INFLUENCE OF DIFFERENT ALTITUDES MAIZE HARVEST SEASONS AND STORAGE AND PRE-STORAGE PRACTICES ON AFLATOXIN OCURRENCE AMONG HOUSEHOLDS IN MAKUENI COUNTY, KENYA

JAMES MWASHEMBE MALUSHA

DOCTOR OF PHILOSOPHY

(Public Health)

JOMO KENYATTA UNIVERSITY OF

AGRICULTURE AND TECHNOLOGY

2016

Influence of Different Altitudes Maize Harvest Seasons and Storage and Pre-Storage Practices on Aflatoxin Ocurrence among Households in Makueni County, Kenya

James Mwashembe Malusha

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Public Health in the Jomo Kenyatta University of Agriculture and Technology

2016

DECLARATION

This Thesis is my original work and has not been presented for a degree in any other University.

Signature…….. Date……

James Mwashembe Malusha

This Thesis has been submitted for examination with our approval as University supervisors.

Signature…….....Date……..

Prof. Mohamed Karama

KEMRI, Kenya

Signature……Date……

Prof. Anselimo O. Makokha

JKUAT, Kenya

DEDICATION

This work is dedicated to my parents Mr. and Mrs. Johnson Chombo, to my wife Peris Wughanga, and to my children Johnson Malusha, Gidnora Mkacharo and Salome Chanya.

ACKNOWLEDGEMENT

This research was made possible through support, assistance and guidance from various resource people and institutions. I therefore, in particular, wish to express special thanks to my supervisors Professor Mohammed Karama, Centre for Public Health Research of Kenya Medical Research Institute and Professor Anselimo Makokha, Department of Food Science and Technology of Jomo Kenyatta University of Agriculture and Technology for their valuable technical support and guidance throughout the entire process of undertaking this research. Further, I am very grateful to Graduate Course co-ordinator of Institute of Tropical Medicine and Infectious Diseases, Principal of College of Health Sciences and Director of Board of Postgraduate Studies of Jomo Kenyatta University of Agriculture and Technology for their useful support and guidance in this research as well as in the course work.

I am also very grateful to my research assistants Mary Somba, Martin Mutua, Elizabeth Kilemba, Jabeshi Muoki, Christopher Kasimbi, Ruth Mutua, Beatrice Wamba and Rosemary Mweke for their assistance particularly in data collection. I am equally thankful to all who participated in focus group discussions, and in interviews, in which without their cooperation this research will not have been realized. Many thanks are also extended to Mr. George Kimani of Bora Biotech Laboratory and University of Nairobi for laboratory analysis of maize samples.

Further, I am equally thankful to Jomo Kenyatta University of Agriculture and Technology for giving me admission to undertake this course, as well as permission to carry out this research and writing of thesis.

I am also greatly indebted to my employer, Ministry of Health for granting me course approval and giving me permission to undertake the study. In particular, i am

particularly grateful to Director of Public Health, Dr. Kepha Ombacho, for his encouragement.

Finally, I wish to thank all lecturers who guided me in my course work, and also to all who assisted me either directly or indirectly through out the research work as well as production of this thesis.

TABLE OF CONTENT

DECLARATION ..... **ii**

DEDICATION ..... **iii**

ACKNOWLEDGEMENT ..... **iv**

TABLE OF CONTENT ..... **vi**

LIST OF FIGURES ..... **xiv**

LIST OF TABLES ..... **xv**

LIST OF APPENDICES ..... **xvii**

ABBREVIATIONS AND ACRONYMS ..... **xix**

DEFINATION OF OPERATIONAL TERMS ..... **xxi**

ABSTRACT ..... **xxiii**

CHAPTER ONE **1**

INTRODUCTION **1**

1.1 Background 1

1.2 Problem Statement 4

1.3 Justification and benefits of the study 7

1.4 Research questions 10

1.5 Study objectives 11

1.5.1 Main Objective 11

1.5.2 Specific objectives 11

1.6 Hypotheses 12

1.7 Conceptual Framework 13

1.8 Variables Studied 14

1.8.1 Independent Variables 14

1.8.2 Intermediate Variables 16

1.8.3 Dependent Variables 16

CHAPTER TWO 17

LITERATURE REVIEW 17

2.1 Aflatoxins and their Causes 17

2.2 Effects of aflatoxins in human health 20

2.3 The burden of aflatoxin problem 23

2.4 Methods and techniques of aflatoxin detoxification 27

2.5 Different altitudes maize seasons and aflatoxin development 29

2.6 Control of aflatoxin 31

2.6.1 Pre-storage practices of maize 31

2.6.2 Storage practices of maize 34

2.6.3 Recent innovations in aflatoxin control at pre-harvest and post-harvest level 38

CHAPTER THREE 41

MATERIALS AND METHODS 41

3.1 Study design 41

3.2. Study sites 42

3.3 Study population 45

3.4 Inclusion criteria 45

3.5 Exclusion criteria 45

3.6 Sample size determination and sampling 46

3.6.1 Sample size determination 46

3.6.2 Sampling and study sites selection 47

3.6.2.1 Households selection and recruitment of study participants 48

3.6.2.2 Maize sampling 50

3.7 Data collection 50

3.7.1 Data collection tools 50

3.7.2 Maize sample analysis 52

3.7.2.1 Moisture content determination 53

3.7.2.2 Aflatoxin determination using Enzyme Linked Immunoassay (ELISA) test 53

3.7.2.3 Determination of aflatoxin using high performance liquid chromatography 56

3.8 Determination of environmental temperature and relative humidity 59

3.9 Data management and analysis 59

3.10 Limitations of study 60

3.11 Ethical considerations 61

CHAPTER FOUR` 62

RESULTS 62

4.1 Descriptive results for socio-economic and demographic characteristics and maize pre- storage and storage practices 62

