

# UNIVERSITY OF NAIROBI

# DEPARTMENT OF CHEMISTRY

# CHEMICAL DEGRADATION OF AFLATOXIN CONTAMINANTS IN MAIZE AS A VALUE ADDITION STRATEGY FOR SELECTED COUNTIES IN KENYA

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# A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY OF THE UNIVERSITY OF NAIROBI

December, 2023

#### DECLARATION

I declare that this thesis is my original work and it has not been submitted anywhere for examination, award of a degree or publication. Where other people or my own work is use has properly been acknowledged in accordance with the University of Nairobi's requirement

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#### DEDICATION

I dedicate this work to my loving family, Dr. Peninah K. Mwenda, Eng. Victor Kimathi and Dr. Neema Kinya, for their continuous love, encouragement and support they offered in the process of this study. In addition, I am grateful to my parents, the late Jacob Kinoti and Janet Mbothu, for their sacrifice and motivation to acquire education. Finally, my utmost gratitude goes to the Almighty God for He has enabled me to reach this far in the academic ladder.

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#### ABSTRACT

Aflatoxin contamination of maize, rice, groundnuts and wheat products is a common phenomenon in Kenya. This result to low nutritional value and huge economic loss of the crops produced. Additionally, consumption of aflatoxin contaminated cereals and nuts above 10 µg/kg can lead to chronic health effects in humans, hence the products are usually destroyed. This study sought to assess aflatoxin prevalence in Busia, Migori, Trans-Nzoia, Nakuru, Nairobi, Kajiado, Machakos, Makueni, Embu and Isiolo counties in Kenya, determine the socio-economic impact and value addition to aflatoxin contaminated maize by use of different chemicals. Structured questionnaires were used to assess the prevalence and socio-economic impact caused by aflatoxin contamination of maize handlers along the value chain from different regions. Aflatoxin B1, B2, G1 and G2 residues in the samples were extracted using 22.7 % methanol and screened by enzyme-linked immunosorbent assay. Confirmatory and characterization tests were done using high performance liquid chromatography equipped with a fluorescence detector. Degradation potential of aflatoxin in maize was conducted using sodium hydrogen sulfite, ferulic acid, ammonium carbonate, sodium hydrogen carbonate and sodium hypochlorite on whole, dehulled and ground maize, catalyzed by hydrogen peroxide, ammonia and methylamine. Ninety-four (94) percent of the maize samples were contaminated with aflatoxins, of which 59.6 % had levels above the East Africa tolerable limit of 10 µg/kg. The mean concentration of aflatoxins ranges was 10.54±1.52 to 50.08±4.42 µg/kg in eastern, 2.65±0.32 to 9.36±0.97 µg/kg in western, 1.90±0.11 to 8.74±0.54 µg/kg in Nairobi, and  $0.95\pm0.05$  to  $4.37\pm0.27$  µg/kg in Rift valley. The Spearman rank correlation test for aflatoxins B1, B2, G1 and G2 showed a strong correlation > 0.75, while the Scheirer Ray Hare test showed statistically significant levels of the four aflatoxins at p < 0.05. Sodium hypochlorite achieved the highest degradation of aflatoxins on whole, de-hulled and ground maize samples ranging from 88.2 to 98.1 %. Ethanol yield from aflatoxin stripped maize was 34.7 % while contaminated maize registered 29.2 % yield. Variances in aflatoxin contamination load across the regions can be attributed to differences in microclimates, handling, environment and maize seeds factors. The results suggest high prevalence of aflatoxin contamination in maize from the 11 counties, which need to be managed to reduce the human health risks. The maize products decontaminated of aflatoxin formed useful raw material for industrial ethanol and briquettes production. There is a need for stakeholders to prioritize cereal crop irrigation, fertilizer management, plant inspection, and pest and disease control to reduce phyto-immunity stresses on maize and post-harvest losses associated with aflatoxin contamination.

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### **ABBREVIATIONS**

AEZ	Agro-ecological zones
AF	Aflatoxins
AIC	Akaike information criterion
AOAC	Association of Official Analytical Chemists
BDL	Below detection limits
BHA	Butylated hydroxyanisole
BIC	Bayesian information criterion
CAC	Codex Alimentarius Commission
CAST	Council for Agricultural Science and Technology
CDC	Centers for Disease Control and Prevention
CGIAR	Consultative Group on International Agricultural Research
CIDP	County Integrated Development Plan
CIMMYT	International Maize and Wheat Improvement Center
CODEX	General Standard for Contaminants and Toxins in Food and Feed
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
ELISA	Enzyme-linked immunosorbent assay
EAC	East African Community
EU	European Union
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FUM	Fumonisins
HPLC	High Performance Liquid Chromatography
HQ	Head quarter

IARC	International Agency for Research into Cancer		
IFPRI	International Food Policy Research Institute		
JECFA	Joint FAO/WHO Expert Committee on Food Additives		
KBS	Kenya Bureau of Standards		
KEPHIS	Kenya Plant Health Inspectorate Services		
LOD	Limit of Detection		
LOQ	Limit of Quantitation		
LSD	Least Significant Difference		
MOA	Ministry of Agriculture		
ΜΥΤΟΧ	Mycotoxins and Toxigenic Molds		
NCPB	National Cereals and Produce Board		
NIV	Nivalenol		
OTA	Ochratoxins		
PBS	Phosphate Buffered Saline		
PMTDI	provisional maximum tolerable daily intake		
RMSE	Root Mean Square Error		
SPSS	Statistical Package for the Social Scientists		
TCA	Tricarboxylic acid		
µg/kg	Microgram per kilogram		
USD	United States of America Dollar		
WHO	World Health Organization		
ZON	Zearalenone		

#### **CHAPTER ONE**

#### **1.0 INTRODUCTION**

#### **1.1.1 Background Information**

Mycotoxins affect quality of many agricultural crops, depreciating market prices leading to economic losses and increased wastes of crops globally. According to WHO, about 1.3 billion tons (25 %) of agricultural products are lost annually due to mycotoxin contamination (WHO, 2018). Sub-Saharan Africa countries are the most affected because of recurring agricultural waste in terms of food and feed losses due to fungal contamination (Udomkun *et al.*, 2017a).

Mycotoxins are a group of natural contaminants produced by toxigenic strains of fungi in agriculturally based foods for humans and animals (Ukwuru *et al.*, 2017; Darwish *et al.*, 2014). These are secondary metabolites mainly produced for self-protection and environmental adaptation with a serious health risk when consumed by humans and animals (Streit *et al.*, 2013; Zain, 2011). These are usually soil and air borne toxins, but may also occur in animal products directly metabolized from feeds.

Mycotoxins differ in terms of chemical structure, properties and bioactivities. The basic structure consists of organic non-protein low molecular weight compounds formed primarily from acetyl coenzyme A, shikimic acids and amino acids (Milosevic *et al.*, 2015). Some mycotoxins have therapeutic benefits such as penicillin and lovastatin, while others have significant human toxicities such as aflatoxins and fumonisins (Cary *et al.*, 2018; Nesic *et al.*, 2014).

Common mycotoxins are produced by aspergillus, penicillium, and fusarium genera, which grow naturally in agricultural crops. The aspergillus and penicillium species mainly grow on foods and feeds under poor storage conditions, while the fusarium species often grow and infect field crops such as wheat, barley and maize. Proliferation of molds or spores to the crops occur at different stages from field to store, in store, and in processing (Peng *et al.*, 2018b; Alshannaq & Yu, 2017; Kimanya *et al.*, 2008).

The types of mycotoxins produced are named after their parent fungal species, and are classified according to toxicity levels they produce. Common classes of mycotoxins are aflatoxins, fumonisins, citrinins, sterigmatocystins, luteoskyrins, patulins, penicillic acids, ochratoxins, trichothecenes, zearalenones, tremorgenic toxins, deoxynivalenols (DON or vomitoxin) and ergot alkaloids (Zain, 2011). Aflatoxins are the most potent and carcinogenic mycotoxins to humans and animals. They are produced by the aflatoxigenic fungis, namely *Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius* (Samson *et al.*, 2014; Khayoon *et al.*, 2010).

These kinds of fungi are widely found in soil and air, where they frequently contaminate agricultural crops at pre and post-harvest handling stages. Generally, mycotoxins contaminate field crops before and after harvesting, and during storage. The most affected crops include cereal grains, nuts, oilseeds, fruits, vegetables, tea, beverage beans, wine, beer, herbs and spices (Puttaswamy & Raveesha, 2016; Farkas & Mohácsi-Farkas, 2014).

The main effect of the mycotoxins contamination in cereals is compromised germination and physiochemical qualities affecting their nutritional content and food safety standards (Kumar *et al.*, 2017). In humans and animals, the effects include acute intoxication, reduced productivity, immunosuppression, genotoxic, nephrotoxic, hepatotoxic, teratogenic, cytotoxic, carcinogenic, and mutagenic, posing a considerable risk to health (Ramírez *et al.*, 2016).

The extent of toxicity depends on the dosage, exposure period, physiological conditions, nutritional status and synergistic effect of other chemical compounds in the body. Acute toxicity may result from consuming 1 mg/kg or more or 20-120  $\mu$ g/kg body weight per day for 1-3 weeks (WHO, 2018). On the other hand, chronic toxicity may result from exposure to low-doses over a long period leading to different organ related ailments (Tsedaley *et al.*, 2016).

When livestock feed on the contaminated agricultural feeds, their health and productivity is affected negatively, disrupting livelihoods and household food security and safety (Enyiukwu *et al.*, 2014; FAO, 2011). Furthermore, mycotoxin residues can also be carried-over from feeds to animal products such as meat, eggs and milk thus increasing health risk for the consumer.

Consumption of mycotoxins such as aflatoxin in contaminated food or inhalation contaminated air or absorption through the skin may lead to bioaccumulation and bio-magnification of the toxins in the food chain (Unnevehr *et al.*, 2013; Cast, 2003). Besides, they may affect DNA, disrupt genetic coding or promote carcinogenesis (Wu, 2013; Wild & Gong, 2010). They cause human diseases such as liver cancer, Reye's Syndrome, Indian childhood cirrhosis, chronic gastritis, hepatocellular carcinoma related ailments and deaths, high prevalence of hepatitis B, and esophageal cancers, kwashiorkor and other occupational respiratory ailments (Darkish *et al.*, 2014; Ferly *et al.*, 2013 Amanda *et al.*, 2013; Kimanya *et al.*, 2012; Zain, 2011).

#### 1.1.2 Global and Regional Outlook on Aflatoxins

Maize (*Zea mays L.*), was domesticated as an agricultural crop more than 5000 years ago (Matsuoka *et al.*, 2002). Maize contains, on average, 72 % starch, 10 % protein, 4 % fat and provides 365 Kcal/100g energy, vitamin Bs, essential minerals and fiber (Nuss &

Tanumihardjo, 2010). These make maize an attractive source of food, beverages, commercial starch, fuels, animal feeds and raw material for industrial processes (Peng *et al.*, 2018 a)

The global maize production amounts to an annual average of 1,127 million tons (2016–18, OECD/FAO, 2019). Maize grows in a wide agro-ecological zone with diverse temperatures, altitudes and latitudes, land and soil types—though with quite different yields per hectare (Grote *et al.*, 2021). The major maize producers are North America, followed by Asia (Noldin *et al.*, 2016). According to FAO (2021), United States of America topped the list of maize producers in the world in 2020, with 360,252 thousand metric tons covering 33.66 %. China, Brazil, Argentina and Ukraine produced 75.38 % of the balance, while 24.62 % was produced by other countries.

In Sub-Saharan Africa, 34 million hectares were under maize cultivation producing 70 million tons of the yield. The estimated yield was 2 tons per hectare, a low maize yield that may be caused by climate change, agronomic practices and heavy post-harvest losses (Koskei *et al.*, 2020; Ranum *et al.*, 2014). The sub-Saharan Africa economy thrive largely from smallholder agriculture and agribusinesses activities with product like maize, wheat, groundnuts and their value chains.

In Sub-Saharan Africa, between 20–30 % maize harvest alone, estimated at US\$4 billion annually is lost or goes to waste. The losses stated are associated with poor postharvest practices, informal marketing systems, unfavorable physical and environmental factors, insect pests and fungal attacks on maize (Koskei *et al.*, 2020). This implies that regular aflatoxin contamination exposes the continent to different challenges including loss of opportunities to access lucrative export and local markets for their agricultural crops and income (Daou *et al.*, 2021; Pulina *et al.*, 2014; Monyo *et al.*, 2012).

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#### 1.1.3 Aflatoxin Situation Analysis in Kenya

Kenya produces about 4000 thousand metric tons of maize from 1.5 million hectares of land (FAO, 2021), accounting for 20 % of arable land and total agricultural production, and provides 25 % of employment (Schroeder *et al.*, 2013; FAO, 2008). Central, Coastal, Eastern, Nyanza, Rift Valley and Western Kenya covers 56 % of land is suitable climatic conditions for maize production (Kirimi *et al.*, 2011). Maize is a key indicator of food security and safety, a basic staple food in Kenya, accounting for 65 % of caloric intake and 36 % of total food caloric intake (Haradhan, 2014). The per capita annual consumption of maize is between 88 -103 kgs and 400 kg per household (Abate *et al.*, 2015; D'Alessandro *et al.*, 2015; Short *et al.*, 2012).

There are insufficient uniform storage methods adapted by the country for harvested maize, but these vary with individual farmer or trader, except for the National and Cereal Produce Board (NCPB) that stores in silos ran by the government. Inadequate uniformity in storage facilities and conditions as well as handling techniques, lead to fungal contamination in some of stored produce in these facilities (Manubolu *et al.*, 2018).

The high consumption rate of maize, estimated at 400 g/person/day with an average total aflatoxin load of  $0.132 \mu g/kg$  (ACDI/VOCA, 2015; Lewis *et al.*, 2005), in Kenya increases chances of exposure to aflatoxin contamination. Past studies tracing back to 1973, reported high incidence of aflatoxin contamination in 93 % of the maize meals and local brew collected from households in Murang'a (Peers and Linsell, 1977). Further, independent studies conducted on a variety of foodstuff collected from other part of Kenya have consistently reported positive aflatoxin contamination cases (Keter *et al.*, 2017; Sirma *et al.*, 2016; Menza *et al.*, 2015; Mutiga *et al.*, 2015, 2014; Daniel *et al.*, 2011; Mwihia *et al.*, 2008; Lewis *et al.*, 2005). The studies in Migori, Busia, Nakuru, and Muranga showed high level of aflatoxin

contamination in maize, peanuts and animal feeds that was above the set limits (KEBS). In 2004, the Center for Disease Control and protection (CDC) reported 317 illness cases linked to aflatoxin contamination in food from Makueni, Kitui, Machakos, Embu and Thika counties (CDC, 2004). According to Center for Disease Control and protection (CDC, 2005), laboratory analysis reports for maize samples collected from the 5 counties had 880 times greater than the set limit of 5-µg/kg aflatoxin B1 contamination level. Other studies have linked malnutrition among children to aflatoxins exposure (Stepman, 2018; Kang'ethe *et al.*, 2017; Kiarie *et al.*, 2016; Malusha *et al.*, 2015; Leroy *et al.*, 2015; El-Tras *et al.*, 2011).

#### **1.1.4 Global Concerns Related to Aflatoxins**

Aflatoxins are carcinogens, particularly associated with liver cancer. Chronic exposure to even low levels of aflatoxins through contaminated food can lead to an increased risk of developing liver cancer. In addition to long-term health risks, high doses of aflatoxins in the short term can cause acute toxicity, leading to symptoms such as nausea, vomiting, abdominal pain, and in severe cases, acute liver failure. Aflatoxin contamination is a significant concern in staple crops, particularly in developing countries where food safety regulations and infrastructure may be insufficient. Contaminated crops can lead to both food safety and food security issues. Aflatoxin contamination can result in the rejection of food exports by importing countries due to safety concerns. This can lead to economic losses for countries heavily dependent on agriculture and food exports. Farmers may experience significant losses due to aflatoxin contamination, affecting their livelihoods and contributing to economic instability. Ensuring compliance with aflatoxin regulations requires robust monitoring systems and effective enforcement mechanisms. Some regions may face challenges in implementing and enforcing regulatory measures to control aflatoxin levels in food. Climate change can influence the prevalence of aflatoxin-producing fungi. Changes in temperature and precipitation patterns 

may influence crop susceptibility to contamination, potentially increasing the risk of aflatoxin presence in food.

Aflatoxin-contaminated crops are used as feed for livestock, leading to the accumulation of toxins in animal products such as milk and meat. This poses risks to both animal health and the safety of the human food supply chain. Increasing public awareness about aflatoxins is crucial for promoting safe food practices. Educating consumers about proper storage, handling, and processing of food can help reduce the risk of aflatoxin exposure.

Efforts to address these concerns include the development and implementation of good agricultural practices, improved storage and processing methods, stringent food safety regulations, and international collaboration to mitigate the impact of aflatoxins on human and animal health.

#### **1.1.5 Problem Statement**

Aflatoxin contamination causes significant loss for farmers, business men, marketers and consumers of varied agricultural crops in Asian, American, Australian, European and African countries (Keeble *et al.*, 2020; Massimo, 2020; Nongoma, 2013). Globally, scientists have not developed an effective and affordable mycotoxin detoxification method(s) (Chauhan *et al.*, 2016).

Most developing countries do not have national budgets to support regulatory mechanisms and enforcement measures for food quality standards (Wild *et al.*, 2015; Njobeh *et al.*, 2017; Chauhan *et al.*, 2016; Bryden, 2012), unlike in developed countries (Udomkun *et al.*, 2017b; Karlovsky *et al.*, 2016; Pulina *et al.*, 2014; Cheli *et al.*, 2013). In East and West African countries, 90 to 99 % of maize grown and consumed has high probability of being aflatoxin contaminated (Chauhan *et al.*, 2016; Umereweneza *et al.*, 2018).

According to Phyllis (2020), the government of Kenya destroyed 107,773 metric tons of contaminated maize and maize flour in 2018 and 2019 that had high level of aflatoxin contamination above  $10 \mu g/kg$  or decolorized. On this incident, the government lost more than 30 million dollars. Kenya recurrent aflatoxicosis episodes with fatalities were reported in central, eastern and western Kenya (Obonyo and Salano, 2018). To date underlying causative factors to the aflatoxicosis have not been adequately delineated and controlled mechanisms instituted.

Economic recovery of aflatoxins contaminated maize to industrial grade materials has attracted research in the recent past (Jalal *et al.*, 2011). The hot and humid environmental conditions experienced in the country promote preferential aflatoxin growth and proliferation in certain agro-ecological zones which results in huge economic losses and threaten food security and safety. However, majority of the Kenyan population have limited information for effective management of mycotoxins (Gash *et al.*, 2019). The situation is aggravated by high poverty levels among the farming communities driving them into consumption of aflatoxins contaminated maize.

Furthermore, processed and packaged food products in supermarket shelves, shops and local food stores are occasionally condemned and destroyed because of aflatoxin contamination by leading loss of billions of Kenya shillings. Complete elimination of aflatoxin contaminants in food products remains a challenge (Marechera *et al.*, 2015; Enyiukwu *et al.*, 2014).

#### 1.2.1 Main Objective

To assess socio-economic impact and prevalence of aflatoxin contamination of maize and design a low-cost industrial process for value addition.

#### **1.2.2 Specific Objectives**

- To assess social-economic impacts of aflatoxin contamination of maize in selected counties of Kenya
- 2. To assess aflatoxin contamination prevalence in maize in selected counties in Kenya.
- 3. To establish chemical process for degrading aflatoxin in contaminated maize.
- 4. To design a low-cost industrial process for utilizing aflatoxin decontaminated maize.

#### **1.3 Research Hypothesis**

Chemical reagents can effectively denature aflatoxin molecules in contaminated maize and reduce the level of aflatoxin contaminants in maize to acceptable levels. The detoxified maize can be a raw material for manufacture of glue, briquettes, industrial alcohol (ethanol), amongst other industrial products.

#### **1.4 Study Justification**

Mycotoxins contamination on food materials is a global problem, particularly aflatoxins, which impact negatively on health and economy especially in tropical and sub-tropical countries. The Figure 1.1 below depicts different types of mycotoxins.



#### Figure 1.1: Global Mycotoxins Overview (Source Taschl, 2018)

Africa food security and safety are a common challenge, occasioned by mycotoxin contaminations of agricultural crops, poverty, diseases, climate change and political turmoil. The problem is aggravated in most African countries by not institutionalizing food safety standards and guidelines, nor allocating adequate resources for conducting research and developing solutions to this challenge.

Many Kenyans have limited information on the consequences of consuming aflatoxincontaminated food because systems for relaying such information are not fully functional. The country has commercial and government laboratories to offer quality assurance services for food destined for export, but inadequate for locally consumed foodstuff.

Furthermore, aflatoxin contamination of agricultural products imposes an economic barrier to market. Socio-economic stress to local farmers can be mitigated by science and technology interventions such as chemical degradation of aflatoxins in maize. In 2014, Kenya faced challenges in terms of collection, transportation and safe destruction of approximately 13,992 metric tons of aflatoxins contaminated maize (EAC policy paper, 2016). There was neither a clear policy direction or legal provisions on approved alternative uses of aflatoxin contaminated produce/ approved disposal methods. This study assessed socio-economic, prevalence and chemical degradation of aflatoxins contaminated maize, and designed a low cost industrial process for utilization of degraded aflatoxin contaminated maize.

#### **CHAPTER TWO**

#### 2.0 LITERATURE REVIEW

#### 2.1 Overview of Mycotoxins

Mycotoxins contamination on food materials is a global problem, particularly aflatoxins, which impact negatively on health and economy especially in tropical and sub-tropical countries. The Figure 2.1 below depicts different types of mycotoxins



Figure 2.1: Global Mycotoxins Overview (Source Taschl, 2018)

Mycotoxins are natural chemical compounds of low molecular weight, produced by filamentous fungi as secondary metabolites. They are a group of toxigenic and heterogeneous compounds whose members have a potential of causing numerous acute and chronic fungal diseases. Among the toxic activities under chronic conditions are carcinogenicity, mutagenicity, teratogenicity, estrogenicity, nephrotoxicity, hepatotoxicity and immunosuppressive in humans and animals (Leroy, 2013; Wu, 2013). Moreover, they are

carcinogenic and/or toxic to higher vertebrates even in low doses, depending on the strain and target condition (Alshannaq & Yu, 2017; Huong *et al.*, 2016).

Many of these chemical compounds target the liver, kidneys, lungs, digestive tract including the colon (Omotayo *et al.*, 2019; Marin *et al.*, 2013). They compromise the immune system, cause stunting growth, reduced productivity and feeding efficiency for the target (Zain, 2011). There are more than 100,000 known species of fungi but a few like *Aspergillus spp.*, *Penicillium spp.*, *Fusarium spp. Cladosporium spp.*, and *Alternaria spp.* are responsible for mycotoxin production of toxigenic importance to humans and animals (Freire & Rocha, 2017).

Mycotoxin produce trichothecenes (Deoxynivalenol (DON) and nivalenol (NIV)), ochratoxins (OTA), aflatoxins (AF), zearalenone (ZON), fumonisins (FUM), patulin, and citrine (Agriopoulou *et al.*, 2020; Taschl, 2018; Abdallah *et al.*, 2015). *Penicillium* fungal species produce antibiotics toxic to bacteria and phytoxins for plants (Alshannaq & Yu, 2017; Hymery *et al.*, 2014). Favorable conditions for mycotoxin production are high temperatures, moisture content and water activity (Tola & Keeble, 2016). Poor hygienic practices during harvest, transportation and storage of agricultural produce also lead mycotoxin production. Cereals and cereal products, fruits and fruit products, legumes, nuts, tea, coffee and cocoa are easily contaminated with mycotoxins naturally from the environments (Marin *et al.*, 2013) hence expose consume to the same. Inhaling toxin contaminated air and dust also exposure human to the contaminations.

Negative social-economic impacts from mycotoxin contamination vary with geographical locality and seasons (Table 2.1). The major social-economic impacts include treatment costs, loss of agricultural produce and low competitiveness of products from presumed mycotoxin prone zones (Rosburg and Menapace, 2018; Kumar *et al.*, 2017; Pereira *et al.*, 2014; Streit *et* **12** | P a g e

*al.*, 2012; Boevre *et al.*, 2012). According to Darkish *et al.*, (2014), mycotoxin incidences and contaminations in agricultural crops and foodstuffs in different African countries exceed the 10  $\mu$ g/kg levels, the set limit for total aflatoxin in most these countries.

Good agricultural practices do not prevent contamination of agricultural products pre-, during and post- harvest processing of products stages (Streit *et al.*, 2012). Contaminated grains in stores usually have reduced germination ability, lower starch and sugar contents, deteriorated qualities and increased fatty acid contents (Marin *et al.*, 2013).

Country	Mycotoxin	Health Problem	Reference
Egypt	Aflatoxins	Primary hepatocellular carcinoma	Ezzat et al., 2021
Ghana	Aflatoxins	Anemia	Shuaib et al., 2010
	Aflatoxins	Immunodeficiency	Jiang <i>et al.</i> , 2005
Gambia	Aflatoxins	Impaired growth	Watson <i>et al.</i> , 2018
		Liver cirrhosis	Kuniholm et al., 2008
Cameroon	Aflatoxins	Primary hepatocellular carcinoma	Tchana <i>et al.</i> , 2010
Kenya	Aflatoxins	Aflatoxicoses	Azziz-Baumgartner et al., 2005
Nigeria	Aflatoxins	Infertility	Uriah et al., 2001
Benin	Aflatoxins	Stunting and being underweight	Gong <i>et al.</i> , 2003
Togo	Aflatoxins	Stunting and being underweight	Gong <i>et al.</i> , 2003
Tunisia	Ochratoxins	Nephropathy	Hmaissia et al., 2012
Côte d'Ivoire	Ochratoxins	Nephropathy	Sangare-Tigori et al., 2006
Tanzania	Fumonisins	Stunting and being underweight	Shirima et al. 2013,

Table 2.1: Mycotoxins-Related Public Health Problems in Selected African Countries

#### 2.2. Mycotoxins Occurrence in Africa

Studies carried out in different African countries have ranked occurrence of major mycotoxins contaminants in food and feed in order of aflatoxins (43.75 %), fumonisins (21.87 %), ochratoxins (12.5 %), Zearalenone (9.38 %), Deoxynivalenol (6.25 %) and beauvericin (6.25 %) deoxynivalenol (Kebede *et al.*, 2020; Udomkun *et al.*, 2017; Darwish *et al.*, 2014). These mycotoxins are associated with poor agricultural practices, and their presence in food products poses significant health risks to both humans and animals.

The contamination of food by mycotoxins can occur at various stages, including production, storage, processing, transportation, and marketing (Darwish *et al.*, 2014). The situation is made worse by the lack of effective control strategies and the frequent exceedance of maximum limits set by regulatory bodies (Kebede *et al*, 2020; Udomkun *et al.*, 2017). Therefore, there is an urgent need for the development and implementation of effective control measures to mitigate the impact of mycotoxin contamination in Africa.

#### 2.2.1 Ochratoxins

Ochratoxins are produced by *Aspergillus* and *Penicillium* species (in particular *Aspergillus ochraceus*) molds under conditions ranging from between 21–28 °C and 25–28 °C, respectively and over 70 % humidity. Ochratoxins are weak organic acids of dihydroisocumarin moiety joined by a peptide bond to 1-phenylalanine (Figure 2.1). The main strains of ochratoxin are A, B, and C, (Malir *et al.*, 2016) where ochratoxin A is the most predominant and toxic compared to B and C. Physical characteristic of the compounds includes being colorless and soluble in organic solvents, alkaline and water (O'Brien and Dietrich, 2005).

Ochratoxins are liposoluble and accumulate in body tissues and cause toxicity. Its toxicity is exacerbated by its ability to enhance lipid peroxidation, a process that can lead to cell damage (Luci *et al.*, 2018). Ochratoxin compounds resemble in structures with essential amino acid phenylalanine. The toxins take advantage of the resemblance to interfere with hydroxylase activity in the kidney and liver to prevent protein synthesis by enzyme phenylalanine (Niaz *et al.*, 2020; Zhu *et al.*, 2016). Storage molds metabolize into ochratoxin compounds under cool-temperate and hot-tropical conditions (Mutlu-Ingok & Karbancioglu-Guler, 2014).

Ochratoxins naturally contaminate maize, wheat, barley, flour, coffee, rice, oats, rye, beans, peas, animal feeds, wine, grape juice, and dried vine fruits (Paterson *et al.*, 2014). Humans and animals get ochratoxin contamination through; foods and feeds, skin and inhalation of mold spores, as well as carry over from animal products such as meat, eggs and milk (Mozaffary *et al.*, 2019; Heussner & Bingle, 2015; Maria Edite *et al.*, 2014). In acidic environment, ochratoxin molecules are stable to most food processing conditions (Adegoke *et al.*, 2018). These studies collectively contribute to our understanding of the chemistry and toxicology of ochratoxins.





#### Figure 2.1: Ochratoxins A, B and C

The International Agency for Research on Cancer (IARC, 2009), has classified ochratoxins as the group 2B human carcinogen (Ostry *et al.*, 2017). Ochratoxin contamination of humans and animals is acutely nephrotoxic, hepatotoxic, and causes immunotoxicity, genotoxicity, neurotoxicity, teratogenicity, and embryo toxicity (Malir *et al.*, 2013). Farm animals ingest these toxins from the feeds and get absorbed into the kidney, liver and blood. They affect livestock productivity through reduced feed conversion, body weight gains and egg production (Martins *et al.*, 2012).

Chronic exposure to ochratoxin A, beyond 200  $\mu$ g/kg causes hepatocellular and renal-cell tumors. There has been no reported health risk for levels below 20  $\mu$ g/kg. Ochratoxins compete with Aflatoxin at production level in foodstuff and in gastrointestinal tract during absorption into the body system (Paterson *et al.*, 2018). *Aspergillus species*, *Brevibacterium species*, *Acinetobacter calcoaceticus*, and *Rhizopus* isolates degrade ochratoxin A, from food and feed to ochratoxin alpha (Rodríguez *et al.*, 2011). These findings suggest the potential for the development of biological detoxification systems to mitigate the health risks associated with ochratoxin A contamination. There is knowledge gap on chemical methods to degrade ochratoxin molecules from food and feed.

#### 2.2.2 Patulin

Patulin mycotoxins are colorless crystalline, unsaturated heterocyclic compounds of low molecular weight (154.121 g/mol) and with molecular formula  $C_7H_6O_4$ . They are heat-tolerant, soluble in water, ethanol, methanol, and acetone. They have a melting point range of between 110-113 °C, are stable in aqueous media and at pH values ranging 3.0 to 6.5 (Guo *et al.*, 2017). Patulin mycotoxins are *Penicillium, Aspergillus* and *Byssochlamys* mold species metabolites (Tannous *et al.*, 2016). They are common contaminants in rotten vegetables and fruits, including apples, pears, peaches, cider, compotes, apricots, grapes, fruit juices, and foods intended for children (Cunha *et al.*, 2014; Omar, 2013; Majid *et al.*, 2005,).

The levels of patulin contaminants in raw apples, apple-based juices and other apple food products are quality indicators (Zhong *et al.*, 2018; Rahimi *et al.*, 2015). The set maximum acceptable level for patulin contamination for apple juice, solid apples, and infant apple-based foods are 50 µg/kg, 50 µg/kg and 10 µg/kg, respectively (De Clercq *et al.*, 2016; Codex Alimentarius, 2014; Kirimi *et al.*, 2008). Consumption of commercial apple, apple juice and other apple products exposes humans to patulin contamination (Heinmaa *et al.*, 2019; Ahmadi-Afzadi *et al.*, 2015; Soliman *et al.*, 2015; Baert *et al.*, 2007).

In the body, the toxins are genotoxic, embryo toxic, and neurotoxic, carcinogenic, mutagenic, teratogenic, immunosuppressant, or may mimic estrogens hence toxic to animal and human health (Alshannaq &Yu, 2017). Patulin molecule undergoes chemical reaction with amino acids and DNA molecules of proteins to form intramolecular and intermolecular links. The toxins use the links to mimic enzyme inhibitor, depletory glutathione and chromosomal aberration (Karlovsky *et al.*, 2016). The lactone ring of patulin molecule (Figure 2.2) opens

easily when exposed to oxidizing reagents causing the molecule to lose its characteristic toxicity and biological activity (Mbundi *et al.*, 2014; Yang *et al.*, 2014; Piqué *et al.*, 2013).



Figure 2.2: Lactone Ring of Patulin Molecule and Rotting Apple Fruits

In recent studies have identified various strategies for biological and chemical degradation of patulin in food commodities. Yeast, bacteria, and fungi have the potential of biological decontaminating patulin contaminants using mild conditions. (Sajid *et al.*, 2019; Dong *et al.*, 2015). Different sulfites degrade patulin into 3-keto-5-hydroxy-pentanal Collin (2008). Use of monochromatic ultraviolet radiation on patulin contaminated fruit juices decontaminates them to useable levels (Zhu *et al.*, 2013).

#### 2.2.3 Fumonisins

Fumonisins are chemical metabolites produced by *Fusarium verticillioides, Fusarium proliferatum, Aspergillus nigri* and other related species of pathogenic fungi (Tola and Kebede, 2016). They are polyketides of four strains of *fusarium* A, B, C and P with the same basic structure but different structural moieties. Their molecular structure has 18-carbon backbone, functionalized with two tricarballylic esters and an alanine derived amine. The fumonisin B<sub>1</sub> has a diester with propane-1,2,3-tricarboxylic acid (TCA) and 2-amino-12,16-dimethyl-
3,5,10,14,15-pentahydroxyleicosane where hydroxyl (OH-) groups at the C-14 and C-15 positions react with the carboxyl groups (-COOH) of TCA.

The strains  $B_2$  and  $B_3$  are dihydroxy analogues of  $B_1$  at C-5 and C-10, respectively (Figure 2.3). The above properties determine the strain toxicity (Alshannaq and Yu, 2017) including ability to inhibit sphingolipid biosynthesis in animals, plants, and yeasts (Burgess *et al.*, 2016). Fumonisin B-series is the most abundant and toxic form that co-exists with other forms.



Fumonisin B<sub>1</sub>



Fumonisin B<sub>2</sub>

# Figure 2.3: Chemical Structure of Fumonisin B1 and B2

Fumonisins contaminate maize and maize-based products, rice, wheat, barley, rye, oat, yams and millet and their products with a significant health implication (Somorin *et al.*, 2012). In humans, these toxins may have adverse health effects such as cancer and birth defects, toxic effects in the liver, hepatocarcinoma, stimulation and suppression of the immune system, neural-tube defects and nephrotoxicity (Omurtag, 2008). Fumonisin toxins synergistically interact with aflatoxin  $B_1$ , to cause ailments in humans and animals (Misihairabgwi *et al.*, 2019).

According to the international agency for Research on Cancer, fumonisin  $B_1$  and  $B_2$  are group 2B possible human carcinogens (IARC, 2015). The toxins have a provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg body weight /day for  $B_1$ ,  $B_2$ , and  $B_3$  alone or in combination set, according to the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2001). The European Union acceptable upper limits for raw materials used in human food production is 800–4000 and 2000–4000 µg/kg  $B_1$  and  $B_2$ , respectively (Torelli *et al.*, 2012, **20** | P a g e

EU Regulation 1126/2007) and is the same for the Americans in cereal-based products (Anfossi *et al.*, 2016).

Correlation studies linking consumption of maize with high level of *Fusarium verticillioides* and *fumonisins* to high incidences of human esophageal carcinomas reported a link between fumonisin B1 incidences to human esophageal cancers in Africa, Central America, and Asia (Mousavi *et al.*, 2019). Other studies have also reported co-existence of Aflatoxin B1 and Fumonisin B<sub>2</sub> in foods particularly in Asia, South and Central America, and Africa (Kumi *et al.*, 2014; Sun *et al.*, 2011).

Fumonisin  $B_1$  reduce uptake of folate in different cell lines of human babies causing neural tube defects (Kagot *et al.*, 2019; Katengesya et al., 2018). Fumonisins cause huge economic losses during *fusarium* outbreak and related fumonisin contamination in maize estimated to be USD 46 million annually. In America routine management of fasarium costs about USD 1–20 million annually (Winter and Pereg, 2019; Chilaka *et al.*, 2017). No sufficient data on the extent of effects to human health due to co-exposure and co-contamination of aflatoxin B1 and fumonisin B<sub>1</sub>.

There are potential enzymatic and chemical methods for decontaminating fumonisins in food and feed. They include; fusion enzyme called FUMDI, bacterial, enzymes, essential oils and ally, benzyl and phenyl isothiocyanate (Li *et al.*, 2022; Xing *et al.*, 2014; Azaiez *et al.*, 2013; Hartinger *et al.*, 2011; Heinl *et al.*, 2010). These studies collectively suggest that enzymatic and chemical methods, such as the use of specific enzymes and essential oils, hold promise in the degradation of fumonisins, potentially reducing health risks.

#### 2.2.4 Aflatoxins

#### **2.2.4.1 General Information**

Aflatoxins was identified in 1961 in United Kingdom in animal feeds after killing 100,000 turkeys and other animals that had consumed the feeds (Filazi & Sireli, 2013). Aflatoxins are secondary metabolites produced mainly by Aspergillus species, *Aspergillus flavus* and *Aspergillus parasiticus*. The *flavus species* is widely distributed in nature, colonizing parts of plants above the ground. *Aspergillus parasiticus* has limited distribution but adapted to soil environments (Wang *et al.*, 2018; Ostry *et al.*, 2017; Marin *et al.*, 2013; De Boevre *et al.*, 2012; Liu & Wu, 2010).

Accumulation of aflatoxins in food materials and even contamination leading to negative impacts on human health is a culmination of events from seed sowing into the soil along the stages up to the plate (Gholami-shabani *et al.*, 2017). The soil borne aflatoxin-producing fungi depend on soil-plant interaction for survival and growth. Aflatoxin producing fungi directly colonize plants in the field or infest harvested crop later in the store. Sometimes, colonizing molds on the contaminated food material are visible (Stroka *et al.*, 2016; Venkateswarlu *et al.*, 2012).

Aflatoxin molecules can resist food processing stages to affect the consumer. A wide-range of aflatoxin contaminations occur in hot and humid conditions (Marroquín-Cardona *et al.*, 2014). Many regions have these conditions and with specifically adapted crops but none has any adaptation to aflatoxin colonization. Among these crops contaminated with fungi before and after harvest are; nuts, soybean, rice, maize and other dried foods, spices and crude vegetable oils and cocoa beans (Marin *et al.*, 2013).

Aspergillus fungi produce different categories of Aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. B<sub>1</sub> is the most common and potent to humans and animals (Gonçalves *et al.*, 2012; Dhanasekaran *et al.*, 2011). The four common strains of aflatoxin under various conditions can also metabolize into other aflatoxins. The contaminants affect food and feed materials for humans and animals causing serious health effects and death depending on dosage (Kumar *et al.*, 2017b). Aflatoxins are toxic, hepatotoxic, carcinogenic, mutagenic, immunosuppressive, genotoxic, cytotoxic and teratogenic agents, with a capacity to penetrate cell membrane, attach to the DNA and induce irreversible cell mutations (Obonyo & Salano, 2018).

The toxins are highly linked to hepatocellular carcinoma incidences and high rate of hepatitis infections in Eastern and South-Eastern Asia, Middle and Western Africa countries (Rawla *et al.*, 2018). Aflatoxins are immune modulators capable of suppressing resistance of secondary infections, affect testicles and sperm quality leading to human and animal infertilities (Khoury *et al.*, 2019; Marin *et al.*, 2013).

Approximately 5 billion people globally, are exposed to aflatoxins through diets, with the tropical and subtropical countries being the most affected (Zyoud, 2019). The ease of food contamination by aflatoxins in these countries is associated with the sub-optimal storage conditions for cereals, spices, and milk, which favour fungi growth (Herrera *et al.*, 2014; De Groote *et al.*, 2013). Aflatoxins contamination effect depends on the consumed dosage and period of exposure (Ahmed *et al.*, 2017).

Developed countries have early detections systems and provide timely solutions for aflatoxin management. On the other hand, developing countries in South-Eastern Asia and sub-Saharan Africa lack similar capacity for early detections and interventions (Battilani *et al.*, 2016; Wu *et al.*, 2011; Leslie *et al.*, 2008).

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#### 2.2.4.2 Some Properties of Aflatoxins

Pure aflatoxin molecules appear in a range of colors from pale yellow to white. When observed under ultraviolet (UV) light the fluorescence varies with aflatoxin subtype;  $B_1$  and  $B_2$  blue,  $G_1$  and  $G_2$  green, and  $M_1$  range from blue–violet (Owuor *et al.*, 2020). They are slightly soluble in water (10-20 µg/ml) and freely soluble in moderately polar solvents such as chloroform, acetone, acetonitrile, menthol and dimethyl sulfoxide. They are unstable in UV light in presence of oxygen and extreme pH (3 < or >10) (Vijaya, 2018). Table 2.2, shows physical and chemical properties of the main strains and metabolites of Aflatoxins.

Aflatoxin	Molecular Formula	Molecular Weight (g)	<i>Melting Point</i> (° <i>C</i> )	Fluorescence emission (nm)
<b>B</b> <sub>1</sub>	$C_{\ 17}  H_{\ 12}  O_{\ 6}$	312	268-269	425
<b>B</b> <sub>2</sub>	C 17 H 14 O 6	314	286-289	425
$G_1$	$C_{17} H_{12} O_7$	328	244-246	450
$G_2$	C 17 H 14 O 7	330	237-240	450
$M_{1}$	C 17 H 12 O 7	328	299	425
M 2	C 17 H 14 O7	330	293	-
B 2A	C 17 H 14 O 7	330	240	-
G 2A	C 17 H 14 O 8	346	190	-

#### **Table 2.2: Some Properties of Aflatoxins**

# 2.2.4.3 Nature of Aflatoxins

Aflatoxins are chemical compounds derived from difuranceoumarins with a coumarin nucleusbased bifuran group one side and a lactone ring (Gs) or a pentanone ring on the other (Bs and Ms) (Bbosa *et al.*, 2013). The pentanone series has 7-member difurceoumarcyclopentenone: B<sub>1</sub>, B<sub>2</sub>, B<sub>2A</sub>, M<sub>1</sub>, M<sub>2</sub>, M<sub>2A</sub> and aflatoxicol. The lactone series has 11-member difurceoumarcolactone compounds: G<sub>1</sub>, G<sub>2</sub>, G<sub>2A</sub>, GM<sub>1</sub>, GM<sub>2</sub>, GM<sub>2A</sub> and B<sub>3</sub> (Table 2.3). Consumption of Aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in contaminated food and feeds by humans and animals, metabolize into B<sub>2A</sub>, M<sub>1</sub>, M<sub>2</sub>, M<sub>2A</sub>, G<sub>2A</sub>, GM<sub>1</sub>, GM<sub>2</sub>, GM<sub>2A</sub> B<sub>3</sub> and aflatoxicol compounds. The strength of aflatoxins' toxicity depends on the structural nature of terminal furan ring; saturated ring is least toxic compared to the unsaturated.

Aflatoxin molecules are highly oxygenated with similar structures (Kitya *et al.*, 2013). The high number of oxygen atoms influence physical-chemical and bio-chemical properties of the toxins. These include high liposoluble property that enables the toxin molecules to be absorbed into the bloodstream through polar and nonpolar sites (Allah Ditta *et al.*, 2019; Cary *et al.*, 2018) including gastrointestinal, respiratory and skin pathways. The molecules circulate in the bloodstream to different body organs including the liver and the kidneys (Ahmed *et al.*, 2017).

The aflatoxins toxic effects in the body depends on the levels of exposure (Waliyar *et al.*, 2009), for example, acute necrosis in the liver, cirrhosis, carcinoma, immune suppression and malabsorption of various food nutrients. Sometimes, the affected animal or human show signs of nutritional deficiencies, impaired immune function, malnutrition and stunted growth (Bourke et al., 2016).

# Table 2.3: Major Aflatoxins Producing Aspergillus Species (Adopted from Bbosa et al.,

2013)

	Aflatoxin Type	Aspergillus specie(s)
Difurocoumarocyclopentenone series	Aflatoxin B <sub>1</sub>	A. flavus, A. arachidicola, A. bombycis, A. minisclerotigenes, A. nomius, A. ochraceoroseus, A. parasiticus, A. pseudotamarii, A. rambellii, Emericella venezuelensis
	Aflatoxin B <sub>2</sub>	A. arachidicola, A. flavus, A. minisclerotigenes, A. nomius, A. parasiticus
	Aflatoxin B <sub>2A</sub>	A. flavus
	Aflatoxin M <sub>1</sub>	A. <i>flavus and parasiticus</i> ; metabolite of aflatoxin $B_1$ in humans and animals and comes from mother's milk
	Aflatoxin M <sub>2</sub>	Metabolite of aflatoxin B <sub>2</sub> in milk of cattle fed on contaminated foods
	Aflatoxin M <sub>2A</sub>	Metabolite of Aflatoxin M <sub>2</sub>
Difurocoumarolactone series	Aflatoxin G <sub>1</sub>	A. arachidicola, A. flavus, A. minisclerotigenes, A. nomius, A. Parasiticus
	Aflatoxin G <sub>2</sub>	A. arachidicola, A. flavus, A. minisclerotigenes, A. nomius, A. parasiticus
	Aflatoxin G <sub>2A</sub>	Metabolite of Aflatoxin G <sub>2</sub>
	Aflatoxin GM <sub>1</sub>	A. flavus
	Aflatoxin GM <sub>2</sub>	Metabolite of Aflatoxin G <sub>2</sub>
	Aflatoxin GM <sub>2A</sub>	Metabolite of Aflatoxin GM <sub>2</sub>
	Aflatoxin B <sub>3</sub>	Aspergillus species not defined

Most frequently, *Aspergillus flavus* produces both subtype  $B_1$  and  $B_2$  aflatoxins in maize. However, to a less extent *Aspergillus parasiticus*, and *Aspergillus nomius*, *Fusarium* and *Penicillium species* also produce them. Figure 2.4 shows the aflatoxin molecules.



B1











M1



M2









Figure 2.4: Aflatoxin Molecules

These aflatoxigenic strains have the capability of growing in maize at any stage, from cultivation, harvesting, drying, storage, transportation, and in the market (Mitchell *et al.*, 2017). The optimum conditions for their growth and the subsequent production of aflatoxins are; above 14 % moisture content, temperature range between 28 and 30 °C and between 0.83 to 0.97 water activity range (Wagacha and Muthomi, 2008). Other conditions necessary are oxygen and carbon dioxide ratio, grain physical integrity, initial presence of fungi spores,

presence of competing molds, pest activity on the grain, grain genetic properties and subsequent aflatoxin contamination (Ng'ang'a *et al.*, 2016).

Moreover, aflatoxin contamination also occurs due to drought stress, level of maturity at the time of harvest, broken and undersized grains and discolored kernels. The grain storage environmental conditions also determine if the aflatoxin contaminants, thus acidic environment favour production while basic one does not (Al-Gabr *et al.*, 2013; Stoev *et al.*, 2013). In summary aflatoxin contamination of any agricultural produce, depend on the factors: biological, pre and post-harvest management, environmental, climatic, agronomic practices, storage, transportation and processing. These conditions are unattainable by majority of farming communities, making aflatoxin an avoidable threat (Mitchell *et al.*, 2017; Amudalat, 2015; Stoev *et al.*, 2013; Wagacha and Muthomi, 2008; Bhatt and Vasanthi, 2003).

Aflatoxins directly influence DNA structure by their carcinogenic and Geno toxigenic nature (Benkerroum, 2020). There are strict regulations in developed countries for aflatoxin concentrations in human foods. These conditions are sparingly adapted in developing countries (De Saeger *et al.*, 2016; Cotty *et al.*, 2008). In developing countries, maximum accepted total aflatoxins level ranges from 10 to 30  $\mu$ g/kg, with 10  $\mu$ g/kg being the most common for maize in most of the regions of the world (Table 2.4).

European Commission however has a lower accepted aflatoxin contamination level set at 2 and 4  $\mu$ g/kg for B1 and total, respectively. These levels have created a trade barrier for leading producers, processers and traders. In addition, the exposure limit is lower at 1  $\mu$ g/kg body weight per day compared to 100  $\mu$ g/kg body weight per day in developing countries. In developing African and Asian countries where exposure is 100-fold, higher, recorded cases are very regular (WHO, 2017). Sometimes, leading producers, processers, and traders incur **28** | P a g e

additional costs to mitigate against these aflatoxin contaminations (Edgar *et al.*, 2021; Codex Alimentarius Commission, 2014; Technical Policy Paper 4, 2015).

Geographical and climatic variation, affect the rate and degree of aflatoxin contamination in food at different stages. This is because toxins are dependent on temperature, humidity, soil, storage and physical condition of food material (Marin *et al.*, 2013). Aflatoxin molecules are highly resistant to most mitigating measures in storage, handling and processing conditions. According to Sirot *et al*, (2013) aflatoxin molecules can withstand cooking temperatures beyond the boiling point and fungi spores can hibernate in the store and stored food material for more than 7 years.

