regarded as the country's greatest threat to maize production since its discovery in 2011.

2.3 Occurrence of Maize Lethal Necrosis Disease globally

The co-infection of two viruses, Maize Chlorotic Mottle Virus (MCMV) and Sugar Cane Mosaic Virus (SCMV), causes maize lethal necrosis disease (MLND).The disease was discovered in 2011 in Kenya's Rift Valley and quickly spread to other eastern African countries between 2012 and 2014 (Wangai *et al*., (2012); Adams *et al*., (2014);and Lukanda *et al*., (2014). In 1974, Peru reported the first occurrence of MCMV (Castillo and Herbert, 1974). In 1978, the disease (MLN) was discovered in the United States (Niblet and Caflin, 1978). Corn lethal necrosis disease was the name given to the disease when it was first discovered in the United States. Following that, it was reported in a number of countries around the world, including Argentina, Thailand, Mexico and China.

A serious disease epidemic was first noted in the Longisa Division of Bomet County in September 2011, and it was ultimately identified as Maize Lethal Necrosis disease (Wangai *et al*., 2012a, b). Several districts in Nyanza, Central, Western, and other regions of the Rift Valley, including Narok, Sotik, Transmara, Naivasha, Bureti, Nakuru, Konoin, South Narok, had also reported symptoms resembling those of Maize Lethal Necrosis Disease (Wangai *et al*., 2012b).

In August 2012, MLND was observed in Tanzania's border regions, particularly in the Northern zone and along Lake Victoria (Makumbi and Wangai, 2013).In October 2012, the disease was discovered in Uganda's border regions of Tororo, Mbale, Kapchorwa, and Busia (Kiruwa *et al*.,2016). Adams *et al*. (2014) confirmed the first occurrence of MLND in Rwanda's Northern Province in February 2013. After that, the disease spread to Rwanda's Western Province (Adams *et al*. (2014). In 2014 and 2015, surveys in Oromia, Benishangul Gumuz, Amahara, and Tigrez revealed the presence of MLND in Ethiopia (Demissie *et al*., 2016).

2.3.1 Causative Pathogens of Maize Lethal Necrosis Disease

2.3.1.1 Maize Chlorotic Mottle Virus (MCMV)

Maize Chlorotic Mottle Virus is the only species in the genus *Machlomovirus* and a member of the family *Tombusviridae*.The *Tombusviridae* family contains 16 virus genera that infect a wide variety of plant hosts (King *et al*., 2012). Maize is a natural host for MCMV, maize necrotic streak virus, and maize white line mosaic virus, among others.

Maize Chlorotic Mottle Virus has a 4.4 kb positive-sense RNA (+RNA) genome that is not capped or polyadenylated (Nutter *et al*., 1989). It is

encapsidated in 30-nm-diameter t = 3 icosahedral virions made up of a single capsid protein (Walkey, 2012). The virus particle is extremely stable, retaining infectivity at 20°C for more than 30 days before thermal inactivation at 80-85°C (Redinbaugh, 2018). In laboratory settings, MCMV is easily mechanically transmissible to maize and other experimental hosts.

The MCMV genome is made up of four major open reading frames (ORFs) and one small ORF, each of which encodes seven proteins via different expression strategies. From the genomic +RNA, the MCMV genome encodes three proteins: ORF1 begins at the first start codon and encodes p32, a protein with an unknown mode of action that increases symptom severity as well as virus establishment and accumulation (Scheets, 2016). ORF2 partially overlaps ORF1 and encodes the replication proteins p50 and p111, which are expressed by reading through a UAG stop codon at the end of p50 (Scheets, 2016). The 3′ MCMV ORFs are expressed from sub genomic RNA1 (sgRNA1), which also expresses p7a from the 5′-most ORF3 and p31 by reading through a UAG stop codon (Scheets, 2000). Another small protein, p7b, is predicted to be encoded by a small ORF following ORF3 and starting with an unusual CUG codon (Scheets, 2016).

Based on sequence similarities to known *Tombusviridae* MPs, proteins p7a and b are predicted movement proteins (MPs).The 25-kDa CP is encoded by ORF4 from the second sgRNA1 start codon (Scheets K. 2000, Scheets. 2016,).

In plants, MCMV causes mosaic, chlorosis, and stunting. Necrosis, severe stunting, low seed set, shortened inflorescences, and premature plant death have been reported in the field with MCMV, but it is unclear whether this is due to MCMV infection alone or the presence of other viruses or stresses. However, the severity of disease caused by MCMV differs between maize genotypes, but the role of environmental factors in symptom development is not well defined.

In comparison to the diversity of *Potyviridae* sequences associated with MLN, the diversity of MCMV sequences across known isolates is extremely limited. Maize Chlorotic Mottle Virus lacks the intermediate genomes and close relatives seen in the *Potyviridae*, and East African populations have very little diversity, with few features distinguishing them from US and other global isolates (Mahuku *et al*., 2015).

Phylogenetic analyses of complete genomes of MCMV isolates from around the world show similar limited diversity (1% to 4% nucleotide sequence divergence), but isolates from Asia and Africa are more similar to each other than

isolates from other parts of the world. To date, no differences in pathogenicity have been reported among MCMV variants.

2.3.1.2 Potyviruses

Maize-infecting viruses in the family *Potyviridae* are ubiquitous worldwide, occurring everywhere maize is grown. Different maize-infecting virus species in the *Potyviridae* (collectively referred to as potyviruses herein) predominate in different regions of the globe, usually with several present in a given locale. Several viruses in the family have been demonstrated to cause MLN in co-infections with MCMV, including Sugar Cane Mosaic Virus (SCMV), Maize Dwarf Mosaic Virus (MDMV), and Johnson grass mosaic virus (JGMV) in the genus *Potyvirus* and Wheat Streak Mosaic Virus (WSMV) in the genus *Tritimovirus* (Niblett and Claflin, 1978; Uyemoto, 1980; Stewart *et al*., 2017).

Maize Dwarf Mosaic Virus and Sugar Cane Mosaic Virus are both common in the United States (Stewart *et al*., 2014). Sugar Cane Mosaic Virus predominates in East Africa, but JGMV and MDMV are also present (Wangai *et al*., 2012; Mahuku *et al*., 2015; Stewart *et al*., 2017). The presence of maize-infecting potyviruses in areas of recent MLN emergence predates reports of MCMV.

Potyviruses have a 10-kb +RNA genome that is encapsulated in a flexuous rod-shaped virion 700-900 nm in length and 11-13 nm in diameter. Potyviruses encode a single large polyprotein from a single ORF, with a small overlapping ORF expressed from P3 via frame shifting transcriptional slippage (Kehoe *et al*., 2014).

Potyviruses are spread by aphids, whereas the Tritimovirus -WSMV is spread by the eriophyid wheat curl mite *Acer tulipae Keifer* (Namikoye *et al*., 2017). In single infections, they cause mosaic, chlorosis, and stunting in maize, symptoms that are very similar to those caused by MCMV infection. Depending on the host-virus-environment interaction, symptoms can be bright or muted.

Unlike MCMV, maize-infecting potyviruses are extremely diverse both within and between species. They exhibit hyper variability in the CP N-terminus, as do other potyviruses. Potyviridae species are distinguished by more than 76% nucleotide sequence identity and more than 82% amino acid identity, as well as biological differences such as insect vector and host range differences (Adams *et al*., 2011). However, potyvirus genome organization and more general biological features are similar (Adams *et al*., 2011).

In East Africa, in addition to the multiple species of potyviruses present, including SCMV, MDMV, and JGMV, CP sequence comparisons reveal several

clades of SCMV, many of which are most similar to the BD8 strain first described in China (Agrios, 2005)

2.3.2 Transmission and spread of Maize Lethal Necrosis Disease

Maize Lethal Necrosis Disease is transmitted by mechanical means as well as through insect vectors. The most common vectors are maize thrips, rootworms and leaf beetles. Potyviruses are frequently spread through non-persistent transmission, especially by aphids (Namikoye *et al*., 2018). As beetles feed, predigested substances known as regurgitants are dispersed on the plant's surface, and when beetles carrying the virus disperse this substance, they also disperse the virus (Lukanda *et al*., 2018).

The disease is also spread by thrips like *Frankliniella williamsi* and stem drills like *Busseola fusca*, *Chilo partellus*, and *Chilo orichal*, as well as beetles like Cereal Leaf Beetles (*Oulema melanopus*), Corn Flea Beetles (*Systena frontalis*), and rootworms like *Diabrotica undecimpunctata*, *Diabrotica longicornis*. Maize Chlorotic mottle virus and the Sugar Cane Mosaic Virus are non-persistently transmitted by several species of aphids (Namikoye *et al*., 2018). Gupta (2018) found that mites continuously transmit WSMV. Infected seeds and soil have also

been noted as potential vectors for the transmission of viruses (Nelson *et al*., 2011).

Farm implements contaminated with the virus can spread it from infected to uninfected fields (Kiruwa *et al*., 2016). Root feeders such as nematodes and others create viral entry points and thus frequently weaken the plants, rendering them unable to defend themselves against infection.

2.3.3 The symptoms of Maize Lethal Necrosis Disease

Symptoms are changes in appearance or behavior that occur in plants as a result of biotic or abiotic stress (Agrios, 2005; Punja *et al*., 2007). Long yellow streaks parallel to the leaf veins and streaks that join to form chlorotic mottling are some of the symptoms of MLND. Streaks usually begins at the base of the young leaves and spreads upward to the leaf tips. Other symptoms of MLND include a "dead heart," plant death, premature plant aging, sterility, and rotting maize cobs (Nelson *et al*., 2011; Wangai *et al*.,2012; Makone *et al*., 2014).

2.3.4 Favorable conditions for Maize Lethal Necrosis Disease occurrence

Environmental elements may influence the occurrence of MLND. According to study, the terrain, temperature, soil humidity, and weather tend to affect the likelihood of MLND (Guadie *et al*., 2018b). The low temperature range

(22^0C-24^0C) in the mid-latitude zones influences the development of MLND (Mudde *et al*., 2018; Guadie *et al*., 2018b). Warm weather promotes the growth of insect populations and MLND viral vectors (Kiyyo, 2015; Guadie *et al*., 2018b).

Cropping practices also have an impact on MLND epidemic expansion and insect vector population dynamics. Compared to mono-cropping, which showed a disease severity of up to 79.81%, intercropped plants have a lower disease development rate (Ramkat *et al*., 2008; Gopal *et al*., 2010; Boudreau, 2013). Intercropping causes increased spatial separation, which has a negative impact on pest and disease production (Terefe and Gudero, 2019). Intercropping also allows for the use of diversity to maximize beneficial effects and reduce outbreaks of pests and weeds that serve as alternate hosts (Ratnadass *et al*., 2012).

Terefe and Gudero (2019) estimated that poorly weeded farms had the highest disease severity (86.59 %). Weeds have a beneficial effect on the development of diseases and insect infestations. They also observed that, removing weeds from fields eliminates alternate hosts as well as potential insect vectors and viruses.

Proper fertilizer application to the soil reduces plant stress vulnerability, increases physical tolerance, and decreases susceptibility to plant pests and diseases (Veresoglou *et al*., 2013).

2.3.5 Economic impacts of Maize Lethal Necrosis Disease

Maize Lethal Necrosis Disease is referred to as the most destructive plant disease responsible for maize's highest yield loss due to its up to 100% yield loss (Mbega *et al*., 2016). Farmers have lost their entire crops at the farm level (Wangai *et al*., 2012). The MLND's malformations and rotting make the crop susceptible to subsequent diseases. Field observations show that almost all commercial maize varieties are infected with MLND, resulting in yield losses ranging from 30 to 100 percent (Wangai *et al*., 2012). Furthermore, as seed companies incur additional costs for seed treatments, the cost of seed production frequently rises.