4:1:1Socio-economic and demographic characteristics of respondents 62

4.1.1.1 Age and sex of study respondents 62

4.1.1.2 Marital status of respondents 62

4.1.1.3 Highest education levels of respondents 63

4.1.1.4 Religious affiliation of respondents 64

4.1.1.5 Main occupations of respondents 65

4.1.1.6 Income of households 66

4.1.2 Quantity of maize stored and utilized in households 68

4.1.2.1 Quantity of maize found in household store during sampling 68

4.1.2.2 Quantity of maize consumed in household 68

4.1.3 Knowledge and awareness of aflatoxin 68

4.1.3.1 Awareness levels 68

4.1.3.2 Source of information for knowledge/awareness of aflatoxin 69

4.1.3.3 Awareness of health problems associated with improperly stored maize 70

4.1.3.4 Knowledge on identification of maize suspected to be contaminated with aflatoxin 71

4.1.3.5 Knowledge of of signs of aflatoxin contamination in maize 71

4.1.3.6 Knowledge of causes of aflatoxin contamination of maize 72

4.1.3.7 Awareness on effect of altitude/climate variation of aflatoxin contamination 73

4.1.3.8 Awareness on effect of different maize harvest seasons on aflatoxin contamination 73

4.1.3.9 Knowledge on prevention of aflatoxin 73

4.1.4 Maize pre-storage practices 74

4.1.4.1 Removal of outer cover of maize cob during harvest and duration in days of maize in field before harvest 74

4.1.4.2 Methods used by households for drying of maize after harvest 75

4.1.4.3 Placing of Maize on top of any material underneath during drying 76

4.1.4.4 Reasons for drying Maize Properly 77

4.1.4.5 Cleaning of Maize Prior to Storage 78

4.1.5 Maize storage practices 79

4.1.5.1 Storage of maize in households 79

4.1.5.2 Proportion of households which stored maize properly 80

4.1.5. 3 Arrangement of maize stored in bags 80

4.1.5.4 Form in which maize is stored in household 81

4.1.5.5 Duration of maize in store and frequency of store cleaning 82

4.1.5.6 Insect pests affecting maize in storage 82

4.1.5.7 Conditions of maize stores 84

4.1.5.8 Reasons for storing maize properly 85

4.2 Maize sample analysis results for aflatoxin and physical parameters 86

4.2.3 Aflatoxin sub-types and quantities for maize harvested in first season 87

4.2.4 Maize physical parameters and aflatoxin levels for second season maize 88

4.2.5 Aflatoxin Sub-types and quantities for maize harvested in second season 89

4.2.6 Temperature and Humidity of Kibwezi and Kilome study sites 90

4.3 Comparison of maize pre-storage and storage practices, physical parameters aflatoxin levels and moisture content 90

4.3.1 Comparison of maize storage and pre-storage practices 90

4.3.2 Comparison of physical parameters of stored maize 93

4.3.3 Comparison of aflatoxin and moisture content of maize 94

4.3.4 Comparison of maize storage practices and aflatoxin positivity 95

4:4 Analytical results for associations and correlations among maize pre-storage and storage practices and aflatoxin occurrence 96

4.4.1 Associations of maize pre-storage practices with aflatoxin occurrence for first season maize 96

4.4.2 Associations of maize storage practices with aflatoxin occurrence for first season maize 96

4.4.3 Association of maize pre-storage practices with aflatoxin occurrence for second season maize 98

4.4.4 Association of maize storage practices with aflatoxin occurrence for second season maize 99

4.4.5 Association of knowledge/ awareness and moisture content with aflatoxin occurrence in maize 100

4.4.6 Association of pre-storage and storage practices with intermediate variables linked to Aflatoxin Occurrence in Maize 101

4.4.7 Focus group discussion results 101

CHAPTER FIVE 107

DISCUSSION 107

5:1 Household socio-demographic and economic characteristics 107

5.2 Knowledge and awareness of aflatoxin 109

5.3 Maize pre-storage and storage practices 113

5.3.1 Pre-storage practices 113

5.3.1.1 Pre-harvesting and harvesting practices 113

5.3.1.2 Drying of maize 115

5.3.2 Maize storage practices 118

5.4. Prevalence of aflatoxin occurrence in maize and maize physical conditions 124

5.4.1 Prevalence of aflatoxin occurrence in maize 124

5.4.2 Maize physical conditions 129

5.5 Comparison of aflatoxin prevalence and maize pre-storage and storage practices in different altitudes and maize harvest seasons 130

5.6 Maize pre-storage and storage factors associated with aflatoxin occurrence 132

5.6.1 Maize pre-storage practices associated aflatoxin occurrence 132

5.6.2 Maize storage practices associated with aflatoxin occurrence 133

5.7 Effects of temperature and humidity on aflatoxin occurrence in maize 136

CHAPTER SIX 139

CONCLUSIONS AND RECOMMENDATIONS 139

6.1 Conclusions 139

6.2 Recommendations 140

REFERENCES 143

APPENDICES 158

LIST OF FIGURES

Figure 1.1 Conceptual Framework of The Study 13
Figure 3.2 Study Design 42
Figure 3.3 Map Showing Study Sites in Makueni County 44
Figure 4.5 Main Occupations of Repondents 66
Figure 4.6 Methods Used by Households to Dry Maize after Harvest 76
Figure 4.7 Arrangement of Bags of Maize in Store 81

LIST OF TABLES

Table 4.1 Marital Status of Respondents 63
Table 4.2 Religious Affiliations of Respondents 65
Table 4.3 Income of Households 67
Table 4.4 Sources of Information for Knowledge/Awareness of Aflatoxin 69
Table 4.5 Awareness of Perceived Health Problems Associated with Improperly Stored Maize 70
Table 4.6 Knowledge on Signs of Aflatoxin Contamination of Maize 71
Table 4.7 Knowledge of Causes of Aflatoxin Contamination of Maize 72
Table 4.8 Knowledge on Prevention of Aflatoxin 74
Table 4.9 Reasons for Placing Material Underneath During Drying of Maize 77
Table 4.10 Reasons for Drying Maize Properly 78
Table 4.11 Types of Maize Storage Practices of Maize in Households 79
Table 4.12 Form in which Maize was Stored in Households 82
Table 4.13 Action Taken to Remove Insect Pests Affecting Maize in Household 83
Table 4.14 Conditions of Maize Stores 84
Table 4.15 Reasons for Storing Maize in well Ventilated Clean Store 85
Table 4.16 Physical Parameters and Aflatoxin Level for first Season Maize 86
Table 4.17 Aflatoxin Sub-Types and Quantities for first Season 87
Table 4.18 Physical Parameters and Aflatoxin Level for Second Season Maize 88
Table 4.19 Aflatoxin Sub-Types and Quantities for Second Season Maize in Kibwezi .. 89
Table 4.20 Comparison of Maize Storage and Pre-Storage Practices between Kibwezi and Kilome for first and second Season Maize 92
Table 4.21 Comparison of Physical Parameters of Maize 93
Table 4.22 Comparison of Aflatoxin Levels between Kibwezi and Kilome for first and Second Season Maize Harvest 94