Country	Food	msl, total aflatoxin, μg/kg
Argentina	Peanut (B1, 5 µg/kg)	20
	Maize (B1, 5 µg/kg)	20
Brazil	Peanut	20
	Maize	20
Colombia	Cereal	30
	Food	20
Peru	All food	10
Uruguay	Peanut	30
	Dried fruits	30
	Baby food	3
Venezuela	Rice flour	5
Kenya	Peanut	15
	Maize	10
EU	Cereals, cereal products, rice (B1, 2 µg/kg)	12
	Species, Dried fruits and nut (B1, 4 µg/kg)	15
	Milk (M1, 0.025-0.05 µg/kg)	0.05
Turkey	Red pepper and nut (B1, 5 µg/kg)	10
Korea	All food (B1, 10 µg/kg)	
Japan	All food (B1, < 10 µg/kg)	
China	Peanut (B1,20 µg/kg)	
US FDA	Cereals, cereal products, rice (B1, 2 µg/kg)	20
	Species, Dried fruits and nut (B1,4 µg/kg)	20
	Milk (M1, µg/kg)	0.5

Table 2.4: Summary of Maximum Set Limit (MSL) of Contaminants in Foods

### 2.2.4.4 Aflatoxins Prevalence

Tropical and sub-tropical regions have higher aflatoxins contamination prevalence in foods and feeds than in other regions (Benkerroum, 2020). South-East Asian and Sub-Saharan Africa report high prevalence of aflatoxins contamination in staple foods almost annually. The pedoclimatic conditions, agricultural practices, cultivars grown, mechanical and pest damage of crops and knowledge of the harmful effects of food-borne toxins on the productivity and food safety are the main reason for high prevalence (Rugemalila, 2015; Strawn et al., 2012; Wu *et al.*, 2011). These seem to agree with the highest incidence of hepatocellular carcinoma and the occurrence of acute aflatoxicosis outbreak episodes in these regions (Amid *et al.*, 2013; Liu *et al.*, 2010).

According to Benkerroum, (2020) control, monitoring systems and standard regulations are poor and not enforced in these regions. Mutegi *et al.* (2018) reports from analysis of studies done in different parts of Kenya since 1960 concluded that high aflatoxins contamination levels in staple foods and other foods lead to human exposure directly or indirectly (Table 2.5). Some studies reported high incidence of hepatocellular carcinoma and occurrences of acute aflatoxicosis outbreak episodes. 

 Table 2.5: Selected Data on Aflatoxin Prevalence in Kenya (1960-date) (Source: Mutegi et al., 2018)

	Maize products (µg/kg)								
LOCATION	Range	Mean	>MSL (%)	Regulatory µg/kg	Population	Reference			
Makueni, Kitui	0-48,000	9.1 Gm	35	20	716	Daniel et al., 2011			
Nairobi	0-88.83	6.7 AM	16	10	99	Kiarie <i>et al.</i> , 2016			
(Korogocho)									
Nairobi (Dagoretti)	0-20	2.97 AM		20	87	Kiarie et al., 2016			
Kitui, Makueni, Machakos, Thika	1.0-46,400	20.53 AM	55	20	342	Lewis et al., 2005			
Eastern, Nyanza	0.01- 9,091.8	46.9 AM	50.3	10	789	Mahuku, 2018			
Western Kenya	0-710		15	10	985	Mutiga <i>et al.</i> , 2015			
Upper and Lower eastern	0-4,839		39	10	1,500	Mutiga et al., 2014			
Makueni	0.0-13,000		35.5	20	104	Mwihia et al., 2008			
Nairobi	0.11-4,593		83	10	144	Okoth and Kola, 2012			
Kwale, Isiolo, Tharaka Nithi, Kisii, Bungoma	<1.0-1,137		26	5 (KEBS)	497	Sirma <i>et al.</i> , 2016			
		Milk prod	ucts (µg	g/kg)					
Eldoret, Machakos, Nyeri, Nakuru, Nairobi	8-600		20	0.05	613	Kang'ethe and Lang'a, 2009			
Makueni	1.4-152.7	0.83	22.2		18	Kang'ethe et al., 2017			
Nandi	0.5-0.8	0.06	9.5		21	Kang'ethe et al., 2017			
Nairobi (Korogocho)	0.002-2.56	0.132	63	0.05	76	Kiarie et al., 2016			
Nairobi (Dagoretti)	0.007- 0.64	0.093	63	0.05	52	Kiarie et al., 2016			
Nairobi	0-1,675		55	0.05	190	Kirino et al., 2016			
Bomet	0-2.93		43.8	0.05	156	Langat et al., 2016			
Kwale, Isiolo, Tharaka Nithi, Kisii, Bungoma	<2-6,999	3.2 Gm	10.4	0.05	512 (farmers)	Senerwa et al., 2016			
		Animal fe	ed prod	ucts (µg/kg)					
Eldoret, Machakos, Nakuru, Nairobi	-		67	5 (KEBS)	830	Kang'ethe and Lang'a, 2009			
Nairobi	5.13-1,123		95	10	72	Okoth and Kola, 2012			
Kwale, Isiolo, Tharaka Nithi, Kisii, Bungoma	<1.0-4,682	9.8 Gm	61.8	5 (KEBS)	102(FM)	Senerwa et al., 2016			

Maize products (µg/kg)

#### 2.2.4.5: Metabolism of Aflatoxin Contamination in Humans and Animals

The main foods consumed throughout the world are maize and groundnuts based. The two products also top in the list of high susceptibility to aflatoxin contamination and the source of exposure to humans. Aflatoxin contamination begins in the farms and enhanced during harvest, transportation and processing depending on moisture content levels (Warnatzsch *et al.*, 2020; Okoth *et al.*, 2016). Silage for farm animal feeds include maize, wheat, beans and groundnuts leftover (Ogunade *et al.*, 2018).

The route way for exposure to contaminants for beef and dairy livestock feeding on the silage. Contaminated feeds directly affect animal health and its productivity, indirectly humans from their products (Kovalsky *et al.*, 2016; Atherstone, *et al.*, 2016; Alonso *et al.*, 2013; Klopfenstein *et al.*, 2013). To address the problem propionate preservatives and binders are incorporated during feeds preparation (Saminathan *et al.*, 2018). Some developed countries allowed up to 300  $\mu$ g/kg of aflatoxin contaminants in grains for animal feeds. This allows traders in these countries to utilize most grain produced (Sirma *et al.*, 2018; De Mil *et al.*, 2015). In these countries, animal feeding situations and long-term cancer risks are not a concern, but protection of susceptible species whose response to mycotoxin contamination is dependent on age, sex, weight, diet and exposure to infectious agents (Kemboi *et al.*, 2020).

Under ordinary conditions, a lactating cow or goat metabolized aflatoxin  $B_1$  and  $B_2$  into aflatoxin  $M_1$  and  $M_2$  7-12 hours after ingesting contaminated feed. The metabolic conversion ratio for  $B_1$  to  $M_1$  is 1–3%. The hydroxylated aflatoxin metabolites  $M_1$  and  $M_2$  are heat resistant (Moreno-Martinez *et al.*, 2011). Human health and animal with deficiency in zinc, iron and vitamin A are more susceptible to aflatoxin effects (Roohani *et al.*, 2013). The body enzymes metabolize mycotoxins into less toxic metabolites (Murugesan *et al.*, 2015; Wang *et al.*, 2011).

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The double bond on carbon number 8, 9 in the terminal furan ring cause the biochemical potency of aflatoxin B1 and G1 molecules (Yazdanpanah & Eslamizad, 2015). The metabolic processes of aflatoxin B<sub>1</sub> include O-dealkylation to aflatoxin P<sub>1</sub>, keto-reduction to aflatoxicol L, epoxidation to B<sub>1</sub>-8,9-epoxide, hydroxylation to aflatoxin M<sub>1</sub>, aflatoxin Q1 and aflatoxin B<sub>2a</sub> (Omar, 2013: Dhanasekaran *et al.*, 2011). The reactive 8,9-epoxide metabolite bind with the cell DNA and proteins using cytochrome P450 enzymes and phospholipids to induce disruption both functions and integrity of the cell (Rushing & Selim, 2017; Zhuang *et al.*, 2016; Santini & Ritieni, 2013; Klaunig *et al.*, 2009; Bedard *et al.*, 2006) (Figure 2.5).

These reactions do not occur with aflatoxin  $B_2$  and  $G_2$  owing to their saturated furan ring. These aflatoxins are less carcinogenic in their natural form but this can change if oxidized in the body to  $B_1$  and  $G_1$ .



Figure 2.5: Metabolism of Aflatoxin in the Liver (modified from Omar, 2013)

#### 2.2.4.6 Reactive Sites and Degradation of Aflatoxin Molecules

Aflatoxins have similar structures and form a unique group of highly oxygenated, naturally occurring heterocyclic compounds (Lalah *et al.*, 2019). They slight variations in terms of double bond position and ketonic groups' nature. Their metabolite products also vary in hydroxy groups and hydroxylation positions. The aflatoxin molecules are slightly soluble in water and other aqueous media. Their high ability to epoxidation reactions influences secretion and toxicity (Owuor *et al.*, 2020).

The molecules are differentiated from other heterocyclic compounds through fluorescence UV light, presence of a double bond on C 8 and 9 in furo-furan ring for  $B_1$  and  $G_1$ , and absence of a double bond at the same position in  $B_2$  and  $G_2$  (Bräse *et al.*, 2013). In general, a molecule of aflatoxin contains four chemically active sites; the lactone ring, furan ring, methoxy connection and cyclopentanone bridge (Figure 2.6).



Figure 2.6: Active Sites of aflatoxin molecule.

Aflatoxin B<sub>1</sub> is a hepatocarcinogen associated with hepatocellular carcinoma (HCC) development. In the body, cytochrome-P450 enzyme metabolically converts aflatoxin into highly reactive intermediate AFB1exo-8, 9-epoxide at its terminal furan double bond. The intermediate covalently bonds with guanine bases in the liver cell deoxyribonucleic acid (DNA) inducing DNA adducts (Hamid *et al.* 2013; Hamilton & Arya, 2012). The reaction of AFB<sub>1</sub> exo-8, 9-epoxide and DNA is through groove binding because of hydrogen bonding to form AFB<sub>1</sub>-N7-GUA (Ma, Wang, and Zhang 2017 (Figure 2.7).



#### Figure 2.7: AFB1 Binding to DNA (Wang et al. 2016).

The lactone ring in the coumarin moiety exert aflatoxin toxicity to the body. Depending on the acidity environment in the liver, the ring opens and decarboxylation reaction occurs (Nazhand *et al.*, 2020; Bond *et al.*, 2016; Al -Gabr *et al.*, 2013) The ring is unstable under extreme pH (<3 or >10) but when the pH adjusts to (3-10) the original structure resumes. The lactone ring in the aflatoxin molecule is a vulnerable site to degradation reactions, which disrupt or remove it all together. If such a chemical, reaction occurs a drastic reduction or loss of its toxicity results. The lactone ring undergoes degradation reactions, which include ring opening, lactone ring cleavage/hydrolysis, decarboxylation and reduction (Figure 2.9 a and b). Any reaction or process not targeting the rings for example, heating, pasteurization and sterilization have no effect on the aflatoxin molecule.





Figure 2.9: Aflatoxin B1 degradation pathways. (Kumar et al., 2022)

Alkalis and basic solutions like ammonia react with Aflatoxin molecules. This occurs through opening of the lactone ring, hydrolysis, decarboxylation and loss of methoxy group attached to the aromatic ring (Owuor *et al.*, 2020; Colović *et al.*, 2019; Lyagin & Efremenko *et al.*, 2019; Hassan & Zhou, 2018; Gholami-Shabani *et al.*, 2017; Hojnik *et al.*, 2017; Kovlasky *et al.*, 2016; Kolosova and Stroka, 2011). The efficacy of ammonia in this the reaction is greater than 99 % and recommendable for decontamination of aflatoxin contaminants in animal feeds. The reaction, however, has limitations to commercialization as an aflatoxin contaminants degradation method because the final products bear ammonia odor and are decolorized. Figure 2.10 below shows Ammoniation process of aflatoxin B1.



Figure 2.10: Ammoniation of aflatoxin B1 (Cucullu et al., 1976).

Ammonia solution being basic may corrode treatment chambers and storage bins increasing the cost of maintenance. Ammonia gas has a likelihood of exploding during application (Rushing & Selim., 2019; Porto *et al.*, 2019). Ammonia gas efficacy increases under conditions of high pressure, high temperature and a narrow range of moisture between 13 and 16 %. Degradation of aflatoxin with ammonia require humidification of feeds before through drying which is costly and decrease the nutritional value content (Luo *et al.*, 2018; Chen *et al.*, 2014; Luo *et al.*, 2011; Jouany, 2007)

Hypochlorite, chlorinating agents, chlorine dioxides and chlorine gas, hydrogen peroxide, and sodium hydrogen sulfite induce lactone ring cleavage (Soni *et al.*, 2020). Toxigenic fungi growth in foods are suppressed in Asian countries with natural products and essential oils from cinnamon, peppermint, basil, oregano, flavoring herb epazote, cloves, and thyme (Agriopoulou *et al.*, 2020). Enzymes in the body system disrupt the lactone ring in aflatoxins such as laccases, peroxidases, reductases, and oxidases (Kim *et al.*, 2017). Aflatoxin molecules' terminal furan ring is directly involved in its toxicity and is another key target for detoxification reactions.

Hydrolytic agents at raised temperatures breaks the double bond in furan ring (Herzallah *et al.*, 2008).

Past studies reported 99 % reduction of aflatoxin load with ozone gas on contaminated cereals, vegetables, and fruits in acidic environments. Ozone degrades by oxidizing the double bond in the furan ring without changing chemical composition and nutritional value of the food materials and with no harmful residues. Ozonation and rearrangement reactions are initiated at the double bond to form less toxic compounds like aldehydes, ketones and organic acids (Soni *et al.*, 2020; Mallakian *et al.*, 2017; Agriopoulou *et al.*, 2016; Chen *et al.*, 2014; Luo *et al.*, 2014). In storage facilities, ozone reduce mold counts of A aflatoxigenic species, flavus and A. parasiticus significantly (Porto *et al.*, 2019).

Dilute mineral or organic acids react with aflatoxin molecules in contaminated food by adding water across the furan ring double bond to form less toxic acetoxyl compounds (Owuor *et al.*, 2020). Similar conversions occur when hydrogen, sodium borohydride and sodium hydrogen sulfite are reacted with aflatoxin  $B_1$  and  $G_1$  to form aflatoxin  $B_2$  and  $G_2$ , respectively. Excess reagents cause more reactions to occur with parts of aflatoxin  $B_1$  targeting the lactone ring of coumarin moiety, the acid group and the cyclopentane ring to form tetrahydroaflatoxin molecules.

Aflatoxin molecules can be degraded completely within two-hour with industrial food additive in low concentrations and acidic conditions. Among the additives are sodium hypochlorite, hydrogen peroxide, sodium sulphite, and sodium hydrogen sulfite. Oxidizing agents; potassium permanganate, chlorine and sodium perborate (Hojnik *et al.*, 2017), destroy double bond at furan ring, the lactone ring and the methoxyl group and alters toxicity properties of aflatoxins. Butylated hydroxyanisole (BHA) and concentrated sodium chloride suppress the development **39** | P a g e of aspergillus, fusarium and penicillium fungi species in food fields and stores. These chemical compounds moderate water activity and ion transport necessary for mold growth (Hymery *et al.*, 2014; Kumar *et al.*, 2007).

#### 2.2.4.6 Aflatoxin Degradation Mechanism

The possible mechanisms for the detoxification of aflatoxins by different chemical reagents involves two distinct pathways, the removal of the double bond positioned at the terminal furan ring and the opening of the lactone ring (Mishra and Das, 2003). Once the lactone ring is open, further reactions may occur to alter the binding properties of terminal furan ring to DNA and proteins. Wang *et al.* (2011) reported that the degradation of aflatoxin AFB1 involve the oxidation of the 8, 9-vinil bond of the toxin to form the aflatoxin AFB1-8,9-epoxide, followed by its hydrolysis to generate the AFB1-8, 9-dihydrodiol (Figure 2.11).



# Figure 2.11: Degradation of aflatoxin AFB1 through oxidation at 8,9-vinil bond of the aflatoxin to form aflatoxin AFB1-8,9-epoxide

This is a main step to reduce affinity of the epoxide with DNA and other enzymes that lead to unwanted transformations (Mishra and Das, 2003). Treatment with reducing and oxidizing chemical reagents can alter aflatoxin's structure and can form non-toxic products (Mishra and Das, 2003).

# **2.2.5 Ethanol Production**

Lactic acid bacteria catalyze ethanol production process and some strains of yeast open the lactone ring in aflatoxin molecules and degrading it (Chaves-López *et al.*, 2020; Wacoo *et al.*, 2019). According to Ezekiel *et al.*, (2015), given optimal conditions at pH range of 4-6, fermentation degrade mycotoxins content in cereals by 76.2–99.9 % range. Alcohol distillation process removes aflatoxin residues regardless of its original contamination level in the substrate like maize (Omemu *et al.*, 2007b). The contaminants residues are then concentrated in the spent grain materials.

Spent grain materials are course-unfermented distiller's grains, and stillage whose content include yeast, fine grain particles and soluble nutrients. In developing countries these fermentation residues are fed to livestock thus risking their health (Sarrocco & Vannacci, 2018;

Reis *et al.*, 2017; Crowley *et al.*, 2016; Wambacq *et al.*, 2016; Rasmussen *et al.*, 2014; Okeke *et al.*, 2013; Inoue *et al.*, 2013; Murthy *et al.*, 2011).

# 2.2.6 Knowledge Gaps for the Study

Several studies on aflatoxin prevalence in Kenya has been summarized (Mutegi *et al.*, 2018). Other studies have linked malnutrition among children to aflatoxins exposure (Stepman, 2018; Kang'ethe *et al.*, 2017; Kiarie *et al.*, 2016; Malusha *et al.*, 2015; Leroy *et al.*, 2015; El-Tras *et al.*, 2011). Economic losses due to aflatoxins contamination in Sub-Saharan countries have been studied (Udomkun *et al.*, 2017a).

This study assessed socio-economic impacts and prevalence of aflatoxin contamination in maize in selected counties in Kenya. Further, chemical degradation of Aflatoxin contaminants in maize was studied, and a low cost industrial process for value addition to decontaminated maize was designed.

# **CHAPTER THREE**

# **3.0 MATERIALS AND METHODS**

# 3.1 Study Site

The study was done in Makeuni, Machakos, Meru, Isiolo, Embu, Nairobi, Kajiado, Nakuru, Trans Nzoia, Busia and Migori counties in Kenya (Figure 3.1).



Figure 3.1: Sampling Sites in Different Counties in Kenya

#### 3.1.1 Field Survey

The study design was a mixed method employing qualitative and quantitative analysis. The questionnaire format is attached (Appendix 2). Five volunteers were identified per county to assist in questionnaire administration to the maize handlers across the value chain. The respondents filled out a standardized consent form (appendix 1) before the oral interview. Focused discussion and survey targeted maize handlers who included NCPB officers, farmers, and small-scale maize traders. The study population was (N=3300) comprised of maize farmers and traders in Makeuni, Machakos, Meru, Isiolo, Embu, Nairobi, Kajiado, Nakuru, Trans Nzoia, Busia, and Migori.

The minimum sample size (n) of maize handlers was determined using the (Biemer & Lyberg, 2003) formula:  $n = [(N) (p) (1 - p)] / [(N - 1) (B/C)^2 + (p) (1 - p)].$ 

Where:

n is the computed sample size needed for the desired level of precision N is the population size; B is the acceptable amount of sampling error or precision set at 0.1, 0.05, or 0.03

C is the Z statistic associated with the confidence level which is 1.96 which corresponds to the 95 % level.

p is the proportion of the population expected to choose a set at 0.5

In this study N = 1,800, p = 0.5, B = 0.05, C = 1.96 for every county. The formula yielded 314 persons where 300 was taken from for questionnaire interview.

The minimum maize sample size (n) was determined using the formula:

$$N_{min} = z^2 \times p \times q / d^2$$

Where N\_min was the minimum sample size required, q = (1 - p), z = 1.96 is the standard error, p = prevalence of condition under study, which was aflatoxin contamination of maize grain in the study area, and d = 0.05 is the absolute precession required for the study at 95 % confidence 44 | P a g e level. The mean prevalence rate of aflatoxin contamination in the study area was 9.3 % (Lauren *et al.*, 2005; CDC, 2004) and was used to determine the sample size.

Factoring in the value of q = (1 - p) = 0.907, p = 0.093,

Then  $n = (1.96)^2 (0.093) (0.907) / (0.05)^2 = 129.61$ .

Maize samples (n=630) were collected from farmers (n=156), retailers (n=156), wholesalers (n=156) and Nation cereals produce board (NCPB) stores (n=162) in eleven counties. From counties in; Eastern Kenya (n=280), Rift Valley (n=147), Western Kenya (n=178), and Nairobi (n=25). Maize samples were collected from National Cereal Produce Board (NCPB) Depots and private stores in different county markets namely Makueni, Machakos, Meru, Isiolo, Embu, Nairobi, Kajiado, Nakuru, Trans Nzoia, Busia and Migori counties attached (Table 1 appendix 3).

# **3.1.2 Sampling**

Bulk maize samples were collected from National Cereal Produce Board (NCPB) Depots and private stores in Makueni, Machakos, Meru, Isiolo, Embu, Nairobi, Kajiado, Nakuru, Trans Nzoia, Busia, and Migori counties in January, April, and October 2017. Maize samples (n=630) were collected from farmers (n=156), retailers (n=156), wholesalers (n=156) and Nation cereals produce board (NCPB) stores (n=162) in eleven counties. From counties in; Eastern Kenya (n=280), Rift Valley (n=147), Western Kenya (n=178), and Nairobi (n=25). In addition, maize samples were collected from selected bags at five intervals using an automatic spear sampler in conformity with the guidelines provided by the European Commission (EC) regulation no. 178/2010 (EC, 2010). A representative sample from a composite of thoroughly mixed five 1 kg samples was collected from each bag, packed in self- sealing brown sugar bags, coded, and transported to the laboratory for storage. In the laboratory, 500 g of each sample was refrigerated at -4 °C until analysis.

#### **3.2 Reagents**

Certified high purity and low concentration standards mixture containing 1.026, 0.311, 1.046, and 0.322  $\mu$ g mL<sup>-1</sup> of Aflatoxins B1, B2, G1, and G2 dissolved in methanol (MeOH), was purchased from Sigma Aldrich (Sigma Aldrich, St. Louis, USA). A working solution of Aflatoxins standard was prepared by mixing 100  $\mu$ L of standard with 900  $\mu$ L of MeOH: H<sub>2</sub>O. The same standard was used for spiking blank samples and preparing calibration standards in 20 % methanol.

A working standard was used for the fortification of samples and preparation of calibration standards (solvent and matrix-matched) in 20 % methanol. All solutions were stored at -20°C in the dark until analysis. Chemicals regents (Sodium Hydrogen Sulfite, Ferulic acid, Ammonium carbonate, Sodium hydrogen carbonate and Sodium hypochlorite, ammonia solution, hydrogen peroxide, acetic acid) and solvents (methanol, methylamine, acetonitrile, and deionized water) used in the study were HPLC grade supplied by Kobian Scientific. Chemo Equip supplied the seal-able 50 ml glass tubes, the enzymes, and aflatest immunoaffinity columns (IAC) and HPLC column (C18).

#### **3.3 Determination of Aflatoxins.**

# **3.3.1 Method Validation**

The method was validated according to SANCO/12571/2013 which demonstrates the conformity of the analytical performances with criteria established in the European Commission (EC) regulation no. 178/2010 (EC, 2010), which provides guidelines for validation procedures for linearity, specificity, limit of detection (LOD), limit of quantitation

(LOQ), accuracy, and precision. Calibration curves were constructed using standard solutions of AFB1, AFB2, AFG1 and AFG2.

The validation was done by spiking an external standard with aflatoxin standards, to quantify the aflatoxins concentrations in the samples. A stock solution was prepared containing 100 µg/mL, and serially diluted into seven different concentrations using methanol/water (4:6, v/v) according to the Association of Official Analytical Chemists (AOAC) reference method Number 994.08. The linearity of the data was tested by external standardization using matrix calibration curves constructed from AFB1, AFB2, AFG1, and AFG2, standard solutions of six different concentrations within the range of 1-100 ng/mL (1, 5, 10, 30, 50, and 100 n/mL). Analytical curves were established by plotting the peak areas used as the analytical signal response (y) versus the concentration of the Aflatoxin (x) (Figure 30). All four strains had linear curves that obeyed the mathematical linear equation y = mx + c where y represented the response signal, m is the gradients of the curve, c is a constant, and y-intercept.



Figure 3.2: External Standardization Calibration Curves A, B, C, D for Aflatoxin B1, B2, G1 and G2

# 3.3.2 Linearity

The capacity of a method to get results is directly proportional to the amount or concentration of the analyte in a defined range. Aflatoxin B1, B2, G1, and G2 were evaluated using  $0.1 - 50 \mu g/kg$  concentrations of standard solutions and plotted as illustrated by the four calibration curves. Method sensitivity is considered according to the LOD and LOQ. The LOD was calculated as the lowest aflatoxins concentration signal response, three times greater than the sample standard error derived from replicate observations. The LOQ was calculated as an

aflatoxin signal response ten times greater than the sample standard error derived from replicate observations. The  $R^2$  values, linear equation, LOD, and LOQ per the aflatoxin calibration curve are in (Table 4.1). The correlation coefficients,  $R^2$ , for the four aflatoxin B1, G1, B2, and G2 calibration curves were all above 0.994, which indicated a good linear fitting for the experimental data as a strong relationship between concentration variables and responses.

Parameter	B1	G1	B2	G2
Linear equation	Y=307.6556x-	Y=225.740x +	Y=257.206x-	Y=81.046x +
	82.709	81.342	102.456	20.705
R <sup>2</sup>	0.9983	0.9961	0.9943	0.9979
LOD / (µg/kg)	0.49	0.36	0. 39	0.45
LOQ / (µg/kg)	1.16	1.34	1.48	1.1

Table 3.1: Linearity Data for Aflatoxin B1, G1, B2 and G2

# 3.3.3 Method Accuracy, Precision and Specificity

Method specificity was evaluated by comparing the retention times in the blank samples spiked with 100  $\mu$ g/kg of AFB1. The sensitivity of the method was determined by determining LOD and LOQ following the EuraChem guide (Magnusson *et al.*, 2014) as required in Commission Regulation (EC) 401/2006 (EC-2006). The LOD was the lowest concentration of the aflatoxin measured which was 3 times greater than the average baseline noise obtained from 10 independent blank samples. The LOQ was the aflatoxin signal response that was 10 times greater than the average baseline noise obtained samples.

Method accuracy and specificity were tested through analysis of spiked blank samples recoveries of aflatoxin standard solution at three different concentrations (20, 40, and 100  $\mu$ g/kg) in replicates according to the method used by Serrano *et al.*, (2012). The percentage recoveries calculated as: Recovery (%) = (S'-S) 100 % /S spiked (2)

Where S' is the concentration of the spiked sample, S is the concentration of the non-spiked sample, and S- spiked is the concentration added.

The method accuracy was tested through recovery studies of 6 replicates of spiked aflatoxin standard solutions at three different concentration levels (equivalent to 20, 40, and 100  $\mu$ g/kg) and a blank. Relative standard deviations (RSDs) were estimated by performing daily repeatability was used to determine the level of precision expressed as the confidence interval of the mean value. Tests were done on each matrix daily for three consecutive days using six replicate concentrations.

Method specificity was evaluated by comparison of retention time for aflatoxin in spiked blank with 100  $\mu$ g/kg aflatoxin standards to control interference with the target analyte The recoveries for the aflatoxins were B1; 70.59-79.46 %, B2; 73.46-85.53 %, G1; 76.71-83.80 % and G2: 80.75-100.63 % with the method hence qualify it for quantification of native contaminants in maize samples. The method precision was calculated by determining the relative standard deviation of recoveries recorded (Table 3.2).

Parameter	<b>Retention Time</b>	Spiked	Recover	ies ( %)		Mean	STD	RSTD
	(min)	(ug/kg)	1	2	3	%		%
B1	4.3	20	83.02	77.33	88.55	79.46	5.61	7.06
		40	79.22	81.75	66.89	75.95	7.95	10.47
		100	74.35	66.35	71.06	70.59	4.02	5.70
B2	4.15	20	73.33	82.65	64.41	73.46	9.12	12.42
		40	78.91	69.23	84.18	77.44	7.58	9.79
		100	94.26	86.17	76.45	85.63	8.92	10.41
G1	4.18	20	81.11	84.78	74.45	80.11	5.24	6.54
		40	91.04	69.62	69.48	76.71	12.41	16.17
		100	83.52	93.44	74.45	83.80	9.50	11.33
G2	4.03	20	81.16	71.89	89.21	80.75	8.67	10.73
		40	81.23	75.41	90.25	82.30	7.48	9.09
		100	101.02	111.32	89.54	100.63	10.90	10.83

 Table 3.2: Retention Time, Recoveries, and Relative Standard Deviation (RSTD) of

 Aflatoxin B1, G1, B2 And G2 Spiked Blank Samples

# .3.4 Calibration Curve

3

An external standard calibration curve was constructed using aflatoxin standards to quantify the aflatoxin concentrations in all the samples. A stock solution was prepared containing 100 ng/mL and serially diluted into seven different concentrations using methanol/water (4:6, v/v). Six different concentrations within the range of 5-100 ng/mL (1, 5, 10, 30, 50, and 100  $\mu$ g/kg) were used. Analytical curves were established by plotting the peak areas, used as the analytical signal response (y) versus the concentration of the Aflatoxin (x).

The peak area of the aflatoxin standards plotted against concentration. From the plot curve, the slope (S) was determined as well as the Y-intercept (a). The level of aflatoxins in the sample was calculated using the formula:

Aflatoxin, 
$$\mu g/kg = \{(L-a)/S\} \times V/W\} \times F.$$
 (Trucksess et al., 2009) (1)

Where L represents the test solution peak area, V is the final volume (mL) of the injected test solution, F is the concentration factor, and W amount of sample passed through the immune-**51** | P a g e

affinity column. The total aflatoxin is the sum of aflatoxin G2, G1, B2, and B1 following the Association of Official Analytical Chemists (AOAC) method 994.08 (Lupo *et al.*, 2010).

# 3.3.5 Sample Preparation for Aflatoxin Screening with Enzyme-Linked Immunosorbent Assay (ELISA) Kit techniques.

A competitive ELISA (Ridascreen® Aflatoxin Total R 4701 and Aflatoxin B1 30/15, R1211, R-Biopharma, Darmstadt, Germany) was used for the screening analysis of the Aflatoxin total and B1. Maize samples for ELISA analysis were prepared according to procedure guides described by the kit manufactures. 20.0 g of each replicate of maize samples was ground to 20-mesh size flour using a hammer mill and 5 grams of replicates were sampled. Aflatoxins were extracted from the flour with 25 mL of 70 % methanol, homogenized for 5 minutes at 250 rpm, centrifuged at 4000 rpm for 10 minutes and a 100  $\mu$ L of the supernatant was drawn for analysis.

Determination of the aflatoxins is done by modified methods described by the kit manufacturers. All analysis reagents were at room temperature. 50  $\mu$ L of standard /samples, aflatoxin enzyme conjugates and anti-aflatoxin antibodies solution in polystyrene micro titer plate wells were incubated for 30 minutes. After 30 minutes, the wells were rinsed five times with 0.15 M phosphate buffer saline at a pH of 7.2. The wells were then treated with 50  $\mu$ l tetra-methyl benzidine and urea peroxide mixture and incubated for a further 30 min in darkness. Followed by the addition of 100  $\mu$ l of reaction termination reagent. Multiskan<sup>TM</sup> FC

microplate photometer microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) was used to the absorbance was read at 450 nm within 30 min.

#### **3.3.6 Sample Preparation for HPLC-FD**

The maize sample was accurately weighed to 20 g, ground into 20 mesh size powder with a hammer mill, and divided into 5 g replicates for aflatoxin extraction. Extraction was done by blending each replicate with 30 mL of extraction solution (a mixture of 24 mL methanol and 4 mL deionized water, 2 mL of acetonitrile, 1 g NaCl, and 4 g of anhydrous magnesium sulfate) for 10 minutes at 120 rpm. The mixture was filtered through Whitman filter paper (No. 4.) 1 mL of the supernatant was diluted with 39 mL of Phosphate Buffer Solution (PBS) at pH=7. 2. The diluent was centrifuged at 3400 RPM for 1 minute, and filtered through nylon membrane filters (pore size, 0.45  $\mu$ m). The 40 mL of the supernatant was passed through the immunoaffinity column at a flow rate of one drop per second, column was washed twice with 10ml of water at a flow rate of two drops per second. The eluate was evaporated to dryness at room temperature under a stream of nitrogen, followed by reconstitution in 400  $\mu$ L of the mobile phase (water/methanol/ acetonitrile, 55/10/35, v/v/v). The sample was transferred into the HPLC vials and refrigerated until analysis. A matrix-matched composed of aflatoxin-free maize was prepared in a similar way to the other maize samples including clean-up and nitrogen evaporation, and was used in making up the matrix-matched calibration standard solutions.

### 3.3.7 Sample Analysis and Quantification for Aflatoxin with HPLC-FLD

Agilent High-Performance Liquid Chromatography (HPLC-FLD) system (Agilent, USA) was used for separation, detection, and quantification of aflatoxins B1, B2, G1, and G2 where 10  $\mu$ L of the sample was injected at a time. The HPLC was equipped with a fluorescence detector

RF–20A, a pump LC-20AT, an autosampler system (SIL-20A), and a column oven- CT 10AS-VP thermo-controller, controlled by lab solutions software. Chromatographic separation was done on a genesis reverse-phase C18 analytical column ( $4.6 \times 250$  mm, 100 Å, and 5 µm particle size; Gloucester, UK) at 40°C under isocratic elution with the mobile phase at a flow rate of 0.9 mL min<sup>-1</sup>.

A KOBRA cell electrochemical post-column derivatization system (R-Biopharm Inc., Marshall, MI) set at 100  $\mu$ A, was applied before the fluorescence detector to enhance the AFB1 and AFG1 fluorescence activity. It consisted of a 254- nm UV lamp and a 0.5 mm i.d. x 10 m PTFE tube fitted around the UV lamp. The fluorescence detector was operating at 360 and 450 nm wavelengths for excitation and emission, respectively. The column was maintained at 40°C temperature. Separation was achieved under isocratic elution using a mixture of acetonitrile/methanol/water (15/30/70 v/v/v) mobile phase. Aflatoxin retention time was used to determine the analyte peaks, and quantification was done by comparing peak areas of the calibration standards and the Aflatoxins analytes in the sample. The selection criteria for aflatoxin in samples were the retention time and peak shape of the analytes observed in matrixmatched calibration solutions.
#### **3.4 Degradation Reactions of Aflatoxin Contaminated Maize**

#### **3.4.1 Sample Preparation for Degradation Reactions**

The sample preparation stage involved drawing a 13500 g representative maize sample from a thoroughly mixed contaminated sample, sub-setting into three equal parts of 4500 g each. The first set was whole maize, the second dehulled maize without a coat and the third was milled into powder. Each set was further divided into 900 sub-samples of 5g each and kept at 20 °C until time for degradation experiments. Degradation reagents were prepared by serial dilution of 1 molar concentration of each reagent with deionized water to 0.5, 0.05, 0.005, and 0.0005 molar, except for ferulic acid which was purchased in required dilutions. Ammonia, hydrogen peroxide, and methylamine were diluted into 2 % by volume. The reagents were labeled and stored ready for degradation reactions.

#### **3.4.2 Degradation Reactions**

Tests were conducted per reagent with a maximum of 60 samples at a time. Each of the 5 g maize was transferred into a separate 50 ml labeled glass tube, 15 ml of 1 molar sodium hydrogen sulfite solution was added to the first three in a row of 12 tubes, and 5 ml of hydrogen peroxide was added from the 4<sup>th</sup> to the 6<sup>th</sup> tube. Similar amounts of ammonia and methylamine were added from the 7<sup>th</sup> to the 9<sup>th</sup> and from the 10<sup>th</sup> to the 12<sup>th</sup> tube, respectively. The procedure was repeated with the second row with 0.5 molar, the third with 0.05 molar, the fourth with 0.005, and five row with 0.0005 molar sodium hydrogen sulfite solution. The catalysts were added separately from the fourth tube to the twelfth in a row as in the first case. The samples were closed tightly and shaken vigorously for two minutes using a laboratory orbital shaker. After this, the samples were heated in a water bath for 5 minutes at 80 ° C. The samples were cooled to room temperature. A gram was pulled out hourly and tested for aflatoxin content until **55** | P a g e

the 5<sup>th</sup>. The procedure was repeated with sodium hydrogen carbonate, sodium hypochlorite, ammonium carbonate, and ferulic acid.

#### **3.4.3 Degradation Rate Determination**

Different chemical kinetic models were tried to determine the best for aflatoxin contaminants degradation reactions. The first-order kinetic model (Equation (1)), was found to best represent the degradation reactions and to test the efficacy of different chemical reagents on the contaminants (Nguyen *et al.*, 2021; Li *et al.*, 2016). The model was used to measure different reaction parameters; reaction rate constant instantons concentration of aflatoxin contaminants and time for each degrading reagent. calculated.  $Ct = Coe^{-kt}$  and In Ct = -kt + In Co (Equation 1). Where Ct is the concentration of aflatoxin at any time, Co is the concentration of aflatoxin at the start of the degradation process, Euler's constant (e) represents the reaction characteristic, (k) is the rate constant and (t) the instantaneous reaction time. The predictive model was verified by conducting additional experiments with reagents with known degradation pathways at optimum points.

The coefficient of determination  $(R^2)$ , root mean square error (RMSE), Akaike information criterion (AIC) test, and the Bayesian information criterion (BIC) were tested and calculated using equations 2 and 3.

$AIC = -2\log L(\theta) + 2k$	2	
$BIC = -2\log L(\theta) + k \log n$	3	

Where,  $\theta$  = the set (vector) of model parameters,  $L(\theta^{2})$  = the likelihood of the candidate model giving the expected result when evaluated at the maximum. The rate of chemical degradation of aflatoxin was determined by measuring the initial and final concentration of aflatoxin contaminants in each sample and the values applied in equation 4.

$$Degradation = \left(\frac{\text{Initial-final}}{\text{Final}}\right) x 100 \%$$
4

#### 3.5 Industrial Process Design for Utilizing Aflatoxin Decontaminated Maize

The aflatoxin-decontaminated maize was tested as raw material for ethanol starch, ink, glue, and briquettes production and briquettes. The yields of ethanol produced from degraded aflatoxin-contaminated maize were tested and compared with striped maize using an enzyme fermentation process. Degradation products were rinsed with distilled water, dried, 100 g weighed in beakers, and hammer milling. Replicates of this powder were mixed with a 1.5 mL mixture of enzymes (endocellulase, exocellulase, xylanase) and 150 mL water, oven heated at 170 ° C for 7 minutes to soften the maize starch for  $\alpha$  - amylase enzyme digestion (Li *et al.*, 2016; Ranum *et al.*, 2014; Omemu *et al.*, 2007a).

The samples were cooled to 60 °C and maintained for 60 minutes. The mixture pH was adjusted to 4.5 and 2 mL of gluco-amylase enzyme was added. After cooling to 34 °C, 2 mL of yeast was added, while stirring on an orbital shaker, and the mixture fermented for 60 hours. The fermented broth was distilled to get ethanol at a temperature range of 77-79 °C. The stillage was dried and baked into briquettes. The percentage ethanol yield was determined for each of the degradation products of maize.

#### **3.6 Data Analysis**

Data processing and statistical analyses were performed using different statistical programs that included IBM SPSS Statistics 26, Microsoft Excel 2019, and R (version 4.0.3, R Core Team). The weighting factors for weighted calibration were selected based on the minimum REsum using the command 'weight\_select' (package 'envalysis', available from https://doi.org/ft9p). Analysis of variances (ANOVA), Fisher's LSD test (p<0.05), and Tukey

Post Hoc Test were used to compare significant differences, confidence levels of different parameters, and the hypothesis of the study. Model assumptions were verified using diagnostic plots i.e. normality of residuals was checked via QQ plots and homoscedasticity of residuals was checked via scale-location-plots (square root of standardized residuals versus predicted values) (Zuur *et al.*, 2009). The statistical significance was set at the level of 95 % taken as (P < 0.05).

#### CHAPTER FOUR

#### 4.0 RESULTS AND DISCUSSION

# 4.1 Socio-Economic Impacts of Aflatoxin Contamination of Maize in Selected Counties of Kenya

## 4.1.1 Results of Field Questionnaires

Field questionnaires were administered to 3300 respondents who were directly involved in the maize value chain. The questionnaires focused on demographic, socioeconomics, pre- and post-harvest processing, trading, pests, diseases, insects, mold, maize contamination, and treatment of contaminated maize data. A total of 2882 participants returned their questionnaires representing 87 % of the targeted population. According to Mugenda and Mugenda, (2003), a response rate of 50 % is adequate, and a more the 70 % response rate is good. Respondents who did not return the questionnaires were in the meeting and participated in oral discussion but because of enormity, it was not easy to associate returned questionnaires to the actual participants.

#### 4.1.2 Demographic Information

# 4.1.2.1 Maize Chain Handlers Gender

Demographic information included gender, age bracket, level of education, and occupation. Two thousand, eight hundred and eighty-two respondents participated in the study. 60 % of respondents were males while females were 40 % (Figure 4.1). The results suggest that male participants were more than females in the maize value chain. However, both genders contributed differently to the value chain activities such as planting, weeding, cutting, shelling, bugging, milling, loading, sorting and sieving, dusting, trading, and transportation among others.



# Figure 4.1: Maize Chain Handlers by Gender

# 4.1.2.2 Respondents Age

The majority of the respondents were in the age bracket between 21-50 years which was at 76 %, 23 % were in the aged between 51-70 years, and 1 % did not reveal their ages (Figure 4.2). This implied that 99 % of the respondents involved in the maize value chain in different counties were adults.



## Figure 4.2: Respondents Age

#### 4.1. 2.3: Respondents' Level of education

This section aimed to establish the level of education for the maize chain players. Analysis of the responses showed 55 % of the respondents had secondary education, 22 % had post-secondary education, and 21.0 % had primary education but 2.0 % of the participants did not reveal their education level (Figure 4.3). The respondents were able to read and interpret interview questions but responses depended on the knowledge of maize chain activities. Some respondents in focused group discussion had a challenge in understanding the problems faced by farmers and traders in their regular practices. The information collected from the participants was only for basic guidance.



Figure 4.3: Participants Education Level.

# 4.1.2.4: Respondents' Occupation

This section sought to establish the respondents' daily occupation or actual engagement in the maize value chain. 82 % percent of the respondents were practicing both farming and maize sales. 13.0 % were in formal employment as officers in the maize chain including accountants, drivers, millers, and farmers' relations officers among others. 4 % of the respondents were practicing cereal business in the trading centers, while 1 % declined to indicate their occupation (Figure 4.4). Key concerns raised were declining maize yields, increasing costs of inputs to manage pests, unreliable weather at the harvest time, and occasional loss of cereals due to rots, insects, and rodents.



## **Figure 4.4: Respondents Occupations**

# 4.1.3 Domestic Sources of Cereal, Vegetables and Animal Feeds

## 4.1.3.1 Sources of Cereal

This section sought to establish the respondent's sources of household food. In the 11 counties, 78 % of the respondents used cereals from their farms, 3 % bought from neighboring farmers 15 % bought the same from open markets and retailer stores in the locality and 4 % of the respondents declined (Figure 4.5). Some farmers cultivated maize for subsistence use and sold the remains to consumers within and without the region. This explains the fact that when maize has any form of contamination, it affects persons within the same region. Moreover, when the contamination is low such that its effect cannot result in an outbreak the chronic contamination continues to affect the populace in the region.



Figure 4.5: Sources of Cereal

# 4.1.3:2 Source of Vegetable

This section targeted the data on the sources of vegetable food for respondents. 67 % of the respondents owned vegetable gardens for household consumption, but also supplied the excess to 7 % of respondents in the neighborhood. 16 % of the respondents bought vegetables from the market and shops, while 10 % did not disclose the source of their cereals (Figure 4.6). The counties under the study provide both cereal and vegetable foods to their households directly from the farms before selling the excess to others.



**Figure 4.6: Respondent Sources of Vegetable** 

# 4.1.3.3 Source of Animal Feed

Maize straw and other materials from the farm were used by 72 % of the respondents as livestock feeds, 12 % bought maize straw and other materials for animal feeds from the neighbors' farms, 14 % bought hey and other materials from the market and 2 % did not respond (Figure 4.7). The majority of livestock feeds in the rural areas constituted farm agricultural waste, which implies that any contaminations of any kind in the feed stocks could be transferred to the livestock and later carried over through livestock products to humans.



**Figure 4.7: Respondents Source of Animal Feeds** 

# 4.1.4 Maize Farm in Ace rage

This section targeted to establish the participants' level of land acreage for maize cultivation in the 11 counties. On the land acreage, 45 % of the respondents owned small farms with sizes that ranged between 0.5-4 hectares and practiced mixed farming. 24 % of the respondents owned small pieces of land of sizes between 0.25 - 4 Ha where they practiced maize monoculture while 6 % of the respondents owned large pieces of land whose sizes were more than 4 hectares, where they practiced mass production of maize. However, 25 % of the respondents were not maize farmers therefore, provided no answer to the question (Figure 4.8). The majority of maize farmers own small land and practice mixed farming. There were reasons for mixed farming according to the maize change handlers key among them was the size of land where farmers wanted to maximize their productivity. There were other reasons, which included recycling of waste, the unreliability of prices, and crop failure.



## Figure 4.8: Maize Growing Land Acreage

## 4.1.5 Maize Maturation Period

This section targeted to establish the period of maturation for maize varieties grown in different counties. In the maize maturity period, 30 % of the respondents planted maize varieties suitable to their counties climatic conditions and matured in 6-10 months. 26 % of the respondents planted maize that matured in 4-5 months while 28 % planted maize that matured in 3-4 months. 16 % of the respondents were not farming maize so provided no clear answer to the question (Figure 4.9). The maize planted in different counties varied in varieties and maturation periods because of differing altitudes, time of sowing and soil fertility, climate, and other factors that are typical and unique for the counties.



#### **Figure 4.9: Maturation Period of Maize**

## 4.1.6. Method of Improve Maize Yield per Hectare

This section targeted to establish how participants improved maize yield per hectare in their farms. On the yield improvement, 40 % of the respondents cited planting maize before the rains, managed weeds, and used fertilizers to improve their soil. 35 % of the respondents planted maize during the rains and did weed management but used manuals from the livestock. 3 % of the respondents planted maize at any time and used irrigation water for the crop. 23 % of the respondents provided no answer to the question about how they improved their maize yield (Figure 4.10).

From the responses to questionnaires and the focus group discussion views, the majority of maize farmers invest in yield improvement. According to some of the respondents, the cost of production of a 90 kg bag of maize varied with agro-climatic zones and moisture index based on the annual rainfall. Generally, respondents quoted in order Ksh. 1093, Ksh. 1086 and Ksh.1066 for Upper highlands, Upper midlands, and lower midlands, for maize growing regions of Kenya.

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Figure 4.10: Methods of Improving Maize Yield per Hectare

# **4.1.7: Maize Handling Procedures**

## 4.1.7.1 Maize Drying

This section sought to find the procedures used by respondents in handling maize from farm to consumer among them are harvesting, drying, shelling, transporting, storing, and selling. In the maize handling procedures, 74 % of the respondents allowed maize to be dried while on the farm to physiological maturity, cut shelled, dusted for pest control, and bagged ready for transportation. 23 % of the respondents cut and transferred physiological maturity maize to their home compounds, dried in stack piles, hand and machine shelled, dusted for pest control, bagged, and stored. 1 % of the respondents dried their maize in heaped stacks at the farms, machine-shelled, and sold to the National Cereals and Produce Board directly and or other buyers as well.

Two percent of the respondents provided unclear procedures including selling products well green and making silage for livestock (Figure 4.11). The majority of the respondents dried their maize on the farm, harvested, shelled and stored it for sale. These procedures created avenues and possibilities for maize contamination in the handling chain. Respondents did not give instances where contamination control measures were taken, like fumigation of maize transporting facilities, carrier bags, compound grounds, and stores.



## Figure 4.11: Drying Maize at the Farm

#### 4.1.7.2 Indicators of Dry Maize

This section targeted to determine indicators of dry maize in the counties; on the dryness of maize indicators, 34 % of the respondents checked by chewing some grains and 29 % used a special tin and salt to measure sound intensity and stickiness on the can wall. 20 % of the respondents followed NCPB guidelines for moisture content determination. A small group of respondents 4 % measured maize weight until constant. 2 % of the respondents kept maize in

direct sunshine for 4-7 days to dry while another 3 % did not determine moisture content at all. 8 % of the respondents did not respond to the question (Figure 4.12).

The majority of the indicator methods employed by the respondents were based on estimation and could not provide accurate data on maize dryness. Based on their experience, however, during focused group discussions, many confirmed that they had done the estimations over the years and few received complain from consumers or buyers. Employ standardized methods for measuring maize moisture content and transfer the cost to the buyers. This will be a permanent solution to chronic contamination of maize by molds due to irregular drying.



