EFFICACY OF PLASMA TECHNOLOGY IN ELIMINATING FUNGI AND AFLATOXINS IN MAIZE IN MAKUENI AND BARINGO COUNTIES, KENYA

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A Thesis Submitted in Fulfilment of the Requirements for the Award of the Degree of Doctor of Philosophy in Food Science and Technology

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

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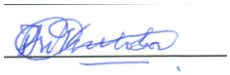

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This thesis is dedicated to all my loved ones especially my children Mary, Philomena, Samuel, Clare, Jude, Magdalene, Bridget, Monica, Michael and Bernadette for their many prayers, amazing patience, encouragement and support. All the glory, power and honour be unto God who has brought me this far!

Psalms 23

"The lord is my Shepherd; there is nothing I shall want"

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ABBREVIATIONS AND ACRONYMS

LTNP	-	Low temperature nitrogen plasma
KAP	-	Knowledge, attitude and practices
BBD	-	Box Benken design
SDG's	-	Sustainable development goals
CDC	-	Centre for disease control
HCC	-	Hepatocellular carcinoma
HBV	-	Hepatitis B virus
FAO	-	Food and Agriculture Organization
EAC	-	East African Community
KEBS	-	Kenya Bureau of Standards
DBD	-	Dielectric barrier discharge
NTP	-	Non-thermal plasma
APP	-	Atmospheric pressure plasma
UV	-	Ultraviolet
MT's	-	Mycotoxins
DNA	-	Deoxyribonucleic acid
ELISA	-	Enzyme linked immunosorbent assay
HPLC	-	High performance liquid chromatography
HIV	-	Human Immunodeficiency virus
EABS	-	East African Bureau of Standards
OR	-	Odds ratio
EC	-	European commission

DEFINITION OF TERMS

Toxin	-	Poisonous or harmful substance produced by a living cell or organism
Mycotoxins	-	Toxic substance produced by fungi capable of causing death or illness to humans or animals
Aflatoxins	-	Group of toxins produced by certain fungi that commonly attack maize, peanuts, cotton seed and tree nuts.
Plasma	-	Emerging technology producing gaseous ions and free radicals that is considered effective against micro- organisms and their associated toxins
Efficacy	-	Ability to achieve an intended result
Toxigenic	-	Produces a toxin or toxic effect
Atoxigenic	-	Does not produce a toxin
Metabolites	-	An intermediate or end product of a metabolic event
Nixtamalization	-	Traditional method of cooking maize in alkali to remove aflatoxins commonly practiced in Mexico and Central America
Extrusion	-	Process where food material is forced to flow through a die or small orifice at very high temperatures
Aflatoxicosis	-	Disease resulting from aflatoxin poisoning
Serum	-	It is the largest protein constituent in human blood
Hepatocellular carcinoma	-	This is the most common type of primary liver cancer
Mutagenic	-	Ability to cause changes in genetic structure
Teratogenic	-	Ability to cause abnormalities in foetal growth and development
Organoleptic	-	Aspects relating to use of sense organs (taste, odour, feel and colour)
Sterilization	-	Process involving complete removal of deactivation of all forms of life
Aerobic	-	Organisms that require oxygen for survival

GENERAL ABSTRACT

Maize (*Zea mays var.* indentata L.) is the most important food security crop in Kenya and plays an important role in human nutrition. Over the years, there has been increased concern over the rising cases of aflatoxin poisoning in Kenya due to contaminated maize especially in Eastern and North Rift parts of the country. This has led to huge losses not only in the country's breadbasket areas but also in the national grain reserves. Aflatoxins are fungal toxic metabolites that naturally contaminate food and feed. Exposure to aflatoxins is associated with various cancers, suppressed immunity, retarded growth, mutations, and aggravation of other existing conditions such as HIV among others. Plasma technology presents a possible solution. Plasma is electrically energized matter in gaseous form that is generated at different conditions of temperature, pressure and ionization power. Low temperature plasma is an emerging technology that is finding space in the food industry particularly in decontamination processes. Use of plasma at low temperature makes the decontamination process practical, inexpensive and suitable for products where high temperatures are not desired.

The main objective of this study was to determine the efficacy of plasma technology in destroying fungi and aflatoxins in maize in Makueni and Baringo counties in Kenya. The specific objectives were: to determine the influence of knowledge, attitude and practices of farmers on aflatoxin contamination of maize in Makueni and Baringo counties in Kenya, to determine the influence of postharvest practices and storage conditions on aflatoxin contamination in maize in the two counties, to isolate and characterize the fungi responsible for contamination in both counties and finally to determine the efficacy of plasma technology in destroying fungi and aflatoxin in maize.

A convergent mixed method study design that combined quantitative and qualitative data collection techniques was used for the knowledge, attitude and practices study. The data collection methods included interviewing, using a pretested questionnaire, focus group discussions and key informant interviews. To screen the aflatoxin levels in the maize samples from both counties, 144 samples were randomly collected and subjected to the ELISA technique for quantitative detection of aflatoxin B1, B2, G1 and G2. Confirmatory test for the ELISA positive samples was carried out using HPLC analysis. Isolation of fungal strains was done using rose bengal selective media which contained chloramphenicol thereby suppressing bacterial growth. Isolated strains were characterised based on their phenotypic

characteristics on the plate and microscopic techniques. Finally, the efficacy of Low temperature nitrogen plasma (LTNP) in destroying fungi and aflatoxin was studied using an experimental design generated using Response Surface Methodology (RSM) of the Box Benken Design (BBD) of the Design Expert software (StatEase, 2020). Independent factors were exposure time, pressure and ionization power whilst percent reduction in both the fungal load and aflatoxin level were the response variables.

The results of the knowledge, attitude and practices (KAP) study revealed a significant difference in the knowledge of factors contributing to aflatoxin contamination in maize. Socio-economic and demographic factors were linear predictors of knowledge (R^2=0.76, p<0.001), whereas they had no effect (R^2=0.043, p=0.076) on the attitude of the maize farmers. Farmers indicated poorly dried maize and poor storage conditions as the main causes of aflatoxin contamination. The aflatoxin analysis on the maize showed that Makueni County had the highest percentage of aflatoxin positive samples with up to 174 ppb. The type of storage condition had a significant effect on the extent of contamination and accounted for 11% of the variation (R^2 =0.11). Gunny bags were the most common type of storage condition and had the highest level of contamination in both counties whilst metallic bins had the lowest contamination. Strains of *Aspergillus flavus, Aspergillus terreus* and *Aspergillus parasitucus* were positively identified after characterization of the isolated strains. Finally the RSM linear model predicted the reduction in fungal load and aflatoxin content with F-values of 7.22 and 15.89 respectively ($P \leq 0.01$). An increase in exposure time and pressure lead to a corresponding decrease in the fungal load and aflatoxin content. Ionization power did not have a significant effect on both response variables. For optimisation of the detoxification process, the RSM model supported process settings of time at 153.58 seconds, pressure of 0.98 Pascals and ionization power of 194.82 Watts.

The findings lead to the conclusion that more awareness creation, training of farmers on good agricultural practices, enhanced market surveillance and laboratory services are needed to educate farmers and the general public on dangers related to exposure to aflatoxins. The type of storage condition significantly affects the aflatoxin level in stored maize, proper drying of maize and storage in hermetic structures offers the best method to prevent aflatoxin contamination. Finally, plasma is efficacious in destroying aflatoxins and fungi in the maize to a reduction of 68.78% and 33.89 log (cfu/g) for aflatoxin content and fungal load, respectively.

Further the research recommends encompassing temperature as an independent variable in the RSM model to fine tune optimisation parameters.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background of the Study

Realisation of the sustainable development goals (SDGs) of achieving zero hunger by the year 2030 not only requires developing countries to increase the quantity of food grains produced but also improve on pre- and postharvest processes. However, food grains when stored under unfavourable conditions are prone to aflatoxin contamination. This subsequently poses a major threat to human health, production and marketing of food grains world over. Data from the Food and Agriculture Organisation (FAO) approximates that 25% of world food crops are affected by aflatoxin. On the other hand, the Centre for Disease Control (CDC) estimates that more than 4.5 billion people in the developing countries are exposed to aflatoxins (Unneveh & Delia, 2013). In Kenya, maize and particularly white dent maize (*Zea mays var*. indentata L.) is an important staple food that plays an important role in human nutrition and food security. However, along the value chain, pre- and postharvest losses occur as a result of insect pests, rodents and pathogens. Stored grain pests include: maize weevils, larger grain borer or "scania", moths and red rust beetle. Rodents include rats and mice whilst pathogens include fungus among others. Various practices such as: timely harvesting when the ear (cob) droops, proper drying of shelled maize before storage, observing storage hygiene and treating the grains with insecticidal dust, are practiced by farmers to alleviate the problem. Aflatoxins are toxic chemical metabolites produced by different species of fungi and are known to have carcinogenic, mutagenic, teratogenic and hepatotoxic effects in both animals and humans (Benkerroum, 2020). The main fungus associated with very high fatalities in many parts of Kenya is *Aspergillus flavus*, which produces the most lethal chemical metabolite, aflatoxin B1. Destruction of aflatoxins by use of conventional methods such as cooking, boiling is difficult due to their heat resistant nature. Detection is a challenge as testing is expensive coupled with

shortage of analytical capacity. Low temperature plasma is one of the emerging technologies for the improvement of food safety. Several studies have shown that plasma is selective and thus does not harm the food material.

1.2 Statement of the Problem

Maize is the most important staple food for the majority of Kenyans. The grain however is vulnerable to myco-toxigenic fungi which not only causes a reduction of its quality by discolouration, but also a reduction of its nutritional value. Mycotoxins cause devastating economic losses all over the world by impacting negatively on human health, animal productivity and trade. Many interventions ranging from physical, chemical to biological approaches have been developed to eliminate aflatoxins from contaminated maize. Biological approaches involve use of atoxigenic strains that are selected from nature through an intense process using microbiological, DNA and field-based methodologies. These strains exclude the aflatoxin producing *A. flavus* and other aflatoxin producers from the crop environment and results in decreased crop aflatoxin contamination. Another key biological method is use of resistant or tolerant varieties to achieve resistance to *A. flavus* through prevention of fungal infection of maize, prevention of subsequent growth once the infection occurs, inhibition of aflatoxin production following infection and degradation of aflatoxins by the plant or fungus. Development of aflatoxin-resistant varieties is, therefore, a very complex process. Physical methods usually involve use of heat; these are mainly nixtamilization and extrusion cooking. Nixtamilization is a process whereby maize is soaked and cooked in an alkaline solution, usually lime water, followed by washing and hulling. The process is known to remove up to 97-100% of aflatoxins from mycotoxin-contaminated maize. Extrusion, on the other hand, involves cooking under very high temperatures and pressure. Both methods are expensive and therefore cannot be economical if used on commercial basis. Finally, chemical methods which

involve use of insecticidal powders on the grain are used before storage to prevent attack by pests and insects that expose the grain to higher chances of developing aflatoxin. However, these pesticides are detrimental to human health hence most of them have been banned from use. Due to setbacks of existing methods, there is need to develop safer, cheaper, efficient and more convenient methods to detoxify grains. Plasma technology has been applied in food processes and has been shown to destroy the pathogen while being selective to the food material. It has been successfully used to sterilize surfaces and reduce the level of aflatoxin in various food materials at ambient temperature and pressure without resulting in any detectable changes in quality. It therefore could be suitable in the destruction of mycotoxins and fungus in food materials.

1.3 Justification of the Study

The efficacy of Low Temperature Plasma (LTP) in various applications has been demonstrated in selected studies. In the food industry, LTP has been identified as a promising intervention to improving food safety and extending shelf life of foods. It has shown ability to remove toxins (N. Misra et al., 2019) and inactivate a wide range of microbes including spores (Feichtinger et al., 2003; Kelly-Wintenberg et al., 1998; K. P. K. Lee et al., 2006) and viruses (Terrier et al., 2009). Another important factor is it is selective to the food material but destroys the pathogen or toxin (Dobrynin & Fridman, 2014). Maize and particularly white dent maize (*Zea mays var.* indentata L.) is the most important food security crop in Kenya and plays an important role in human nutrition. Unfortunately, due to various setbacks, aflatoxin contamination in maize leads to huge losses in the country's bread basket and also in the national grain reserves. Aflatoxins are heat resistant and their destruction difficult by use of the conventional food processes. Their detection is further complicated by lack of analytical capacity as the required

equipment and reagents are expensive. One of the possible methods for control of the aflatoxin menace in maize would be through the use of plasma technology.

1.4 Aim of the Study

To contribute towards improving the safety of maize using plasma technology as well as mitigate against losses as a result of aflatoxin contamination.

1.5 Purpose of the Study

The purpose of the study will be to contribute towards information on knowledge, attitude and practices, prevailing levels of aflatoxin in maize and the strains responsible in Makueni and Baringo counties and ways of mitigating the occurrence of aflatoxicosis by use of plasma technology.

1.6 Objectives

1.6.1 General objective

To determine the efficacy of plasma technology in eliminating fungi and aflatoxins in maize for increased food safety in Makueni and Baringo counties.

1.6.2 Specific objectives

a) To establish the influence of knowledge, attitude and practices of farmers on aflatoxin contamination of maize in Baringo and Makueni counties
b) To determine the influence of storage conditions and postharvest practices on aflatoxin contamination in maize in Makueni and Baringo counties
c) To isolate and characterize *Aspergilli* species in maize grain in Baringo and Makueni counties
d) To determine the efficacy of plasma technology to destroy fungi and aflatoxin in maize

1.7 Hypotheses

a) Knowledge, attitude and practices of communities in Makueni and Baringo counties do not lead to aflatoxin contamination in maize.

b) Storage conditions and postharvest practices do not have an influence on aflatoxin contamination of maize in Makueni and Baringo counties.

c) Myco-toxigenic *Aspergilli* strains of fungi are not present in maize grain grown in Makueni and Baringo counties.

d) Low temperature plasma does not have the efficacy to destroy fungi and aflatoxins in maize.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mycotoxins and their Negative Effects

Mycotoxins (MTs) are fungal toxic metabolites which naturally contaminate food and feed (Daou et al., 2021). When ingested, inhaled or adsorbed through the skin, even in very small concentrations, are associated with various cancers, retarded growth, suppressed immunity and mutations among other complications (Umesha et al., 2017). Presently, there are slightly over 300 known mycotoxins with varying characteristics associated with their origin, chemical structure, function and biological effects. This notwithstanding, only a few of these strains have a significant effect in on health and agriculture (Alshannaq & Yu, 2017; Umesha et al., 2017). Aflatoxins especially aflatoxin B1, are considered the most lethal in the group of more than three hundred known mycotoxins.

2.2 Conditions that Promote Growth of Mycotoxigenic Fungi

Mycotoxigenic fungi are a common occurrence in food crops all over the globe. Their proliferation and growth is promoted by various factors especially the environmental conditions (Richard et al., 2003). The contamination with mycotoxins can occur at any point of the value chain in an accumulative way especially in the field, during harvesting, drying and eventually in storage (Richard et al., 2003). However, the presence of mycotoxigenic fungi in a food material, does not guarantee mycotoxin contamination as fungi require certain favourable environmental factors for them to grow and thrive (Kochiieru et al., 2020; Perdoncini et al., 2019). Likewise, complete removal of fungi in the food material does not eliminate the risk of mycotoxin production as fungi due to their resistant chemical nature (Daou et al., 2021). There are six major factors that promote mycotoxin production; namely, temperature, water activity, relative humidity, pH, fungal strain and substrate.

2.2.1 Temperature, water activity and relative humidity

Environmental factors play a key role in fungal growth and mycotoxin production. Most fungal growth and subsequent mycotoxin production is promoted by a temperature range of between 25-30^{o}C, water activity of greater than 0.78 and a relative humidity of between 88-95% (Thanushree et al., 2019). For the *Aspergillus spp*, that mainly produce aflatoxins, the conditions that promote germination are also conducive for fungal growth which in turn leads to mycotoxin production in the crop at the different pre- and postharvest stages (Mannaa & Kim, 2017).

2.2.2 Medium pH

The pH of the medium in which the fungi exists plays a key role in its growth and mycotoxin production. Fungi have the intrinsic ability to regulate their environment by producing acids or even alkali in order to enhance their survival and existence in the food material. For instance, *Penicillium* and *Aspergillus spp*. can acidify their environment by producing gluconic and citric acids (Vylkova, 2017). Aflatoxin production requires a pH of 4.0 or lower which also enhances its production (Perdoncini et al., 2019; Reverberi et al., 2010).

2.2.3 Fungal strain

The production and level of toxicity of the different mycotoxins is dependent on the type of fungal strain in a food material. Aflatoxin B1 for example is majorly produced by *Aspergillus flavus, Aspergillus parasitucus, Aspergillus pseudotamarii* and more rarely *Aspergillus nomius* (Frisvad et al., 2019). There is also a variation in the optimum growth conditions for different species. The optimal conditions for *Aspergillus* flavus is 15-44^{o}C which varies with the other strains of the same genera (Mannaa & Kim, 2017).

2.2.4 Substrate type

Mycotoxigenic fungi thrive on a wide range of food materials as the nutrients they require are mainly carbon and nitrogen which are abundantly found in many food materials especially those rich in carbohydrates (Kokkonen et al., 2005). Cereals such as maize are particularly prone as they are high in carbohydrates.

2.3 Aflatoxins and their Toxic Effects

Aflatoxin is one of the most studied mycotoxins in the world. It is a toxic metabolite produced by aflatoxigenic fungi of the *Aspergillus* species (Frisvad et al., 2019). Across the value chain, aflatoxins contaminate food and feed leading to devastating acute and chronic effects to huge populations (Rahimi et al., 2010). These crops include maize grains, peanuts, cereals and animal feeds. There are six out of the 18 identified aflatoxins that have been identified as important. These are B_1, B_2, G_1, G_2, M_1 and M_2 (Dors et al., 2011). They differ in terms of molecular characteristics and even how they are identified. For instance, the B group will exhibit a blue fluorescence under UV light and the G group a yellow-green fluorescence. Globally, aflatoxin B_1 is the most widespread (Cullen et al., 1993; Kok, 1994) and most common (Hussein & Brasel, 2001) of all aflatoxins and accounts for over 75% of all contamination in food and feed (Ayub & Sachan, 1997). Aflatoxin M_1 and M_2 are found in milk and are hydroxylation derivatives of aflatoxin B_1 and B_2 respectively. They end up in the milk after the cows consume aflatoxin contaminated feed. Even after milk processing, they still remain stable and this poses a serious public health concern.

2.4 Prevalence of Aflatoxins in Foods

Globally, it is estimated that about 25% of agricultural produce is lost due to aflatoxin contamination. This mainly affects cereals especially maize and ground nuts (Belayhun et al.,

2019). Aflatoxins also pose a serious threat to human health (Umoh et al., 2011) and are considered a serious challenge globally (Kumar et al., 2017). In 2008, aflatoxins were put on a Rapid Alert System for Food and Feed (RASFF) of the European Union due to its devastating effects (EC, 2009). Aflatoxin B1 was later categorized as a group 1 carcinogen for humans (Min et al., 2011). In Kenya, the government with the support of non-governmental organizations has continued to support farmers through many programs trying to address this challenge. Despite these interventions, aflatoxins continue to be a serious threat to food and agricultural products (Kumar et al., 2017).

2.5 Aflatoxins and Risks Posed to Food Security

Food security is defined as "a situation when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" (FAO, 2001). Aflatoxins contaminate a huge array of foods which include maize, peanuts and tree nuts rendering the produce unsuitable for both human and livestock consumption (Massomo, 2020). The aflatoxins pose a more serious problem to maize grains in Kenya as this leads to enormous losses in the country's bread basket and this poses a threat to food security. According to data collected by the Famine Early Warning System Network, during the 2020 short rains assessment, it estimates that about 1.4 million Kenyans are facing a food crisis. This is attributed to poor rainfall resulting in poor harvest (Network, 2021). Nonetheless, the potential of maize as a food security crop in this country has not been fully realized because of limited raw material and product diversity, and persistent aflatoxin contamination of the maize value chain.

2.6 Regulatory Policies on Aflatoxins in Kenya

Due to risks associated with acute and chronic exposure to aflatoxins, Kenya just like the rest of the world has set maximum limits permitted in food and feed. This is through development

of standards. Standards are documents that provide requirements, specifications, guidelines or characteristics for various materials, products, processes and services. This involves multi-stakeholder consultations pooled from both public and private entities (Y. Y. Gong et al., 2015). The regulatory arm of the government also works alongside other International partners such as East African Community (EAC) through the East African Bureau of Standards (EABS) and Codex Alimentarius Commission (Codex). Consequently, harmonized standards have been developed that promote free and fair trade between countries. Additionally, consumer confidence is enhanced when standards are in place as they guarantee safe, reliable and good quality food products. The regulatory limit set by Kenya through the Kenya Bureau of Standards (KEBS) is 5 ppb for aflatoxin B1 and 10 ppb for total aflatoxins (B_1, B_2, G_1, G_2) (Y. Y. Gong et al., 2015).

2.7 Maize Production in Kenya and Associated Risks of Aflatoxin Contamination

In Kenya, maize is produced in both small and large farms with fluctuating overall production trends over the years. In 2019, 95% of the 3,800 tonnes of maize produced was utilized at subsistence level. Aflatoxin contamination is a common occurrence in specific regions of the country especially the North Rift and Eastern parts of Kenya (Okoth et al., 2017). These areas have varying weather conditions with the Rift valley region majorly experiencing cool and humid conditions. The Eastern region is mainly hot and dry most parts of the year, conditions that are conducive for aflatoxin proliferation (S. Mutiga et al., 2015). The growth of myco-toxigenic fungi and subsequent production of toxins in the field is promoted by high humidity, high temperature, drought conditions and insect infestation. During storage, growth may be accelerated if there is sub-optimal harvesting, poor drying and storage conditions (Belayhun et al., 2019). These regions usually experience erratic rains that may increase the moisture of the maize during harvesting and during storage. Poor storage conditions and post-harvest practices

are common in most small scale farms in Kenya which also contribute to aflatoxin contamination in maize (Stasiewicz et al., 2017). For instance, in 2010, the Kenyan government declared over 2.3 million bags of maize unfit for human consumption due to the high levels of aflatoxins (Mutegi et al., 2018).

2.8 Prevalence and Episodes of Aflatoxicosis in Kenya

Kenya is among the leading countries that has experienced some of the most devastating cases of human aflatoxin poisoning in the world (Kilonzo et al., 2014; Mehl & Cotty, 2010; Okioma, 2008). Exposure by ingestion of high concentrations greater than 6000 mg/kg will lead to liver failure and death after 1-2 weeks (Groopman et al., 1988). Typical signs of aflatoxicosis include: oedema, vomiting, abdominal pain, convulsions, jaundice and in the worst case scenario, death. The first ever recorded case of aflatoxicosis in Kenya was in 1960 where 16,000 ducklings succumbed after consumption of contaminated feed (Peers & Linsell, 1973). Later, in 1981, the first serious recorded case of human aflatoxicosis occurred. Patients presented with several symptoms including abdominal pain, anorexia and fever. Out of the 20 cases admitted, 12 developed liver failure and succumbed after 1-12 days of hospitalization. However, the most historic and tragic case occurred in 2004 in Eastern Province. Out of the 317 cases that were reported, 125 fatalities resulted. Since then, there have been recurring episodes of aflatoxicosis annually mainly in Eastern and Central parts of Kenya. A study in 2010 reported one of the highest ever recorded levels of aflatoxin B1 in human serum in the world (Unnevehr & Grace, 2013).

2.9 Fungi Associated With Aflatoxin Contamination

Aflatoxins are a group of mycotoxins produced by a group of at least 20 fungal strains of the *Aspergillus* section Flavi, Nidulantes and Ochraceorosei (Baranyi et al., 2013; Villers, 2014).

The main fungi (*Aspergillus flavus*) which produce these mycotoxins thrive under favourable conditions on a wide range of foods and feed such as maize and groundnuts/peanuts, and are a world-wide problem. Aflatoxin contamination can occur before harvest when the crop undergoes drought stress due to elevated temperatures at the grain filling stages and when wet conditions occur at harvest periods. Contamination also occurs when there is insect damage, delayed harvesting and high moisture levels during storage and transportation (Tola & Kebede, 2016). Grains (cereals and oilseeds) and nuts in general are subject to mould attack, in pre-harvest and postharvest. Among moulds that can attack these foods, *A. flavus*, and *A. parasiticus* are the most important because they can produce aflatoxins that are considered a potent natural toxin (Turner et al., 2005). Aflatoxins are produced mainly by different *Aspergillus* species, but *Emiricella* and *Petromyces* have also been reported as aflatoxin producers (Frisvad et al., 2019).

2.10 Health Effects of Aflatoxin Contamination in Humans and Animals

Aflatoxins are highly toxic, cancer causing fungal metabolites known to cause immune-system suppression, growth retardation, liver disease, and even death in both humans and domestic animals.

2.10.1 Carcinogenic, mutagenic and hepatotoxic effects

Aflatoxin B1 is the most potent of all aflatoxins and is associated with liver cancer particularly causing hepatocellular carcinoma (HCC) in humans and a variety of animal species. Concurrent exposure to both aflatoxin and hepatitis B virus (HBV) is a common occurrence in developing countries and greatly increases an individual's risk of developing HCC (Y. Liu & Wu, 2013). Recent studies have suggested an almost multiplicative relationship between presence of detectable aflatoxin biomarkers, HBV infection and HCC. The risk of developing HCC grew

6 times for individuals with detectable aflatoxin biomarkers as opposed to those without. This risk increased to 11 times for those with HBV infection and further increased to 73 times for individuals with both HBV infection and detectable aflatoxin biomarkers (Y. Liu et al., 2012). Studies to estimate the global burden of liver cancer attributable to aflatoxins have been conducted. (Y. Liu & Wu, 2013) used a quantitative cancer approach in their analysis that include 5 billion individuals around the world, estimated that 25,200-155,000 liver cancer cases annually could be attributed to aflatoxin exposure. A latter study by *Liu et al,* (2012) estimated that about 23% of all HCC cases in Africa and Asia were attributable to aflatoxin, for a total of up to 172,000 cases per year. HCC is the third leading cause of cancer deaths worldwide, which only goes to show the contribution of aflatoxins to these deadly cancers.