Table 4.23 Maize Storage Practice and Aflatoxin Positivity 95
Table 4.24 Association of Maize Storage Practices with Aflatoxin Occurrence for first Season 97
Table 4.25 Association of Maize Pre-Storage Practices with Aflatoxin Occurrence for Second Season Maize 99
Table 4.26 Association of Maize Storage Practices with Aflatoxin Occurrence for Second Season 100
Table 4.27 Kibwezi Focus Group Discussion Results 103
Table 4.28 Kilome Focus Group Results 105

LIST OF APPENDICES

Appendix 1: Informed consent form in english language 158
Appendix 2: Informed consent translated into kikamba language 162
Appendix 3: Questionaire for house holds in english language 166
Appendix 4: Questionaire for house holds in kikamba language 177
Appendix 5: Observation checklist for maize storage 184
Appendix 6: Form for collection of maize sample for aflatoxin analysis 186
Appendix 7: Form for collection of data on temperature and humidity 187
Appendix 8: Focus group discussion guide for commnity informants in english language 188
Appendix 9: Focus group discussion guide translated into kikamba language 189
Appendix 10: In-depth interview schedule for agricultural and public health officials . 190
Appendix 11: Kibwezi ELISA test results and physical conditions for maize harvested in first season 191
Appendix 12: Kilome elisa test results and physical conditions for maize harvested in first season 193
Appendix 13: Kibwezi elisa test results and physical conditions for maize harvested in second season 195
Appendix 14: Kilome elisa test results and physical conditions for maize harvested in second season 197
Appendix 15: Certificate of training on protecting human research subjects 199
Appendix 16: Kemri scientific steering committee study approval 200
Appendix 17: Kemri ethical review committee study approval 201
Appendix 18: Research authorization from national council for science and technology 202
Appendix 19: Approval of ph.d study proposal and supervisors 203
Appendix 20: Local household maize crib 204

Appendix 21: Improved household maize crib 205
Appendix 22: Map of makueni county 206
Appendix 23: Cover page and abstract of published scientific paper on east african medical journal march 2015 issue 207
Appendix 24: Cover page and abstract for published scientific paper on east african medical journal may 2015 ssue 209

ABBREVIATIONS AND ACRONYMS

AFB1	Aflatoxin type B1
AF B2	Aflatoxin type B2
AFG1	Aflatoxin type G1
AFG2	Aflatoxin type G2
AOAC	Association of Analytical Chemists
CAST	Council for Agriculture Science and Technology
CDC	Centre for Disease Control
CI	Confidence Interval
ELISA	Enzyme Linked Immunosorbent assay
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FSRIO	Food Safety Research Information Office
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
HAA	High altitude Area
HL	High Lands
KEBS	Kenya bureau of standards

KBS	Kenya Bureau of Statistics
KBZ	Kibwezi
KLM	Kilome
LAA	Low altitude Area
LL	Low Lands
MPND	Ministry of Planning and National Development
NCPB	National Cereal and Produce Board
NGO	Non Governmental Organization
PPb	Parts per billion
SDA	Seventh Day Adventist
SPSS	Statistical Package for Social Sciences
µg/Kg)	Micrograms per Kilogram

DEFINATION OF OPERATIONAL TERMS

Aflatoxins They are secondary metabolites from a number of mould of The *Aspergillus* family which include *Aspergillus flavus and Aspergillus parasticus*

Aflatoxin Contamination Presence of aflatoxin in maize as determined by analysis

Aflatoxin Development Production of mycotoxins in maize known as aflatoxins by fungi of *Aspergillus* species.

Aflatoxin Occurrence Presence of aflatoxin in maize produced by fungi of *Aspergillus* species

Intermediate Variable A variable lying between independent variable and dependent variable and capable of being influenced by independent variable as well as influencing dependent variable

Storage Practices Keeping of maize in any place, structure or facility within the household for later use for human consumption.

Pre-Storage Practices Pre-harvest, harvest and post harvest activities or actions undertaken to maize prior to storing for later use for human consumption.

First Season Maize Harvest Maize grown during long rains of April/May and harvested in August/September also referred to as August/September maize harvest season

Second Season Maize Harvest Maize grown during short rains of October/November and harvested in February/March also referred to as February/March maize harvest season

Low Altitude Area/Land located below 920 Metres above sea level also referred to as Lowlands, in this study represented by Kibwezi study site

High Altitude Area/Land located above 1700 Metres above sea level also referred to as High lands, in this study represented by Kilome study site

Household Head The main provider for the household and someone who is familiar with all activities and household members, and could be male or female

Mature Maize Maize which has attained physiological maturiry, ready for harvest

ABSTRACT

Aflatoxicosis resulting from consumption of highly contaminated maize poses a challenge to public health as outbreaks have occurred in a number of countries including Kenya. It is caused by aflatoxins which are secondary metabolites from mould of the *Aspergillus* species that include among others *Aspergillus parasiticus* and *Aspergillus flavus*. Fungal spoilage and aflatoxin contamination have been known to be of major concern in cereals and other foodstuffs including maize. The main objective of the study was to determine the influence of maize storage and pre-storage practices on aflatoxin occurrence among maize stored in households in Makueni County which had previously experienced aflatoxicosis outbreaks. Aflatoxin levels of maize harvested in different altitudes and different seasons were also compared. The design of the study, which was conducted in Kibwezi and Kilome sub-counties of Makueni County, was comparative analytical study. Two different sites were selected for this study in which 240 households from each site were enrolled in the study. Data was collected using questionnares, observation checklists, and focus group discussions. Maize samples were collected from sub-sampled households for analysis of aflatoxin. Results showed that maize harvested in first season had higher moisture content (12.9%) and aflatoxin positivity (25.0%) in lower altitude area than higher altitude area which had 12.8% and 4.2%, respectively. Maize harvested in second season had higher moiture content (13.6%) and aflatoxin positivity (33.3%)) in lower altitude area than maize harvested in higher altitude area which had 13.5% and 12.5%, respectively. Maize harvested in second season had higher moiture content and aflatoxin positivity than maize harvested in first season. The most common sub-types of aflatoxin affecting maize were AFB1 and AFB2. Over 70% of households' stored their maize in raised wooden platforms with some storing in traditional cribs. Storage of maize in bags directly on the floor had higher aflatoxin positivity in low altitude area at 33.3% and 37.5% for first and second