Figure 4.12: Checking Maize Dryness Indicators

# 4.1.7.3 Modes of Marketing Maize

This section sought to establish the flow of maize from the farm to the consumer. Maize is the main staple food crop and most important cereal grain. It contributes significantly to food security by providing roughly a third of the caloric intake for most people in Kenya. Dry cereals

are marketed from the farms in different ways; among them are brokers (intermediaries) at the farms as cited by 60 % of the respondents. 10 % of the respondents sold also maize when green to brokers while 26.0 % sold directly to the National Cereals and Produce Board and millers. 2 % of the respondents were not maize farmers while another 2 % had no response (Figure 4. 13). Farmers in the counties market their produce mostly through brokers (intermediaries).

The reason for this action is that brokers provided market information about maize availability and surplus in specific regions. The respondents indicated during a focused group discussion that this market arrangement saved both time and money by enabling farmers to dispose of their produce at prices offered by brokers in large volumes. NCPB purchased maize mainly from medium and large farms, but the small-scale farmers did not enjoy that facility.



Figure 4.13: Modes of Marketing Maize

## 4.1.8 Maize Contamination

#### 4.1.8.1 Observable Maize Contaminant

This section targeted to find out the general understanding by respondents about different changes or appearances observed on the maize grains freshly picked from the farms or in the stores. The changes included color, the presence of pest destruction (warms, weevils, borers, rodents, and birds), virtual growth of molds, and physical damage. The question targeted to establish the frequency of food contamination (maize) or incidences observed in the last 5 years.

The responses required were daily, weekly, monthly, and rarely. 52 % of the respondents rarely witnessed cases of maize contamination in the last 5 years, 30 % gave no response to the question, while 5 % had witnessed frequent maize contamination daily. 2 % observed maize contamination weekly. 12 % cited monthly contamination of maize (Figure 4.14). Most of the respondents had no specialization to identify maize contaminations, so rarely witnessed any changes. According to the responses received, contamination of maize is an issue that required more sensitization to the respondents who are in maize value chain handlers.



Figure 4.14: Observed maize contamination

# 4.1.8.2 Pests Infestation in maize

This section sought more information on the contamination of maize by establishing from respondents the frequency of pest infestation in maize. The expected responses were rodents, weevils, borers, birds, and warms. 71 % percent of the respondents cited the presence of black weevil, rodents, borer, warm and bird bites in/on maize fresh the farm and in-store. 5 % of the respondents cited grain borers and rodents in maize especially in the store while 24 % did not respond to the question. In the maize-growing regions weevil, borer, and rodent and bird pests were common contaminants and widely observed (Figure 4.15). Pest injury or vectors for fungal contaminants in maize if other conditions like water activity, temperature, and content favor their growth and colonization of maize kernels.



## Figure 4.15: Observed Pests in Maize

## 4.1.8.3 Fungi in Maize

Molds are abundant in nature and grow almost anywhere, indoors and outdoors, some species of fungi play a substantial role in spoilage of foodstuffs. This question is targeted to establish the level of knowledge for identifying mold infestation in maize. 30 % of the respondents reported having observed mold contaminants in maize, 55 % did not respond to the question, and 15 % were not sure about mold contamination in maize (Figure 4.16). The large percentage of no response indicated the difficulty in noticing fungi contamination in maize without any instrument. Knowledge of molds also varied with the level of education, with those without or with low formal education experiencing difficulties in identifying mold contamination. The fractional with the ability to identify molds closely agrees with the education level in section 4.1.2.3



Figure 4.16: Presence of Mold in Maize Identified

# 4.1.8.4 Observed Color Change in Maize

This question targeted to establish awareness of contamination signs on maize by the respondents. Contaminated maize may have a color change but not always and maize may have a color change due to other factors. 57 % of the respondents did not provide any response to the color change question, 32 % observed color change in maize, and 11 % of the respondents were not sure (Figure 4.17). Contaminated maize may have a slight color change not observed easily. Contamination is not the only cause of color change but also environmental conditions in the growing and drying stages. Most of the respondents could not see the color change; partly they were not sure or not keen to observe color change.



Figure 4. 17: Observed Colour Change

# 4.1.8.5 Presence of Rot in Foodstuff

This question targeted to determine if the respondents knew the linked between maize rot and fungi contamination. Occasionally rots are associated with poor storage methods. Sixty-five percent of the respondents did not respond to this question, 27 % of the respondents observed rot on maize kernel tips but 8 % were not sure (Figure 4.18). According to the findings, most of the respondents were not keen on details in observing maize kernels well or they were unsure if rots are linked to mycotoxin contamination signs. A high number of respondents were not sure or indicated the low awareness state of the counties. In addition, the occurrence of symptomless kernel rot infection in most maize samples implied that people in these areas were likely consuming higher levels of contaminants. Hence mitigation measures to control mycotoxins to alleviate health issues related to mycotoxins (Mukanga *et al* 2010).



Figure 4.18: Percentage of Respondent Observed Rot in Maize

# 4.1.9 Determining Contamination in Maize

This question is targeted to determine ways used to deter contamination in maize. According to the responses from the respondents, 62 % of them compared maize color. 24 % of respondents sent maize samples for laboratory analysis, 17 % used food taste, and 12 % gave no responses to the question. Seven percent physically sorted out contaminated grains and weighed the remains. Five percent thought that no food contamination, another 5 % never measured, 2 % were not sure and 1 % used both smell and taste to measure contamination (Figure 4.19). Most respondents employed methods that cannot conclusively determine the contamination of foods. The respondents were non--specialists in matters related to mycotoxin and food contamination. The responses showed the perception in the counties regarding maize contamination.



# **Figure 4.19: Maize Contamination Determination in the Counties**

## 4.1.10 Contamination Impacts and Awareness

This section targeted the respondent's ways of measuring impacts and awareness of mycotoxin contamination in foodstuff.

# 4.1.10.1 Food Safety-Related Organizations

The question sought to find out if food safety-related organizations were in the counties. The expected responses were yes or no. 61 % of the respondents indicated not knowing any food safety-related organizations in the county, 24 % reported being aware of several food safety organizations in the county and 15 % did not provide any response to the question (Figure 4.20). According to the findings food safety-related organizations are few in the counties with

little impact in the maize chain. Focus group discussion revealed knowledge of some institutions involved in food safety such as the Kenya Bureau of Standards (KEBS), University laboratories, Kenya Plant Health Inspectorate Service (KePHIS), the Consultative Group on International Agricultural Research (CGIAR) centers, and Government Chemist Department.



Figure 4.20: Knowledge of Food Safety Organizations

# 4.1.11 Training on Food Safety

The question targeted to determine the presence and utilization of services from food safety organizations. 64 % of the respondents had attended different workshops on food safety programs organized by Universities, NGOs, and Millers associations. 29 % of the respondents had no information on food safety training, workshops, or seminars. 7 % of the respondents did not respond to the question (Figure 4.21). The majority of the respondents knew about food safety training, workshops, and seminars. Staple food crops are highly predisposed to toxic contaminations, underpinning the importance of training farmers on food safety guidelines necessary to minimize common exposures and related health hazards.



# Figure 4.21: Food Safety Awareness Training

# 4.1.12 Knowledge of Aflatoxin Contamination

The question sought to determine the level of awareness of aflatoxin contamination of maize chain players. Knowledge of aflatoxin contamination was clear to 68 % of the respondents, 19 % had no information on aflatoxin contamination and 13 % had no answer to the questions (Figure 4.22). The majority of the respondents had information on aflatoxin contamination in maize. The number of respondents who were not aware of aflatoxin contamination was also big and showed the need for awareness creation. The information on aflatoxin contamination, prevention, and control is important to prevent health-related effects, economic losses, and a decline in livestock productivity.



Figure 4.22: Level of Aflatoxin Contamination Knowledge in the Counties

## 4.1.13 Aflatoxin Contamination Prevention

The question targeted to determine prevention methods applied by respondents to control aflatoxin contamination. The respondents suggested different methods of controlling aflatoxin contamination in maize; 60 % suggested drying maize to prevent contamination, and 5 % said appropriate storage methods. One percent suggested good agricultural practices and 3 % suggested regular seminars and workshops (sensitization) to maize farmers. Other respondents suggested planting certified seeds while another ground suggested appropriate harvesting and storage bags. The two groups represented 2 % each of the total respondents. In addition, 28 % of the respondents did not respond to the question (Figure 4.23). The majority of the respondents believed the best way of preventing aflatoxin contamination was by drying maize to the NCPB-recommended moisture content of 13 %. The respondents however may not attain the moisture level because of variations in weather, seasons, and harvesting time.



# Figure 4.23: Suggested methods for Prevention of Aflatoxin Contamination in Maize

#### 4.1.14 Effects of Consuming Aflatoxin Contaminated Foods

The question sought to determine the level of knowledge on the effect of consuming aflatoxincontaminated foods. 70 % of the total respondents were aware of the effects of consuming aflatoxin-contaminated maize. 23 % were not aware if aflatoxin contamination in maize had any possible effect, while 7 % provided no answer (Figure 4.24). The majority of the respondents were aware of the effects of consuming aflatoxin-contaminated maize. Equally, a large number of community members required information about the effects of consuming aflatoxin-contaminated maize.



Figure 4.24: Knowledge of 5he Effects of Consuming Aflatoxin-Contaminated Maize 4.1.15 Causes of Aflatoxin Contamination in Cereals

This question evaluated the respondents' awareness of possible causes of aflatoxin contamination in cereals. 69 % of the respondents suggested poor post-harvest handling methods were the leading cause of contamination in maize. 25 % of the respondents suggested poor storage contributed to aflatoxin contamination in cereal. 4 % and 2 % of the respondents did not provide any response and did not know the causes of aflatoxin contamination respectively (Figure 4.25). The majority of the respondents were aware of the causes of aflatoxin contamination in cereals, which included harvesting techniques and storage methods.



Figure 4.25: Suggested Causes of Aflatoxin Contamination in Cereals

# 4.1.16 Management of Aflatoxin Contaminated Maize

This question sought to determine methods used by respondents to manage aflatoxincontaminated maize in the counties. 85 % of the respondents suggested blending with good maize to dilute contamination for sell in the market. 3 % of the respondents recommended seizing aflatoxin-contaminated maize and putting it in custody for burning and burying. One percent recommended dehulling and cooking with Magadi soda. Another 1 % suggested feeding the affected maize to livestock, 2 % were not sure and 8 % did not provide any response (Figure 4.26). The results show that the majority of the respondents supported alternative methods, which included blending and selling, feeding to livestock, or using Magadi soda rather than burying or burning. The mixing of contaminated maize with uncontaminated to dilute the contamination level was not a good solution. This showed the level of ignorance or poverty in the farming members who hate losing capital from previous crops.



## Figure 4.26: Management of Aflatoxin-contaminated Maize

## 4.1.17 Effects Consuming Aflatoxin Contaminated Maize

The question sought to determine the respondents' level of awareness of diseases or ailments caused by consuming aflatoxin-contaminated cereals. 39 % of the respondents cited cancer as a likely ailment resulting from eating aflatoxin-contaminated cereals. 3 % of the respondents cited stomachaches, diarrhea, and death by 7 %. Another 8 % of the respondents cited deformities and stunted growth in children while 10 % cited infertility. Among the participants, 33 % did not respond to the question (Figure 4.27). The findings revealed that most respondents were aware of one or more diseases or ailments resulting from ingesting aflatoxin-contaminated food with cancer being the most cited. In addition, the large number of respondents who gave no response indicates a low level of awareness of any diseases or ailments associated with the consumption of aflatoxin-contaminated maize.



#### Figure 4.27: Effects Consuming Aflatoxin Contaminated Maize

## 4.1.18 Decontamination Methods of Aflatoxin Contaminated Maize

The question sought to determine methods used by respondents to remove aflatoxin contaminants in maize. 42 % of the respondents did not know any method that could control aflatoxin contamination. 10 % of the respondents suggested harvesting at physiological maturity and drying of maize to the accepted moisture content of between 12- 15 % and ensuring good ventilation. 13 % of the respondents suggested the use of biocontrol including pest control to prevent contamination of maize by aflatoxigenic fungi. 25 % of the respondents suggested burning or burying the affected maize, to prevent further contamination. Another group of respondents 2 % suggested training of farmers in good agricultural practice and land preparation to avoid maize contamination. 3 % of respondents suggested testing of soil quality to control aflatoxin infection in maize. Still another group of respondents 2 % suggested planting certified seeds would control aflatoxin contamination. The last group of respondents 3 % suggested the use of chemicals to react with aflatoxin contaminants in maize as a method to reduce contamination (Figure 4.27).

A large group of maize chain players has little knowledge of methods that would control aflatoxin contamination in maize. The methods suggested needed scientific input for them to be adapted as a solution to aflatoxin contamination in maize. This might be ineffective, expensive and therefore, predisposing consumers to aflatoxin toxicity dangers.



Figure 4.27: County Suggested Methods to Control of Aflatoxin Contamination in Maize

## 4.1.19 Summary of the Findings from the Questionnaires

The first objective assessed the socioeconomic impacts of aflatoxin contamination in maize on the people living in the eleven counties selected. From the result of the analysis of completed questionnaires, most participants were aware of the various effects of consuming aflatoxincontaminated food, however, the control measures and awareness creation activities were not adequate to prevent health risks associated with aflatoxin and management of the impacts of the toxicants on micro-economies.

Post-harvest handling practices and procedures varied greatly from the field to the store. Other contributing factors included storage conditions such as aeration, light, moisture content, and levels of atmospheric gases in the elements. The majority of the respondents had information on aflatoxin contamination in maize but 32 % did not. A large population lacked awareness of aflatoxin contamination, prevention, and management of aflatoxin-affected crops and by-products. Lack of knowledge was associated with health-related effects, economic losses, and a decline in livestock productivity.

#### 4.2 Aflatoxin Contamination Prevalence in Maize for Selected Counties in Kenya

## 4.2. 1 Analysis and Quantification of Aflatoxin Contaminants in Maize

Maize samples used in the study were collected from selected counties in Eastern, Rift Valley Western parts of Kenya, and Nairobi county. Maize matures at different times of the year in each of these regions with Western and Rift Valley planting in February and harvest in December, Eastern region maize is grown and harvested twice in a year; grown in March and harvested in July –August and in November and harvested in March. Farming in Nairobi is limited to very few people having land to grow maize, especially in areas near Kajiado and Kiambu counties. National Cereal and Produce Board's headquarters is in Nairobi where maize is sometimes stored before selling to local millers.

Nairobi is the main market for green and dry maize for all maize growers in the country directly or indirectly (De Groote & Kimenju, 2012). Nairobi being the capital city and business hub has a population of about 5 million people who depend on food brought in from other parts of the

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country. Private distribution stores have similar characteristics, with a holding capacity from 1 to 100 metric tons for commercial purposes. Government-controlled depots have a holding capacity of more than 100 metric tons for national food security.

Maize samples were collected from the eleven maize-growing counties selected from Eastern, Nairobi, Rift Valley, and Western parts of Kenya.

Six hundred and thirty (630) maize samples each weighing 1 kg were randomly collected from the national cereals produce board deports, open markets, retail, and farmer stores for aflatoxin analysis and quantification. 594 (94 %) out of 630 samples collected and analyzed had total aflatoxin contaminants that varied in frequency and concentration from one store type to another and county and region. 354 (59.6 %) of the contaminated samples had a total aflatoxin contamination load above the East Africa tolerable level of 10  $\mu$ g/kg.

The total aflatoxin levels detected in the samples ranged from below detectable level (reported in Nakuru and Busia counties) to 198.45  $\mu$ g/kg (reported in Makueni county) Eastern region. The eastern region had the highest total aflatoxin levels compared to the other three regions. Maize samples from the five counties selected in eastern Kenya had total aflatoxin contamination in order of ranked from highest to lowest Makueni> Isiolo>Machakos> Meru > Embu. The total aflatoxin contamination in maize collected from other counties in Rift Valley, Western Kenya, and Nairobi, had much lower values as shown in (Table 1).

County	Sampled site	B1	B2	G1	G2
Kajiado	NCPB Depot	8.50±0.88	4.34±0.86	5.36±0.34	3.93±0.57
	Market store	4.01±0.41	1.84±0.01	3.03±0.52	1.72±0.21
	Retail store	5.55±0.30	2.27±0.00	4.53±0.51	1.92±0.03
	Farmer store	2.99±0.45	$0.84 \pm 0.00$	2.55±0.95	1.08±0.19
County	Sampled site	B1	B2	G1	G2
-------------	------------------	-------------	------------	------------	------------
Nairobi	NCPB HQ store	2.83±0.53	0.58±0.00	1.24±0.00	0.17±0.00
	NCPB Depot	5.61±0.46	1.98±0.07	3.19±0.66	1.63±0.00
	Market store	10.4±0.25	4.51±0.61	6.55±0.78	2.36±0.22
	Retail store	16.33±0.91	4.81±0.86	14.24±1.72	3.46±0.19
Nakuru	NCPB depot	BDL	BDL	BDL	BDL
	Market store	1.84±0.74	0.16±0.03	0.03±0.00	0.82±0.00
	Retail store	2.25±0.17	0.02±0.00	1.17±0.36	0.15±0.00
	Farmer store	2.49±0.65	0.83±0.00	0.46±0.00	BDL
Busia	NCPB Depot	0.50±0.00	BDL	BDL	BDL
	Farmer store	1.45±0.50	0.99±0.00	0.15±0.00	BDL
	Market store	1.60±006	BDL	BDL	BDL
	Retail store	1.74±0.00	0.66±0.00	1.04±0.04	0.21±0.00
Migori	NCPB Depot	3.04±0.79	1.05±0.04	1.15±0.33	0.65±0.00
	Market store	18.60±1.46	4.28±0.91	10.51±0.37	2.28±0.51
	Farmer store	23.75±2.21	7.43±0.76	21.02±1.48	9.16±1.39
	Retail store	4.83±0.30	15.48±2.41	17.67±2.14	5.60±0.57
Trans Nzoia	NCPB Depot	9.37±0.81	4.64±0.69	8.74±0.74	3.65±0.73
	Market store	4.36±0.87	3.06±0.98	4.57±0.58	2.33±0.04
	Farmer store	9.48±0.38	2.88±0.36	5.81±1.23	2.95±0.03
	retail store	33.54±4.72	8.98±1.66	16.17±1.84	5.02±0.49
Isiolo	NCPB Depot store	46.23±2.93	19.49±1.40	26.63±1.95	10.05±0.4
	Market store	51.27±3.28	14.64±1.19	36.25±2.41	10.95±0.58
	Retail store	60.51±8.48	12.33±0.56	32.68±1.49	12.69±1.77
	Farmer store	82.33±2.95	16.86±0.63	14.71±1.45	5.11±0.11
Meru	NCPB Depot store	55.08±2.91	4.16±1.23	22.58±1.83	4.32±0.91
	Market store	46.02±3.87	23.96±2.87	23.48±1.47	5.34±0.64
	Retail store	36.90±2.25	30.21±1.92	25.63±1.39	6.38±0.99
	Farmer store	41.13±2.15	26.01±0.48	22.93±1.82	8.59±0.96
Embu	NCPB Depot store	6.26±0.14	2.16±0.06	5.19±0.56	3.42±0.45
	Market store	4.02±0.84	0.95±0.00	3.41±0.49	1.51±0.76
	Retail store	24.31±3.96	13.64±1.93	4.96±1.13	4.86±0.52
	Farmer store	40.24±5.54	15.53±0.05	38.50±3.34	25.65±4.72
Makueni	NCPB Depot store	92.66±5.78	4.88±1.15	52.12±1.25	4.87±1.19
	Market store	83.67±10.41	19.13±1.02	45.27±6.73	22.67±4.76
	Retail store	82.91±10.56	22.23±1.47	53.33±1.70	17.15±5.91
	Farmer store	73.02±3.37	26.33±1.79	46.81±2.73	21.41±2.93
Machakos	NCPB Depot store	38.27±2.75	9.60±0.79	42.62±3.55	9.37±1.91
	Market store	36.34±6.26	15.15±0.22	38.22±3.16	9.94±0.08
	Retail store	54.81±1.44	12.08±0.63	25.76±1.11	5.16±0.11
	Farmer store	45.62±8.41	23.33±1.32	26.38±1.59	5.42±0.81

The other counties were Kajiado, Nakuru, and Trans- Nzoia for Rift Valley, Migori and Busia for western Kenya, and Nairobi County. The total aflatoxin per store for the county is represented in figure 4.28 below. The underlying factors responsible for the observed trends include climatic, environmental, seeds planted, agronomic practices, and biotic and abiotic factors that vary from one region to another (Mutegi *et al.*, 2012).





4.2.2 Comparison of Aflatoxin Contamination in Maize Samples

#### 4.2.2.1 Aflatoxin Strains Regional Comparison, Mean and Probability Distribution

Samples collected were from the 11 counties and analyzed for the presence of the four-aflatoxin strains, mean and probability distribution determined and compared in regions. Aflatoxin B1 had the highest median that was in the middle quarter while B2, G1, and G2 had their mean in

the lower quarter as shown in the violin plot (Figure 4.29) below. The whisker lines and symbols inside the boxes represent the interquartile range and median respectively. The probability distribution for the four toxins varied from horizontal to vertical as seen in the same figure. The figure has multiple layers; the outer shape represents all possible results. The next layer inside might represent the values that had 95 % occurrence. Aflatoxins B1 and G1 seem to have a higher prevalence than B2 and G2 but with a positive skewness.





## **4.2.2.2** Correlation Between Aflatoxins and Distribution of the Contaminants in Maize Samples

The correlation tests between aflatoxins B1, B2, G1, and G2 showed that all were strongly correlated (> 0.75) among themselves. The Spearman rank correlation between them was aflatoxin B1 and B2 = 0.88, B1 and G 1= 0.92, B1 and G2 = 0.87, between B2 and G1 = 0.89,

B2 and G2 = 0.86 and G1 and G2 = 0.96. The distribution had a positive skew to the left and the lower quantile according to the Kernel density estimation plots (Figure 4.30). On the distribution of contaminants in maize using the ranks shown in the same histogram plots (Figure 4.30).; aflatoxin B1 had more than 80  $\mu$ g/kg in 20 %, G1 had more than 50  $\mu$ g/kg in only 10 %, B2 had less than 30  $\mu$ g/kg, and G2 more than 25  $\mu$ g/kg, both in only 5 % of the estimated rank.



Figure 4.30: Correlation between Aflatoxins and distribution of the contaminants in maize samples

Regional analysis of contamination revealed that the Eastern region had the highest aflatoxin B1, B2, G1, and G2 strains compared to the other regions. The aflatoxin B1, B2, G1 and G2 mean values for the region were  $50.08 \pm 4.42$ ,  $17.26 \pm 1.08$ ,  $30.17 \pm 2.06$  and  $10.54 \pm 1.52$  (µg/kg). The mean values for aflatoxin B1, B2, G1 and G2 strains in the western part were  $9.36 \pm 0.97$ ,  $4.12 \pm 0.65$ ,  $7.24 \pm 0.73$ , and  $2.65 \pm 0.32$  µg/kg, respectively, followed by Nairobi at  $8.74 \pm 0.54$ ,  $2.97 \pm 0.37$ ,  $6.31 \pm 0.79$  and  $1.90 \pm 0.11$  µg/kg. The least mean concentrations of aflatoxin B1, B2, G1, and G2 strains in Rift valley were  $4.37 \pm 0.27$ ,  $1.48 \pm 0.19$ ,  $3.15 \pm 0.40$  and  $0.95 \pm 0.05$  µg/kg, respectively (Figure 4.31).



Figure 4.31: Comparison of Aflatoxin Contamination in Maize Samples from Selected Counties

# 4.2.3: Regional Comparison of Aflatoxin Strains in Terms of Mean and Probability Distribution

Samples were analyzed for the presence of the four aflatoxin strains in terms of regions. The mean and probability distribution were determined and compared. Aflatoxin B1 had the highest median that was in the middle quarter while B2, G1, and G2 had their mean in the lower quarter as shown in the box plot (Figure 4.32) below. The counties in the Eastern region showed a high concentration of the contaminants compared to the other three regions. The whisker lines and symbols inside the boxes represent the interquartile range and median respectively. The probability distribution for the four toxins varied from horizontal to vertical as seen in the same figure. The figure has multiple layers; the outer shape represents all possible results. The next layer inside might represent the values that had 95 % occurrence.



### Figure 4.32: Regional Comparison of Aflatoxin Strains Median Contaminants Level and Probability Distribution

Different regional microclimatic conditions, soil profiles, and agronomic practices some extent contributed to the observed prevalence of aflatoxin contamination in cereals. Climatic and environmental factors fluctuation influence crop growth directly. They also influence the growth of mycotoxigenic fungi communities responsible for contaminating farm products (Camaro *et al.*, 2019; Shekhar *et al.*, 2018; Medina *et al.*, 2015). Consequently, comparative threats and incidences of certain fungi and mycotoxicosis were reported.

Several regions have witnessed toxigenic molds and pest invasion of crops owing to climatic conditions and other factors. The targeted crops are maize, wheat, groundnuts, sorghum, and cassava (Elshafie *et al.*, 2011). Other studies also have linked aflatoxin development to oxidative stress from abiotic and biotic plant stressors (Fountain *et al.*, 2019; Venkateswarlu *et al.*, 2012; Kebede *et al.*, 2012; Jayashree & Subramanian, 2000). *Aspergillus flavus* usually colonizes stressed plants with broken and injured grain kernels in the field, threshing, and during transportation. Cereal grains in this state have a high chances of aflatoxin infestation (Patterson and Lima, 2010).

Minimizing these effects could be through preventing mycotoxin contamination at the cereal development and handling stage (Shiferaw *et al.*, 2011). Isiolo, Makueni, Machakos, Embu, and Meru counties in August – October experience hot and dry weather. Sometimes droughts which affect soil and air temperatures hurt crop production in terms of quantity and quality (Gichangi *et al.*, 2015). Soils in Isiolo county are undeveloped, and loss and depletion of nitrogen and other nutrients are fast (Mora-Vallejo *et al.*, 2008). This increases stress on field crops like maize.

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Farmers in Migori and Isiolo counties practice mixed cropping without observing spacing hence overcrowded plant populations that also increase their stress. The food crops in such practices compete for nutrients and occasionally are stressed reducing their phyto -immunity. In some cases, drought, wind, competition for nutrients with weeds, pests, and crop diseases are common in counties where extension services are not intensive (Soares *et al.*, 2019).

The height above the sea level, nature, and type of soil determine the seed varieties appropriate for each region and sub-region. Other facts that have contributed to the observation include different technical and financial abilities, post-harvest handling practices, and mode of transportation and storage conditions (Koskei *et al.*, 2020). Observed were huge variations of aflatoxin strains B1, B2, G1, and G2 contamination levels in cereals collected in 11 counties. Nine, out of these 11 counties have similar rainfall patterns, received from October to May with peaks in March-May and October -December.

These counties are Nairobi, Kajiado, Machakos, Makueni, Embu, Meru, Busia, and Migori while Nakuru and Trans Nzoia receive rainfall from March to November. All the counties have hot and dry months at different times of the year with occasional droughts experienced in Eastern counties and Kajiado. Rainfall patterns are part of microclimates in the regions that have different precipitation, relative humidity, and temperature (Sserumaga *et al.*, 2020; Van der Fels-Klerx *et al.*, 2019; Alvarado *et al.*, 2017). Some of the mitigation measures for drought and related include planting varieties with short maturity periods and irrigation. This measure significantly reduces crop water stress hence minimizing chances of aflatoxin contamination incidences (Kebede *et al.*, 2012; Young *et al.*, 2012).

Aflatoxin molecules are exceedingly resilient to transportation, food handling, and processing and storage conditions. Maintaining optimal temperatures and moisture content favors **98** | P a g e mycotoxin production. That is maximum moisture content levels of 25 % at 30 ° C temperature and minimum relative humidity of between 83 % and 88 % are good conditions for aflatoxins production in maize kernels (Houssou *et al.*, 2009; Kaaya *et al.*, 2006; Hell *et al.*, 2000b). Reduced oxygen level from 1- 10 % repress slows down the growth and production of aflatoxins in aerobic conditions (Gnonlonfin *et al.*, 2013).

Pre- and post-harvest pests and insect attacks are vectors for fungal and bacterial diseases spreading to uncontaminated cereals or are vectors to the same (Nduti *et al.*, 2017; Waliyar *et al.*, 2015; Gnonlonfia *et al.*, 2013). Fumonisin and aflatoxins are among the common mycotoxins that take advantage of insect damage on maize kernels. According to Matumba *et al.* (2015), the relative significance of insect damage relies on the population of the insects in the region, which may affect the plant resistance mechanisms of particular fungi and the environmental conditions that favor the growth of the fungi. Insect damage on the crop increases the surface area upon which mycotoxin fungi colonize (Tola & Kebede, 2016).

Contaminating fungi on maize affects germination qualities, digests and produces volatile metabolites, utilizes maize starch for energy, and degrades lipids and proteins. The metabolites sometimes are involved in the competition mechanism and play an important role in systemic resistance against predators, parasites, and diseases (Siddiquee et al., 2012). High total aflatoxin contamination levels reported in the eastern region of Kenya show how aflatoxin exposure varied from one region to another (Jacob *et al.*, 2020; Obonyo *et al.*, 2018).

The regional high levels of aflatoxins probably could be associated with the prevailing conditions which include; the presence of different strains of *A*. flavus, climatic conditions, period of storage, and uncoordinated post-harvest management. These findings suggest that a majority of maize farmers, harvested, shelled, and stored the product in their stores, and at **99** | P a g e

times they sold it in the local county markets, retail traders, and NCPB stores (Jacob *et al.*, 2020). Daniel *et al.* (2011) had earlier reported aflatoxin contamination levels of 48,000  $\mu$ g/kg in maize collected from a farmer's store eastern region.

According to this study, high levels of aflatoxin contamination occur in any form of maize storage, suggesting that toxigenic fungi may be airborne or soil borne. Variations in aflatoxin contamination occurrence observed across the counties are attributed to environmental conditions such as precipitation, relative humidity, temperature, and drought that are unique to the region. The high prevalence rate is attributed to the warmer and drier microclimates depending on the zoning (Hell *et al.*, 2003).

Variations in environmental factors may lead to the occurrence of illnesses and the emergence of newer strains of diseases (Medina *et al.*, 2014). Spatial climatic patterns have an impact on food contamination with a direct influence on the *Aspergillus flavus* fungi growth (Cotty & Jaime-Garcia, 2007; Gnonlonfin *et al.*, 2013). Aflatoxin B1 contamination level was high in all farmers' stores in the five counties out of the eleven.

The use of insecticides in the field, purchase of low-quality maize for storage, and reuse of storage bags were associated with high levels. More reasons were untimely control of storage pests and store fumigation to control host toxigenic fungi spores that sprout to colonize new crops when conditions favor (Mwangi *et al.*, 2017, Nyakio, 2015). Invasion of crops by toxigenic blights aided by vectors, for instance, insect pests equally move concerning the climatic changes (Zain 2011; Paica *et al.*, 2013; Marechera and Ndwiga, 2014). Rare store fumigation before new harvest is stored (Mwangi *et al.*, 2017). The farmers' livelihoods revolve round selling their farm products; hence, the reason for finding similar contamination levels in maize from open county markets or individual retailers (Ren *et al.*, 2020). Colonized **100** | P a g e

and contaminated grain kernels by *Aspergillus flavus* had earlier suffered stresses (Kumar *et al.*, 2017).

The five counties in the eastern region are within the agro-climatic conditions of the moist transitional, moist mid-altitude, and dry mid-altitude zones. These zones have temperatures above 30 °C and relative humidity between 83 % and 88 %. These conditions are ideal for the growth of *Aspergillus species* to produce aflatoxin when maize is not dried to the required moisture content (Houssou *et al.*, 2009; Kaaya *et al.*, 2006). Most maize stores are located in towns and along the roads, some time with heavy traffic, contributed partly to maize stress. High contamination levels observed in some NCPB depots could be associated with ventilations or storage conditions that favor mycotoxins under aerobic conditions (Gnonlonfin *et al.*, 2013).

Physical and mechanical threshing methods used by farmers caused shocks, breakage, and cracks to the cereal grains that increased susceptibility to mycotoxins infestation. Storage, drying, and transportation modes may be inappropriate leading to post-harvest contaminations (Kang'ethe *et al.*, 2017; Yard *et al.*, 2013; Zain, 2011; Daniel *et al.*, 2011; Lewis *et al.*, 2005). All four store types had a high frequency of aflatoxin incidences, a finding that agreed with previous studies about the mycotoxin hotspots in eastern Kenya (Kaaya *et al.*, 2005). The results paint the negative impact of aflatoxin contamination on the regional maize market and household consumption of the grains (Kang'ethe *et al.*, 2017).

#### 4.2.4 Aflatoxin Contamination in Maize from selected Counties Eastern Kenya

Eastern Kenya is a vast area with a different climatic condition, attracting various economic activities such as agricultural (crop and animal farming), horticulture, trade and tourism. The

region according to the Kenya population and housing census (2019) has 6,821,049 people, distributed in eight counties. The counties are Marsabit, Isiolo, Meru, Embu, Kitui, Tharaka Nthi, Makueni and Machakos. The counties grow maize with the main growers being Meru, Embu, Tharaka Nthi, Kitui, Makueni, and Machakos. The study focused on five out of seven main growers (Figure 4.33).



Figure 4.33: Aflatoxin Contamination in Maize Samples from Counties in Eastern Kenya

#### 4.2.4.1 Isiolo County

The samples collected from selected stores in Isiolo were analyzed and quantified for aflatoxin strains B1, B2, G1, and G2. The mean value for B1 -farmers store  $82.33\pm2.95 \ \mu g/kg$ , retailer

stores 60.51±8.48 µg/kg, open market stores 51.27±3.28 µg/kg and NCPB depot 46.23±2.93 µg/kg. For aflatoxin B2 the mean values for NCPB depot 19.49±1.40 µg/kg, farmers store 16.86±0.63 µg/kg, open market stores 14.64±1.19 µg/kg, and retailer stores 12.33±0.56 µg/kg. The mean value for G1 - open market stores 36.25±2.41 µg/kg, retailer stores 32.68±1.49 µg/kg, NCPB depot 26.63±1.95 µg/kg and farmers store 14.71±1.45 µg/kg.

The mean value for G2 were retailer stores  $12.69\pm1.77 \ \mu g/kg$ , open market stores  $10.95\pm0.58 \ \mu g/kg$ , NCPB depot store  $10.05\pm0.20 \ \mu g/kg$  and farmers store  $5.11\pm0.11 \ \mu g/kg$ . All stores in Isiolo had high aflatoxin levels for B1, B2, G1 and G2 contamination. The farmers' store recorded the highest mean levels of aflatoxin. Maize samples collected from the county had aflatoxin contamination mean levels above the guideline limit of 4  $\mu g/kg$  for aflatoxin B1, and  $10 \ \mu g/kg$  for total aflatoxin (FAO/WHO, 1995, 2015) (Figure 4.33).

Variation in findings from one store to another supports the fact that storage and other handling conditions were different including handling techniques. Maize farms had different soil qualities including a pH range of 5.90-8.60, seed varieties were different, and harvesting and shelling methods differed. Isiolo climate ranges from Semi-Arid to Arid, the implication was that the maize crop might have experienced various stresses during its maturation cycle from dry, hot, windy, and drought conditions. The County of Isiolo is 1095 meters above sea level, with 23.3 °C and about 714 mm average annual temperature and precipitation falls, respectively.

The varieties of maize grown in the region require 1,200 - 2,500 mm annual rainfall, a huge imbalance hence high possibilities of stress on maize grown exposed to *Aspergillus flavus* vulnerabilities and later aflatoxin contaminations. Observed high values of aflatoxin B1 in the maize stores agree with the expected observation and soil properties that affect water retention **103** | P a g e

and nutrient availability. These influence plant health, susceptibility to fungal colonization, and aflatoxin production (Smith *et al.*, 2016).

#### 4.2.4.2 Meru County

Maize samples collected from Meru stores were analyzed for the aflatoxin strains B1, B2, G1 and G2. The mean value for aflatoxin B1 was NCPB depot  $55.08\pm2.91 \ \mu\text{g/kg}$ , open market stores  $46.02\pm3.87 \ \mu\text{g/kg}$ , farmers store  $41.13\pm2.15 \ \mu\text{g/kg}$ , and retailer stores  $36.90\pm2.25 \ \mu\text{g/kg}$ . The mean value for aflatoxin B2 was retailer stores  $30.21\pm1.92 \ \mu\text{g/kg}$ , farmers store  $26.01\pm0.48 \ \mu\text{g/kg}$ , open market stores  $23.96\pm2.87 \ \mu\text{g/kg}$  and NCPB depot  $20.16\pm1.23 \ \mu\text{g/kg}$ . The mean value for aflatoxin G1 was retailer stores  $25.63\pm1.39 \ \mu\text{g/kg}$ , open market stores  $23.48\pm1.47 \ \mu\text{g/kg}$ , farmers store  $22.93\pm1.82 \ \mu\text{g/kg}$  and NCPB depot  $22.58\pm1.83 \ \mu\text{g/kg}$ .

The mean value for aflatoxin G2 was farmers store  $8.59\pm0.96 \ \mu\text{g/kg}$ , retailer stores  $6.38\pm0.99 \ \mu\text{g/kg}$ , open market stores  $5.34\pm0.64 \ \mu\text{g/kg}$  and NCPB depot  $4.32\pm0.91 \ \mu\text{g/kg}$ . All stores in Meru had high aflatoxin B1, B2, G2, and G1 contaminants for the tested maize samples. Aflatoxin B1 was high for all the stores with NCPB depot recording the highest mean contamination. These values were way above the guideline limit of 4  $\mu\text{g/kg}$  for aflatoxin B1 and 10  $\mu\text{g/kg}$  for total aflatoxin for human foods (Figure 4.33).

The spatial location for Meru County is on the windward side and foot of Mt. Kenya, the second tallest mountain in Africa and home to Nyambene hills. Climatic factors and environmental factors might have influenced aflatoxin contamination in maize. Slightly more than half of Meru County has sufficient rainfall ranging from 370 mm -2,800 mm, fertile volcanic soils with pH ranging from 4.2-8.6, temperatures between 8 °C and 28 °C, and favorable altitude between 600 -2,145 meters above the sea level. Most of the county is wet, with high water activity and climatic conditions influence fungi growth in maize. Nevertheless, part of the **104** | P a g e

county experiences drought stress, particularly during flowering and early grain-filling stages, a condition associated with increased aflatoxigenic fungal infection and proliferation in maize (Omara *et al.*, 2021; Waldman *et al.*, 2019).

Despite the spatial difference in the county, maize matures for harvest during the long rain season in March–May months. Crop harvested at the time has high moisture content and storage may result in fungal colonization and aflatoxin contamination. Farmers also contribute to pressure on maize by overcrowding with other crops to maximize land due to high population and land scarcity. The observed contamination levels in maize collected from different stores in Meru indicator abiotic and biotic factors on the ground that are linked directly to aflatoxigenic fungal infection.

#### 4.2.4.3 Embu County

Maize samples collected from different stores in Embu County were analyzed for aflatoxin strains B1, B2, G1, and G2. The mean value for aflatoxin B1 in the stores was  $40.24\pm5.54$  µg/kg, retailer stores  $24.31\pm3.96$  µg/kg, NCPB depot  $6.26\pm0.14$  µg/kg, and open market stores  $4.02\pm0.84$  µg/kg. The mean value for aflatoxin B2 for farm stores was  $15.53\pm0.05$  µg/kg, retailer stores  $13.64\pm1.93$  µg/kg, NCPB depot  $2.16\pm0.06$  µg/kg, and open market stores  $0.95\pm0.00$  µg/kg. The mean value for aflatoxin G1 in farm stores was  $38.50\pm3.34$  µg/kg, retailer stores  $20.96\pm1.13$  µg/kg, and NCPB depot  $5.19\pm0.56$  µg/kg, while open market stores had  $3.41\pm0.49$ .

The mean levels of aflatoxin G2 contaminants in farm stores were  $25.65\pm4.72 \ \mu g/kg$ , retailer stores  $4.86\pm0.52 \ \mu g/kg$ , NCPB depot  $3.42\pm0.45 \ \mu g/kg$ , and open market stores  $1.51\pm0.76 \ \mu g/kg$ . Samples from different stores in the county had high aflatoxin B1, B2, G1, and G2 contaminant levels. Farm and retail stores had higher levels of contaminants compared to the **105** | P a g e other two outlets. Farm stores had the highest Aflatoxin B1 contamination level probably due to lack of extension services in the county. Therefore, mitigation measures, moisture meters, and aflatoxins testing equipment would assist farmers in monitoring grain moisture content and overall surveillance. Weather information and early warning systems necessary to assist farmers in planning were not sufficient. Communication on Aflatoxins standards to ensure optimal safety for maize consumers was ad hoc. Contamination levels were above the accepted limit of 4  $\mu$ g/kg for aflatoxin B1 and 10  $\mu$ g/kg for total aflatoxin in human foods (Figure 4.33).

Embu County is located on the windward side of Mt. Kenya with sufficient rainfall, ranging between 600 - 2,500 mm annually, and fertile volcanic soils with pH ranging from 4.1-8.6, temperatures between  $12 \,^{\circ}$ C -  $30 \,^{\circ}$ C and altitude between 515 - 5,199 m above sea level. These conditions are not only perfect for crop farming but also good for fungi proliferation. However, the lower Embu experiences drought stress during the flowering and early grain-filling stages, a condition associated with increased aflatoxigenic fungal infection in maize.

Common field pests include stem borers and birds which injure maize ears creating room for fungi spores on maize grains. Maize crops mature during the long rains in March–May at the time when grains have high moisture content. The field conditions lead to increased fungal colonization on maize and aflatoxin contamination during storage. Other parts of the county experience sub-optimal nitrogen availability, and coupled with crowded crops, exert pressure on land and stressed maize crops increasing chances of aflatoxin contamination.

The observed levels of aflatoxin contamination in maize samples collected from different county stores confirm the effect of abiotic and biotic factors on aflatoxigenic fungal infection in maize. However, NCPB deports and market stores that applied good handling procedures had low levels of aflatoxin contamination in maize.

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#### 4.2.4.4 Makueni County

Maize samples collected from different stores in Makueni County were analyzed for the aflatoxin strains B1, B2, G1, and G2. The mean levels for aflatoxin B1 were NCPB depot store 92.66- $\pm$ 5.78 µg/kg; open market stores 83.67  $\pm$ 10.41 µg/kg, retailer stores 82.91 $\pm$ 10.56 µg/kg, and farmers store 73.02 $\pm$ 3.37 µg/kg. The mean values for aflatoxin B2 contaminants were: farmers store 26.33  $\pm$ 1.79 µg/kg, retailer stores 22.23 $\pm$ 1.47 µg/kg, NCPB depot store 20.88 $\pm$ 1.15 µg/kg, and open market stores 19.13  $\pm$ 1.02 µg/kg.

The mean concentrations for aflatoxin G1 were: retailer stores  $53.33 \pm 1.70 \ \mu\text{g/kg}$ , NCPB depot store  $52.12 \pm 1.25 \ \mu\text{g/kg}$ , farmers store  $46.81 \pm 2.73 \ \mu\text{g/kg}$ , and open market stores  $45.27 \pm 6.73 \ \mu\text{g/kg}$ . For aflatoxin G2 mean concentrations were: open market stores  $22.67 \pm 4.76 \ \mu\text{g/kg}$ , farmers store  $21.41 \pm 2.93 \ \mu\text{g/kg}$ , NCPB depot store  $20.87 \pm 1.19 \ \mu\text{g/kg}$ , and retailer stores  $17.15 \pm 5.91 \ \mu\text{g/kg}$ . These values were way above the accepted limit of  $4 \ \mu\text{g/kg}$  for aflatoxin B1 and  $10 \ \mu\text{g/kg}$  for total aflatoxin in human foods (Figure 4.33).

All samples collected from different stores in Makueni County had aflatoxin B1, B2, G1 and G2. Aflatoxin B1 was found in all the samples from the four store types with NCPB deport observed to have the highest contaminants. The capacity to manage aflatoxin contamination needs was required to be cascaded to the lowest administration units.

#### 4.2.4.5 Machakos County

Maize samples collected from different stores in Machakos County revealed aflatoxin strains B1, B2, G1 and G2. The mean values for aflatoxin B1 were: retailer stores  $54.81\pm1.44 \ \mu\text{g/kg}$ , farmers store  $45.62\pm8.41 \ \mu\text{g/kg}$ , NCPB depot  $38.27\pm2.75 \ \mu\text{g/kg}$ , and open market stores  $36.34\pm6.26 \ \mu\text{g/kg}$ . The mean values for aflatoxin B2 contamination were: farmers store

 $23.33\pm1.32 \ \mu\text{g/kg}$ , open market stores  $15.15\pm0.22 \ \mu\text{g/kg}$ , retailer stores  $12.08\pm0.63 \ \mu\text{g/kg}$ , and NCPB depot  $9.60\pm0.79 \ \mu\text{g/kg}$ .

The mean aflatoxin G1 contamination for NCPB depot 42.62±3.55 µg/kg, open market stores 38.22±3.16 µg/kg, farmers store 26.38±1.59 µg/kg, and retailer stores 25.76±1.11 µg/kg. Aflatoxin G2 contamination in open market stores was 9.94±0.08 µg/kg, NCPB depot store 9.37±1.91 µg/kg, farmers store 5.42±0.81 µg/kg, and retailer stores 5.16±0.11 µg/kg. In general, the samples from Machakos had contamination above the accepted level of 4 µg/kg for aflatoxin B1 and 10 µg/kg for total aflatoxin (Figure 4.33).

Machakos County is located in an arid and semi-arid climatic zone in eastern Kenya, with elevation ranging from 400 to 2100 meters above sea level. It receives about 500 mm of annual rainfall with variations depending on altitude. For example, Kangundo and Iveti highlands receive about 1000 mm of annual rainfall. According to the CIDP, the annual rainfall received in the county ranges between 500 -1300 mm and 18 - 29 °C (Machakos County, 2015).

The county has a warm climate and sometimes prolonged drought. Experienced fluctuations in weather conditions at the critical stage of flowering and grain filling of maize crops tend to affect yield and increase the susceptibility to *Aspergillus flavus* attacks. Maize is very sensitive to water deficit during the critical stages for two reasons: high water is requirement in terms of evapotranspiration and high physiological sensitivity when determining its main yield components such as the number of ears per plant and number of kernels per ear.

The warm climate and prolonged drought conditions have a great likelihood of fungi infection in maize. These conditions influence insect and other pests' activities as well as different types of aflatoxigenic fungi in soil. The harvested grains may have mold growth and aflatoxin contamination due to climatic factors and storage conditions including moisture content, injuries, the presence of mold spores from non-fumigated stores, and storage facility aeration. The factors were the reason for the observed level of contamination in maize from these county stores.

#### 4.2.5: Aflatoxin Contamination in Maize from Nairobi Region

Nairobi is the smallest county in Kenya, by size. It has a climatic pattern compared to the surrounding regions Eastern, Rift Valley, and Central. Being a business and political capital city, Nairobi has attracted various economic activities key among them are; agribusiness (crop and animal products), manufacturing, trade, hospitality, and tourism. The region according to the Kenya population and housing census (2019) has 4,556,000 people, distributed in a 686 Km<sup>2</sup> county area. The region sources more than 80 % of food consumed from other regions. Food distribution is done through depots, wholesale markets, retail markets, and direct orders from farmers. The region has a constant supply round the year from other regions and neighboring countries. Less than 20 % of food in the region is produced in kitchen gardens and green houses.



Figure 4.34: Aflatoxin Contamination in Maize Samples from Nairobi County

The individual aflatoxin strains B1, B2, G1 and G2 in Nairobi County stores, mean measured values for aflatoxin B1 were: retail store  $16.33\pm0.91\mu$ g/kg, county market stores  $10.20\pm0.25\mu$ g/kg, farmers store  $5.61\pm0.46\mu$ g/kg and NCPB store  $2.83\pm0.53\mu$ g/kg. The mean aflatoxin B2 were: retailer stores  $4.81\pm0.86\mu$ g/kg, open market stores  $4.51\pm0.61\mu$ g/kg, farmers store  $1.98\pm0.07\mu$ g/kg, and NCPB store  $0.58\pm0.00\mu$ g/kg. The mean aflatoxin G1 were: retail store  $14.24\pm1.72\mu$ g/kg, county market stores  $6.55\pm0.78\mu$ g/kg, farmers store  $3.19\pm0.66\mu$ g/kg, and NCPB store  $1.24\pm0.00\mu$ g/kg. The mean aflatoxin G2 were: county market stores

 $2.36\pm0.22\mu$ g/kg, farmers store  $1.63\pm0.00\mu$ g/kg, NCPB store  $0.17\pm0.00\mu$ g/kg, and retail store  $3.46\pm0.19\mu$ g/kg. In general, the samples from Nairobi County had levels of aflatoxin contamination above the accepted level of 5 µg/kg for aflatoxin B1 and 10 µg/kg for total aflatoxin in all stores (Figure 4.34).

Nairobi County lies in between the edges of the Rift Valley region to the south and eastern region to the east and central to the North. The county has a subtropical highlands climate influenced by the altitude that rises 1800 m above sea level. The County receives a bimodal rainfall with an average annual of 610 mm. The long rains are in March and May and the short rains are in November – December and strongly vary from year to year. Temperatures fluctuate from the lowest of 16.5 °C in July to the highest of 27.5 °C in February.

Nairobi is the major market for all foods grown in other counties in Kenya. It has market and storage facilities, owned privately or by the government for food distribution including cereals. Maize is transported from farms, other deports, and holding stores across the country and beyond the borders. The transportation, storage conditions, climate, and environmental factors contributions to the observed data on aflatoxin contamination in maize.