There is a negative environmental impact as farmers embark on the management option of controlling the vectors that cause MLND through the use of insecticides. With the decrease in the quantity and quality of maize used as both food and cash crops, there is a risk of increased insecurity in the farming community.

Low wages increase stress because farmers are unable to meet their basic needs, resulting in more school dropouts as school fees become unaffordable and thus an increase in child labor. Therefore it is important to find an effective solution to properly manage MLND to minimize losses and optimize output (Kiruwa *et al.,* 2016).

2.3.6 Maize Lethal Necrosis Management Techniques

This is the use of appropriate management techniques to reduce MLND to a low level. Some of the fundamental principles of disease control are the reduction of initial inoculums and the reduction of infection rates (Mawishe and Chacha, 2013).

2.3.6.1 Reduction of initial inoculum

2.3.6.1.1 Pathogen exclusion and strict quarantine

Pathogen exclusion or rigorous quarantine helps prevent the spread of illness in areas where it hasn't been before. Pathogen exclusion, according to Kiruwa *et al.* (2016), is a key objective of plant quarantine around the world and is achieved by examining maize seed prior to entry and exit to prevent disease spread. One of the greatest approaches to control the spread of MCMV, according

to Adam *et al*. (2014), is quarantine as opposed to treating the endemic Sugar Cane Mosaic Virus.

2.3.6.1.2 Pathogen eradication

Pathogen eradication entails cleaning infected field equipment and machinery, as well as ensuring that the field is free of infected plant debris and any other material that may harbor the pathogens (Mawishe and Chacha, 2013). Planting a non-host crop during crop rotation significantly reduces virus density (Narayanasamy, 2013).

2.3.6.2 Reducing the rate of infection of Maize Lethal Necrosis Disease

2.3.6.2.1 Avoidance

Avoidance involves the use of certified seeds, timely maize planting at the beginning of the main rainy season, as opposed to planting during the short rainy season, in order to create a gap in maize growing seasons.

2.3.7.6.2 Plant protection

This entails guarding against contagious germs infecting the host (maize). Plant protection has been accomplished using pesticides and proper plant nutrition. To control vectors, insecticide formulations may be employed. Imidacloprid, Abamectin, Permethrin, Thiamethoxam, Deltamethrin, Endosalphan, and

Dimethoate are some examples of insecticides (Mekureyaw, 2017). For efficient vector control, insecticides should be used every one to two weeks, and every month, different chemicals should be alternated to prevent the target vector from acquiring immunity (Awata, 2019). Other defense strategies include the use of compost, basal fertilizers, and top-dressing fertilizers to boost plants' resilience to disease and pests (Wangai *et al*., 2012).

2.3.6.3 Resistant or tolerant varieties to Maize Lethal Necrosis Disease

One of the pillars of modern agriculture is the use of disease resistant varieties. In some cases, resistant varieties are the only way to control plant diseases. The variable costs of farming are now being heavily emphasized. Fungicide inputs are being scrutinized, particularly in light of the emergence of fungicide-sensitive or resistant pathogen isolates (Karanja *et al*., 2018).

2.4.0 Contamination of Maize Lethal Necrosis infected maize by mycotoxigenic fungi

Maize Lethal Necrosis Disease infected maize frequently develops grain malformations such as wrinkles, discoloration, and rotting as a result of increased moisture retention, making the crop vulnerable to other pests and diseases (Wangai e*t al.*, 2012). Furthermore, MLND-infected maize grains are more likely to shatter

during harvest, shelling, drying, and shipping, potentially serving as a point of entry for fungal infection.

Not only do fungal infections cause significant economic losses, but they also have an impact on human and animal health (Moretti, 2017). It has been discovered that fungal infection in maize reduces the grain's processing and nutritional value (Oliveira *et al*., 2014).

Fungi contamination of cereal is a common additive process. The process begins in the field and continues through harvesting, drying, and storage. A useful classification has traditionally been established that divides fungal contaminants in maize and other cereals into two categories: pathogenic and non-pathogenic (saprophytic). *Fusarium* spp. are common in the field, while *Penicillium* spp. and *Aspergillus* spp. are common in storage (Bryden, 2009).

2.4.1 Mycotoxin

These are a group of toxins produced by various fungal genera that have toxic effects on humans and animals, resulting in mycotoxicosis (Zain, 2011). More than 300 million pathogenic fungi have been present on Earth for more than 1.6 million years and can be found in a variety of environments (Campell, 2013).

Mycotoxin contamination occurs throughout the food chain, including in the field prior to harvesting, harvesting, drying, refining, and storage. As a result of this occurrence, managing and controlling mycotoxins has become a major challenge, particularly in developing countries (Anukul *et al*., 2013). Climate, commodity type, and poor agricultural practices, such as inadequate drying, handling, and storage, all contribute to mycotoxin production. They can occur in both temperate and tropical climate zones, with the most severe cases occurring in tropical and subtropical climate zones (Suleiman *et al*., 2013).

Mycotoxin classification is based on fungal cells, mode of action, and structure (Money, 2016). *Fusarium*, *Aspergillus*, and *Penicillium* are the three known fungi that produce mycotoxin in cereals. *Aspergillus flavus* and *Aspergillus parasiticus* fungi produce aflatoxin and ochratoxin, while Fusarium fungi produce deoxynivalenol, fumonisin, and zearalenone.

Mycotoxin contamination of food and feed poses a serious health risk, which can be acute or chronic. From central nervous system damage to the induction of hepatocellular carcinoma, cancer, reproductive, respiratory, and gastrointestinal effects, cardiovascular effects, and sudden death, the effects are numerous (Suleiman *et al*., 2013).

According to a Food and Agriculture Organization report (FAO, 2004), more than 100 countries have established regulatory limits on appropriate and acceptable levels of mycotoxins in human and animal feed. However, due to insufficient grain control, inadequate testing equipment, and a lack of inspection and monitoring systems, regulatory limits are rarely enforced (Duarte *et al*., 2010; Wild and Gong, 2010; Wu and Guclu, 2012).

2.4.2. Types of Mycotoxins

2.4.2.1 Aflatoxin

Aflatoxin's negative effects on human and animal health are a major source of concern (Zinedine and Manes, 2009). They are toxins produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Okun, 2015). The four major structural chemotypes of aflatoxins are aflatoxins groups B1, B2, G1, and G2 (Jolly *et al*., 2008).

The appearance of fluorescence under ultraviolet light distinguishes aflatoxins from many other heterocyclic fluorescence compounds (Suleiman *et al*., 2015). The letters B and G stand for blue and green colors, respectively, and the numbers 1 and 2 represent the migration distances of the aflatoxins as seen on a thin-layer chromatographic plate and under UV light.

According to several studies, the most toxic chemotype is aflatoxin B1, which suppresses immunity, causes some cancer types, stunts growth, and inhibits many metabolic processes in humans and other animal species (Bluma and Etcheverry, 2008; Zinedine and Manes, 2009).

2.4.2.2 Fumonisin

Fumonisins are mycotoxins produced by *Fusarium* species, primarily *Fusarium verticillioides* (also known as *Fusarium moniliforme*) and *Fusarium proliferatum* (Gelderblom *et al*., 1988; Marin *et al*., 2013). It is a common contaminant of maize and maize products. Fumonisins are toxic to horses, pigs, cattle, rats, and mice (Marasas *et al*., 1988; Hussein and Brasel, 2001). Fumonisins are toxic to many domesticated and wild animals' vital organs. It is most important in veterinary medicine as a cause of porcine pulmonary edema and equine leukoencephalomacia.

2.4.2.3 Ochratoxin

Ochratoxins are produced by a variety of *Aspergillus* and *Penicillium* species. Ochratoxin is classified into three types: A, B, and C. The most dangerous natural contaminant found in many agricultural crops, including coffee, wheat, barley maize, peanut cassava, grapes, and beer, is ochratoxin A (Zain, 2010).

According to Bennett and Klich (2003), ochratoxin has teratogenic, mutagenic, genotoxic, and immunotoxic effects. A toxin known as a class 2B carcinogen, according to Murphy *et al*. (2006) and Erkekolu *et al*. (2008), induces reproductive toxicity and other adverse effects in a variety of animal species.

2.4.2.4 Trichothecenes

They are a type of mycotoxin produced by a variety of fungi, the majority of which are *Fusarium* species. Trichothecenes can be found in maize, wheat, other cereals, vegetables, and animal feed all over the world (Eriksen, 2004).

2.4.2.5 Deoxynivalenol

Fusarium graminearum and *Fusarium culmorum* contain deoxynivalenol, a non-fluorescent mycotoxin (Anukul *et al*., 2013). Deoxynivalenol, also known as "vomitoxin," is a well-studied mycotoxin that causes maize and wheat contamination in both tropical and temperate climates. Deoxynivalenol causes anorexia, which is defined as a loss of appetite that results in weight loss and nutritional effectiveness (Pestka *et al*., 2005; Anukul *et al*., 2013).

2.4.2.6 Zearalenone

Zearalenone, an estrogenic mycotoxin, is produced by *Fusarium graminearum, Fusarium culmorum, Fusarium cerealis, Fusarium equiseti*, and

Fusarium crookwellense (Anukul *et al*., 2013). Zearalenone, also known as 6-(10-hydroxy-6-ox-trans-1-undecenyl) resorcylic acid -lactone, is a phytoestrogen with the chemical formula 6-(10-hydroxy-6-ox-trans-1-undecenyl) resorcylic acid (Hussein and Brasel, 2001).

Zearalenone is well-known for its negative effects on the urinary tract in animals, as well as neuroendocrine disruption via estrogen receptor binding (Richard, 2007). It has been linked to lower serum testosterone, testicular weight loss, spermatogenesis, fetal reabsorption, abortion, smaller litter sizes, and low birth weights in pigs. In cows, zearalenone has been linked to infertility, reduced milk production, and hyperestrogenism (Zinedine *et al*., 2007).

2.4.3 Factors influencing mycotoxin infection

2.4.3.1 Factors in the field

Plant stress encourages mycotoxin-producing fungi to invade maize kernels. Mycotoxin contamination occurs during food production, harvesting, storage, and processing (Reverberi *et al*., 2010). Drought, high temperatures, plant nutrient shortages, and agronomic activities such as cropping patterns, variety and seed selection, planting dates, delayed drying and storage conditions, and tillage all contribute to high mycotoxin levels. Some of the biotic factors associated with

mycotoxin production include insects, weeds, and plant diseases (Diao *et al*., 2015).

2.4.3.2 Abiotic stresses

The effects of heat and drought on some physiological traits in plants, such as photosynthetic pigments, membrane stability, and photosystem II efficiency, are associated with oxidative stress (Mannaa and Kim, 2017). Previous research found that aflatoxin and fumonisin contamination levels were higher in kernels from non-irrigated corn plants than in those grown on irrigated land (Munkvold, 2014).

High temperatures and drought conditions have a significant impact on pathogenic fungi development and their ability to produce harmful mycotoxins. There is compelling evidence that drought stress and high temperatures are major contributors to elevated aflatoxin levels (Wagacha and Muthomi, 2008). Heat stress causes stress in maize plants, making the invading fungus more advantageous (Guo *et al*., 2008).

Limited nutrient availability in soils causes plant stress, exposing the plant to fungal infection and increased aflatoxin production compared to plants receiving optimal nutrients (Munkvold, 2014).