season, respectively. Certain maize pre-storage practices such as length of stay of maize in field before harvest and duration of drying were found to be associated with aspects of aflatoxin contamination of maize ($P<0.05$). Similarly, maize storage practices including storage time, proper storage, frequency of store cleaning were associated with mould and insect pests infestation, discolouration and aflatoxin occurrence in maize ($P<0.05$). Moisture content was negatively correlated with aflatoxin occurrence. The results of this study will help in development of better policies and strategies of reducing aflatoxin contamination of maize and resultant aflatoxicosis. As maize consumers are likely to be exposed to risk of aflatoxicosis, there is need for government authorities to regularly monitor levels of aflatoxin contamination of household maize for timely intervention should afatoxin exceed permissible levels of 10 ug/kg set by regulatory authorities. Further research is recommended to determine the effects of household specific storage and pre-storage practices on aflatoxin contamination of maize in other different regions and seasons.

CHAPTER ONE
INTRODUCTION

1.1 Background

Aflatoxicosis resulting from consumption of contaminated maize poses a significant public health problem in many countries including Kenya. Aflatoxins are secondary metabolites from mould of the *Aspergillus* family that include among others *Aspergillus parasiticus* and *Aspergillus flavus*. Aflatoxins are also of various types which include Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2, out of which B1 is the most common, most toxic and the most potent in terms of causing liver cancer in humans (Yau, 2012). Fungal spoilage and aflatoxin contamination have been known to be of major concern in cereals and other foodstuffs.

Although aflatoxin occurrence in maize can be minimized by sound agricultural, pre-harvest, harvest, drying and storage practices since aflatoxins can develop rapidly in fungal infected maize, previous studies revealed that aflatoxicosis was acquired from eating contaminated maize attributed to improper harvesting, drying and storage of maize (Nyikal *et al.*, 2004; Lewis *et. al*., 2005). In addition, despite infrastructure and grain storage and pre-storage practices being able to prevent post-harvest development of mycotoxins in developed countries, it has been noted that this aspect still remains a threat in developing countries, especially in tropical areas (FSRIO, 2009).

A previous study found out that varying sources of maize, inadequate households with homegrown maize as well as different storage practices of maize were main challenge to effective storage practices (Hell *et.al*., 1995). Further this study indicated that most maize found in household was purchased and not home grown thus making it difficult to determine relationship between storage practices and aflatoxin levels.

More over, although various factors have been implicated in causation of contamination of maize with aflatoxin, maize storage and pre-storage practices and their association with aflatoxin contamination have not been properly explored and documented. In addition comparative analysis of prevalence of aflatoxin contamination of maize

harvested and stored in different altitude areas as well as maize harvested and stored in different seasons have also not been properly explored and documented. These aspects when properly explored could help authorities in developing appropriate aflatoxin control and prevention strategies.

Consumption of maize contaminated with aflatoxin poses a serious challenge to public health. The aflatoxin problem was first recognized in 1960, during severe outbreak of disease which was referred as "Turkey 'X' disease in UK, in which 100,000 turkey poults died (Reddy *et. al.*,2000). Since then a number of cases have been reported in different parts of the world. Food and Agriculture Organisation (FAO) estimates that 25% of the world's food crops are affected by mycotoxins, particularly aflatoxins (Food Safety Research Information Office, 2009). It is estimated that 4.5 billion people living in developing countries could be chronically exposed to aflatoxin through their diet (CDC, 2014). Children have been found to be most sensitive to food contaminated with aflatoxins (Hell *et al*., 2007), and maize consumption is considered to be an important source of aflatoxin exposure to children (Egal *et al*.,2005). In Kenya acute aflatoxin poisoning is estimated to cause liver failure and death in up to 40% of cases of aflatoxicosis.

Outbreaks associated with acute aflatoxicosis from consumption of highly contaminated maize have occurred in a number of countries including Kenya, India, and Thailand [Council for Agriculture Science and Technology (CAST) 2003]. In Kenya maize contaminated with aflatoxins has been implicated in deadly outbreaks three times since 1981 (Nyikal *et al*., 2004; Lewis *et al*., 2005; Klich, 2007).

The eastern region of Kenya, particularly Makueni, has been mostly affected by aflatoxicosis outbreaks resulting from consumption of maize contaminated with aflatoxins. There have been three major outbreaks in this region since 1981 to date (CDC, 2004; CDC, 2014; Nyikal., *et al*.,2004 ; Lewis *et al*.,2005; Klich, 2007). The

outbreaks, especially the 2004 which was the most severe, caused significant human mortality and morbidity resulting in 317 cases and 125 deaths (CDC, 2004) as well as causing widespread socio-economic impact (Lewis *et al*., 2005; Walker, *et.al.*,2013). Epidemiologic investigations conducted during that time revealed that the 2004 outbreak was the result of aflatoxin poisoning from ingestion of contaminated maize (CDC) 2004). Other aflatoxicosis outbreaks that had occurred previously in the area, were also acquired from eating contaminated maize attributed to improper drying and storage (Ngindu *et al*. 1982; Nyikal *et al.,* 2004; Lewis *et.al*., 2005).

Most of the affected local population engaged in small-scale, subsistence mixed farming that included some livestock with maize being the primary dietary staple and the main crop produced. This outbreak affected more than seven districts mainly in eastern and central regions. Out of the 317 cases, 89% resided in four Counties (Makueni, Kitui, Machakos and Kiambu. Of the four counties, Makueni and Kitui were most heavily affected (representing 47% and 32% of cases, respectively, followed by Machakos (6 %of cases) and Thika (4% of cases) (CDC 2004). The outbreak also resulted in significant mortality among livestock as well as widespread socio-economic impact (Lewis *et al*., 2005)

Overall, the affected area had a rural population that is primarily from the indigenous community. Maize is the primary dietary staple and the main food crop produced, and at harvest, farmers store most of their maize for household consumption and sell the rest to meet other household needs.

Aflatoxin occurrence in maize can be minimized by sound agricultural, pre-harvest, harvest, drying and storage practices since aflatoxins can develop rapidly in fungal infected maize. Previous studies had revealed that aflatoxicosis was acquired from eating contaminated maize suspected to be attributed to improper harvesting, drying and storage of maize (Nyikal *et al.,* 2004; Lewis *et. al*., 2005).