#### 4.2.6 Aflatoxin Contamination in Maize from Selected Counties in Rift Valley

The Rift Valley region of Kenya is a vast area with different climates due to varied features like escapements, valleys, Mau Ranges, Nandi hills, Cherangany hill, Ngong hills, Aberdare Ranges, Mt. Kilimanjaro, Mt. Elegon, and others. Fresh water and salt-water lakes including geothermal fountains found in the region. Most of the soils are volcanic and loamy, good forming. The region attracts many economic activities key among them are agricultural (crop and animal farming), horticulture, floral culture, Apiculture, aquaculture, trade, hospitality and tourism, mining, and energy production.

The region according to the 2019 Kenya population and housing census has 12,752,966 people, distributed in 13 counties. The counties are Turkana, West Pokot, Samburu, Trans-Nzoia, Uasin Gishu, Elgeyo Marakwet, Nandi, Baringo, Laikipia, Nakuru, Kajiado, Kericho, and Bomet with a total area of 173,854 km<sup>2</sup>. Whereas all the counties grow maize, the major growers are Trans-Nzoia, Uasin Gishu, Elgeyo Marakwet, Nandi, Baringo, Laikipia, Nakuru, Kajiado, Kericho and Bomet. The study focused on three out of the 13 counties.



Figure 4.35: Aflatoxin Contamination in Maize Samples from Selected Counties in Rift Valley

#### 4.2.6.1 Kajiado

The four strains of aflatoxin analyzed were B1, B2, G1, and G2. Aflatoxin B1 contamination was: NCPB depot store  $8.50\pm0.88$  µg/kg, retailer stores  $5.55\pm0.30$  µg/kg, market stores  $4.01\pm0.41$  µg/kg and farmers store  $2.99\pm00$  µg/kg. The mean levels for aflatoxin B2 were: farmers store  $0.84\pm0.00$  µg/kg, open market stores  $1.84\pm0.01$  µg/kg, retailer stores  $2.27\pm0.00$  µg/kg, and NCPB depot store  $4.34\pm0.86$  µg/kg. Aflatoxin G1 registered contamination levels with NCPB depot store  $5.36\pm0.34$  µg/kg, retailer stores  $4.53\pm0.51$  µg/kg, open market stores

 $3.03\pm0.52$  µg/kg and farmers store  $2.55\pm0.95$  µg/kg. The mean values for aflatoxin G2 contamination were: NCPB depot store  $3.93\pm0.57$  µg/kg, retailer stores  $1.92\pm0.03$  µg/kg, open market stores  $1.72\pm0.21$  µg/kg and farmers store  $1.08\pm0.19$  µg/kg. In general, Kajiado samples had aflatoxin contamination above the accepted level of 5 µg/kg for aflatoxin B1 and 10µg/kg for total aflatoxin (Figure 4.35).

Kajiado County is located in an arid and semi-arid zone in the rift valley. It consists of hills and small plateaus with elevations ranging from 500 to 2500 meters above sea level in Ngong Hills. The county receives a bi-modal rainfall pattern with an average of 500 - 750 mm per year. These variations depend on the regional altitude and seasons. The short rains fall between October and December while the long rains fall between March and May. The monthly rainfall ranges from as low as 300 mm to as high as 1250 mm in Ngong hills and the slopes of Mt. Kilimanjaro. Temperatures fluctuate from the lowest of 10 °C in July and August to the highest of 34 °C from November to April depending on the altitude and seasons.

The county is known for livestock keeping and also for crop production which is mainly rainfed. The practice benefits from a high amount of rainfall being on the windward side of Mt Kilimanjaro. The impact of climate change includes decreased rainfall, increased drought, and a moderate increase in mean temperatures (Magan *et al.*, 2011). In addition, a remarkable increase in day heat stress, high variability, a slight decrease in precipitation, and increased flooding risk harm crop agriculture (Hooda *et al.*, 2016; Ongoma, 2013). Parts of Kajiado have good soils for crop production as well as poor soils that cannot support crop agriculture. These conditions total stress on sensitive crops like maize. These stress factors were the reason for the observed level of contamination in maize from all store types in Kajiado County.

#### 4.2.6.2 Nakuru

The mean values for aflatoxin B1 contamination were: NCPB depot store BDL, retailer stores 2.25 $\pm$ 0.17 µg/kg, market stores 1.84 $\pm$ 0.74 µg/kg and open farmers store 2.49 $\pm$ 0.65 µg/kg. The mean aflatoxin B2 contamination was farmers store 0.83 $\pm$ 0.00 µg/kg, open market stores 0.16 $\pm$ 0.03 µg/kg, retailer stores 0.02 $\pm$ 0.00 µg/kg, and NCPB depot store BDL. The mean aflatoxin G1 contamination were: NCPB depot store BDL, retailer stores 1.17 $\pm$ 0.36 µg/kg, open market stores 0.03 $\pm$ 0.00 µg/kg and farmers store 0.46 $\pm$ 0.00 µg/kg. The mean aflatoxin G2 contamination was NCPB depot store BDL, retailer stores 0.15 $\pm$ 0.00 µg/kg open market stores 0.82 $\pm$ 0.00 µg/kg and farmers store BDL. In general, samples collected from different stores in Nakuru County had aflatoxin contamination levels below the maximum guideline limits of 4 µg/kg for aflatoxin B1 and 10 µg/kg for total aflatoxin (Figure 4.35).

The Agro-Ecological Zones (AEZ) for Nakuru county are influenced by the altitude from 1480-1550 to 2980-3050 m above sea level. The county experiences bimodal rainfall patterns that vary between 500-1800 mm annually. The county receives short rains in October – December and long rains in March-May. Complex, soil distribution patterns fund in the county are influenced by climatic conditions, volcanic activities, and underlying rock type. The county has well-drained and fertile soils, good for crop agriculture. Also has poorly drained, infertile, and unproductive soils, which are not good for farming (GoK, 2013). Challenges encountered in Nakuru County are influenced by environmental and climatic factors, which include crop pests and diseases. These challenges are linked to mold growth and aflatoxin contamination but at a lower rate in comparison to the counties in eastern Kenya. These factors were the reason for the observed level of contamination in maize collected from all store types in the County.

#### 4.2.6.3 Trans Nzoia County

The mean value for aflatoxin B1 was retailer stores was  $33.54 \pm 4.72 \ \mu g/kg$ , open farmers stores  $9.48 \pm 0.38 \ \mu g/kg$ , NCPB depot stores  $9.37 \pm 0.81 \ \mu g/kg$ , and open market stores  $4.36 \pm 0.87 \ \mu g/kg$ . Aflatoxin B2 contamination were: retailer stores  $8.98 \pm 1.66 \ \mu g/kg$ , NCPB depot store  $4.64 \pm 0.69 \ \mu g/kg$ , open market stores  $3.06 \pm 0.98 \ \mu g/kg$ , and farmers store  $2.88 \pm 0.36 \ \mu g/kg$ . The mean value for aflatoxin G1 contamination was NCPB depot store  $8.74 \pm 0.74 \ \mu g/kg$ , retailer stores  $16.17 \pm 1.84 \ \mu g/kg$ , open market stores  $4.57 \pm 0.58 \ \mu g/kg$  and farmers store  $5.81 \pm 1.23 \ \mu g/kg$ . The mean values for aflatoxin G2 contamination were: NCPB depot store  $3.65 \pm 0.73 \ \mu g/kg$ , retailer stores  $5.02 \pm 0.49 \ \mu g/kg$ , open market stores  $2.33 \pm 0.04 \ \mu g/kg$  and farmers store  $2.95 \pm 0.03 \ \mu g/kg$ . In general, the samples from Trans Nzoia had aflatoxin contamination above the accepted level of  $4 \ \mu g/kg$  for aflatoxin B1 and 10  $\mu g/kg$  for total aflatoxin (Figure 4.35).

Trans Nzoia County rises from an altitude of 1,800 meters above sea level on average. However, specifically, the county's altitude varies from 4,313 meters above sea level in Mt. Elgon and gradually drops to 1,400 meters towards the north. The agro-ecological zones have three major zones, the Upper Highland Zones, Upper Midland Zones, and the Lower Highland Zones. The annual rainfall ranges from 1000 mm to 1700 mm distributed into the long rainfall season March, April, and May, the Intermediate season- June-July-August, and the short rainfall season- October-November-December. The long and intermediate seasons are more reliable for agricultural production as compared to the short rainfall season (Gnonlonfin *et al.*, 2013).

The impacts of climate change (Ongoma, 2013), drought, dry spells, unstable rains, and flood hazards have increased in frequency and complexity, and have affected agriculture. Sensitive

crops like maize experience stress during development to grain maturity and increased storage. Soil patterns are also complex in distribution, influenced by climatic conditions, volcanic activities, and underlying rock types. The soils range from well-drained and fertile soils for crop agricultural activities to poorly drained and infertile soils unproductive for crop agriculture (Abera *et al.*, 2016). Over use of acidic fertilizers and poor soil management practices by small farmers have affected soil pH. These factors have contributed to the observed aflatoxin contamination in maize to above limits.

#### 4.2.7 Aflatoxin Contamination in Maize from Selected Counties in Western Kenya

Western Kenya is a vast area with different climates influenced by Lake Victoria and other geographical formations in the surroundings. Various economic activities key among them are agricultural (crop and animal farming), fishing, mining, trade, hospitality, and tourism. The region according to the Kenya population and housing census, (2019) has 11,291,362 people, distributed in 10 counties namely Kisumu, Homabay, Migori, Kisii, Nyamira, Siaya, Vihiga, Busia, Bungoma and Kakamega, and a total area of 19,877 km<sup>2</sup>. All the counties grow maize mostly for domestic consumption rather than commercial. A lot of maize was brought in from other counties and neighboring countries through Busia and Migori border towns. The study focused on the two counties that maize flows through.



#### Figure 4.36: Aflatoxin Contamination in Maize Samples from Western Counties

#### 4.2.7.1 Busia.

Maize samples collected from different stores in Busia County were analyzed for the aflatoxin strains, quantified, and categorized as aflatoxin B1, B2, G1, and G2. The mean value for aflatoxin B1 contamination was retailer stores  $1.74\pm0.00 \ \mu\text{g/kg}$ , market stores  $1.60\pm0.06 \ \mu\text{g/kg}$  open farmers store  $1.45\pm0.50 \ \mu\text{g/kg}$ , and NCPB depot store  $0.50\pm0.00 \ \mu\text{g/kg}$ . The mean value for aflatoxin B2 contamination was farmers store  $0.99\pm0.00 \ \mu\text{g/kg}$ , retailer stores  $0.66\pm0.00 \ \mu\text{g/kg}$ , open market stores BDL and NCPB depot store BDL. The mean value for aflatoxin G1

contamination was retailer stores  $1.04\pm0.04 \ \mu g/kg$ , farmers store  $0.15\pm0.00 \ \mu g/kg$ , open market stores BDL and NCPB depot store BDL. The mean values for aflatoxin G2 contamination were retailer stores  $0.21\pm0.00 \ \mu g/kg$ , NCPB depot store BDL, open market stores BDL, and farmers store BDL. In general, the samples from Busia had aflatoxin contamination below the maximum guideline limits of 4  $\mu$ g/kg for aflatoxin B1 and 10  $\mu$ g/kg for total aflatoxin (Figure 4.36).

Busia County rises from the altitudes 1140-1500 meters above sea level with four Agro-Ecological Zones (AEZ) covering different regions. The county has a mean temperature of about 21-27 °C with the lowest according to GoK (2013a) ranging between 14 and 22 °C and the highest are between 29-30 °C. The county has a bimodal rainfall pattern with an average annual rainfall of about 750-2000 mm (GoK, 2014a). The long rains are received in March -May and short rains in August – October. The rainfall, however, varies across the county with areas near Lake Victoria receiving the least amount of rainfall about 760-1015 mm.

According to Jaedzold *et al.* (2007), Western Kenya soils vary in their physicochemical properties. Soil types and precipitation patterns governed by climate, parent material, biota, relief, and age determine agricultural production. The county has soils and climatic and environmental factors that are good for maize production. The maize experiences less abiotic stresses but biotic stresses depend on the agronomic practices. The farming is done on a small scale thus allowing fewer management challenges hence low mold growth resulting to low aflatoxin contamination.

#### 4.2.7.2 Migori

The mean value for aflatoxin B1 contamination was farmers store  $23.75\pm2.21 \ \mu g/kg$ , open market stores  $18.60\pm1.46 \ \mu g/kg$ , retailer stores  $4.83\pm0.30 \ \mu g/kg$ , and NCPB depot  $3.04\pm0.79$ 

 $\mu$ g/kg. The mean aflatoxin B2 contamination was in retailer stores 15.48±2.41  $\mu$ g/kg, farmers store 7.43±0.76  $\mu$ g/kg, open market stores 4.28±0.91  $\mu$ g/kg and NCPB depot store1.05±0.04  $\mu$ g/kg. The mean aflatoxin G1 contamination was in farmers' stores 21.02±1.48  $\mu$ g/kg, retailer stores 17.67±2.14  $\mu$ g/kg, open market stores 10.51±0.37  $\mu$ g/kg, and NCPB depot 1.15±0.33  $\mu$ g/kg. The mean aflatoxin G2 contamination was in farmer's stores 9.16±1.39  $\mu$ g/kg, retailer stores 5.60±0.57  $\mu$ g/kg, open market stores 2.28±0.51  $\mu$ g/kg, and NCPB depot 0.65±0.00  $\mu$ g/kg. In general, maize samples collected from different stores in Migori County had aflatoxin contamination levels above the accepted level of 5 $\mu$ g/kg for aflatoxin B1 and 10  $\mu$ g/kg for total aflatoxin (Figure 4.36).

Migori County has six different Agro-Ecological Zones (AEZ). It has undulating hills, plains, and ranges rising from 1135 to 1700 m above sea level. The county has a mild inland equatorial-type climate, modified by relief and altitude owing to its proximity to Lake Victoria. It receives a bimodal rainfall that varies from 700 mm to 1800 mm annually, long rain from March to May, and short rains from October to November. It has a mean annual temperature of 21.2  $^{\circ}$ C. The lowest temperature mean was experienced in July at 13.3  $^{\circ}$ C and the highest in February-March at 29.2  $^{\circ}$ C.

Soils in Migori County vary in their physicochemical properties such that agricultural productivity is affected. The soil types are of medium fertility because of precipitation patterns received in the region as well as climate, biota, relief, and age. Maize grown in the county has both abiotic and biotic stresses depending on the agronomic and storage practices. Maize crops in the county seemed to have storage and farm challenges that contributed to the growth of molds that contaminate maize with aflatoxin hence the observation.

#### 4.2.8 Effect of County and Maize Store Type on Aflatoxin Contamination

The national cereals and produce board ensures continuous supply, controls prices, food security and safety, and stores maize harvested in most parts of Kenya. In the counties, farmers store some of the maize for food security and price speculation. Traders buy from the farmers during harvesting at lower prices and store for price speculation. Storage conditions, however, vary from NCPB to traders to farmers due to the financial implications of storage logistics. Farmers occasionally reuse old bags, and dust with low-quality chemicals and use available and affordable storage space.

Some have the maize harvest vulnerable to damage by grain borers, weevils, and rodents. Retail traders, use polypropylene bags new, reused, and plastic containers to store maize at home or shops in trading centers without assured aeration of the stored product. Maize in such stores are prone to pest damage and fungal infestation. Wholesalers and brokers are the intermediaries between the farmers and retailers or NCPB stores in the trade. Some own large warehouses for cereal storage in major county markets. They dust maize with phostoxin and actellic super, have better-aerated space, and use polypropylene bags.

National cereals and produces board (NCPB) can hold large volumes of maize in improved conditions and fumigation is done regularly. Maize handlers in the four store types may not have similar storage knowledge hence variations in terms of maize quality. The government-managed NCPB depots have standards and guidelines for storage, trained staff, and regular

supervision done in the stores. The other three store types do not enjoy such opportunities but business or routine to earn a living.

#### 4.2.8.1 Effect of County and Store Type on Aflatoxin Occurrence in Maize

The effect of different county and maize store types on aflatoxin occurrence was tested for possible interaction with the Scheirer Ray Hare (SRH) test. It was statistically significant for the four strains. The result showed p value > 0.05 for both county and store, which meant that counties and stores were independent from each other without interaction. The effect of the county alone was significant P< 0.05 (Table 4.3).

Aflatoxin	Predictor	Difference	Hare test	P-value
<b>B1</b>	County	10	101.55	P< 0.01
	Store type	3	3.51	0.32
	Interaction	30	15.11	0.99
B2	County	10	95.82	P< 0.01
	Store type	3	5.44	0.14
	Interaction	30	16.43	0.98
G1	County	10	97.01	P< 0.01
	Store type	3	4.71	0.198
	Interaction	30	15.10	0.98
G2	County	10	91.71	P< 0.01
	Store type	3	0.66	0.88
	Interaction	30	18.95	0.94

 Table 4.3: Scheirer Ray Hare (SRH) test on the effect of county and store type on

 Aflatoxin occurrence

#### 4.2.8.2 Interaction of County and Performance of Maize Store Type

The interaction and pattern ranks were used to compare the aflatoxin contaminants in maize and to find the relationship in different counties. This is being considered in terms of high and low ranks:

High ranks; aflatoxin B1 had high rank values from 60> for farm, market, retail and NCPB stores in Isiolo, Embu, and Makueni. Aflatoxin G1 had high-rank values from 45 > for farm,
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market, retail, and NCPB stores in Embu and Makueni. Aflatoxin B2 had high ranks from 25 > for farm, market, retail, and NCPB stores in Makueni, Meru, and Migori. Higher rank values for aflatoxin G2 were from 20 > for farm, market, retail, and NCPB stores in Embu, Makueni, and Isiolo. In comparison, the ranks for aflatoxin contaminants in the counties were in order B1>G1>B2>G2 with the values 60>45>25>20.

When the displayed patterns were compared, Isiolo and Embu had similar patterns for aflatoxin B1 while Makueni had an opposite pattern, while for aflatoxin G1 the pattern was opposite for farms and market stores and similar for NCPB and retail stores. Opposite patterns were observed in Makueni and Meru for aflatoxin B2. Similar patterns were observed for Makueni and Migori for the same stores. Three different patterns were observed for aflatoxin G2, for stores in Makueni, Isiolo, and Embu counties which contrasted in farms, markets, and NCPB stores but the retail stores were similar. Embu and Isiolo counties had similar patterns opposite to those observed for Makueni. But in general, Embu County consistent patterns were observed for the four aflatoxins (Figure 4.37).



Figure 4.37: Interaction of County and Performance Maize Store Type

Low ranks; aflatoxin B1 had low-rank values from <25, for farm, market, retail, and NCPB stores in Busia, Kajiado, Nairobi, Nakuru, Trans-Nzoia, Embu, and Migori counties. Aflatoxin G1 had low-rank values from < 20, for farm, market, retail, and NCPB stores in Busia, Embu, Kajiado, Isiolo, Machakos, Nairobi, Nakuru, Trans-Nzoia, and Migori counties. Aflatoxin B2 has low-rank values < 10, for farm, market, retail, and NCPB stores in Busia, Kajiado, Migori, Nairobi, Nakuru, and Trans-Nzoia and Embu counties. Aflatoxin G1 had low-rank values <10, for farm, market, retail, and NCPB stores in Busia, Kajiado, Migori, Nairobi, Nakuru, and Trans-Nzoia and Embu counties. Aflatoxin G1 had low-rank values <10, for farm, market, retail, Kajiado, Isiolo, Machakos, Nairobi, Nakuru, Trans-Nzoia and Embu counties. Aflatoxin G1 had low-rank values <10, for farm, market, retail, and NCPB stores in Busia, Kajiado, Nairobi, Nakuru, Trans-Nzoia, Meru, and Migori counties (Figure 4.36).

The order of aflatoxin contaminants in maize for the low ranks in the counties was B1>G1>B2=G2 thus 25>20>10=10. Embu, Busia, and Nakuru had a consistent pattern in the four contaminants. The Market and NCPB stores were more consistent in patterns for the four aflatoxins in low ranks compared to farms and retail stores.

The ranking of performance for the 11 counties in terms of the effect of store type and contamination of maize by the four aflatoxin strains did not show any interactions. Stores and aflatoxin strains may have correlations in the patterns of contamination that depend on the local conditions. When the ANOVA test was performed on the results of the contaminants in the four regions, there was a significant difference with the other regions P < 0.05 as observed from pairwise comparisons using the Wilcoxon rank sum test (Table 4.4).

	Predictor	Eastern	Nairobi	Rift Valley	Western
Aflatoxin					
B1	Eastern	-	2.4×10 <sup>-5</sup>	8.1×10 <sup>-12</sup>	3.7×10 <sup>-9</sup>
	Nairobi	$2.4 \times 10^{-5}$	-	0.13	0.13
	Rift Valley	$8.1 \times 10^{-12}$	0.13	-	0.85
	Western	3.7×10 <sup>-9</sup>	0.13	0.85	-
B2	Eastern	-	1.3 ×10 <sup>-5</sup>	2.7 ×10 <sup>-11</sup>	4.2 ×10 <sup>-6</sup>
	Nairobi	$1.3 \times 10^{-5}$	-	0.55	0.55
	Rift Valley	2.7 ×10 <sup>-</sup>	0.55	-	0.76
		11			
	Western	$4.2 \times 10^{-6}$	0.55	0.55	-
G1	Eastern	-	3.7 ×10 <sup>-6</sup>	$3.2 \times 10^{-12}$	$3.2 \times 10^{-12}$
	Nairobi	3.7 ×10 <sup>-6</sup>	-	0.79	0.16
	Rift Valley	3.2 ×10 <sup>-</sup>	0.79	-	0.39
		12			
	Western	$3.2 \times 10^{-12}$	0.16	0.39	-
G2	Eastern	-	2.2 ×10 <sup>-5</sup>	1.5 ×10 <sup>-9</sup>	1.7 ×10 <sup>-7</sup>
	Nairobi	2.2 ×10 <sup>-5</sup>	-	0.91	0.15
	Rift Valley	1.5 ×10 <sup>-9</sup>	0.91	-	0.12
	Western	1.7×10 <sup>-7</sup>	0.15	0.12	-

Table 4.4: Pairwise Comparisons Using Wilcoxon Rank Sum Test

#### 4.3 Chemical Processes for Degrading Aflatoxin in Contaminated Maize

#### 4.3.1 Degradation of Aflatoxin contaminants in maize

This study sought to establish the degradation potency of sodium hydrogen sulfite, ferulic acid, ammonium carbonate, sodium hydrogen carbonate, and sodium hypochlorite on 50 g of aflatoxin-contaminated whole maize, de-hulled maize, and ground maize. The process was repeated with each chemical agent and enhanced with hydrogen peroxide, ammonia solution, and methylamine as catalysts. The reaction mixtures were heated to reduce the time taken to complete the reaction process. Some of the reagents used in the study are used commercially as additives, treatments, or antioxidants thus justifying their use. Other studies also reported efficacy in controlling mycotoxins with some chemical reagents and catalysts to degrade mycotoxins or promote the reactions (Nunes *et al.*, 2021; Pankaj *et al.*, 2018; Magan & Olsen, 2004)

Sodium hydrogen sulfite is a permitted food additive and a strong reducing agent taken to be safe for human consumption. Hydrogen peroxide is used commercially in the dairy industry for milk preservation (Arefin *et al.*, 2017). Previous studies have reported success with 1 % sodium hydrogen sulfite, in reducing mycotoxins in maize and dried fig fruits by 25 % at 25 °C in 72 hours (Karlovsky *et al.*, 2016). In the same study, raising the temperature to 0.2 % of  $H_2O_2$  degraded a higher percentage of mycotoxin in a shorter time. Ammonia gas reduced aflatoxin in maize under atmospheric pressure and ambient temperature conditions in previous studies (Colovic' *et al.*, 2019) but was not tried in ammonia solution.

Previous studies have reported success in reducing aflatoxin in food by 62 % and 82 % at room temperature by  $H_2O_2$  or sodium hypochlorite respectively in storage conditions for 168 hours

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(Colovic', 2019). Methylamine in previous studies, successfully degraded mycotoxins in food at 1.25 % concentration and 100  $^{\circ}$ C in 1.5 hours reducing the toxin to trace levels. Ammonium carbonate, sodium carbonate, and ferulic acid have been used in other studies to test their efficacy in reducing mycotoxin growth in maize and other stored foods (Gautier *et al.*, 2020, Jacob *et al.*, 2020, Shekhr *et al.*, 2009).

Chemical reactions target the lactone ring, difuran ring, and oxygen bonds in the methoxy link to the benzene ring. A synergy of the chemical reagent and the catalyst to degrade the toxins rings and links were determined in the degradation experiments. The rate of degradation was measured by determining the concentration of total aflatoxin (B1, B2, G1, and G2) in maize at regular intervals of 30 minutes. The degradation data was fitted into reaction kinetic law equations to determine reaction order, rate constant, and half-life. The experiments tested the impact of maize coating cover, size, concentration of chemical reagent, and time taken to degrade.

#### 4.3.2 Degradation of Aflatoxin Contaminants in Maize with Different Concentrations of Sodium Hydrogen Sulfite and Catalysts

Tests were carried out to investigate the degradation rate of aflatoxin contaminants in maize with different concentrations of sodium hydrogen sulfite and three different catalysts. Contaminated maize samples were divided into three subsets: whole maize (WM), dehulled maize (DM), and ground maize (GM). The three were reacted with 1, 0.5, 0.05, 0.005, and 0.0005 M of sodium hydrogen sulfite. Tests were repeated separately with sodium hydrogen sulfite and 4 mL of 2 % hydrogen peroxide, 2 % ammonia, and 2 % methylamine. Heating the reaction mixture to 80 °C and cooling to 25 °C was done to increase the efficacy of degradation.

The Aflatoxin was concentration measured in intervals of 30 minutes for five hours (Appendix 4, Table 2).

Sodium hydrogen sulfite of 1 M degraded aflatoxin contaminants in whole maize, de-hulled maize, and ground maize flour by mean percentages of 68.0 %, 70.28 %, and 70.2 %, respectively. The procedures were repeated with sodium hydrogen sulfite and 4 mL of 2 % hydrogen peroxide solution. Sodium hydrogen sulfite of concentration 1 M with 4 mL of hydrogen peroxide degraded aflatoxin in whole, de-hulled, and ground maize by 79.2 %, 81.22 %, and 80.92 %, respectively. The Aflatoxin reduction by 1 M sodium hydrogen sulfite catalyzed by 4 mL of 2 % ammonia solution in whole, de-hulled, and ground maize by 84.64 %, 86 %, and 85.76 %, respectively. The use of sodium hydrogen sulfite catalyzed by 4 mL of 2 % methylamine reduced aflatoxin in whole, de-hulled, and ground maize by 72.04 %, 72.04 %, and 71.52 %, respectively.

Sodium hydrogen sulfite of 0.5 M, reduced aflatoxin contaminants in whole, dehulled, and ground maize by 67.08 %, 67.06 %, and 70.4 %, respectively. The same concentration of sodium hydrogen sulfite with 4 mL of 2 %) hydrogen peroxide reduced aflatoxin in whole, dehulled, and ground maize by 79.38 %, 79.36 %, and 82.78 %, respectively. Using ammonia instead of hydrogen peroxide, the aflatoxin contaminants in whole, dehulled, and ground maize were reduced by 85.1 %, 85.1 %, and 86.26 %, respectively. When methylamine was used in place of the other two catalysts, 0.5 M sodium hydrogen sulfite reduced aflatoxin in whole, dehulled, and ground maize by 71.42 %, 71.88 %, and 71.88 %, respectively.

Sodium hydrogen sulfite solution of 0.05 M reduced aflatoxin contaminants in whole, dehulled, and ground maize by 70.52 %, 70.48 %, and 71.9 %, respectively. Coupled with 4 mL of 2 % hydrogen peroxide, it reduced aflatoxin in whole, dehulled, and ground maize by a mean **128** | P a g e

percentage of 82.12 %, 82.06 %, and 84.1 %, respectively. Using 0.05 M sodium hydrogen sulfite with 4 mL of ammonia solution reduced aflatoxin in whole, dehulled, and ground maize by 86.76 %, 86.76 %, and 91.42 %, respectively. Using 0.05 M sodium hydrogen sulfite with 4 mL of methylamine solution reduced aflatoxin contaminants in whole, dehulled, and ground maize by 73.16 %, 73.14 %, and 74.96 %, respectively.

Sodium hydrogen sulfite of 0.005 M as oxidant reduced aflatoxin contaminants in whole, dehulled, and ground maize by 71.52 %, 71.5 %, and ground maize by 73.36 %, respectively. The presence of 4 mL hydrogen peroxide as a catalyst reduced aflatoxin in whole, dehulled, and ground maize by 84.14 %, 84.12 %, and 86.24 %, respectively. Using 4 mL of ammonia solution, 0.005 M sodium hydrogen sulfite reduced aflatoxin in whole, dehulled, and ground maize by 87.32 %, 87.32 % and 88.1 %, respectively. Using 4 mL of methylamine solution as a catalyst reduced aflatoxin by 74.52 % for whole maize, 74.52 % for dehulled maize, and 76.26 % for ground maize.

Sodium hydrogen sulfite solution of 0.0005 M achieved a reduction of aflatoxin contaminants by 71.52 % for whole maize, 70.32 % for dehulled maize, and 72.0 % for ground maize. When 0.0005 M sodium hydrogen sulfite was combined with 4 mL of hydrogen peroxide, it reduced the aflatoxin contaminants by 84.14 % for whole maize, 80.16 % for dehulled maize and 83.06 % for ground maize. Using 4 mL of 2 % ammonia solution as a catalyst reduced aflatoxin by 87.32 % for whole maize, 84.44 % dehulled maize, and 86.1 % ground maize, while the use of 4 mL methylamine solution as a catalyst achieved aflatoxin reduction of 74.52 % for whole, 74.46 % for dehulled and 74.36 % for ground maize.

The nature of maize such as whole, dehulled, and ground, concentration of sodium hydrogen sulfite, and type of catalyst affected the rate of degradation of aflatoxin contaminants in maize. **129** | P a g e The catalysts improved the rate of degradation reaction thus taking a short time to reach 10 ppm or 5 ppm, the maximum total aflatoxin tolerated in maize for most countries and European Union. These are shown with continuous and dotted lines, respectively in the plots for all maize subtypes. The graphs summarize these effects (Figure 4.38 A, B, C, and D) and Appendix 4 (Table 3).









The degradation results for different concentrations of sodium hydrogen sulfite and those of various catalysts fitted into a kinetic reaction model. The assumption was that sodium hydrogen sulfite reacted with aflatoxin molecules to produce new products that made the aflatoxin molecules lose their fluorescence under UV-light

Aflatoxin + NaHSO<sub>3</sub> — Aflaproducts

The measured loss of aflatoxin contamination in maize samples over time appeared to obey the decay to  $Ct = Coe^{-kt}$ .....Equation 3

Where Ct is the concentration of aflatoxin at any time, Co is the concentration of aflatoxin at the start of the degradation process, Euler's constant (e) reaction characteristic, k is the rate constant, and t is the instantaneous reaction time.

On rearranging the equation by dividing through with Co the reaction reduced to

Applying natural logarithm on all sides of equation 4 resulted in equation 5

In Ct-InCo = -kt----- Equation 5

On further rearrangement of equation 5 reduced to

In Ct = -kt + In Co ..... Equation 6

The linear form of y = mx + b equation in six shows the y and x relationship in the study, m the gradient of the equation, and the y-intercept b. When a semi-log plot of equation 5 is done for values of InCt against changing time t, a straight-line graph is produced with a slope of -k and y-intercept of InCo. The rate constant is given by the gradient for degradation of aflatoxin in each curve.

To determine the rate at which degradation occurred equation 6 was applied when the total aflatoxin contaminants had reduced by half the starting concentration Ct = 2Co /the half- life and I determined by In1/2Co-InCo = -kt1/2 relationship. Equation 7

On applying Ct = I/2Co into equation 7 reduced to  $In \frac{Co}{2Co} = -kt1/2$  Equation 8.

In $2 = k t 1/2$	Equation 9
In $2 = 0.693 = \text{kt}1/2$ by getting the natural logarithm of 2	Equation 10
Make time the subject $T1/2 = 0.693/k$	Equation 11

Since Co cancels out and is divided by (-1), equation 8 reduced to

Regression analysis was done to determine the strength and characteristic relationship between the decontamination of aflatoxin-contaminated maize with time when 1 M sodium hydrogen sulfite was used (Figure 4.39). It produced an exponential decay curve for the relationship that was difficult to calculate its slope and character. This ruled out zero zero-order relationship but according to equation 11, the degradation process followed a first-order kinetic (n=1). It implied that results for the 60 degradation tests followed differently same kinetic reaction pathways.





The results applied to equation 6 and the respective regression curves plotted. A linear relationship is generated with its equation, slope,  $R^2$  value, and y-intercept (Figure 4.40).

Appling the slope value into equation 12; generated the half- life. The implication was degradation of aflatoxin contaminants in maize followed first-order kinetics.



#### Figure 4.40: Degradation of Aflatoxin Contamination in Whole Maize with Sodium Hydrogen Sulfite

The 60 regression curves for degradation of aflatoxin contaminants in maize with different concentrations of sodium hydrogen sulfite and the catalysts fitted into equations 6. The regression equations, k-values, correlation co-efficient ( $\mathbb{R}^2$ )-values, half-life values, and y-intercept values, and the data about the degradation process are attached in appendix 4 (Table 2) and (Table 4.3) below. There were statistical implications for each set of values in the study. The linear regression equation y = mx + b, where the natural logarithm y- of aflatoxin concentration (InCt) in maize, m- the gradient or slope (k) of the curve or reciprocal, calculated by determining the change in concentration over time.

Degradation, x- the variation in time (t) during the degradation process and b- the y-intercept value at the start time of the degradation process, represented the initial concentration of aflatoxin in maize (InCo). The rate constant k-value for each degradation reaction varied with 135 | P a g e

the concentration of sodium hydrogen sulfite, the nature of maize (whole, dehulled, and ground) and the catalyst used. The lower the k value, the steeper the curve slope and the faster the degradation reaction for the stated concentration and nature of maize at the time.

The coefficient R<sup>2</sup> determines the percentage fitting of the experimental data into the regression equation model. It also measured the degradation effectiveness of sodium hydrogen sulfite on aflatoxin contaminants in maize which ranged from 78.8 % to 90.3 %. The range of 9.7- 21.2 % was the influence of other factors including temperature, sodium hydrogen sulfite concentration, the nature of maize, particle sizes, particle orientation, compatibility of the reagents in the degradation process, and the activation energy. Half-life (T1/2) was the time taken to reach half the initial amount of aflatoxin contaminant in maize. A shorter time implied a fast rate of degradation of aflatoxin contaminants in maize to new products. 0.5 M Sodium hydrogen sulfite had a fast rate of degradation of aflatoxin in whole and dehulled maize both at 57.76 minutes. 1 M sodium hydrogen sulfite was the slowest in dehulled maize at 82.52 minutes.

The  $R^2$  value ranged from 86.6 % to 92.0 % and other factors influence ranged from 8.0- 14.4 %. These factors were; the concentration of sodium hydrogen sulfite, catalyst, nature of maize, particle size, particle orientation and compatibility with chemicals during degradation reaction, and the activation energy. The half-life (T1/2) for the rate of degradation reaction was fastest with 0.05 M concentration on dehulled maize at 37.67 minutes. The rate was slow with 1 M concentration on dehulled maize at 52.23 minutes.

When the concentration of sodium hydrogen sulfite was varied with a constant volume of 4 mL of 2 % Hydrogen peroxide, the aflatoxin degradation reaction was found to vary with the nature of the maize. The coefficient of determination ( $\mathbb{R}^2$ ) value ranged from 74.3 to 93.4 %. The influence of other factors including temperature, nature of maize, the particle sizes, particle **136** | P a g e

orientation, compatibility during degradation in the reaction, and the activation energy ranged from 6.6-25.7 %. The half-life (T1/2) for the degradation reaction was fastest in 0.05 M sodium hydrogen sulfite in dehulled maize at 47.48 minutes.

The factors that influenced the rate of degradation included the concentration of sodium hydrogen sulfite with 4 mL of 2 % methylamine solution, the catalyst, the nature of maize, the particle size, the particle orientation, and the activation energy. The half-life (T1/2) for the rate of degradation reaction was fastest with 0.005 M concentration on dehulled and ground maize at 43.32 minutes. The rate was slow with a 1 M concentration of sodium hydrogen sulfite on dehulled maize at 68.63 minutes.

Regression curves for degradation of aflatoxin in maize with different concentrations of sodium hydrogen sulfite with 4 mL of 2 % ammonia solution had different regression equations, k-value, correlation co-efficient ( $\mathbb{R}^2$ )-value, half-life value, and y-intercept value. The  $\mathbb{R}^2$  value ranged from 86.6 to 92.0 % for the reactions. The influence of concentration of hydrogen sulfite, catalyst, temperature, nature of maize, particle size, particle orientation, and the activation energy ranged from 8.0- 14.4 %. These factors were. The half-life (T1/2) for the degradation reaction was fastest with 0.05 M sodium hydrogen sulfite and the catalyst on dehulled maize at 37.67 minutes.

Regression curves for the aflatoxin degradation reaction in maize with different concentrations of sodium hydrogen sulfite with 4 mL of 2 % methylamine solution fitted the model. Each curve had a different regression equation, k-value, correlation co-efficient ( $R^2$ )-value, half-life value, and y-intercept value. The  $R^2$  ranged from 83.9 to 98.7 %. The degradation reaction half-life (T1/2) was fastest with 0.005M sodium hydrogen sulfite and catalyst in dehulled and ground maize at 43.32 minutes but slow with 1M sodium hydrogen sulfite and catalyst in dehulled **137** | P a g e

maize at 68.63 minutes. The nature of maize, catalyst, and concentration influenced directly the degradation of aflatoxin contaminants in maize. The summary of degradation regression data for decontamination of aflatoxin in maize with different concentrations of sodium hydrogen sulfite and catalysts (Table 4.5).

 Table 4.5: Summary of Regression Degradation Data for Aflatoxin Content in Maize with

 Different Concentrations of Sodium Hydrogen Sulfite, Catalysts.

Maize	Reagent	[Con]	Catalyst	Slope	Intercept	R^2	AIC	BIC	RMSE	5µg/kg	10µg/kg
WM	NaHSO <sub>3</sub>	1	no	-0.0106	5.5778	0.786	22	23	0.492	6.2	5.1
	NaHSO <sub>3</sub>	0.5	no	-0.0084	5.3974	0.8921	8	9	0.261	7.5	6.1
	NaHSO <sub>3</sub>	0.05	no	-0.0109	5.5606	0.822	4	21	0.452	6	5
	NaHSO <sub>3</sub>	0.005	no	-0.0124	5.6871	0.8153	23	24	0.524	5.5	4.5
	NaHSO <sub>3</sub>	0.0005	no	-0.0119	5.6772	0.8708	18	19	0.411	5.7	4.7
DM	NaHSO <sub>3</sub>	1	no	-0.0104	5.233	0.9651	-1	0	0.177	5.8	4.7
	NaHSO <sub>3</sub>	0.5	no	-0.0121	5.3163	0.9401	9	10	0.274	5.1	4.2
	NaHSO <sub>3</sub>	0.05	no	-0.0118	5.2151	0.9726	-1	0	0.178	5.1	4.1
	NaHSO <sub>3</sub>	0.005	no	-0.0134	5.3406	0.9664	4	6	0.224	4.6	3.8
	NaHSO <sub>3</sub>	0.0005	no	-0.0108	5.2351	0.9598	2	3	0.199	5.6	4.5
GM	NaHSO <sub>3</sub>	1	no	-0.0108	5.3487	0.9256	9	10	0.275	5.8	4.7
	NaHSO <sub>3</sub>	0.5	no	-0.0124	5.3915	0.9519	7	8	0.251	5.1	4.2
	NaHSO <sub>3</sub>	0.05	no	-0.0149	5.5104	0.9456	12	13	0.32	4.4	3.6
	NaHSO <sub>3</sub>	0.005	no	-0.0152	5.4939	0.9418	13	15	0.339	4.3	3.5
	NaHSO <sub>3</sub>	0.0005	no	-0.0124	5.4045	0.9135	14	15	0.342	5.1	4.2
WM	NaHSO <sub>3</sub>	1	$H_2O_2$	-0.0113	5.312	0.7684	24	25	0.55	5.5	4.4
	NaHSO <sub>3</sub>	0.5	$H_2O_2$	-0.0086	5.0553	0.8529	12	13	0.32	6.7	5.3
	NaHSO <sub>3</sub>	0.05	$H_2O_2$	-0.0113	5.1998	0.791	23	24	0.517	5.3	4.3
	NaHSO <sub>3</sub>	0.005	$H_2O_2$	-0.0112	5.0621	0.8966	14	15	0.342	5.1	4.1
	NaHSO <sub>3</sub>	0.0005	$H_2O_2$	-0.0094	5.0874	0.9062	8	10	0.271	6.2	4.9
DM	NaHSO <sub>3</sub>	1	$H_2O_2$	-0.0169	5.4827	0.8982	22	24	0.508	3.8	3.1
	NaHSO <sub>3</sub>	0.5	$H_2O_2$	-0.0173	5.4834	0.8962	23	24	0.527	3.7	3.1
	NaHSO <sub>3</sub>	0.05	$H_2O_2$	-0.0165	5.3737	0.9024	21	23	0.486	3.8	3.1
	NaHSO <sub>3</sub>	0.005	$H_2O_2$	-0.0163	5.3589	0.9135	4	21	0.45	3.8	3.1
	NaHSO <sub>3</sub>	0.0005	$H_2O_2$	-0.0163	5.4163	0.9061	21	22	0.469	3.9	3.2
GM	NaHSO <sub>3</sub>	1	$H_2O_2$	-0.0169	5.4706	0.8983	22	24	0.508	3.8	3.1
	NaHSO <sub>3</sub>	0.5	$H_2O_2$	-0.0178	5.5141	0.904	23	24	0.519	3.7	3

	NaHSO <sub>3</sub>	0.05	$H_2O_2$	-0.0183	5.4903	0.9052	23	25	0.532	3.5	2.9
	NaHSO <sub>3</sub>	0.005	$H_2O_2$	-0.0187	5.477	0.9076	23	25	0.534	3.4	2.8
	NaHSO <sub>3</sub>	0.0005	$H_2O_2$	-0.0174	5.4215	0.9217	4	21	0.456	3.7	3
WM	NaHSO <sub>3</sub>	1	NH <sub>3</sub>	-0.0151	5.441	0.854	24	26	0.556	4.2	3.4
	NaHSO <sub>3</sub>	0.5	NH <sub>3</sub>	-0.0147	5.3605	0.8738	22	23	0.501	4.3	3.5
	NaHSO <sub>3</sub>	0.05	NH <sub>3</sub>	-0.0151	5.3229	0.8782	22	23	0.502	4.1	3.3
	NaHSO <sub>3</sub>	0.005	NH <sub>3</sub>	-0.0138	5.1543	0.9114	16	17	0.386	4.3	3.4
	NaHSO <sub>3</sub>	0.0005	NH <sub>3</sub>	-0.0154	5.4304	0.872	23	24	0.527	4.1	3.4
DM	NaHSO <sub>3</sub>	1	NH <sub>3</sub>	-0.045	5.4482	0.9142	25	26	0.562	3.1	2.6
	NaHSO <sub>3</sub>	0.5	NH <sub>3</sub>	-0.0211	5.4331	0.9217	24	25	0.551	3	2.5
	NaHSO <sub>3</sub>	0.05	NH <sub>3</sub>	-0.0221	5.4822	0.9256	25	26	0.562	2.9	2.4
	NaHSO <sub>3</sub>	0.005	NH <sub>3</sub>	-0.0233	5.4551	0.9419	23	24	0.518	2.8	2.3
	NaHSO <sub>3</sub>	0.0005	NH <sub>3</sub>	-0.0233	5.5304	0.9427	23	24	0.516	2.8	2.3
GM	NaHSO <sub>3</sub>	1	NH <sub>3</sub>	-0.021	5.3884	0.9347	22	23	0.497	3	2.4
	NaHSO <sub>3</sub>	0.5	NH <sub>3</sub>	-0.0222	5.4616	0.9501	4	21	0.457	2.9	2.4
	NaHSO <sub>3</sub>	0.05	NH <sub>3</sub>	-0.0222	5.3948	0.9431	21	23	0.488	2.8	2.3
	NaHSO <sub>3</sub>	0.005	NH <sub>3</sub>	-0.0234	5.4108	0.9551	4	21	0.455	2.7	2.2
	NaHSO <sub>3</sub>	0.0005	NH <sub>3</sub>	-0.0227	5.4033	0.951	4	21	0.463	2.8	2.3
WM	NaHSO <sub>3</sub>	1	CH <sub>3</sub> NH <sub>2</sub>	-0.0101	5.4598	0.8409	17	18	0.391	6.4	5.2
	NaHSO <sub>3</sub>	0.5	CH <sub>3</sub> NH <sub>2</sub>	-0.0096	5.3735	0.8722	13	14	0.329	6.5	5.3
	NaHSO <sub>3</sub>	0.05	CH <sub>3</sub> NH <sub>2</sub>	-0.0105	5.4365	0.8446	17	18	0.403	6.1	5
	NaHSO <sub>3</sub>	0.005	CH <sub>3</sub> NH <sub>2</sub>	-0.0105	5.4365	0.8446	17	18	0.403	6.1	5
	NaHSO <sub>3</sub>	0.0005	CH <sub>3</sub> NH <sub>2</sub>	-0.0112	5.4817	0.8863	15	16	0.359	5.8	4.7
DM	NaHSO <sub>3</sub>	1	CH <sub>3</sub> NH <sub>2</sub>	-0.0155	5.2592	0.975	4	5	0.223	3.9	3.2
	NaHSO <sub>3</sub>	0.5	CH <sub>3</sub> NH <sub>2</sub>	-0.0154	5.44	0.9744	4	6	0.225	3.9	3.1
	NaHSO <sub>3</sub>	0.05	CH <sub>3</sub> NH <sub>2</sub>	-0.0155	5.1614	0.9779	3	4	0.21	3.8	3.1
	NaHSO <sub>3</sub>	0.005	CH <sub>3</sub> NH <sub>2</sub>	-0.016	5.1892	0.9819	1	3	0.196	3.7	3
	NaHSO <sub>3</sub>	0.0005	CH <sub>3</sub> NH <sub>2</sub>	-0.0154	5.1706	0.9809	1	2	0.193	3.9	3.1

GM	NaHSO <sub>3</sub>	1	CH <sub>3</sub> NH <sub>2</sub>	-0.0153	5.2374	0.9757	4	5	0.218	4	3.2
	NaHSO <sub>3</sub>	0.5	CH <sub>3</sub> NH <sub>2</sub>	-0.0155	5.1987	0.977	3	5	0.214	3.9	3.1
	NaHSO <sub>3</sub>	0.05	CH <sub>3</sub> NH <sub>2</sub>	-0.0156	5.1391	0.9813	1	2	0.193	3.8	3
	NaHSO <sub>3</sub>	0.005	CH <sub>3</sub> NH <sub>2</sub>	-0.016	5.1707	0.9858	-1	0	0.173	3.7	3
	NaHSO <sub>3</sub>	0.0005	CH <sub>3</sub> NH <sub>2</sub>	-0.0153	5.1672	0.9835	-1	1	0.179	3.9	3.1

#### 4.3.3: Degradation of Aflatoxin Contaminants in Maize with Ferulic Acid and Catalysts

The effect of ferulic acid and different catalysts on the degradation rate of aflatoxin in contaminated maize was. Different samples of whole maize (WM), dehulled maize (DM), and ground maize (GM) were reacted with 1.0, 0.5, 0.05, 0.005, and 0.0005 M ferulic acid. The reactions were repeated separately with ferulic acid combined with 4 mL of 2 % concentration of different catalysts namely hydrogen peroxide, ammonia, and methylamine. Each reaction mixture was s heated to 80 ° C and cooled to 25 ° C. The decrease in aflatoxin concentration in maize was tested at intervals of 30 minutes for five hours.

Ferulic acid of 1 M reduced the aflatoxin contaminants by 73.72 % for whole maize, 73.72 % for dehulled maize, and 76.62 % for ground maize. When the same concentration of ferulic acid was combined with 4 mL of 2 % hydrogen peroxide, the mixtures reduced aflatoxin by 87.56 % for whole maize, 87.56 % for dehulled maize, and 89.8 % for ground maize. Using 2 % ammonia solution as the catalyst reduced aflatoxin by 83.64 % for whole, 83.64 % for dehulled, and 85.36 % for ground maize. Using 4 mL methylamine solution as the catalyst reduced aflatoxin by 86.48 % for whole, 86.48 % for dehulled, and 86.88 % for ground maize.

Ferulic acid of 0.5 M reduced aflatoxin contaminants by 75.0 % for whole, 75.0 % for dehulled, and 79.4 % for ground maize, respectively. Using 4 mL of 2 % hydrogen peroxide as a catalyst reduced aflatoxin by 84.64 % for whole, 84.64 % for dehulled, and 86.04 % for ground maize,

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respectively. The reaction with 4 mL of ammonia solution as the catalyst reduced aflatoxin by 88.94 % for whole, 88.94 % for dehulled, and 90.72 % for ground maize, respectively. When 4 mL of methylamine was used with 0.5M ferulic acid with 4 mL of methylamine solution as the catalyst reduced aflatoxin by 87.24 % for whole, 87.22 % for dehulled, and 87.6 % for ground maize.