In animals, mutagenic effects have been reported. These are related to changes in the DNA structure resulting in breaking, rearrangement or even complete loss of chromosomes. In foetuses, teratogenic effects have been observed and have presented as abnormalities in the development of the skeletal structure, skull and other features (Feitah et al., 2014).

2.10.2 Immunotoxic effects

There is also evidence pointing that aflatoxins modulate the immune system (Xu et al., 2021) and is also associated with stunting in children (Alamu et al., 2020). It is also associated with aggravation of other existing diseases such as HIV/AIDS, Kwashiorkor, Tuberculosis and Hepatitis B (Keenan et al., 2011; Obuseh et al., 2011; Wangia et al., 2019). In animals, aflatoxins are known to cause reduced immunity making the animal susceptible to a many bacterial, viral, fungal and parasitic infections (Meissonnier et al., 2008; Oswald et al., 2005; Pierron et al., 2016; Rushing & Selim, 2019; Yunus et al., 2011).

2.10.3 Nephrotoxic effects

Damage to the kidneys has been reported due to prolonged exposure to aflatoxins in animals. In poultry, for instance, exposure to AFB1 resulted in decreased levels of calcium, inorganic phosphate, sodium and potassium whilst urea levels increased. Heart damage was also reported (Yilmaz et al., 2018). A study on mice also concluded adverse effects as a result of exposure AFB1 and AFM1 (Huiying et al., 2018).

2.10.4 Reproductive effects

In animals, aflatoxins have been associated with reduced fertility by adversely affecting the spermatozoa and oocytes. Aflatoxins have spermatotoxic effects, consequently destroying the morphology and physiology of spermatozoa. In females, oocyte maturation is affected and may lead to reduced egg production and quality in poultry. Exposure to the foetus resulted in retarded growth and reduced foetal lengths (Jia et al., 2016; J. Liu et al., 2015; Rawal et al., 2010).

2.10.5 Gastrointestinal (GIT) malfunctions

Aflatoxins affect the GIT in many ways with the most significant being changing its morphology, reduced digestive ability, intestinal immunity and microflora. Consequently, this leads to malnutrition as the integrity of the GIT is compromised as a result of malabsorption and micronutrient deficiencies. A study on aflatoxins in body fluids in Nigerian children concluded that higher aflatoxin biomarkers were found in body fluids of children with protein energy malnutrition (PEM). This was attributed to reduced excretion and/or increased exposure (Onyemelukwe et al., 2012). Other similar studies have reported similar results (Mupunga et al., 2017; Omara et al., 2021; Wangia-Dixon et al., 2020; Wangia et al., 2019; Xu et al., 2021).

In animals, many studies have shown a link between the GIT effects and aflatoxins (Gallo et al., 2015; Mughal et al., 2017).

2.11 Methods of Detection and Quantification

One of the key mitigation strategies of aflatoxin contamination lies in accurate detection and quantification of samples. Sampling is the most sensitive stage during the aflatoxin testing process. It is imperative to ensure that the sub-sample is a true representative of the entire lot (Whitaker, 2003). Sampling protocols have been developed and are in use in the over 50 KEBS certified aflatoxin testing labs in Kenya. They use Gafta methods (No. 130, 24:1) and EAS 79. Across the globe, there is yet another emerging challenge in aflatoxin testing. This is associated with "masked mycotoxins" as they can neither be identified nor detected using ordinary analytical techniques (Kamle et al., 2019). They are modified biologically by fungi and plant enzymes during the infection stage and thus changing their structures. In Kenya, the methods used for aflatoxin detection and quantification are: Enzyme-linked immunosorbent assay (ELISA), high performance liquid chromatography (HPLC), thin layer chromatography (TLC), flourimetry, liquid chromatography-tandem mass spectroscopy (LC- MS/MS), tandem quadrupole mass spectroscopy (TQMS), ultra-high-pressure liquid chromatography (UHPLC) and finally lateral flow immune-chromatography (LFI). The most widely used of all these methods is the ELISA techniques as it is highly sensitive, convenient, inexpensive, requires minimal sample clean up and doesn't pose a health hazard as it uses enzyme labels (Wacoo et al., 2014).

2.12 Current Methods Used in Detoxification and Decontamination of Mycotoxins

Early control and prevention of mycotoxin contamination along the value chains is very important especially in all critical production stages (N Hojnik et al., 2017; Pankaj et al., 2018).

This is because initial prevention from contamination is better and assures of better quality products. However, this is not always the case as contamination usually occurs at critical stages while the crop is in the field, during harvesting and storage. In order to detoxify and decontaminate these grains, several method are used that can be classified as physical, microbial, chemical and enzymatic (Karlovsky et al., 2016). The level of decontamination of these methods ranges between 14% reduction to complete eradication of the toxin. The degree of elimination depends on the method of decontamination used, toxin concentration and the type of toxin in the food (Gomez-Salazar et al., 2021). Physical methods used include hand sorting to remove visibly infected grain, washing, dehulling, polishing and even classification by colour based fluorescence under UV light rays. The challenge with these methods is they have to be applied mainly at an industrial level using optical density sorting equipment (Karlovsky et al., 2016). Chemical methods used include ozone treatment, alkali, use of mycotoxin binders and even use of organic and inorganic acids (Sipos et al., 2021). These have a challenge of the possibility of the subsequent products formed after treatment rebuilding back in the body once the treated maize is consumed leading to poisoning. They may also change the organoleptic properties of the food. Microbial methods involve use of dominating microflora that out compete the aflatoxigenic fungi. Enzymatic methods also generally employ the use of enzymes that interfere with the metabolism of the fungi making it impossible for the pathogenic fungi to thrive (Sipos et al., 2021). These methods are not only expensive and tedious but also time consuming making their implementation quite challenging.

2.12.1 Physical methods

2.12.1.1 Sorting to remove contaminated grains

The first step in the processing of most agricultural produce involves sorting, washing or milling (Grenier et al., 2014). Washing involves partial removal of mycotoxins found on the

outer surface of the grain but the solubility of the mycotoxin should be considered (Pleadin et al., 2019). The broken and damaged grains are often prone to more fungal attack and hence contain high levels of mycotoxins (Johansson et al., 2000). In industrial applications, sorting is done using optical sorting equipment that use UV light technology. A bright greenish-yellow fluorescence is observed in contaminated grains and they are automatically removed from the batch. This glow does not originate from the aflatoxins but from a kojic acid derivative formed during the process of the toxin formation (Karlovsky et al., 2016). However, some mycotoxins do not show any visible symptoms as they accumulate which may pose a challenge when using the optical sorting equipment. A recent study found that aflatoxin content did not reduce even after sorting was done (S. K. Mutiga et al., 2014).

2.12.1.2 Sieve cleaning

Sieve cleaning is applied where there are broken grains that may be infected or can act as a source of spoilage as they are more prone to fungal attack as compared to the whole grain (Peng et al., 2018). Removal of smaller kernels by use of an industrial screen siever and a gravity table reduced the aflatoxin content significantly (Shi et al., 2017).

2.12.1.3 Floatation and density segregation

Fungi damaged maize grains can be separated from the healthy ones using density segregation and fractionation on gravity tables. Since damage is largely by a broad group of fungi, this method does not specifically target a specific toxin (Karlovsky et al., 2016). However, some studies have shed light on reduction of specific toxins. For instance, a study on effect of saturated sodium chloride solution removed 3 % of the damaged grain and 74% of the total aflatoxin in the maize (Huff & Hagler, 1985).

2.12.1.4 Washing of grains

Mycotoxins that are soluble in water can be partially washed off the surface of contaminated grain thereby reducing the aflatoxin content. Washing medium that have been used include water and sodium carbonate solutions (Rotter et al., 1995). One of the greatest challenges of using this method is that the grain must be dried afterwards before it can be stored (Karlovsky et al., 2016). This further exposes the grain to more attack by fungi and production of mycotoxins.

2.12.1.5 Steeping of grains

This is the first step in the wet milling operations with the intention of separating the germ from the rest of the kernel and breaking down the protein part. It involves soaking the maize in water with concentrations ranging between 0.1 - 0.2% SO_2 for 36-50 hours at 50^{o}C (Karlovsky et al., 2016). A study involving steeping of maize found that half of the aflatoxin content in the maize was found in the steep water (Aly, 2002). Other studies on sorghum have reported similar results (Lefyedi & Taylor, 2006).

2.12.1.6 Dehulling of grains

Dehulling is also an effective method for fungal removal and aflatoxin reduction (Peng et al., 2018) that has been found to be more significant compared to floating and washing (Fandohan et al., 2005; L. Matumba et al., 2015; Mutungi et al., 2008). It involves removal of the outer layer of the grain before eventual milling is done. A study on *'muthokoi'*, a traditional maize dish made from dehulled maize found that the aflatoxin content was reduced by 46.6% (Mutungi et al., 2008). This subsequently lowered the dietary exposure to aflatoxins which is the case with consumption of whole grains (Kilonzo et al., 2014).

2.12.1.7 Heat treatment

Heat treatment is another method that has been applied but with limitations. Aflatoxins are very heat resistant with a decomposing temperature of above 235ºC (Pankaj et al., 2018; Peng et al., 2018; Ryu et al., 2008). This is the why sun drying does not decrease the aflatoxin content in stored grain. However, heat treatments at higher temperatures and longer periods have successfully reduced the microbial load and aflatoxin content. For instance, soy bean treated at 100 and 150ºC for 90 minutes resulted in a decrease of 41.9 and 81.2% respectively (J. Lee et al., 2015). Similar results were reported when peanuts and pistachio were roasted at temperatures ranging 90-150ºC for 30-120 minutes. This resulted in a percent reduction in the aflatoxin content of 57-90 and 93% for peanuts and pistachio respectively (Arzandeh & Jinap, 2011; Rastegar et al., 2017). Drying at higher temperatures and longer duration was also found to reduce the microbial load and aflatoxin content in wheat (Hwang & Lee, 2006). Other innovative decontamination methods that use heat treatment include steaming, infrared, microwave, radiofrequency and extrusion cooking. Extrusion of maize meal has achieved decontamination levels of 80.5-83.7% and 74.7-87.1% in aflatoxin B ($_{1,2}$) and aflatoxin G ($_{1,2}$) respectively (Massarolo et al., 2021).

2.12.1.8 Milling of grains

Milling reduces the level of mycotoxins to a large extent. It has been observed that for small grains contained a higher level of mycotoxins as opposed to the milled flour (Cheli et al., 2013; Tibola et al., 2015). Mycotoxins on a kernel of maize are found concentrated on the non-starch fraction of the grain. This is why wet milling provides even more benefits as the mycotoxins leach out of the non-starch parts of the grain moving into the steep water (Karlovsky et al., 2016). Dry milling also removes most of the aflatoxins that are concentrated in the germ and bran sections of the kernel (Bullerman & Bianchini, 2007).

2.12.1.9 Mycotoxin binders

These have been used in decontamination of animal feeds but can be used in food meant for human consumption (Jans et al., 2014). Some studies have reported positive results on use of mycotoxin binders in mitigation of extreme effects caused by aflatoxins. However, up until now, there have been limited reports on use of mycotoxin binders in foods.

2.12.1.10 Irradiation of contaminated grains

This method has potential for application in large scale set ups and can be used to partially decontaminate and detoxify food products. Maize grain contaminated with *A.flavus* will easily be identified under UV light and removed as the contaminated grains emit a bright greenish-yellow light thus making the separation possible. The fungi and toxins inside the grain are not visible under UV light (Pasikatan & Dowell, 2001). The challenge with this method is the public may not embrace this technology due to the fear of irradiated foods. This notwithstanding, the European commission approved the use of 10 kGy as a maximum permissible limit after it was proved harmless by the FAO/IAEA/WHO Expert Committee (Pleadin et al., 2019).

2.12.1.11 Cold plasma technology

This is an emerging technology that is considered effective against fungi and their associated toxins. Plasma comprises of ionized gases containing metastable atoms and molecules at zero electrical charge. The mycotoxin degradation ability of plasma is associated with the presence of free radicals (mainly O- and -OH) (N. Misra et al., 2019). Plasma also has antimicrobial effects and has been used on experimental basis to sterilize fragile and surfaces that are

temperature sensitive. It has been found to be affordable and environmentally friendly compared to other existing detoxification methods (N Hojnik et al., 2017).

2.12.2 Chemical methods

There have been many studies that have explored the use of chemical processing aids in detoxifying or decontaminating foods. Despite these numerous studies, use of chemicals in foods meant for human consumption remains banned within the EU and other jurisdiction (Karlovsky et al., 2016). Thus their use would require approval but the relevant regulatory agencies to guarantee consumer safety. In 2015, the European commission (EC, 2015) developed a set of guidelines for approval of detoxification techniques. Their mode of application ranges from mixing, immersion, packing and fumigation (Karlovsky et al., 2016).

2.12.2.1 Treatment with acids

Majority of mycotoxins are resistant to weak acids. Strong acids, however, interfere with the biological activity of AFB1 and AFG1 converting them to less toxic metabolites (Karlovsky et al., 2016). A recent study exploring the combined effect of diluted acids (acetic acid, citric acid and lactic acid) and cooking on aflatoxins found that the treatment with lactic acid was the most efficient. AFB_1 was reduced to the less toxic AFB_2 and AFB_{2a} (Aiko et al., 2016). Additionally, the carboxylic acids formed had a preservative effect by interfering with the fungal growth.

2.12.2.2 Treatment with bases

Alkaline conditions make aflatoxins unstable by converting it to less toxic metabolites. This reaction should be completed till the end as this process is reversible and can lead to rebuilding of the original toxin (Karlovsky et al., 2016). Nixtamilization which involves soaking or

cooking of maize or other grain in an alkaline solution (usually calcium hydroxide) for 8-24 hours has been shown to partially remove aflatoxins. The grain is thereafter washed and hulled, a process commonly used in the production of tortillas and other maize based food products (Sergio et al., 2019). However, the danger lies in the subsequent rebuilding up of the original toxin when the conditions become conducive.

2.12.2.3 Treatment using oxidizing agents

Oxidizing agents such as ozosne and hydrogen peroxide have been shown to degrade mycotoxins such as AFB_1. For instance, ozone was shown to reduce AFB_1 and AFG_1 by 77 and 80% respectively in peanuts after treatment at 75^{o}C for 10 minutes. A 51% reduction was observed for AFB_2 and AFG_2 (Proctor et al., 2004). Hydrogen peroxide and ozone have been used commercially in various applications to detoxify corn and other food matrices (Ferreira et al., 2020; Ismail et al., 2018; Loi et al., 2020; J. Silva et al., 2018; Torlak et al., 2016). This process has not shown any sadverse effects in the food quality (Tiwari et al., 2010; Zhu, 2018) but may lead to production of by products which may not be safe and thus caution must be taken (Deng et al., 2020) .

2.12.2.4 Treatment with reducing agents

Sodium bisulphite ($NaHSO_3$) has been found to reduce the aflatoxin content in various applications. The content of black pepper reduced by 96-100% after sodium bisulphite was applied at a rate of 0.25-2% with a combination of atmospheric and high pressures (Jalili & Jinap, 2012). The aflatoxin content in white and black pepper reduced after treatment with several compounds of acidic, alkaline and salt nature. These include: phosphoric acid, calcium hydroxide, sodium, potassium, sodium bicarbonate, sodium chloride, sodium hydrosulphite and sodium sulphate. These treatments resulted in between 18-51% reduction in the aflatoxin content (Jalili et al., 2011).

2.12.2.5 Treatment with food ingredients and medical plants

It has been proved that certain spices, herbs and other cooking ingredients destroy mycotoxins. For instance, an Asian cooking spice, carom, an extract of ajwan was shown shown to detoxify mycotoxins (Velazhahan et al., 2010). Other studies have shown similar positive results (Panda & Mehta, 2013; Vijayanandraj et al., 2014). A recent review also summarized all the most recent studies of detoxification of aflatoxins using Asian spices and herbs (Aiko & Mehta, 2015).

2.12.3 Enzymatic methods

This detoxification method employs the use of enzymes such as amylases, glucanases and proteases as the most common ones (N. Misra et al., 2019). An exceptional feature with enzymes is that they are highly specific. The potential use of enzymes in detoxification processes has been generally reviewed (Vanhoutte et al., 2016) and their potential use in production of food (Karlovsky, 2014). Their application in detoxification processes has been tested using laccases and peroxidases (J. F. Alberts et al., 2009; J. Wang et al., 2011). However, there are certain challenges associated with use of enzymes. First, enzymes are proteins in nature and thus may cause allergic reactions in the food material. Secondly, they have the potential to change or destroy valuable food nutrients. However, there has not been any approval of use of any enzyme for decontamination processes in the EU (Karlovsky et al., 2016).

2.12.4 Biological methods

This form of detoxification involves use of microorganisms such as algae, bacteria, yeast and moulds. It became popular after there was need to avoid some physical and chemical methods and opt for more natural treatments (Daou et al., 2021). Their mode

of action is primarily by degrading the mycotoxin by several modes of action. These may by binding or by modifying the original toxin to a less toxic form either by acetylation, glucosylation, deamination, hydrolysis or decarboxylation especially in the animal feeds industry (N Hojnik et al., 2017). For instance, lactic acid bacteria and yeast are commonly used in detoxification of mycotoxins by binding them on cell wall surfaces and resultantly converting them to less toxic compounds (Pleadin et al., 2019). This method is considered cheap and environmentally friendly as it does not present any danger to the environment. However, it is considered impractical, time consuming and it cannot be applied in the case of multiple mycotoxins (Patriarca & Fernandez Pinto, 2017).

2.13 Application of Low Temperature Plasma (LTP) in Food Systems

The last two decades have presented a lot of advancement in technology relating to thermal sciences such as plasma technology. Low temperature plasma (LTP) is a promising intervention in food processing to improve food safety and increase shelf life of foods (N. Misra et al., 2019). It is also denoted as non-thermal or cold plasma. Plasma technology is used in production of many products and processes such as plasma science (Nageswaran et al., 2019), microbiology (Segura-Ponce et al., 2018), biotechnology (Bekeschus et al., 2018; Julak et al., 2017; Laroussi, 2018; Simoncicova et al., 2019) and food sciences (Coutinho et al., 2018; Scholtz et al., 2015). In the food industry, LTP has been applied in food decontamination, enzyme inactivation, removal of toxins, food packaging applications and treatment of wastewater (Misra et al., 2016). The main guiding factors for its increasing application are high

demand for high product quality, improved productivity, environmental compatibility, precision and flexibility (Kuloba et al., 2014). Plasma is the fourth state of matter formed by ionization of elements and gases among others. It comprises electrons, protons, and positive and negative ions, neutral molecules, and atoms and a variety of other particles all existing in the same environment. The purpose and behaviour of the produced plasma is influenced by the manner in which the charged and neutral particles interact. Due to these factors, their utilization in biosystems, biochemical and bioengineering processes is playing a very vital role where conventional methods could not have been possible (Kuloba et al., 2014). Among other fields where useful applications have been made are medical treatment especially in sterilization, surgery, material treatment/ surface coating and waste treatment, namely decomposition of compounds containing NO_3, NH_3 or CN_x groups as an environmental management technique. Others are catalytic reactions in chemical processes, bioprocesses in agriculture and food as a nonchemical gas phase disinfection agent, nanotechnology and biomaterials (Osamu, 2008). Plasma can be generated in different forms: low [non-thermal] or high temperature, high and low pressure. Hence, plasma can be created in various types that include Low Temperature Plasma (LTP) and Low-Pressure Plasma (LPP) (Kuloba et al., 2014).

While there has been some progress in the interaction of plasmas with organic materials, the study of plasma-living tissue interaction is an almost unexplored field (Kushner, 2008). Two areas where interaction between plasma and living tissue have been exploited are categorized as destructive and non-destructive; destructive sterilization of medical devices, surgery etcetera and non-destructive treatment of wheat and oat seeds to enhance their germination and early growth (Osamu, 2008). Low temperature plasma did not harm the living cells of the seed and thus implied that this can also be employed in other foods to improve bioavailability or even biosafety such as in the case of aflatoxin in maize. Low temperature plasma has also been used

for the treatment of wool fabric in which the wool characteristics of wettability were changed (F. F. Chen, 1994). Low temperature plasma is usually free of complicated magnetic fields and ultraviolet ray emissions are negligible*;* thus, it can be used in the field of food processing (Mastwijk & Nierop, 2010).

2.14 Application of Low Temperature Plasma Science in Controlling Aflatoxicosis

The microbiology and safety of grains, seeds, nuts and their products remain very important due to their extensive use as human food and in livestock feeds. The fungal attack in cereal grains is caused by field fungi, which attack grains at high moistures or storage fungi, which attack grains stored at relatively low moisture (Karlovsky et al., 2016). *Alternaria, Cladosporium* and *Fusarium* are typical examples of field fungi whilst *Eurotium, Aspergillus* and *Penicillium* are storage fungi. *Aspergillus spp*. is associated with the aflatoxin poisoning. Aflatoxins are heat resistant and their detoxification from food is not possible by use of normal food processing temperatures. Methods for their detection are also expensive and complicated thus making them unavailable to many. Aflatoxin B1, B2, G1 and G2 are produced by some strains of *Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius* (Sipos et al., 2021) with aflatoxin B1 being the most common. Use of Atmospheric Pressure Plasma (APP) also commonly referred to as Non-Thermal Plasma (NTP) in the food industry has opened up doors for improvement in the area of food safety. It involves the use of a neutral ionized gas that is ionised by use of high electric current resulting in an environment comprising many reactive species. These include positive ions, negative ions, electrons, photons and molecules (excited or non-excited) at or near room temperature. There are also changes in pressure that give different forms of low- or high-pressure plasma. In the fresh and processed foods industry, plasma has been used in inactivation of microbes albeit at an experimental level. Uses of plasma activated water (Los

et al., 2018) and plasma packaging have also been implemented in the food industry. The limitation for full upscaling of the plasma technology has been the limited knowledge on the effects of the NTP on the chemical and nutritional characteristics of the food after treatment with plasma. Therefore, more studies to investigate the safety and cost implications of the use of this technology are needed in order to open more avenues for its uptake in many food applications. Several studies have reported the inactivation of fungi and lowering of aflatoxin in grains and nuts (Basaran et al., 2008; Iqdiam et al., 2020; Sen et al., 2019; Shi et al., 2017). A study on the effect of low temperature plasma on *Aspergillus flavus* and *Aspergillus parasitucus* showed a decrease of 5.48 and 5.20 $\log_{10}$ CFU/g respectively after 5 min plasma treatment (Dasan et al., 2016).

2.15 Microbial Inactivation Mechanism of Plasma

The use of plasma as a sterilization method was first patented in 1968 and the plasma made from oxygen was first applied in 1989 (Basaran et al., 2008). Since then, several studies have been carried out to assess the use of plasma for microbial inactivation. The result was that interacting plasma agent with biological matter contributed to lethal action. Plasma treatment could effectively inactivate a wide range of microorganisms including spores and viruses. Effect of plasma on different microorganisms can be completely selective, meaning that it can damage pathogenic microorganisms without damaging food material or it can activate different pathways in different organisms (Sen et al., 2019). The reactive species in plasma have been known to cause the oxidative effects on the outer surface of microbial cells. Nitrogen and oxygen gas plasma are good sources of reactive oxygen-based and nitrogen-based species such as O., O_2, O_3, OH, NO., NO_2. Chemical rate constant of atomic oxygen for oxidation at room temperature is higher than that of molecular oxygen (Iqdiam et al., 2020). Cold plasma species destroy fungi through various pathways finally resulting in their inactivation as summarised in

Figure 1. These pathways include destruction of cellular protein, fragmentation and release of DNA, deformation of mycelial tips and accumulation of body lipids (N. Misra et al., 2019). The reactive species in the plasma environment change the DNA of the microorganism and therefore prevent the cells from replicating. The role of UV photons in inactivation of microorganisms when they are subjected to plasma was reviewed in detail by Boudam (Dobrynin & Fridman, 2014). Many studies have found that reactive species had the most important role in inactivation of microorganisms whereas the role of UV photons in plasma was minor (Critzer et al., 2007). However, results from these studies demonstrated that more research needed to be done over the role of UV photons in plasma. Contribution of each of the above-mentioned mechanisms in inactivation of microorganisms depends on plasma characteristics and type of microorganism. The duration required for inactivation of the microorganism is dependent on the type of device producing the plasma, gas pressure, gas composition, voltage, and the distance of the microbe from the discharge glow. The types of microorganisms include Gram-positive, Gram-negative and spores (Boudam et al., 2006).

The efficacy of different gas compositions and temperatures was studied using *Bacillus spp*. Spores (Perni et al., 2007). They found that oxygen-based plasma was more efficient than pure argon plasma. Another study compared the efficiency of exposure of the substrate to plasma (Moisan et al., 2002). Findings showed that the amount of heat energy transferred to a substrate was less in remote exposure as compared to direct exposure. Many of the short-lived reactive species in the plasma environment did not reach the substrate which made the treatment very inefficient in microbial inactivation. In summary, the inactivation of fungi by low temperature plasma occurs as a result of one or more of the following cellular events:

2.15.1 Loss of cell membrane functionality

Fungal cells are able to weather harsh environmental conditions due to the integrity of their cell membranes. However, when this is lost, they become exposed and can be easily attacked (Wu et al., 2021). The mode in which low temperature operates is by destroying the cell membrane by etching by the many reactive species within the generated plasma or by accumulation of the reactive species on the cell membrane surface (Lopez et al., 2019; N. Misra et al., 2011). Further action by the plasma reactive species results in breaking up of bonds and morphological changes (Bourke et al., 2017; Klampfl et al., 2012). It is also associated with electrostatic interference which readily destroys the cell membrane surface as its force is much higher than the tensile strength of the membrane (Hertwig et al., 2018). A study on the effect of LTP on membrane functionality of *A. flavus* and *A. Parasiticus* spores demonstrated its viability in destroying the cell membranes (Dasan et al., 2016). Once the cells rapture, there is further destruction of the cell contents leading to further decontamination.