In an effort to limit human exposure to aflatoxin contaminated maize, regulatory authorities have set limits of aflatoxin contamination in foods including maize to 10ppb in order to control aflatoxicosis which is caused by aflatoxin poisoning (KEBS, 1988; FDA, 1997). Put in another way, aflatoxin contaminated grains above 10pbb are rendered unsuitable for human consumption (Songa *et. al.*, .2010). Besides causing ill health, aflatoxin together with damage caused by insect pests as a result of poor storage causes significant grain loses thus adversely affecting food security. It is estimated that post harvest loses account for about 30% of stored grains (Bett *et. al*., 2007; Songa *et.al*. 2010). Aflatoxins have also been known to compromise food security in many vulnerable groups of people in some countries in Africa (Kell *et al.,* 2007*).* It thus reduces food availability leading to famine and malnutrition.

In addition to maize being a staple food, it is one of the cereals which has been found to be most susceptible to aflatoxin contamination (Wilson *et al.* 2006). Further, maize being highly consumed in Africa, ranging from 85kg/per year per person to 105 kg/year per person and coupled with elevated aflatoxin levels, could lead to high aflatoxin risks (FAO, 2005; Hell *et. al.,* 2007).

This study was therefore carried out with the main objective of determining the

influence of different altitudes, harvest seasons and storage and pre-storage practices of maize among households on aflatoxin occurrence in Makueni County. The design of this study, which was conducted in Kibwezi and Kilome sub-counties of Makueni County, was comparative analytical study.

1.2 Problem Statement

Despite numerous efforts to control aflatoxin, aflatoxin still remains a worldwide threat to public health as it continues to affect many countries including Kenya. It is estimated that about 155,000 people worldwide have been affected by liver cancer associated with

consumption of aflatoxin contaminated products, and out of this affected population 40% occur in Africa (Emitati *et al*., 2013). The extent of aflatoxin contamination in food is postulated to be high in developing countries particularly sub-saharan Africa (AATF, 2014). In Kenya the eastern parts such as Makueni, Kitui and Machakos counties have been affected by outbreaks of aflatoxicosis on several occasions. Makueni which was the area for this study has experienced the most severe outbreaks of aflatoxicosis since 1981, with the latest outbreak in 2004 affecting 317 people and claiming 125 lives (CDC, 2004; Klich, 2007).

The risk of aflatoxicosis is still potentially imminent given that it is estimated 25% of food crops are affected by mycotoxins, particularly aflatoxins (Food Safety Research Information Office, 2009; Smith *et al*., 1994). In addition, aflatoxins and other mycotoxins are considered to be the main source of morbidity and mortality in Africa, Asia and Latin America (Smith *et al.,* 1994). Thus, some population could be exposed to unacceptable levels of aflatoxin throughtout their lives including prenatal exposure of the fetus and the consequences may have been inadequately addressed (AATF, 2014). This shows that aflatoxin is still a major problem and a potential burden to people living in risk areas and relying on maize as staple food.

Consumption of maize contaminated with aflatoxin could affect human health with consequent several adverse health effects resulting in suffering and loss of life. These adverse health effects may be acute or chronic depending on the dose and duration of aflatoxin exposure (Bhat *et.al.,* 1997; Groopman *et al*., 2008; Kuniholm *et al*., 2008). The acute form of aflatoxicosis is characterized by vomiting, abdominal pain, pulmonary edema, convulsions, coma and cerebral edema, among other symptoms. While the chronic form resulting from prolonged exposure to aflatoxin is characterized by hepatotoxicity, immune suppression, stunted growth and underweight in children (Gong *et. al*., 2003). Aflatoxin could put babies also at risk through their mother's breast milk,

and in young children aflatoxins can cause poor cognitive development and greater susceptibility to infectious diseases (PAEPARD, 2015). Moreover, aflatoxins have been found to be among the most potent carcinogens and have particularly been implicated as the cause of primary liver cancer known as hepatocellular carcinoma (Food Safety Research Information Office, 2009).

In addition to causing ill health, aflatoxin and pests also cause considerable post-harvest losses. These post-harvest losses attributed to pests and other hazards are estimated to about 30%, while maize post-harvest losses in the tropics mainly due to pests have been estimated to about 20% (Likhayo *et al*., 2004; Bett *et al*., 2007). These post-harvest losses are enormous leading to food shortages and adversely affecting food security. Inadequate and poor post-harvest storage is one of the contributors to food insecurity. Besides post-harvest losses, aflatoxin lowers the nutrition value of food thus affecting its nutritional status of consumers (Mboya *et al*., 2011). It also negatively affects palatability of meal made from maize (Mboya *et al*., 2011). Furthermore, the long-term impact of aflatoxin exposure is enormous given that infants are exposed to aflatoxins through their diet of complementary foods (Shabani *et al*., 2015). In addition, people in Africa are exposed to aflatoxins before birth and throughout their lives with serious effects on their health (Williams *et al*., 2004).

Despite several studies having previously been done, there is still inadequate documented information to enable implementation of appropriate preventive interventions and strategies at household level. Particularly, althougth the impact of aflatoxin contamination can be reduced by improving community household maize storage and pre-storage practices, the extent of influence of these practices on aflatoxin contamination is not well documented, particularly under the Kenyan practices in the affected areas. Further it had been observed that information about other risk factors associated with outbreaks of aflatoxicosis is limited (Edurado *et al*., 2004; Unger *et al.*

1997). Specifically there is very limited documentation of maize storage and pre-storage practices and their association with aflatoxin occurrence at household level in Kenya, and particularly Makueni which is mostly affected by aflatoxin problem.

1.3 Justification and benefits of the study

The recurrent perennial problem of aflatoxicosis in Makueni coupled with inadequate documentation regarding aflatoxin control and prevention, call for more studies to be done including study on influence of maize storage and pre-storage practices on aflatoxin occurrence in different regions and harvest seasons. In addition, the persistent high level of aflatoxin contamination in maize and dietary exposure to infants and children as well as to adults is still of great concern (Shabani *et al*., 2015, Food safety Research Information, 2009). Furthermore, the suffering and loss of life as well as food and other economic losses attributed to aflatoxin neccessitate seeking solutions to this perennial aflatoxin problem. Even though considerable research efforts have been made over the years to prevent or reduce aflatoxin contamination in maize, aflatoxin control still remains a challenge. A study done in Makueni associated aflatoxicosis with eating homegrown maize stored improperly in damp conditions (Lewis *et al*., 2005). This suggested the need for a study on home grown maize storage practices and aflatoxin occurrence.