2.4.2.3 Biotic stresses

Conidia attached to insect bodies are transferred to various parts of the plant while the insect feeds and are also spread through defecation. Stem borers, fall army worms, and ear worms are some of the insects that spread fungi. While feeding, these insects frequently cause plant and maize kernel injuries, providing fungal infection sites. Weed competition is also a major contributor to aflatoxin-related stress problems. Weeds compete with plants for nutrients, moisture, and space, causing the plant to suffer from a lack of available resources. When a plant is stressed, it is more likely to be attacked by fungi and thus produce mycotoxin.

2.4.2.4 Factors in storage

Temperature, moisture content, maize condition, and storage time all affect fungal invasion and aflatoxin development in maize during storage. Drying maize grain with a moisture content of about 13% is the most effective method of preventing fungal invasion and aflatoxin infection. The fungus *Aspergillus flavus* grows well at a minimum grain moisture content of 17.5% (Munkvold, 2014).

2.4.4 Mycotoxin contamination in Kenya

Aflatoxin outbreaks have occurred around the world since the 1980s, with some areas and age groups being more vulnerable than others. Kenya experienced its first outbreak in 1981 as a result of the populace's consumption of maize grain.

However apart from outbreaks, a population study in Kenya discovered increased levels of aflatoxin exposure (Mehl and Cotty, 2010).

Kenyans, particularly those in the country's eastern region, have previously had the highest levels of aflatoxin exposure, as evidenced by previous outbreaks. According to the CDC (2004), the most serious outbreak of aflatoxicosis in Kenya occurred in the Eastern Province in 2004. During January–June 2004, the Kenya Ministry of Health (MOH) and partners identified 317 cases of acute hepatic failure in eastern Kenya; 125 cases occurred in persons who subsequently died during the illness.

Aflatoxin prevalence in Kenya has been assessed in population studies conducted outside areas of reported outbreaks in areas such as the Rift Valley, Nyanza, and Western Kenya, with the Eastern Province having the highest aflatoxin exposure (Okoth, 2012). According to Mahuku *et al*. (2015), aflatoxin levels in maize samples collected at various points along the value chain (pre-harvest and storage) and in selected markets in the upper-east, lower-east, and south-west regions were higher than expected at levels above the legal limit of 10ppb. Sirma *et al*. (2015) and Nduti *et al*. (2017) discovered aflatoxin levels of contamination ranging from 0.17 to 5.3µg/kg in 67% of maize samples collected in

the Rift Valley regions. These findings suggest that the population in Kenya, particularly in the Eastern, Coastal, Central, Nairobi, Western, and Rift Valley regions, is at risk of aflatoxin contamination.

2.4.5 Methods used to determine aflatoxin contamination

The development of efficient and trustworthy analytical technologies used to detect and quantify mycotoxins has increased as a result of worries about the safety of human food and animal feed. Many of these processes are standardized, and agencies like the FDA, USDA, EPA, and AOAC strictly regulate the amount of mycotoxin in food and feed.

2.4.5.1 Chromatographic techniques

The two most widely used chromatography methods for routine mycotoxin testing are high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) (Shephard *et al*., 2012). Thin-layer is frequently used because it is affordable, simple to use, and speeds up the examination of several samples on a single plate (Sherma, 2003). Aflatoxin quantification via TLC is based on immunoaffinity columns, which stay away from substances that could interfere with the experiment and enable quantification of aflatoxins at 1 ng/g levels (Stroka *et al*., 2004). Both the normal and reverse HPLC phases can be

employed, although the reverse phase is recommended since it provides better separation of aflatoxins and more precision (Stroka *et al*., 2004).

Post-column photochemical derivatization and time-based programming of the proper wavelengths are used for fluorescence detection (Shephard *et al*., 2012). More sophisticated methods, such as high selectivity and sensitivity liquid chromatography combined with mass spectrometry (LC-MS/MS), have been developed for multi-toxin analysis (Bueno *et al*., 2015).

2.4.5.2 Immunological techniques

Using immunological methods like VICAM and enzyme-linked immunosorbent assay (ELISA) testing, mycotoxins may now be measured and analyzed. The basis for ELISA is the antibody's capacity to differentiate between various mycotoxins in maize (Zhang *et al*., 2018). Since mycotoxins rather than antigens are the target substances in ELISA tests, this has the drawback that substances with identical chemical groups can interact with antibodies (Waltman, 2008). Vicam is a quantitative analytical technique that reacts to a particular mycotoxin using a particular antibody. VICAM is the trusted aflatoxin test that produces precise numerical results. Using monoclonalantibody-based affinity chromatography, VICAM can isolate aflatoxins B1, B2, G1, and G2 from feeds,

food, grains,and nuts, and M1 from dairy products. VICAM is safe and simple. It can be performed in less than 10 minutes and requires no special skills. Results may be recorded using a digital fluorometer readout or automatic printing devices (Patel, 2004).

2.4.5.3 Capillary electrophoresis

Capillary electrophoresis is an electrophoretic procedure that rapidly separates components by utilizing charge- and mass-dependent movement in electrical fields. To find minute amounts of mycotoxin, capillary electrophoresis and fluorescence detection are used.

2.4.5.4 Nucleic acid based techniques

Polymerase chain reaction (PCR) technology is used to identify and categorize mycotoxigenic fungus (Niessen, 2007). The PCR method enables the rapid and precise detection of microorganisms in a variety of substrates ((Varga, 2003).

Additionally, more advanced methods have been created, such as quantitative real-time PCRs, which are precise and extremely sensitive. However, a replication step is required before the PCR test in order to prevent or address issues of false positive results (Watzinger *et al*., 2006).

2.5.0 Methods used to prevent aflatoxin contamination in maize

2.5.1 Pre harvest management practices

To avoid the production of mycotoxin during drying and storage, it is critical to be cautious throughout the maize production process, because the majority of contamination by mycotoxins occurs in the field and persists during storage (Strosnider *et al*., 2006).

Improving crop growing conditions, such as providing adequate moisture through irrigation, reduces plant stress, lowering the level of fungal contamination. Good agronomic practices, such as providing adequate nutrients, promote plant health and thus reduce fungal contamination (Munkvold, 2014).

2.5.2 Biological control

The use of various microorganisms reduces aflatoxin contamination in agricultural products and many other food commodities. In the emulative environment, biological agents such as bacteria, yeasts, molds, and algae have varying abilities to degrade aflatoxin. Aflatoxin detoxification using biological agents is divided into two steps: absorption and enzymatic decadence (Jard *et al*., 2011). Aflatoxin can be absorbed directly by microorganisms by concatenating to their cell wall contents via effective internalization or congregation (Motawe *et al*.,

2014). Dead microorganisms can also absorb aflatoxins; this property is useful for creating bio-filters in the form of probiotics and finds application in fluid decontamination (Motawe *et al.,* 2014).

Different bacteria strains, however, were also discovered to be effective at deteriorating the AFB1. Aflatoxin B1 is strongly degraded (91.5%) in the bacterium *Bacillus velezensis* DY3108's cell-free supernatant (Shu *et al*., 2018).When used as a microbial consortium, thermophilic bacterial strains (*Geobacillus* and *Tepidimicrobium*) also play an important role in AFB1 degradation (Zhao, *et al*., 2017). Furthermore, inoculation of antagonistic strains of *Pseudomonas, Trichoderma, Ralstonia, Lactobacilli, Burkholderia,* and *Bacillus* spp. reduced *A. flavus* growth significantly in pre-harvest crops (Akocak *et al*., 2015). Surprisingly, aflatoxin contamination was also found to be reduced when non-toxigenic strains of *A. flavus* and other molds were used as the primary controlling agent (Udomkun *et al*., 2017).

The atoxigenic strain is used once during the growing season when there is only a small amount of *Aspergillus flavus* inoculum in the field, allowing it time and favorable exposure to the crop as well as an edge over toxic strains when vying for crop resources (Bandyopadhyay *et al*., 2016).

Through a collaboration between the International Institute of Tropical Agriculture (IITA), the USDA-Agricultural Research Service (USDA-ARS), and several National Agricultural Research Services, including the Kenya Agricultural and Livestock Research Organization (KALRO), biocontrol technology has recently been adapted to reduce aflatoxin in maize and groundnut in Kenya and several other African countries (Bandyopadhyay and Cotty, 2013; Bandyopadhyay *et al*., 2016). Aflasafe^{TM} with a country-specific suffix is the name given to aflatoxigenic *A. flavus*-based aflatoxin biocontrol products aimed at various regions in Africa. Each Aflasafe product contains active ingredients from atoxigenic strains native to the target country. Aflasafe KE01^{TM} (previously known as KE01) has been registered with Kenya's Pest Control Products Board (PCPB) as a Kenya-specific aflatoxin bio control product.

2.5.3 Chemical control

When employed in the proper quantity, a number of substances, including acids, alkalis, oxidizing agents, aldehydes, and various gases, have also been shown to reduce aflatoxigenic fungus growth and aflatoxin production (Udomkun *et al*., 2017). Ozone was discovered to be the most efficient gas for enhancing aflatoxin degradation on grains and legumes through an electrophilic attack on the

carbon bonds of the furan ring (Jalili, 2016). Ozone treatment is less useful for treating crops after harvest, though, because of its high cost.

The ammoniation procedure in corn and other food commodities can also prevent the development of aflatoxins (Karlovsky *et al*., 2016). The ammoniation process has a positive side effect in that high-pressure ammoniation (0.25, 0.5, 1.5, and 2%) reduces the time required to reduce aflatoxin production in a variety of crops and food commodities (Temba *et al.*, 2016).

Certain chemicals, such as Sodium bisulfite, Calcium hydroxide, formaldehyde, Sodium hypochlorite, Sodium borate, and sorbents, also significantly reduce aflatoxin levels in many food commodities (Carvajal & Castillo, 2009). Aflatoxin was removed from groundnut cake and poultry feed by using sodium bisulfite (0.5%) and sodium hydroxide (1%) (Bedi and Agarwal, 2014).

Some food additives are also used in conjunction with physical factors such as temperature and moisture to inhibit fungal growth and aflatoxin production. Citric acid treatment combined with high temperature and pressure inhibits fungal growth as well as aflatoxin production in sorghum (Méndez Albores *et al*., 2009).

Some scientists discovered that some food preservatives, such as propionic acid, crystal violet, p-amino benzoic acid, benzoic acid, boric acid, and sodium acetate, inhibited the growth of *A. flavus* and the production of aflatoxin (Aiko and Mehta, 2015). Production of AFB1 was also inhibited in peanut and maize by sodium chloride (10%), acetic acid (5%), and propionic acid (5%) treatments (Kabak *et al*., 2015). Even under favorable conditions for *A. flavus* growth, several propionic acid-like calcium, sodium propionate salts were able to reduce aflatoxin formation in maize (Hassan *et al*., 2015).

Azole fungicides are also used to control fungus growth and aflatoxin production, with prochloraz being more effective than tebuconazole (Mateo *et al*., 2017).

2.5.4 Cultural control

Controlling or reducing infection, as well as regulating the factors that increase the risk of contamination in maize fields, will go a long way toward reducing aflatoxins. Timely planting, optimal plant densities, proper plant nutrition, avoiding drought stress, controlling other plant pathogens, weeds, and insect pests, and proper harvesting are all management practices that reduce the incidence of mycotoxin contamination in the field (Bruns, 2003).

Crops in Africa are grown under rainfed conditions, with little fertilizer and almost no pesticide application. These management practices encourage *A. flavus* infection in fertile plants. Aflatoxin contamination will be reduced by any action taken to reduce the possibility of silk and kernel infection (Diener *et al*., 1987)

Pre-harvest measures that reduce aflatoxin levels are the same as those that increase yields. Crop rotation and crop residue management are also important in controlling *A. flavus* infection in the field. Tillage practices, crop rotation, fertilizer application, weed control, late season rainfall, irrigation, wind, and pest vectors can all have an impact on the source and level of fungal inoculum in maize, thereby maintaining the disease cycle (Diener *et al*., 1987).