2.15.2 DNA destruction

Studies on this have revealed that UV photons cause a breakdown in the bonds that further breaks down of the DNA strand (Lopez et al., 2019)and consequently leads to death of the fungal cells (Puligundla & Mok, 2018). The loss of integrity of the cell membrane after exposure to the plasma reactive species also further exposes the DNA material in the fungal cells to more destruction and with a higher efficacy (Simoncicova et al., 2018).

2.15.3 Oxidation of proteins

Proteins play a very important role in the normal functioning of a cell and particularly chemical and metabolite transport between cells (Jayan et al., 2020). Once the protein cells are destroyed, death of the fungal cells is inevitable. The reactive species disrupt the cell membranes and

change the three dimensional structure of proteins and enzymes (Liao et al., 2017) not forgetting the conformation of other key features. This consequently results in loss of cell integrity and stops the fungal growth (Kim et al., 2014).

2.15.4 Cell apoptosis

According to most research, this is the main agent that is used in most decontamination processes (Ishikawa et al., 2012). In the case of LTP, the reactive species find their way into the fungal cells and destabilizing the cell equilibrium (N. Misra et al., 2019; Niedzwiedz et al., 2019). This results in cell apoptosis or necrosis which leads inactivation of fungal cells, an observation that has corroborated with results of several studies (Hashizume et al., 2015; Panngom et al., 2014).

2.15.5 Peroxidation of lipids

When lipid peroxide accumulates, it causes cell death by stopping the proper functioning of that cell (Hashizume et al., 2015). The cell membrane is disrupted causing permeability and fluidity changes that eventually cause morphological changes. Once the membrane has been invaded, the lipids undergo a chain of peroxidation reactions that result in harmful products, majorly, malondialdehyde. This may destroy the cell further and curtails normal functions.

2.16 Mycotoxin Degradation Using Plasma

This being an emerging area of research, the mechanism of degradation of mycotoxins by use of plasma technology is not fully understood and not much literature is available. However, more recent studies have shown that plasma can indeed lower and, in some cases; completely destroy mycotoxins in different foods as shown in Table 1. A recent study by (Shi et al., 2017) showed a 62 and 82% decrease in aflatoxin in corn with 1 and 10 min treatment respectively at

40% humidity. Air and a modified high oxygen mixture (65% O_2, 30% CO_2, 5% N_2) was used with a combination of different relative humidity levels (5, 40 and 80%). Plasma has also been applied in other food applications such as hazel nuts, pistachio, peanuts, date palm fruits and rice extracts and showed promising results (Table 1).

Table 1: Summary of most recent studies related degradation of aflatoxins in food matrices

Matrix	Type of aflatoxin	Plasma type	Process settings	Study conclusions	Reference	Year
N/A	Aflatoxin B1, deoxynivalenol and nivalenol	Low temperature plasma generated using microwave energy	Argon gas at a flow rate of 100L/min for 1-10s	The mycotoxins were completely degraded after 5s exposure to plasma	(Park et al., 2007)	2007
Hazelnuts, peanuts, pistachio nuts	Aflatoxins B1, B2, G1,G2	Low temperature plasma at low pressure generated using a dielectric barrier discharge (DBD)	Air at 300W ionization power, voltage of 20kV for 5-20 minutes	Upto 50% reduction in the level of total aflatoxins after exposure of between 5-20 minutes	(Basaran et al., 2008)	2008
Bearing plate	Aflatoxin B1	Low temperature plasma – radio frequency plasma	Plasma at 15 pascals pressure, 100-300W power, exposure time ranged between 2-10 minutes	Up to 88.3% of aflatoxin B1 degraded after 10 minutes of exposure to plasma	(S. Wang et al., 2015)	2015
Hazel nuts	Aflatoxins B1, B2, G1,G2	Low temperature plasma generated using a (DBD)	A mixture of pure nitrogen and oxygen at ionization power of 0.4-2 kW, exposure time ranging 1,2,4,12 minutes	Up to 70% reduction in the level of aflatoxins with more effectiveness in decreasing the level of Aflatoxins B1 and G1 as opposed to B2 and G2	(Siciliano et al., 2016)	2016
Maize	Aflatoxins B1, B2, G1,G2	Low temperature plasma generated using a (DBD)	Air and modified atmosphere exposed to 50Hz of ionization power, 90 kV voltage for 1-30 minutes	Aflatoxins reduced by 62-80% for 1 and 10 minutes treatment respectively: a much greater reduction observed in more humid conditions (80% RH) than in the dry air (5%RH)	(Shi et al., 2017)	2017

Table 1: Summary of most recent studies related to degradation of aflatoxins in food matrices (continued)

Glass cover slip	Aflatoxin B1	Low temperature plasma generated using a static induction thyristor	Nitrogen gas at 0.5 atmosphere for 0-30 min	The aflatoxin reduction was up to 90% after exposure of 15 minutes	(Sakudo et al., 2017)	2017
Hazel nuts	Aflatoxins B1, B2, G1,G2	Low temperature plasma generated at low pressure	Dry air exposed to 655W ionization power, voltage of 13.56 kHz, pressure < 0.25mbar for 30 minutes	A reduction in the level of aflatoxin B1 of between 72-73%	(Sen et al., 2019)	2019
Wheat, rice, glass slides	Aflatoxin B1	Low temperature plasma generated using corona discharge plasma jet	Air at 20kV voltage, ionization power of 58kHz for 5,10,15,20,25,30 minute intervals	Over 95% of aflatoxin B1 destroyed after 30 minutes of exposure	(Puligundla et al., 2017)	2019
N/A	Aflatoxin B1, Hep G2 cells	High voltage low temperature plasma generated using a DBD	Plasma generated at 85kV and exposure time of 0,2,5,10, 20 minutes	There was significant reduction in the cytotoxicity of aflatoxin B1, showing potential plasma as possible safe decontamination method	(Nishimwe et al., 2021)	2021

In 2007, a study on the effect of atmospheric pressure cold argon plasma that was generated using microwave energy showed that aflatoxin B1, deoxynivalenol (DON, vomitoxin) and nivalenol were destroyed completely after 5 seconds of treatment (Park et al., 2007). In 2008, another study concluded that 20 minutes of air plasma reduced the concentration of aflatoxins (B_1, B_2, G_1 and G_2) by up to 50% (Basaran et al., 2008). Treatment of AFB1 with 300W plasma for 10 minutes degraded up to 88% of AFB1. The resultant compounds were also found to be less toxic than the original toxin (S. Wang et al., 2015). Later in 2016, hazelnut inoculated with aflatoxins underwent a 70% reduction of total aflatoxins after 12 minutes of treatment. Distinctively, AFB1 and AFG1 were more responsive to the plasma environment than AFB2 and AFG2. The authors recommended that the cold plasma could be incorporated in the hazelnut processing preferably after the dehulling and before the roasting stages of production (Siciliano et al., 2016).

In 2017, a study on the effect of LTP on maize found that a 62-80% reduction in total aflatoxins was achieved after 1-10minutes of treatment. A higher reduction was noted in more humid conditions as compared to using dry air (Shi et al., 2017). Nitrogen induced LTP was exposed a glass cover slip contaminated with AFB1 for 15 minutes. A reduction of up to 90% was achieved (Sakudo et al., 2017). In yet another study, the effect of low temperature plasma on aflatoxins (B1, B2, G1, G2) on hazel nuts was studied. A reduction of between 72-73% was achieved (Sen et al., 2019). Another study by Puligundla et al found that over 95% of aflatoxin B1 was degraded after 30 minutes in wheat, rice and glass slides exposed to LTP generated using corona discharge plasma jet (Puligundla et al., 2017). Finally, in a more recent study, high voltage LTP reduced the cytotoxicity of AFB1 significantly suggesting the possibility of use of plasma as possible decontamination method (Nishimwe et al., 2021).

2.17 Mycotoxin Degradation Mechanism of Plasma

Degradation pathways are associated with molecular structure, the nature of the plasma and most importantly, the interaction of the toxin molecules with the activated plasma species (Larussi, 2005). The release of O* and OH* free radicals during the treatment is highly associated with the degradation of the mycotoxins during plasma treatment (Hury et al., 1998). Several studies investigating the effect of cold plasma on aflatoxin B1 (AFB1), have shown a breakdown at C8 and C9 double bond of the dihydrofuran rings (R. Chen et al., 2014; Pankaj et al., 2018). The loss of the double bonds at the terminal furan ring is associated with the reduced toxicity and carcinogenicity of AFB1 as it is very characteristic of these two functions.

2.17.1 Factors affecting detoxification efficiency of low temperature plasma

Several factors influence the efficiency of LTP to detoxify mycotoxins and this varies between the different mycotoxins. These are type of mycotoxin, plasma source, storage environment, process conditions, gas type used, sample stirring, exposure time, ionization power used and humidity (Devi et al., 2017; Jablonowski et al., 2018; Ouf, Basher, et al., 2015; Ouf, Mohamed, et al., 2015; Shi et al., 2017; Siciliano et al., 2016; Ten Bosch et al., 2017; Tsehaye et al., 2018; Wei et al., 2017).

2.17.1.1 Mycotoxin structure

Several studies have pointed to the fact that the structure of the mycotoxin will determine the detoxification process. The molecular mass is independent. For instance, two toxins with similar molecular masses showed different decay rates after being subjected to the same conditions of LTP. i.e. Ennaiatin B and Sterigmatocystin (Ten Bosch et al., 2017). It has also been reported that the sensitivity of aflatoxins B1 and G1 is higher than that of B2 and G2 (Siciliano et al., 2016). This is associated with the existence of C8-C9 double bond in the

structure of aflatoxin B1 and G1, which does not exist in B2 and G2. The opening up of these double bonds leads to the formation of primary ozonides and subsequent production of their derivatives such as organic acids, aldehydes and ketones (Jalili, 2016).

2.17.1.2 Humidity, gas type, ionization intensity and exposure time

The efficacy of the generated plasma is believed to be linked to the factors adopted for its discharge. However, these studies have given conflicting opinions (Yousefi et al., 2021). For instance, modified air (65% O_2, 30% CO_2, 5% N_2) was found to have a higher efficacy of detoxifying corn as compared to air (78% N_2, 22% O_2). The aflatoxin content reduced from 420 ± 21 ppb to 102 ± 17 and 161 ± 15 ppb after one minute treatment and under air at 40% relative humidity respectively (Shi et al., 2017). This efficacy was attributed to the release of a higher number of reactive species under the modified atmosphere as compared to that of air.

Similarly higher relative humidities increased the efficacy of the generated plasma. This was attributed to the higher generation of hydroxyl molecules which have higher oxidation potency than ozone leading to more efficacies in detoxification (Tsehaye et al., 2018; Wei et al., 2017). An increase in the relative humidity from 5 to 40% lead to higher efficacy of between 143 ± 24 and 102 ± 17 ppb after one minute of treatment.

The ionization power also plays a big role in the efficacy of the produced plasma. An increase in the ionization power for 40W to 60W led to an increased reduction in aflatoxin B1. Longer exposure periods also increased the efficacy of detoxification although complete detoxification was not achieved (Jablonowski et al., 2018; Ouf, Basher, et al., 2015). These studies have not given consistent observations and each study differs from the other and thus need for further study (Yousefi et al., 2021).

2.18 Potential Application in Food

Non-thermal plasma (NTP) has found use in the food industry in several applications including decontamination of food products. The most recent studies on destruction of fungi and aflatoxin degradation in maize using cold plasma were systematically reviewed (Table 1).

In 2007, (Park et al., 2007) studied the effect of low temperature plasma on aflatoxin B1 among other mycotoxins. The study concluded that the mycotoxins and their cytotoxicity were completely degraded after 5 seconds of treatment. Later, (Basaran et al., 2008) carried out a similar study on hazel nuts, pea nuts and pistachio nuts contaminated with aflatoxins (B1, B2, G1 and G2) and applying low temperature plasma. The total aflatoxins were reduced by 50% after 20 minutes of air plasma treatment. In 2015, another study by (S. Wang et al., 2015) found that 88.3% of aflatoxin B1 was degraded from a bearing plate after 10 minutes of treatment with low temperature radio frequency plasma. (Siciliano et al., 2016) concluded that up to 70% detoxification of aflatoxin B1 was achieved using a combination of gases [nitrogen and oxygen] in generating the plasma. The efficacy of destruction was better on aflatoxin B1 and G1 as opposed to B2 and G2. In 2017, (Ten Bosch et al., 2017) found that pure mycotoxins were completely degraded after 60 seconds exposure to low temperature plasma. (Shi et al., 2017) also carried out a study on degradation of aflatoxins in maize by use of low temperature plasma. There was a 62 -82% decrease in the aflatoxin content after exposure for 1 and 10 minutes respectively. Another subsequent study by (Sen et al., 2019) revealed a reduction in the level of aflatoxin B1 of between 72-73%. Over 95% of aflatoxin B1 was destroyed after 30 minutes of exposure to low temperature plasma according to a study by (Puligundla et al., 2017). In 2021, (Nishimwe et al., 2021) carried out a study on the effect of low temperature plasma on the cytotoxicity of aflatoxin. The findings were that there was significant reduction in the cytotoxicity of aflatoxin B1, and thus showing the potential of plasma as a possible safe decontamination method.

2.19 A discussion on the Future Prospects of Atmospheric Pressure Plasma

Atmospheric Pressure Plasma (APP) has shown a promising future in decontamination of foods and feed. A combination of APP and other non-thermal methods of decontamination could be the breakthrough the world has been waiting for. In this case, synergistic effects may be considerable; however, scaling up this technology remains a challenge to be solved. One of the constraints of experimental work on APP is that treatment must not have negative impact on the organoleptic and nutritional properties of food. Nevertheless, there have been limited investigations on this aspect of treatment. At room temperature, the activated species of cold plasma selectively destroy the pathogen without causing any chemical residues (Dasan et al., 2016). However, more studies should investigate the effect of NTP on the nutritional, chemical as well as shelf life of food and feed. Most importantly, risk assessment of the process is necessary to ascertain the food products are free of toxic residues in future studies. The estimated costs and safety of the gas used in the plasma treatment should also be investigated (Song et al., 2009). Non-thermal plasma is an emerging technology for reducing microbial population on the surface of fresh and processed foods. Various reactive species of plasma interact with biological cell to cause changes on cell wall and morphology of the microorganisms that lead to death. Because of the limited information about the nutritional and chemical changes in food products treated with this technology, especially, sensitive food which has high content of lipid and vitamins, additional issues concerning food quality and safety must be considered (S. Wang et al., 2015). Non-thermal plasma is a promising technology that has the potential to destroy fungi and also detoxify food and feed by degrading the toxins produced. The method could present a more sustainable and cheaper method for decontamination of food and feed. For scaling up of this technology to be possible, several concerns need to be addressed. Cold plasma systems should be tailor made to handle food and feed in bulk either in batch or continuous systems which should be explored in future studies.

Since mycotoxins that are formed on grains such as cereals [maize] are found on the surfaces, they can easily be destroyed by use of cold plasma while ensuring the nutritional integrity of the food or feed (N. Misra et al., 2019).

CHAPTER THREE

KNOWLEDGE, ATTITUDE AND PRACTICES (KAP) OF FARMERS ON POSTHARVEST AFLATOXIN CONTAMINATION OF MAIZE IN MAKUENI AND BARINGO COUNTIES, KENYA

Abstract

Aflatoxin contaminated home grown maize has been a perennial problem in Kenya especially in the Eastern and North Rift parts of the country. This study focused on investigating the influence of knowledge, attitude and practices of farmers on aflatoxin contamination of maize in Makueni and Baringo counties in Kenya. A convergent mixed method study design combined quantitative and qualitative data collection techniques in maize producing areas of Baringo and Makueni Counties in Kenya. These methods included questionnaire administration, focus group discussions and key informant interviews. Of the 220 farmers who participated in the survey, 67.27% were male and 32.73% female in Baringo County whilst 45.45% male and 54.55% female in Makueni County. Majority of the farmers were in a marital union and were between the ages of 40-54 years. The average KAP score for knowledge was 57.6% for both counties. The average knowledge score for Makueni was 37.70% and 77.2% for Baringo County. The average KAP attitude of the farmers in both counties was 77.1%. There was a significant difference in the knowledge of factors contributing to aflatoxin in maize, as to the point where contamination begins, the signs of aflatoxin contamination and the consequences of aflatoxin exposure in both counties ($p<0.005$) The individual county scores were 76.5% and 77.7% in Makueni and Baringo counties respectively. Socio-economic and demographic factors were linear predictors of knowledge ($R^2=0.76$, $p<0.001$), whereas they had no effect ($R^2=0.043$, $p=0.076$) on the attitude of the maize farmers. Farmers from Makueni County (Eastern Region of Kenya) were more likely (OR=1.24) to have higher knowledge scores on aflatoxin contamination than those from Baringo County (Rift Region of Kenya). On

the contrary, with increasing age the maize farmers were less likely (OR=0.01) to have higher scores of knowledge. Farmers associated poorly dried maize and poor storage conditions as the maize cause of aflatoxin contamination. The study findings revealed a significant difference in knowledge and attitude between the two counties. This consequently had an effect on the practices of the farmers. There is need for increased awareness creation on dangers posed by consumption of aflatoxin contaminated maize grain within the communities. Training of farmers on good agricultural and management practices is also of utmost importance. This coupled with regular surveillance and enhancement of laboratory capacities can also significantly reduce the occurrence of aflatoxicosis in Kenya.

3.1 Introduction

The global production of maize is estimated to be 717 metric tons/year with United States, China and Brazil being the leading maize producing countries in the world with an estimated production of 563 metric tons/year (Ranum et al., 2014). It is also estimated that about 25% of this production is lost due to aflatoxin contamination (WHO, 2018). This data after extrapolation shows that a 60 kg adult on consumption of 233g of maize per day with a mean contamination of 17 ng/g (Arithmetic mean contamination of maize from four Agro-ecological Zones (AEZ) will translate to about 66 ng/kg of body weight (Sirma et al., 2018). Consumption of aflatoxin contaminated food in humans is associated with liver cancer, retarded growth and compromised immunity and aggravates infectious diseases such as tuberculosis, hepatitis and Human Immunodeficiency Syndrome (HIV) (brief, 2018). Therefore, control of aflatoxin contamination in maize is of utmost importance. The control should be addressed both at the pre- and post- harvest levels. Understanding the farmers' perceptions, as the producer, is key to coming up with solutions. Several knowledge, attitude and practices (KAP) studies have been carried out in Kenya and Africa as a whole (Belayhun et al., 2019; Gichohi-Wainaina et

al., 2020; L. Matumba et al., 2016; Udomkun et al., 2018). In Congo for instance (Udomkun et al., 2018) farmers associated high levels of aflatoxin contamination to high humidity, improper storage practices and poor soils. This then led to discoloration of the grain and accompanied by unacceptable change in the organoleptic properties. This resulted in difficulty in selling the grain. In Ethiopia (Belayhun et al., 2019), majority of the respondents associated aflatoxin contamination to stomach related disorders, liver ailments including cancer. In Malawi (Gichohi-Wainaina et al., 2020), the farmers had relatively low knowledge scores on both pre- and post- harvest practices that lead to aflatoxin contamination. Higher knowledge scores were observed on issues related to income loss and less knowledge on the factors leading to occurrence of aflatoxins in produce. More educated households had higher knowledge scores as compared to those less educated. Pre- and post-harvest practices were recommended as a way of reducing cases of aflatoxin contamination in the produce. A similar study in Malawi (L. Matumba et al., 2016), reported that about 33% of consumers bought mouldy maize despite the associated dangers of consuming aflatoxin contaminated foods. Aflatoxin contamination is a very perennial problem in Kenya as well with many reported cases that have resulted in many reported cases and even deaths (Lewis et al., 2005; Sirma et al., 2018). The objective of this study was to investigate the factors that influence the knowledge, attitude and practices of farmers on aflatoxin contamination in Makueni and Baringo counties in Kenya.

3.2 Methodology

3.2.1 Study sites

The survey was carried out in Baringo and Makueni counties. Baringo County is located in the Rift Valley region of Kenya. It borders eight counties geographically. To the North are Turkana

and Samburu Counties and Laikipia County to the East. It also borders Elgeyo Marakwet and Pokot Counties to the West, Nakuru and Kericho Counties to the South and finally, Uasin Gishu County to the South West. It covers 11,015.3Km^2 of which 165km^2 is surface water. Agriculture is the main economic activity in the county with 80% proportion (GOK, 2013b). Baringo County consists of three major ecological zones: Highlands, mid and lowlands. The soils in the highlands are basically well drained and fertile making these areas suitable for agriculture and improved livestock development. The lowlands are semi-arid in nature with complex soils where essentially pockets of rain-fed subsistence agriculture is practiced and also under irrigation in some areas. On the other hand, Makueni County is located in the Eastern part of Kenya. It borders Machakos County to the North, Taita Taveta County to the South, Kitui County to the East and Kajiado County to the West. Most of the 8.034.7km^2 is either arid or semi-arid (GOK, 2013a). The main income generating activity in the county is agriculture. The long rains are received in April and May while the short rains in November and December. The county is exposed to serious climatic challenges that include drought, heat stress, moisture stress, increased precipitation and temperatures (20.2^{o}C -35.8^{o}C). Due to these challenges, food insecurity is prevalent. Aflatoxicosis has been a big challenge as well since the first major recorded case in the 2004 in the county which resulted in 317 cases and 125 deaths (Lewis et al., 2005). Since then, the county has recorded several major and minor incidences. The survey targeted these new hot spots to unearth the underlying issues contributing to the aflatoxicosis cases. The study sites were purposively selected due to the higher occurrence of aflatoxin-related cases compared to the other counties in Kenya. The specific sub-counties were chosen based on the fact that these areas where mixed farming is carried out and maize growing is an important economic activity therefore likelihood of finding maize in such homesteads. The survey was carried out in Baringo North, Baringo South, Baringo Central and Eldama Ravine

sub-counties in Baringo County. In Makueni, the sub counties sampled were Makueni and Mbooni which had recently registered some mild cases of aflatoxicosis (Figure 1).

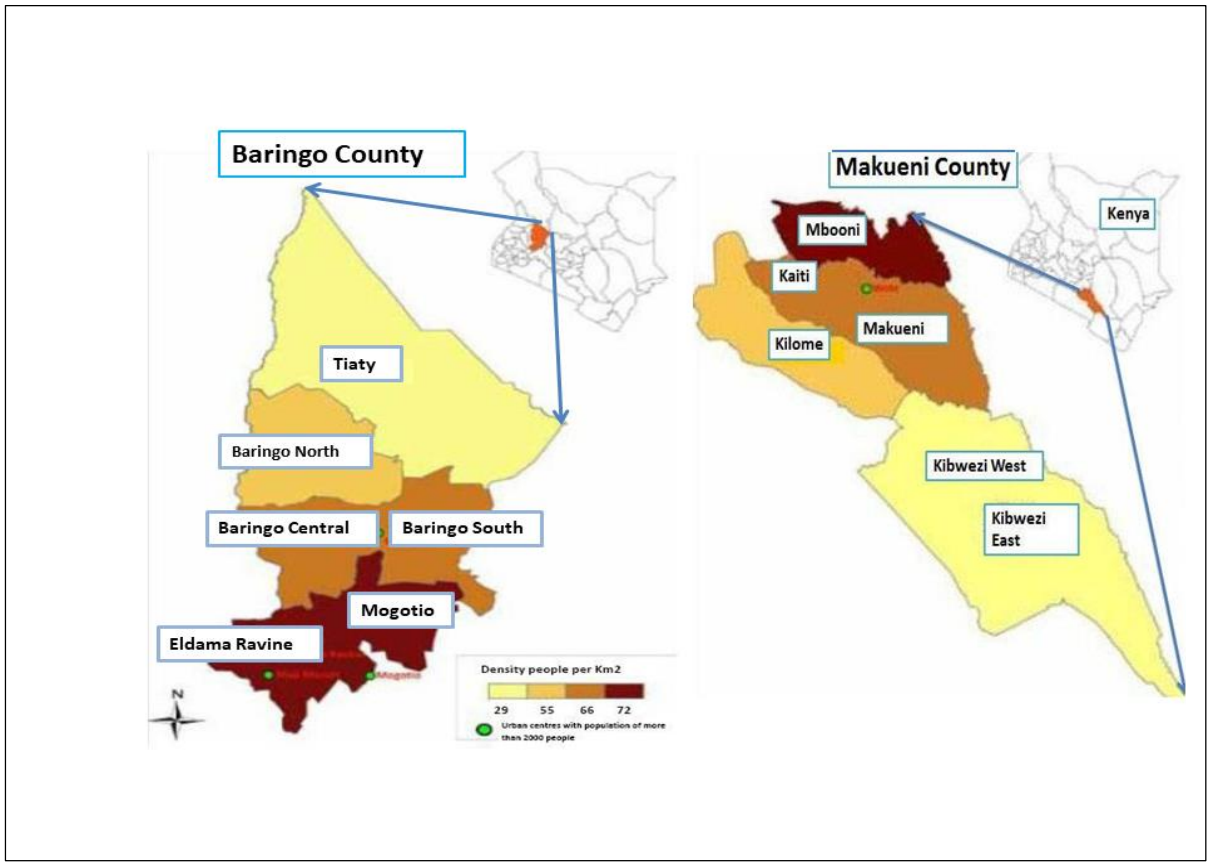


Figure 1: Map of the study areas (Makueni and Baringo Counties, Kenya)

3.2.2 Determination of sample size

The sample size was determined using the Fischer's formula (Fischer et al., 1991):

$$n = \frac{Z^2pq}{d^2}$$

where n is the sample size, z is the normal deviation (1.96) corresponding to 95% confidence interval, p (0.5) is the estimated prevalence of aflatoxin in the county, q is 1-p and d is the degree of the desired accuracy (5%). This yielded a total of 196 households plus a 10% attrition giving a sample size of 216 households.