Ferulic acid of 0.05 M reduced aflatoxin contaminants in maize by 79.8 % for whole, 79.78 % for dehulled, and 81.46 % for ground maize, respectively. Using 4 mL 2 % hydrogen peroxide solution as the catalyst reduced aflatoxin by 86.04 % for whole, 86.04 % for dehulled, and 87.68 % for ground maize, respectively. When 4 mL ammonia solution was used as the catalyst could degraded aflatoxin content in maize by 89.52 % for whole, 89.52 % for dehulled, and 91.42 % for ground maize, respectively. Using 4 mL of methylamine solution as the catalyst degraded aflatoxins by 88.36 % for whole, 88.36 % for dehulled, and 89.12 % for ground maize.

Ferulic acid of 0.005 M reduced aflatoxin contaminants by 80.86 % for whole, 80.84 % for dehulled, and 81.48 % for ground maize, respectively. Using 4 mL 2 % hydrogen peroxidase the catalyst achieved aflatoxin reduction by 86.8 % for whole, 86.81 % for dehulled, and 88.26 % for ground maize, respectively. 4 mL of ammonia solution as the catalyst reduced aflatoxin by 91.58 % for whole, 91.56 % for dehulled, and 92.58 % for ground maize, respectively. 4 mL methylamine solution as the catalyst reduced aflatoxin by 88.66 % for whole, 88.66 % for dehulled, and 89.48 % for ground maize.

Ferulic acid of 0.0005 M reduced aflatoxin contaminants content in whole, dehulled and ground maize by 73.62 %, 73.6 %, and 78.22 %, respectively. The presence of 4 mL 2 % hydrogen peroxide as the catalyst reduced aflatoxin by 85.4 % for whole, 85.4 % for dehulled, and 88.0 % for ground maize. With 4 mL 2 % ammonia as catalyst reduced aflatoxin by 90.9 % for 142 | P a g e

whole, 91.9 % for dehulled, and 92.08 % for ground maize, respectively. 4 mL of (2 %) methylamine as the catalyst reduced aflatoxin by 88.0 % for whole, 88.0 % for dehulled, and 88.72 % for ground maize, respectively. The results showed that dehulling and grinding maize affected aflatoxin degradation by ferulic acid as well as concentration and catalysts (Figure 4.41 A, B, C, and D).







Figure 4.41: Effect of Concentration, Nature of Maize, and Catalysts on the Rate of Degradation of Aflatoxin contaminants in Maize with Ferulic Acid

The degradation of aflatoxin in maize fitted into the first-order kinetics equation. The catalysts improved the rate of degradation reaction thus taking a shorter time to reach 10 ppm or 5 ppm, the maximum total aflatoxin and B1 levels tolerated by maize in most countries and the European Union shown in Figures 4.44, with continuous and dotted lines respectively. Different concentrations of aflatoxin collected at regular times from the reaction mixtures gave linear regression curves. The slope,  $R^2$  value, and y-intercept were generated as summarized in (Table 4.6 and appendix 4, Table 4).

### Table 4.6: Regression Data for Degradation of Aflatoxin Contaminants in Maize with Different Concentrations of Ferulic Acid and Catalysts.

Reagent	[Con]	Catalyst	Slope	Intercept	R^2	AIC	BIC	RMSE	5µg/kg	10µg/kg
$C_{10}H_{10}O_4$	1	no	-0.0098	5.3863	0.9753	-6	-5	0.14	6.4	5.2

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C10H10O4	0.5	no	-0.0125	5.5329	0.948	8	9	0.264	5.2	4.3
C10H10O4	0.05	no	-0.0143	5.5278	0.9512	10	11	0.291	4.6	3.8
C10H10O4	0.005	no	-0.0153	5.573	0.9529	11	12	0.305	4.3	3.6
C10H10O4	0.0005	no	-0.0144	5.7445	0.8391	25	26	0.562	4.8	4
C10H10O4	1	no	-0.0117	5.5188	0.8599	18	19	0.422	5.6	4.6
C10H10O4	0.5	no	-0.0119	5.4757	0.8949	15	16	0.364	5.4	4.4
C10H10O4	0.05	no	-0.0125	5.4792	0.9407	9	10	0.281	5.2	4.2
C10H10O4	0.005	no	-0.0128	5.4863	0.9518	8	9	0.259	5	4.1
C10H10O4	0.0005	no	-0.0119	5.545	0.8881	16	17	0.379	5.5	4.5
C10H10O4	1	no	-0.0117	5.5359	0.8883	15	17	0.372	5.6	4.6
C10H10O4	0.5	no	-0.012	5.5113	0.9045	14	15	0.348	5.4	4.5
$C_{10}H_{10}O_4$	0.05	no	-0.0124	5.4652	0.9413	9	10	0.278	5.2	4.3
C10H10O4	0.005	no	-0.0128	5.4595	0.9502	8	9	0.262	5	4.1
$C_{10}H_{10}O_4$	0.0005	no	-0.0118	5.4411	0.9327	10	11	0.285	5.4	4.4
C10H10O4	1	$H_2O_2$	-0.0165	5.526	0.8944	22	24	0.508	4	3.3
$C_{10}H_{10}O_4$	0.5	$H_2O_2$	-0.0164	5.4598	0.9012	21	23	0.486	3.9	3.2
C10H10O4	0.05	$H_2O_2$	-0.0163	5.4031	0.9072	4	22	0.466	3.9	3.2
$C_{10}H_{10}O_4$	0.005	$H_2O_2$	-0.0158	5.347	0.9152	19	4	0.431	3.9	3.2
C10H10O4	0.0005	$H_2O_2$	-0.0159	5.4042	0.9094	4	21	0.451	4	3.3
$C_{10}H_{10}O_4$	1	$H_2O_2$	-0.0096	5.1425	0.8964	10	11	0.292	6.1	4.9
C10H10O4	0.5	$H_2O_2$	-0.0101	5.0919	0.9301	7	8	0.249	5.7	4.6
C10H10O4	0.05	$H_2O_2$	-0.0098	5.0109	0.9257	7	8	0.248	5.8	4.6
C10H10O4	0.005	$H_2O_2$	-0.0103	5.0179	0.945	8	10	0.271	5.5	4.4
C10H10O4	0.0005	$H_2O_2$	-0.0095	5.0338	0.9376	4	5	0.219	6	4.8
$C_{10}H_{10}O_4$	1	$H_2O_2$	-0.0092	5.1394	0.8847	10	12	0.295	6.4	5.1
$C_{10}H_{10}O_4$	0.5	$H_2O_2$	-0.0092	5.0529	0.9016	9	10	0.271	6.2	5
$C_{10}H_{10}O_4$	0.05	$H_2O_2$	-0.0091	4.9738	0.9093	7	9	0.257	6.2	4.9
$C_{10}H_{10}O_4$	0.005	$H_2O_2$	-0.0097	4.9761	0.8719	13	14	0.331	5.8	4.6
$C_{10}H_{10}O_4$	0.0005	$H_2O_2$	-0.0089	5.0225	0.8916	9	10	0.276	6.4	5.1
$C_{10}H_{10}O_4$	1	NH <sub>3</sub>	-0.045	5.4673	0.9123	25	26	0.571	3.1	2.6
$C_{10}H_{10}O_4$	0.5	NH <sub>3</sub>	-0.0212	5.468	0.9155	25	26	0.576	3	2.5
$C_{10}H_{10}O_4$	0.05	NH <sub>3</sub>	-0.0222	5.5119	0.946	25	27	0.583	2.9	2.4
$C_{10}H_{10}O_4$	0.005	NH <sub>3</sub>	-0.0234	5.5013	0.9336	24	26	0.559	2.8	2.3
$C_{10}H_{10}O_4$	0.0005	NH <sub>3</sub>	-0.0234	5.5692	0.9369	24	25	0.545	2.8	2.3
$C_{10}H_{10}O_4$	1	NH <sub>3</sub>	-0.0092	5.0736	0.9279	5	6	0.229	6.3	5
$C_{10}H_{10}O_4$	0.5	NH <sub>3</sub>	-0.009	4.9658	0.9273	5	6	0.226	6.2	4.9
$C_{10}H_{10}O_4$	0.05	NH <sub>3</sub>	-0.009	4.8951	0.9398	2	4	0.45	6.1	4.8
$C_{10}H_{10}O_4$	0.005	NH <sub>3</sub>	-0.0093	4.8906	0.9467	2	3	0.198	5.9	4.6
$C_{10}H_{10}O_4$	0.0005	NH <sub>3</sub>	-0.0087	4.8904	0.9289	4	5	0.217	6.3	5
C10H10O4	1	$NH_3$	-0.0095	5.0453	0.966	-3	-2	0.159	6	4.8
C10H10O4	0.5	NH <sub>3</sub>	-0.0094	4.9604	0.9621	-2	-1	0.167	5.9	4.7
C10H10O4	0.05	NH <sub>3</sub>	-0.0097	4.9261	0.9582	0	1	0.182	5.7	4.5
C10H10O4	0.005	NH <sub>3</sub>	-0.0098	4.9076	0.9621	-1	0	0.174	5.6	4.4
C10H10O4	0.0005	NH <sub>3</sub>	-0.045	5.4673	0.9123	25	26	0.571	3.1	2.6

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C10H10O4	1	$CH_3NH_2$	-0.0152	5.2654	0.9769	3	4	0.21	4	3.2
$C_{10}H_{10}O_4$	0.5	$CH_3NH_2$	-0.0154	5.2282	0.9775	3	4	0.21	3.9	3.2
$C_{10}H_{10}O_4$	0.05	$CH_3NH_2$	-0.0155	5.1835	0.9788	2	4	0.45	3.8	3.1
$C_{10}H_{10}O_4$	0.005	$CH_3NH_2$	-0.016	5.481	0.9817	1	3	0.197	3.7	3
C10H10O4	0.0005	$CH_3NH_2$	-0.0154	5.489	0.9811	1	2	0.192	3.9	3.1
$C_{10}H_{10}O_4$	1	$CH_3NH_2$	-0.0117	5.4247	0.9216	11	12	0.305	5.4	4.4
$C_{10}H_{10}O_4$	0.5	$CH_3NH_2$	-0.0117	5.3707	0.9347	9	10	0.277	5.4	4.4
$C_{10}H_{10}O_4$	0.05	$CH_3NH_2$	-0.012	5.3436	0.9502	6	8	0.248	5.2	4.2
$C_{10}H_{10}O_4$	0.005	CH <sub>3</sub> NH <sub>2</sub>	-0.0123	5.3284	0.9539	6	7	0.243	5	4.1
$C_{10}H_{10}O_4$	0.0005	CH <sub>3</sub> NH <sub>2</sub>	-0.0121	5.4015	0.939	9	10	0.276	5.2	4.3
$C_{10}H_{10}O_4$	1	CH <sub>3</sub> NH <sub>2</sub>	-0.012	5.5244	0.9238	11	13	0.309	5.4	4.5
$C_{10}H_{10}O_4$	0.5	CH <sub>3</sub> NH <sub>2</sub>	-0.0123	5.4937	0.9465	8	9	0.263	5.3	4.3
$C_{10}H_{10}O_4$	0.05	CH <sub>3</sub> NH <sub>2</sub>	-0.0124	5.4424	0.964	3	5	0.215	5.2	4.2
$C_{10}H_{10}O_4$	0.005	CH <sub>3</sub> NH <sub>2</sub>	-0.0127	5.4473	0.9682	3	4	0.47	5	4.1
C10H10O4	0.0005	CH <sub>3</sub> NH <sub>2</sub>	-0.0124	5.5005	0.9665	3	4	0.48	5.2	4.3

#### 4.3.4 Degradation of Aflatoxin in Contaminated Maize with Different Concentrations of Sodium Hydrogen Carbonate and Catalyst

Three maize subsets comprising whole (WM), dehulled maize (DM), and ground maize (GM) were reacted with 1, 0.5, 0.05, 0.005, and 0.0005 molar of sodium hydrogen carbonate. The tests were repeated separately in combination with 4 mL of 2 % hydrogen peroxide, 2 % ammonia, and 2 % methylamine. The reaction mixture was oven heated to 80 °C, and cooled to 25 °C. Aflatoxin concentration was tested at intervals of 30 minutes for five hours. The results of degradation are summarized in appendix 4 (Table 6) and (Figure 4.42) below.







## Figure 4.42: A, B C, and D. Effect of sodium hydrogen carbonate and catalysts on the degradation rate of aflatoxin contaminants in maize

The rate of degradation was fast with 0.5 M and 0.05M concentrations on whole and ground maize at 48.81 minutes. The degradation rate was slowest in 0.05 M sodium hydrogen carbonate in whole maize at 58.25 minutes. Hydrogen peroxide with the bicarbonate, half-life (T1/2) for the rate of degradation was fastest with 0.005 M in dehulled maize at 53.32 minutes and slow with 0.0005 M in whole maize, at 62.45 minutes. In the presence of ammonia, the degradation half-life (T1/2) was fastest with 0.005 M in ground maize at 23.58 minutes and slowest with 0.5 M in dehulled maize at 31.51 minutes. However, for methylamine, half-life (T1/2) for the rate of degradation was fastest at 1 M and 0.5 M in ground maize at 23.1 minutes and slowest for 0.5 M and 0.005 M in dehulled maize, at 42.79 minutes. The results showed that concentration, nature of maize, and catalyst influenced the rate of degradation for aflatoxin in contaminants in maize (Table 4.7 below).

Maiz e	Reagent	[Con]	Catalyst	Slope	Intercept	R^2	AIC	BIC	RMSE	5µg/kg	10µg/kg
WM	NaHCO3	1	no	-0.0132	5.6875	0.939	11	12	0.302	5.1	4.3
	NaHCO3	0.5	no	-0.0133	5.6174	0.9475	9	10	0.281	5	4.2
	NaHCO3	0.05	no	-0.0134	5.5695	0.9551	8	9	0.261	4.9	4.1
	NaHCO3	0.005	no	-0.0142	5.5529	0.9721	3	5	0.216	4.6	3.8
	NaHCO3	0.0005	no	-0.0137	5.5912	0.9716	3	4	0.21	4.8	4
DM	NaHCO3	1	no	-0.0213	5.2849	0.8803	29	31	0.704	2.9	2.3
	NaHCO3	0.5	no	-0.0214	5.288	0.8873	29	30	0.682	2.9	2.3
	NaHCO3	0.05	no	-0.0213	5.2438	0.8925	28	29	0.663	2.8	2.3
	NaHCO3	0.005	no	-0.0221	5.3292	0.9047	27	29	0.642	2.8	2.3
	NaHCO3	0.0005	no	-0.016	4.9847	0.9679	8	9	0.262	3.5	2.8
GM	NaHCO3	1	no	-0.0211	5.178	0.9112	26	27	0.59	2.8	2.3
	NaHCO3	0.5	no	-0.021	5.1383	0.9144	25	26	0.576	2.8	2.3
	NaHCO3	0.05	no	-0.0191	4.9529	0.9486	17	18	0.399	2.9	2.3
	NaHCO3	0.005	no	-0.0278	5.7058	0.835	39	41	1.103	2.5	2

 Table 4.7: Summary Regression Equation Data for each Degradation Reaction with

 Different Concentrations of Sodium Hydrogen Carbonate.

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	NaHCO3	0.0005	no	-0.0188	5.0988	0.975	8	10	0.27	3.1	2.5
WM	NaHCO3	1	H2O2	-0.0118	5.2416	0.9828	-6	-5	0.141	5.1	4.2
	NaHCO3	0.5	H2O2	-0.0117	5.1784	0.9769	-3	-2	0.161	5.1	4.1
	NaHCO3	0.05	H2O2	-0.0118	5.1558	0.9742	-1	0	0.172	5	4
	NaHCO3	0.005	H2O2	-0.012	5.1331	0.9779	-3	-2	0.162	4.9	3.9
	NaHCO3	0.0005	H2O2	-0.0111	5.1358	0.9492	5	6	0.23	5.3	4.3
DM	NaHCO3	1	H2O2	-0.0155	4.6532	0.9457	13	14	0.334	3.3	2.5
	NaHCO3	0.5	H2O2	-0.0155	4.5925	0.9446	13	15	0.338	3.2	2.5
	NaHCO3	0.05	H2O2	-0.024	5.1833	0.8933	31	32	0.741	2.5	2
	NaHCO3	0.005	H2O2	-0.0155	4.5099	0.9351	15	16	0.367	3.1	2.4
	NaHCO3	0.0005	H2O2	-0.0149	4.4942	0.9294	15	16	0.368	3.2	2.5
GM	NaHCO3	1	H2O2	-0.017	4.7737	0.9495	14	15	0.351	3.1	2.4
	NaHCO3	0.5	H2O2	-0.0173	4.7521	0.9487	15	16	0.36	3	2.4
	NaHCO3	0.05	H2O2	-0.0261	5.3677	0.8819	34	35	0.854	2.4	2
	NaHCO3	0.005	H2O2	-0.0185	4.7578	0.953	15	16	0.368	2.8	2.2
	NaHCO3	0.0005	H2O2	-0.0179	4.7163	0.948	16	17	0.376	2.9	2.2
WM	NaHCO3	1	NH3	-0.026	5.8046	0.9012	32	33	0.771	2.7	2.2
	NaHCO3	0.5	NH3	-0.0221	5.4046	0.933	23	25	0.532	2.9	2.3
	NaHCO3	0.05	NH3	-0.0261	5.6053	0.9237	29	30	0.673	2.6	2.1
	NaHCO3	0.005	NH3	-0.0261	5.5832	0.9234	29	30	0.674	2.5	2.1
	NaHCO3	0.0005	NH3	-0.0256	5.5183	0.9291	27	28	0.635	2.5	2.1
DM	NaHCO3	1	NH3	-0.0146	4.199	0.8707	22	23	0.503	3	2.2
	NaHCO3	0.5	NH3	-0.0152	4.446	0.8822	22	23	0.495	2.8	2.1
	NaHCO3	0.05	NH3	-0.0231	4.7122	0.8879	30	32	0.733	2.2	1.7
	NaHCO3	0.005	NH3	-0.015	4.0667	0.8622	23	25	0.535	2.7	2
	NaHCO3	0.0005	NH3	-0.0148	4.0931	0.8579	24	25	0.537	2.8	2
GM	NaHCO3	1	NH3	-0.0161	4.3143	0.9229	18	19	0.418	2.8	2.1
	NaHCO3	0.5	NH3	-0.0181	4.4034	0.9143	22	23	0.496	2.6	1.9
	NaHCO3	0.05	NH3	-0.0261	4.943	0.9	32	33	0.778	2.1	1.7
	NaHCO3	0.005	NH3	-0.0177	4.288	0.9091	22	23	0.502	2.5	1.9
	NaHCO3	0.0005	NH3	-0.0178	4.3645	0.9005	23	24	0.529	2.6	1.9
WM	NaHCO3	1	CH3NH2	-0.019	5.945	0.9013	25	26	0.562	3.8	3.2
	NaHCO3	0.5	CH3NH2	-0.0193	5.9368	0.8987	25	26	0.579	3.7	3.1
	NaHCO3	0.05	CH3NH2	-0.0194	5.8874	0.9028	25	26	0.569	3.7	3.1
	NaHCO3	0.005	CH3NH2	-0.0195	5.858	0.9036	25	26	0.569	3.6	3
	NaHCO3	0.0005	CH3NH2	-0.0192	5.8938	0.9117	23	25	0.534	3.7	3.1
DM	NaHCO3	1	CH3NH2	-0.0165	4.3762	0.8619	26	27	0.59	2.8	2.1
	NaHCO3	0.5	CH3NH2	-0.0162	4.347	0.8604	25	27	0.584	2.8	2.1
	NaHCO3	0.05	CH3NH2	-0.016	4.2997	0.8609	25	26	0.575	2.8	2.1
	NaHCO3	0.005	CH3NH2	-0.0162	4.32	0.8644	25	26	0.571	2.8	2.1
	NaHCO3	0.0005	CH3NH2	-0.0164	4.3897	0.8706	25	26	0.566	2.8	2.1
GM	NaHCO3	1	CH3NH2	-0.03	5.379	0.9545	26	27	0.588	2.1	1.7
	NaHCO3	0.5	CH3NH2	-0.0299	5.347	0.9541	26	27	0.59	2.1	1.7
	NaHCO3	0.05	CH3NH2	-0.0284	5.18	0.9634	22	23	0.498	2.1	1.7

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NaHCO3	0.005	CH3NH2	-0.0327	5.5332	0.9418	30	31	0.728	2	1.6
NaHCO3	0.0005	CH3NH2	-0.0271	5.1223	0.9699	19	4	0.43	2.2	1.7

### 4.3.5 Degradation of Aflatoxin Contaminated Maize with Different Concentrations of Sodium Hypochlorite

Degradation of aflatoxin in maize using different concentrations of sodium hypochlorite on whole maize (WM), dehulled maize (DM), and ground maize (GM) was tested for 1.0, 0.5, 0.05, 0.005, and 0.0005 M sodium hypochlorite. The concentration of aflatoxin contaminants was measured in intervals of 30 minutes for five hours. The maize samples were heated to 80 ° C and cooled to 25 ° C before degradation reactions were initiated. The results are summarized in Appendix 4 (Table 8).

Sodium hypochlorite of 1 M reduced aflatoxin contaminants by 92.38 %, 93.04 %, and 93.76 % for whole, dehulled, and ground maize, respectively. The presence of 4 mL 2 % hydrogen peroxide solution as the catalyst reduced aflatoxin by 94.1 %, 94.24 %, and 94.48 % for whole, dehulled, and ground maize, respectively. Using 4 mL of ammonia solution as the catalyst reduced aflatoxin by 96.06 %, 96.44 %, and 96.32 % for whole, dehulled and ground maize with 4 mL methylamine solution reduced aflatoxin content in whole, dehulled, and ground maize by 95.1 % for whole, 95.62 % for dehulled and 95.72 % for ground maize.

Applying 0.5 M of sodium hypochlorite, degraded aflatoxin contaminants by 91.78 %, 95.88 %, and 93.54 % for whole, dehulled, and ground maize, respectively, while using 4 mL 2 % hydrogen peroxide solution as the catalyst reduced aflatoxin by 94.2 %, 94.58 % and 94.44 % for whole, dehulled and ground maize, respectively. Using 4 mL ammonia solution as catalysts reduced aflatoxin by 96.62 %, 96.66 %, and 96.78 % for whole, dehulled, and ground maize,

respectively. On the other hand, using 4 mL methylamine reduced aflatoxin by 95.26 % for whole, 92.76 % for dehulled, and 96.0 % for ground maize.

Sodium hypochlorite of 0.05 M reduced aflatoxin contaminants by 91.96 %, 91.96 %, and 93.88 % for whole, dehulled, and ground maize, respectively. The addition of 4 mL hydrogen peroxide solution as a catalyst reduced aflatoxin by 94.72 %, 94.72 %, and 95.02 % for whole, dehulled, and ground maize, respectively. On the other hand, the use of 4 mL ammonia solution as the catalyst reduced aflatoxin content by 96.86 % for whole, 96.86 % for dehulled, and 97.0 % for ground maize use of 4 mL methylamine solution as the catalyst reduced the aflatoxin content in maize by 95.2 % for whole, 95.2 % for dehulled and 96.12 % for ground maize, respectively.

Sodium hypochlorite of 0.005 M, degraded aflatoxin contaminants by 92.06 %, 93.4 %, and 93.68 % for whole, dehulled, and ground maize, respectively. The same concentration of sodium hypochlorite combined with 4 mL of hydrogen peroxide solution, reduced aflatoxin content in maize samples by 94.6 %, 94.86 %, and 94.92 % for whole, dehulled, and ground maize, respectively. Using 4 mL of ammonia solution as the catalyst reduced aflatoxin content in maize by 96.8 %, 96.84 %, and 97.02 % for whole, dehulled, and ground maize, respectively, while the use of 4 mL methylamine solution as a catalyst reduced aflatoxin contaminants by 94.84 %, 95.9 % and 95.96 % for whole, dehulled and ground maize, respectively.

Sodium hypochlorite of 0.0005 M, degraded aflatoxin contaminants to 90.84 %, 92.02 %, and 93.68 % for whole, dehulled, and ground maize, respectively. The addition of 4 mL hydrogen peroxide solution as the catalyst reduced aflatoxin content by 94.48 % for whole, 94.44 % for dehulled, and 94.86 % for ground maize. The presence of 4 mL of ammonia as a catalyst degraded aflatoxin by 96.6 %, 96.84 %, and 96.74 % for whole, dehulled, and ground maize,

respectively. Using 4 mL of methylamine reduced the aflatoxin content by 94.68 %, 96.64 %, and 95.68 % for whole, dehulled, and ground maize, respectively.

Dehulling and grinding of the maize were found to directly affect the rate of degradation of aflatoxin in maize. The concentration of sodium hypochlorite was found to increase the rate of degradation of aflatoxin in maize. Figure 4.43 A, B, C, and D below shows the summary of the degradation results.









Figure 4.43: Effect of Sodium Hypochlorite and Catalysts on the Degradation Rate of Aflatoxin Contaminants in Maize A, B, C, and D

The sodium hypochlorite and catalyst degradation reaction fitted into the kinetic reaction firstorder kinetics. The assumption was that sodium hypochlorite reacted with the contaminating aflatoxin molecules in maize to produce aflatoxicol products. The half-life (T1/2) for aflatoxin contaminants in maize under sodium hypochlorite was fastest with 0.5 M concentration on whole maize at 24.67 minutes and the slowest was for 0.005 molar on whole maize at 43.32 minutes. The half-life (T1/2) for the rate of decontamination of aflatoxin reaction was fastest at 0.05 M sodium hypochlorite and hydrogen peroxide on ground maize at 26.56 minutes and slowest with 1 M in whole maize, at 60.27 minutes. With ammonia catalyst, the shortest half-life (T1/2) for the rate of degradation reaction was in 0.05 molar concentration with ground maize at 26.56 minutes and the longest was in one molar concentrations in whole and dehulled maize at 47.48 minutes. The half-life (T1/2) for degradation reaction was fastest with 0.005 M sodium hypochlorite coupled with methylamine in dehulled maize at 27.62 minutes. The slowest with 0.0005 molar in ground maize at 42.01 minutes. The nature of maize, its concentration, and the catalyst influenced the degradation rate of aflatoxin contaminants in maize (Table 4.8)

 Table 4.8: Regression Data for each Degradation Reaction With Different Concentrations of Sodium Hypochlorite

Maize	Reagent	[Con]	Catalyst	Slope	Intercept	R^2	AIC	BIC	RMSE	5 μg/kg
WM	NaOCl	1	no	-0.0211	5.3181	0.871	30	31	0.726	2.9
	NaOCl	0.5	no	-0.0212	5.3395	0.8733	30	31	0.722	2.9
	NaOCl	0.05	no	-0.0193	5.1576	0.9014	25	26	0.57	3.1
	NaOCl	0.005	no	-0.0281	5.9689	0.7973	42	43	1.259	2.6
	NaOCl	0.0005	no	-0.019	5.4035	0.9308	4	22	0.465	3.3
DM	NaOCl	1	no	-0.0116	5.2625	0.7814	24	25	0.544	5.2
	NaOCl	0.5	no	-0.0104	5.1294	0.8088	4	21	0.448	5.6
	NaOCl	0.05	no	-0.0146	5.4332	0.714	33	34	0.813	4.4
	NaOCl	0.005	no	-0.0119	5.0609	0.8674	18	19	0.415	4.8
	NaOCl	0.0005	no	-0.0098	5.1044	0.927	6	8	0.246	5.9
GM	NaOCl	1	no	-0.0119	5.2847	0.8172	22	23	0.502	5.1
	NaOCl	0.5	no	-0.0115	5.2106	0.8131	22	23	0.492	5.2
	NaOCl	0.05	no	-0.0117	5.1387	0.8326	21	22	0.468	5
	NaOCl	0.005	no	-0.013	5.1088	0.9068	16	17	0.373	4.5
	NaOCl	0.0005	no	-0.0123	5.2354	0.8496	4	21	0.461	4.9
WM	NaOCl	1	$H_2O_2$	-0.0155	4.6691	0.941	14	15	0.348	3.3
	NaOCl	0.5	$H_2O_2$	-0.0155	4.626	0.9412	14	15	0.348	3.2
	NaOCl	0.05	$H_2O_2$	-0.024	5.2124	0.8897	31	32	0.756	2.5
	NaOCl	0.005	$H_2O_2$	-0.0156	4.5518	0.9388	15	16	0.358	3.1
	NaOCl	0.0005	$H_2O_2$	-0.015	4.5033	0.9324	15	16	0.362	3.2
DM	NaOCl	1	$H_2O_2$	-0.0181	5.6182	0.8598	28	29	0.654	3.7
	NaOCl	0.5	$H_2O_2$	-0.0177	5.5699	0.8707	26	28	0.61	3.7
	NaOCl	0.05	$H_2O_2$	-0.0184	5.5708	0.876	27	28	0.62	3.6
	NaOCl	0.005	$H_2O_2$	-0.0179	5.4422	0.9044	23	24	0.521	3.6
	NaOCl	0.0005	$H_2O_2$	-0.0178	5.5473	0.8856	25	26	0.572	3.7
GM	NaOCl	1	$H_2O_2$	-0.0179	5.6093	0.8514	28	29	0.666	3.7

	NaOCl	0.5	$H_2O_2$	-0.0176	5.5453	0.8664	27	28	0.616	3.7	
	NaOCl	0.05	$H_2O_2$	-0.0181	5.5257	0.879	26	27	0.6	3.6	
	NaOCl	0.005	$H_2O_2$	-0.0169	5.3704	0.9138	4	22	0.466	3.7	
	NaOCl	0.0005	$H_2O_2$	-0.0182	5.5763	0.8752	26	28	0.613	3.6	
WM	NaOCl	1	NH <sub>3</sub>	-0.0146	4.2569	0.8708	22	23	0.504	3	
	NaOCl	0.5	NH <sub>3</sub>	-0.015	4.1764	0.8645	23	25	0.532	2.9	
	NaOCl	0.05	NH <sub>3</sub>	-0.0231	4.7374	0.8845	31	32	0.748	2.3	
	NaOCl	0.005	NH <sub>3</sub>	-0.015	4.0999	0.8588	24	25	0.542	2.8	
	NaOCl	0.0005	NH <sub>3</sub>	-0.0148	4.1192	0.858	24	25	0.538	2.8	
DM	NaOCl	1	NH <sub>3</sub>	-0.0107	5.4823	0.8477	17	18	0.404	6	
	NaOCl	0.5	NH <sub>3</sub>	-0.0099	5.3724	0.8813	12	14	0.325	6.3	
	NaOCl	0.05	NH <sub>3</sub>	-0.0108	5.4136	0.8616	16	17	0.385	5.9	
	NaOCl	0.005	NH <sub>3</sub>	-0.0115	5.4478	0.8634	17	19	0.408	5.6	
	NaOCl	0.0005	NH <sub>3</sub>	-0.0109	5.4231	0.8954	13	14	0.333	5.8	
GM	NaOCl	1	NH <sub>3</sub>	-0.01	5.443	0.8451	16	17	0.382	6.4	
	NaOCl	0.5	NH <sub>3</sub>	-0.0095	5.3065	0.8796	12	13	0.313	6.5	
	NaOCl	0.05	NH <sub>3</sub>	-0.0104	5.3543	0.8516	16	17	0.386	6	
	NaOCl	0.005	NH <sub>3</sub>	-0.0114	5.408	0.8234	21	22	0.469	5.6	
	NaOCl	0.0005	NH <sub>3</sub>	-0.0109	5.4306	0.8716	16	17	0.375	5.8	
WM	NaOCl	1	$CH_3NH_2$	-0.046	4.8182	0.9033	26	27	0.604	2.6	
	NaOCl	0.5	$CH_3NH_2$	-0.045	4.7852	0.9014	26	27	0.607	2.6	
	NaOCl	0.05	$CH_3NH_2$	-0.0192	4.6588	0.9023	25	26	0.564	2.6	
	NaOCl	0.005	$CH_3NH_2$	-0.0236	5.0699	0.9069	29	30	0.676	2.4	
	NaOCl	0.0005	CH <sub>3</sub> NH <sub>2</sub>	-0.018	4.6658	0.8883	25	26	0.571	2.8	
DM	NaOCl	1	$CH_3NH_2$	-0.0235	4.8465	0.9288	25	27	0.583	2.3	
	NaOCl	0.5	$CH_3NH_2$	-0.0227	4.829	0.9453	22	23	0.49	2.4	
	NaOCl	0.05	$CH_3NH_2$	-0.041	4.6235	0.9403	4	21	0.454	2.5	
	NaOCl	0.005	$CH_3NH_2$	-0.0252	5.147	0.9359	26	27	0.591	2.3	
	NaOCl	0.0005	$CH_3NH_2$	-0.041	4.8006	0.938	4	21	0.463	2.6	
GM	NaOCl	1	$CH_3NH_2$	-0.0246	5.0905	0.965	18	19	0.421	2.4	
	NaOCl	0.5	$CH_3NH_2$	-0.0237	4.9849	0.9454	22	24	0.51	2.4	
	NaOCl	0.05	CH <sub>3</sub> NH <sub>2</sub>	-0.025	5.1345	0.9	31	32	0.744	2.4	
	NaOCl	0.005	$CH_3NH_2$	-0.022	4.9801	0.9604	17	18	0.4	2.6	

# 4.3.6 Degradation of Aflatoxin Contaminants in Maize with Ammonium Carbonate (NH4)<sub>2</sub>CO<sub>3</sub> and Catalysts

The degradation rate of aflatoxin-contaminated in maize with different concentrations of ammonium carbonate using 1.0, 0.5, 0.05, 0.005, and 0.0005 M with three different catalysts. Maize samples in three sets whole maize (WM), dehulled maize (DM), and ground maize (GM)

heated to 80 °C and cooled to 25 °C. The decrease in concentration of aflatoxin was tested in intervals of 30 minutes for five hours.

Ammonium carbonate solution of 1 M decreased aflatoxin contaminants by 97.62 %, 73.44 %, and 71.5 % for whole, dehulled, and ground maize, respectively. With 4 mL hydrogen peroxide solution, the mixture reduced aflatoxin contamination by 79.02 %, 79.74 %, and 79.32 % for whole, dehulled, and ground maize, respectively. Using 4 mL ammonia solution achieved aflatoxin reduction by 72.1 %, 75.64 %, and 74.34 % for whole, dehulled, and ground maize, respectively.

Ammonium carbonate solution of 0.5 M reduced aflatoxin contaminants by 72.0 % for whole maize, 75.46 % for dehulled maize, and 72.14 % for ground maize. Combined with 4 mL hydrogen peroxide as a catalyst reduced aflatoxin by 80.7 % for whole maize, 82.12 % for dehulled maize, and 81.02 % for ground maize. 0.5 M ammonium carbonate combined with 4 mL ammonia reduced aflatoxin by 80.88 % whole maize, 82.6 % dehulled maize, and 83.26 % ground maize, while 0.5 M ammonium carbonate with 4 mL of methylamine reduced aflatoxin contaminants by 72.84 % whole maize, 77.5 % dehulled maize and 76.04 % ground maize by 72.26 % for whole maize, 77.08 % for dehulled maize and 75.48 % for ground maize.

Ammonium carbonate solution of 0.05 M combined with 40 mL hydrogen peroxide solution achieved a mean reduction in aflatoxin by 82.66 % for whole maize, 83.80 % for dehulled maize, and 82.98 % for ground maize. 0.05 M ammonium carbonate combined with 4 mL ammonia reduced aflatoxin contaminants by 83.32 % whole maize, 84.08 % dehulled maize, and 85.1 % ground maize. Using 0.05 M ammonium carbonate solution with 4 mL 2 % methylamine achieved aflatoxin contaminants reduction by 75.72 % for whole maize, 77.08 % for dehulled maize, and 77.62 % for ground maize.

Ammonium carbonate solution of 0.005 M concentration degraded aflatoxin contaminants by 75.26 % for whole, 77.2 % for dehulled, and 77.74 % for ground maize. Using 0.005 M ammonium carbonate combined with 4 mL of hydrogen peroxide solution reduced aflatoxin by 83.86 % for whole, 84.62 % for dehulled, and 83.86 % for ground maize. 0.005 M ammonium carbonate solution combined with 4 mL 2 % ammonia solution achieved aflatoxin reduction by 83.32 % for whole, 84.62 % for dehulled, and 85.22 % for ground maize. Using 0.005 M ammonium carbonate solution combined with 4 mL 2 % ammonia solution achieved aflatoxin reduction by 77.3 % for whole, 79.68 % for dehulled, and 78.76 % for ground maize.

Ammonium carbonate solution of 0.0005 M degraded aflatoxin by 72.1 % for whole, 74.68 % for dehulled, and 76.3 % for ground maize. Combined with 4 mL of hydrogen peroxide the solution achieved aflatoxin reduction by 81.5 %, 82.84 %, and 81.5 %, for whole, dehulled, and ground maize, respectively. Ammonium carbonate of 0.0005 M with 4 mL of ammonia solution reduced aflatoxin by 82.92 % for whole, 83.78 % for dehulled, and 84.52 % for ground maize. 0.0005 M ammonium carbonate combined with 4 mL methylamine reduced aflatoxin contamination in maize by 73.74 %, 78.26 %, and 77.7 % for whole, dehulled, and ground maize, respectively.

Maize cover, particle size, reagents concentration, and catalyst had a directly exponential effect on the degradation of aflatoxin contaminant in maize. The degradation effect of different ammonium carbonate concentrations on aflatoxin contaminants in maize is shown in (Figure 4.44 A, B, C, and D).








# Figure 4.44 Effect of Ammonium Carbonate and Catalyst on Degradation of Aflatoxin Contaminants in Maize

The degradation results with ammonium carbonate fitted into the first-order reaction kinetics. The assumption was that ammonium carbonate reacted with aflatoxin molecules in maize to produce aflatoxin-ammonium products not detected as aflatoxin during the periodic tests.

Aflatoxin + 
$$(NH_4)_2CO_3$$
 — Aflaproducts

Test results for different concentrations of ammonium carbonate fitted equation 6. Respective regression curves were plotted to generate corresponding linear relationships (Figure 4.21) and data in the appendix Table 11. The linear regression equation y = mx + b, where y- is the natural logarithm of concentration (InCt) of aflatoxin in maize, and m- is the gradient or slope (k) of the curve. These are determined by the change in concentration over the change in time of degradation, x- is the variation in time (t) during the degradation process, and b- is the y-intercept value at the start of the degradation process representing the concentration of aflatoxin in the sample (InCo). The k-value in the study represented the rate constant for each degradation reaction that varied with the concentration of ammonium carbonate concentration and the nature of maize for whole, dehulled, and ground maize.

The coefficient of determination  $R^2$  value showed the percentage of how well the experimental results fit into the regression model. The effectiveness of degradation of aflatoxin contaminants in maize with ammonium carbonate was in the range from 88.18 % to 97.08 %. The shortest half-life (T1/2) was 54.15 minutes for ground and dehulled maize under 0.005 M ammonium carbonate followed by 60.80 minutes for 1 M on whole maize.

Regression curves for the rate of degradation of aflatoxin contaminants in maize with the five concentrations of ammonium carbonate combined with 4 mL of (2 %) hydrogen peroxide solution, had a coefficient of determination ( $R^2$ ) value ranging from 84.21 % to 98.12 %. The influence of other factors accounted for 1.98- 15.79 %. The half-life (T1/2) for the fastest degradation reaction was with 0.005M ammonium carbonate in dehulled maize at 54.58 minutes slowest with 0.0005M concentration in whole maize at 79.67 minutes.

Repeated with the same concentrations of ammonium carbonate combined with ammonia, ( $R^2$ ) values ranged from 90.42 % to 94.67 %. Other factors' influence was in the range of 5.33 – 9.98 %. The half-life (T1/2) for the rate of degradation reaction was fastest with 0.005 M concentration in dehulled maize at 70.73 minutes and slowest with 0.5, 0.05, and 0.005 M concentrations in whole maize, at 83.51 minutes. Regression curves were generated for the degradation of aflatoxin content in maize with different concentrations of ammonium carbonate combined with 4 mL of 2 % methylamine solution. The coefficient of determination ( $R^2$ ) values ranged from 90.60 % to 96.61 % and the influence of other factors was 3.39- 9.40 %. The half-life (T1/2) for the rate of degradation was fastest with 0.05 M ammonium carbonate in ground maize at 35.55 minutes and slowest with one molar concentration in whole maize at 57.28 minutes. The nature of maize, concentration, and the catalyst influenced the rate of degradation of aflatoxin contaminants in maize, the data summarized in (Table 4.9).

Maiz e	Reagent	[Con]	Catalyst	Slope	Intercept	R^2	AIC	BIC	RMSE	5µg/kg	10µg/kg
WM	$(NH_4)_2CO_3$	1	no	-0.0114	5.592	0.8818	16	17	0.374	5.8	4.8
	$(NH_4)_2CO_3$	0.5	no	-0.0116	5.5319	0.9112	12	14	0.325	5.6	4.6
	$(NH_4)_2CO_3$	0.05	no	-0.012	5.4821	0.9423	8	9	0.265	5.4	4.4
	$(NH_4)_2CO_3$	0.005	no	-0.0127	5.5341	0.9572	6	7	0.242	5.2	4.2
	$(NH_4)_2CO_3$	0.0005	no	-0.0116	5.6036	0.9097	13	14	0.328	5.7	4.7
DM	$(NH_4)_2CO_3$	1	no	-0.0133	5.5172	0.9307	12	14	0.324	4.9	4
	$(NH_4)_2CO_3$	0.5	no	-0.0132	5.408	0.9327	12	13	0.318	4.8	3.9
	$(NH_4)_2CO_3$	0.05	no	-0.0135	5.3949	0.9507	9	10	0.275	4.7	3.8
	$(NH_4)_2CO_3$	0.005	no	-0.0142	5.4289	0.9554	9	10	0.276	4.5	3.7
	$(NH_4)_2CO_3$	0.0005	no	-0.0131	5.43	0.9457	9	10	0.281	4.9	4
GM	$(NH_4)_2CO_3$	1	no	-0.0132	5.5576	0.9414	10	12	0.295	5	4.1
	$(NH_4)_2CO_3$	0.5	no	-0.0133	5.4979	0.9583	7	8	0.249	4.9	4
	$(NH_4)_2CO_3$	0.05	no	-0.0134	5.4567	0.965	5	6	0.23	4.8	3.9
	$(NH_4)_2CO_3$	0.005	no	-0.0139	5.4528	0.9708	4	5	0.217	4.6	3.8
	$(NH_4)_2CO_3$	0.0005	no	-0.0132	5.4648	0.9685	3	4	0.214	4.9	4
WM	$(NH_4)_2CO_3$	1	$H_2O_2$	-0.0089	5.1141	0.8676	11	13	0.31	6.6	5.3
	$(NH_4)_2CO_3$	0.5	$H_2O_2$	-0.0088	5.0231	0.8883	9	10	0.28	6.5	5.2
	$(NH_4)_2CO_3$	0.05	$H_2O_2$	-0.0087	4.9472	0.8841	9	11	0.282	6.4	5.1
	$(NH_4)_2CO_3$	0.005	$H_2O_2$	-0.0094	4.9593	0.8429	15	16	0.36	5.9	4.7
	$(NH_4)_2CO_3$	0.0005	$H_2O_2$	-0.0087	5.0076	0.8838	9	11	0.281	6.5	5.2
DM	$(NH_4)_2CO_3$	1	$H_2O_2$	-0.0129	5.2708	0.9764	0	1	0.18	4.7	3.8
	$(NH_4)_2CO_3$	0.5	$H_2O_2$	-0.0127	5.478	0.976	-1	1	0.179	4.7	3.8
	$(NH_4)_2CO_3$	0.05	$H_2O_2$	-0.0129	5.183	0.9805	-3	-1	0.163	4.6	3.7
	$(NH_4)_2CO_3$	0.005	$H_2O_2$	-0.013	5.1519	0.9812	-3	-2	0.162	4.5	3.7
	$(NH_4)_2CO_3$	0.0005	$H_2O_2$	-0.0121	5.1542	0.978	-3	-2	0.163	4.9	3.9
GM	$(NH_4)_2CO_3$	1	$H_2O_2$	-0.012	5.2169	0.9811	-5	-3	0.15	5	4
	$(NH_4)_2CO_3$	0.5	$H_2O_2$	-0.0117	5.1451	0.9765	-3	-1	0.164	5	4
	$(NH_4)_2CO_3$	0.05	$H_2O_2$	-0.0118	5.116	0.9758	-2	-1	0.167	5	4
	$(NH_4)_2CO_3$	0.005	$H_2O_2$	-0.0119	5.0702	0.9749	-2	0	0.172	4.8	3.9
	$(NH_4)_2CO_3$	0.0005	$H_2O_2$	-0.0114	5.1075	0.9659	1	2	0.192	5.1	4.1
WM	$(NH_4)_2CO_3$	1	NH <sub>3</sub>	-0.0084	5.0605	0.9216	4	5	0.22	6.8	5.5
	$(NH_4)_2CO_3$	0.5	NH <sub>3</sub>	-0.0083	4.9735	0.9255	3	4	0.212	6.8	5.4
	$(NH_4)_2CO_3$	0.05	NH <sub>3</sub>	-0.0083	4.9011	0.9112	5	6	0.232	6.6	5.2
	$(NH_4)_2CO_3$	0.005	NH <sub>3</sub>	-0.0083	4.8492	0.9158	4	6	0.224	6.5	5.1
	$(NH_4)_2CO_3$	0.0005	NH <sub>3</sub>	-0.0083	4.9099	0.9137	5	6	0.228	6.6	5.2
DM	$(NH_4)_2CO_3$	1	NH <sub>3</sub>	-0.026	5.768	0.9042	31	32	0.757	2.7	2.2
	$(NH_4)_2CO_3$	0.5	NH <sub>3</sub>	-0.022	5.3615	0.9357	23	24	0.518	2.8	2.3
	$(NH_4)_2CO_3$	0.05	NH <sub>3</sub>	-0.0261	5.5707	0.9267	28	29	0.658	2.5	2.1
	$(NH_4)_2CO_3$	0.005	NH <sub>3</sub>	-0.0261	5.5552	0.9257	28	29	0.662	2.5	2.1
	$(NH_4)_2CO_3$	0.0005	NH <sub>3</sub>	-0.0258	5.5889	0.922	29	30	0.673	2.6	2.1

 Table 4.9: Regression Data on Degradation of Aflatoxin Contaminated Maize with

 Ammonium Carbonate and Catalysts

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GM	$(NH_4)_2CO_3$	1	NH <sub>3</sub>	-0.0262	5.7182	0.9199	29	30	0.693	2.6	2.2
	$(NH_4)_2CO_3$	0.5	NH <sub>3</sub>	-0.0226	5.4009	0.9467	21	22	0.481	2.8	2.3
	$(NH_4)_2CO_3$	0.05	NH <sub>3</sub>	-0.0261	5.5461	0.9284	28	29	0.649	2.5	2.1
	$(NH_4)_2CO_3$	0.005	NH <sub>3</sub>	-0.0264	5.5393	0.9331	27	28	0.634	2.5	2
	$(NH_4)_2CO_3$	0.0005	NH <sub>3</sub>	-0.0294	5.8831	0.9436	28	29	0.646	2.4	2
WM	$(NH_4)_2CO_3$	1	CH <sub>3</sub> NH <sub>2</sub>	-0.0121	5.6177	0.9183	12	13	0.322	5.5	4.6
	$(NH_4)_2CO_3$	0.5	CH <sub>3</sub> NH <sub>2</sub>	-0.0121	5.5509	0.9401	9	10	0.275	5.4	4.5
	$(NH_4)_2CO_3$	0.05	CH <sub>3</sub> NH <sub>2</sub>	-0.0123	5.4675	0.9599	4	6	0.225	5.2	4.3
	$(NH_4)_2CO_3$	0.005	CH <sub>3</sub> NH <sub>2</sub>	-0.0127	5.4951	0.9661	3	5	0.214	5.1	4.2
	$(NH_4)_2CO_3$	0.0005	CH <sub>3</sub> NH <sub>2</sub>	-0.0124	5.5751	0.9531	7	8	0.248	5.3	4.4
DM	$(NH_4)_2CO_3$	1	CH <sub>3</sub> NH <sub>2</sub>	-0.0139	5.3951	0.9572	8	9	0.264	4.5	3.7
	$(NH_4)_2CO_3$	0.5	CH <sub>3</sub> NH <sub>2</sub>	-0.014	5.3285	0.9467	11	12	0.298	4.4	3.6
	$(NH_4)_2CO_3$	0.05	CH <sub>3</sub> NH <sub>2</sub>	-0.0139	5.2666	0.9404	12	13	0.313	4.4	3.6
	$(NH_4)_2CO_3$	0.005	$CH_3NH_2 \\$	-0.0138	5.2426	0.9376	12	13	0.32	4.4	3.6
	$(NH_4)_2CO_3$	0.0005	$CH_3NH_2$	-0.0138	5.2754	0.9369	12	13	0.321	4.4	3.6
GM	$(NH_4)_2CO_3$	1	$CH_3NH_2 \\$	-0.0191	5.8683	0.906	24	25	0.55	3.7	3.1
	$(NH_4)_2CO_3$	0.5	$CH_3NH_2$	-0.0192	5.81	0.9158	23	24	0.522	3.6	3
	$(NH_4)_2CO_3$	0.05	$CH_3NH_2 \\$	-0.0195	5.8121	0.9227	22	23	0.505	3.6	3
	$(NH_4)_2CO_3$	0.005	$CH_3NH_2 \\$	-0.0194	5.7454	0.9175	23	24	0.521	3.6	3
	$(NH_4)_2CO_3$	0.0005	CH <sub>3</sub> NH <sub>2</sub>	-0.0191	5.7469	0.9212	22	23	0.5	3.6	3

#### 4.3.7 Comparative Analysis

# **4.3.7.1** Comparative Analysis for Effect of Reagents, Concentrations, and Catalysts on the Degradation Rate of Aflatoxin Contaminants in Maize.