Further, while various technologies have been developed to reduce post-harvest and pre-harvest losses, their effectiveness in reducing aflatoxin contamination in maize and exposure in humans is still poorly understood (PAEPARD, 2015). In addition, despite various efforts to improve maize storage and pre-storage practices, aflatoxin continued to be a problem requiring more investigations. This could lead to further understanding of aflatoxin levels and and their association with maize storage and pre-storage practices in the household level which is important as it will go along way in addressing aflatoxin problem.

In particular, Makueni County having been the epicenter of aflatoxicosis owing to severe previous outbreaks which had occurred in the area requires further studies for evidenced based interventions. Moreover, knowing the current level of aflatoxin problem is important for appreciation of risk posed by consumption of aflatoxin contaminated maize and this may trigger scaling up of adoption of preventive and control measures.

Although several studies have been done with the aim of finding ways and means of reducing aflatoxin contamination of maize in Makueni, aflatoxin is still a problem and very few studies have been done with none specifically documenting household maize storage and pre-storage practices and their influence on aflatoxin occurrence. In addition no study had been done comparing aflatoxin levels in different geographical zones as well as in different maize harvest seasons taking into account different climatic conditions, despite the area having had the greatest burden of aflatoxicosis outbreaks. In addition factors relating to aflatoxin contamination of maize have not been adequately documented.

Indeed, some previous studies have recommended further studies to investigate risks associated with homegrown maize with the aim of informing development of culturally and locally appropriate strategies for prevention of aflatoxicosis (Eduardo *et. al*., 2005; Mwihia *et.al*. 2008). Mwihia *et al*. (2008) also recommended further study for other maize harvest seasons to determine their effects on aflatoxin contamination.

Hell *et al*. (2010) also recommended investigations on effects of different pre-and post harvest crop management practices on aflatoxin contamination in different agro-ecologies in Africa so that practices that result in a significant reduction in aflatoxin levels could be promoted.

Furthermore, there has been limited awareness on aflatoxin risks at all levels as well as insufficient knowledge on options to reduce aflatoxin coatamination (Hell *et al*., 2010). Thus, given the inadequate documentation of aflatoxicosis prevention information, and aflatoxicosis still remains a problem in Makueni, there was need for this study to be undertaken in this area. The study, which was done in two different geographical zones and comparing aflatoxin occurrence in different maize seasons, identifyed and compared maize storage and pre-storage practices, and associated these practices with aflatoxin occurrence levels. In the process it illuminated certain aspects, which may not have been clearly understood regarding household maize storage and pre-storage practices and their association with aflatoxin occurrence. This contributed to identifying positive practices to be promoted and negative practices to be discouraged.

The findings of this study will further aid in understanding the extent and current situation of aflatoxin occurrence in the area. This could greatly contribute to better understanding of the causes and prevention of aflatoxin among the community and hence promote better maize storage and pre-storage practices. The study could further contribute to identification of high risk areas and seasons of aflatoxin occurrence and thus enable early interventions for its control and prevention. This, coupled with other strategies, will go along way in alleviating the burden of aflatoxin in the community.

The expected impact outccomes out of implementation findings of this study include raising level of awareness which could translate into reduced aflatoxin contamination in stored maize through adoption of improved storage and pre-storage practices which in the long term could lead to reduced exposure of aflatoxin to people particularly children, women and the poor who are more vulnerable and at higher risk. In essence, besides preventing aflatoxicosis outbreaks the findings of the study go along way in reducing long-term exposure to aflatoxins.

The findings of the study which will be disseminated and shared with local community, policy makers and implementers concerned with improvement of food safety and quality as well as prevention of aflatoxin contamination of maize, will increase awareness on aflatoxin prevention. Thus, the findings will be useful to policy makers in government and non governmental organisations for developing appropriate strategic interventions in afflatoxin control/prevention taking into account geographical, seasonality and socio-cultural context of the area. This will go a long way in preventing ill health as well as reducing maize spoilage which is a key issue in food security.

At the county level, the results will be expected to assist in development of aflatoxin control plans in order to control and prevent outbreaks of aflatoxicosis, taking into account socio-cultural situation. This could greatly contribute towards the Government target of scaling up aflatoxin control and prevention.

At the community, households are expected to benefit from adopting improved methods/practices of maize harvesting, drying and storage which could result in reduced morbidity and mortality attributed to consumption of aflatoxin contaminated maize as well as reducing food losses, and thus contribute to improvement in their health as well as their socio-economic status.

1.4 Research questions

1. What are storage and pre-storage practices of homegrown maize in Kibwezi and Kilome sub-counties of Makueni County?

2. What is the prevalence of aflatoxin occurrence in maize in two different altitudes in the two study areas?

3. What is the difference in aflatoxin levels in maize between two consequent maize harvest seasons in the two study areas?

4. Which storage and pre-storage practices are associated with aflatoxin occurrence in the two study areas?

1.5 Study objectives

1.5.1 Main Objective

To determine the influence of different altitudes, harvest seasons and storage and pre-storage practices of maize among households on aflatoxin contamination in Kibwezi and Kilome sub-counties of Makueni County.

1.5.2 Specific objectives

The study had the following specific objectives:

1. To determine storage and pre-storage practices of household maize in Kibwezi and Kilome sub-counties of Makueni County by sampling households and collecting data using questionnaires and observation checklists.

2. To determine and compare the prevalence of aflatoxin contamination of maize at two different altitudes in the two study areas by collecting maize samples and analyzing them for aflatoxin levels and subtypes.

3. To determine and compare aflatoxin levels in maize harvested in two consecutive harvest seasons in the two study areas by collecting maize samples and analyzing them for aflatoxin levels.

4. To determine association between maize storage and pre-storage practices and aflatoxin occurrence in the two study areas by analyzing and determing relationships among identified practices and aflatoxin occurrence in maize.

1.6 Hypotheses

Null Hypotheses

HO: 1 There is no difference in aflatoxin occurrence in maize between the two study areas.

HO: 2 There is no difference in aflatoxin occurrence between two consecutive maize harvest seasons.

HO: 3 There is no difference in aflatoxin occurrence among the different maize storage and pre-storage practices in households in the two study areas.

HO: 4 There is no association between maize storage and pre-storage practices and aflatoxin occurrence in maize in the two study areas.