3.2.3 Sampling procedure

A mixed methods approach to data collection was used which included the use of semi-structured pre-tested questionnaires, focus group discussions and key informant interviews. Five enumerators in each county were selected and trained to aid in data collection. They were recruited based on their previous experience in similar research work and ability to understand and write in the local dialects, Kiswahili and English. Quantitative data was collected by administering a semi-structured questionnaire. A total of 220 questionnaires were randomly administered and shared equally between the two counties. Simple random sampling technique was used to identify the households. A list of households was created and a random number generator was used to identify the households to be sampled. The interviews were conducted in a combination of languages: local, Kiswahili and English. The semi-structured questionnaire was used to collect information on the farmer's demographics and knowledge, attitude and practices leading to aflatoxin contamination. A total of four focus group discussions were conducted with four farmer groups with each group consisting of 12 members divided equally between the two counties. Gender was balanced with each panel comprising of 6 men and 6 women. Key informants interviewed included senior ministry of agriculture officials, health officials and non-governmental organizations.

3.2.4 Study tools

The following study tools were used: A structured questionnaire, focus group discussion guide and key informant interview guide. Knowledge, attitude and practices were determined by questions in the questionnaire as follows:

i. **Knowledge**: It is the awareness of the community about aflatoxins. It was measured by calculating the mean score of 13 items and categorized as knowledgeable (if participants

scored ≥ mean score of the correctly answered questions) or not knowledgeable (if participants score < mean score of the correctly answered questions).

ii. **Attitude**: The way a community thinks and behaves toward aflatoxin contaminated maize. It was measured by 13 questions with a five point like Likert's scale. All individual answers to attitudinal questions were computed to obtain total scores; then, mean score was calculated to categorize as having good attitude (if participants scored ≥ mean score) or poor attitude (if participants score < mean score).

iii. **Practice**: The behavior of a community that prevents or causes aflatoxin contamination. It was measured by 19 questions. All individual answers to practice questions were computed to obtain a total mean score and categorized as good practice (if participants scored ≥ mean score) or poor practice (if participants scored < mean score).

3.2.5 Quality control

Before administration of the questionnaire, farmers signed a consent form accepting to participate in the survey. The completed questionnaires were then checked for quality assurance before leaving the field.

3.2.6 Ethical considerations

Due to the corona virus pandemic, social distancing was maintained, masks and sanitizers were provided for all the study participants. Consent was sought from the respondents before proceeding with the questionnaire administration. A permit to undertake the work was granted by the National Commission for Science, Technology and Innovation (NACOSTI).

3.2.7 Data analysis

The data was then analysed using R software (version 4.0.3). A 5% level of significance was used throughout the study. Any independent variable with a p value of less than 0.05 was considered statistically significant in association to the outcome variable.

3.3 Results and Discussion

3.3.1 Socio-demographic characteristics of farmers

Of the farmers who participated in Baringo County, 67.27% were male and 32.73% female whilst in Makueni, the proportion of male and female respondents was 45.45% and 54.55% respectively. Those below the ages of 24 years were less than 2% in all the counties. Majority of the farmers were between the ages of 40-54 years and most were married, 82.73% and 81.82% in Makueni County and Baringo County, respectively. Most of the farmers engaged in farming as well as business in both counties. A small proportion was also in formal employment. Most of the farmers had inherited land on which they were farming with a proportion of 59.09% and 90% in Baringo and Makueni counties respectively. There was a similar trend in the sizes of land owned in both counties with majority owning 1-5 ha of land. This stood at 87.27% in Makueni and 81.82% in Baringo counties. A larger proportion of the farmers in both counties had a family size of between 1 and 5 family members. There was a significant difference in the gender, education, source of income, housing and land ownership between Makueni and Baringo counties (Table 2).

Table 2: Socio-demographic characteristics of farmers (N=220)

Farmer characteristics	Makueni (%)	Baringo (%)	p-value (X^2, df)
Gender			
Male	45.45	67.27	0.0018 (9.78,1)
Female	54.55	32.73	
Age			
≤24	1.82	1.82	0.7852 (46.52, 55)

25-39	28.18	33.64	
40-54	40.00	37.27	
≥55	30.00	27.27	
Marital status			
Married	82.73	81.82	0.4656 (2.55, 3)
Separated	0.91	1.82	
Single	6.36	2.73	
Widowed	10.00	13.64	
Education			
Adult education	1.82	1.82	<0.001 (46.65, 6)
College/University	14.55	37.27	
Completed primary	6.36	12.73	
Completed secondary	27.27	29.09	
Dropped from primary	12.73	3.64	
Dropped from secondary	36.36	7.27	
Illiterate	0.91	8.18	
Income source			
Farming	65.45	72.73	0.0096 (13.37, 4)
Farming & Business	32.73	19.09	
Farming, Business & formal employment	1.82	8.18	
Land ownership			
Freehold title (inherited)	90.00	59.09	<0.001 (34.17, 6)
Freehold title (purchased)	10.00	21.82	
Community land	0.00	8.18	
Leased	0.00	6.36	
Rented	0.00	4.55	
Farm size (ha)			
≤1	1.82	3.64	0.0662 (35.15, 24)
1 – 5	87.27	81.82	
6 – 10	8.18	13.64	
≥ 11	2.73	0.91	
Family size			
1 – 5	47.27	50.91	0.0987 (22.36, 15)
6 – 10	44.55	38.18	
≥ 11	8.18	10.91	

Table 3: Socio-demographic characteristics of farmers (N=220)….Continued

Farmer characteristics	**Makueni (%)**	**Baringo (%)**	**p-value (X^2, df)**
Housing (Floor/Wall/Roof)			
Cemented/Iron sheets/Iron sheets	32.73	0.00	<0.001 (101.41, 5)
Cemented/Stone/Iron sheets	24.55	85.45	
Cemented/Stone/Tiles	2.73	2.73	
Cemented/Timber/Iron sheets	8.18	0.00	
Earth/Mud/Iron Sheets	17.27	0.00	
Earth/Mud/Thatch	14.55	11.82	

Similarly, there was a significant difference on the asset ownership of some assets between the two counties. These were the television and private wells. No farmer in Baringo County had a private well but 10.91% of farmers in Makueni County did. On the other hand more farmers in Baringo (65.45%) owned a television as compared to Makueni County (43.64%) (Table 3).

Table 4: Asset ownership of maize farmers in Makueni and Baringo counties (N=220)

Item	Baringo (%)	Makueni (%)	p-value (X^2, df)
Motor vehicle (commercial)	0.91	5.45	0.1244 (2.36, 1)
Motor vehicle (Private)	19.09	18.18	1 (0, 1)
Tuk tuk	0.00	0.91	1 (0, 1)
Bicycle	21.82	27.27	0.4334 (0.61, 1)
Radio	93.64	87.27	0.1686 (1.90, 1)
TV	65.45	43.64	0.0018 (9.70, 1)
Mobile phone	88.18	94.55	0.1498 (2.07, 1)
Fixed phone	1.82	0.91	1 (0, 1)
Generator	12.73	7.27	0.2612 (1.26, 1)
Well (Private)	0.00	10.91	0.0011 (10.67, 1)
Water pump	6.36	8.18	0.7952 (0.07, 1)
Bore hole	2.73	8.18	1 (0, 1)
Water tanks	55.45	52.73	0.7867 (0.07, 1)
Livestock	82.73	75.45	0.2458 (1.35, 1)

The most commonly accessed farm tool was the bull/donkey drawn plough which was highly significant with 84.55% and 14.55% in Makueni and Baringo counties respectively. The plough was mainly used for land preparation. Owning a wheel barrow was common in both counties (Table 4).

Table 5: Accessibility of farming tools among maize farmers in Makueni and Baringo counties (N=220)

Item	Baringo	Makueni	p-value (X^2, df)
Trailer (Tractor)	1.82	0.91	1 (0, 1)
Harrow (Tractor)	1.82	0.00	0.4775 (0.51, 1)
Plough (Tractor)	7.27	7.27	1 (0, 1)
Trailer (bull/donkey)	0.91	0.00	1 (0, 1)
Harrow (bull/donkey)	0.91	2.73	0.6138 (0.25, 1)
Plough (bull/donkey)	14.55	84.55	<0.001 (105.03, 1)
Wheel barrow	40.00	20.91	0.34 58, 1)

3.3.2 Knowledge assessment

There was a significant difference in the knowledge of factors contributing to aflatoxin in maize, as to the point where contamination begins, the signs of aflatoxin contamination and the consequences of aflatoxin exposure in both counties ($p<0.05$) (Table 5). Baringo County had relatively higher frequency of farmers knowledgeable on aflatoxin contamination and the precipitating effects of such contamination.

Table 6: Households' Knowledge about aflatoxin (N=220)

Parameter	Baringo			Makueni			p-value (X2, df)
	Yes (%)	No (%)	Don't Know (%)	Yes (%)	No (%)	Don't Know (%)	
Practices leading to aflatoxin contamination in maize:	100	0.00	0.00	95.45	2.73	1.82	0.078 (5.12, 2)
Poorly dried or wet maize	95.45	4.55	0.00	36.36	61.82	1.82	<0.001 (85.508, 2)
Poor storage of maize	63.64	36.66	0.00	6.36	91.82	1.82	<0.001 (79.94. 2)
Drying maize on the ground	83.64	16.36	0.00	21.82	76.36	1.82	<0.001 (84.57, 2)
Shelling wet maize							
Contamination begins:							
In the field when growing	4.55	95.45	0.00	13.64	85.45	0.91	0.037 (6.61, 2)
During harvest	91.82	8.18	0.00	27.27	71.82	0.91	<0.001 (95.16, 2)
After harvest	95.45	4.55	0.00	56.36	42.73	0.91	<0.001 (45.99, 2)
In-storage	94.55	5.45	0.00	9.09	90.00	0.91	<0.001 (86.11, 2)
Improper drying	91.82	8.18	0.00	30.91	68.18	0.91	<0.001 (160.88, 2)
Not grading maize	53.64	46.36	0.00	0.00	99.09	0.91	<0.001 (81.03, 2)
Wet storage conditions	70.91	29.09	0.00	1.82	92.27	0.91	<0.001 (113.67, 2)

Table 7: Households' Knowledge about aflatoxin (N=220)…..continued

Signs of aflatoxin contamination:							
Discolouration	99.09	0.91	0.00	76.36	22.73	0.91	<0.001 (26.39, 2)
Mouldiness and wetness	98.18	1.82	0.00	38.18	60.91	0.91	<0.001 (91.27, 2)
Presence of insects	56.36	43.64	0.00	2.73	96.36	0.91	<0.001 (76.40, 2)
Mouldy smell	90.00	10.00	0.00	30.00	69.09	0.91	<0.001 (82.56, 2)
Aflatoxin exposure leads to:							
Stunting in children	49.09	50.91	0.00	1.82	96.36	1.82	<0.001 (65.72, 2)
Immunity suppression	76.36	23.64	0.00	17.27	80.91	1.82	<0.001 (77.53, 2)
Low productivity in livestock	71.82	28.18	0.00	8.18	90.00	1.82	<0.001 (93.25, 2)
Liver cirrhosis (Liver cancer)	73.64	26.36	0.00	23.64	74.55	1.82	<0.001 (55.58, 2)
Loss of income	57.27	42.73	0.00	86.36	11.82	1.82	<0.001 (102.21, 2)
Death	100.00	0.00	0.00	95.45	2.73	1.82	<0.001 (27.75, 2)

The average KAP score for knowledge of maize producing farmers in the two arid counties was 57.6%. The average knowledge score for Makueni was 37.70% and 77.2% for Baringo County. At the sub-county level, there was a very significant difference in both counties. In Makueni County, Mbooni sub-county had a larger proportion of farmers with knowledge scores of between 26-50%, followed by Makueni sub-county (Figure 2).

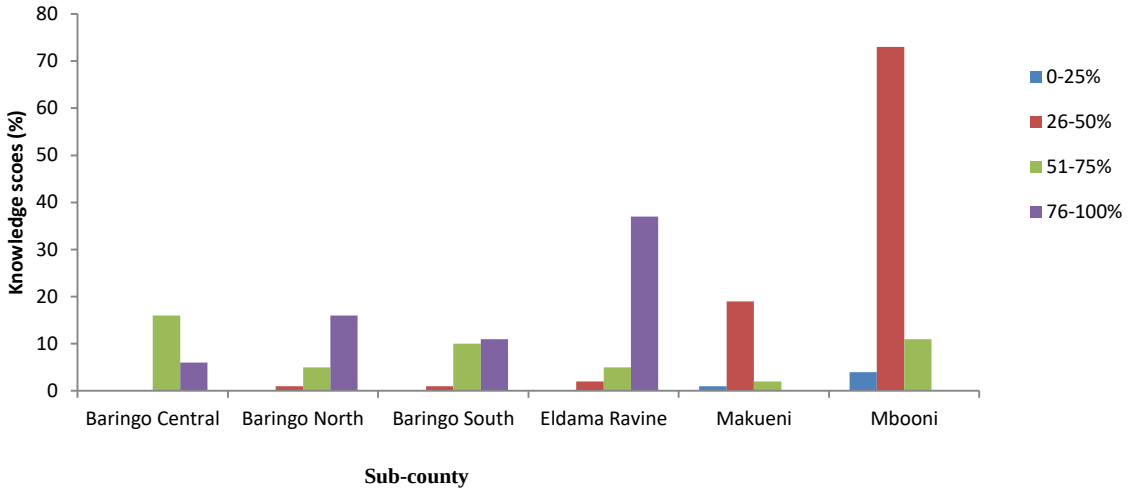


Figure 1: Knowledge scores of farmers in Makueni and Baringo counties

Wote (Makueni sub-county) can be considered a more peri-urban area as compared to Kisau Kiteta and Kako Woiya (Mbooni sub-county) which are remotely located. Outreach programs and projects by the Ministry and non-governmental organizations (NGO's) that train farmers on best practices mainly choose to work with farmers who are near the centres. This can explain the lower knowledge scores in Mbooni as compared to Makueni sub-county. Despite farmers in Baringo County having higher knowledge scores as compared to Makueni County, there was a variance at the sub-county level. Eldama Ravine sub-county had the highest number of farmers with between 76-100% knowledge scores. This can be explained by the fact that maize farming in Eldama Ravine sub-county is done under large scale production and under irrigation. There are over 30 irrigation schemes in Eldama Ravine under contract farming. Seed companies usually contract farmers and undertake very close monitoring and supervision

of the crop to ensure high quality seeds are produced. Additionally, they also train farmers on the best practices which could have led to the huge variation compared to the other sub-counties in Baringo ($p<0.001$).

Socio-economic and demographic factors were linear predictors of knowledge ($R^2=0.76$, $p<0.001$) (Table 6. Farmers from Baringo County (Rift Region of Kenya) were more likely (OR=1.24) to have higher knowledge scores on aflatoxin contamination than those from Makueni County (Eastern Region of Kenya). On the contrary, with increasing age the maize farmers were less likely (OR=0.01) to have higher scores of knowledge.

Table 8: Linear model of predictor factors of knowledge scores of farmers on aflatoxin contamination of maize (N=220)

Variable category	Beta	Std error	P value	Odds ratios
(Intercept)	0.63	1.79	<0.001	1.88
Gender Male	-0.18	0.07	0.726	0.84
Age	-5.26	6.74	0.015	0.01
Marital status Separated	-4.78	3.90	0.436	0.01
Marital status Single	1.84	2.82	0.221	6.32
Marital status Widowed	-8.96	6.27	0.514	0.00
Education College/University	-9.65	6.35	0.155	0.00
Education Completed primary	-10.02	6.11	0.130	0.00
Education Completed secondary	-12.66	6.45	0.102	0.00
Education Dropped from primary	-11.79	6.09	0.051	0.00
Education Dropped from secondary	-0.84	6.80	0.054	0.43
Education Illiterate	2.99	1.96	0.901	19.93
Source of income Farming & Business	2.24	4.46	0.128	9.36
Source of income Farming & Employment	1.11	11.77	0.616	3.04
Source of income Farming, Business, Employed	0.23	0.34	0.092	1.26
Farm size	-37.78	1.81	0.491	0.00
County Makueni	0.21	0.32	<0.001	1.24
Family size	0.63	1.79	0.505	1.88

Adjusted $R^2=0.760$, $p<0.001$

3.3.3 Attitude assessment

The average KAP attitude of the farmers in both counties was 77.1%. The individual county scores were 76.5% and 77.7% in Makueni and Baringo counties respectively. Farmers in Mbooni sub-county had a better attitude on control of aflatoxin compared to Makueni sub-county. Eldama Ravine sub-county also had a higher proportion of farmers with a good attitude on control of aflatoxins compared to other sub-counties in Baringo County (Figure 3). The knowledge of the farmers on aflatoxin had a similar effect on his/her attitude with the exception of Makueni County (Figure 3). In Makueni, farmers with lower knowledge scores had a higher attitude scores which could be due to the high number of cases that have occurred in the county some of which have led to many fatalities (Daniel et al., 2011; IFPRI, 2020; Lewis et al., 2005; Mwihia et al., 2008). There was a significant difference in the attitude scores in both counties ($p<0.003$).

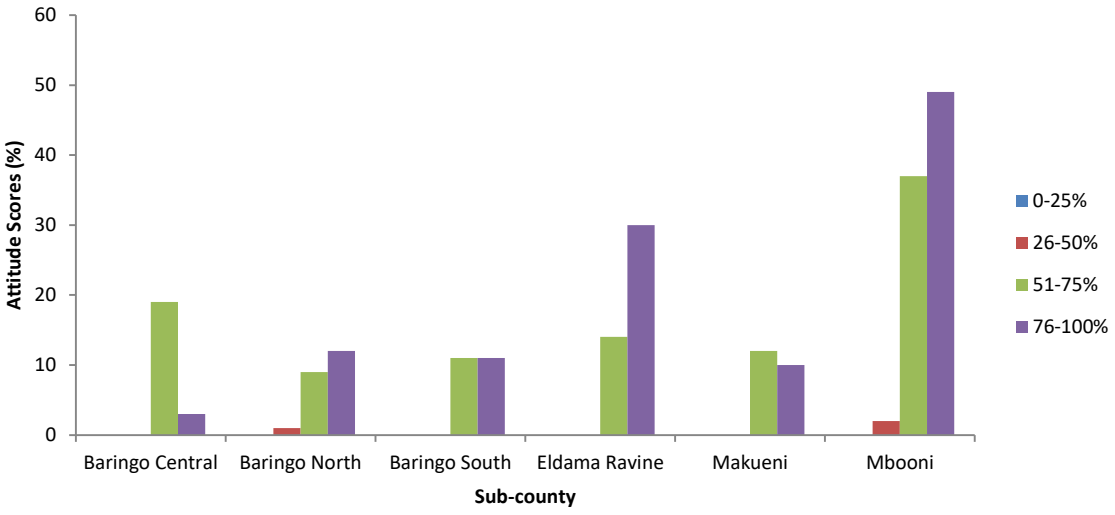


Figure 2: Attitude scores of respondents in Makueni and Baringo counties

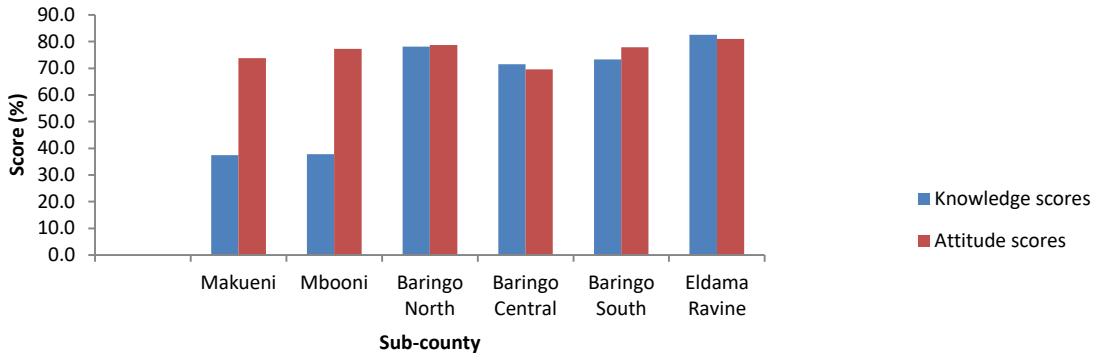


Figure 3: Relationship between knowledge and attitude of farmers in Makueni and Baringo counties (N=220)

Socio-economic and demographic factors had no effect on attitude of the maize farmers (R2=0.043, p=0.076) (Tables 7).

Table 9: Linear model of predictor factors of attitude scores of farmers on aflatoxin contamination of maize (N=220)

Variable category	Beta	Std error	P value	Odds ratios
(Intercept)	76.54	5.86	<0.001	<0.001
Gender Male	1.98	1.45	0.172	7.260
Age	0.00	0.06	0.943	1.000
Marital status Separated	1.24	5.44	0.821	3.440
Marital status Single	-2.83	3.15	0.371	0.006
Marital status Widowed	-0.04	2.28	0.986	0.961
Education College/University	2.13	5.07	0.675	8.370
Education Completed primary	3.59	5.13	0.484	36.40
Education Completed secondary	2.59	4.93	0.599	13.40
Education Dropped from primary	3.96	5.21	0.448	52.60
Education Dropped from secondary	2.29	4.92	0.641	9.920
Education Illiterate	6.12	5.49	0.267	456.00
Source of income Farming & Business	-1.89	1.58	0.232	0.150
Source of income Farming & Employment	4.26	3.60	0.238	70.90

Table 10: Linear model of predictor factors of attitude scores of farmers on aflatoxin contamination of maize (N=220)…continued

Source of income Farming & Family support	14.49	9.51	0.129	>1000
Source of income Farming, Business, Employed	8.38	6.66	0.210	>1000
Farm size	-0.83	0.27	0.003	0.436
County Makueni	0.35	1.46	0.811	1.420
Family size	-0.06	0.26	0.808	0.939

Adjusted R^2=0.043, p=0.076

3.3.4 Practices assessment

Harvesting was exclusively done manually by farmers in Makueni County and 95.45% in Baringo County. Manual use of labour in production of maize and use of artisanal tool were the most dominant practices (Table 8). Manual harvesting is not only labour intensive but also time consuming. However, over 80% of farmers in both counties had an average farm size of between 1-5ha, thus harvesting would not take such a long time. There was significant difference in the mode of handling, drying, shelling and storage of maize in both counties.

Table 11: Summary of practices of respondents in Makueni and Baringo counties (N=220)

Practice	**Baringo (%)**	**Makueni (%)**	**p-value (X^2, df)**
Mode of harvesting			
Hand	95.45	100.00	0.070 (3.27, 1)
Machine	4.55	0.00	
Mode of handling			
Maize stovers stacked in heaps	3.64	27.27	<0.001 (21.74, 1)
Maize cob removed while stovers standing	96.36	72.73	
Drying			
On ground with canvas	51.82	39.09	<0.001 (71.60, 10)
On ground without canvas	28.18	60.91	
Left to dry in field	19.09	0.00	
In an open store	0.91	0.00	

Table 12: Summary of practices of respondents in Makueni and Baringo counties (N=220)…continued

Shelling			
By hand	13.64	49.09	<0.001 (157.43, 11)
Use of machine	81.82	10.91	
Pounding manually in gunny bags	4.55	40.00	
Storage			
Gunny bags	38.18	63.64	0.0003 (13.26, 1)
Pics bags	51.82	40.00	0.105 (2.64, 1)
Granary/Thatch	24.55	7.27	0.0009 (11.01, 1)
Granary/ Iron sheets	30.91	31.82	0 (1, 1)
Air tight bins	0.00	0.91	0 (1, 1)
Hermetic storage	33.64	0.00	8.633 (42.11, 1)
Mode of preservation			
Insecticides (Actellic)	82.73	76.36	0.251 (5.38, 4)
Ash	0.00	4.55	
None	17.27	19.09	

3.3.5 Focus group discussions

The farmers had a wealth of knowledge on the different methods that were used to control aflatoxin in maize from time in memorial. Aflatoxin, also known as *'mbuuka'* in Kamba language in the Eastern part of Kenya was a term that was initially used to refer to maize that was not fit for human consumption. Due to discolouration, the maize, locally known as *'mbemba'* would be set aside as it had a bitter taste and would be fed to chicken (Figure 10). The farmers did not know that this was also poisonous but chose to set it aside due to organoleptic challenges. Farmers were also still using the traditional methods of preservation such as smoking and use of ash. Maize meant for seed or *'mbeu ya mbemba'* would be hung on the kitchen roof where it would be smoked gradually (Figure 5). This kept the maize intact free of insect attack as well as aflatoxin contamination until the next season.

Figure 5: Seed maize hang on ceiling of kitchen

Figure 6: Traditional thatch granary

Figure 7: Pics bags (hermetic bags)

Figure **4**: Modern iron sheet roofed granary

Figure **5**: Air tight bins (hermetic storage)

Figure **6**: Mouldy maize/ *'maozo'*

Maize meant for household consumption was on the other hand mixed with ash, also known as *'mouu'* and kept in the traditional granary (Figure 6), also known as *'ikumbi'* or *'ndaali'*. Modern methods (Figure 7, 8 and 9) are also used currently such as use of insecticides such as *actellic* to prevent insect attack especially weevils or *'ngulu'*. Maize in the *'keinga'* would dry fast within the store as they left spaces in between the walls to allow for free air flow . Farmers attributed the high cases of aflatoxin contamination to early harvesting, labour challenges due to too much work when airing the maize, poor onset of rains caused by climate change, ignorance of the communities on dangers of consuming contaminated maize, poverty, insecurity, interference by wildlife and differences in varieties. Maize is exposed to unfavourable conditions in the house due to human and animal interference such as wild pigs also known as *'nguuwe'*. Farmers associated diseases to aflatoxin contamination in the community. These were linked to liver cancer, swelling of legs, joint pains and early child mortality. In children, farmers associate aflatoxin contamination with jaundice, also referred to as *'muuku'* which also clinically presents with vomiting and diarrhoea. It has also been associated with *'kwashiokor'* in children as children would be very skinny and swollen

abdomen. Farmers were aware of the issue of bio-transfer of aflatoxin from contaminated chicken meat and even mothers milk. Maize stalks are usually left in the farm and start rotting when in the field. This contaminated feed is then fed to dairy cows and this ends up in the cow's milk. Their concern was that the community need more sensitization on the dangers of continued exposure to aflatoxin as a result of consumption of aflatoxin contaminated food and feed. Farmers suggested use of community health workers to educate the communities and also use of posters in schools, churches and health facilities.