The rate of degradation for sodium hydrogen sulfite, ferulic acid, ammonium carbonate, sodium hydrogen carbonate, and sodium hypochlorite and the three catalysts were compared. The concentrations were 1.0, 0.5, 0.05, 0.005, and 0.0005 M for the reagents and catalysts volume at 4 mL and 2 % concentration.

The reagents had a different half-life that implied the rates of degradation of aflatoxin in maize were different. The chemical degradation reaction of aflatoxin primarily was an addition at the double bond of the furan ring and oxidation involving phenol formation and opening of the lactone ring (Gonçalves *et al.*, 2018; Owuor *et al.*, 2019; Colović *et al.*, 2019) (Figure 4.45).



Figure 4.45: Main Active Sites of Aflatoxin Molecules

The terminal double bond in the dihydrofuran ring of aflatoxins B1 and G1 was susceptible to oxidizing agent attack. The same was lacking in aflatoxin B2 and G2 therefore; they were resistant to the reaction with oxidizing agents. The oxidizing agents instead opened the lactone ring in the four-aflatoxin sub-types. Sodium hypochlorite had the shortest time over all in the measured half-life. It meant sodium hypochlorite had the best degrading effect of aflatoxin contaminants in maize compared to sodium carbonate, ferulic acid, ammonium carbonate, and sodium hydrogen sulfite.

Sodium hydrogen sulfite had the longest time in degradation reaction and the least effective. Sodium hypochlorite and sodium hydrogen sulfite induce cleavage of the lactone ring and the addition of chlorite and sulfite groups at the terminal furan ring. The reagents sodium carbonate, ferulic acid, and ammonium carbonate targeted the methoxyl group, lactone, and furan ring reading to decarboxylation, hydration at the double bond of the terminal furan ring, and opening of the ring to form polyphenols (Manubolu *et al.*, 2018; Gholami-Shabani *et al.*, 2017; Hojnik *et al.*, 2017; Karlovsky *et al.*, 2016; Kolosova and Stroka, 2011) The effect of the rate of three catalysts on the degradation rate of aflatoxin contaminants in maize with sodium hydrogen sulfite compared by values of half-half time per set of kinetic reactions. Each set of reactions had different half-life values, different from those without catalysts. The catalyst affected the degradation rate of aflatoxin contaminants in maize. The concentrations of sodium hydrogen sulfite remained at 1.0, 0.5, 0.05, 0.005 and 0.0005 M. The reaction sites were still addition at the furan ring double bond and oxidation involving phenol formation and lactone ring opening.

The reaction was on total aflatoxins, the presence of B1 and G1 terminal double bond and aflatoxin B2 and G2 without the terminal double bond affected the reaction hence making the reacting fast or slow depending on catalysts. The catalyst also participated in the opening of the ring, adding across the double bond of the furan ring. The oxidizing agents in the reaction opened the lactone ring in the four-aflatoxin subtypes. Ammonia solution had the shortest time in measured half-life and was the best in catalyzing the degrading effect compared to the other two catalysts. Sodium hydrogen sulfite alone took the longest time in degradation reaction and hence was the worst when compared to the combined with the catalysts.

The three catalysts affected the degradation rate of aflatoxin contaminants in maize with ferulic acid. This is according to the half-half values per the set of kinetic reactions. The concentrations of ferulic acid were maintained at 1.0, 0.5, 0.05, 0.005, and 0.0005 M. Each reaction set had a different half-life value, the variation indicated the effect of catalyst on the degradation rate of aflatoxin contaminants in maize. The reaction sites remained the same as in the case mentioned with sodium hydrogen sulfite. Ammonia solution had the shortest half-life measured, which implied the best ability to catalyze the degrading effect in comparison with the other two catalysts. Ferulic acid alone took the longest time to degrade aflatoxin contaminants in maize.

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The degradation rate of aflatoxin contaminants in maize with sodium hydrogen carbonate combined differently with the three catalysts were effected as observed in the half-half time for a set of kinetic reaction at all concentrations of the 1.0, 0.5, 0.05, 0.005, and 0.0005 M. Each set of reactions had different half-life values that indicated the effect of each catalyst on the degradation rate. Chemical degradation involved the same sites as the mentioned reactions above. Ammonia solution was the best catalyst for the degradation reaction; it had the shortest half-life compared to the other two catalysts. Methylamine was next in the list and hydrogen peroxide was worse than the sodium hydrogen carbonate alone except for 0.05 M concentration. The observation may mean a possibility of hydrogen peroxide interfered with the molecular reaction of sodium hydrogen carbonate.

The effect of catalysts namely ammonia, hydrogen peroxide, and methylamine on the degradation rate of aflatoxin contaminants in maize with various concentrations of sodium hypochlorite at concentrations of 1.0, 0.5, 0.05, 0.005, and 0.0005 M. The half-half for each kinetic reaction was determined and varied with the concentration, catalyst, and nature of maize. The chemical degradation reaction of aflatoxin targeted the C8-C9 double bond of the furan ring, lactone ring, and methoxyl connection to the benzene and the cyclopentenone-bridge.

Oxidation reaction involving phenol formation and opening of the lactone ring (Soni *et al.*, 2020; Mallakian *et al.*, 2017). Aflatoxins B1 and G1 contain a double bond in the terminal hydro- furan ring which was susceptible to attack by oxidizing agents. Aflatoxin B2 and G2 lack the terminal double bond and therefore resisted oxidation reaction. The sodium hypochlorite reacted with the lactone ring and the cyclopentenone-bridge in the four-aflatoxin sub-types. Sodium hypochlorite alone and with methylamine solution had the shortest half-life, hence the best in reducing aflatoxin contaminants in maize compared to the other two catalysts.

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Hydrogen peroxide and ammonia catalysts performed poorly compared to sodium hypochlorite alone. 0.05M performed better than sodium hypochlorite because of the possibility of sodium hypochlorite competing for the same reaction site with other species.

Degradation rate aflatoxin contaminants in maize with ammonium carbonate was affected by combined with any of the three catalysts as in the half-half time values for each set of kinetic reactions. Ammonium carbonate concentrations were maintained at 1.0, 0.5, 0.05, 0.005 and 0.0005M. Chemical degradation of aflatoxin molecules was primarily at the terminal furan ring, double bond, and oxidation process that formed phenol-involved lactone ring opening as earlier stated. The terminal double bond in the dihydrofuran ring of aflatoxins B1 and G1 broke easily with the degrading reagents. Aflatoxin B2 and G2 lacked the terminal double bond in the furan ring and thus resisted degrading reaction at position C8-C9.

The degradation reagents, however, opened the lactone ring in all four aflatoxin strains (Agriopoulou *et al.*, 2016; Chen *et al.*, 2014, Luo *et al.*, 2014). The lone pairs of electrons on nitrogen in ammonia improved the catalyst, it was a better catalyst compared to the other two catalysts and had the shortest half-life to qualify the effect on the degradation reaction. The other two catalysts, in comparison to ammonium carbonate alone, hydrogen peroxide was still poorer improving the rate of degradation of aflatoxin contaminants in maize. Ammonium carbonate and hydrogen peroxide might have interfered with each other in the cause of the reaction.

The reagents when ranked according to the best performer in the degradation reaction of aflatoxin contaminants in maize, sodium hypochlorite formed better than all other four, and ammonium carbonate was the worst. Ammonia solution greatly improved sodium hydrogen sulfate by oxidation and reduction at the same time. Ranking the catalysts in the degradation 170 | P a g e

reaction, ammonia performed better in three reactions (60 %) while methylamine in two (40 %). The concentration of the reagent affected the reaction differently, especially with ammonia (60 %) the lowest concentration 0.0005M was the best of three reagents (Figure 4.46).



# Figure 4.46: Comparative Analysis of Catalysts' Effect on Degradation of Aflatoxin in Contaminated Maize with Sodium Hydrogen Sulfite

#### 4.3:8 Hypothesis Testing

Univariate analysis for variance was used to test the hypothesis with the Tukey Post Hoc test, a confirmatory test run to minimize the chances of rejecting the alternative hypothesis hence committing the type one error (rejecting a true null hypothesis). The null hypothesis that aflatoxin in maize could be chemically degraded to a useable level was retained. The alternate hypothesis was that aflatoxin molecules are stable and resist chemical reactions hence difficult to degrade in food (feed) materials.

## 4.3.8.1 Chemical Reaction of Aflatoxin in Contaminated Maize with Sodium Hydrogen Sulfite and Catalysts

The univariate analysis of variance for the effect of sodium hydrogen sulfite and catalysts on contaminated whole maize, de-hulled maize, and ground maize showed statistically significant results at p < 0.01, as indicated in Table 4.10.

Source	<b>Type III Sum of Squares</b>	df	Mean Square	F	Sig.
Corrected Model	1622.134a	3	540.711	137.092	0.000
Intercept	14487.97	1	14487.97	3673.281	0.000
Chemical reaction	1622.134	3	540.711	137.092	0.000
Error	31.553	8	3.944		
Total	16141.66	12			

Table 4.10: Sodium Hydrogen Sulfite and Catalysts Tests of Between-Subject Effects

Dependent Variable: Aflatoxin concentration levels

a R2 = 0.981 (Adjusted R2 = 0.974)

The Tukey Post Hoc Test showed the means categorized in three subsets indicating statistically significant differences as shown in Table 4.11 below. The results indicated that sodium hydrogen sulfite and the catalysts degraded aflatoxin contaminants in whole maize, de-hulled maize, and ground maize to usable levels. Thus, the null hypothesis was retained and the alternate hypothesis was rejected.

Types of Chemical Reactions	N	Subset		
		1	2	3
Ammonia solution + sodium hydrogen sulfite	3	21.2833		
Hydrogen peroxide + sodium hydrogen sulfite	3	25.56		
Methylamine + sodium hydrogen sulfite	3		43.0267	
Sodium hydrogen sulfite	3			49.1167
Sig.		0.111	1	1

#### Table 4.11: Sodium Hydrogen Sulfite and Catalysts Tukey SD Test

#### 4.3.8.2 Chemical Catalysts

Univariate analysis of variance run for the effect of ferulic acid and catalysts on contaminated whole, de-hulled, and ground maize yielded results that were statistically significant at p < 0.01 as indicated in (Table 4.12).

<b>Fable 4.12: Ferulic Acid and</b>	Catalysts Tests	s of Between-Subj	ect Effects
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Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	535.023a	3	178.341	151.654	0.000
Intercept	4836.469	1	4836.469	4112.731	0.000
Chemical reaction	535.023	3	178.341	151.654	0.000
Total	5380.899	12			

Dependent Variable: Aflatoxin concentration levels

a  $R^2 = 0.983$  (Adjusted  $R^2 = 0.976$ )

The Tukey Post Hoc test results showed three subsets statistically significant (Table 4.13). The degradation of aflatoxin strains in whole maize, de-hulled maize, and ground maize was possible with Ferulic acid and catalysts. Thus, the null hypothesis was retained, while the alternate hypothesis was rejected.

Types of chemical reaction	Ν	Subset			
		1	2	3	4
Ammonia solution + ferulic acid	3	12.5033			
Methylamine + ferulic acid	3		16.6167		
Hydrogen peroxide + ferulic acid	3			4.6833	
Ferulic acid	3				30.5
Sig.		1	1	1	1

 Table 4.13: Ferulic Acid and Catalysts Tukey SD Test

## 4.3.8.3 Chemical Reaction of Aflatoxin in Contaminated Maize with Ammonium Carbonate and Catalysts

The univariate analysis of variance test for the effect of ammonium carbonate and catalysts on contaminated whole maize, de-hulled maize, and maize flour. The results were statistically significant at p < 0.01 as indicated in (Table 4.14).

1 able 4.14: Ammonium Cardonale and Calarysis Tests of Between-Subject Effect	Table	<b>4.14:</b> A	Ammonium	<b>Carbonate and</b>	Catalysts	Tests o	f Between-	Subject	Effects
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Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	150.986a	3	50.329	18.194	0.001
Intercept	12288	1	12288	4442.048	0.000

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Chemical reaction	150.986	3	50.329	18.194	0.001
Error	22.13	8	2.766		
Total	12461.12	12			

Dependent Variable: Aflatoxin concentration levels

a  $R^2 = 0.872$  (Adjusted  $R^2 = 0.874$ )

The Tukey Post Hoc test showed the means in three different subsets indicating a statistically significant difference as shown in (Table 4.15). The results showed that ammonium carbonate and the three catalysts could degrade whole maize, de-hulled maize, and ground maize to usable levels. Thus, the null hypothesis was retained while the alternate hypothesis was rejected.

Types of chemical reaction	Ν	Subset	
		1	2
Ammonia solution + ammonium carbonate	3	27.9733	
Hydrogen peroxide + ammonium carbonate	3	29.28	
Methylamine + ammonium carbonate	3		33.9667
Ammonium carbonate	3		36.78
Sig.		0.774	0.241

 Table 4.15: Ammonium Carbonate and Catalysts Tukey SD Test

## 4.3.8.4: Chemical Reaction of Aflatoxin in Contaminated Maize with Sodium Hydrogen Carbonate and Catalysts

Univariate analysis of variance test for the effect of sodium hydrogen carbonate and catalysts on aflatoxin in whole, de-hulled, and ground maize. The results were statistically significant at p < 0.01 as indicated in shown in Table 4.16.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected model	641.198a	3	213.733	66.118	0.000
Intercept	6798.232	1	6798.232	2103.018	0.000
Chemical reaction	641.198	3	213.733	66.118	0.000
Error	25.861	8	3.233		
Total	7465.291	12			

Table 4.16: Sodium Hydrogen Carbonate and Catalysts Tests of Between-Subject Effects

Dependent Variable: Aflatoxin concentration levels

a  $R^2 = 0.961$  (Adjusted  $R^2 = 0.947$ )

The Tukey Post Hoc Test showed the means in three subsets indicating a statistically significant difference as shown in Table 4.17. The results showed that sodium hydrogen carbonate and catalysts can degrade aflatoxins content in whole, de-hulled, and ground maize.

 Table 4.17: Sodium Hydrogen Carbonate and Catalysts Tukey SD Test

Types of reactions	Ν	Subset		
		1	2	3
Ammonia solution + sodium carbonate	3	11.86		
Hydrogen peroxide + sodium carbonate	3		24.87	
Methylamine + sodium carbonate	3		26.9067	26.9067
Sodium carbonate	3			31.57
Sig.		1	0.54	0.052

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# 4.3.8.5: Chemical reaction of aflatoxin in contaminated maize with sodium hypochlorite and catalysts

Univariate analysis of variance of sodium hypochlorite and catalysts on contaminated whole, de-hulled, and ground maize. The results were statistically significant at p <0.01 as indicated in (Table 4.18).

Source	<b>Type III Sum of Squares</b>	Df	Mean Square	F	Sig.
Corrected Model	56.189a	3	18.73	17.155	0.001
Intercept	694.489	1	694.489	636.11	0.000
Chemical reaction	56.189	3	18.73	17.155	0.001
Error	8.734	8	1.092		
Total	759.412	12			

Table 4.18: Sodium hypochlorite and catalysts tests of between-subject effects

Dependent Variable: Aflatoxin concentration levels

a  $R^2 = 0.865$  (Adjusted  $R^2 = 0.815$ 

The Tukey Post Hoc Test results showed three different subsets indicating a statistically significant difference as shown in (Table 4.19). The results, therefore, indicate that sodium hypochlorite and catalysts can degrade whole maize, de-hulled maize, and maize flour to usable levels. The null hypothesis was retained while the alternate hypothesis was dropped.

 Table 4.19: Sodium hypochlorite and catalysts Tukey SD Test

Types of chemical reaction	Ν	Subset		
		1	2	3
Ammonia solution + sodium hypochlorite	3	5.2833		
Methylamine + sodium hypochlorite	3	6.0833	6.0833	
Hydrogen peroxide + sodium hypochlorite	3		8.19	8.19
Sodium hypochlorite	3			10.8733
Sig.		0.786	0.14	0.054

4.4 Design of a Low-Cost Industrial Process for Utilizing Aflatoxin Decontaminated Maize.

Most of the industrial designs available process clean/striped maize destined for human and animal consumption, for example, hammer and roller mills, Oil pressers, and crushers. In this study, a fermenter was deigned to convert decontaminated maize into ethanol, and the remaining materials were converted into briquettes. The reactor design included a mixing container, temperature



Figure 4.47: Flow Diagram for Briquettes and Ethanol production from Degraded Aflatoxin Contaminated Maize.

The most important storage reserve of carbohydrates in staple foodstuffs is starch. Carbohydrate content in different sources varies widely. For instance, cereal grains (40–90 % dry weight), roots (30–70 % dry weight), and tubers (65–85 % dry weight). In the food industry, starch is used as a thickener, gelling agent, bulking agent, and water retention agent. Dry maize kernel contains 71.5–73 % carbohydrate, composed of macromolecular granules of amylose

and amylopectin starch in the ratio 1:3 and other contents such as proteins 10.3 %, fats 3.8 % ash 1.3 % and moisture 13.1 % (Spier *et al.*, 2013).

Degradation of aflatoxin contaminants in maize improved the starch properties by increasing carboxyl and carbonyl content, pasting, enzymatic susceptibility, depolymerization, hydrolysis, and relative crystallinity. Among the alternative uses of contaminated maize is improved starch (Spier *et al.*, 2013; Singh *et al.*, 2013). Maize farmers are depressed communities, economically, while other maize value chain actors benefit through access to the market for their maize irrespective of quality and income through employment in the ethanol industry (Bothast & Schlicher, 2005). Clean maize samples were degraded in a similar method as aflatoxin-contaminated maize samples using the five reagents and catalysts. The residues could be used as binders for briquettes (Zubairu & Gana 2014)

#### 4.4:1 Application of Maize Residue in Industrial Ethanol Production.

#### 4.4.2 The Ethanol Yield from Aflatoxin Decontaminated and Clean Maize

After fermentation and distillation processes, the ethanol yields from contaminated maize, decontaminated maize with and without catalysts, and clean maize were compared. The yields for each feed namely, whole maize (WM), dehulled (DM), and ground maize (GM) (Figure 4.48) were measured.



Figure 4.48: Ethanol Yield Variation from Aflatoxin decontaminated and Clean Maize

The mean volume of ethanol yield from untreated contaminated maize was;  $31.85 \pm 3.42$  mL whole maize,  $33.24 \pm 2.12$  mL dehulled maize, and  $32.16 \pm 1.97$  mL ground maize. The mean volume of ethanol yield from clean maize before any treatment was;  $38.19 \pm 3.21$  mL for whole maize,  $39.83 \pm 3.92$  mL dehulled maize, and  $37.46 \pm 1.79$  mL for ground maize, respectively. The mean volume of ethanol produced from decontaminated maize with sodium hydrogen sulfite on whole maize, dehulled maize, and ground maize was;  $28.26 \pm 5.72$  mL,  $29.11 \pm 4.11$  mL, and  $27.17 \pm 4.05$  mL, respectively.

The mean volume of ethanol yield from remains of degradation of contaminated maize with sodium hydrogen sulfite combined with hydrogen peroxide was;  $26.97 \pm 2.89$  mL for whole maize,  $28.00\pm 2.90$  mL dehulled and  $28.33 \pm 1.51$  mL ground maize, respectively. Using clean maize with sodium hydrogen sulfite combined with hydrogen peroxide gave ethanol yield of  $37.27\pm 1.75$  mL for whole maize,  $33.62 \pm 3.44$  mL dehulled maize, and  $33.82 \pm 3.65$  mL for **180** | P a g e

ground maize, respectively. The mean volume of ethanol yield from contaminated maize products of degradation with sodium hydrogen sulfite combined with ammonia was;  $24.76 \pm 5.57$  mL for whole maize,  $25.98 \pm 2.46$  mL for dehulled maize, and  $25.39 \pm 2.26$  mL for ground maize.

The test on degradation products of clean maize with sodium hydrogen sulfite combined with ammonia gave ethanol yield was;  $32.27 \pm 4.75$  mL for whole maize  $33.60 \pm 3.12$  mL dehulled maize and  $33.77 \pm 3.20$  mL ground maize, respectively. The mean volume of ethanol yield from degraded products of contaminated maize with sodium hydrogen sulfite combined with methylamine was  $29.57 \pm 2.93$  mL for whole maize,  $29.94 \pm 2.65$  mL for dehulled and  $29.43 \pm 2.40$  mL for ground maize, respectively. Sodium hydrogen sulfite combined with methylamine on clean maize gave ethanol yield of  $33.25 \pm 3.74$  mL for whole maize,  $34.47 \pm 2.92$  mL dehulled maize, and  $34.92 \pm 1.19$  mL ground maize, respectively.

The fermentation process with products of ferulic acid on clean maize yielded ethanol at 33.89  $\pm$  2.45 mL for whole maize, 34.34  $\pm$  1.62 mL for dehulled maize, and 34.45  $\pm$  1.31 mL for ground maize, respectively. Ethanol yields from degradation residues of contaminated maize with ferulic acid were 29.81  $\pm$  3.33 mL for whole maize, 29.78  $\pm$  1.91 mL for dehulled maize, and 30.01  $\pm$  4.22 mL for ground maize, respectively. Tests with products of clean maize and ferulic acid combined with hydrogen peroxide, ethanol yield volume was 34.92  $\pm$  2.77 mL for whole maize, and 34.42  $\pm$  2.02 mL dehulled maize and 33.44  $\pm$  3.75 mL ground maize respectively.

The mean volume of ethanol yield from the remains of the reaction between contaminated maize with ferulic acid combined and hydrogen peroxide was  $28.18 \pm 2.35$  mL from whole maize,  $28.59 \pm 1.65$  mL for dehulled maize, and  $28.03 \pm 2.35$  mL for ground maize, respectively. The **181** | P a g e

mean volume of ethanol yield from products of contaminated maize reacted with ferulic acid combined and ammonia was;  $27.36 \pm 5.16$  mL for whole maize,  $27.03 \pm 2.15$  mL for dehulled maize, and  $27.13 \pm 1.07$  mL for ground maize, respectively.

Tests with products from the reaction of ferulic acid with clean maize combined with ammonia, the mean volume of ethanol yield was  $36.98 \pm 2.88$  mL for whole maize, and  $36.94 \pm 2.24$ mL dehulled maize and  $32.99 \pm 4.62$  mL ground maize respectively. The mean volume of ethanol yield from degradation remains of contaminated maize with ferulic acid combined with methylamine was;  $26.08 \pm 1.89$  mL for whole maize,  $26.40 \pm 4.27$  mL for dehulled maize, and  $26.09 \pm 2.49$  mL for ground maize, respectively. Products of ferulic acid with clean maize combined and methylamine tested, the mean volume of ethanol yield was;  $30.45 \pm 5.81$  mL for whole maize,  $36.49 \pm 2.78$ mL dehulled maize, and  $35.54 \pm 1.68$  mL ground maize respectively. In experiments with products of clean maize and sodium hydrogen carbonate, the mean volume of ethanol yield was  $31.92 \pm 1.21$  mL for whole maize,  $34.65 \pm 2.28$  mL dehulled maize, and  $34.62 \pm 1.11$  mL ground maize respectively.

The degradation products contaminated maize with sodium hydrogen carbonate, the mean volume ethanol yielded was;  $29.17 \pm 3.32$  mL whole maize,  $30.16 \pm 2.72$  mL dehulled maize, and  $28.47 \pm 1.30$  mL ground maize respectively. Repeated tests with products of clean maize and sodium hydrogen carbonate combined with hydrogen peroxide, the mean volume of ethanol yield was;  $34.64 \pm 2.65$  mL from whole maize,  $34.68 \pm 2.15$  mL for dehulled maize, and  $34.22 \pm 2.55$  mL for ground maize respectively.

The mean volume of ethanol yield from products of contaminated maize with sodium hydrogen carbonate in combination with hydrogen peroxide was;  $28.51 \pm 2.48$  mL for whole maize,  $26.54 \pm 2.97$  mL for dehulled maize, and  $28.79 \pm 3.97$  mL for ground maize, respectively. The results **182** | P a g e

of ethanol yield from product-contaminated maize reaction with sodium hydrogen carbonate combined with ammonia solution were;  $27.69 \pm 1.33$  mL for whole maize,  $28.10 \pm 2.06$  mL for dehulled maize, and  $28.02 \pm 3.99$  mL for ground maize, respectively. Repeated tests with products of clean maize with sodium hydrogen carbonate combined with ammonia, the mean volume ethanol yield was;  $35.34 \pm 2.23$  mL for whole maize,  $35.36 \pm 2.20$ mL dehulled maize, and  $35.12 \pm 1.67$  mL ground maize respectively.

The mean volume ethanol yield for the products of contaminated maize with sodium hydrogen carbonate combined with methylamine was;  $27.02 \pm 2.11$ mL for whole maize,  $27.39 \pm 1.45$  mL for dehulled maize, and  $27.26 \pm 1.65$  mL for ground maize, respectively. In experiments with products of clean maize with sodium hydrogen carbonate combined with methylamine, the mean volume of ethanol yield was;  $35.98 \pm 3.11$  mL for whole maize,  $35.94 \pm 2.99$  mL dehulled maize, and  $33.93 \pm 2.28$  mL ground maize respectively.

The use of clean maize with sodium hypochlorite gave ethanol yield of  $32.92 \pm 4.90$  mL for whole maize,  $33.65 \pm 3.86$  mL dehulled maize, and  $34.21 \pm 4.01$  mL ground maize, respectively. The mean volume of ethanol yield from products degraded contaminated maize with sodium hypochlorite was  $27.77 \pm 2.45$  mL,  $29.67 \pm 2.02$  mL, and  $28.27 \pm 2.10$  mL whole, dehulled, and ground maize, respectively. Residues of clean maize reacted with sodium hypochlorite combined with hydrogen peroxide gave ethanol yield of  $35.12 \pm 2.77$  mL for whole maize,  $35.02 \pm 2.02$  mL for dehulled maize, and  $35.47 \pm 3.75$  mL for ground maize.

Fermentation of the residues of contaminated maize reacted with sodium hypochlorite combined with hydrogen peroxide gave ethanol yield of  $25.89 \pm 5.90$  mL for whole maize,  $26.81 \pm 2.42$  mL dehulled maize and  $25.56 \pm 2.01$  mL ground maize, respectively. Fermentation of the remains of the reaction between contaminated maize with sodium hypochlorite combined **183** | P a g e

with ammonia yielded ethanol at  $28.33 \pm 5.57$  mL whole maize,  $28.66 \pm 2.46$  mL dehulled maize, and  $28.51 \pm 2.26$  mL ground maize.

Using clean maize remains from the reaction between sodium hypochlorite combined with ammonia yielded  $34.97 \pm 2.10$  mL for whole maize,  $34.49 \pm 2.85$  mL dehulled maize, and  $33.45 \pm 4.06$  mL ground maize, respectively. The mean volume of ethanol yield from degradation residues from contaminated maize reacted with sodium hypochlorite combined with methylamine was  $28.84 \pm 0.92$  mL for whole maize,  $28.80 \pm 1.45$  mL for dehulled maize, and  $28.89 \pm 3.93$  mL for ground maize, respectively. Clean maize reacted with sodium hydrogen carbonate combined with methylamine yielded  $34.22 \pm 3.61$  mL for whole maize,  $35.27 \pm 2.39$  mL dehulled maize and ammonium carbonate degradation on clean maize yielded  $29.94 \pm 1.21$  mL for whole maize,  $32.44 \pm 2.28$  mL dehulled maize and  $35.14 \pm 1.11$  mL ground maize, respectively.

Using residues of contaminated maize reacted with ammonium carbonate yielded  $26.55 \pm 2.29$  mL for whole maize,  $26.67 \pm 2.29$  mL for dehulled maize, and  $26.82 \pm 1.60$  mL for ground maize, respectively. Residues of clean maize reacted with ammonium carbonate catalyzed by hydrogen peroxide yielded  $30.92 \pm 2.72$  mL for whole maize,  $33.46 \pm 2.18$  mL for dehulled maize, and  $33.78 \pm 3.71$  mL for ground maize, respectively.

Fermentation of the residues of contaminated maize reacted with ammonium carbonate and catalyzed hydrogen peroxide yielded  $28.12 \pm 3.35$  mL for whole maize,  $27.81 \pm 2.78$  mL for dehulled maize, and  $27.71 \pm 4.75$  mL for ground maize, respectively. The reaction residues of the same reaction catalyzed by ammonia yielded  $26.75 \pm 1.12$  mL for whole maize,  $26.22 \pm 2.95$  mL for dehulled maize, and  $27.48 \pm 1.16$  mL for ground maize, respectively. Tests with products of reaction between clean maize and ammonium carbonate combined with ammonia, the mean **184** | P a g e

volume of ethanol yield was;  $34.72 \pm 2.38$  mL for whole maize,  $33.73 \pm 2.44$  mL dehulled maize, and  $36.11 \pm 2.07$  mL for ground maize, respectively.

The mean volume of ethanol from the reaction catalyzed by methylamine was  $28.64 \pm 1.63$  mL for whole maize,  $28.77 \pm 1.48$  mL for dehulled maize, and  $28.14 \pm 1.87$  mL for ground maize, respectively. Clean maize reacted with ammonium carbonate combined with methylamine yielded  $35.12 \pm 2.81$  mL for whole maize  $34.92 \pm 3.06$  mL for dehulled maize and  $34.76 \pm 3.38$  mL for ground maize, respectively.

#### 4.4.3 Comparison of Ethanol Yield, Mean and Probability Distribution

The tests for the hypothesis that aflatoxin-contaminated maize could be degraded to a usable level were done by checking the homogeneity of variance for the data collected using Levene's test which was highly significant at p < 0.01. Due to non-homogenous variance, the Welch test was used instead of the ordinary t-test for independent variables in the degradation and fermentation process data, there was high significant at p < 0.01. Ethanol yield from clean maize had the highest ethanol yield at 34.7 % followed by contaminated maize at 29.2 % as shown in the violin plot (Figure 4.49) below. The whisker lines and symbols inside boxes represent the interquartile range median and means, respectively.



Figure 4.49: Comparison of Ethanol Yield, Mean, and Probability Distribution

# 4.4.4: Comparative of the Effect of Reagents, Catalyst, Contamination, and Nature of Maize On Mean Volume of Ethanol Yield

The variations in the mean volume of ethanol produced depended on the type of oxidant used for degradation, the catalyst, the extent of maize contamination, and the nature of maize. The mean volume of ethanol produced from contaminated maize was the reference for comparison of the degradation products in whole maize, dehulled maize, and ground maize. The mean volume of ethanol yield from clean maize before any treatment was also used as a reference for comparison of the degradation products in whole, dehulled, and ground maize.

The mean volume of ethanol produced from residues of degradation of contaminated maize with sodium hydrogen sulfite decreased by 11.3 % for whole, 12.4 % for dehulled, and 15.5 % for ground maize, respectively, compared to the reference. The volume of ethanol yield from residues of clean maize degradation by sodium hydrogen sulfite reduced by 12.0 % for whole, 12.5 % for dehulled, and 6.8 % for ground maize, respectively, compared to the referenced. In the two reactions, the most affected were contaminated ground and dehulled clean maize, while the least affected were contaminated whole and ground clean maize.

Comparison of the mean volume of ethanol yield from residues of degradation of contaminated maize with sodium hydrogen sulfite using hydrogen peroxide catalyst to the reference showed a decrease of 15.3 % for whole maize, 15.8 % dehulled, and 11.9 % ground maize, respectively. Comparison of the ethanol yield from products of the reaction between clean maize with sodium hydrogen sulfite catalyzed by hydrogen peroxide to the reference declined by 3.1 % whole, 15.6 % dehulled, and 9.7 % ground maize, respectively. Dehulled contaminated and dehulled clean maize products were more affected than ground contaminated and ground clean maize products. **186** | P a g e

Ethanol yield from contaminated maize reaction with sodium hydrogen sulfite catalyzed by ammonia decreased by 22.3 % for whole maize, 21.9 % for dehulled maize, and 21.1 % for ground maize. In a comparison of degradation products of clean maize with sodium hydrogen sulfite combined with ammonia to the reference, there was a decrease in volume of ethanol yield by 15.5 % for whole maize 15.6 % dehulled maize, and 9.9 % ground maize respectively. Hence the most affected were whole contaminated and dehulled clean maize products compared to ground contaminated and ground clean maize.

Ethanol yield from residues of contaminated maize reacted with sodium hydrogen sulfite catalyzed by methylamine reduced by 7.2 % for whole, 9.9 % for dehulled, and 8.5 % for ground maize, respectively. For clean maize reacted with sodium hydrogen sulfite catalyzed by methylamine, ethanol yield decreased by 12.9 % for whole 13.5 % dehulled and 8.1 % ground maize, respectively. This showed that the most affected were dehulled contaminated and dehulled clean maize products, while the least affected were whole contaminated and ground clean maize.

The volume of ethanol yield from the fermentation of the products of contaminated maize reacted with ferulic acid compared to the reference showed a decline of 6.4 % whole, 10.4 % dehulled, and 6.7 % ground maize, respectively. The same reaction was compared for the mean volume of ethanol yield from degradation remains of clean maize with ferulic acid to the volume of the reference, there was a decrease of 11.3 % whole, 13.8 % dehulled, and 8.0 % ground maize, respectively. Dehulled contaminated and dehulled clean maize products were the most affected while whole contaminated and ground clean maize products were the least affected.

The mean volume of ethanol from the reaction between contaminated maize with ferulic acid combined and hydrogen peroxide was lower than the reference by 11.5 % whole, 14.0 % 187 | P a g e

dehulled, and 12.8 % ground maize, respectively. The product of clean maize and ferulic acid catalyzed by hydrogen peroxide dropped by 8.6 % for whole, 13.6 % dehulled, and 10.7 % ground maize, respectively, compared to the reference. Fermentation of the reaction products of dehulled contaminated and dehulled clean maize were the most affected while whole contaminated and whole clean maize products were the least affected. Ethanol yield from the products of contaminated maize reacted with ferulic acid catalyzed by ammonia declined by 14.1 % for whole, 18.7 % dehulled, and 15.6 % ground maize, respectively, compared to the reference.

The products for the reaction of ferulic acid and clean maize catalyzed with ammonia compared, the mean volume of ethanol yield reduced by 3.2 % whole maize, 7.2 % dehulled maize, and 11.9 % ground maize, respectively.

The residues of dehulled contaminated and ground clean maize were more affected, while whole contaminated and whole clean maize products were the least affected. Ethanol yield from degradation products of contaminated maize with ferulic acid catalyzed by methylamine reduced by 18.4 % for whole, 20.6 % for dehulled, and 18.9 % for ground maize, respectively. The residues of clean maize with ferulic acid catalyzed by methylamine had ethanol yield declined by 20.3 % for whole, 8.4 % for dehulled, and 5.1 % for ground maize, respectively, compared to the reference yield. The results show that dehulled contaminated and whole clean maize products were more affected, while whole contaminated and ground clean maize products were the least affected.

In comparison of the mean volume of ethanol yield from the reaction products of contaminated maize with sodium hydrogen carbonate to the yield of the reference, there was a reduction in volume by; 8.4 % for whole, 9.3 % for dehulled and 11.5 % for ground maize, respectively. The **188** | P a g e

mean volume of ethanol yield from degradation products of clean maize with sodium hydrogen carbonate was reduced by 16.4 % for whole, 13.0 % for dehulled, and 7.6 % for ground maize, respectively, compared to the reference. Sodium hydrogen carbonate, ground contaminated and whole clean maize products were the most affected while whole contaminated and ground clean maize products were the least affected.

When the mean volume of ethanol yield from the products of contaminated maize with sodium hydrogen carbonate in combination with hydrogen peroxide compared to the volume of the reference, it dropped by 10.5 % for whole, 20.2 % for dehulled and 10.4 % for ground maize, respectively. Ethanol volume from the products of clean maize and sodium hydrogen carbonate catalyzed by hydrogen peroxide yielded reduced by 9.2 % for whole, 12.9 % for dehulled, and 8.6 % ground maize, respectively. Dehulled contaminated and dehulled clean maize products were the most affected while ground contaminated and ground clean maize products were the least affected.

Ethanol yield from the product of contaminated maize reaction with sodium hydrogen carbonate catalyzed with ammonia solution was reduced by 13.1 % for whole, 15.5 % for dehulled, and 12.9 % for ground maize, respectively. Ethanol yield with clean maize with sodium hydrogen carbonate combined with ammonia declined by 7.5 % for whole, 11.2 % for dehulled, and 6.2 % for ground maize, respectively. Sodium hydrogen carbonate reactions with dehulled contaminated and dehulled clean maize products were more affected compared to ground contaminated and ground clean maize products.

The mean ethanol yield from the reaction contaminated maize with sodium hydrogen carbonate catalyzed by methylamine reduced by 15.1 % for whole, 17.6 % for dehulled, and 15.2 % for ground maize, respectively. For clean maize reaction with sodium hydrogen carbonate catalyzed **189** | P a g e

by methylamine dropped in ethanol yield by 5.8 % for whole, 9.8 % for dehulled, and 9.4 % for ground maize, respectively. Hence dehulled contaminated and dehulled clean maize products were more affected while whole contaminated and whole clean maize products were less affected.

Ethanol yield from the products of degradation of contaminated maize with sodium hypochlorite had yielded less than the reference by 14.4 % for whole, 10.4 % for dehulled, and 12.1 % for ground maize, respectively. The product of clean maize reaction with sodium hypochlorite yielded lower ethanol by 13.8 % for whole, 15.5 % for dehulled, and 8.7 % for ground maize, respectively. Whole contaminated and dehulled clean maize products were more affected compared to dehulled contaminated and ground clean maize. Ethanol yield from the products of the reaction between clean maize and sodium hypochlorite combined catalyzed by hydrogen peroxide was compared to the reference was lower by 18.7 % for whole, 19.4 % for dehulled and 20.5 % for ground maize, respectively. The products of the reaction for clean maize with sodium hypochlorite combined with hydrogen peroxide, the mean ethanol yield that was; 8.0 % whole maize, 12.1 % dehulled maize, and 5.3 % ground maize lower than the reference in that order.

In the two reactions of sodium hypochlorite, ground contaminated and dehulled clean maize products were the most affected while whole contaminated and ground clean maize products were the least affected. When the mean volume of ethanol yield from the remains of the reaction between contaminated maize with sodium hypochlorite combined with ammonia was compared with the reference yield the volume was lower by; 11.1 % for whole, 13.7 % for dehulled and 11.3 % for ground maize.

Ethanol yield from clean maize from sodium hypochlorite catalyzed by ammonia, decreased by 8.4 % for whole maize, 13.4 % for dehulled maize, and 10.7 % for ground maize, respectively. Dehulled contaminated and dehulled clean maize products were more affected than whole contaminated and whole clean maize. The mean ethanol yield from degradation products of contaminated maize with sodium hypochlorite catalyzed by methylamine decreased by 9.4 % for whole maize, 13.4 % for dehulled maize, and 10.2 % for ground maize, respectively.

The mean ethanol yield volume from the reaction products of clean maize with sodium hydrogen carbonate catalyzed by methylamine decreased by 10.4 % for whole, 11.4 % for dehulled, and 11.1 % for ground maize. Dehulled contaminated and dehulled clean maize products were more affected than whole contaminated and whole clean maize. Using ammonium carbonate and contaminated maize yielded lower ethanol by 16.6 % for whole, 19.8 % for dehulled, and 16.7 % for ground maize, respectively. In this case, the product of clean maize yielded a lower yield by 21.6 % for whole, 18.6 % for dehulled, and 6.2 % for ground maize. The results showed that the effect of ammonium carbonate on dehulled contaminated and whole clean maize was more than on whole contaminated and ground maize. The mean ethanol yield from the products of contaminated maize with ammonium carbonate catalyzed by hydrogen peroxide decreased by 11.8 % for whole, 16.3 % for dehulled, and 13.8 % for ground maize.

Ethanol yield from the products of the reaction between clean maize with ammonium carbonate catalyzed by hydrogen peroxide was lower by 19.0 % for whole, 16.0 % for dehulled, and 9.8 % for ground maize, respectively, suggesting that dehulled contaminated and whole clean maize were more affected than whole contaminated and ground maize products. For the same reaction catalyzed with ammonia, the ethanol yield decreased by 16.1 % for whole, 21.1 % for dehulled,

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and 14.5 % for ground maize, respectively. Using clean maize and ammonium carbonate catalyzed by ammonia recorded lower ethanol yield by 9.1 % for whole, 15.3 % for dehulled, and 3.6 % for ground maize, respectively.

The results showed that dehulled contaminated and dehulled clean maize products were more affected than ground contaminated and ground clean maize. The mean volume of ethanol from the products of the reaction between ammonium carbonate catalyzed by methylamine decreased by 10.1 % for whole maize, 13.4 % for dehulled maize, and 12.5 % for ground maize, respectively. Ethanol yield from the products of the reaction between the clean maize with ammonium carbonate catalyzed by methylamine was 8.0 % for whole, 12.3 % for dehulled, and 7.2 % for ground maize, respectively. The results showed that dehulled contaminated and dehulled clean maize products were more affected by the ammonium carbonate with methylamine than whole contaminated and whole clean maize products.

# 4.4.5 Comparison of Interaction Effect of Maize Type, Reagents, and Catalyst on Ethanol Yield from Aflatoxin decontaminated Maize

There was a marked interaction among the maize type, the catalysts, and oxidants used in the degradation of aflatoxin in maize, before ethanol production, and this affected ethanol yields. The maize type interacted differently with the catalyst and the oxidants, partly due to the coating on the grains and the particle size. Dehulled maize had the best interaction with the catalysts and the oxidants and exhibited higher yields. Ammonium carbonate interaction with the dehulled maize showed the worst performance without catalysts and minimal performance with ammonia, but slightly better performance with methylamine, sodium hypochlorite, and sodium hydrogen sulfite.

Ferulic acid with methylamine also performed poorly for dehulled maize whereas improvement in ethanol yield was recorded without catalysts and with hydrogen peroxide. The performance of sodium hydrogen sulfite with dehulled maize was very good with methylamine and hydrogen peroxide, but also very poor with ammonia and without catalyst. Sodium hypochlorite performed well with ammonia and methylamine, but poor in both hydrogen peroxide and without a catalyst

#### 4.4.6 Commercial implication

Maize production has been fluctuating over the years (GOK, 2015) from 150 kg per capita in the mid-1970s, to 70 kg per capita, which is less than the estimated consumption of 103 kg per capita, necessitating regular imports (Kang'ethe *et al.*, 2017; Kimenju *et al.*, 2005). Based on the Kenyan population of 47.5 million people (KPHC, 2019) more than 48 million bags of maize are required annually. Animal feeds, starch, and oil industrial production also compete with food reserves (Kiama *et al.*, 2016; FAO, 2014) while mycotoxin contaminants waste 20–30 % annually. Kenya loses between 9.6 and 14.4 million bags annually to mycotoxins.

This study found that it is possible to degrade contaminated maize chemically to a usable level or at least to recover 30 % of the condemned maize for industrial ethanol production and spent grain as binders for briquettes. The market price of 98 % ethanol is Ksh. 250 per liter and costs between Ksh 50 -100 to produce the same depending on the production volumes. The industrial process would generate = 96 00000(250-100)  $\times$  30/100 to 14400000(250-100)  $\times$  30/100=Ksh. 432 - 648 million annually. The estimated amount of money lost through mycotoxin if enhanced through the proposed recovery method would translate to income and other benefits for the country.

#### **CHAPTER FIVE:**

#### 5.0 CONCLUSIONS AND RECOMMENDATIONS

#### **5.1 Conclusions**

- More than 32 % of the population in the selected county lacked awareness of aflatoxin contamination, prevention, and management of aflatoxin-affected crops and by-products. Lack of knowledge was associated with the huge health burden-related effects of consuming aflatoxin of the respondents. The decline of livestock productivity for feeding contaminated maize and maize by-product from the farm contaminated maize. Incurred economic losses from
- High aflatoxin contamination prevalence in maize ranked by counties from highest to lowest was Makueni> Is iolo>Machakos> Meru > Embu and low prevalence by counties from high to lowest was Trans Nzoia> Migori >Nairobi>Kajiado>Nakuru > Busia.
- Ninety-four percent of maize samples collected for aflatoxin contamination screening had aflatoxin contamination, of these 59.6 % were contaminated above the set limit of 10 μg/kg for human consumption in Kenya.
- Maize collected from wholesale/ market and NCPB stores in Embu, Migori, Nakuru, Busia, Trans- Nzoia counties had low concentrations of aflatoxin contaminants. The

good post-harvest management procedures resulted in slightly low aflatoxin contamination in maize.

- The use of catalysts increased the rate of degradation of aflatoxin in whole, dehulled, and ground maize. Catalyzed sodium hypochlorite performed better than the other reagents.
- Decontaminated maize products can be utilized as feedstock for industrial ethanol production with locally assembled boilers and distillers. The average yield of ethanol from decontaminated maize by the process was 30 % (v/m).

#### 5.2 Recommendations

- 1. Need for advocacy and training on health risks associated with poor post-harvest management of agricultural produce, effective management of aflatoxin-contaminated products in community, and public sensitization about aflatoxin control, standard guidelines, and regulations.
- 2. Regular monitoring of aflatoxin in farm products protects the public from the consumption of contaminated food. The government of Kenya through the ministries of agriculture, trade, and industries should develop the capacity to detect and quantify aflatoxin and related mycotoxin in foods to protect the public from risks associated with aflatoxin contamination.
- 3. More studies are required on fates and sinks in the environment for the metabolites generated during the degradation.
- 4. More studies are needed on the design and application of future degradation of other forms of aflatoxin contaminated produce among them groundnuts, millets and sorghum.

5. The average yield of ethanol from decontaminated maize by designed method was 30 % (v/m), further work is required optimal conditions maximum yields

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#### APPENDIX 1:

#### INFORMED CONSENT

My name is Nicholas J. Mwenda, a PhD student at the University of Nairobi. I am carrying out a study on how to develop a Method for Degrading Aflatoxin in Contaminated Maize. In this study, I request you to collaborate with me in the study by allowing me to use samples of your stored maize. This study will provide information on the best method to apply in decontaminating aflatoxins from contaminated maize. From the study, possible uses of decontamination products of maize will have been suggested like industrial alcohol production, starch, glue, and briquettes. The study findings will provide a solution to the aflatoxin problem in terms of reducing stockpiles of contaminated maize public silos and creating room for storing clean maize, the blending of contaminated and selling in the local market endanger the consumers and also creates a market for the maize which would otherwise be wasted. The findings will also contribute knowledge on how to manage aflatoxins and mycotoxins in the Country.

If you agree to participate in this study, we expect of you the following:

- $\checkmark$  Sign the consent form attached.
- $\checkmark$  Allow us to access your maize store and take samples at your terms.
- $\checkmark$  Not to be maize sample analyzing charges.
- ✓ We pledge to hold all information regarding your maize status and store ownership as confidential information hence anonymization criteria will be used to code samples for study purposes only.

Before sample analysis, you are free to withdraw at any time from the study. Thank you

### CONSENT FORM

I, Mr. / Mrs. / Miss ..... am the legal owner of a maize store known as .....

I hereby freely agree to participate in this study, as explained to me. I understand that my participation in this study will not affect my business in any way. I also understand the strictest confidence required about the information regarding my store and me.

Name of the storeowner ...... Sign..... Date.....

Witness.....Date.....

Appendix 2.Survey Questionnaire on the Impact of Aflatoxin

Introduction; this questionnaire is designed to collect information on the social economic impact of aflatoxin on the people in this area. The information gathered will be confidentially treated and not to use the gathered data to discriminate on the use of products from the area. Any questions you feel uncomfortable answering please do so and you do not have to give a reason for your decision. You are welcome to participate in the study.

## Section A: Identification

1. Respondent identif	ication
Date	
Enumerator's Name	
County Name	
Farmer	
Trader	
NCPB Staff	
2. Respondent gender	(tick where applicable)
Male	
Fema	le
Rathe	er not say
3. Respondent age (tio	ck where applicable)
21 - 50	
51 - 70	
Rather	not say
4. Respondent education	ion level (tick where applicable)
Primar	У
Second	lary

Post- Secondary

Rather not say

5. What is your main occupation? (tick where applicable)

Farmer

Employed

Business

Rather not say

### **Section B: Source of food**

1. From what source do you get your food? (tick where applicable)

Grains

My farm	local farmers	market and	d shops
Vegetables			
My farm	local farmers	market and	d shops
Livestock products			
My farm	local farmers	market and	d shops
Other (specify)			
2. What maize seed variety	do you plant in your area? I	Please tick how y	ou plant.
Variety		Months Tick	
H517-H6128, H614D, WH	E1101	6-9	Plantation alone
H511-H516, PHB 3253, Du	uma 43	4-5	Small area alone
PH1-PH4		3-4	Small area mixed

3-4

(specify)

Others

Other

DLC 1, DH 01 -02

Tick

3. How long does your maize take to mature?

Tick Mature in months

6-9

4-5

3-4

Other (specify)

4. How do you improve your yield per hectare?

Tick Improve yield

Plant early, care, and apply fertilizers

Plant early and care

Plant and irrigate

Other (specify)

5. How do you process your maize? (tick where applicable)

Drying in the farm, remove with cobs and store

Cut and stack in the farm to dry for one month, shred the kernels mechanically, and store

Cut and stack in the farm to dry for one month, shell the kernels mechanically, and sell to NCPB and other buyers.