Because prolonged field drying of maize can result in significant grain losses during storage, it is recommended that maize be harvested immediately after physiological maturity to combat aflatoxin problems (Kaaya *et al*., 2006). According to Kaaya *et al*. (2006), aflatoxin levels increased by about four times by the third week and more than seven times when maize harvest was delayed for four weeks. However, products must be dried after early harvesting to prevent fungal growth. Leaving the harvested crop to dry in the field before storing it encourages fungal infection and insect infestation; this is a common practice in

Africa, often due to labor constraints and the need to allow the crop to dry completely before harvest (Udoh *et al*., 2000).

The growth of toxigenic fungi in stored commodities is influenced by moisture and temperature. When field harvested maize is stored with a high moisture content for three days, aflatoxin contamination can increase tenfold (Hell *et al*., 2008). The general recommendation is to dry harvested commodities as soon as possible to safe moisture levels of 10-13% for cereals.

2.5.5 Post-harvest management practices

Strategies for reducing quantitative and qualitative post-harvest losses have been developed (Hell *et al*., 2008). These improved postharvest technologies have been used successfully to reduce aflatoxin, exposure in individuals from intervention villages (Turner *et al*., 2005).

Traditional storage methods in Africa are classified into two types: temporary storage, which is primarily used to dry the crop, and permanent storage, which occurs in the field or on the farm. Containers made of plant materials (woods, bamboo, and thatch) or mud placed on raised platforms and covered with thatch or metal roofing sheet are examples of the latter. The grain storage facilities are designed to keep insects and rodents out and moisture out of the grains. Due to

the high cost of new storage technologies, such as the use of metal or cement bins, it is difficult to promote them to small-scale farmers.

Many farmers now store their grains in non-airtight bags, particularly polypropylene, but there is evidence that this method promotes fungal contamination and aflatoxin development (Udoh *et al*., 2000).There are currently efforts in Africa to market improved hermetic storage bags based on triple bagging developed for cowpea that has been or is being tested for other commodities (Hell *et al*., 2010).

2.5.6 Breeding for resistance

Several screening tools have been developed and used to aid in the development of germplasm resistant to fungal growth and/or aflatoxin contamination in corn (Brown *et al.*, 2003). Although sources of *Aspergillus* infection and aflatoxin contamination in corn have been identified, commercial hybrids have not been developed. This is largely due to the difficulty in locating elite lines that produce high yields while remaining resistant in a variety of environments (Clements and White, 2004).

Many new biotechnologies-based strategies for increasing host plant resistance to aflatoxin are being investigated (Brown *et al*., 2013). These methods

involve the development and production of maize plants that reduce the occurrence of fungal infection, limit the growth of toxigenic fungi, or prevent toxin accumulation. In the long run, identifying compounds that inhibit aflatoxin biosynthesis would greatly improve mycotoxin control.

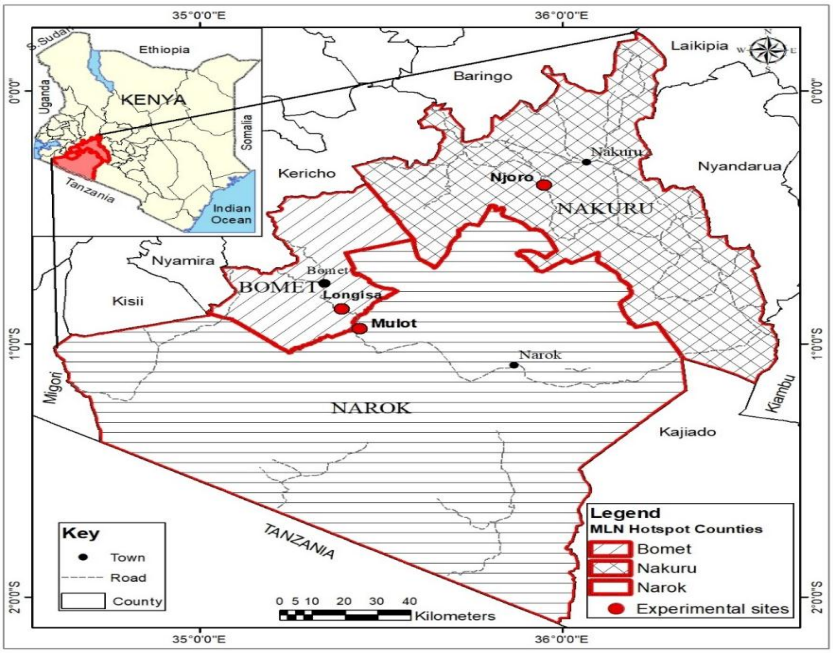
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Sites

This study was carried out in the Rift Valley counties of Nakuru (Njoro), Bomet (Longisa), and Narok (Mulot), which had recently reported outbreaks of Maize Lethal Necrosis Disease (MLND) infections and are known to be areas with high prevalence of Maize Lethal Necrosis Disease (Wangai *et*

al.,2012)(Figure3.1).



QGIS 201 software version 3.20.0.

Figure 3. 1: The map of Kenya showing location of the study sites

3.1.1 Nakuru County

Nakuru County has a total area of 7, 495.1km^2, of which 5,039.40 km^2 is arable land, 852.1 km2 is non-arable land, and 202 km^2 is water mass (that covers Nakuru, Naivasha and Elementaita lakes).With a total population of 2,162,202 people, the county is divided into 11 subcounties: Njoro, Molo, Kuresoi North,

Kuresoi South, Bahati, Naivasha, Subukia, Nakuru Town East, Nakuru Town West, and Rongai (KNBS, 2019).

The county's agro-ecological zones range from tropical alpine to lower midlands. The altitude varies significantly (1400-2970 m). Long rains fall in March, April, May, and June, while short rains fall in October and November, with an annual average range of 500-1900mm.

Njoro Sub County where the study was carried out has an altitude of 1,600 to 2,000 m above sea level and about 20 km southwest of Nakuru city (Mainuri and Owino 2013). The main activity of Njoro sub- County is farming.

3.1.3 Bomet County

Bomet County is one of Kenya's seven most promising maize production zones, and agriculture is the county's primary economic activity (Olwande *et al*., 2009). Bomet County has an area of 1,997.9 km^2 and a population of 875,689 (2019 census).

Maize, beans, and cowpeas are the most common crops in Bomet, with maize playing a significant role in food and nutrition security as well as income generation (Nyoro *et al*., 2014).

The county is in Kenya's semi-humid agro-ecological zone, with an annual maximum temperature of 28°C (Bryan *et al*., 2013). The average annual rainfall ranges from 500 to 2000 mm. Rainfall in Bomet peaks twice a year: in March-May (during the long rainy season) and in September-November (short rainy season).

The vast majority of farmers in Bomet regard the long rainy season as their primary maize cropping season, and the continuous cropping-system has long been known as the dominant cropping system in Bomet. However, fewer farmers grow maize during the short rainy season. The area under monocropping and intercropping systems varies according to the length of the rainy season (Ochieng *et al*., 2020). In areas where rainfall patterns are unpredictable, the majority of farmers intercrop maize and beans. Furthermore, irrigated farming is practiced in areas adjacent to major rivers (Kimani *et al*., 2011).

3.1.3 Narok County

Narok county covers an area of 17,933.1 Km^2 with a population of 1,157, 873 (KNBS 2019). Administratively, Narok County is divided into six sub-counties namely; Transmara West, Transmara East, Narok North, Narok South, Narok West and Narok East. The county has a robust ecological system that residents depend on for agriculture, tourism, water and many other benefits.

The climatic condition of Narok County is strongly influenced by the altitude and physical features. The county has four agro-climatic zones namely: humid, sub-humid, semi-humid to arid and semi-arid. Two-thirds of the county is classified as semi-arid (Njeru *et al*., 2016). Temperatures range from 20^0C (January-March) to 10^0C (June-September) with an average of 18^0C. Long rains are experienced between the months of February and June while the short rains are experienced between August and November. Rainfall ranges from 2,500 mm in wet season to 500 mm during the dry season. The main economic activities in the county include pastoralism, crop farming, tourism and trade among other activities undertaken in small scale. The main crops grown in the county are wheat, barley, maize, beans, Irish potatoes and horticultural crops.

3.2 Seed selection and field experimental design

Field trials were conducted in plots in Longisa, Bomet County, Mulot, Narok County, and Njoro, Nakuru County. Six maize hybrid, breeder's seeds were selected by KALRO breeders based on data from Karanja *et al*.,(2018); Karanja *et al*.,(2020) and Prasanna *et al.*,(2020) and grown in the named three sites during the April-October 2018 growing season. The treatments in each of the three experimental units were arranged in a randomized complete block design and replicated three times. Recommended crop management practices were applied. Pesticides were not used to allow for natural infestation by MLND vectors.

Table 3. 1: Field layout for a correlation between maize lethal necrosis disease and mycotoxin experiment in Bomet, Narok and Nakuru counties

FIELD LAYOUT						
		IM PATH				
	5M		5M		5M	
1M PATH	BLOCK1	IM PATH	**B**LOCK2	1M PATH	BLOCK3	1M PATH
	PLOT 1 **WEMA 5140**		PLOT 12 **KATEH16-02**		PLOT 13 **DUMA 43**	
	PLOT 2 **PH30G-20**		PLOT 11 **PH30G-20**		PLOT 14 **WEMA 5140**	
	PLOT 3 **KATEH16-02**		PLOT 10 **WE5135**		PLOT 15 **PH30G-20**	
	PLOT 4 **DK777**		PLOT 9 **DUMA 43**		PLOT 16 **DK777**	
	PLOT 5 **DUMA 43**		PLOT 8 **WEMA 5140**		PLOT 17 **WE5135**	
	PLOT 6 **WE5135**		PLOT 7 **DK777**		PLOT 18 **KATEH16-02**	

3.3. Testing for severity of Maize Lethal Necrosis Disease

Severity of MLND was determined on a scale of 1-5 according to Shekha and Kumar (2012 and https://mln.cimmyt.org/, where: 1= Resistant (No Symptoms, very slightly infected, one or two restricted lesion on lower leaves or trace); 2=Moderately Resistant- slightly to moderate infection on lower leaves, a few scatter necrosis on lower leaves; 3= Moderately Susceptible- abundant necrosis on leaves; 4= Susceptible- abundant necrosis on lower and middle leaves extending to upper leaves; 5= Highly Susceptible (plant dead completely). The disease scores were recorded at 3, 6, 10 and 15 weeks, after germination.

Data was collected from the middle row of 20 plants, and the mean of the three replicates was used to ensure data consistency. In Bomet, Nakuru, and Narok counties, 354, 340, and 352 plants were examined, for a total of 1044 plants. Bioassay data were arcsine transformed prior to computing area under disease progress curve (AUPDC) (Jeger and Rollison, 2001) for each replicate of the maize varieties.

3.4 Sample collection for fungal and mycotoxin analysis

Maize was harvested six months after planting for fungal and aflatoxin analysis. Samples were taken from the middle row consisting of approximately 20

cobs. Each maize variety was threshed separately to avoid contamination. Every time a fresh variety was introduced for threshing, the threshing apparatus (basins) and hands were washed and disinfected using 70% ethanol.

From each of the six varieties of maize, roughly 1 kg of seed was collected, put in khaki paper bags, properly labeled and taken to the National Agricultural Research Laboratory (NARL)-Plant Pathology Laboratory. Samples were appropriately labeled with the location, variety, and date of harvesting, stapled, and kept at 40°C. From each site a total of 18 samples were collected bringing to a total of 54 samples.