The farmers have been advised by the ministry of agriculture to harvest and heap in the farm for 2-3 weeks to allow for complete drying. However, they still find it had to practice this due to seasonality challenges. Early onset of rains sometimes makes them harvest the maize prematurely and this has led to an influx of cases of aflatoxin contamination in maize. Attack by army worm attack and birds whilst still on the farm are also associated with contamination. Indigenous varieties locally known as *'kikamba'* or *'kinyanya'* were not susceptible to attack by fungi compared to the modern varieties. Hand harvesting is widely practiced while threshing is sometimes mechanized. The most common is use of gunny bags. Hermetic bags, which are locally referred to as *'kinga njaa'* are regarded as expensive and has challenges as they are not able to test the moisture content at the point of storage. If maize is not continually aired, this has led to cases of poisoning. Since most farmers are not able to afford a moisture meter, they have been taught by extension officers on how to use salt in a glass to assess whether the maize is adequately dried. Drying is done on tarpaulins (10%) and the other 90% of the population dries their maize on the ground due to lack of resources to buy and dependence on donations through outreach programs.

In Baringo County on the other hand, farmers had a wealth of traditional knowledge on preservation of maize. Similarly as in Makueni County, maize meant for seed *'sixtabut'or 'keswek'* was smoked in order to extend its shelflife till the next planting season. The maize which is locally referred by the Kalenjin community as *'bantek'* to as was hung on the central pillar/ *'tolkta* or *saina'* of the kitchen. The traditional store also known as *'choke'* is still widely used by farmers to store their maize. Ash is also added to the maize before storage to discourage attack by weevils and other insects. This is not widely practiced as compared to use of insecticides such as actellic. The farmers also feed contaminated grain to livestock as is the case in Makueni County.

3.3.6 Key informant interviews

Interviews with key informants in the county revealed many underlying issues. They pointed out that about two thirds of Baringo County receives erratic rains and cereals do not do well. Grains are thus imported from neighbouring counties such as Keiyo, Kericho, Bomet, Nandi, Trans Nzoia, Uasin Gishu and Nakuru. Maize from the neighbouring counties is often of very poor quality as they sell what has been rejected by National Cereals and Produce Board. Trucks lie in wait for buyers in markets under wet conditions at times due to erratic rains. Maize flour from these markets is bitter in taste due to aflatoxin contamination but is still widely consumed. Some of the markets include: Eldama Ravine, Mogotio, Kabarnet town, Barrwessa, Koloa, Chemalingot, Nginyang, Marigat, Mochongoi, Kabel, Kaptara, Kabartonjo and Kipsaraman. At Kaptara, there is barter trade of maize grain and livestock. Baringo county is also one of the tourist destinations due to some of its remarkable land and lake features such as Lake Bogoria, Lake Baringo, hot springs, flamingo's, lake 94 which is since sub-merged. There are many others. Maize is grown all across the six sub-counties; however, in large scale, this is in: Eldama Ravine, Baringo South – under irrigation and Mochongoi which is an island in Baringo South

Sub-county. There are over 30 irrigation schemes in Baringo South under contract farming. These schemes are under the management of seed companies, who undertake very close monitoring and supervision of the crop to ensure high quality seeds are produced. Hence, aflatoxin is not such a big issue in these areas. However, this is a very serious issue for domestic subsistence farmers. The areas of concern are: Eldama Ravine, Marigat and Mochongoi. The current season was harvesting time for the lowlands where maize takes a very short time to grow as compared to the highlands. Maize in the low lands (Marigat) takes 3-4 months to mature whilst that in the highlands (Mochongoi) takes approximately 9 months to mature. Due to the climatic conditions, excessive heat in the lowlands hastens germination as compared to the highlands.

In Baringo County, the aflatoxin issue is very serious. This has been attributed to the rising cases of cancer in the county. This is due to lack of knowledge among the population. Maize is mainly consumed in form of *'ugali'* and fermented milk, commonly referred to as *'mursik'*. Another dangerous trend is the use of the rotten maize by farmers, locally known as '*maozo*' to make livestock feed or *'dairy meal'*. It is estimated to have approximately 90% aflatoxin and is bitter when used in cooked form or '*ugali'.* It is also used to prepare traditional fermented brews such as '*busaa* 'and '*chang'aa*' so when farmers get a lot of '*maozo*' after harvest they are usually very excited. Interestingly, in some cases, domestic animals like chicken, goats and cows refuse to consume it as it is unpalatable. Farmers often mill the '*maozo*' and mix with bran to improve its taste converting it to feed. There has been a lot of disconnect between government agencies such as the county agricultural extension officers and the public health department. This is because mothers are advised to consume whole grains which are often contaminated with aflatoxins. This in turn finds its way into the breast milk and this has been

shown to cause serious side effects to the offspring. This include but not limited to reduced immunity, stunted growth, liver cancer and in more severe cases, death occurs.

Aflatoxicosis had been a big problem in Makueni County dating back to the year 2004 when some major cases were reported in Makindu and surrounding areas. Generally, whenever the area experienced long rainy seasons, they also experienced high levels of aflatoxin in maize. Since then, a number of development partners had come in to intervene and offer solutions. Some interventions have involved introduction of a strain to the soil to deter the growth of the *Aspergillus* strain. The carrier material used is sorghum which is broadcasted in the maize field during the growth stage. Other programs have been promoting postharvest practices among the farmers through various interventions: Farmers have often been encouraged to use tarpaulin to dry their maize and not on the soil as well as use of hermetic storage (bags/ containers). At times, the arrangement also includes cost sharing purchase of inputs – seeds, fertilizer, agro-chemicals, tarpaulin and hermetic bags. At the time of the survey, the current season activity was land preparation.

3.4 Conclusions

The average knowledge score is higher in Baringo County compared to Makueni County. This does not directly have an effect on the attitude and practices of the farmers. Despite the knowledge on the dangers associated with consumption of contaminated grain and the bio-transfer effect of aflatoxin such as to meat, poultry and even breast milk, some farmers still consume the maize. However, a large percentage of the farmers practice good agricultural practices such as drying maize adequately before storage, storing maize on a raised surface and even using hermetic bags to store the maize. Training of farmers on the good agricultural practices is therefore not enough to curb the recurrence of aflatoxicosis in these counties. It is

imperative to also give recommendations on the methods that the farmers can use that are simple and affordable. Use of hermetic bags has been largely promoted in these counties but still remains a hurdle for most farmers due to high cost of the bags. There is also the challenge of ensuring the maize is at the right moisture content. Methods such as use of salt in a glass or bottle are simple and can be used by the farmers without difficulty. Farmers should also be encouraged to store their grain in aerated stores that are built on raised surfaces. Sensitization of farmers through community forums, posters in health centres and even schools can be useful in raising awareness on the dangers of consumption of contaminated grain.

3.5 Recommendations

Aflatoxin control and reduction in maize in Kenya requires a concerted effort by all the key players across the value chain. More importantly is to begin right from the production stage and this primarily revolves around the farmer. Hence, training of farmers on good agricultural and management practices is of utmost importance. There is need for increased awareness creation on dangers posed by consumption of aflatoxin contaminated maize grain within the communities. This coupled with regular surveillance and enhancement of laboratory capacities can also significantly reduce the occurrence of aflatoxicosis in Kenya.

CHAPTER FOUR

STORAGE CONDITIONS AND POSTHARVEST PRACTICES LEAD TO AFLATOXIN CONTAMINATION IN MAIZE IN TWO COUNTIES (MAKUENI AND BARINGO) IN KENYA

Abstract

Aflatoxins are known to cause devastating acute and chronic effects in humans and animals. The objective of the study was to determine the influence of postharvest practices and storage conditions on aflatoxin contamination in maize in two counties. Aflatoxin levels in 142 maize samples from different maize storage conditions were determined. At sampling, a structured questionnaire was also administered to evaluate the farmer postharvest practices. Makueni County had the highest percentage of aflatoxin positive samples with up to 174ppb attributed to the long storage under unfavorable conditions. On the other hand, Baringo County had lower positivity associated with the harvesting season at the time of sample collection. The type of storage condition had a significant effect on the extent of contamination and accounted for 11% of the variation (R^2 =0.11). Gunny bags were the most common type of storage condition and had the highest level of contamination in both counties. Metallic bins had the lowest level of contamination. Aflatoxin G_1 and G_2 were predominant in samples from Baringo county while aflatoxin B_1 and B_2 in those from Makueni county. The study concluded that the type of storage condition significantly contributes to the aflatoxin contamination in the stored maize. Proper drying of maize to the recommended moisture content and subsequent storage in hermetic structures will reduce the cases of aflatoxin contamination.

4.1 Introduction

Globally, maize also referred to as 'corn' has the highest production and is utilized in food, feed and fuel. It is the most preferred cereal grain in Southern and Eastern Africa, Central America and Mexico (Ranum et al., 2014). In Africa, maize is regarded as a cash crop and

in most cases; the highest quality is set aside for export or sale to milling companies whilst the poor quality is left behind for home consumption, preparation of local brews or sold in the informal markets (L. M. Matumba et al., 2014). In Kenya, maize is grown in both large and small farms and is the most important food security crop. In 2019, 95% of the 3,800 thousand tonnes of maize was utilized for subsistence needs (Jeffrey & Maria, 2013; Okoth et al., 2017). According to the WHO (World Health Organization statistics), Kenya is ranked among the countries with the highest maize consumption (171g/person/day). Maize is prone to aflatoxin contamination that leads to huge losses threatening the country's breadbasket (Ranum et al., 2014). In fact, for the last four decades Kenya has been documented as one of the leading countries with the most severe and highest incidence of human aflatoxin exposure in the world. In 2004, an outbreak in the Eastern part of the country led to 125 deaths and 317 cases of infection. There were other outbreaks that followed in the year 2005 and 2006 due to fluctuating weather patterns (Mehl & Cotty, 2010; Muthomi et al., 2010; Njeru et al., 2019). Aflatoxin contamination in maize is a common occurrence in Kenya especially in specific counties in the Eastern and North rift parts of the country (Koskei et al., 2020; Omara et al., 2021; Onesmus & Roselyne, 2019). In 2010, the Kenyan government declared over 2.3 million bags of maize unfit for human consumption due to the high levels of aflatoxins (Mutegi et al., 2018). One of the most studied mycotoxins in the world is aflatoxin, which is a toxic metabolite produced by aflatoxigenic fungi particularly *Aspergillus flavus, Aspergillus parasitucus* and the more rare *Aspergillus nomius* (Birgen et al., 2020; Wacoo et al., 2014). Aflatoxins contaminate food and feed across the value chain including maize grains, peanuts, cereals and animal feeds among others. Across the globe, a huge population is chronically exposed to aflatoxins (Rahimi et al., 2010).

For instance, aflatoxin M_1 was detected in samples of breast milk in Ghana, Kenya, Nigeria and Sierra Leone (Bhat & Vashanti, 1990; Groopman et al., 2008). In Benin, 99% of the children had the highest level of aflatoxin biomarkers ever observed in humans indicating a very high exposure level (Y. Gong, Hounsa, Egal, Turner, Hall, et al., 2002). Several studies have also intuited a close interaction of chronic exposure to mycotoxins with retarded growth, suppressed immunity, malnutrition and diseases such as malaria and HIV/AIDS. Acute exposure to aflatoxin contamination has been associated with liver failure, hepatitis and even death in some instances (Y. Gong, Hounsa, Egal, Turner, Sutcliffe, et al., 2002; Katerere et al., 2008; Khlangwiset et al., 2011; Kimanya, 2015; L. M. Matumba et al., 2014; Strosnider et al., 2006; Warth et al., 2012). Aflatoxin exposure has also been linked to infertility according to a study carried out in Benin (Ibeh et al., 1994).

High level of aflatoxins in stored maize is fuelled by various factors such as fungal load, insect infestation, environmental factors (climate, humidity, temperature, O_2, CO_2), pre-harvest and most importantly poor post-harvest practices by farmers (Daou et al., 2021; Gnonlonfin et al., 2013). If harvesting is done during a rainy season, this may predispose the maize to humid conditions that may facilitate growth of aflatoxigenic fungi during storage due to the increased moisture content in the maize (Stasiewicz et al., 2017). The type of storage condition and storage practices usually influence the state of the grain after storage and is paramount is ensuring the integrity of the maize. This research sought to investigate the influence of type of storage condition and postharvest practices on aflatoxin contamination in Makueni and Baringo counties.

4.2 Materials and Methods

4.2.1 Study sites

Makueni and Baringo counties were purposively selected due to numerous reported and unreported cases of aflatoxicosis (Kangethe et al., 2017; Okoth et al., 2012; Ouko, 2014) (Figure 1). Makueni County is located within the lower midland agro-ecological zones LM2, LM3, LM4 and LM5. Annually, it receives between 200-1200 mm rainfall also characterised by sporadic droughts which results in crop failure (Kangethe et al., 2017). Baringo County on the other hand is divided into three agro ecological zones: highlands, midlands and lowlands. Over 80% of the population depends on maize as the main food and cash crop with most of them being farmers (GOK, 2013b). The aflatoxin analysis work was carried out at the Mycotoxin Research Centre located at the Department of Public Health, Pharmacology and Toxicology (PHPT) of the University of Nairobi, Kenya.

4.2.2 Sample collection and preparation

A total of 144 maize samples (1 kg each) were randomly collected from four different types of storage conditions in Makueni and Baringo counties. These included gunny bags, metallic bins, open storage and pics (Purdue Improved Crop Storage) bags; 36 samples from each storage condition. Each sample was homogenized before the analysis began. Milling was done using a knife mill (GRINDOMIX GM200, GERMANY) before further analysis. The mill was thoroughly cleaned and dried using paper towels after every mill to avoid cross contamination between samples.

4.2.3 Determination of postharvest practices

At sampling a questionnaire was also administered with the purpose of relating the postharvest practices to the aflatoxin content of samples collected from each farmer. They included mode of harvesting, drying, shelling, preservation, grading and sorting, disposal of contaminated grain and storage practices.

4.2.4 Aflatoxin analysis

4.2.4.1 Enzyme immunoassay for total aflatoxin (ELISA)

Screening of samples was carried out using the competitive enzyme-linked immunoassay for quantitative detection of aflatoxin B1, B2, G1 and G2 (10). The aflatoxin kits (HELICA Total Aflatoxin Assay) were sourced from the United States (Helica biosystems Inc). The samples were prepared according to the manufacturer's recommendations. All the reagents were brought to room temperature before use. Five (5) g of the ground maize samples was mixed with 25ml of 70% methanol. The ratio of the sample to extraction solvent used was 1:5 (w/v). The extracted sample was thereafter mixed with 200µL of HRP conjugated aflatoxin. 100µL of each standard and sample was then added to appropriate mixing well containing conjugate and mixed three times. The mixture (100µL) was then transferred to a corresponding antibody coated microtiter well and incubated for 15 minutes at room temperature. The well contents were then discarded into a discard basin and the micro wells washed five times by filling each well with PBS (Phosphate buffered saline) - Tween wash buffer. Absorbent towels were used to dry the wells (face down) before introduction of 100µL of substrate reagent. Finally, 120µLof stop solution was added to each micro well. The optical density of each micro well was read using a Spectrophotometer - model type - 355, manufactured by Thermo Fisher Scientific (Shanghai, China). The readings were taken using a 450nm filter. The limit

of detection of the kits was 20ppb. Samples that had more than 20ppb were further diluted with 70% methanol and retested to obtain the accurate total aflatoxin level.

4.4.2.2 High Performance Liquid Chromatography (HPLC) analysis

Confirmatory test for the ELISA positive samples were carried using HPLC analysis. The respective standards for each aflatoxin were prepared accordingly (Asao et al., 1990). The Evira method for determination of aflatoxins B_1, B_2, G_1 and G_2 was used (Romer Labs.). This involved extraction, filtration, clean up, elution and drying. Extraction of the aflatoxins was done by adding 5g of ground maize sample to 25 ml of 70% methanol and shaking for 2 hours followed by filtration using filter paper (Whatman No.1). Cleaning was done by taking 9ml of the mixture and drying using nitrogen to < 0.5 ml. This was then diluted to 10ml using phosphate buffer solution (PBS), 1 ml of mixture was passed through immunoaffinity columns placed on vacuum manifold. This was then washed with 2 x 10 ml water. Derivatization followed by drying the elute using a nitrogen stream. 200 µL of trifluoro acetic acid was then added vortexed for1 minute and incubated for 30 minutes away from light. The sample was then filtered using a 0.2 µm membrane filter (GHP) before injection into the HPLC machine (NEXERA UHPLC SHIMADZU – JAPAN). The type of column used for the analysis was Nova-pak C18 4µm x 150mm (WATER CORP – IRELAND). The following operating conditions were observed during the process were: run time – 30minutes; injection volume - 10µl; column temperature – 35^{o}C, velocity – 1.0 ml/minute). Aflatoxins were analysed as their trifluoro acetic acid derivatives (TFA) and identified according to their retention times. Quantification was done by use of external standard curves.

4.2.5 Data analysis

Data entered in Microsoft Excel spread sheets was analysed with R software (version 4.0.3). Means and standard deviations were calculated from duplicate sample readings. A one way ANOVA was used to compare means of the aflatoxin levels from the different storage conditions. The means were separated using Tukey's HSD method (Tukey's honestly significant difference test). Independent variables with a p value <0.05 were considered statistically significant to the outcome variable. Model selection was based on the Akaike Information Criteria (AIC) method (Snipes & Taylor, 2014).

4.3 Results

4.3.1 Results of ELISA analysis

Makueni County had the highest percentage of positive samples. The levels of contamination ranged between 0.3 to 174 ppb (Table 9). The location of the sub-county as to being local or urban did not influence the level of aflatoxin contamination in the maize grain. There was no significant difference between the mean values of the aflatoxin levels between the sub counties (P=0.39) and also comparing the two counties (P=0.60). However, more than half of the samples collected from Wote (urban, 59.1%) and Kisau Kiteta (rural, 68%) sub-counties were above the Kenya regulatory limit and WHO/FAO maximum limit (ML) allowed in food of 10 ppb (Sirma et al., 2018).

Table 13: Summary of Mean Total Aflatoxin Levels of maize (ELISA) from Makueni and Baringo Counties

County	Sub-county	Locality	% Positive (exceeding 10 ppb)	Range ppb
Makueni	Wote	Urban	59.1	0.3 to 100
	Kako Woiya	Rural	24.0	2 to 172
	Kisau Kiteta	Rural	68.0	0.2 to 105
Baringo	Baringo Central	Urban	14.3	4 to 160
	Baringo North	Rural	45.5	6 to 171
	Baringo South	Rural	42.9	2 to 166
	Eldama Ravine	Rural	16.7	3 to 174

Four models were explored to determine which factors had the largest effect on the level of aflatoxin contamination in the maize grain. The main effects of locality and the type of storage (Model 1) were chosen as they accounted for up to 81% of the variation (Table 10).

Table 14: Model selection for analysis of storage data

	AICcValue	AICc Delta	AICcWeight
Model 1	7 1481.29	0.00	0.81
Model 2	9 1484.61	3.32	0.15
Model 3	3 1488.46	7.17	0.02
Model 4	6 1489.86	8.57	0.01

***Model selection based on Akaike Information Criteria (AIC)**
Model 1- Locality + Type of storage,
Model 2- Locality * Type of storage
Model 3- Locality
Model 4- Type of storage

The type of storage had a significant effect ($p<0.001$) on the extent of contamination and accounted for 11% of the variation ($R^2 =0.11$). Gunny bags had the highest level of contamination in both counties. Metallic bins on the other hand had the lowest level of contamination. The mean level of contamination was 3.8ppb and 47.3 ppb for metallic bins and gunny bags respectively. There was a similarity in the levels observed in maize samples collected from pics bags, metallic bins and in open storage (Figure 11).

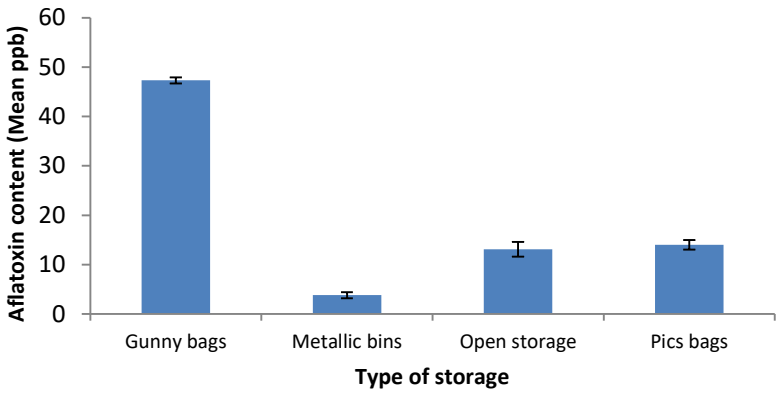


Figure 7: Aflatoxin levels of maize stored in different types of storages

Metallic bins and Pics bags had lower levels of aflatoxin contamination in the stored maize as compared to gunny bags and open storage. Metallic bins and pics bags are forms of hermetic storage. Hermetic storage has been found to be very effective against the *Aspergillus spp*. which produces aflatoxin in conducive environments (Ng'ang'a et al., 2016; Pretari et al., 2019). Effectiveness of improved drying and storage practices of maize was studied in Senegal. The study concluded that hermetic storage greatly improved the integrity of the stored maize by extending storage period by 3-4 months (Bauchet et al., 2020). Proper post-harvest management and practices are promising ways of reducing or even completely eliminating aflatoxin contamination of maize (Dövényi-Nagy et al., 2020; Marete et al., 2020). Harvesting for instance by 97.9% of farmers was by stacking maize in heaps (Table 3). This is a stage where aflatoxin contamination can easily occur especially if the maize is left in the field for a long period before drying. Infection of maize by *Aspergillus spp*. begins in the field and therefore control strategies need to also begin pre-harvest (Mahuku et al., 2018; Nabwire et al., 2020). Erratic rains that are common in this region are possible cause of aflatoxin contamination of the maize during harvest. Timely harvesting is also critical to prevent contamination. A study on effect of delayed harvesting of maize after maturity revealed that the aflatoxin contamination increased by 4-7 fold after 3-4 weeks (Hell et al., 2008). Drying of maize on a tarpaulin/ canvas

was practiced by over 50% of the farmers in both counties. Interesting to note was 47. 1% of farmers in Makueni county dried maize on the ground and this is associated with the high levels of positivity in the samples. Drying of maize on the ground is highly discouraged as the soil contains the mycotoxigenic strains responsible for aflatoxin contamination. Shelling was mainly by hand (38.2%) and pounding manually in gunny bags (47.1%) in Makueni County.

The farmers in Baringo County mainly employed use of a mechanised sheller. The use of the appropriate shelling methods to reduce grain damage, control of insects in the store, sorting to remove damaged grain and use of clean well aerated stores are recommended as a good post-harvest practices (Misihairabgwi et al., 2019; Negash, 2018). When the grain is damaged, it is more prone to aflatoxin contamination. This could also be a contributing factor to the high positivity of samples from Makueni County as pounding of the maize grain in the gunny bags makes it prone to aflatoxin contamination by the spoilage fungi. Some of the farmers did not dispose off the contaminated maize as is recommended but rather used it in various ways. The larger proportion of farmers used it to make alternative products such as local alcoholic based beverages (*busaa, chang'aa*) and some fed it to livestock. Another group still sold it in local markets after blending with 'clean' or uncontaminated maize grain.

The association of the level of aflatoxin contamination was also related to the postharvest practices of the farmers. This is shown in Table 11. However, none of these post-harvest factors investigated in this study significantly contributed to aflatoxin contamination (P >0.001).