Other (specify)

6. Where do you sell your farm produce? (tick where applicable)

Don't sell

Local market

Brokers

NCPB

Other specify)

7. How do you dry maize (tick where applicable)?

Don't dry but sell green

Sell directly from the farm to brokers

Solar dry and sell to NCPB

Convert everything to hey

Other (specify)

8. How do you know that maize is dry (tick where applicable)?

Chew some grains

Use a tin to measure the sound produced

Check the weight of maize

Measure moisture content and compare with NCPB guidelines

Other (specify)

#### Section C: Foodstuff contamination

1. In the last Five (5) years, how often have you witnessed contamination on the following foodstuff? (tick where applicable,)

Grains	daily	weekly	monthly	rarely
Maize Gains				
Beans Grains				
Wheat grains				
Sorghum				
Barley				
Rice				
Groundnuts				

## 2. What contaminants do you find in the foodstuff? (tick where applicable)

Contaminants	Yes	Not sure
Weevils		
Fungi		
Color change		
Rot		
Others specify		

3. How do you measure the level of contaminants in foodstuff? (tick where applicable)

No contaminants

Count and calculate the percentage

Send to the laboratory for analysis

Compare the color

Taste

Other (specify)

#### Section D: Contamination impacts and awareness

1. Are there any food safety-related organizations in your community?

If yes, what do they do?

2. Have you attended any training forum on food safety? (tick where applicable)

Yes

No

3. Have you heard about aflatoxin contamination? (tick where applicable)

Yes, if yes indicate where.....

No

4. What method do you suggest be used to control or prevent aflatoxin contamination?

.....

5. Do you know any effect caused by eating aflatoxin-contaminated foods (tick where applicable)?

Yes

No

If yes, explain------

6. In your opinion how does a flatoxin contamination occur? (tick where applicable)

Poor cooking

From handling methods after harvest

Poor storage only

Compare the color

I don't know

7. In your view, of what you suggested, name a used for aflatoxin-contaminated maize. (tick where applicable);

Feed to livestock

Remove the topic skin, wash in Magadi soda, and cook

Dilute with good maize and sell in the market

Keep the maize in custody for destruction

Other (specify)

- 8. What are the names of ailments/ diseases coursed by eating aflatoxin-contaminated foods in your community? (list)
- 9. What do you think can treat aflatoxin-contaminated grains?

#### Conclusion

It has been good interacting with you and we will keep in touch for more information.

Thank you very much.

## Annex 3:Table 2: Instantaneous Data on Degradation of Aflatoxin Contaminants in Maize with Sodium Hydrogen Sulfite and Catalysts

#### Sodium hydrogen sulfite

Time	1 M			0.5 M			0.05 N	М		0.005	М		0.0005M		
	WM	DM	GM	WM	DM	GM	WM	DM	GM	WM	DM	GM	WM	DM	GM
(Min															
)															
0	198.45	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.
		45	45	45	45	45	45	45	45	45	45	45	45	45	45
30	124.03	98.2	124.	125.	95.6	121.	130.	99.4	115.	136.	102.	101.	142.	99.6	114.
00	12.1.00	3	03	02	5	15	98	2	30	03	00	1011	88	2	70
(0)	11000	5	100	110	01.0	101	117	2	09.4	14.0	00	42	105	2	09.5
00	110.09	90.0	100.	118.	91.0	101.	11/.	90.4	98.4	14.0	95.0	98.2	125.	89.5	98.5
		5	61	08	9	21	09	5	3	6	5	7	82	0	3
90	103.59	80.3	68.4	105.	75.6	64.3	101.	76.0	63.1	106.	73.2	61.8	108.	77.4	64.4
		7	7	58	1	2	41	1	1	17	3	4	16	0	8
14	93.27	53.7	59.5	95.4	52.5	57.6	82.3	50.6	54.2	81.7	58.5	58.4	97.0	61.8	59.6
		8	5	5	9	5	6	0	8	6	4	4	4	6	5
150	83.35	43.0	52.1	83.1	42.1	43.9	63.5	26.7	37.9	70.6	28.1	35.9	90.4	41.9	41.9
		6	9	5	7	6	0	9	3	5	8	4	9	1	1
180	69.46	40.4	46.2	72.4	28.1	28.4	61.5	22.2	21.6	53.7	18.0	19.7	43.6	26.0	37.4
		8	6	3	8	2	2	3	7	8	6	1	6	0	7
210	49.61	23.9	30.9	54.5	26.0	24.3	53.5	18.8	18.1	42.6	17.8	17.3	35.7	24.8	24.5
		1	6	7	0	1	8	5	6	7	6	8	2	1	1
240	34.73	16.4	19.3	28.7	12.3	12.9	27.7	7.94	7.46	24.8	5.95	5.36	23.8	17.2	16.7
		7	7	8	0	8	8			1			1	7	7
270	9.92	10.3	6.75	17.8	4.66	4.56	9.92	6.65	2.38	8.14	4.41	2.22	6.95	6.81	3.95
		2		6											

300	3.97	6.35	6.19	11.9	4.37	4.39	3.77	6.59	2.23	2.18	4.19	2.22	4.17	6.79	3.79
				1											
Sodiun	n hydroge	en sulfit	e with l	hydrog	en pero	oxide									
0	198.45	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.
		45	45	45	45	45	45	45	45	45	45	45	45	45	45
20	04.04	01.6	70.6	05.0	70.4	77.4	02.2	75.4	75.4	01.0	-	72.0	00.0	70.7	77.0
30	84.34	81.6	79.6	85.3	79.4	77.4	83.3	75.4	75.4	81.3	74.7	73.8	89.3	79.7	77.9
		6	0	3	2	0	5	1	1	6	4	6	0	8	0
60	76.40	75.8	74.2	69.4	73.0	72.4	65.4	64.3	64.1	64.5	67.8	61.7	67.4	71.2	61.8
		1	2	6	3	3	9	0	8	0	7	6	7	4	2
90	63.90	64.4	64.0	61.9	61.9	63.9	55.5	59.9	61.7	49.6	58.9	56.3	61.7	61.7	59.7
			0	2	0	2	7	7	4	1	4	~	2	7	7
			0	2	8	2	/	/	4	1	4	0	2	/	/
14	61.52	47.0	46.1	57.7	44.6	43.8	50.6	40.1	38.4	42.0	40.0	35.1	59.7	48.0	35.3
		3	1	5	5	9	0	4		7	9	6	3	2	7
150	57.55	29.7	30.3	51.4	28.1	28.2	43.6	28.4	24.3	30.9	28.3	22.2	50.8	30.1	24.5
		7	6	0	8	3	6		0	6	6	9	0	6	0
		,	0	0	0	5	0		0	0	0	-	0	0	
180	43.66	24.8	22.0	46.6	22.0	19.8	37.7	22.0	18.1	27.9	4.04	17.8	39.8	22.0	19.2
		1	9	4	7	7	1	3	6	8		6	9	5	7
210	41.67	12.1	13.3	38.7	12.5	12.1	32.5	12.1	11.5	22.8	11.8	11.0	29.3	12.6	11.8
		1	0	0	0	1	5	3	5	2	7	Q	7	0	Q
		1	0	0	0	1	5	5	5	2	/	-	/	0	
240	22.82	1.59	1.59	24.8	1.39	1.39	21.8	1.61	1.19	18.8	1.75	1.19	22.0	1.81	1.79
				1			3			5			3		
270	7.94	1.57	1.55	15.8	1.35	1.33	8.53	1.59	1.11	4.17	1.72	0.91	8.14	1.79	1.37
				8											
						0									
300	1.98	1.55	1.49	5.95	1.33	0.99	1.79	1.59	0.79	3.97	1.67	0.69	7.74	1.79	0.91
Sodiun	n hydroge	en sulfit	e with a	ammon	ia										
0	198.45	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.

0	198.45	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198
		45	45	45	45	45	45	45	45	45	45	45	45	45	45

30	82.36	81.7	79.5	81.9	81.5	77.6	81.7	79.7	77.5	79.3	78.1	78.7	84.3	80.3	76.8
		6	8	6	6	5	6	8	9	8	9	8	4	7	5
60	68.47	67.6	64.5	66.4	65.6	62.5	62.1	61.5	59.7	60.0	59.5	57.6	68.2	64.3	64.3
		7	0	8	9	1	1	2	9	3	4	0	7	0	0
90	59.93	57.9	57.5	57.9	55.9	55.9	53.1	52.1	51.8	48.6	46.6	47.8	63.7	61.7	57.9
		5	9	5	6	6	8	9	0	2	4	7	2	4	7
14	55.76	46.2	51.8	53.7	46.0	49.8	44.7	42.1	43.9	39.9	35.7	39.9	61.5	39.6	53.6
		6	0	8	6	1	5	8	7	3	4	3	4	7	0
150	53.38	45.4	51.4	44.8	44.6	40.8	40.0	40.0	35.7	26.9	26.9	23.0	40.8	40.8	35.8
		5	0	3	3	6	9	9	6	9	9	2	0	0	3
180	23.81	22.2	22.0	22.8	22.1	4.84	4.22	4.08	17.9	4.06	18.0	16.8	4.04	19.8	17.0
		3	7	2	3				0		8	7		2	9
210	22.03	18.0	18.2	4.84	16.8	18.3	4.64	14.6	16.5	4.14	14.1	16.1	21.4	15.4	17.2
		6	8		7	6		9	5		9	9	3	8	8
240	2.28	2.08	2.28	2.48	2.38	2.48	2.18	1.98	2.18	2.98	2.28	2.98	2.18	2.40	2.18
270	2.28	0.86	0.92	2.48	0.98	0.97	2.18	0.80	0.85	2.98	0.90	1.18	2.18	0.97	0.85
300	2.28	0.71	0.74	2.48	0.79	0.78	2.18	0.62	0.66	2.98	0.68	0.86	2.18	0.78	0.71
0	198.45	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.
		45	45	45	45	45	45	45	45	45	45	45	45	45	45
30	82.36	81.7	79.5	81.9	81.5	77.6	81.7	79.7	77.5	79.3	78.1	78.7	84.3	80.3	76.8
		6	8	6	6	5	6	8	9	8	9	8	4	7	5
Sodiun	n hydroge	en sulfit	e with 1	nethyla	amine										
0	198.45	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.
		45	45	45	45	45	45	45	45	45	45	45	45	45	45
30	121.45	89.9	81.6	106.	83.9	77.7	111.	81.9	75.6	112.	87.9	87.3	121.	87.0	81.9
		0	6	17	4	9	13	6	1	92	1	2	45	8	2
60	104.19	75.4	74.4	100.	71.6	71.2	99.2	69.6	64.2	98.2	69.6	62.5	100.	71.7	65.9
		1	2	22	8	4	3	9	2	3	8	5	02	4	8

90	99.23	66.4	61.8	97.4	63.7	61.9	97.0	62.1	59.7	96.2	59.1	57.6	95.4	59.5	61.9
		8	2	4	0	4	6	1	9	5	4	9	5	3	0
14	91.68	39.8	39.7	89.5	37.1	36.7	79.6	29.1	29.3	77.6	28.0	27.7	77.4	30.1	29.9
		9	1	0	1	1	8	7	7	3	1	9		6	9
150	62.55	19.8	22.6	61.5	18.3	18.0	57.9	17.3	16.8	50.8	16.3	15.8	62.7	17.7	17.6
		6	2	2	1	9	5	8	7	0	2	8	1	2	6
180	59.63	8.93	9.33	58.5	8.14	8.93	55.7	7.94	8.53	49.8	7.58	8.14	48.0	7.94	9.27
				4			6			1			2		
210	42.67	5.36	5.56	40.6	5.16	5.16	39.6	4.76	5.00	38.7	4.37	4.58	33.9	5.46	5.71
				8			9			0			3		
240	34.73	4.21	4.96	28.7	4.76	4.56	27.7	4.56	4.19	24.8	4.37	4.37	23.8	3.97	5.16
				8			8			1			1		
270	10.91	3.81	3.57	11.9	3.53	3.31	11.1	3.11	2.98	9.72	2.46	2.38	7.74	3.47	3.18
				1			1								
300	5.16	1.85	1.73	6.55	1.81	1.65	3.95	1.81	1.61	2.38	1.71	1.59	4.36	1.92	1.63
0	198.45	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.
		45	45	45	45	45	45	45	45	45	45	45	45	45	45

KEY; WM = Whole Maize DM= Dehulled Maize GM= Ground Maize CM=Clean Maize.

Table 3: Summary Regression	1 Equations	and	Data	On	Degradation	Reaction	with
Sodium Hydrogen Sulfite and (	Catalysts						

Sodium hydrogen sulfite without catalyst												
Concentration (M)	Maize	Relation Equation	Slope k	R <sup>2</sup>	Half-life T1/2	y-intercept						
1	WM	y = -0.0106x + 5.5778	-94.340	0.807	65.39	5.578						
	DM	y = -0.0084x + 5.3974	-119.050	0.903	82.52	5.397						
	GM	y = -0.0109x + 5.5606	-91.743	0.840	63.59	5.561						
0.5	WM	y = -0.0124x + 5.6871	-83.333	0.834	57.76	5.687						
	DM	y = -0.0119x + 5.6772	-83.333	0.884	57.76	5.677						
	GM	y = -0.0116x + 5.6257	-86.47	0.788	59.75	5.626						
0.05	WM	y = -0.0108x + 5.4637	-92.593	0.840	64.18	5.464						
	DM	y = -0.0112x + 5.403	-89.286	0.861	61.89	5.403						

	GM	y = -0.0115x + 5.4123	-86.957	0.873	60.27	5.412
0.005	WM	y = -0.0116x + 5.5476	-86.47	0.900	59.75	5.548
	DM	y = -0.0102x + 5.4992	-98.039	0.827	67.96	5.499
	GM	y = -0.0094x + 5.4161	-106.383	0.876	73.74	5.416
0.0005	WM	y = -0.0102x + 5.4483	-98.039	0.876	67.96	5.448
	DM	y = -0.0112x + 5.5058	-89.286	0.891	61.89	5.506
	GM	y = -0.0108x + 5.5231	-92.593	0.881	64.18	5.523
With Hydrogen per	oxide					
1	WM	y = -0.0113x + 5.3121	-88.4956	0.7915	61.34046	5.3121
	DM	y = -0.0071x + 4.9193	-140.845	0.8936	97.62636	4.9193
	GM	y = -0.0113x + 5.1998	-88.4956	0.8119	61.34046	5.1998
0.5	WM	y = -0.0112x + 5.0621	-89.2857	0.9069	61.88814	5.0621
	DM	y = -0.0094x + 5.0874	-106.383	0.9156	73.73906	5.0874
	GM	y = -0.0116x + 5.2625	-86.469	0.8033	59.75407	5.2625
0.05	WM	y = -0.0104x + 5.1294	-96.1538	0.8279	66.64877	5.1294
	DM	y = -0.0146x + 5.4332	-68.4932	0.7426	47.47583	5.4332
	GM	Y=-0.0119x + 5.0609	-84.0336	0.8807	58.24766	5.0609
0.005	WM	y= -0.0098x + 5.1044	-102.041	0.9343	70.7293	5.1044
	DM	y = -0.0119x + 5.2847	-84.0336	0.8355	58.24766	5.2847
	GM	y = -0.0115x + 5.2106	-86.9565	0.8318	60.27367	5.2106
0.0005	WM	y = -0.0117x + 5.1387	-85.4701	0.8493	59.24335	5.1387
	DM	y = -0.013x + 5.1088	-76.9231	0.9161	53.31901	5.1088
	GM	y = -0.0123x + 5.2354	-81.3008	0.8646	56.35343	5.2354
With ammonia						
1	WM	y = -0.0151x + 5.441	-66.2252	0.8686	45.90379	5.441
	DM	y = -0.0181x + 5.6182	-55.2486	0.8738	38.29542	5.6182
	GM	y = -0.0179x + 5.6093	-55.8659	0.8663	38.72331	5.6093
0.5	WM	y = -0.0147x + 5.3605	-68.0272	0.8864	47.15287	5.3605
	DM	y = -0.0177x + 5.5699	-56.4972	0.8836	39.16086	5.5699
	GM	y = -0.0176x + 5.5453	-56.8182	0.8797	39.38336	5.5453
0.05	WM	y = -0.0151x + 5.3229	-66.2252	0.8904	45.90379	5.3229
	DM	y = -0.0184x + 5.5708	-54.3478	0.8884	37.67104	5.5708
	GM	y = -0.0181x + 5.5258	-55.2486	0.8911	38.29542	5.5258
0.005	WM	y = -0.0138x + 5.1544	-72.4638	0.943	50.22806	5.1544
	DM	y = -0.0179x + 5.4423	-55.8659	0.914	38.72331	5.4423

	GM	y = -0.0169x + 5.3704	-59.1716	0.9224	41.01463	5.3704
0.0005	WM	y = -0.0154x + 5.4304	-64.9351	0.8848	45.00956	5.4304
	DM	y = -0.0178x + 5.5473	-56.1798	0.8971	38.94085	5.5473
	GM	y = -0.0182x + 5.5762	-54.9451	0.8877	38.08501	5.5762
With methylamine						
1	WM	Y = -0.0101X + 5.4598	-99.0099	0.8568	68.62843	5.4598
	DM	Y = -0.0155X + 5.2592	-64.5161	0.9775	44.71917	5.2592
	GM	Y = -0.0153X + 5.2375	-65.3595	0.9781	45.30374	5.2375
0.5	WM	Y = -0.0096X + 5.3735	-104.167	0.885	72.4283	5.3735
	DM	Y = -0.0154X + 5.44	-64.9351	0.9769	45.00956	5.44
	GM	Y = -0.0155X + 5.1987	-64.5161	0.9793	44.71917	5.1987
0.05	WM	Y = -0.0105X + 5.4365	-95.2381	0.8601	66.01402	5.4365
	DM	Y = -0.0155X + 5.1614	-64.5161	0.9801	44.71917	5.1614
	GM	Y = -0.0156X + 5.1391	-64.1026	0.9832	44.43251	5.1391
0.005	WM	Y = -0.0116X + 5.5026	-86.469	0.8395	59.75407	5.5026
	DM	Y = -0.016X + 5.1892	-62.5	0.9837	43.3217	5.1892
	GM	Y = -0.016X + 5.1707	-62.5	0.9872	43.3217	5.1707
0.0005	WM	Y = -0.0112X + 5.4817	-89.2857	0.8977	61.88814	5.4817
	DM	Y = -0.0154X + 5.1706	-64.9351	0.9829	45.00956	5.1706
	GM	Y = -0.0153X + 5.1671	-65.3595	0.9852	45.30374	5.1671

# Table 4: Instantaneous Degradation Rate of Reaction for Aflatoxin Contaminated Maize After of Ferulic Acid and Catalysts.

Time	1 M			0.5 M			0.05 M			0.005 N	I		0.0005N	1
(Min)	WM	DM	GM	WM	DM	GM	WM	DM	GM	WM	DM	GM	WM	DN
0	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	19
30	148.84	121.25	119.09	142.88	119.27	119.49	121.05	119.67	117.19	127.01	124.63	115.52	134.55	12
60	138.92	105.18	115.70	136.93	99.29	118.30	111.13	98.43	114.43	103.19	98.03	103.67	128.99	96
90	89.30	89.90	97.50	81.36	92.28	95.45	75.41	83.35	81.76	71.44	90.29	90.39	97.24	94
14	61.52	89.50	97.44	60.53	82.56	98.15	54.57	80.77	79.06	58.54	88.55	78.63	73.43	93
150	53.58	53.58	48.22	44.65	41.67	37.13	38.70	51.60	37.57	36.12	35.72	37.25	43.66	81
180	47.63	41.73	41.93	31.75	38.70	34.73	23.81	33.14	31.16	19.85	25.80	25.4	39.29	39

210	33.74	28.78	28.38	29.97	26.79	24.01	4.84	18.06	18.26	17.86	4.04	18.00	36.71	22
240	19.85	24.49	24.41	13.89	4.24	22.07	7.94	11.71	15.68	5.95	10.72	10.52	17.86	18
270	11.91	11.11	8.93	4.96	10.12	9.78	2.98	9.37	9.37	2.38	8.33	9.84	2.18	9.3
300	11.91	2.58	3.37	4.96	2.78	3.18	2.98	3.18	2.98	2.38	3.18	2.98	2.18	3.3
			Hydroge	en Peroxio	le									
0	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	19
30	89.30	81.66	79.60	83.35	79.42	77.40	81.36	75.41	75.41	75.41	73.86	74.74	83.35	79
60	79.38	75.81	74.22	75.41	73.03	72.43	67.47	64.30	64.18	63.50	61.76	67.87	69.46	71
90	69.46	64.4	64.00	65.69	61.98	63.92	63.50	59.97	61.74	59.54	56.36	58.94	64.50	61
14	53.58	47.03	46.11	49.61	44.65	43.89	44.65	40.14	38.4	42.67	35.16	40.09	47.63	48
150	33.74	29.77	30.36	29.77	28.18	28.23	28.97	28.4	24.30	28.18	22.29	28.36	29.97	30
180	25.80	24.81	22.09	23.81	22.07	19.87	22.82	22.03	18.16	4.84	17.86	4.04	23.81	22
210	13.89	12.11	13.30	13.49	12.50	12.11	12.30	12.13	11.55	11.71	11.09	11.87	12.50	12
240	1.79	1.59	1.59	1.79	1.39	1.39	1.79	1.61	1.19	1.94	1.19	1.75	1.98	1.8
270	1.79	1.57	1.55	1.79	1.35	1.33	1.79	1.59	1.11	1.94	0.91	1.72	1.98	1.7
300	1.79	1.55	1.49	1.79	1.33	0.99	1.79	1.59	0.79	1.94	0.69	1.67	1.98	1.7
						Ammon	iia							
0	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	19
30	85.33	83.81	70.05	81.76	79.62	75.81	79.58	77.42	73.62	75.21	75.17	73.23	83.75	81
60	75.61	75.61	57.97	65.09	65.09	57.15	62.31	62.31	51.87	57.95	57.95	50.01	64.50	64
90	64.50	62.02	54.08	62.13	61.74	54.4	59.93	57.59	50.01	55.37	49.41	49.41	62.51	61
14	43.06	41.08	39.09	40.68	35.82	31.36	38.4	37.71	30.26	22.82	4.84	4.84	22.62	4.0
150	19.98	19.85	18.00	4.84	18.85	17.36	4.76	17.95	15.57	4.68	17.94	12.98	4.86	18
180	2.58	2.58	2.58	2.38	2.38	2.38	2.28	2.28	2.28	2.08	2.08	2.08	2.38	2.3
210	1.39	1.39	1.39	1.35	1.35	1.35	1.23	1.23	1.23	1.17	1.17	1.17	1.25	1.2
240	0.99	0.99	0.99	0.79	0.79	0.79	0.60	0.60	0.60	0.40	0.40	0.40	0.42	0.4
270	0.99	0.99	0.79	0.79	0.79	0.60	0.60	0.60	0.56	0.40	0.40	0.39	0.42	0.4
300	0.99	0.99	0.60	0.79	0.79	0.40	0.60	0.60	0.48	0.40	0.40	0.33	0.42	0.4

				Methyla	imine									
0	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198
30	89.30	101.01	125.22	83.35	99.62	133.16	81.36	99.60	130.78	87.32	99.36	144.67	86.92	101
60	75.41	97.46	105.38	73.23	91.51	101.41	70.65	90.51	99.42	69.85	90.12	98.05	72.43	90.
90	68.47	95.87	99.42	67.67	86.35	95.45	66.08	83.79	81.76	64.89	84.98	85.73	69.26	97.
14	41.67	86.35	95.45	37.71	81.60	89.70	29.77	81.52	79.74	28.18	77.44	77.44	31.16	85.
150	21.83	46.04	46.44	18.85	43.86	35.92	17.86	34.13	37.11	16.27	30.76	30.76	17.66	47.
180	9.72	40.09	36.12	9.13	31.95	29.97	8.73	25.4	27.19	8.33	4.24	4.24	9.33	23.
210	5.95	22.82	28.78	5.56	4.24	24.25	4.96	18.26	19.15	4.56	17.86	18.50	5.66	18.
240	5.36	14.09	14.09	4.86	14.69	13.10	4.58	12.11	12.11	4.43	10.32	11.55	4.17	11.
270	3.77	10.4	10.16	3.37	9.94	9.55	3.10	9.33	9.53	2.44	9.17	9.09	3.37	9.7
300	1.92	3.75	3.57	1.85	3.61	3.57	1.81	3.61	3.57	1.73	3.57	3.57	1.96	3.7

 Table 5 Summary Regression Data for Degradation Reaction for Decontaminants with

## Ferulic Acid and The Catalysts.

	Ferulic ac	id without catalysts				
Concentration (M)	Maize	Relation Equation	Slope k	R²	Half-life T1/2	y-intercept
1	WM	y = -0.0098x + 5.3901	-102.04	0.971	70.73	5.39
	DM	y = -0.0125x + 5.5329	-80.00	0.953	55.45	5.533
	GM	y = -0.0143x + 5.5278	-69.93	0.956	48.47	5.528
0.5	WM	y = -0.0153x + 5.573	-65.36	0.958	45.3	5.573
	DM	y = -0.0144x + 5.7445	-69.44	0.855	48.14	5.744
	GM	y = -0.0104x + 5.233	-96.15	0.969	66.65	5.233
0.05	WM	-0.0121x + 5.3163	-82.64	0.946	57.28	5.316
	DM	y = -0.0118x + 5.2151	-84.75	0.975	58.74	5.215
	GM	y = -0.0134x + 5.3405	-74.63	0.970	51.73	5.34
0.005	WM	y = -0.0108x + 5.2351	-92.59	0.964	64.18	5.235
	DM	y = -0.0102x + 5.4992	-98.04	0.827	67.96	5.499
	GM	y = -0.0094x + 5.4161	-106.38	0.876	73.74	5.416
0.0005	WM	y = -0.0102x + 5.4483	-98.04	0.876	67.96	5.448
	DM	y = -0.0112x + 5.5058	-89.29	0.891	61.89	5.506
	GM	y = -0.0108x + 5.5231	-92.59	0.881	64.18	5.523

		With hydrogen peroxide				
1	WM	y = -0.0165x + 5.526	-60.6061	0.9049	42.00892	5.526
	DM	y = -0.0169x + 5.4827	-59.1716	0.9084	41.01463	5.4827
	GM	y = -0.0169x + 5.4706	-59.1716	0.9084	41.01463	5.4706
0.5	WM	y = -0.0164x + 5.4598	-60.9756	0.911	42.26507	5.4598
	DM	y = -0.0173x + 5.4834	-57.8035	0.9066	40.06631	5.4834
	GM	y=-0.0178x + 5.5141	-38.9409	0.9136	56.17978	5.5141
0.05	WM	y = -0.0163x + 5.4031	-61.3497	0.9165	42.52437	5.4031
	DM	y = -0.0165x + 5.3737	-60.6061	0.9121	42.00892	5.3737
	GM	y = -0.0183x + 5.4904	-53.1915	0.9146	36.86953	5.4904
0.005	WM	y = -0.0158x + 5.347	-63.2911	0.9237	43.87007	5.347
	DM	y = -0.0163x + 5.3589	-61.3497	0.9222	42.52437	5.3589
	GM	y = -0.0187x + 5.4771	-53.4759	0.9169	37.06669	5.4771
0.0005	WM	y = -0.0159x + 5.4041	-62.8931	0.9185	43.59416	5.4041
	DM	y = -0.0163x + 5.4164	-59.5238	0.9155	41.25876	5.4164
	GM	y = -0.0174x + 5.4215	-57.4713	0.9295	39.83604	5.4215
		With ammonia				
1	WM	y = -0.045x + 5.4673	-48.7805	0.921	33.8146	5.4673
	DM	y = -0.045x + 5.4481	-48.7805	0.9228	33.8146	5.4481
	GM	y = -0.021x + 5.3883	-47.1698	0.9412	32.69562	5.3883
0.5	WM	y = -0.0212x + 5.468	-44.4444	0.924	30.80654	5.468
	DM	y = -0.0211x + 5.4332	-47.3934	0.9296	32.85058	5.4332
	GM	y = -0.0222x + 5.4616	-45.045	0.9551	31.22285	5.4616
0.05	WM	y = -0.0222x + 5.5118	-45.045	0.9286	31.22285	5.5118
	DM	y = -0.0221x + 5.482	-45.2489	0.933	31.36413	5.482
	GM	y = -0.0234x + 5.5013	-43.4783	0.9402	30.13683	5.5013
0.005	WM	y = -0.0234x + 5.5013	-43.4783	0.9402	30.13683	5.5013
	DM	y = -0.0233x + 5.4552	-42.9185	0.9478	29.74881	5.4552
	GM	y = -0.0234x + 5.4107	-42.735	0.9596	29.62167	5.4107
0.0005	WM	y = -0.0234x + 5.5694	-42.735	0.9432	29.62167	5.5694
	DM	y = -0.0233x + 5.5306	-42.9185	0.9484	29.74881	5.5306
	GM	y = -0.0227x + 5.4034	-44.9035	0.9559	31.12471	5.4034
		With methylamine				
1	WM	y = -0.0152x + 5.2654	-65.7895	0.9792	45.60179	5.2654
	DM	y = -0.0117x + 5.4247	-85.4701	0.9294	59.24335	5.4247

	GM	y = -0.012x + 5.5244	-83.3333	0.9314	57.76227	5.5244
0.5	WM	y = -0.0154x + 5.2282	-64.9351	0.9797	45.00956	5.2282
	DM	y = -0.0117x + 5.3707	-85.4701	0.9412	59.24335	5.3707
	GM	y = -0.0124x + 5.4424	-81.3008	0.9676	56.35343	5.4424
0.05	WM	y = -0.0155x + 5.1835	-64.5161	0.9809	44.71917	5.1835
	DM	y = -0.012x + 5.3436	-83.3333	0.9552	57.76227	5.3436
	GM	y = -0.0124x + 5.4424	-80.6452	0.9676	55.89897	5.4424
0.005	WM	y = -0.016x + 5.481	-62.5	0.9835	43.3217	5.481
	DM	y = -0.0123x + 5.3284	-81.3008	0.9585	56.35343	5.3284
	GM	y = -0.0124x + 5.4424	-78.7402	0.9676	54.57852	5.4424
0.0005	WM	y = -0.0154x + 5.489	-64.9351	0.983	45.00956	5.489
	DM	y = -0.0121x + 5.4015	-82.6446	0.9451	57.28489	5.4015
	GM	y = -0.0124x + 5.5005	-80.6452	0.9698	55.89897	5.5005

## Table 6 Instantaneous Concentrations of Aflatoxin in Maize after Degradation with Sodium Hydrogen Carbonate

				Sodium	hydroger	n carbonat	te								
Time	1M			0.5 M			0.05 M			0.005 M			0.0005 N	Л	
	WM	DM	GM	WM	DM	GM	WM	DM	GM	WM	DM	GM	WM	DM	GM
min.															
0	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45
30	154.79	49.61	45.64	138.72	47.63	44.45	135.54	47.23	45.88	136.53	66.06	59.77	151.42	69.68	67.47
60	138.92	32.15	31.16	134.95	36.12	34.33	117.09	33.32	32.33	113.12	31.45	34.73	110.54	41.92	38.4
90	106.17	24.26	24.31	99.03	24.03	4.28	94.46	22.45	4.22	82.36	22.05	4.26	99.42	36.12	34.13
14	91.49	21.23	4.06	83.94	21.06	19.86	75.41	4.46	18.06	71.84	4.28	18.10	75.61	22.03	19.31
150	43.66	18.06	13.32	37.90	17.28	11.14	37.51	17.09	10.74	32.15	14.11	9.11	43.66	14.14	10.15
180	33.93	13.89	8.93	30.56	12.90	8.18	26.99	12.33	8.16	25.60	12.11	8.10	27.39	12.13	8.31
210	22.23	4.17	3.97	17.86	4.21	4.37	16.67	3.37	3.57	11.31	3.14	2.58	12.30	5.56	4.01
240	17.86	1.63	1.59	17.07	1.61	1.59	15.68	1.67	1.79	10.32	1.65	1.79	9.92	1.81	2.00
270	5.95	0.18	0.4	5.76	0.18	0.4	5.16	0.18	0.40	4.56	0.16	0.02	6.15	1.85	0.60
300	3.39	0.18	0.4	3.31	0.18	0.4	3.16	0.18	0.40	3.00	0.16	0.02	3.77	1.45	0.60
					With Hy	/drogen pe	roxide								
0	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45
30	115.10	102.80	101.57	99.03	93.25	92.46	95.85	90.95	91.74	96.84	89.06	88.07	91.88	86.58	91.51
60	83.94	81.82	83.57	81.76	79.62	79.42	81.17	77.57	73.61	80.77	77.53	73.04	82.75	80.77	80.77
90	66.48	62.21	65.72	65.29	62.10	61.32	62.71	59.57	57.87	60.53	57.65	55.67	61.32	57.73	59.73

14	57.75	55.76	55.76	56.16	50.21	54.18	54.57	48.62	52.59	51.99	46.04	50.01	57.35	47.43	51.40
150	38.10	32.15	36.12	33.93	27.98	31.95	33.54	27.58	31.55	28.18	24.21	26.4	41.48	37.71	37.88
180	4.04	17.88	18.06	18.65	18.26	17.44	17.07	16.27	16.77	15.68	14.29	14.31	19.45	18.65	15.48
210	14.29	15.12	14.29	14.09	13.91	14.09	12.11	11.93	12.11	11.49	11.35	11.49	12.48	12.30	12.48
240	12.11	10.12	12.11	13.10	11.11	12.50	12.11	9.74	11.93	9.92	8.97	10.52	17.86	11.21	12.30
270	6.15	4.17	5.97	5.93	4.15	5.76	5.91	4.13	5.76	5.93	4.01	5.76	6.55	6.17	6.57
300	6.15	3.97	5.15	5.93	3.97	5.43	5.91	3.97	5.21	5.93	3.99	5.23	6.55	4.19	6.55
						With A	mmonia								
0	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45
30	97.04	95.45	87.12	91.49	89.50	83.55	83.94	83.55	79.98	81.92	81.56	79.41	81.96	81.95	82.08
60	71.24	67.27	65.72	67.27	65.72	61.80	64.50	61.77	58.05	63.31	61.76	55.94	60.92	63.31	56.70
90	60.13	59.59	56.16	49.41	47.86	45.45	48.02	47.85	42.07	44.85	43.89	40.88	40.68	45.63	43.06
14	43.66	42.07	26.39	26.4	22.46	26.00	24.21	22.27	25.60	22.23	4.08	23.62	4.24	27.82	26.4
150	15.68	14.13	14.88	10.52	10.15	10.15	8.93	8.17	7.12	10.32	10.14	6.95	8.55	8.36	8.71
180	3.18	2.98	3.18	2.96	2.84	2.96	2.80	2.60	2.80	2.52	2.42	2.52	3.19	3.18	3.19
210	0.29	0.29	0.29	0.65	0.65	0.65	0.25	0.25	0.25	0.24	0.24	0.24	0.26	0.26	0.26
240	0.29	0.29	0.28	0.65	0.65	0.62	0.25	0.25	0.24	0.24	0.24	0.22	0.26	0.26	0.24
270	0.29	0.29	0.25	0.65	0.65	0.60	0.25	0.25	0.24	0.24	0.24	0.22	0.26	0.26	0.22
300	0.29	0.29	0.23	0.65	0.65	0.46	0.25	0.25	0.23	0.24	0.24	0.4	0.26	0.26	0.03
						With Mo	ethylamine	e							
0	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45
30	142.49	42.27	38.10	134.75	40.15	35.92	125.62	37.73	35.92	122.64	42.05	37.29	141.49	41.88	37.67
60	130.98	21.23	21.23	109.15	4.06	4.06	101.01	4.10	4.10	90.69	21.93	22.00	92.42	24.26	24.26
90	90.00	4.09	4.55	69.44	19.09	18.68	64.22	18.28	18.08	61.82	18.28	17.88	69.48	4.24	17.88
14	48.84	18.06	19.27	83.94	16.12	17.70	75.41	16.30	16.50	71.84	14.13	16.12	75.61	16.14	16.70
150	43.66	2.78	3.4	43.48	2.80	3.79	43.44	3.04	3.00	42.90	2.80	2.80	44.06	3.02	2.53
180	22.03	1.65	1.76	18.65	1.69	1.71	17.07	1.73	1.71	16.45	1.75	1.71	17.54	1.71	1.75
210	8.18	1.61	0.44	7.14	1.63	0.44	6.63	1.65	0.36	6.49	1.67	0.30	7.18	1.63	0.58
240	4.37	1.45	0.16	4.34	1.49	0.16	4.19	1.53	0.18	3.99	1.55	0.18	4.19	1.51	0.4
270	3.41	1.41	0.03	2.56	1.41	0.03	2.23	1.43	0.04	2.03	1.43	0.01	2.21	1.43	0.06
300	0.34	1.25	0.02	0.33	1.29	0.02	0.32	1.33	0.04	0.30	1.35	0.01	0.38	1.31	0.06

 Table 7 Summary of Regression Data on Degradation Reaction with Sodium Hydrogen Carbonates and The Catalysts.

Sodium hydrogen carbonate

Concentration (M)	Maize	Relation Equation	Slope k	R <sup>2</sup>	Half-life T1/2	y-intercept
1	WM	y = -0.0132x + 5.6875	-75.7576	0.9451	52.5112	5.6875
	DM	y = -0.0133x + 5.6174	-75.1880	0.9528	52.1163	5.6174
	GM	y = -0.0134x + 5.5695	-74.6269	0.9596	51.7274	5.5695
0.5	WM	y = -0.0142x + 5.5529	-70.4225	0.9749	48.8132	5.5529
	DM	y = -0.0137x + 5.5913	-72.9927	0.9745	50.5947	5.5913
	GM	y = -0.0132x + 5.5172	-75.7576	0.9377	52.5112	5.5172
0.05	WM	y = -0.0119x + 5.4757	-84.0336	0.9054	58.2477	5.4757
	DM	y = -0.0125x + 5.4792	-80.0000	0.9467	55.4518	5.4792
	GM	y = -0.0142x + 5.4289	-70.4225	0.9598	48.8132	5.4289
0.005	WM	y = -0.0131x + 5.43	-76.3359	0.9511	52.914	5.4300
	DM	y = -0.0132x + 5.5576	-75.7576	0.9473	52.5112	5.5576
	GM	y = -0.0133x + 5.4979	-75.1880	0.9625	52.1163	5.4979
0.0005	WM	y = -0.0134x + 5.4568	-74.6269	0.9685	51.7274	5.4568
	DM	y = -0.0139x + 5.4528	-71.9424	0.9737	49.8667	5.4528
	GM	y = -0.0132x + 5.4649	-75.7576	0.9716	52.5112	5.4649
		With hydrogen peroxide				
1	WM	y = -0.0118x + 5.2417	-84.7458	0.9845	58.74129	5.2417
	DM	y = -0.0129x + 5.2708	-77.5194	0.9788	53.73234	5.2708
	GM	y = -0.012x + 5.2169	-83.3333	0.983	57.76227	5.2169
0.5	WM	y = -0.0117x + 5.1784	-85.4701	0.9792	59.24335	5.1784
	DM	y = -0.0127x + 5.478	-78.7402	0.9784	54.57852	5.478
	GM	y = -0.0117x + 5.1451	-85.4701	0.9788	59.24335	5.1451
0.05	WM	y = -0.0118x + 5.1558	-84.7458	0.9767	58.74129	5.1558
	DM	y = -0.0129x + 5.1831	-77.5194	0.9825	53.73234	5.1831
	GM	y = -0.0117x + 5.1451	-85.4701	0.9788	59.24335	5.1451
0.005	WM	y = -0.012x + 5.1331	-83.3333	0.9801	57.76227	5.1331
	DM	y = -0.013x + 5.1519	-76.9231	0.9831	53.31901	5.1519
	GM	y = -0.0119x + 5.0702	-84.0336	0.9774	58.24766	5.0702
0.0005	WM	y = -0.0111x + 5.1358	-90.0901	0.9542	62.44569	5.1358
	DM	y = -0.0121x + 5.1542	-82.6446	0.9802	57.28489	5.1542
	GM	y = -0.0114x + 5.1075	-87.7193	0.9693	60.80238	5.1075
		With ammonia				
1	WM	y = -0.026x + 5.8048	-38.4615	0.9111	26.65951	5.8048
	DM	y = -0.026x + 5.7682	-38.4615	0.9137	26.65951	5.7682

	GM	y = -0.0262x + 5.718	-38.1679	0.9278	26.456	5.718
0.5	WM	y = -0.0221x + 5.4047	-45.2489	0.9397	31.36413	5.4047
	DM	y = -0.022x + 5.3615	-45.4545	0.9421	31.50669	5.3615
	GM	y = -0.0226x + 5.4007	-44.2478	0.952	30.67023	5.4007
0.05	WM	y = -0.0261x + 5.6052	-38.3142	0.9313	26.5574	5.6052
	DM	y = -0.0261x + 5.6052	-38.3142	0.9313	26.55736	5.6052
	GM	y = -0.0261x + 5.5706	-38.3142	0.934	26.55736	5.5706
0.005	WM	y = -0.0269x + 5.5831	-37.1747	0.9311	25.76755	5.5831
	DM	y = -0.0266x + 5.5551	-37.594	0.9331	26.05816	5.5551
	GM	y = -0.0262x + 5.5783	-38.1679	0.9356	26.456	5.5783
0.0005	WM	y = -0.0256x + 5.5183	-39.0625	0.9362	27.07606	5.5183
	DM	y = -0.0258x + 5.5889	-38.7597	0.9298	26.86617	5.5889
	GM	y = -0.0294x + 5.883	-34.0136	0.9492	23.57643	5.883
		With methylamine				
1	WM	y = -0.019x + 5.9449	-52.6316	0.9112	36.48143	5.9449
	DM	y = -0.0165x + 4.3762	-60.6061	0.8757	42.00892	4.3762
	GM	y = -0.03x + 5.3809	-33.3333	0.959	23.10491	5.3809
0.5	WM	y = -0.0193x + 5.9366	-51.8135	0.9089	35.91436	5.9366
	DM	y = -0.0162x + 4.347	-61.7284	0.8744	42.78686	4.347
	GM	y = -0.03x + 5.3489	-33.3333	0.9586	23.10491	5.3489
0.05	WM	y = -0.0194x + 5.8876	-51.5464	0.9125	35.72924	5.8876
	DM	y = -0.016x + 4.2998	-62.5	0.8749	43.3217	4.2998
	GM	y = -0.0285x + 5.1819	-35.0877	0.967	24.3495	5.1819
0.005	WM	y = -0.0195x + 5.8582	-51.2821	0.9131	35.54601	5.8582
	DM	y = -0.0162x + 4.32	-61.7284	0.878	42.78686	4.32
	GM	y = -0.0327x + 5.54	-30.581	0.947	21.19716	5.54
0.0005	WM	y = -0.0192x + 5.8938	-52.0833	0.946	36.10142	5.8938
		-				
	DM	y = -0.0164x + 4.3897	-60.9756	0.8835	42.26507	4.3897

Table 8 Instantaneous Concentrations of Aflatoxin in Maize After Degradation withDifferent Concentrations of Sodium Hypochlorite.