1.7 Conceptual Framework

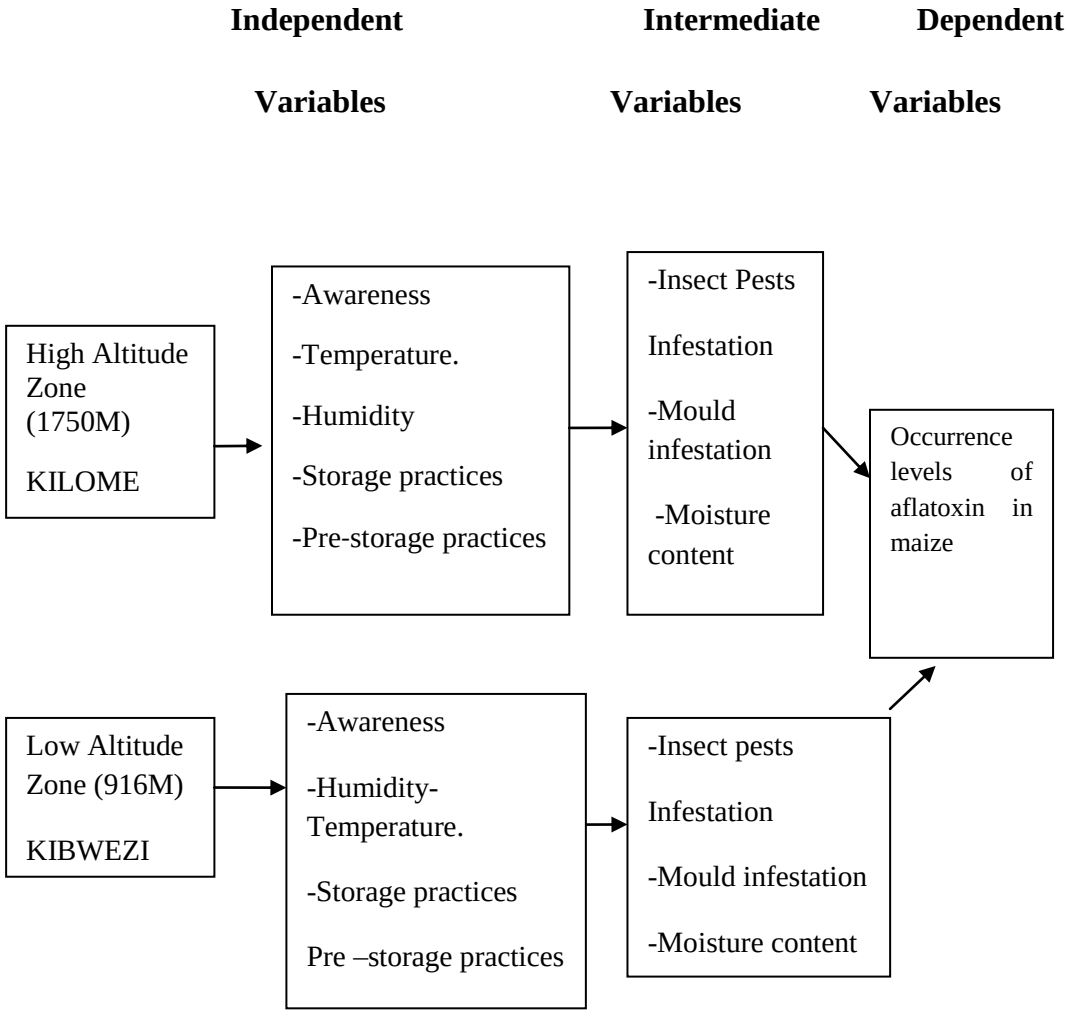


Figure 1.1 Conceptual framework of the study

Factors such as temperature and humidity, maize pre-storage practices (pre-harvest, harvest, post-harvest) and storage practices affect insect infestation and fungi growth, and subsequent aflatoxin occurrence in maize.

1.8 Variables Studied

1.8.1 Independent Variables

The independent variables include:

1) Climatic variables- temperature and humidity.

2) Pre-storage practices- duration of stay of maize in the field before harvest after maturity, and whether de-husked or not de-husked during harvest, duration of drying before storage in days, method of drying, facilities used for drying and whether maize is cleaned prior to storage, and

3) Storage practices- type of storage system/structure used, storage form, storage period in days, conditions of the store.

Other independent variables also studied included the following socio-cultural variables because they were thought to have some aspects which might influence uptake of maize storage and pre-storage practices:

Age: Age determines maturity of a person and quite often it is believed to influence decision making including issues regarding health. In this study age of respondent was postulated to influence undertstanding of aflatoxin and its prevention either negatively or positively. This variable was measured by asking respondents to state their age.

Education level: Education increases general knowledge and awareness and is believed to influence health behaviour of a person. In this study level of education of respondent was postulated to influence undertstanding of aflatoxin and its prevention either negatively or positively. This variable was measured by asking respondents to state the highest level of education attained.

Knowledge/awareness of aflatoxin: Knowledge/awareness of a condition is believed to influence its adoption and practice. In this study knowledge/wareness of aflatoxin of respondent was postulated to influence its prevention either negatively or positively. This variable was measured by asking respondents whether they were aware of aflatoxin.

Marital status: Parents have joint responsibility of giving care to their children. They can also pool their resources and efforts together for the good of their children. This variable was measured by asking respondents their marital status.

Occupation: Occupation often constitutes source of income and different occupations have different incomes. It might also influence understanding on certain issues and hence influence behavior. In this study occupation of respondent was postulated to influence undertstanding of aflatoxin and its prevention either negatively or positively. This variable was measured by asking respondents what they did to earn a living.

Income: As storage practices have cost implications, it is expected that income in a household will have a bearing on pre-storage and storage practices. In this study income of household was postulated to influence adoption of pre-storage and storage practices for aflatoxin prevention. This variable was measured by asking respondents to state their total house hold income in a month.

Household size: The size of household has economic implications depending on the number of dependants in the household. Larger households require more income than smaller households for maintenance and other needs. This variable was measured by asking respondents the number of people residing in the house and sharing meals and other needs.

Quantity of maize consumed in Household: The respondents were asked to estimate the quantity of maize or maize products (e.g. muthokoi, ugali, Githeri, uji etc.) they consumed per day as an individual. The purpose of this question was to get a clue on the likelihood of exposure risk of aflatoxicosis.