At NARL, Samples from each variety were mixed to homogenize fully by shaking them in khaki paper bags, then divided into two parts; one portion (500g) for fungal isolation and characterization and a second portion (500g) for aflatoxin quantification. For aflatoxin analysis nine samples from each variety from the three study sites, each weighing 30grams were weighed from the second portion representing 54 samples from the three experimental sites.

3.5 Fungal isolation and identification

Five hundred grams of maize grain samples obtained from each hybrid maize was thoroughly mixed and milled into fine flour using a Laboratory Milling

machine. Ten grams of the ground sample was mixed with 100ml sterile water to make a stock solution and serially diluted up to dilution 10^3.

Isolation media was prepared by weighing 39g of PDA into 1 litre of water. The mixture was autoclaved for 15 minutes at 121^{o}C and 15 PSI pressure. The media was allowed to cool to about 50^{o}C and then amended with 25ng/l of streptomycin and tetracycline (Mutegi, 2010) to inhibit bacterial growth. Petri dishes to be used were labelled appropriately and a millilitre of the diluted sample was poured into a sterile petri dish aseptically and swirled gently to mix (Muriithi, 2014). The plates were incubated at room temperature for 5 to 14 days.

3.5.1 Identification of *Aspergillus* species

Purification was done by sub culturing *Aspergillus* species identified on PDA and Rose Bengal Medium following the protocols from Mutegi (2010) and Mutegi (2012). Sub culturing of the fungal isolate on PDA was done to differentiate *Aspergillus* section Flavi from the other species. Pure cultures of each *Aspergillus* isolated were made on PDA media. *Aspergillus* section Flavi species obtained were sub cultured on Rose Bengal Medium. Microscopy on *Aspergillus* was done using modified Riddell mounts (Muriithi, 2014) to allow detailed study of the conidia. Presence of sclerotia after seventh day of incubation, the pattern of

sclerotia growth, colony colour, spore formation on the conidiophores, size of the sclerotia and growth rate was used to differentiate the colonies. Colony number for each species obtained was recorded on the data sheet.

3.5.2. Identification of *Fusarium* species

Fusarium colonies were plated on low-strength PDA and synthetic nutrient agar (SNA) according to Reddy *et al*. (2008) and Muriithi (2014).

Low strength PDA was made by weighing 10g agar, 17g PDA, 1g potassium dihydrogen phosphate (KH_2PO_4), 1g potassium nitrate (KNO_3), 0.5g magnesium sulphate anhydrous ($MgSO_4$), and 0.5g potassium chloride (KCl) in 1000ml water (H_2O). After autoclaving and cooling to 40^0C, the media was supplemented with 25ng/kg streptomycin and tetracycline (Reddy *et al*., 2008).

Synthetic nutrient agar was prepared by weighing 0.2g sucrose, 0.2g glucose, 20g agar, 1g Potassium dihydrogen phosphate (KH_2PO_4), 1g Potassium nitrate (KNO_3), 0.5g Magnesium sulphate anhydrous ($MgSO_4$), 0.5g Potassium chloride (KCl), in 1000ml water (H_2O). The mixture was then cooled to about 50^oC and then amended with 25ng/l of streptomycin and tetracycline (Muriithi, 2014)

For sporulation to occur, the plates were incubated in the dark for 14 days. Cultures grown on PDA were incubated at 25°C for two weeks, while SNA cultures were incubated at 25°C under near UV light for two weeks. The growth characteristics of the low strength PDA plates were noted and used for identification. From the SNA plated, tape and squash mounts were made and observed under a 100 lens light microscope (Nissen, 2007).

The observed features of microconidia, macroconidia, chlamydiospores, and phialides were used to identify the *Fusarium* species obtained, as described in the laboratory manual by Nelson *et al*. (1983) and Leslie *et al*. (2006).

Each sample was replicated three times and the total fungal expressed as a colony forming unit per gram of sample (CFU/g). The relative density of each fungal species was calculated as follows:

$$\text{The relative density \%} = \frac{\text{Number of isolates of a fungal species}}{\text{Total number of fungal species}} \times 100$$

3.6 Aflatoxin quantification.

The materials, after being weighed into 100 g, were homogenized, ground in a Romer mill (Union, 11, USA) and thoroughly mixed. They were then divided

into two equal parts to create a representative subsample for the study. The subsample (50 g) was then mixed with 250 ml water/methanol 40:60 v/v and then vortexed at high speed.

The sample was then centrifuged at 3600rpm for 10 minutes, and the supernatant was collected in a filtration funnel by filtering the material through a Whatman paper No1.To remove the fatty components, the supernatant was treated with 30ml of sodium chloride and 50ml of hexane. To dilute the methanol before analyzing the material with the Enzyme Linked Immunosorbent Assay (ELISA), 10% potassium buffered sulphate was added to the mixture.

The Helica Biosystems Inc. ELISA kits were used in the experiment. Mutiga *et al*. (2014) validated these kits for aflatoxin quantification. The manufacturer of the kit set the lower limit for quantification at 1µ g/kg and the upper limit at 20 µg/kg. The optical density of the samples was measured using an ELISA reader (BioTek Instruments, Inc.) with a 450 nm absorbance filter. The readings were taken, and the test values were interpreted using the experiment's provided standards.

3.8. Statistical analysis

To attain normal distribution, data were transformed using the most appropriate methods. For the Maize lethal necrosis disease severity experiment, bioassay data were arcsine transformed prior to computing area under disease progress curve (AUPDC) for each replicate of a maize varieties (Jeger and Rollison, 2001). For the fungal count experiment, the fungal population were cube root transformed prior to statistical analysis. Aflatoxin data from the experiments were transformed to log (ng/g + 1).

The Maize Lethal Necrosis Disease incidence and severity, fungal species abundance and mycotoxin concentration were analysed separately using Genstat Statistical Programme 11th Ed (Payne *et al.,* 2008). The means were separated using a Least Square Difference test for significance at P=0.05. Pearson's correlation coefficient was calculated to determine the relationship between Maize Lethal Necrosis Disease and Fungal genera. Relative fungal densities were analyzed using ANOVA to assess the magnitude of fungal infection of selected maize varieties.

CHAPTER FOUR

4.0 RESULTS

4.1 Maize lethal necrosis disease severity

The MLND-affected varieties DUMA43 and PH30G-20 had the highest mean Bioassay scores of 4.83 and 4.5, respectively. DK777 had the lowest mean Bioassay score of 2.16, followed by KATE16-02, WEMA5140, and WEMA5135, which had mean Bioassay scores of 2.58, 2.25 and 2.08, respectively. There was significant difference between varieties (p =0.001) (Figure 4.1).

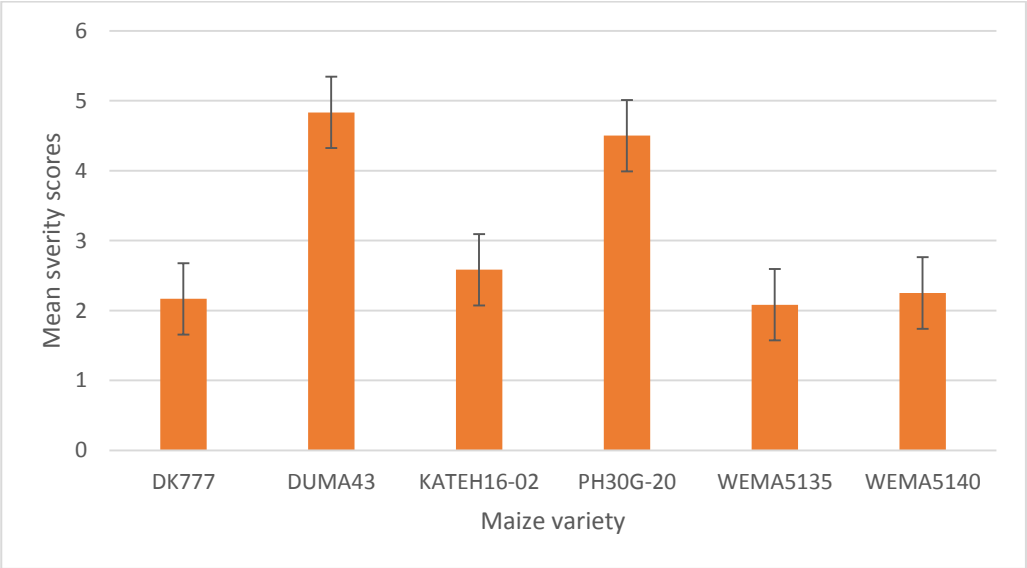
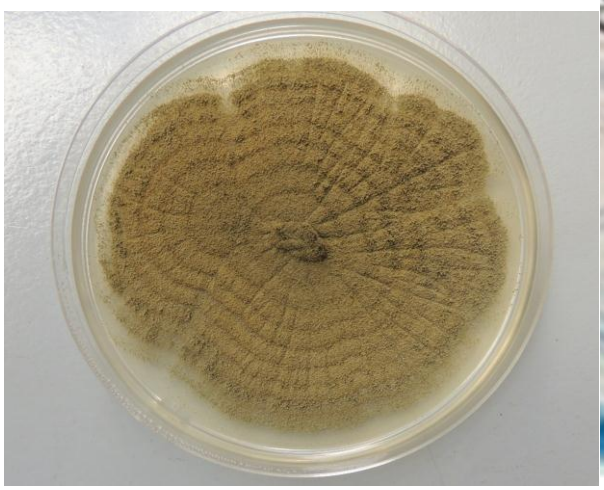


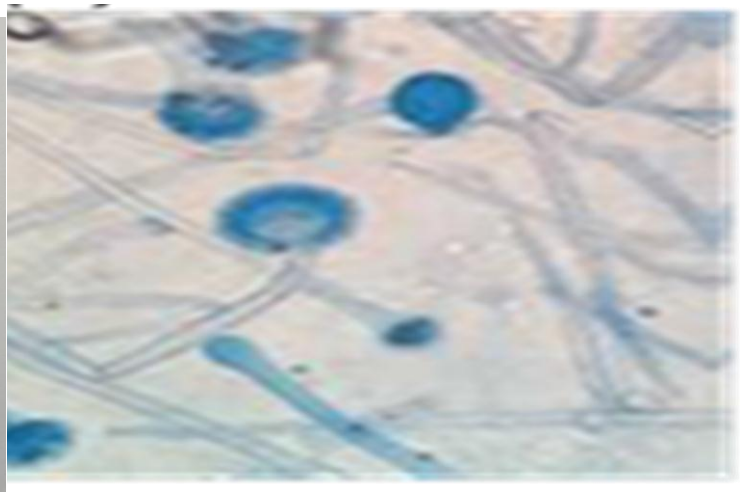
Figure 4. 1: Maize Lethal Necrosis Disease in 6 maize varieties.

4.2. Fungal species isolated and identified in the crop infected with maize lethal necrosis disease

The species were identified on PDA based on their conidial colour and head serration were *A. Flavus;* yellow green surface and numerously biseriated (Fig 4.2 a (i) and a (ii)); *A. niger*, black surface and brownish, relatively long and smooth conidiophore (Fig 4.2 b (i) and b (ii)); *Fusarium verticillioides* isolates were pink in color with white aerial mycelia that had a powdery appearance. Abundant conidia were observed, oval in shape, slightly flattened at the end (Fig 4.2 c (i) and c (ii)).