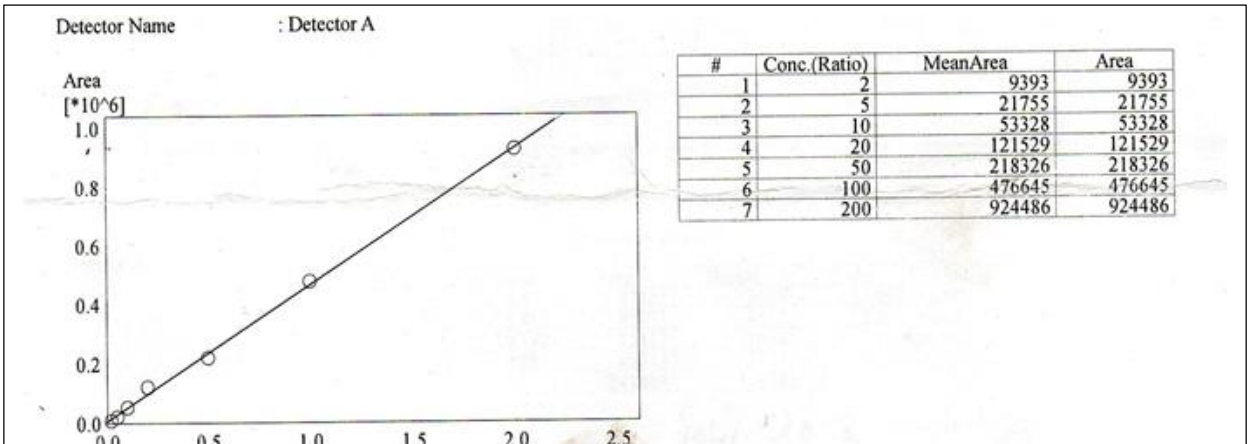
Table 15: Association of Postharvest practices and Aflatoxin Contamination

Postharvest practices	Makueni Yes(%)	Baringo Yes(%)	p-value (R^2, df)

Harvesting	Maize stovers stacked in heaps	77.9	97.9	0.73 (0.001,114)
	Maize cob removed while stovers standing	22.1	2.1	
Drying	Drying maize on the ground	47.1	11.8	0.32 (0.081, 107)
	Drying maize on a tarpaulin mat/ canvas	51.5	76.5	
	Open store	0.0	3.9	
	Left to dry in the field	1.5	0.0	
Shelling	By hand	38.2	6.3	0.88 (0.052, 104)
	Using a machine (Sheller)	14.7	85.4	
	Pounding manually in gunny bags	47.1	8.3	
Preservation	Using insecticides (actellic)	76.5	79.2	0.05 (0.065, 112)
	Using ash	2.9	0.0	
	None	20.6	20.8	
Grading and sorting	Grading based on colour and size	32.4	23.0	0.37 (0.007, 114)
	Grading to improve quality	22.1	33.4	
	No grading	45.5	43.6	
Disposal	Feeding livestock	29.4	56.3	0.57 (0.002, 114)
	Throw away	35.3	23.0	
	Consume in different forms	36.8	58.3	
	Sell in markets	5.9	23.0	
	Destroy	36.8	16.7	
	Give away	0.0	12.5	
Storage	Store bags on wooden pallets	86.8	58.3	0.23 (0.012, 114)
	Store on the ground	13.2	41.7	

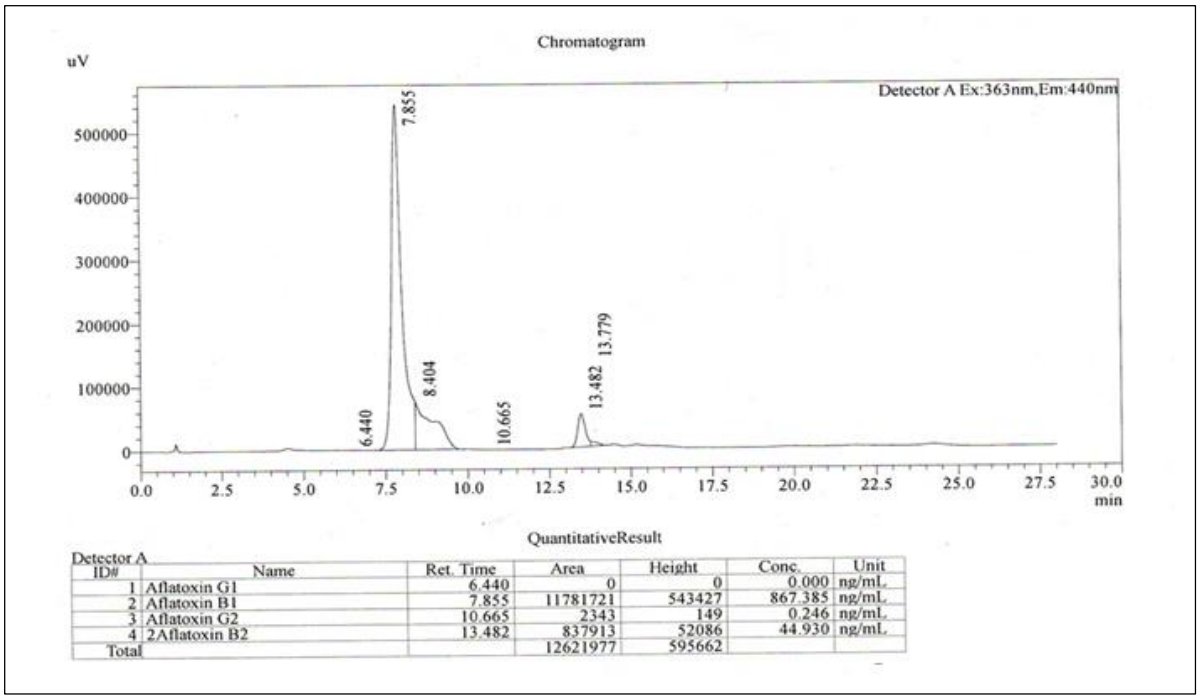
4.3.2: Results of HPLC analysis

A linearity curve for aflatoxin (AF) B_1, B_2, G_1 and G_2 was generated as shown in Figure 12. The average retention times for AFG_1 and AFG_2 were 6.440 min and 10.497 min respectively. On the other hand, those of AFB_1 and AFB_2 were 7.838 min and 13.438 min respectively (Figure 13 and 14).



#	Conc.(Ratio)	MeanArea	Area
1	2	9393	9393
2	5	21755	21755
3	10	53328	53328
4	20	121529	121529
5	50	218326	218326
6	100	476645	476645
7	200	924486	924486

Figure 8: Calibration curve for aflatoxin B_1



QuantitativeResult

Detector A

ID#	Name	Ret. Time	Area	Height	Conc.	Unit
1	Aflatoxin G1	6.440	0	0	0.000	ng/mL
2	Aflatoxin B1	7.855	11781721	543427	867.385	ng/mL
3	Aflatoxin G2	10.665	2343	149	0.246	ng/mL
4	2Aflatoxin B2	13.482	837913	52086	44.930	ng/mL
Total			12621977	595662		

Figure 9: Chromatogram of a maize sample from Baringo County

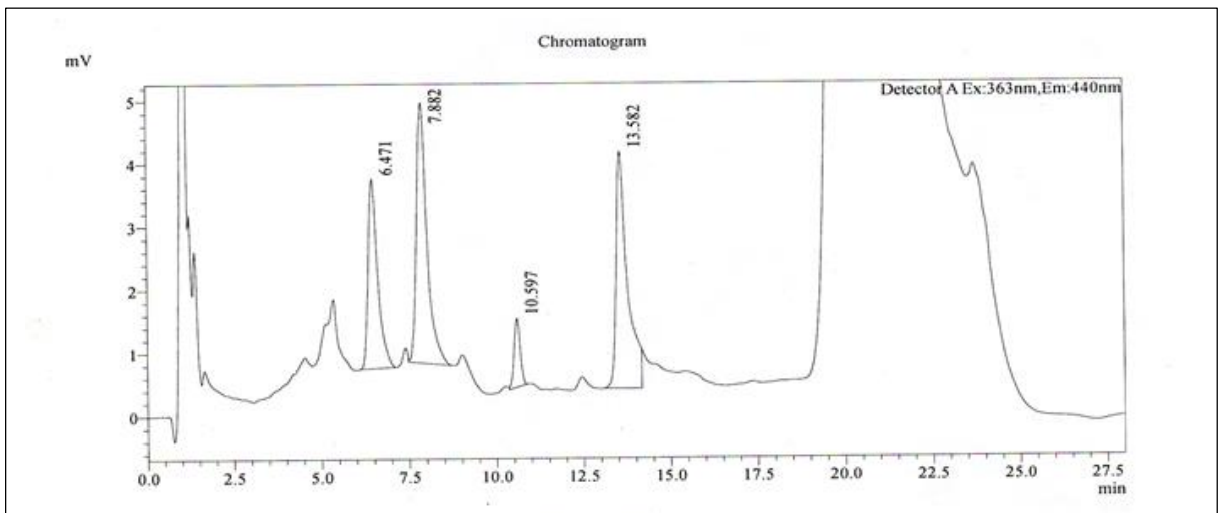


Figure 10: Chromatogram of a maize sample from Makueni County

4.4 Discussion

The results of the Elisa analysis for the maize samples revealed a high level of contamination of the maize grains in these sub-counties especially those in Makueni County. This may have been due to the long period of storage as the samples were collected at the onset of another planting season. Baringo County however experienced lower percentage of samples with maximum permissible limits. This was attributed to the fact that the samples had been fresh from harvest at the time of collection among other factors. The highest and lowest levels of contamination were gunny bags and metallic bins respectively. A similar study on the influence of storage conditions on aflatoxin contamination in wheat and mustard showed a high incidence of *Aspergillus flavus* and high aflatoxin levels in samples collected from gunny bags (Ranjan et al., 1992). The low levels noted in the samples stored in metallic bins and in pics bags can be explained by the integrity of the packing in preventing uptake of moisture by the grains after proper drying. The optimum conditions that favour the development *A. flavus* are temperature (86^{o}F), relative humidity (85%) and kernel moisture (18%). When temperatures are below 65^{o}F and kernel moisture ranging between 12-13%, the growth of the fungus usually stops. Hermetic storage arrests the further growth of the colonies by curtailing respiration (Villers, 2014). Gunny bags being plastic in nature allow

moisture into the grain and this facilitates spoilage. Sisal bags are recommended for use in place of gunny bags (Turner et al., 2005). Proper post-harvest management and practices are promising ways of reducing or even completely eliminating aflatoxin contamination of maize. Harvesting for instance by 97.9% of farmers was by stacking maize in heaps (Table 4). This is a stage where aflatoxin contamination can easily occur especially if the maize is left in the field for a long period before drying. Erratic rains that are common in this region are possible cause of aflatoxin contamination of the maize during harvest. Timely harvesting is also critical to prevent contamination.

A study on effect of delayed harvesting of maize after maturity revealed that the aflatoxin contamination increased by 4-7 fold after 3-4 weeks (Hell et al., 2008). Drying of maize on a tarpaulin/ canvas was practiced by over 50% of the farmers in both counties. Interesting to note was 47. 1% of farmers in Makueni County dried maize on the ground associated with the high levels of positivity in the samples. Drying of maize on the ground is highly discouraged as the soil contains the mycotoxigenic strains responsible for aflatoxin contamination. Shelling was mainly by hand (38.2%) and pounding manually in gunny bags (47.1%) in Makueni County. The farmers in Baringo County mainly employed use of a mechanised sheller. The use of the appropriate shelling methods to reduce grain damage, control of insects in the store, sorting to remove damaged grain and use of clean well aerated stores are recommended as a good post-harvest practices (Misihairabgwi et al., 2019; Negash, 2018). When the grain is damaged, it is more prone to aflatoxin contamination. This could also be a contributing factor to the high positivity of samples from Makueni County as pounding of the maize grain in the gunny bags makes it prone to aflatoxin contamination by the spoilage fungi. Some of the farmers did not dispose off the contaminated maize as is recommended but rather used it in various ways. The larger

proportion of farmers used it to make alternative products such as local alcoholic based beverages (*busaa, chang'aa*) and some fed it to livestock. Another group still sold it in local markets after blending with 'clean' or uncontaminated maize grain.

There was a distinct variation in the levels of aflatoxin B_1, B_2, G_1 and G_2 detected in samples from Makueni and Baringo counties. Aflatoxins B_1 and B_2 were more predominant in samples from Makueni county whilst aflatoxins G_1 and G_2 in samples from Baringo County. This can be explained by the strain variation of the two ecological zone. For instance, confirmatory tests on a sample from Kako Woiya, in Makueni County showed a level of 867 ppb and 45ppb for aflatoxins B_1 and B_2 respectively (figure 4). During one of the most severe reported cases in the last 20 years that occurred in 2004 in Makueni county, maize samples were found to have extremely high levels of aflatoxin B (Lewis et al., 2005). The level of aflatoxin B_1 was 4400ppb, 440 times the maximum permissible limit of 10ppb by Kenya Bureua of Standards.

On the other hand, a test on a sample from Baringo North revealed a level of 11ppb and 2ppb for aflatoxin G_1 and G_2 respectively (Figure 5). *Aspergillus flavus* strains can be grouped into two groups based on their morphology: L and S strains. The morphology of the L strains is characterised by numerous cinidiospores and sclerotia and are larger in size of up to 400 µm. The S strains, on the other hand have fewer and are smaller in size. The S strain isolates are not only more stable but also produce higher amounts of aflatoxin as compared to the L strain isolates (Chang et al., 2006).Recent studies have revealed a very high presence of the toxigenic *A.flavus* S strains in Makueni County and the less toxigenic L strains in Nandi County, which is a county that borders Baringo County. All the *A.flavus* strains isolated from Makueni and Baringo were the S type and L type respectively. The S strains primarily

produce the more toxic B toxins whilst the L strains produce more of the less toxic G toxin (Okoth et al., 2012). This explains the higher cases of aflatoxicosis reported in (Eastern Kenya) Makueni County as compared to (North Rift parts of Kenya) Baringo County. However, the distribution of aflatoxins B_1, B_2, G_1 and G_2 between the two counties was relatively the same despite the different geographical locations and environmental conditions. These results are similar to findings of earlier studies in the areas that found a similar pattern of occurrence of *A.flavus* which eventually produces the B toxins (Okoth et al., 2012). This similarity was also observed in Nigeria (Atehnkeng et al., 2008).

4.5 Conclusions

Very high levels of aflatoxin contamination of stored maize occur in Makueni County whilst Baringo County has lower cases. The most common aflatoxin in Makueni is aflatoxin B1, which is the most lethal of the aflatoxins produced by the S strains of *A. flavus.* Being aware of aflatoxin contamination does not refrain farmers from utilising them. The main uses of contaminated maize include manufacturing of animal feeds and traditional alcoholic beverages such as *'busaa'* and *'changaa'*. The type of storage condition is a determinant of the level of contamination in the grain. Based on the levels of aflatoxins in the stored maize, the storage conditions were rated from the best to the worst in the following order: (Metallic bins - Pics bags - Open storage - Gunny bags).

4.6 Recommendations

There is need for promotion of appropriate postharvest management strategies which have a major impact on reducing or even eliminating aflatoxin contamination in maize. Sensitization

of the farmers at the farm level on Hazard Analysis Critical Control Points (HACCP) and Good Agricultural Practices (GAP) can help reduce the chances of contamination at the production stage. Better postharvest practices can almost solve the problem to a large extent. In the case of contaminated maize grain, farmers need to be sensitized on safe disposal instead of the common practices of use in animal feeds as well as in the manufacture of traditional alcoholic beverages. To protect the public from consumption of contaminated maize grain, surveillance and monitoring of the maize along the value chain by the regulatory authorities is critical.

CHAPTER FIVE

ISOLATION AND IDENTIFICATION OF *ASPERGILLUS* SPECIES IN MAIZE GRAINS IN MAKUENI AND BARINGO COUNTIES, KENYA

Abstract

The objective of this study was to isolate and characterise *Aspergillus* strains from maize samples collected from Makueni and Baringo counties. A cross-sectional research design was employed and samples randomly collected from maize storage structures from different sub-locations. This was done alongside a study on the levels of aflatoxins in the different storage conditions in the two counties. The isolation of the *Aspergillus* spp. was done using Rose-Bengal media with Chloramphenicol. Identification of the isolated strains was based on their macro and micro-morphological characteristics and carried out using taxonomic keys. Strains of *Aspergillus flavus, Aspergillus terreus* and *Aspergillus parasitucus* were positively identified. These results corroborated well with the results of the aflatoxin analysis which showed higher levels of aflatoxin B1 that are directly linked to the presence of these strains

5.1 Introduction

Aspergilli are a group of approximately 180 species that were first described by Pier Antonio (Ainsworth, 1976; A. W. Alberts, 1998). They are largely aerobic and mainly grow on substrates rich in carbon (Nyongesa et al., 2015). *Aspergillus flavus* and related species are known to produce aflatoxin, one of the most devastating of all mycotoxins (Taniwaki et al., 2018). The most common identification tool used for identification of the *Aspergillus* species involves use of macro and micro-morphological features. However, more recently, molecular methods have been developed that have made identification of strains much easier (Warris, 2001). Taxonomic keys have been used to identify the *Aspergillus spp* using their macro and morphological features (Klich, 2002; Raper & Fennel, 1965). The macro-morphological

features used include: colony colour and texture, development of sclerotia, presence of exudates, development of soluble pigments by the fungi and formation of reverse colour of the plate. The micro-morphological features include features of the conidia and sclerotia if developed by the fungi. The objective of this study was to isolate and characterise *Aspergillus* strains from maize samples collected from Makueni and Baringo counties.

5.2 Materials and Methods

5.2.1 Study sites

Makueni and Baringo counties were purposively selected (Figure 1) due to the expected strain variation due to differences in the geographical locations among other factors (Kangethe et al., 2017; Okoth et al., 2012; Ouko, 2014).

5.2.2 Sample collection

A total of 80 maize samples (500 g each) were randomly collected from different storage conditions in Makueni and Baringo counties (40 for each County). This included gunny bags, metallic bins, open storage and pics (Purdue Improved Crop Storage) bags – 10 samples for each storage condition. The samples were thereafter placed in sterile bags and stored at 4^{o}C before transportation to the laboratory for analysis. The analysis was done at the microbiology laboratory at the Department of Plant Science and Crop Protection, University of Nairobi.

5.2.3 Sample preparation

Each sample was homogenized before the analysis began. Milling was done aseptically using a knife mill (GRINDOMIX GM200, GERMANY) before further analysis. The mill

was thoroughly cleaned and dried using paper towels after every milling cycle to avoid cross contamination between samples.

5.2.4 Preparation of media

Isolation and enumeration of the *Aspergillus* species was done using selective media. Rose Bengal media with the following composition was used: Glucose – 10g, peptone – 5g, KH_2PO_4 – 1g, $MgSO_4.7H_20$ – 0.5g, Rose Bengal – 0.05g, Chloramphenicol – 0.1, agar – 15.50g per litre of the prepared media according to the manufacturer's instructions. The media was then autoclaved at 121^oC and 15 PSI pressure for 15 minutes. Thereafter, it was cooled to 40^oC before use.

5.2.5 Isolation and identification of *Aspergillus* species

The dilution method was used for isolation. Each milled sample was weighed (1g), suspended in 9 ml of diluent and serial dilutions prepared. 1 ml was drawn from 10^{-3} to 10^{-5} into petri dishes, swirled to mix with the media and then left to solidify. This was done in duplicates. The plates were then sealed with parafilm and then stored in an inverted position for 3-5 days. Observations were done after every 24 hours from day 2 at 26 ± 2^oC (Embaby et al., 2015). Identification of the isolated strains was done using macro and micro-morphological characteristics exhibited by the colonies. For microscopy, the fungal spores were picked from the plate using adhesive tape, placed on a microscopic slide and a drop of water added. The slide was then observed under a compound microscope (X400 magnification) for identification. The morphological characteristics used were colony colour, colony texture, and colony growth. The identification of the *Aspergillus* was done according to (Klich, 2002). The colonies were then counted and recorded and this was used to calculate the colony forming units (CFU).

$$CFU/g = \frac{A*10^n}{V}$$

Where:

A = Number of colonies

10^n = Dilution level counted

V = Volume of inoculation (ml)

5.3 Results

The fungal load differed for the different storage types and study sites. The fungal load expressed as colony forming units (CFU/g) of the isolates is as shown in Table 12.

Table 16: Fungal load of different storage types in Makueni and Baringo counties

Type of storage	Mean CFU/g		P value (F-value, df)
	Makueni	Baringo	
Open storage	78.15	101.70	0.39(1.07, 7)
Metallic bins	42.15	49.65	
Gunny bags	159.90	202.50	
Pics bags	26.55	35.1	

The *Aspergillus* isolates were thereafter identified using phenotypic and morphological features as shown in Table 13 below. *Aspergillus flavus*, *Aspergillus parasitucus* and *Aspergillus terreus* were positively identified.

Table 17: Phenotypic and morphological characteristics used to identify isolated strains

Aspergillus species	Phenotypic and Morphological features			
	Colour	**Texture**	**Shape**	**Conidia surface**
A flavus	Pale brown, roughened	Quietly spherical	Glubose ellipsoid	Smooth, finely roughened
A parasitucus	Colourless	Finely roughened	Glubose	Smooth walled
A terreus	Colourless	Smooth walled	Glubose	Smooth walled

The frequency of the occurrence of the different *Aspergillus* species also differed (Table 14). The occurrence of *A. flavus* was highest in Makueni County at 60.0%. The frequency of *A. flavus* was minimal in Baringo County with a frequency of 15.0%. The other *Aspergillus* species ranged between an occurrence frequencies of between 5.0 – 13.8%. There were also traces of other species of fungi: namely *Fusarium* and *Penicillium* species.

Table 18: Frequency of *Aspergillus* species isolated from maize samples from Makueni and Baringo counties

Aspergillus species	County				
	Makueni		**Baringo**		**Total (%)**
	No of plates	**%**	**No of plates**	**%**	
A. flavus	48	60.0	12	15.0	37.5
A. parasitucus	11	13.8	8	10.0	11.9
A. terreus	5	8.8	4	5.0	6.9
Penicillium	7	6.3	12	15.0	10.7
Fusarium	2	2.5	22	27.5	15.0

5.4 Discussion

The highest microbial load was found in the gunny bags with an average of 159.90 and 202.5 CFU/g for Makueni and Baringo counties respectively. Open storage recorded higher microbial loads compared to metallic bins and pics bags. The lowest microbial loads were in pics bags with 26.55 and 35.10 CFU/g for Makueni and Baringo counties respectively. The high microbial loads in gunny bags and open storage were attributed to the exposure of the maize to humid conditions. Even when proper drying is done, open types of storage tend to allow moisture to accumulate in the maize resulting in aflatoxin contamination. Moreover, farmers store this maize on the ground as opposed to using pallets thus resulting in water uptake by the grain.

Samples from Makueni County had higher levels of *Aspergillus flavus* and *Aspergillus parasitucus* at 60.0% and 13.8% respectively. These strains especially *A. flavus* are predominantly known to produce more of the more fatal B toxins. The types of strains that thrive in eastern parts of Kenya are the S strains that primarily produce the B toxins. On the other hand, North rift parts of Kenya are known to harbour more of the L strains which predominantly produce the G toxins (Okoth et al., 2012). The factors that promote the growth and proliferation pre- and post-harvest vary and include temperature, humidity, crop varieties prone to infection, crop rotation systems among others (Mutegi et al., 2018). Other factors that increase likelihood of aflatoxin contamination include soil types, drought, genotype and insect activity. When poor postharvest practices occur during the handling of the grain right from handling pre-harvest, post-harvest fungal growth occurs resulting in mycotoxin contamination (Njeru et al., 2019).

The phenotypic and morphological characteristics helped in positively identifying the Aspergilli species. Also identified were *Fusarium* and *Penicillium* strains as the media was for

broad spectrum fungal isolation. These were however set aside as they were not the fungi of interest in this study.

5.5 Conclusions

This study confirmed the presence of mycotoxigenic fungal strains in maize found in both Makueni and Baringo counties. Strains of *Aspergillus flavus*, *Aspergillus parasitucus* and *Aspergillus terreus* were positively identified. The storage conditions usually influence the type of microflora found in stored maize.

5.6 Recommendations

In order to completely lock out moisture and prevent growth of the mycotoxigenic strains, hermetic storage should be promoted. These fungi are aerobic and when oxygen supply is curtailed thus their growth is stopped. Proper drying to the appropriate moisture content and storage under hermetic structures will curtail growth of the mycotoxigenic *Aspergilli* species and prevent aflatoxin contamination.

CHAPTER SIX

EFFICACY OF LOW TEMPERATURE NITROGEN PLASMA IN DESTROYING FUNGI AND AFLATOXINS IN MAIZE

Abstract

Globally, aflatoxin contamination in maize remains a huge burden despite many interventions put in place. Use of low temperature plasma to decontaminate the maize offers a possible solution by ensuring safety and extended shelf life of the grain. Plasma can be defined as an ionized gas containing a mixture of special molecules with the ability to destroy the pathogen and toxins while leaving the food material unharmed. This study investigated the efficacy of low temperature nitrogen plasma (LTNP) in destroying fungi and aflatoxin with exposure time, pressure and ionization density as independent variables. The study generated 17 experimental runs using the Response Surface Methodology (RSM) of the Box Benken Design (BBD) in the Design Expert software (StatEase, 2020). RSM linear model predicted the reduction in fungal load and aflatoxin content with F-values of 7.22 and 15.89 respectively ($P \leq 0.01$). An increase in exposure time and pressure led to a corresponding decrease in the fungal load and aflatoxin content. Ionization power did not have a significant effect on both response variables. For optimisation of the detoxification process, the RSM model supported process settings of time at 153.58 seconds, pressure of 0.98 Pascals and ionization power of 194.82 Watts. The results lead to the conclusion that LTP is capable of achieving a reduction of 68.78% and 33.89 log (cfu/g) for aflatoxin content and fungal load, respectively. Inclusion of temperature as an independent factor will help fine tune these optimised conditions. Piloting is also necessary to assess the performance on a large scale before upscaling. Finally, testing of the maize for safety is paramount after treatment.

6.1 Introduction

Maize is one of the most important food security crops in Kenya. Despite maize being a very important food security crop in Kenya, farmers face numerous challenges due to postharvest losses due to aflatoxin contamination (Short et al., 2012). Globally, Kenya has posted one of the most numerous incidences of acute toxicity since the initial captured outbreak of 1981 (Unneveh & Delia, 2013). Subsequently, there have been other studies that have shown that the exposure to aflatoxins of the general population is still very high (Leroy et al., 2015). Aflatoxins are produced by aflatoxigenic fungi particularly *Aspergillus flavus*, *Aspergillus parasitucus* and more rarely *Aspergillus nomius* (Wacoo et al., 2014). Fungi are able to grow on a broad spectrum of foods such as cereals, fruit, vegetables, meat, fats and many others which eventually leads to production of toxin, development of off-flavour, rotting, changes in colour and growth of pathogens (Pitt & Hocking, 2009).

Low temperature plasma on the other hand is a new emerging technology that is attracting a lot of interest in the food industry. This is particularly due to its ability to rapidly decontaminate a food matrix at ambient temperature and pressure while leaving the food without any detectable changes in quality. Use of air in place of other commonly used gases such as Helium, Argon, Nitrogen and Heliox makes it cheaper and hence sustainable in the long run (N. Misra et al., 2019).

Fungal decontamination is a major challenge worldwide due to overreliance on chemical disinfectants such as Virkon that have negatively affected the environment and also result in build-up of toxic residues in the treated food. Cold plasma has been found to have more superior results of fungal inactivation than Virkon (Nataša Hojnik et al., 2019). The mechanism of inactivation of fungi by LTP is through chemical interaction of the plasma species with the specimen, destruction of the membranes as well as the internal cellular structures and finally breaking of the DNA strands of the fungi (Bolshakov et al., 2004; Gallagher et al., 2007;

Moisan et al., 2002). Similar to that of fungi, the mechanism of decontamination of mycotoxins using LTP is not exhaustively studied. However, the mycotoxin degradation is associated with their molecular structure and the type of plasma which consequently affects the kind of interaction that results. The properties of the type of plasma produced are dependent on several factors: the type of gas used, matrix under treatment, process parameters and equipment type (Shi et al., 2017).