				Sodiu	ım Hy	pochlo	orite								
Time	e 1M			0.5M			0.05N	Л		0.005	М		0.000	5M	
Min	W	DM	GM	W	DM	GM	W	DM	GM	W	DM	GM	W	DM	GM
	Μ			М			М			М			М		
0	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.
	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45
30	49.6	82.3	81.5	47.6	81.9	81.3	47.2	81.7	79.2	77.4	79.4	79.4	87.3	89.9	81.6
	1	6	6	3	6	8	3	6	2	0	0		2	0	6
60	33.7	68.4	74.4	39.6	64.5	64.0	37.7	64.1	61.7	36.7	63.7	61.7	44.6	68.0	61.8
	4	7	2	9	0	0	1	0	8	1	0	7	5	7	0
90	24.8	61.7	61.7	24.2	59.5	57.5	23.6	52.9	46.3	24.0	48.5	41.9	43.6	61.8	59.5
	1	4	4	1	6	7	2	9	1	1	1	0	6	9	6
14	24.0	53.7	56.9	24.0	51.8	50.9	24.0	48.7	44.1	24.0	39.7	39.6	26.0	59.1	49.6
	1	8	8	1	0	8	1	2	7	1	1	9	0	4	3
15	21.8	46.2	41.9	21.8	44.1	44.2	21.8	39.9	39.6	21.8	26.3	24.2	24.4	47.3	41.9
0	3	2	1	3	7	3	3	1	9	3	1	6	1	7	0
18	15.8	40.0	38.1	15.8	39.9	38.1	15.8	35.1	32.8	16.0	24.2	19.8	17.4	35.7	38.3
0	8	1	0	8	2	7	8	1	2	7	9	9	6	4	2
21	4.37	37.1	33.9	4.37	29.9	33.6	3.57	24.6	26.5	2.98	19.9	17.8	5.76	28.0	18.8
0		3	3		9	4		1	2		4	6		2	1
24	1.59	22.2	18.1	1.59	24.0	16.1	1.79	19.6	17.2	1.79	18.0	13.7	2.00	19.6	16.8
0		3	6		4	2		7	9		8	9		2	9

27	0.4	6.75	6.75	0.4	11.5	8.16	0.40	4.82	6.81	0.02	2.40	3.97	0.60	9.15	6.40
0					1										
30	0.4	1.79	1.79	0.4	2.58	1.85	0.40	0.38	1.77	0.02	4.29	1.63	0.60	6.18	1.66
0															
		_	_	_	With	Hydro	gen Pe	roxide	_	_	_	_	_	_	_
0	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.
	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45
30	41.6	39.7	42.8	39.6	41.8	35.7	37.7	38.1	33.7	37.1	37.1	32.7	37.1	37.2	32.0
	7	1	7	9	7	2	1	0	4	1	9	4	1	9	9
60	29.7	26.7	28.7	29.3	28.1	28.2	28.7	27.8	26.1	27.9	26.0	26.0	28.3	28.4	26.3
	7	9	8	7	8	4	8	2	0	8	0	0	8	2	9
90	23.8	23.8	24.2	22.2	4.48	22.2	22.0	19.8	22.0	4.04	17.8	4.04	4.04	18.6	4.04
	1	1	1	3		3	3	7	3		8			7	
14	1 18.0	1 18.0	1 17.8	3	15.8	3 17.2	3	7	3	16.0	8 15.8	16.0	16.4	7 15.9	16.4
14	1 18.0 6	1 18.0 6	1 17.8 6	3 17.2 7	15.8 9	3 17.2 7	3 16.2 9	7 16.1 0	3 16.2 9	16.0 7	8 15.8 8	16.0 7	16.4 9	7 15.9 4	16.4 9
14	1 18.0 6 14.0	1 18.0 6 14.0	1 17.8 6 12.1	3 17.2 7 11.9	15.8 9 11.1	3 17.2 7 11.9	3 16.2 9 10.1	7 16.1 0 9.92	3 16.2 9 10.1	16.0 7 9.92	8 15.8 8 9.84	16.0 7 9.70	16.4 9 9.91	7 15.9 4 9.92	16.4 9 9.91
14 15 0	1 18.0 6 14.0 9	1 18.0 6 14.0 9	1 17.8 6 12.1 1	3 17.2 7 11.9 1	15.8 9 11.1 7	3 17.2 7 11.9 1	3 16.2 9 10.1 2	7 16.1 0 9.92	3 16.2 9 10.1 2	16.0 7 9.92	8 15.8 8 9.84	16.0 7 9.70	16.4 9 9.91	7 15.9 4 9.92	16.4 9 9.91
14 15 0 18	1 18.0 6 14.0 9 7.94	1 18.0 6 14.0 9 7.94	1 17.8 6 12.1 1 7.34	3 17.2 7 11.9 1 7.94	15.8 9 11.1 7 6.85	3 17.2 7 11.9 1 7.94	3 16.2 9 10.1 2 6.95	7 16.1 0 9.92 6.64	3 16.2 9 10.1 2 6.95	16.0 7 9.92 6.75	8 15.8 8 9.84 6.38	16.0 7 9.70 6.75	16.4 9 9.91 6.35	7 15.9 4 9.92 7.37	16.4 9 9.91 6.35
14 15 0 18 0	1 18.0 6 14.0 9 7.94	1 18.0 6 14.0 9 7.94	1 17.8 6 12.1 1 7.34	3 17.2 7 11.9 1 7.94	15.8 9 11.1 7 6.85	3 17.2 7 11.9 1 7.94	3 16.2 9 10.1 2 6.95	7 16.1 0 9.92 6.64	3 16.2 9 10.1 2 6.95	16.0 7 9.92 6.75	8 15.8 8 9.84 6.38	16.0 7 9.70 6.75	16.4 9 9.91 6.35	7 15.9 4 9.92 7.37	16.4 9 9.91 6.35
14 15 0 18 0 21	1 18.0 6 14.0 9 7.94 4.17	1 18.0 6 14.0 9 7.94 4.17	1 17.8 6 12.1 1 7.34 3.99	3 17.2 7 11.9 1 7.94 3.77	15.8 9 11.1 7 6.85 3.57	3 17.2 7 11.9 1 7.94 3.77	3 16.2 9 10.1 2 6.95 3.37	7 16.1 0 9.92 6.64 3.18	3 16.2 9 10.1 2 6.95 3.37	16.0 7 9.92 6.75 2.82	8 15.8 8 9.84 6.38 2.78	16.0 7 9.70 6.75 2.82	16.4 9 9.91 6.35 2.84	7 15.9 4 9.92 7.37 2.84	16.4 9 9.91 6.35 2.84
14 15 0 18 0 21 0	1 18.0 6 14.0 9 7.94 4.17	1 18.0 6 14.0 9 7.94 4.17	1 17.8 6 12.1 1 7.34 3.99	3 17.2 7 11.9 1 7.94 3.77	15.8 9 11.1 7 6.85 3.57	3 17.2 7 11.9 1 7.94 3.77	3 16.2 9 10.1 2 6.95 3.37	7 16.1 0 9.92 6.64 3.18	3 16.2 9 10.1 2 6.95 3.37	16.0 7 9.92 6.75 2.82	8 15.8 8 9.84 6.38 2.78	16.0 7 9.70 6.75 2.82	16.4 9 9.91 6.35 2.84	7 15.9 4 9.92 7.37 2.84	16.4 9 9.91 6.35 2.84
14 15 0 18 0 21 0 24	1 18.0 6 14.0 9 7.94 4.17 4.17	1 18.0 6 14.0 9 7.94 4.17 1.41	1 17.8 6 12.1 1 7.34 3.99 1.49	3 17.2 7 11.9 1 7.94 3.77 1.45	15.8 9 11.1 7 6.85 3.57 1.45	3 17.2 7 11.9 1 7.94 3.77 1.25	3 16.2 9 10.1 2 6.95 3.37 0.14	7 16.1 9.92 6.64 3.18	3 16.2 9 10.1 2 6.95 3.37	16.0 7 9.92 6.75 2.82 1.41	8 15.8 8 9.84 6.38 2.78 1.41	16.0 7 9.70 6.75 2.82 1.21	16.4 9 9.91 6.35 2.84 1.71	7 15.9 4 9.92 7.37 2.84 1.71	16.4 9 9.91 6.35 2.84 1.31
14 15 0 18 0 21 0 24 0	1 18.0 6 14.0 9 7.94 4.17 1.49	1 18.0 6 14.0 9 7.94 4.17 1.41	1 17.8 6 12.1 1 7.34 3.99 1.49	3 17.2 7 11.9 1 7.94 3.77 1.45	15.8 9 11.1 7 6.85 3.57 1.45	3 17.2 7 11.9 1 7.94 3.77 1.25	3 16.2 9 10.1 2 6.95 3.37 0.14	7 16.1 0 9.92 6.64 3.18 0.14	3 16.2 9 10.1 2 6.95 3.37 0.14	16.0 7 9.92 6.75 2.82 1.41	8 15.8 8 9.84 6.38 2.78 1.41	16.0 7 9.70 6.75 2.82 1.21	16.4 9 9.91 6.35 2.84 1.71	7 15.9 4 9.92 7.37 2.84 1.71	16.4 9 9.91 6.35 2.84 1.31

 1.49 1.21 1.49 1.45 1.45 1.19 0.14 0.14 0.12 1.41 1.41 0.81 1.71 1.71 1.19 

**30** 1.49 0.62 1.49 1.45 1.45 0.52 0.14 0.14 0.03 1.41 1.41 0.32 1.71 1.71 0.33 

	With Ammonia														
0	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.
	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45
30	21.8	22.4	22.2	19.8	22.0	24.4	17.8	18.0	18.6	17.2	17.3	17.4	17.6	17.7	17.7
	3	4	3	5	5	1	6	6	5	7	6	0	6	6	6
60	17.8	15.8	17.2	16.2	17.0	16.2	15.8	16.2	15.8	16.6	16.1	16.6	17.2	17.3	17.2
	6	8	7	7	7	7	8	7	8	7	2	7	7	0	7
90	15.8	15.4	15.0	15.0	14.4	14.2	14.0	13.7	13.9	14.4	13.3	13.9	15.6	15.6	14.2
	8	8	8	8	9	9	9	1	1	9	6	4	8	8	7
14	13.6	12.2	13.1	12.1	11.3	11.9	11.7	11.1	11.3	10.3	9.93	10.1	11.1	11.1	10.7
	9	8	0	1	3	3	3	3	3	2		4	1	9	2
15	14.0	11.9	8.14	11.9	7.54	10.3	10.1	6.97	8.14	9.92	6.95	7.74	9.91	7.94	9.33
0	9	1		1		2	2								
18	4.76	4.37	4.4	2.78	2.21	2.58	2.98	2.21	2.38	2.38	2.21	2.21	2.50	2.38	2.50
0															
21	2.18	1.99	2.18	1.98	1.98	2.38	1.98	1.98	2.58	1.97	1.97	1.95	1.98	1.98	1.88
0															
24	1.41	1.41	1.41	1.25	1.25	1.25	0.13	0.13	0.13	1.21	1.21	1.21	1.31	1.31	1.31
0															

0															
30	1.41	1.41	0.60	1.25	0.4	1.25	0.13	0.02	0.13	1.21	0.22	1.21	1.31	0.4	1.31
0															
					With	Methy	lamine	;							
0	100	100	100	100	100		100	100	100	100	100	100	100	100	100
0	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.
	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45
30	43.6	39.6	40.6	41.6	33.6	34.6	39.8	35.8	35.8	47.4	41.4	41.4	47.9	42.9	42.9
	6	6	6	7	7	7	9	9	9	3	3	3	1	1	1
60	25.4	23.4	22.4	24.0	22.0	19.0	24.3	23.3	4.39	29.4	27.4	24.4	30.2	34.2	28.2
				3	3	3	9	9		7	7	7	1	1	1
90	24.8	22.8	4.81	23.8	4.85	17.8	23.6	21.6	16.6	24.0	25.0	4.01	24.2	26.2	23.2
	1	1		5		5	2	2	2	1	1		1	1	1
14	1 4.04	1 21.0	17.0	5 19.6	17.6	5 15.6	2 19.4	2 15.4	2 13.4	1 18.1	1	17.8	1 18.1	1 4.12	1 18.9
14	1 4.04	1 21.0 4	17.0 4	5 19.6 9	17.6 9	5 15.6 9	2 19.4 7	2 15.4 7	2 13.4 7	1 18.1 0	1 16.1 0	17.8 0	1 18.1 2	1 4.12	1 18.9 2
14	1 4.04 2.18	1 21.0 4 2.18	17.0 4 4.18	5 19.6 9 2.18	17.6 9 2.18	5 15.6 9 6.18	2 19.4 7 2.18	2 15.4 7 2.18	2 13.4 7 8.18	1 18.1 0 2.18	1 16.1 0 2.18	17.8 0 7.18	1 18.1 2 2.44	1 4.12 2.44	1 18.9 2 12.4
14 15 0	1 4.04 2.18	1 21.0 4 2.18	17.0 4 4.18	5 19.6 9 2.18	17.6 9 2.18	5 15.6 9 6.18	2 19.4 7 2.18	2 15.4 7 2.18	2 13.4 7 8.18	1 18.1 0 2.18	1 16.1 0 2.18	17.8 0 7.18	1 18.1 2 2.44	1 4.12 2.44	1 18.9 2 12.4 4
14 15 0 18	1 4.04 2.18 1.59	1 21.0 4 2.18 1.59	17.0 4 4.18 2.59	5 19.6 9 2.18 1.59	17.6 9 2.18 1.59	5 15.6 9 6.18 2.59	2 19.4 7 2.18 1.59	2 15.4 7 2.18 1.59	2 13.4 7 8.18 2.59	1 18.1 0 2.18 1.61	1 16.1 0 2.18 1.61	17.8 0 7.18 2.61	1 18.1 2 2.44 1.75	1 4.12 2.44 1.75	1 18.9 2 12.4 4 2.75
14 15 0 18 0	1 4.04 2.18 1.59	1 21.0 4 2.18 1.59	17.0 4 4.18 2.59	5 19.6 9 2.18 1.59	17.6 9 2.18 1.59	5 15.6 9 6.18 2.59	2 19.4 7 2.18 1.59	2 15.4 7 2.18 1.59	2 13.4 7 8.18 2.59	1 18.1 0 2.18 1.61	1 16.1 0 2.18 1.61	17.8 0 7.18 2.61	1 18.1 2 2.44 1.75	1 4.12 2.44 1.75	1 18.9 2 12.4 4 2.75
14 15 0 18 0 21	1 4.04 2.18 1.59 4.37	1 21.0 4 2.18 1.59 0.37	17.0 4 4.18 2.59 1.37	5 19.6 9 2.18 1.59 4.37	17.6 9 2.18 1.59 1.37	5 15.6 9 6.18 2.59 1.37	2 19.4 7 2.18 1.59 3.57	2 15.4 7 2.18 1.59	2 13.4 7 8.18 2.59 1.57	1 18.1 0 2.18 1.61 2.98	1 16.1 0 2.18 1.61	17.8 0 7.18 2.61 1.98	1 18.1 2 2.44 1.75 5.76	1 4.12 2.44 1.75	1 18.9 2 12.4 4 2.75 2.16
14 15 0 18 0 21 0	1 4.04 2.18 1.59 4.37	1 21.0 4 2.18 1.59 0.37	17.0 4 4.18 2.59 1.37	5 19.6 9 2.18 1.59 4.37	17.6 9 2.18 1.59 1.37	5 15.6 9 6.18 2.59 1.37	2 19.4 7 2.18 1.59 3.57	2 15.4 7 2.18 1.59 1.57	2 13.4 7 8.18 2.59 1.57	1 18.1 0 2.18 1.61 2.98	1 16.1 0 2.18 1.61	17.8 0 7.18 2.61 1.98	1 18.1 2 2.44 1.75 5.76	1 4.12 2.44 1.75 1.74	1 18.9 2 12.4 4 2.75 2.16
14 15 0 18 0 21 0 24	1 4.04 2.18 1.59 4.37	1 21.0 4 2.18 1.59 0.37	17.0 4 4.18 2.59 1.37	5 19.6 9 2.18 1.59 4.37	17.6 9 2.18 1.59 1.37	5 15.6 9 6.18 2.59 1.37	2 19.4 7 2.18 1.59 3.57	2 15.4 7 2.18 1.59 1.57	2 13.4 7 8.18 2.59 1.57	1 18.1 0 2.18 1.61 2.98	1 16.1 0 2.18 1.61 1.38	17.8 0 7.18 2.61 1.98	1 18.1 2 2.44 1.75 5.76	1 4.12 2.44 1.75 1.75	1 18.9 2 12.4 4 2.75 2.16 2.06

1.41 1.41 1.21 1.25 1.19 1.25 0.13 0.12 0.13 1.21 1.01 1.21 1.31 1.21 1.31

| P a g e

27	0.26	0.26	0.16	0.26	0.26	0.16	0.42	0.42	0.22	0.10	0.10	0.40	0.64	0.64	1.94
0															
30	0.26	0.25	0.06	0.26	0.09	0.06	0.42	0.33	0.02	0.10	0.05	0.10	0.64	0.31	0.64
0															

## Table 9 Summary of Regression Equation Data for each Degradation Reaction withSodium Hypochlorite and Catalyst.

Concentration (M)	Maize	Relation Equation	Slope k	R <sup>2</sup>	Half-life T1/2	y-intercept
1	WM	y = -0.0211x + 5.3176	-47.3934	0.8840	32.8506	5.3176
	DM	y = -0.0212x + 5.339	-47.1698	0.8860	32.6956	5.3390
	GM	y = -0.0193x + 5.1576	-51.8135	0.9112	35.9144	5.1576
0.5	WM	y = -0.0281x + 5.9707	-35.5872	0.8173	24.6672	5.9707
	DM	y = -0.019x + 5.4033	-52.6316	0.9377	36.4814	5.4033
	GM	y = -0.0212x + 5.2502	-47.1698	0.8872	32.6956	5.2502
0.05	WM	y = -0.0213x + 5.2225	-46.7290	0.9007	32.3901	5.2225
	DM	y = -0.0214x + 5.2266	-46.9484	0.9013	32.5421	5.2266
	GM	y = -0.0221x + 5.3296	-45.2489	0.9142	31.3641	5.3296
0.005	WM	y = -0.016x + 4.9848	-62.5000	0.9711	43.3217	4.9848
	DM	y = -0.0211x + 5.1775	-47.3934	0.942	32.8506	5.1775
	GM	y = -0.021x + 5.1378	-47.6190	0.9230	33.0070	5.1378
0.0005	WM	y = -0.0191x + 4.9529	-52.3560	0.9537	36.2904	4.9529
	DM	y = -0.0279x + 5.7076	-35.8423	0.8512	24.8440	5.7076
	GM	y = -0.0188x + 5.0987	-53.1915	0.9775	36.8695	5.0987
		With hydrogen peroxide				
1	WM	y = -0.0155x + 4.669	-86.9565	0.9469	60.27367	4.669
	DM	y = -0.0155x + 4.6532	-64.5161	0.9512	44.71917	4.6532
	GM	y = -0.017x + 4.7737	-58.8235	0.9545	40.77336	4.7737

#### Sodium hypochlorite
0.5	WM	y = -0.0155x + 4.6261	-64.5161	0.9471	44.71917	4.6261
	DM	y = -0.0155x + 4.5926	-64.5161	0.9501	44.71917	4.5926
	GM	y = -0.0173x + 4.7521	-57.8035	0.9538	40.06631	4.7521
0.05	WM	y = -0.024x + 5.2126	-41.6667	0.9007	28.88113	5.2126
	DM	y = -0.0155x + 4.5926	-64.5161	0.9501	44.71917	4.5926
	GM	y = -0.0261x + 5.368	-38.3142	0.8936	26.55736	5.368
0.005	WM	y = -0.0156x + 4.5518	-64.1026	0.945	44.43251	4.5518
	DM	y = -0.024x + 5.1836	-41.6667	0.904	28.88113	5.1836
	GM	y = -0.0185x + 4.7581	-54.0541	0.9577	37.46742	4.7581
0.0005	WM	y = -0.015x + 4.5033	-66.6667	0.9391	46.4981	4.5033
	DM	y = -0.0155x + 4.5099	-64.5161	0.9416	44.71917	4.5099
	GM	y = -0.0179x + 4.7161	-55.8659	0.9532	38.72331	4.7161
		With ammonia		1		
1	WM	y = -0.0146x + 4.2568	-68.4932	0.8837	47.47583	4.2568
	DM	y = -0.0146x + 4.199	-68.4932	0.8836	47.47583	4.199
	GM	y = -0.0161x + 4.3143	-62.1118	0.9306	43.05262	4.3143
0.5	WM	y = -0.015x + 4.1764	-66.6667	0.878	46.4981	4.1764
	DM	y = -0.0152x + 4.446	-65.7895	0.8939	45.60179	4.446
	GM	y = -0.0181x + 4.4031	-55.2486	0.9229	38.29542	4.4031
0.05	WM	y = -0.0231x + 4.737	-43.29	0.896	30.00637	4.737
	DM	y = -0.0231x + 4.7118	-43.4783	0.8992	30.13683	4.7118
	GM	y = -0.0261x + 4.9437	-38.3142	0.9099	26.55736	4.9437
0.005	WM	y = -0.015x + 4.1	-66.6667	0.8729	46.4981	4.1
	DM	y = -0.015x + 4.0668	-66.6667	0.876	46.4981	4.0668
	GM	y = -0.0177x + 4.2878	-56.4972	0.9183	39.16086	4.2878
0.0005	WM	y = -0.0148x + 4.1192	-67.5676	0.8722	46.83427	4.1192
	DM	y = -0.0148x + 4.0931	-67.5676	0.8721	46.83427	4.0931
	GM	y = -0.0178x + 4.3642	-56.1798	0.9106	38.94085	4.3642

		With methylamine				
1	WM	y = -0.046x + 4.8182	-48.5437	0.9129	33.64792	4.8182
	DM	y = -0.0235x + 4.8464	-42.5532	0.9359	29.49562	4.8464
	GM	y = -0.0246x + 5.0906	-40.6504	0.9685	28.17671	5.0906
0.5	WM	y = -0.045x + 4.7852	-48.7805	0.9113	33.8146	4.7852
	DM	y = -0.0227x + 4.8291	-44.0529	0.9507	30.53512	4.8291
	GM	y = -0.0237x + 4.9849	-42.1941	0.9509	29.24672	4.9849
0.05	WM	y = -0.0192x + 4.6589	-52.0833	0.9121	36.10142	4.6589
	DM	y = -0.041x + 4.6237	-49.7512	0.9463	34.48493	4.6237
	GM	y = -0.025x + 5.1367	-40	0.9095	27.72589	5.1367
0.005	WM	y = -0.0236x + 5.07	-42.3729	0.9163	29.37064	5.07
	DM	y = -0.0251x + 5.1192	-39.8406	0.9425	27.61543	5.1192
	GM	y = -0.022x + 4.9804	-45.4545	0.9643	31.50669	4.9804
0.0005	WM	y = -0.018x + 4.6657	-55.5556	0.8995	38.50818	4.6657
	DM	y = -0.041x + 4.8007	-49.7512	0.9442	34.48493	4.8007
	GM	y = -0.0169x + 4.6843	-59.1716	0.9428	41.01463	4.6843

**Table 10: Instantaneous Concentrations of Aflatoxin in Maize after Degradation with** 

#### **Ammonium Carbonate And Catalysts**

Ammonium carbonate

Time	1M			0.5 M			0.05 N	Л		0.005	М		0.0005	5 M	
		DM	CN		DM	<u>C</u> ) (		DM			DM	CN		DM	
min.	W	DM	GM	WM	DM	GM	WM	DM	GM	WM	DM	GM	WM	DM	GM
	м														
0	198	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.
	15	4.5	4.5	4.5	4.5	4.5	15	4.5	17	17	4.5	4.7	4.5	4.5	4.5
	.45	45	45	45	45	45	45	45	45	45	45	45	45	45	45
30	140	115.	118.	137.	99.0	114.	129.	95.8	111.	144.	96.8	108.	143.	97.8	112.
	.90	10	43	13	3	11	59	5	73	47	4	95	48	4	92
<i>c</i> 0	107	101	110	101		110		01.0	100	105	00.5	10.5	115	04.4	100
60	125	101.	113.	121.	92.8	112.	111.	91.0	108.	107.	90.6	106.	116.	94.4	108.
	02	01	12	05	7	72	13	9	33	16	9	31	49	0	27
	.02		12		,	12	1.5		55	10		51			21

90	105	90.0	100.	97.2	89.2	81.1	83.3	88.8	80.5	96.2	87.6	80.3	102.	89.3	93.2
	.18	0	22	4	8	7	5	3	7	5	2	7	4	2	9
14	101	68.6	83.5	91.2	67.9	81.6	79.3	67.0	68.2	91.6	66.7	65.9	97.2	71.0	70.2
	.21	8	5	9	3	1	8	8	7	8	2	3	4	6	7
150	53.	44.0	40.0	41.6	27.9	35.9	51.6	25.6	31.5	35.7	24.2	32.1	81.3	42.0	41.9
	58	6	9	7	8	2	0	0	5	2	1	5	6	7	1
180	45.	27.9	27.9	38.7	22.6	26.5	33.1	22.0	25.0	25.8	24.0	21.6	39.2	25.4	21.9
	64	8	8	0	2	9	4	3	0	0	1	3	9	0	9
210	31.	4.08	21.8	27.7	16.6	15.8	19.8	16.1	14.0	21.2	10.1	10.5	29.7	13.7	12.1
	75		7	8	7	8	5	0	9	3	5	2	7	3	1
240	27.	15.4	13.8	23.8	15.6	12.2	17.8	11.4	12.5	11.9	8.97	10.1	4.04	12.9	11.1
	78	6	9	1	0	2	6	9	0	1		6		0	1
270	11.	5.56	5.36	11.1	5.52	5.16	10.3	4.86	4.76	9.13	4.61	4.37	10.1	6.24	5.96
	91			1			2						2		
300	3.5	2.40	3.4	3.57	2.32	3.29	3.57	2.56	3.12	3.57	2.4	3.12	4.37	2.78	3.89
	7														
					With I	Hydroge	en perox	ide							
0	198	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.
	.45	45	45	45	45	45	45	45	45	45	45	45	45	45	45
30	121	89.9	81.6	106.	83.9	77.7	111.	81.9	75.6	112.	87.9	87.3	121.	87.0	81.9
	.45	0	6	17	4	9	13	6	1	92	1	2	45	8	2
60	104	75.4	74.4	100.	71.6	71.2	99.2	69.6	64.2	98.2	69.6	62.5	100.	71.7	65.9
	.19	1	2	22	8	4	3	9	2	3	8	5	02	4	8
90	99.	66.4	61.8	97.4	63.7	61.9	97.0	62.1	59.7	96.2	59.1	57.6	95.4	59.5	61.9
	23	8	2	4	0	4	6	1	9	5	4	9	5	3	0
14	91.	39.8	39.7	89.5	37.1	36.7	79.6	29.1	29.3	77.6	28.0	27.7	77.4	30.1	29.9
	68	9	1	0	1	1	8	7	7	3	1	9		6	9

150	62.	19.8	22.6	61.5	18.3	18.0	57.9	17.3	16.8	50.8	16.3	15.8	62.7	17.7	17.6
	55	6	2	2	1	9	5	8	7	0	2	8	1	2	6
180	59.	8.93	9.33	58.5	8.14	8.93	55.7	7.94	8.53	49.8	7.58	8.14	48.0	7.94	9.27
	63			4			6			1			2		
210	42.	5.36	5.56	40.6	5.16	5.16	39.6	4.76	5.00	38.7	4.37	4.58	33.9	5.46	5.71
	67			8			9			0			3		
240	34.	4.21	4.96	28.7	4.76	4.56	27.7	4.56	4.19	24.8	4.37	4.37	23.8	3.97	5.16
	73			8			8			1			1		
270	10.	3.81	3.57	11.9	3.53	3.31	11.1	3.11	2.98	9.72	2.46	2.38	7.74	3.47	3.18
	91			1			1								
300	5.1	1.85	1.73	6.55	1.81	1.65	3.95	1.81	1.61	2.38	1.71	1.59	4.36	1.92	1.63
	6														
						With	Ammor	nia							
0	198	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.
	.45	45	45	45	45	45	45	45	45	45	45	45	45	45	45
30	82.	81.7	79.5	81.9	81.5	77.6	81.7	79.7	77.5	79.3	78.1	78.7	84.3	80.3	76.8
	36	6	8	6	6	5	6	8	9	8	9	8	4	7	5
60	68.	67.6	64.5	66.4	65.6	62.5	62.1	61.5	59.7	60.0	59.5	57.6	68.2	64.3	64.3
	47	7	0	8	9	1	1	2	9	3	4	0	7	0	0
90	59.	57.9	57.5	57.9	55.9	55.9	53.1	52.1	51.8	48.6	46.6	47.8	63.7	61.7	57.9
	93	5	9	5	6	6	8	9	0	2	4	7	2	4	7
14	55.	46.2	51.8	53.7	46.0	49.8	44.7	42.1	43.9	39.9	35.7	39.9	61.5	39.6	53.6
	76	6	0	8	6	1	5	8	7	3	4	3	4	7	0
150	53.	45.4	51.4	44.8	44.6	40.8	40.0	40.0	35.7	26.9	26.9	23.0	40.8	40.8	35.8
	38	5	0	3	3	6	9	9	6	9	9	2	0	0	3
180	23.	22.2	22.0	22.8	22.1	4.84	4.22	4.08	17.9	4.06	18.0	16.8	4.04	19.8	17.0
	81	3	7	2	3				0		8	7		2	9

210	22.	18.0	18.2	4.84	16.8	18.3	4.64	14.6	16.5	4.14	14.1	16.1	21.4	15.4	17.2
	03	6	8		7	6		9	5		9	9	3	8	8
240	2.2	2.08	2.28	2.48	2.38	2.48	2.18	1.98	2.18	2.98	2.28	2.98	2.18	2.40	2.18
	8														
270	2.2	0.86	0.92	2.48	0.98	0.97	2.18	0.80	0.85	2.98	0.90	1.18	2.18	0.97	0.85
	8														
300	2.2	0.71	0.74	2.48	0.79	0.78	2.18	0.62	0.66	2.98	0.68	0.86	2.18	0.78	0.71
	8														
						With I	Methyla	mine			1				
0	198	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.	198.
	.45	45	45	45	45	45	45	45	45	45	45	45	45	45	45
30	121	89.9	81.6	106.	83.9	77.7	111.	81.9	75.6	112.	87.9	87.3	121.	87.0	81.9
	.45	0	6	17	4	9	13	6	1	92	1	2	45	8	2
60	104	75.4	74.4	100.	71.6	71.2	99.2	69.6	64.2	98.2	69.6	62.5	100.	71.7	65.9
	.19	1	2	22	8	4	3	9	2	3	8	5	02	4	8
90	99.	66.4	61.8	97.4	63.7	61.9	97.0	62.1	59.7	96.2	59.1	57.6	95.4	59.5	61.9
	23	8	2	4	0	4	6	1	9	5	4	9	5	3	0
14	91.	39.8	39.7	89.5	37.1	36.7	79.6	29.1	29.3	77.6	28.0	27.7	77.4	30.1	29.9
	68	9	1	0	1	1	8	7	7	3	1	9		6	9
150	62.	19.8	22.6	61.5	18.3	18.0	57.9	17.3	16.8	50.8	16.3	15.8	62.7	17.7	17.6
	55	6	2	2	1	9	5	8	7	0	2	8	1	2	6
180	59.	8.93	9.33	58.5	8.14	8.93	55.7	7.94	8.53	49.8	7.58	8.14	48.0	7.94	9.27
	63			4			6			1			2		
210	42.	5.36	5.56	40.6	5.16	5.16	39.6	4.76	5.00	38.7	4.37	4.58	33.9	5.46	5.71
	67			8			9			0			3		
240	34.	4.21	4.96	28.7	4.76	4.56	27.7	4.56	4.19	24.8	4.37	4.37	23.8	3.97	5.16
	73			8			8			1			1		

270	10.	3.81	3.57	11.9	3.53	3.31	11.1	3.11	2.98	9.72	2.46	2.38	7.74	3.47	3.18
	91			1			1								
300	5.1	1.85	1.73	6.55	1.81	1.65	3.95	1.81	1.61	2.38	1.71	1.59	4.36	1.92	1.63
	6														

# Table 11. Summary of Regression Equation and Data for Degradation Reaction with<br/>Different Concentrations of Sodium Hypochlorite and Catalyst

Concentration	Maize	Relation Equation	Slope k	R <sup>2</sup>	Half-life T1/2	y-intercept
( <b>M</b> )						
1	WM	y = -0.0114x + 5.592	-87.7193	0.8936	60.8024	5.59
	DM	v = -0.0116x + 5.5319	-86.469	0.941	59.7541	5.53
		0.010 - 5.4001	00.000	0.0401	57.7511	5.55
	GM	y = -0.012x + 5.4821	-83.3333	0.9481	57.7623	5.48
0.5	WM	y = -0.0127x + 5.5341	-78.7402	0.9615	54.5785	5.53
	DM	y = -0.0116x + 5.6036	-86.469	0.9187	59.7541	5.6
	GM	y = -0.0117x + 5.5188	-85.4701	0.8739	59.2433	5.52
0.05	WM	y = -0.0119x + 5.4757	-84.0336	0.9054	58.2477	5.48
	DM	y = -0.0125x + 5.4792	-80	0.9467	55.4518	5.48
	GM	y = -0.0128x + 5.4863	-78.125	0.9566	54.1521	5.49
0.005	WM	y = -0.0119x + 5.545	-84.0336	0.8993	58.2477	5.55
	DM	y = -0.0117x + 5.536	-85.4701	0.8994	59.2433	5.54
	GM	y = -0.012x + 5.5113	-83.3333	0.9141	57.7623	5.51
0.0005	WM	y = -0.0124x + 5.4652	-80.6452	0.9471	55.899	5.47
	DM	y = -0.0128x + 5.4596	-78.125	0.9551	54.1521	5.46
	GM	y = -0.0118x + 5.441	-84.7458	0.9394	58.7413	5.44
		With hydrogen peroxide				
1	WM	y = -0.0089x + 5.1141	-112.36	0.8808	77.88171	5.1141
	DM	y = -0.0121x + 5.6177	-82.6446	0.9265	57.28489	5.6177
	GM	y = -0.0092x + 5.1394	-108.696	0.8962	75.3448	5.1394
0.5	WM	y = -0.0088x + 5.0231	-113.636	0.8995	78.76673	5.0231
	DM	y = -0.0121x + 5.5509	-82.6446	0.9461	57.28489	5.5509
	GM	y = -0.0092x + 5.0529	-108.696	0.9115	75.3448	5.0529

#### Ammonium carbonate

0.05	WM	y = -0.0087x + 4.9472	-114.943	0.8957	79.6749	4.9472
	DM	y = -0.0123x + 5.4675	-81.3008	0.9639	56.35343	5.4675
	GM	y = -0.0091x + 4.9738	-109.89	0.9184	76.17002	4.9738
0.005	WM	y = -0.0094x + 4.9593	-106.383	0.8586	73.73906	4.9739
	DM	y = -0.0127x + 5.4951	-78.7402	0.9695	54.57852	4.974
	GM	y = -0.0097x + 4.9761	-103.093	0.8847	71.45847	4.9741
0.0005	WM	y = -0.0087x + 5.0076	-114.943	0.8954	79.6749	4.9742
	DM	y = -0.0124x + 5.5751	-80.6452	0.9578	55.89897	4.9743
	GM	y = -0.0089x + 5.0225	-112.36	0.9024	77.88171	4.9744
		With ammonia	1	1		
1	WM	y = -0.0084x + 5.0605	-119.048	0.9295	82.51752	5.0605
	DM	y = -0.0092x + 5.0736	-108.696	0.9352	75.3448	5.0736
	GM	y = -0.0095x + 5.0453	-105.263	0.9694	72.96286	5.0453
0.5	WM	y = -0.0083x + 4.9735	-14.482	0.9329	83.51171	4.9735
	DM	y = -0.009x + 4.9658	-111.111	0.9346	77.01635	4.9658
	GM	y = -0.0094x + 4.9604	-106.383	0.9659	73.73906	4.9604
0.05	WM	y = -0.0083x + 4.9011	-14.482	0.941	83.51171	4.9011
	DM	y = -0.009x + 4.8951	-111.111	0.9458	77.01635	4.8951
	GM	y = -0.0097x + 4.9261	-103.093	0.9624	71.45847	4.9261
0.005	WM	y = -0.0083x + 4.8492	-14.482	0.9242	83.51171	4.8492
	DM	y = -0.0093x + 4.8906	-107.527	0.952	74.53195	4.8906
	GM	y = -0.0098x + 4.9076	-102.041	0.9659	70.7293	4.9076
0.0005	WM	y = -0.0083x + 4.9099	-14.482	0.9224	83.51171	4.9099
	DM	y = -0.0087x + 4.8904	-114.943	0.936	79.6749	4.8904
	GM	y = -0.0093x + 4.928	-107.527	0.967	74.53195	4.928
		With methylamine				
1	WM	y = -0.0121x + 5.6177	-82.6446	0.9265	57.28489	5.6177
	DM	y = -0.0139x + 5.3951	-71.9424	0.9615	49.8667	5.3951
	GM	y = -0.0191x + 5.8682	-52.356	0.9155	36.29043	5.8682
0.5	WM	y = -0.0121x + 5.5509	-82.6446	0.9461	57.28489	5.5509
	DM	y = -0.014x + 5.3285	-71.4286	0.952	49.51051	5.3285

#### Appendix 3:

	GM	y = -0.0192x + 5.8099	-52.0833	0.9243	36.10142	5.8099
0.05	WM	y = -0.0123x + 5.4675	-81.3008	0.9639	56.35343	5.4675
	DM	y = -0.0139x + 5.2666	-71.9424	0.9464	49.8667	5.2666
	GM	y = -0.0195x + 5.8123	-51.2821	0.9303	35.54601	5.8123
0.005	WM	y = -0.0127x + 5.4951	-78.7402	0.9695	54.57852	5.4951
	DM	y = -0.0138x + 5.2426	-72.4638	0.9439	50.22806	5.2426
	GM	y = -0.0194x + 5.7456	-52.6316	0.9257	36.48143	5.7456
0.0005	WM	y = -0.0124x + 5.5751	-80.6452	0.9578	55.89897	5.5751
	DM	y = -0.0138x + 5.2754	-72.4638	0.9432	50.22806	5.2754
	GM	y = -0.0191x + 5.7468	-52.356	0.929	36.29043	5.7468

# Table 1: The Sampling sites in the counties and number of samples taken

County	Latitude	Longitude	Sampled Site	Number of samples
Kajiado	2.5521° S	36.7839° E	NCPB Depot store	6
	1.6727° S	36.8425° E	County Market	6
	1.4252° S	36.6937° E	Retail store	6
	2.9248° S	37.5081° E	Farmer store	7
Nairobi	1.2939° S	36.8971° E	NCPB Depot store	6
	1.3061° S	36.8627° E	County Market	6
	1.2877° S	36.8338° E	Retail store	6
	1.2823° S	36.7524° E	Farmer store	7
Nakuru	0.3031° S	36.0800° E	NCPB Depot store	6
	0.3721° S	35.9479° E	County Market	6
	0.7172° S	36.4310° E	Retail store	7
	0.2488° S	35.7324° E	Farmer store	6
Busia	0.4608° N	34.1115° E	NCPB Depot store	6
	0.6362° N	34.2783° E	County Market	6

	0.3042° N	34.2060° E	Retail store	6
	0.4493° N	34.2519° E	Farmer store	7
Migori	1.0707° S	34.4753° E	NCPB Depot store	6
	1.2448° S	34.4767° E	County Market	6
	1.1940° S	34.6165° E	Retail store	6
	0.7553° S	34.5999° E	Farmer store	7
Trans Nzoia	1.0191° N	35.0023° E	NCPB Depot store	6
	0.9414° N	34.9465° E	County Market	6
	1.0677° N	34.8597° E	Retail store	6
	0.8767° N	35.1200° E	Farmer store	7
Isiolo	0.3344° N	37.5785 ° E	NCPB Depot store	6
	0.3547° N	37.5864° E	County Market	6
	0.3402° N	37.6480° E	Retail store	7
	0.3467° N	37.5885 ° E	Farmer store	6
Meru	0.0515° N	37.6456° E	NCPB Depot store	6
	0.0647° S	37.6679° E	County Market	6
	0.0136° S	37.7688° E	Retail store	6
	0.1570°S	37.9778° E	Farmer store	7
Embu	0.4524° S	37.7895° E	NCPB Depot store	6
	0.5388° S	37.4596° E	County Market	7
	0.4541° S	37.5854° E	Retail store	7
	0.5369° S	37.4550° E	Farmer store	7
Makueni	1.7791° S	37.6290° E	NCPB Depot store	6
	1.7886° S	37.6333° E	County Market	7
	2.4101° S	37.9656° E	Retail store	7
	2.0797° S	37.4731° E	Farmer store	6
Machakos	1.5177° S	37.2634° E	NCPB Depot store	7
	1.5155° S	37.2584° E	County Market	7

	1.5288° S	37.2572° E	Retail store	6
	1.4605° S	37.4388° E	Farmer store	7
Total				280

	Sar	nple	Aflatoxin B1 Method	Qualifier (315.1 -> 259.1) Results								
CALIBRATION LEVELS	Data File	Туре	Acq. Date-Time	Level	Dil.	Exp. Conc.	RT	Resp.	Calc. Conc.	Final Conc.	Accuracy	Ratio
Control	9.d	Sample	05-Oct-1806:26 PM		5							
Blank	2.d	Sample	05-Oct-18 05:03 PM		1							
1ppb AF	3.d	Cal	05-Oct-1805:07 PM	L1	1	1	4.3	314.5	0.82458462	0.82458462	82.45846	56.14
3ppb AF	4.d	Cal	05-Oct-1805:27 PM	L2	1	3	4.3	832.9	2.83995357	2.83995357	94.66512	69.02
9ppb AF	5.4	Cal	05-Oct-18 05:39 PM	L3	1	9	4.3	2411	8.9736129	8.9736129	99.70681	75.4
30ppb AF	6.d	Cal	05-Oct-1805:50 PM	L4	1	30	4.3	8725	33.5223722	33.5223722	111.7412	66.05
50ppb AF	7.d	Cal	05-Oct-1806:02 PM	L5	1	50	4.3	12782	49.2985201	49.2985201	98.59704	71
100ppb AF	8.d	Cal	05-Oct-18 06:06 PM	L6	1	100	4.3	23927	92.6284373	92.6284373	92.62844	70.84
1ppb AF	14.d	Cal	05-Oct-1807:25 PM	L1	1	1	4.3	370.6	1.04240609	1.04240609	104.2406	64.34
3ppb AF	15.d	Cal	05-Oct-1807:29 PM	L2	1	3	4.3	771	2.59931129	2.59931129	86.64371	67.73
9ppb AF	16.d	Cal	05-Oct-1807:48 PM	L3	1	9	4.3	2730	10.2157828	10.2157828	113.5087	70.95
30ppb AF	17.d	Cal	05-Oct-18 08:00 PM	L4	1	30	4.3	8932	34.3284607	34.3284607	114.4282	70.74
50ppb AF	18.d	Cal	05-Oct-1808:12 PM	L5	1	50	4.3	13388	51.655114	51.655114	103.3102	69.23
100ppb AF	19.d	Cal	05-Oct-1808:24 PM	L6	1	100	4.3	25327	98.0714445	98.0714445	98.07144	70.65

## Calibration table for Aflatoxin B1

### Calibration table for Aflatoxin B2

			**									
CALBRATION LEVELS	Data File	Туре	Acq. Date-Time	Level	Dal	Exp. Conc.	RT	Resp.	Calc. Conc.	Final Conc.	Accuracy	Ratio
Control	9.4	Sample	05-Oct-18 06:26 PM		5		4.15	20.26	0	0		104.1
Blank	2.d	Sample	05-Oct-1805:03 PM		1							
lppb AF	3.d	Cal	05-Oct-1805:07 PM	L1	1	1	4.15	309.7	1.01156696	1.01156696	101.1567	78.08
3ppb AF	4.d	Cal	05-Oct-1805:27 PM	L2	1	3	4.15	738.2	2.90996437	2.90996437	96.99881	81.13
9ppb AF	5.4	Cal	05-Oct-1805:39 PM	L3	1	9	4.15	2360	10.0957676	10.0957676	112.1752	84.92
30ppb AF	6.d	Cal	05-Oct-1805:50 PM	L4	1	30	4.15	7280	31.8884704	31.8884704	106.2949	84.06
50ppb AF	7.d	Cal	05-Oct-1806:02 PM	L5	1	50	4.15	10945	48.1260531	48.1260531	96.25211	86.47
100ppb AF	8.d	Cal	05-Oct-1806:06 PM	L6	1	100	4.15	21177	93.4513001	93.4513001	93.4513	82.68
1ppb AF	14.d	Cal	05-Oct-1807:25 PM	L1	1	1	4.15	229.8	0.65749217	0.65749217	65.74922	95.87
3ppb AF	15.d	Cal	05-Oct-1807:29 PM	L2	1	3	4.15	770.9	3.05448978	3.05448978	101.8163	80.94
9ppb AF	16.d	Cal	05-Oct-1807:48 PM	L3	1	9	4.15	2390	10.2273374	10.2273374	113.6371	81.42
30ppb AF	17.d	Cal	05-Oct-1808:00 PM	L4	1	30	4.15	7394	32.3953383	32.3953383	107.9845	89.82
50ppb AF	18.d	Cal	05-Oct-1808:12 PM	L5	1	50	4.15	11888	52.3016818	52.3016818	104.6034	80.78
100ppb AF	19.d	Cal	05-Oct-1808:24 PM	Ló	1	100	4.15	22628	99.8805379	99.8805379	99.88054	81.74

## Calibration table for Aflatoxin B1 and B2



Aflatoxin G1 Method	Aflatoxin	G1 Rest	ılts			Qualifier (329.1 -> 311.1) Results									
Calibration Levels	Data File	Туре	Level	Dil.	Exp. [C]	RT	Resp.	Cale. [C].	МІ	Final [C].	Accuracy	Ratio	MI		
Control	9.d	Sple		5		4.1	20.26	0	F	0		104.1	F		
Blank	2.d	Sple		1					F				F		
1ppb AF	3.d	Cal	L1	1	1	4.1	309.7	1.01156696	Т	1.01156696	101.1567	78.08	F		
3 ppb AF	4.d	Cal	L2	1	3	4.1	738.2	2.90996437	F	2.90996437	96.99881	81.13	F		
9 ppb AF	5.d	Cal	L3	1	9	4.1	2360	10.0957676	F	10.0957676	112.1752	84.92	F		
30 ppb AF	6.d	Cal	L4	1	30	4.1	7280	31.8884704	F	31.8884704	106.2949	84.06	F		
50 ppb AF	7.d	Cal	L5	1	50	4.1	10945	48.1260531	F	48.1260531	96.25211	86.47	F		
100 ppb AF	8.d	Cal	L6	1	100	4.1	21177	93.4513001	F	93.4513001	93.4513	82.68	F		
1 ppb AF	14.d	Cal	Ll	1	1	4.1	229.8	0.65749217	F	0.65749217	65.74922	95.87	F		
3 ppb AF	15.d	Cal	L2	1	3	4.1	770.9	3.05448978	F	3.05448978	101.8163	80.94	F		
9 ppb AF	16.d	Cal	L3	1	9	4.1	2390	10.2273374	F	10.2273374	113.6371	81.42	F		
30 ppb AF	17.d	Cal	L4	1	30	4.1	7394	32.3953383	F	32.3953383	107.9845	89.82	F		
50 ppb AF	18.d	Cal	L5	1	50	4.1	11888	52.3016818	F	52.3016818	104.6034	80.78	F		
100ppb AF	19.d	Cal	L6	1	100	4.1	22628	99.8805379	F	99.8805379	99.88054	81.74	F		

## Calibration table for Aflatoxin G1

Sample				Aflatoxin G2 Method	Aflato	win G2 F	tesuits	Qualifier (331.1 -> 285.1) Results					
Cal. std L	Data File	Туре	Level	Dil	Exp. Conc.	Rt	Resp.	Calc. Conc.	мі	Final Conc.	Accuracy	Ratio	Mi
Blank	2.d	Sple	-	1		-			F				F
1ppb AF	3.4	Cal	L1	1	1	3.94	108.9	1.08871668	F	1.08871668	108.8717	77.33	F
3ppb AF	4.d	Cal	L2	1	3	3.94	259.6	2.94744485	F	2.94744485	98.24816	80.07	F
9ppb AF	5.d	Cal	L3	1	9	3.94	770	9.24524953	F	9.24524953	102.725	83.3	F
30ppb AF	6.d	Cal	L4	1	30	3.94	2544	31.1314605	F	31.1314605	103.7715	81.3	F
50ppb AF	7.d	Cal	L5	1	50	3.94	3885	47.6778258	F	47.6778258	95.35565	84.95	F
100ppb AF	8.d	Cal	L6	1	100	3.94	7711	94.8838315	F	94.8838315	94.88383	91.6	F
lppb AF	14.d	Cal	Ll	1	1	3.97	91.71	0.87610916	F	0.87610916	\$7.61092	78.78	F
3ppb AF	15.d	Cal	L2	1	3	3.93	265.7	3.02346479	F	3.02346479	100.7822	83.55	F
9ppb AF	16.d	Cal	L3	1	9	3.93	719.6	8.62358533	F	8.62358533	95.81761	101.4	F
30ppb AF	17.4	Cal	L4	1	30	3.94	2527	30.926837	F	30.926837	103.0895	87.02	F
50ppb AF	18.d	Cal	L5	1	50	3.93	4338	53.2685353	F	53.2685353	106.5371	83.33	
100ppb AF	19.d	Cal	L6	1	100	3.94	8312	102.306939	F	102.306939	102.3069	86.9	F
				1	1	1	1		1				

# Calibration table for Aflatoxin G2

### Calibration Curve for Aflatoxin G1 and G2





Figure 1: External standardization calibration curves A, B, C, D for Aflatoxin B1, B2,

G1 and G2









		AFLAT	OXIN G1	L 13.73 yg/kg		A	FLAT	DXIN G1	29.73 ug/	/k	
	4	Acquisitio	n Time (m	iin)	_	Acc	quisitio	n Time (m	in)		
	3.5	4	4.5	5	-0.5	3.5	4	4.5	5		
-0.2					0						
0.2											
0.4					0.5	~ <b>~</b>	~		- <u>-</u>		
0.6	m		1000	~~	- 1						
0.8					1.0					_	
1					15					-	
1.2					2					-	
1.4					2.5						
1.6											
18					- 3						
2.2					3.5						
2.4											
2.6					A-						
2.8					4.5						
3					0					-	
3.2					Ű -						
3.4					- 5		*4.30 r	nin.			
x IU = _		*4.30 1	nin.		x 10 <sup>_2</sup>						
v10 2					+ MRM (329.1 -> 243.1) 12.d						
MRM (329	1 -> 243	1) 14.d									

# Annex 4: Table 1. Comparative mean volume and standard deviation of ethanol from decontaminated and uncontaminated maize.

		MEAN (mL)			STD (mL)	
	WM	DM	GM		DM	GM
NaHSO3	26.32	30.95	31.18	5.75	4.11	4.05
C10H10O4	29.65	31.68	32.75	3.33	1.91	4.24
(NH4)2CO3	24.92	30.25	30.82	2.29	2.49	1.60
Na2CO3	24.62	31.85	29.92	3.32	2.72	1.30
NaOCl	28.02	31.02	29.25	2.45	2.02	2.10
СМ	34.82	34.38	35.68	3.21	3.92	1.79
NaHSO3 + H2O2	28.38	29.98	28.95	2.89	2.90	1.51
C10H10O4 +H2O2	27.72	29.25	25.72	2.35	1.65	2.32
(NH4)2C03 +H2O2	26.12	29.08	27.65	3.35	2.78	4.75
Na2CO3 + H2O2	25.48	28.32	25.85	2.48	2.97	3.97
NaOCl +H2O2	27.98	27.78	27.08	5.90	2.44	2.01
CM + H2O2	30.48	31.05	30.65	2.37	5.13	2.88
NaHSO3 +NH3	28.08	29.28	27.42	5.57	2.46	2.26
C10H10O4 +NH3	27.12	28.62	28.78	5.16	2.15	1.07
(NH4)2C03+NH3	29.68	28.08	27.88	1.12	2.95	1.16
Na2CO3 +NH3	29.22	28.22	26.92	1.33	2.06	3.99
NaOCl + NH3	28.45	29.52	27.45	2.69	3.69	1.59
CM + NH3	29.98	29.92	29.68	1.46	2.31	4.89
NaHSO3 + CH3NH2	27.42	29.55	28.82	2.93	2.65	2.40
C10H10O4 + CH3NH2	28.78	28.88	27.95	1.89	4.27	2.49
(NH4)2CO3+ CH3NH2	27.88	27.82	27.35	1.63	1.48	1.87
Na2CO3 + CH3NH2	26.92	29.18	26.72	2.11	1.45	1.65
NaOCl + CH3NH2	27.45	27.02	27.52	0.92	1.45	3.93
CM+ CH3NH2	29.68	28.25	28.88	3.79	3.68	3.13

#### Possible degradation pathways of aflatoxin B1



Orbitrap MS-MS spectra and proposed fragmentation (insets) of degradation products of AFB1 by HVACP.

Study Outcome

#### **Publications**

 Jacob, N. Mwenda., Shem O. Wandiga, David K. Kariuki and Vincent O. Madadi. (2022).
 Prevalence of aflatoxins in stored maize in Busia and Migori counties of Kenya, Africa Journal of Agricultural Physical Sciences, Volume (7), 72–88.

Jacob, N. Mwenda., Shem O. Wandiga, David K. Kariuki and Vincent O. Madadi. (2022).
 Occurrence and Prevalence of Aflatoxins Contamination in Stored Maize Grains from the Rift
 Valley, Kenya, Africa Journal of Agricultural Physical Sciences, Volume (7), 54–71

Jacob, N. Mwenda., Shem O. Wandiga, David K. Kariuki and Vincent O. Madadi. (2020).
 Occurrence and Distribution of Aflatoxin in Maize from Selected Counties, Eastern Region,
 Kenya. Journal of Agricultural Policy, 3(2), 7–20.

4. Nicholas M. Jacob, Shem O. Wandiga, David K. Kariuki and Vincent O. Madadi (2020).
Degradation of aflatoxin in maize using Ferulic acid (phydroxy-3-methyl cinnamic acid)
catalyzed by Hydrogen peroxide. International Journal of Food Science, Vol.3, Issue No.1, pp 1 - 17, 2020.

#### **Conference Presentation**

The 6-8th Annual International Scientific Research Conferences in 2018, 2019 and 2021

African Pesticide and Toxicology Network (Tanzania) (2020); Oral Presentation of a conference

paper

3. Monthly ecotoxicology conference in Germany in July 2021.