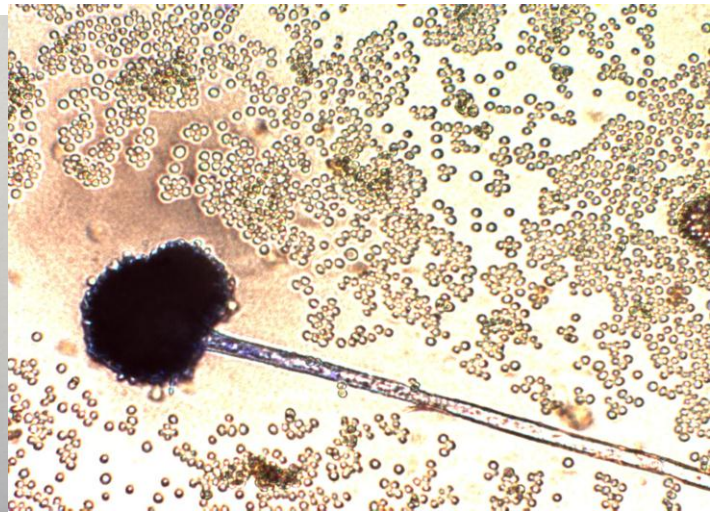


a (i)

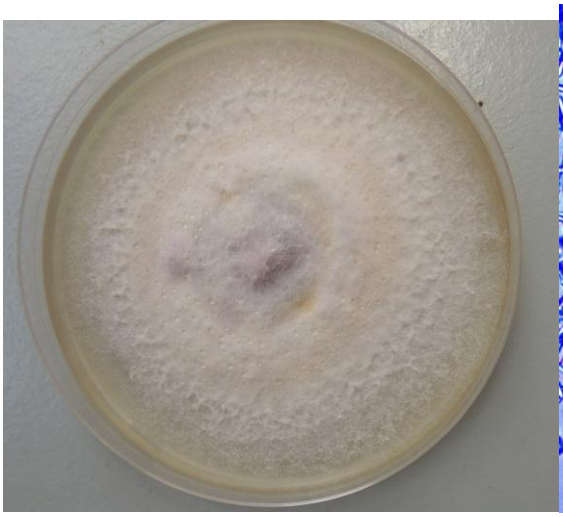


a(ii)

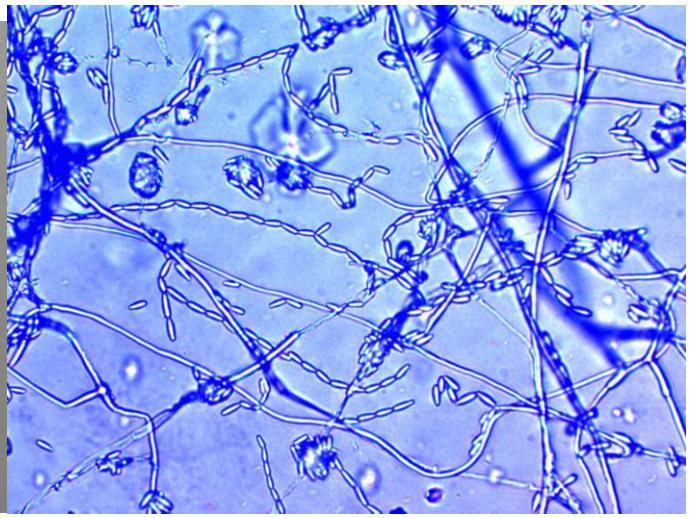
b (i)



b(ii)



c (i)



c(ii)

Plate 4.1: Cultural and morphological traits of the fungi growing on PDA after 14 days of incubation. a(i)*A.flavus* greenish yellow surface,a(ii)a biseriate conidial head with a globose vesicle of *A.flavus* (Mg=1000x);b(i)*A.niger* black surface;b(ii)brownish, relatively long and smooth conidiophores of *A. niger* (Mg=400x);c(i)*Fusarium*, pink in colour color with white aerial mycelia ;c(ii) Abundant conidia were observed, oval in shape, slightly flattened at the end.

4.3 Total fungal contamination in maize grain samples

The results of fungal contamination showed that all the maize samples were infected with *Aspergillus, Fusarium* and *Penicillium* species of the fungi. *Fusarium* was the dominant species of the three fungal isolates in the three regions (Figure 4.2).

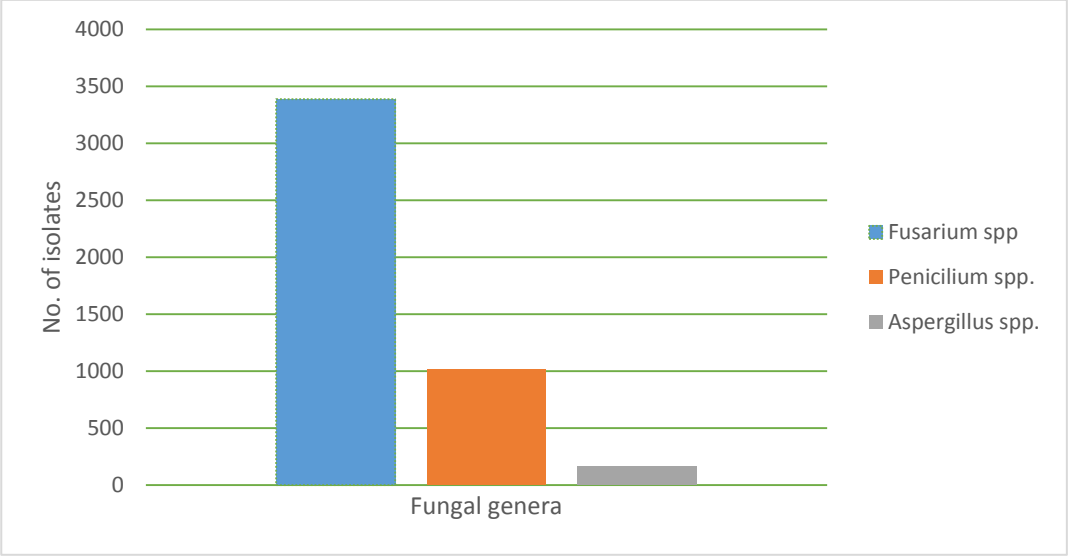


Figure 4.2: Total number of isolates of each fungal genera isolated from maize samples.

4.4 Fungal contamination in relation to the selected maize varieties

PH30G-20 was the most affected, particularly by *Fusarium* fungi, followed by KATE16-02. Overall, *Fusarium* produced high readings in all maize varieties.Variety WE5135 had the highest *Penicilium* reading, while KATE16-02 had the lowest.DK777 did not have *Aspergillus* contamination, but DUMA43 had the most *Aspergillus* contamination.

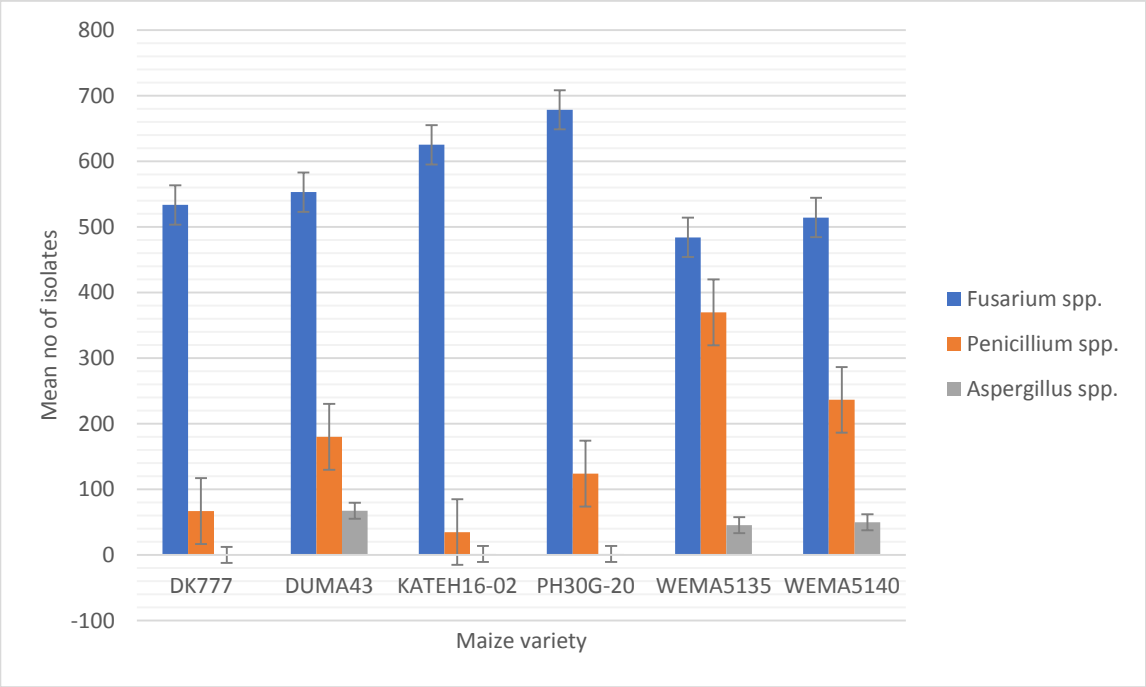


Figure 4. 3: Fungal contamination in relation to the selected maize varieties

4.5 The relative density of fungi in Maize Lethal Necrosis Disease infected grains

Fusarium was the most common fungal genus (79.75%), followed by *Penicillium* (16.03%) and *Aspergillus* (1.933%). In Bomet County, the commercial maize checks DUMA43, PH30G-20, and DK 777 had a mean relative density of 69 %, 82% , and 56 % for *Fusarium*, respectively, while the newly released MLN disease-tolerant maize varieties WE5135 and WE5140 had a relative density of 66.2 % and 58.2 %, respectively. KATE16-02, an MLN disease resistant variety, had 39.6 % relative density for *Fusarium*. There was no significant difference in relative density for *Fusarium* in the six hybrid maize varieties (p=0.88).

With the exception of WE5135, which had a relative density mean of 4%, there was no significant difference in *Aspergillus* contamination at p=0.458. At the same time, there was no significant difference when the sampled varieties were found to be infected with *Penicillium* at p=0.44, with the varieties having a mean relative density of 15.3 %. The maize variety KATE16-02 had a *Penicillium* relative density of zero, while WE5140 had the highest *Penicillium* relative density of 18.2 %.

The highest relative density for *Fusarium* was recorded in variety PH30G-20 in the counties of Narok and Nakuru, at 87.5 and 99.1 %, respectively. WE 5140 and WE5135 had the lowest relative density for *Fusarium* at 31.1 and 32.7 % respectively. The mean relative density of *Fusarium* pollution in maize varieties did not differ significantly between Narok and Nakuru, with p=0.648 and p=0.42, respectively.

There was no significant difference (p=0.453) for *Aspergillus* contamination in the county of Narok, with two varieties WE 5140 and WE5135 recording a relative density of 3.77 % and 2.77 %, respectively, while the other varieties recorded a zero mean for Aspergillus in the county of Narok.

In Nakuru County, there was no significant difference between varieties of *Aspergillus* (p=0.42).The counties of Nakuru and Narok had no significant differences in the incidence of *Penicillium* (p=0.126 and p=065) (Table 4.1).