Plasma is simply defined as the fourth state of matter that consists of an ionized gas comprising several reactive species (RS) that include electrons, photons, negative and positive ions, free radicals and molecules (Ekezie et al., 2018; Han et al., 2019; Muhammad et al., 2018). These RS facilitate a rapid decontamination process and are selective in nature thereby damaging the pathogen leaving the food material unharmed. This occurs at relatively low temperature and pressure without causing any major changes in the food quality. The process is also cheap as limited costs are incurred (N. Misra et al., 2019). Additionally, LTP has shown potential of replacing the conventional decontamination methods in the food industry because of its high efficacy and efficiency (Babra et al., 2017; Gavalian & Cullen, 2019; N Hojnik et al., 2017; Lopez et al., 2019). The free radicals that result from the ionisation are linked to the degradation of the mycotoxins (Pankaj et al., 2018).

Optimization experiments often follow the one-factor at a time technique which requires volumes of data to identify the optimum level, is time consuming and is regarded as unreliable (Sahu et al., 2009). Consequently, such experiments do not represent a combined effect of many factors. RSM experimental designs on the other hand, take into account the combined effect of several factors which sheds light on their interactions and gives resultant statistical models (Alam et al., 2007). The optimization process using RSM largely involves three distinct steps: Generation of statistically designed experimental designs, co-efficient estimation using mathematical modelling and lastly response prediction and testing of significance of the model

to the experiment (Mahalik et al., 2010). The objective of this study was to develop a more efficacious method of decontaminating maize by optimizing the decontamination parameters.

6.2 Materials and Methods

6.2.1 Materials

a) Sample collection

Maize samples (25g each) were drawn from a blended sample obtained from Makueni and Baringo counties that were naturally contaminated with a fungal load range of (3 – 210 cfu/ml) and aflatoxin content (66.12 – 105.98 ppb) before treatment.

b) Microbial analysis: Media and Equipment

Rose Bengal Media with Chloramphenicol (Himedia, Nashik, India), petri dishes (60mm diameter), culturing loop, laminar flow chamber (UMS, UK), colony counter, ethanol GPR and Parafilm (4 In x 125 ft) were used.

c) Aflatoxin analysis: Reagents and equipment

Enzyme-linked immunosorbent assay (ELISA) kits (Total Aflatoxin Assay), United States (Helica biosystems Inc) and High Performance Liquid Chromatography (HPLC) machine (NEXERA UHPLC SHIMADZU – JAPAN) were used. The type of column used for the analysis was Nova-pak C18 4µm x 150mm (WATER CORP – IRELAND). The following operating conditions were observed during the process: run time – 30minutes; injection volume - 10µl; column temperature – 35^{o}C, velocity – 1.0 ml/minute). Main reagents included Methanol Hplc grade, acetonitrile Hplc grade, and Triflouroacetic acid – AR.

d) Plasma experiment: Equipment and process settings

Plasma unit (diener electronic GmbH + Co.KG, Ebhausen, Germany) was used. Petri dishes (100mm diameter) were also used. The runs varied in time, pressure and ionization density.

6.2 Methods

6.2.1 Optimization of the decontamination process

The experimental design was generated using the RSM models of the Box Benken Design (BBD) of the Design Expert 11 software (StatEase, 2020). The optimization formula used was as shown in Equation 1 (Behera et al., 2018). The parameters considered for optimization were reduction in the fungal load and percent reduction in the aflatoxin content. The variable factors were time, pressure and ionization power.

Equation 1: $y= f(x_1, x_2, x_3)$

Where ***y*** represents the response variables; either percent reduction in the aflatoxin content or percent reduction in the fungal load, whilst $\boldsymbol{x}_{(1\text{-}3)}$ represents the independent variables time, pressure and ionization power. Their maximum and minimum values (Table 15) were chosen based on similar studies carried out by (Basaran et al., 2008; Dasan et al., 2016; Sakudo et al., 2017; Shi et al., 2017).

Table 19: Minimum and Maximum values of factors selected in the Box Benken Design (BBD)

Factor	Units	Minimum	Maximum
Time	Seconds	5	1,800
Pressure	Pascals	0.1	1.7
Ionization power	Watts	60	200

The process flow is summarised in Figure 15 and Figure 16 (a-d)

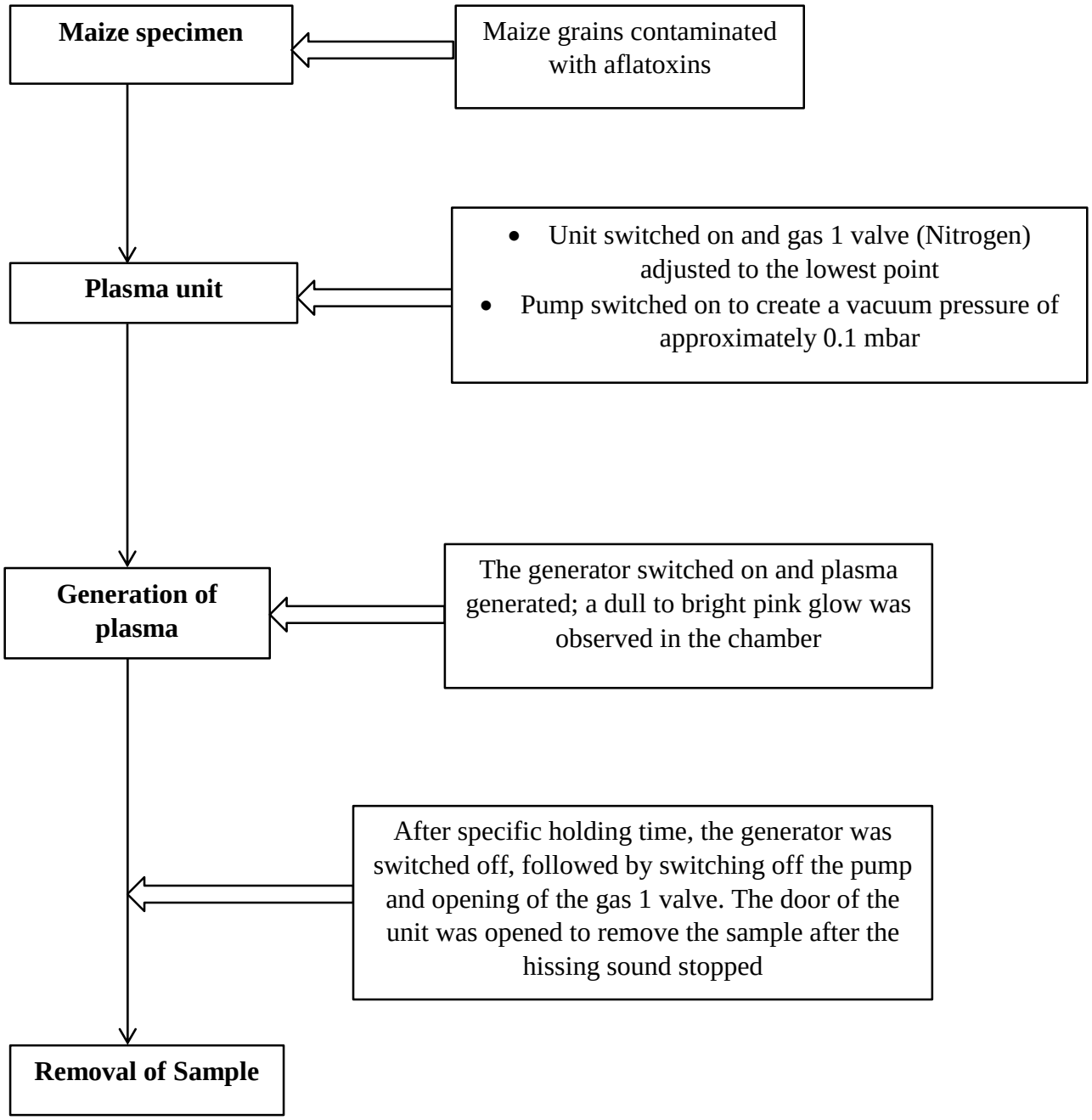


Figure 11: Schematic Flow Chart of the Low Temperature Nitrogen Plasma (LTNP) Treatment

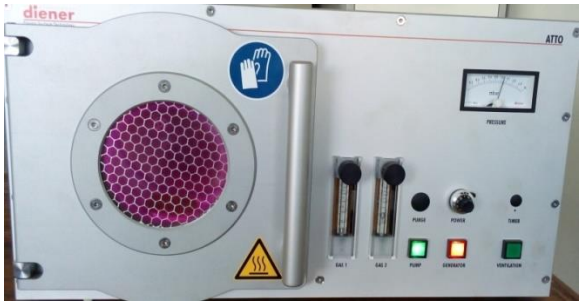


Figure 16a: Plasma unit

Figure 16b: Nitrogen gas cylinder

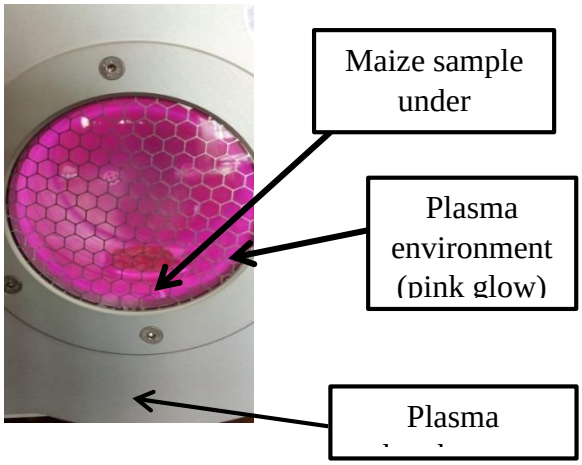


Figure 16c: Maize sample under treatment

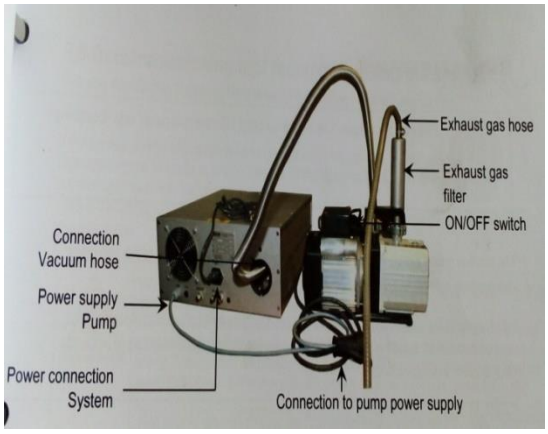


Figure 16d: In-bulit structure of plasma unit

The percent reduction in the aflatoxin content and fungal load was derived using Equation 2 according to (Behera et al., 2018).

Equation 2: $N=3^n + 3n + n_c$

Where ***N*** represents the total number of experimental runs, ***n*** is the number of factors and $\boldsymbol{n_c}$ is the total number of central points that resulted. The total percent reduction for aflatoxin and fungal load was calculated according to the Equations 3 and 4 respectively.

Equation 3:

$$\textit{Aflatoxin percent reduction} = \frac{\textit{Initial aflatoxin level} - \textit{Level of aflatoxin after treatment}}{\textit{Initial aflatoxin level}} \times 100$$

Equation 4:

$$\textit{Fungal load percent reduction} = \frac{\textit{Initial fungal load} - \textit{Fungal load after treatment}}{\textit{Initial fungal load}} \times 100$$

6.2.2 Enzyme immunoassay for total aflatoxin (ELISA)

Aflatoxin analysis of the samples was carried out using the competitive enzyme-linked immunoassay for quantitative detection of aflatoxin B1, B2, G1 and G2 (Wacoo et al., 2014). The aflatoxin kits (HELICA Total Aflatoxin Assay) were sourced from the United States (Helica Biosystems Inc). The samples were prepared according to the manufacturer's recommendations. All there agents were brought to room temperature before use. Five (5) g of the ground maize samples was mixed with 25 ml of 70% methanol. The ratio of the sample to extraction solvent used was 1:5 (w/v). The extracted sample was thereafter mixed with 200 µL of HRP conjugated aflatoxin 100µL of each standard and sample was then added to appropriate mixing well containing conjugate and mixed three times. The mixture (100 µL) was then transferred to a corresponding antibody coated microtiter well and incubated for 15 minutes at room temperature. The well contents were thereafter discarded into a basin and the micro wells washed five times by filling each well with PBS (Phosphate buffered saline) - Tween wash buffer. Absorbent towels were used to dry the wells (face down) before introduction of 100µL of substrate reagent. Finally, 120µL of stop solution was added to each micro well. The optical density of each micro well was read using a Spectrophotometer - model type - 355, manufactured by Thermo Fisher Scientific (Shanghai, China). The readings were taken using a 450 nm filter. The limit of detection of the kits was 20 ppb. Samples that had more than 20 ppb were further diluted with 70% methanol and retested to obtain the accurate total aflatoxin level.

6.2.3 Statistical analysis

To study the combined effect of the independent factors as well as their interactions with process variables and responses, the data from the decontamination experiment was subjected to ANOVA (Analysis of Variance) for quadratic models. This was done using RSM models of

the Box Benken Design (BBD) of the Design Expert software (StatEase, 2020). Other analysis done regression analysis, plotting of responses and contour plots at optimized conditions. The F test was conducted to deduce the statistical significance of the factors. The coefficient of R^2 was used to check for accuracy of the fitted polynomial model factors. Finally, the level of significance of the factors was evaluated using the (P value) at 95% confidence interval (CI).

6.3 Results

6.3.1 Aflatoxin and fungi reduction in experimental runs

The RSM methodology modelled an experimental design with time, pressure and ionization power as the independent variables. The percent reduction in the fungal load and aflatoxin content were the response variables. Using the RSM, seventeen experimental runs were generated (Table 16).

Table 20: Box Benken Design for optimization of the decontamination process

Standard	Run	Predictor variables			Response variables	
		Time (Sec)	Pressure (Pascals)	Ionization power (Watts)	% reduction in fungal load	% reduction in aflatoxin level
11	1	902.5	0.9	130	19.4444	70.2012
8	2	902.5	0.9	130	20	73.8032
5	3	902.5	1.7	60	63.6364	73.5777
15	4	902.5	0.1	60	55.5556	74.8986
4	5	902.5	1.7	200	58.3333	75.668
3	6	1800	0.9	60	83.3333	82.5354
17	7	902.5	0.1	200	87.5	82.5967
2	8	1800	1.7	130	58.3333	80.8688
7	9	1800	0.1	130	93.3333	77.8233
1	10	5	1.7	130	27.2727	64.3686
14	11	902.5	0.9	130	66.6667	73.2935
10	12	5	0.9	200	20	68.589
13	13	902.5	0.9	130	55.5556	75.1734
6	14	5	0.9	60	8.57143	66.3047
9	15	5	0.1	130	45.4545	63.6917
12	16	902.5	0.9	130	35.2941	71.2399
16	17	1800	0.9	200	100	80.1297

6.3.2 Optimal fungal load reduction parameters using RSM modelling

The normality of the data obtained was assessed in order to determine its viability to generate the predicted model. The data was found to be normally distributed with the observed points closely aligning to the line of best fit. A correlation was observed between the observed and the predicted values and the latter were closely aligned to the line of best fit; demonstrating that the data was normally distributed (Figure 17). This indicated that the factors used in the prediction of the response variables were credible and thus gave credit to the use of the response surface methodology (RSM) in the experimentation. The linear model was significant ($P \leq 0.01$) and was adopted as it accounted for the highest R^2. The model predicting percent fungal

load had an F value of 7.22. Time was a significant factor in predicting the fungal load explaining the linear model with an R^2 of 53.85. The model was significant with a 0.43% chance fungal load would increase with time increase (Table 17). Models were not significantly linear and cubic factors were not explaining the variation in the fungal load. A three dimensional approach of the combined effect of all the factors was also tested. Increase in exposure time and pressure led to a corresponding decrease in the fungal load (Figure 18 and 19). In the case of increase or decrease in ionization power, the effect was not significant. The predicted and observed values of percent fungal load reduction did not align to the line of best fit implying that the values were not statistically significant.

Table 21: Analysis of Variance (ANOVA) for linear model prediction of percent reduction in fungal load

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	7891.01	3	2630.34	7.22	0.0043	significant
A-Time	6827.04	1	6827.04	18.74	0.0008	
B-Pressure	689.46	1	689.46	1.89	0.1921	
C-Ionization power	374.51	1	374.51	1.03	0.3291	
Residual	4734.75	13	364.21			
Lack of Fit	2938.84	9	326.54	0.7273	0.6835	not significant
Pure Error	1795.92	4	448.98			
Cor Total	12625.76	16				

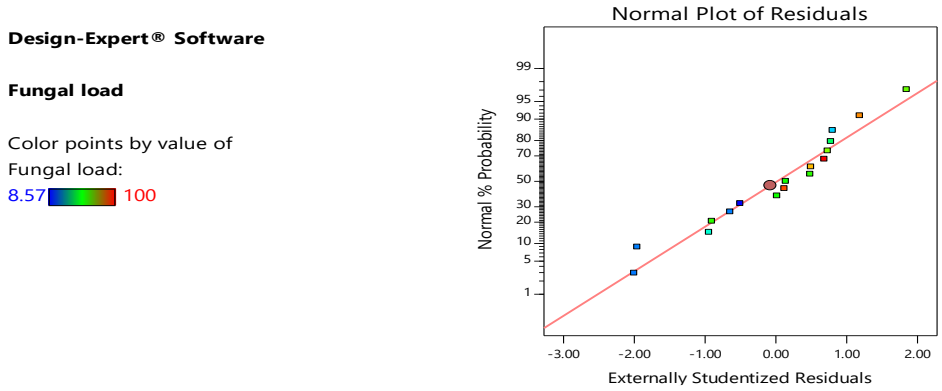


Figure 17: Normal distribution of observed values

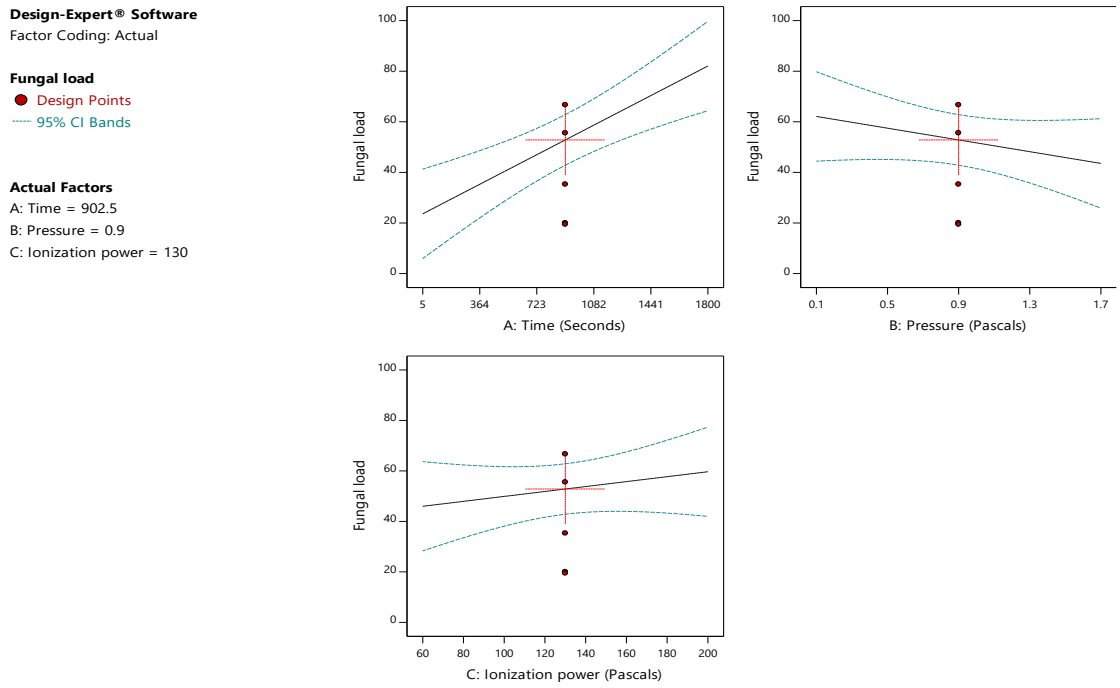


Figure 18*12*: Optimisation of individual factors (time, pressure and ionization power) on reduction in fungal load

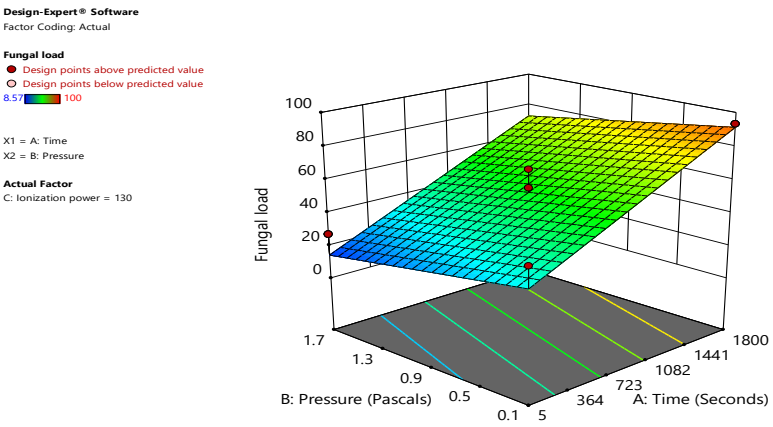


Figure 19: Combined effect of pressure and time on percent fungal load reduction

The final equation for the prediction of the percent reduction in fungal load is as shown in equation 5.

Equation 5:

Fungal load (Percent reduction) = 5.1042 - 0.0009 Time + 0.2850 Pressure – 0.0079 Ionization Power

6.3.3 Optimal aflatoxin reduction parameters using RSM modelling

The obtained data was tested for normality and its adequacy for generating the predicted model. The observed and predicted values were found to cluster along the line of best fit thus proving normality of the data (Figure 20). Additionally, there was little variation between the predicted and observed values demonstrating the model was significant. In the analysis, the linear model was adopted as it accounted for the highest R^2 with an F-value of 15.89 implying that the model was significant ($P \leq 0.01$). This meant that there was only a 0.01% chance that an F-value this large could occur due to noise (residual error).The non-significant lack of fit with an F-value of 2.88 meant that there was 16.02% chance of residual error thus demonstrating model fitting.

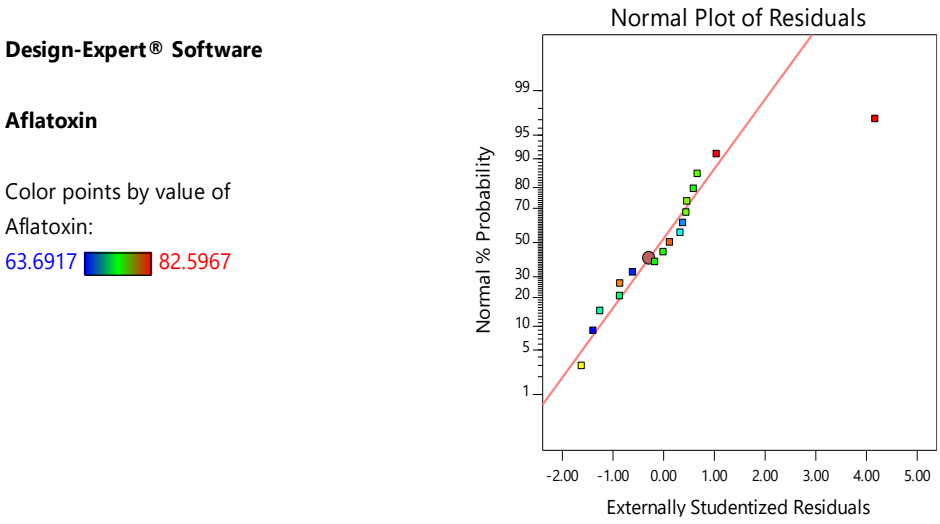


Figure 20: Normal distribution of observed values

The individual and combined effects of factors (time, pressure and ionization power) on the percent reduction in the aflatoxin content were studied using the RSM methodology. The model predicting the effect of time, pressure and ionization power on aflatoxin content was significant ($P<0.001$) as shown in Table 18. With increasing time and pressure the % reduction in aflatoxin content increased (Figure 21). The relationship of the combined effect of the predictors on the reduction of aflatoxin in maize is as shown in Figure 22.