Table 4. 1: Mean relative density of fungal genera in grains infected with MLND in the counties of Nakuru, Bomet and Narok

Counties	Varieties	Fusarium	Aspergillus	Penicillium
	DUMA 43	69.14a	0.0a	8.51a
	PH 30G-20	81.81a	0.0a	27.34a
	KATEH 16-02	39.64a	0.0a	0 a
	WE 5140	58.19a	0.0a	18.21a
	DK 777	55.6a	0.0a	11.13a
BOMET	WE 5135	66.2	4.0a	26.54a
	MEAN	61.8	0.67	15.3
	L.SD(P≤0.05)	74.97	5.032	32.53
	CV (%)	68.2	424.3	119.8
	DUMA 43	70.11a	0.00a	29.8a
	PH 30G-20	87.52a	0.00a	13.5b
	KATEH 16-02	56.87a	0.00a	9.8b
	WE 5140	31.1a	3.77a	31.8a
	DK 777	55.5a	0.00a	11.1b
NAROK	WE 5135	59.5a	2.77a	37.7a
	MEAN	60.1	1.09	22.3
	L.SD(P≤0.05)	69.34	5.264	45.71
	CV (%)	64.9	271.7	115.3
	DUMA43	56a	22.3a	21.7ab
	PH30G-20	99.1a	0.5a	0.5b
	KATEH16-02	66.7a	0.0a	0b
	WE 5140	58.4a	12.7a	28.8ab
	DK777	84.8a	0.4a	1.8b
NAKURU	WE5135	32.7a	8.3a	58.9a
	MEAN	66.3	7.4	18.6
	L.SD(P≤0.05)	69.08	34.01	48.63
	CV (%)	58.6	258.9	146.9

Mean Values within a column followed by the same letter do not differ at p≤0.05 (Fisher's protected LSD Test**.**

4.6 Correlation between Maize Lethal Necrosis bioassay scores versus fungal species

There was a positive significant correlation between the MLND bioassay score and *Penicilium* infection (r = 0.429, p =0.001). In addition, the *Aspergillus* infection also showed a positive correlation with MLND infection on maize (r = 0.275, P =0.5) (Table 4.2).

Table 4. 2 :Correlations between maize lethal necrosis and different fungal genera (*Fusarium*, *Aspergillus* and *Penicillium*)

		MLN bioassay SCORE	*Fusarium*	*Penicillium*	*Aspergillus*
MLND bioassay SCORE	Pearson Correlation	1			
	N	54			
	p-value	0.657			
	N	53	53		
PENICILIUM	Pearson Correlation	0.068	o.429**	1	
	p-value	0.627	0.001		
	N	53	53	53	
ASPERGILLUS	Pearson Correlation	0.081	0.275*	0.119	1
	p-value	0.566	0.046	0.397	
	N	53	53	53	53

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

4.6 Aflatoxin levels in maize grain samples

Aflatoxin contaminated 50% of the maize samples in Bomet County, and 33.3 percent of the samples in Narok and Nakuru Counties. The maize variety PH30G-20 from Narok County had the highest aflatoxin contamination of the three counties, with a mean aflatoxin value of 2.9 g/kg. There was a significant difference in aflatoxin levels between maize varieties tested for aflatoxin ($p = 0.001$)(Table 4.3).Total aflatoxin levels in maize samples from Narok were highest, followed by Bomet and Nakuru, with mean values of 6.9 g/kg, 6.68 g/kg, and 6.64 g/kg, respectively. The levels of aflatoxin in this study were below the national legal recommended standards of 10µg/kg.

Table 4. 3: Aflatoxin levels in maize varieties per county

Counties	Varieties	n	Positive samples	Range (µg/kg)	Mean
NAKURU	KATEH 16-02	9	0	0-0.921	0.523b
	DK 777	9	0	0.31-0.494	0.392b
	WE 5140	9	3	0.632-0.7	0.781b
	WE 5135	9	3	0.521-0.91	1.023b
	DUMA 43	9	9	1.40-2.81	2.024a
	PH30G-20	9	3	0.48-0.762	1.91a
BOMET	KATEH16-02	9	0	0-0.92	0.52b
	DK 777	9	3	0.27-1.1	0.60b
	WE 5140	9	3	0.47-1.04	0.81b
	WE 5135	9	3	0-62-1.3	0.734b
	DUMA 43	9	9	1.13-3.3.01	2.044a
	PH3OG-20	9	9	1.72-249	1.977a
NAROK	KATEH16-02	9	0	0.142-0.527	0.358b
	DK 777	9	0	0-0.834	0.509b
	WE 5140	9	0	0.314-0.492	0.389b
	WE 5135	9	0	0-0.707	0.342b
	DUMA 43	9	9	2.209-3.356	2.49a
	PH3OG-20	9	9	2.366-3.421	2.79a

Mean values within a column followed by the same letter do not differ significantly at $p \leq 0.05$ (Fisher's protected LSD Test). Values ≥1 are positive, value less than 1 are negative

4.7 Aflatoxin levels in relation to traits

In this experiment, cumulative sample data from the three study sites showed that high levels of contamination of aflatoxin with a mean of 2.1µg/kg were produced by the maize varieties that were susceptible to MLN disease. MLN tolerant maize varieties were second with mean aflatoxin contamination of 1µg/Kg and lastly resistance maize varieties had a mean aflatoxin contamination of 0.45µg/Kg. This analysis showed a significant positive difference at 0.1 per cent (Figure 4.4)

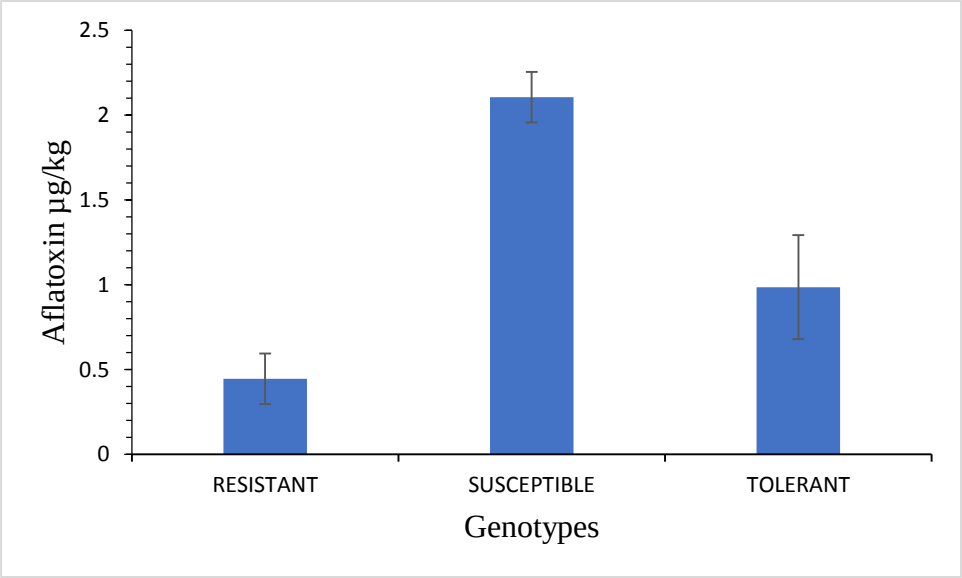
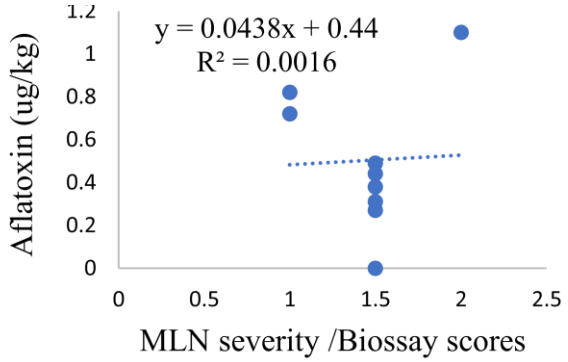


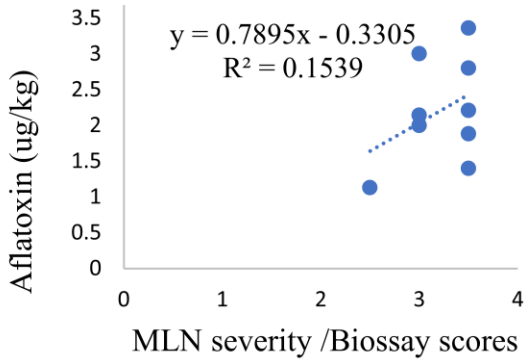
FIgure 4. 4: Aflatoxin levels in relation to resistance, tolerance and susceptibility of maize varieties to MLND

4.8 Correlation for each variety

There was a positive correlation for varieties DK777, DUMA43, PH30G-20, WE5135 and WE5140 but a negative correlation for variety KATE16-02 (Fig 4.5)



(a)



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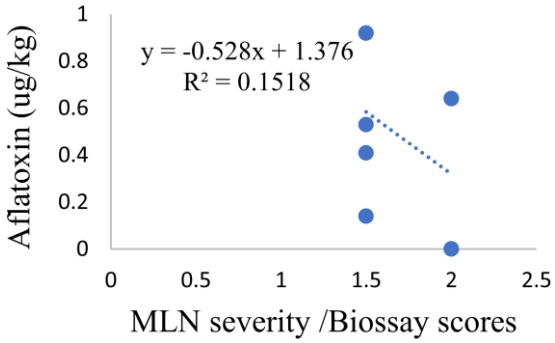

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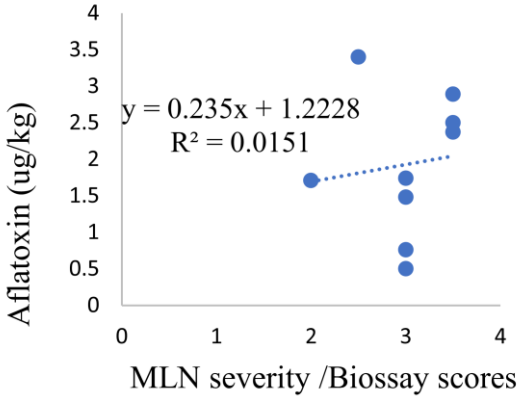

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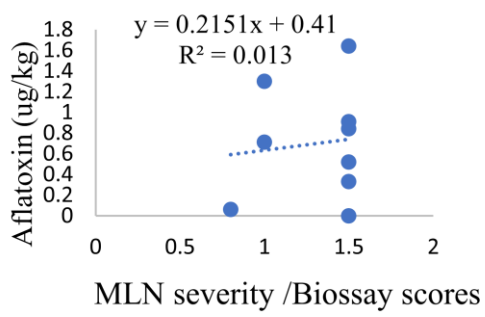

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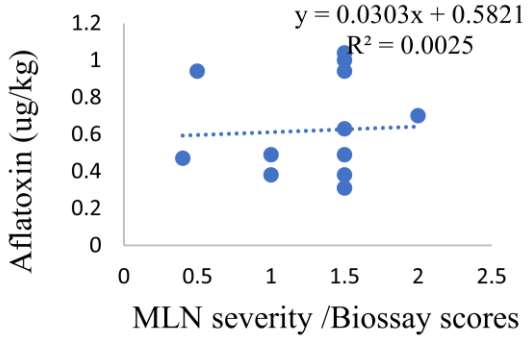
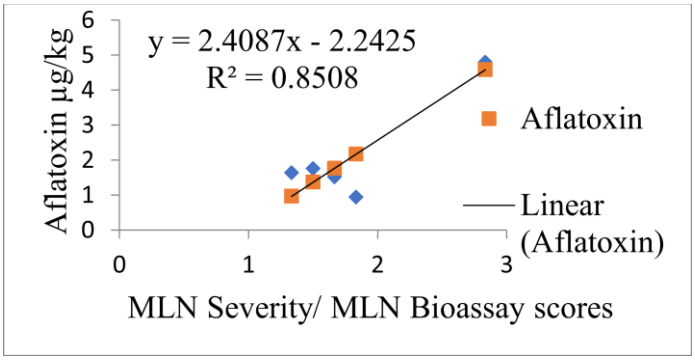


(f)

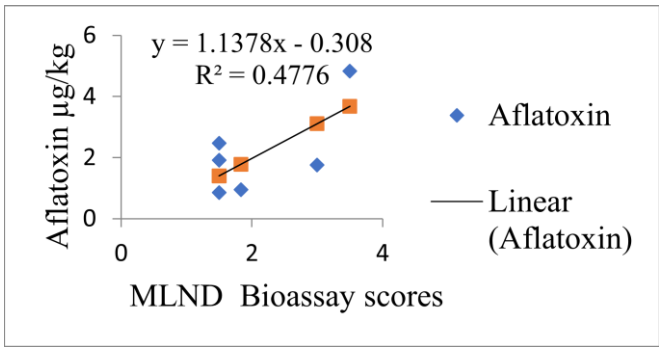
FIgure 4. 5: Regression of MLND against aflatoxin in relation to varieties; (a) DK777, (b) DUMA43, (c) KATE16-02, (d) PH30G-20, (e) WE5135, (f) WE5140

4.9 The relationship between MLN and aflatoxin in the three counties

The research indicated that low MLND bioassay levels resulted in low amounts of aflatoxins in maize samples and high MLND bioassay levels resulted in high amounts of aflatoxins. High correlation was observed in the county of Nakuru (r^2=0.931), followed by the county of Bomet (r^2=0.850) and the county of Narok (r^2=0.477). Acumulative analysis of samples from three counties showed a positive correlation (r^2 =0.5537) (Figure 2). Positive correlation between MLND intensity and aflatoxin content (r^2=0.5471) was established. The overall correlation was significant at p=0.01(Figures 4.6)



(a)



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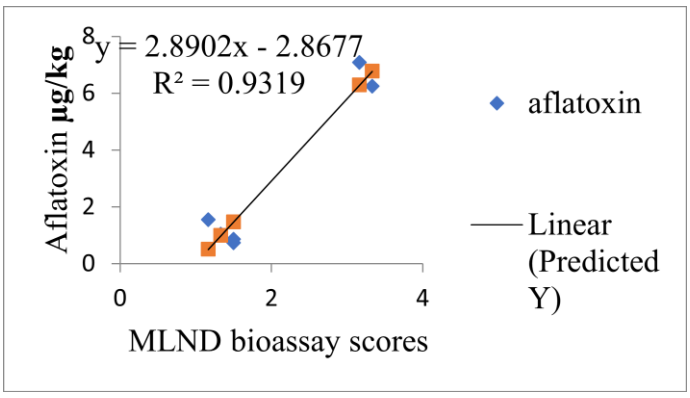

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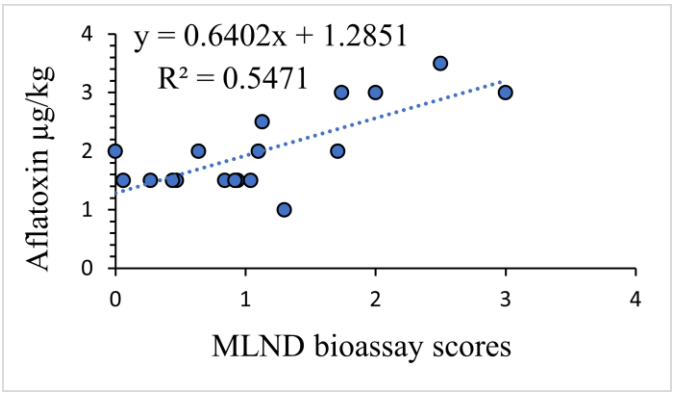

(d)

FIgure 4. 6 Regression of MLND against aflatoxin in the three counties. (a) Bomet (Longisa), (b) Narok (Mulot), (c) Nakuru (Njoro),(d) overall correlation in the three counties.

CHAPTER FIVE

5.0 DISCUSSION

In Kenya, there has not been a study on the relationship between maize lethal necrosis disease (MLND) infection and fungal contamination of maize. The results of the fungal identification show that all corn varieties, whether resistant, tolerant or susceptible, were infected with fungi, particularly *Fusarium*, *Penicillium* and *Aspergillus*. Similar results have been reported elsewhere in tropical regions of Africa (MacDonald and Chapman, 1997; Kapindu *et al*., 1999; Bigirwa *et al*., 2007). The results showed that *Fusarium* species were the most widespread, followed by *Penicilium* and *Aspergillus*. The presence of *Aspergillus* species such as *A.flavus* and *A.niger*, *Fusarium* species and *Penicillium* spp. in the samples is consistent with the findings of other researchers such as Alakonya (2009) and Mutiga *et al*., 2015

Maize Lethal Necrosis Disease infection affected fungal infection as the results show that susceptible lines were more affected by the fungus than resistant and tolerant lines. According to Wangai *et al*. (2012) and https://mln.cimmyt.org/, MLND reduces maize resistance to other opportunistic pathogens. High levels of fungal contamination were reported in susceptible MLND varieties (PH30G-20

and DUMA 43), which also displayed more severe MLND symptoms such as necrosis, rotting, grain deformities, and discoloration.

The disease (MLND) thrives in areas with high rainfall, warm temperatures, and high relative humidity (Mudde *et al*., 2018). This climate, which prevailed in the three study countries, could have provided an ideal environment for MLND and field fungal contamination. Previous research had identified *Fusarium* species, *Aspergillus* species and *Penicillium* species as the most common fungi infecting maize in Kenya (Alakonya, 2009; Nooh *et al*., 2014).

Many factors contribute to *Fusarium* fungal infection of maize and hence mycotoxin contamination, including climatic conditions, insect damage, agronomic and post-harvest activities (Marasas *et al*., 2001). These factors do not affect infection individually, but through interactions (Dragich and Nelson, 2014).

The high *Aspergillus* population in Nakuru can be attributed to the moderately high temperature between 24°and 27 °C, which predisposes maize to fungal contamination. *Aspergillus* thrives in warm, humid conditions with an optimal growth temperature of 37 °C, but the fungus grows easily between 25^0 and 42 °C (Wagacha and Muthomi, 2008).

In this study, the most commonly isolated aflatoxigenic fungi in maize samples were *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus niger*. *Aspergillus flavus* is the most prevalent aflatoxigenic fungus in Kenya and has been commonly associated with foodborne toxicity in the country (Mutegi *et al*., 2012).

Aflatoxin production is mainly determined by moisture availability and temperature (Bandyopadhyay, 2014). Warm and humid conditions encourage *Aspergillus parasticus* and *Aspergillus flavus* to infest maize kernels, leading to production and accumulation of aflatoxins (Wagacha and Muthomi, 2008; Bandyopadhyay, 2014). The distribution of aflatoxigenic *Aspergillus* and aflatoxin was also found to be MLND dependent and varied between resistant/tolerant and susceptible maize varieties. With the susceptible varieties having the most of the contamination. These results agree with Wagacha and Muthomi, 2008, who attributed the spread of *Aspergillus flavus* and its respective mycotoxins to plant stress from abiotic or biotic factors.

In this study, *Penicillium* populations were not as abundant as *Fusarium* species and there were no significant population differences between maize genotypes. *Penicillium* was low in variety KATEH16-02 which can be attributed

to less MLND damage as the variety is resistant. The high *Penicillium* population in DUMA 43 can be attributed to MLND that predisposes maize to fungal attack.

Contamination of maize grains by the three fungal species reduces maize quality, making it susceptible to mycotoxin poisoning caused by some *Aspergillus* and *Fusarium* species (Wagacha and Muthomi, 2008). The presence of the three toxigenic fungi in maize raises the risk of multiple mycotoxins.

Aflatoxin levels in maize samples tested in this study were low, well below the recommended legal limit of 10µg/kg, which is consistent with Alakonya *et al*. (2009)'s Rift Valley study. Despite the low levels of aflatoxin a stronger link was found between Maize Lethal Necrosis Disease and aflatoxins. Maize Lethal Necrosis Disease susceptible varieties had the highest contamination, followed by tolerant varieties, and finally susceptible varieties. This is consistent with the findings of Chen *et al*. (2004), who discovered that aflatoxin contamination is positively correlated to plant stress.

Despite the fact that aflatoxin-tested maize samples indicated levels below the Kenya Bureau of Standards limits for acute aflatoxicosis, chronic exposure to these toxins can pose a high risk to consumers. There are other studies that have revealed widespread aflatoxin exposure in the country at a time when no outbreaks

had been recorded (Mutiga *et al*., 2015). This indicates the need for regular mycotoxin surveillance and tracking. In this research, maize samples that were susceptible to MLND infection were more affected by fungal attacks and aflatoxins compared to tolerant and resistant maize varieties, suggesting that MLND predisposes maize to fungal and mycotoxin contamination.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

All of the selected maize cultivars were found to be infected by the three fungal genera in this study (*Fusarium*, *Penicillium* and *Aspergillus*).In comparison to tolerant and resistant maize varieties, susceptible varieties were the most affected. *Fusarium* was the most prevalent fungus, followed by *Penicillium* and *Aspergillus*.

The levels of aflatoxin in the corn samples tested in this study were low, well below the legal limit of 10 µg/kg.

The study discovered a strong link between aflatoxin and Maize Lethal Necrosis Disease. Aflatoxin levels were higher in maize lines susceptible to MLND, implying that the disease may be a precursor to the development of aflatoxin and other mycotoxins.

It is uncontestable that aflatoxins pose a serious health risk to both livestock and human beings world-over, particularly in the tropics where climatic conditions (high moisture and temperature) spur the growth of moulds. The Eastern Province of Kenya as a case in point has encountered frequent outbreaks of aflatoxicoses. There is no cure for aflatoxin B1 poisoning and it damages vital

organs in the body including the liver, kidneys and lungs. Other than the direct health risk, economic losses arising from aflatoxicoses are equally enormous. Therefore controlling mould growth and aflatoxin production is very important. First step in mould control is to ensure that the maize grains are healthy, stress free and avoiding other pathogens that can act as a precursor to secondary infections of maize.

Although the findings from this study were focused on aflatoxins, there is a strong likelihood of other mycotoxins occurring in maize grains in higher proportions.

RECOMMENDATIONS

- There is need to carry out research on the relationship between MLND and other maize mycotoxins like zearalenone, ochratoxins, fumonisin and trichothecene.
- Regular public awareness campaigns on the dangers of using or feeding MLND-infected corn should be carried out.
- Continued surveillance of fungal infection and aflatoxin levels in Kenyan maize is necessary to identify risks and prevent infections.

- Breeders to include mycotoxin resistance as a criteria for selecting MLND tolerant germplasm.

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APPENDICES

Appendix 1.0: Analysis of variance

Appendix 1. 1: One-way anova for MLND scores versus varieties

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Variety	5	31.41204	6.28241	63.86	<.001	***
Residual	48	4.72222	0.09838			
Total	53	36.13426				

Appendix 1. 2: One-way anova for MLND versus MLND in counties

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
County	2	0.2315	0.1157	0.16	0.849
Residual	51	35.9028	0.704		
Total	53	36.1343			

Appendix 1. 3: One-way anova for MLND versus *Fusarium*_%_fungal _incidence in Bomet. County

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	5	2524	505	0.34	0.888
Residual	48	71833	1497		
Total	53	74357			

Appendix 1.4: One way anova for MLND versus Fusarium % fungal instances in Narok County.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Variety	5	8420.1	1684	3.24	0.013	*
Residual	48	24981.7	520.5			
Total	53	33401.9				

Appendix 1. 4:One-way anova for MLND versus fusarium_%fungal_incidence in Nakuru County.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	5	498.3	99.7	0.81	0.547
Residual	48	5892	122.8		
Total	53	6390.4			