Table 22: Analysis of Variance (ANOVA) for linear model prediction for reduction in aflatoxin content

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	440.61	3	146.87	15.89	0.0001	Significant
A-Time	426.36	1	426.36	46.13	< 0.0001	
B-Pressure	2.56	1	2.56	0.2772	0.6074	
C-Ionization power	11.68	1	11.68	1.26	0.2813	
Residual	120.15	13	9.24			
Lack of Fit	104.10	9	11.57	2.88	0.1602	not significant
Pure Error	16.05	4	4.01			
Cor Total	560.76	16				

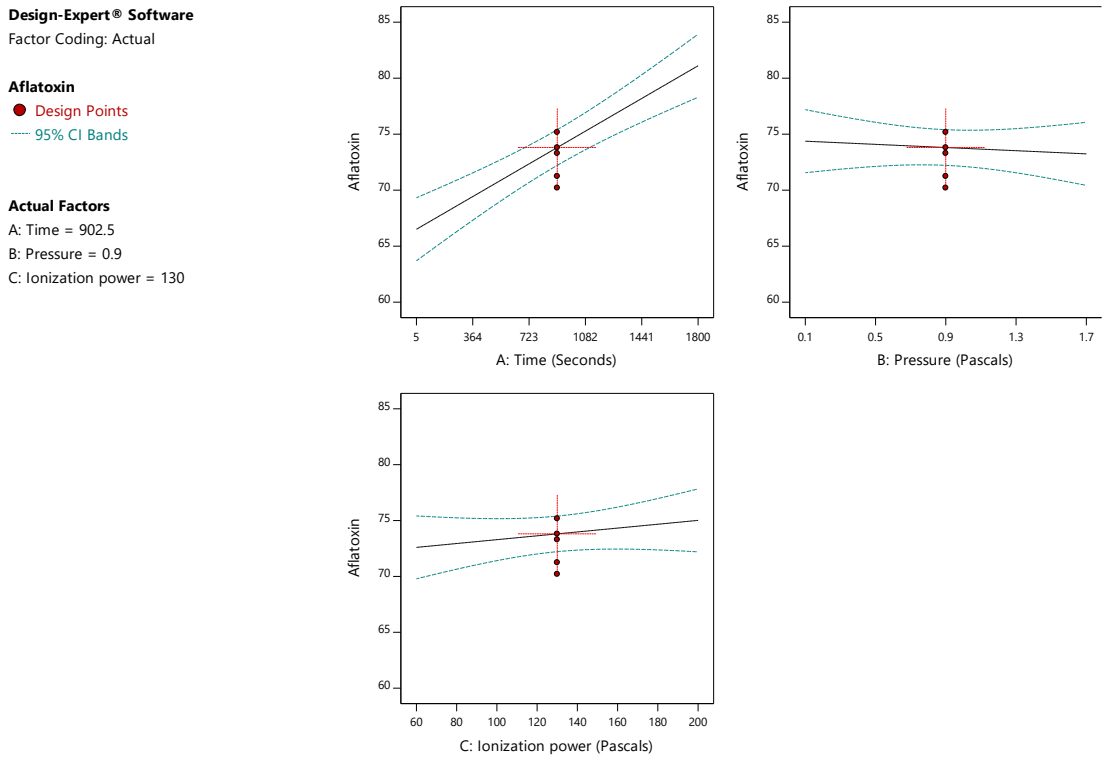


Figure *21*: Effect of optimisation of individual factors (time, pressure and ionization power) on reduction in aflatoxin

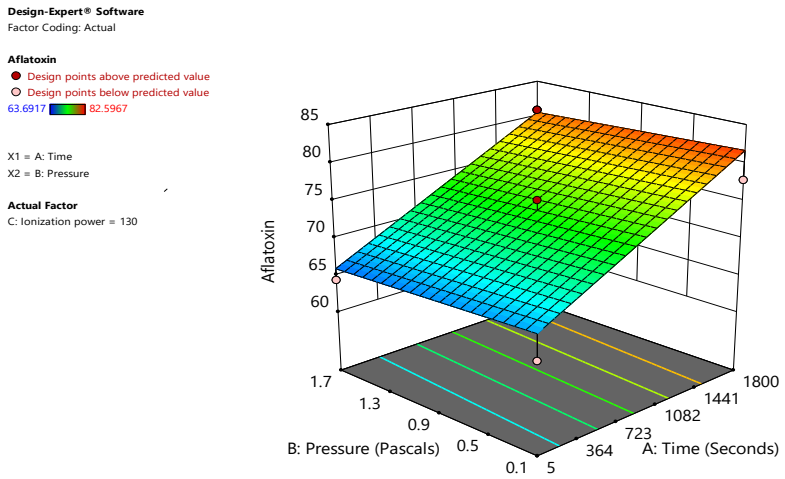


Figure *13*: Combined effect of pressure and time on percent reduction in aflatoxin content

The final equation for the prediction of the percent reduction in the aflatoxin level was thus summarized as shown in equation 6.

Equation 6:

Aflatoxin (Percent reduction) = 64.861 + 0.008 Time - 0.707 Pressure + 0.017 Ionization Power

6.3.4 Optimal factors generated by the Response Surface Methodology

The optimal points for each factor were suggested after the RSM analysis are as shown in Figure 23. These were time being 153.58s, pressure of 0.98 and ionization power of 194.82. In turn, the program also suggested the resultant reduction levels for each response variable. The percent reduction in the fungal load was deduced to be 33.89% and percent reduction of 68.78% in the aflatoxin content.

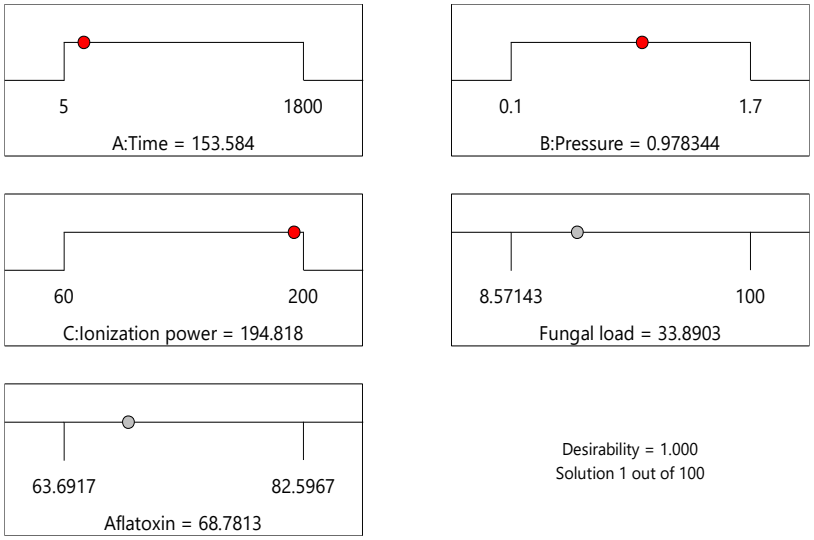


Figure 14: Optimal points as generated using the RSM methodology

6.4 Discussion

The linear model generated by RSM methodology predicting the percent reduction in fungal load revealed that time and pressure were significant factors. Ionization power on the other hand was not significant. An increase in the time and pressure lead to a corresponding decrease in the percent fungal load. The model predicting percent fungal load had an F value of 7.22 ($P \leq 0.01$) with a 0.43% chance of noise (residual error). The same applied for the model predicting reduction in the aflatoxin content which had an F-value of 15.89 ($P \leq 0.01$) with a 0.01% chance that an F value this large could occur due to noise. A large F value with a corresponding low P value, implies more significance of the corresponding coefficients (Yi et al., 2010). The results showed that LTP plasma has efficacy to reduce the total aflatoxin content and fungal load by 68.78% and 33.89% respectively. Some studies on efficacy of LTP to inactivate *Aspergillus sp*. have reported its effectiveness. A similar study on effect of LTP on fungi in maize (B. A. Silva et al., 2020) concluded that LTP demonstrated potential to inactivate fungi in maize by 33.33%. The plasma plant conditions were 360 W for ionization power and for 30 minutes exposure; pressure was not one of the experimental factors. This was also reported in yet another study (J. R. Silva et al., 2018) on the effect of cold plasma on storage

toxigenic fungi – *Aspergillus flavus*. There was no growth of the fungus after 6 days of incubation for samples treated for 15 – 20 minutes. Growth was slowed for the samples treated for 2-12 minutes as compared to the control sample. The plasma conditions were not specified but LTP was used. This supports the results obtained in the present study as the treatment time found to be most optimal was 153.58 seconds (approximately 5 minutes). In 2016, (Dasan et al., 2016) reported significant reductions of between 4.50 log (cfu/g) in *A.flavus* and 4.19 log (cfu/g) in *A.parasitucus* after 5 minutes of treatment at 655 W ionization power. In 2018, (Hosseini et al., 2018) studied the of LTP on *Aspergillus* sp. and complete inactivation of the fungi was noted after 15 minutes exposure at 60 W. The findings of the present study partially corroborate the findings of these authors.

The mechanism of action of LTP on fungi is linked to the reactive species (oxygen) in the plasma. These are thought to interfere with several pathways of the fungal structure leading to its destruction. Some of the functions curtailed by LTP plasma are inhibition of the cell membrane function, apoptosis (Hashizume et al., 2015), intracellular nanostructural changes, morphological changes in cell membrane and increased permeability (Dasan et al., 2016) and finally through oxidation of intracellular organelles (Kang et al., 2014; Panngom et al., 2014). The role of reactive nitrogen species (RNS) and UV light has not been exhaustively researched and further research is needed to understand their role in fungal inactivation (N. Misra et al., 2019).

Similarly, the linear model predicting percent reduction in the aflatoxin content had an F-value of 15.89 with a 0.01% possibility of error ($P \leq 0.01$) and hence its significance. A percent reduction of 68.78% was predicted at the optimal factors generated (Figure 10). As time and pressure increased, there was a corresponding decrease in the aflatoxin content. Change in the ionization power did not have any significant effect on the aflatoxin level. However, this showed that plasma has the potential to destroy aflatoxin inherent in maize. The findings of the

present study were partially similar to other related studies that have explored the potential of plasma technology to destroy aflatoxin in different food substrates. In 2017, (Shi et al., 2017) studied the effect cold plasma on aflatoxin in corn (maize). A 62-82% decrease in aflatoxin content was achieved in 1-10 minutes of treatment. The linear model proposed 5.1 minutes to achieve a 68.78% reduction which corroborates the present study. The ionization power was 200 W and also corresponds with the proposed power of 195W deduced by RSM linear model in the present study. Another study in 2017 (Ten Bosch et al., 2017) on effect of cold plasma on pure mycotoxins in rice extracts found that pure mycotoxins were degraded completely after 60 s of treatment. Degradation of mycotoxins is dependent on their structure and the presence of the matrix; pure toxins are degraded faster than a mixture of several mycotoxins (Ten Bosch et al., 2017). In 2016, (Siciliano et al., 2016) conducted a study on the efficacy of plasma on hazelnuts and reported a maximum detoxification efficacy of 70% using nitrogen gas. This relates well with what the RSM linear model recommended (68.8%). Other more recent studies have also reinforced the efficacy of plasma in eliminating aflatoxin (Puligundla et al., 2020; Sen et al., 2019; Wielogorska et al., 2019).

In order to understand the efficacy of plasma on aflatoxin, the knowledge of the contents of LTP is required. LTP comprises three aspects: heat, UV radiation and the reactive species produced during ionization of the reactive gas (Yousefi et al., 2021). The conditions in heat ($<60^{o}C$) and UV radiation (50 $\mu W/cm^{-2}$) do not meet the threshold for degradation of the mycotoxins. In order to degrade mycotoxins higher UV intensities are required of as high as 800 $\mu W/cm^{-2}$ (R. Liu et al., 2011). Thus, in this case the degradation is attributed to the action of the reactive species on the functional groups, different active rings and the double and triple bonds in the structure of the mycotoxins. This in turn leads to production of lesser toxic compounds as compared to the original mycotoxin. Increase in the power, pressure and

exposure time suggests higher production of reactive species and thus higher efficacy of the generated LTP (Yousefi et al., 2021).

6.5 Conclusions

The results lead to the conclusion that LTP has efficacy to reduce the fungal load and aflatoxin in maize. For optimal process conditions, an exposure time of 153 sec, pressure of 0.98 Pascals and ionization power of 194.82 is required to reduce aflatoxin content of 68.78% and fungal load of 33.89 log (cfu/g). During the plasma treatment, an increase in exposure time and pressure resulted in a corresponding decrease in both fungal load and aflatoxin content. Change in the ionization power did not have a significant effect on both aflatoxin content and fungal load.

6.6 Recommendations

The results of this study lead to the following recommendations: initial piloting to up-scale the technology while applying implementation research or science to perfect maize detoxification parameters. Additionally, more research on the potential organoleptic, physical and chemical changes in the food matrices after treatment is needed. In future studies, incorporation of temperature as an independent factor will perfect the optimization of the decontamination process.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General Discussion

The main aim of this study was to contribute towards enhancing food safety of maize using plasma technology. This consequently would lead to reduced losses due to aflatoxin contamination. Two study sites were purposively selected representing two geographical areas in Kenya where aflatoxin contamination of maize is rife. There were four main hypotheses for this study. First, that knowledge, attitude and practices of communities in Makueni and Baringo counties do not lead to aflatoxin contamination of maize; Secondly, maize grains produced in Makueni and Baringo counties are free of aflatoxins; Thirdly, that myco-toxigenic strains of fungi are not present in maize grain grown in Makueni and Baringo counties and finally, that low temperature plasma does not have efficacy to destroy fungi and aflatoxins in maize.

The results of the study addressed all these research questions and gave a much- needed insight on all these critical issues. Data collected during the qualitative study on the knowledge, attitude and practices of maize farmers in Makueni and Baringo counties on aflatoxin contamination supported that the three factors played a key role. Socio-economic and demographic factors were linear predictors of knowledge ($R2=0.76$, $P<0.001$). Farmers from Baringo county were more likely (OR=1.24) to have higher knowledge scores on aflatoxin contamination than their counterparts from Makueni county. However, as age of the maize farmers increased, they became less likely (OR=0.01) to have higher scores of knowledge leading to aflatoxin contamination. A significant difference ($P<0.05$) was detected in the knowledge of factors contributing to aflatoxin contamination of maize. These factors were on the point where contamination began, signs of contamination and the repercussions of exposure to aflatoxin. Makueni had a higher number of farmers who scored higher in terms of

knowledge on these factors compared to Baringo County. However, overall, Baringo County had higher knowledge scores than Makueni County. Despite farmers being aware of the associated dangers of exposure to aflatoxins, they still consumed contaminated grain and even fed their livestock the same. Majority of farmers adopted good agricultural practices such as proper drying of maize before storage, storage on raised surfaces and even use of hermetic storage structures. Maize samples from Makueni County had higher levels of aflatoxin B1 and B2 whilst those from Baringo County had higher levels of aflatoxin G1 and G2. Overall, hermetic storage and particularly storage in metallic bins offered the best protection from contamination as opposed to gunny bags which had the highest aflatoxin contamination levels. The data collected from the microbial analysis showed high levels of contamination in the different storage conditions. Strains of *Aspergillus flavus, Aspergillus terreus* and *Aspergillus parasitucus* were positively identified. The variation between the different storage structures was not significant ($P<0.39$). However, the average microbial load was highest in gunny bags at 159.9 CFU/g and 202.5 CFU/g for Makueni and Baringo counties respectively. On the other hand, hermetic storage and particularly pics bags had the lowest microbial loads at 26.5 CFU/g and 35.1 CFU/g for Makueni and Baringo counties respectively. Plasma experimentation supports that LTP has efficacy to reduce aflatoxin and microbial load in maize. A percent reduction of 68.78% was achieved for aflatoxin and 33.89 log (CFU/g) for microbial load. The optimised processing conditions obtained by the Response Surface Methodology were an exposure time of 153 seconds, pressure of 0.98 Pascals and ionization power of 194.82W. Any increase in both exposure time and pressure resulted in a corresponding decrease in the response variables. Change in the ionization power did not have a significant effect on the response variables.

The results obtained in this study correlate with results of other previous studies. For instance, the higher knowledge scores in Baringo could be explained by higher presence of support

networks in the County as compared to Makueni County. Baringo is home to many seed companies who employ a lot of extension services in educating the farmers on good agricultural practices (Authority, 2021). Perkerra irrigation scheme found in Baringo south near Marigat township is one of the largest irrigation schemes in the region producing over 270,000 kg of maize seed per year. Other irrigation schemes include: Eldume, Sandai, Kamoskoi, Kapkuikui and Mosuro. The higher attitude scores in Makueni county are associated with higher cases of aflatoxin poisoning experienced in the county (Daniel et al., 2011; IFPRI, 2020; Mwihia et al., 2008). Higher levels of aflatoxin B1 and B2 were observed in maize samples from Makueni County. Those from Baringo County, on the hand, had higher levels of aflatoxin G1 and G2. This can be explained by the strain variation between the two geographical locations. Despite the fact that these toxins are produced by *Aspergilli* species, the strains that predominantly produce these specific toxins are different (Monda et al., 2020). Hermetic storage was found to have lower levels of contamination as opposed to other forms of storage the farmers practiced. In particular, metallic bins provided the best of protection to the maize from aflatoxin contamination. Proper drying of the maize was also practised along with storage under hermetic conditions. Aspergilli species which predominantly produce the aflatoxins do not thrive when denied oxygen and thus this could possibly explain the variation in the contamination levels between the different storage types (Ng'ang'a et al., 2016; Pretari et al., 2019).

This is further reinforced by the results of the microbial analysis where three strains of *Aspergillus flavus, Aspergillus terreus* and *Aspergillus parasitucus* were positively identified. Samples from Makueni County had distinctly high levels of *Aspergillus* flavus which produces more of the B toxins (Monda et al., 2020). Similar to the results of the aflatoxin analysis, hermetic storage also had lower microbial loads which correlated well with the aflatoxin levels deduced. In the RSM experiment, pressure and exposure time were significant factors. The increase in exposure time was characteristic in reducing the microbial load and aflatoxin

content. This can be attributed to the fact that the longer the treatment time, the more effective the treatment, which also is based on the optimised combination of the independent factors.

Some of the limitations of the study are in the plasma experimentation; temperature was not included as an independent factor. This could perhaps help in giving more precise optimisation factors as well as optimising the decontamination studies. Additionally, due to funding limitations, the sample size was also limited. Future studies could encompass a larger study on the aflatoxin levels in the different storage structures and compare with the results of this study.

7.2 Conclusions

The knowledge, attitude and practices of farmers have an effect on aflatoxin contamination in maize. Low knowledge and attitude scores subsequently result in poor practices and hence aflatoxin contamination occurs. Secondly, proper drying and storage of maize grain under hermetic conditions offers the best protection against aflatoxin contamination. If stored under poor conditions, contamination will result. Aflatoxigenic fungi are present in maize samples from Makueni and Baringo counties are associated with aflatoxin contamination in these regions. Finally, plasma has efficacy to decontaminate aflatoxin and fungi in maize.

7.3 Recommendations

The recommendations of the study are that there needs to be a more enhanced outreach programs to educate farmers on dangers of exposure to aflatoxins and the need to employ preventive measures at the production and postharvest levels. Currently, these programs are taking place through government projects and non-governmental organizations but the message has not been hammered enough. Surveillance by the respective regulatory agencies is also very key to protect consumers. Aflatoxin testing is also very expensive and the government should come up with ways of bringing this cost down. This may include introducing a zero-taxation

policy on aflatoxin testing reagents, kits and equipment, not taxing the testing laboratories and so forth. For optimal prevention from aflatoxin contamination, proper drying of maize and subsequent storage under hermetic conditions should be encouraged. The safe disposal of contaminated grain by burning and burying should be promoted as most farmers still feed it to livestock leading to health risks to consumers. Plasma has efficacy to decontaminate aflatoxins and fungi in maize. Further piloting is necessary in order to build on this technology. As for opportunities for future research, temperature should be incorporated in the RSM modelling in optimising the cold plasma decontamination process. Lastly, the wholesomeness of the maize for human consumption after treatment should be guaranteed.

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APPENDIX I: CONSENT FORM

EFFICACY OF PLASMA TECHNOLOGY IN ELIMINATING FUNGI AND AFLATOXINS IN MAIZE IN MAKUENI AND BARINGO COUNTIES, KENYA

I_____ agree to participate in the research project titled '**Efficacy Of Plasma Technology In Eliminating Fungi And Aflatoxins In Maize In Makueni And Baringo Counties, Kenya'** conducted by team led by Ms. Hannah Kamano who has (have) discussed the research project with me.

I have had the opportunity to ask questions about this research and I have received satisfactory answers. I understand the general purposes, risks and methods of this research.

I consent to participate in the research project and the following has been explained to me:

- my participation is completely voluntary
- whom I should contact for any complaints with the research or the conduct of the research
- my participation is completely voluntary
- that my safety is guaranteed
- I am able to request a copy of the research findings and reports
- Security and confidentiality of my personal information
- Publication of results from this study on the condition that my identity will not be revealed.

Name: ____________________________

Signature: _________________________

Date: _____________________________

APPENDIX II: QUESTIONNAIRE

KNOWLEDGE, ATTITUDE AND PRACTICES SURVEY OF COMMUNITIES IN MAKUENI AND BARINGO COUNTIES, 2020

Study county …….

Season ……

A) Demographics and land ownership

Name of interviewer _____

Date of interview _____

Respondent's identification no. _____

QN S/No	1.Gender -codes-	2.Age (years) (optional)	3.Marital Status -codes-	4.Education -codes-	5.Source of Income -codes-	6.Land ownership -codes-
Gender	**7.Family size**	**8.Farm size**	**Marital status**	**Education**	**Source of income**	**Land ownership**
1=Male 2=Female			1=Married 2=Separated 3=Widowed 4=Single 5=Divorced 6=N/A	1=College/University 2=Completed secondary 3=Dropped from secondary 3=Completed primary 4=Dropped from primary	1= Farming 2= Business 3= Other (specify)	1= Freehold title (purchased) 2= Freehold title (Inherited) 3= Leased 4= Rented 5= Community land

				5=In primary 6=In secondary 7=Adult education 8=Illiterate 9=N/A (Pre-school)		6= Other *(Specify)*

B) Household location and GIS positioning

9.County	**10.Sub-county**	**11.Ward**	**12.Village**	**13.Latitude**	**14.Longitude**	**15.Elevation**
Makueni						
	1.Makueni 2. Mbooni	1.Wote 2.Kisau Kiteta 3.Kako Woiya				
County	**Sub-county**	**Ward**	**Village**	**Longitude**	**Latitude**	**Elevation**
Baringo						
	1.Eldama ravine 2. Baringo South 3.Mogotio	1. Marigat 2. Mochongoi 3. Eldama ravine 4. Koibatek 5.Kiserian 6. Eldama Ravine 7.Lembus Perkerra 8. Eldume				

C) Property ownership

16.Assets owned	17.Units	18.Est. (KES)	19.Farming Tools	20.Units	21.Est. (KES)	22.Housing (Floor/Wall/Roof)	23.Est. (KES)
Motor vehicle (commercial)			Trailer (Tractor)			Earth/Mud/ Thatch	
Motor vehicle (Private)			Harrow (Tractor)			Earth/Mud/ Iron sheets	
Tuk tuk			Plough (Tractor)			Cemented/Iron sheets/Iron sheets	
Bicycle			Trailer (bull/donkey)			Cemented /Timber/ Iron sheets	
Radio			Harrow (bull/donkey)			Cemented/Stone/ Thatch	
TV			Plough (bull/donkey)			Cemented/Stone/ Iron sheets	
Mobile phone			Wheel barrow			Cemented/Stone/ Tiles	
Fixed phone			Other (Specify)			Other (Specify)	
Generator							
Well (Private)							
Water pump							
Bore hole							
Water tanks							
Livestock							
Other (Specify)							

D) Knowledge Assessment

24. What are aflatoxins?	25.Where are they found	26. What causes aflatoxins in maize?	27. Aflatoxin exposure in humans leads to:
1 = Toxins in maize 2= Do not know	1 = Maize alone 2= others, please specify	1= Poorly dried or wet maize 2= Poor storage of maize 3= Drying maize on the ground 4= Shelling wet maize 5= Others (Specify)	1= Stunting in children 2= Immunity suppression 3= Low productivity in livestock 4= Liver cirrhosis (Liver cancer) 5= Loss of income 6= Death 7 = others (specify)

28.At which point does contamination begin	29. How do you tell that maize has been affected by aflatoxins?	30.Stability of the mould toxins (Strongly agree, Agree, Neither agree or disagree, Disagree, Strongly disagree)	31.How do you receive information on aflatoxins
1= The field when growing 2= During harvest 3= After harvest 4= In-storage 5= Improper drying 6= Not grading maize 7= Wet storage conditions	1= Discolouration 2=Mouldiness and wetness 3=Presence of insects 4= Mouldy smell 5= Other, please specify	1= Normal cooking destroys aflatoxin 2= use of alkaline solutions destroys aflatoxin 3= High temperature causes increase in toxin 4= High humidity causes build-up of toxin	1= Radio 2= Television 3= Newspapers 4=Extension workers 5= Internet 6=Other(s) Please specify

Statement. Please state (Strongly Agree, Agree, Neither agree or disagree, Disagree, Strongly disagree)	
32. Does aflatoxin contamination lead to income loss?	
33. Should aflatoxin contaminated grain be fed to livestock?	
34. Aflatoxin contaminated feed reduces livestock productivity?	
35. Consumption of aflatoxin contaminated grain causes stunting	
36. Aflatoxin contaminated maize will be rejected in the market	

E) Attitude Assessment

37. How important is it to prevent aflatoxin occurrence in maize?	**38. How important is it to dry maize properly before storage?**	**39. How serious is it to consume maize contaminated with aflatoxins?**	**40. What state do you think your maize grain is in, in your store?**	**41. Do you think consumers would be willing to pay more for aflatoxin-free maize?**
1= Not important 2= Important 3= Not sure Please explain	1= Not important 2= Important 3= Not sure If not important, why?	1= Not really serious 2= Moderately serious 3= Serious If serious, please give your reasons why	1= Not good 2= You're not sure 3= Good If not good, please state the reason	1= Yes 2= No If yes or no, please explain

42.Why do you feed on/sell or feed livestock with grade outs	43. Why do you not dry your maize on a mat/ tarpaulin/ canvas?	44. Why do you not adequately dry your maize before storage?

F) Practices Assessment

45.Mode of Harvesting	46.Mode of Handling	47.Drying	48.Shelling	49.Storage
1=Hand 2=Machine 3=Both	1= Maize stovers stacked in heaps 2= Maize cob removed while stovers standing	1= on ground with canvas 2= on ground without canvas 3= on ground on cob 4= left to dry in field	1= by hand 2= Use of machine 3= pounding manually in gunny bags	1= Gunny bags 2= Pics bags 3= Granary/Thatch 4= Granary/ Iron sheets 5= Air tight bins 6= Hermetic storage

50.Mode of preservation	**51.Amount of maize grain consumed/week (kg)**	**52.Yields (Bags/ Kg)**	**53.Spoilt (Bags/ Kg)**	**54. What do you do to prevent occurrence of aflatoxins in maize?**	**55.Disposal of grade outs (Bags/ Kg)**
1= insecticides (Actellic) 2= Ash 3= None				1= Drying maize properly 2= Storing it in hermetic conditions 3= Storing in well aerated store 4= Proper sorting before storage 5= Changing eating habits 6 = Other, specify	1= Throw away 2= Feed to livestock 3= Consume in different forms 4= Sell in markets 5= Seed 6= Destroy 7= Give away

Which of these do you practice? *(please tick appropriately)*		**Seeking solutions (Strongly Agree, Agree, Neither agree or disagree, disagree, Strongly disagree)**	
56.Store bags on floor		63. Are you willing to pay for services that will improve postharvest handling?	
57.Dry produce on tarpaulin sheet/mat		64. Would you be willing to take up a new technology to stop the development of aflatoxin in maize?	
58.Dry produce on bare soil/roof		65. What other crops would you grow to supplement maize which doesn't get infected by aflatoxins?	
59.Grade grains			
60.Grade grains to improve quality			
61.Grade grains to separate them based on colour and size			
62.Store bags on wooden palates			