1.8.2 Intermediate Variables

These are variables which were thought to enhance chances of occurrence of aflatoxin in maize (dependent variable), and therefore they are predisposing factors to aflatoxin production. They included insect pests' infestation, mould infestation and moisture content.

1.8.3 Dependent Variables

The dependent variable included aflatoxin occurrence in maize. This was measured by randomly collecting samples of homegrown maize and analyzing them for aflatoxin levels.

CHAPTER TWO
LITERATURE REVIEW

2.1 Aflatoxins and their Causes

Aflatoxins which are natural toxins, are produced by *Aspergillus* fungi species mainly *Aspergillus flavus* and *Aspergillus parasticus* that grow on a wide variety of grains and nuts (Patten, 1981). They are a group of mycotoxins, fungal metabolites, which contaminate agricultural products and threaten food safety as well as result in food losses. The other groups of mycotoxins of public health importance being fumonisins and ochratoxins produced mainly by *Fusarium* and *Penicillium* species, respectively. *Aspergillus* species as well as *Fusarium* and *Penicillium* species have been identified as the most important fungi that attack stored maize, and have been associated with production of mycotoxins that cause serious health problems to both humans and animals (Mboya *et al*., 2011; Montes *et al.,* 2009). Aflatoxins though mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus*, can also be produced by *Aspergillus nomius* and *Aspergillus Niger* (Cornell University, 2014). The aflatoxin producing fungus was identified as *Aspergillus flavus* in 1961 following outbreak of "Turkey X disease" in 1960, and was given the name Aflatoxin by virtue of it having originated from Aspergillus flavus (Cornell University, 2014).

There are different types of naturally occurring afatoxins which include aflatoxin B1, aflatoxin B2, aflatoxin G1, and aflatoxin G2. Among these aflatoxins, aflatoxin B1 is the most common, the most toxic and most potent in terms of causing liver cancer in humans, followed by aflatoxin G1 (Akowuah *et al.*, 2015). The toxicities of B2 and G2 are relatively weak. Thus, Aflatoxins in order of decreasing strength in terms of causing cell mutations are B1, G1, B2, and G2. Aflatoxins M1 and M2 are mostly found in milk and milk products (Arthur Yau, 2012), and they are produced when cattle feed on

commodities contaminated with aflatoxin B1 or B2 (Emitati *et al.*, 2013). Aflatoxin B2 and G2 were established as the dihydroxy derivatives of B1 and G1, respectively, and M1 is 4-hydoxy aflatoxin B1 and M2 is 4- dihydroxy aflatoxin B2 (Cornnel University, 2014). Aflatoxin B1 and B2 resulted from the exhibition of blue florescence under UV-light and their molecular formulas as established from elementary analysis and spectrometric determination are C17 H12 O6 and C17 H14 O6 respectively. Aflatoxin G1 and G2 exhibits yellow-green fluorescence and their molecular formulas are C17 H12 O7 and C17 H14 O7 respectively (Cornell University, 2014).

It has been found that the occurrence of aflatoxins is influenced by certain environmental factors such as temperature and humidity (Cornell University, 2014). The contamination will therefore vary with geographic location and agricultural practices, and the susceptibility of commodities to fungal invasion during pre-harvest, drying and storage, as well as during processing (Cornnel University, 2014). The mycotoxins of greatest concern in maize are aflatoxins, deoxynivalenol, fumonisins and zearalenone, although these are not the only ones that occur in maize (Channaiah *et al*., 2014).

However, aflatoxin is the most common mycotoxin known to occur in maize (Channaiah *et al*., 2014). It is a potentially deadly carcinogenic, but odouless, tasteless and colourless, its recognition relying mainly on signs of contamination and chemical analysis (ICIPE Kenya., 2014). Since aflatoxins cannot be seen by the naked eye, infected maize may look normal and this makes it difficult to tell if maize is infected (Hosney, 2015). However, while aflatoxin itself is invisible and tasteless, its presence may be correlated with other attributes resulting from fungal growth which include physical damage of outer protective coat of kernel, discoloration, and change of taste quality (Hoffman *et al*., 2013). Aflatoxin contamination can occur in various stages of maize production including when they are maturing in the field, during harvest, drying, transportation and storage (Wilson *et al*., 1992).

However, contamination is more likely to occur during post-harvest stage if not done properly to minimize fungal invasion and growth (Mwihia *et al*., 2008).

The two important mycotoxigenic fungi mostly found associated with stored maize are *Aspergillus flavus* that produces aflatoxins (Hell *et al.,* 1995) and *Fusarium verticillioides* (previously known as *F*. *moniliforme* , which produces fumonisins (Marasas *et al*., 1995). The fungus can be recognized by a gray-green or yellow-green mold growing on corn kernels in the field or in storage (Summer *et. al*.,2009). This aspect of awareness on recognition, if properly disseminated, can help in early detection of mould contaminated maize during harvest and storage.

The development of fungi and production of aflatoxin requires favorable conditions to be in place. The favorable optimum conditions for *Aspergilus growth* and aflatoxin development are: Temperature 30 degrees centigrade (Range 26.7-43.3), Relative humidity 85 %(Range 62-99%), Kernel moisture 18% (Range 13-20%) (Summer *et al.* 2009). However, Milani, (2013), specifically puts optimum conditions for aflatoxin production at 33^{o}C and water activity at 0.99, and for growth of *Aspergillus* species at 35^{o}C and water activity at 0.95. Moisture, temperature and relative humidity influences the growth of toxigenic mould and aflatoxin production in maize. Thus, aflatoxin contamination can increase ten-fold in 3-day period when harvested maize is stored with high moisture content coupled with high temperature and humidity above optimal levels (Hell *et al*., 2010). However, *Aspergillus favus* and *Aspergillus parasiticus* cannot grow or produce aflatoxin at water activity of less than 0.7 and relative humidity below 70% (Emitati *et al*., 2013). Thus, measures to control fungal growth and aflatoxin production should focus on storing maize on conditions below these levels.

Moreover, knowledge of these optimum conditions for aflatoxins development are important in determining appropriate pre-storage and storage systems of maize to

prevent fungal growth and aflatoxin production. Fungal growth in maize is affected by plant stress due to drought or heat which in turn promotes aflatoxin development (Mboya *et al*., 2011; Summer *et al.,* 2009). Aflatoxins can develop in maize when still growing in the shamba and before it is harvested (Hosney, 2015). Thus, farmers need to harvest their maize early, immediately after attaining maturity.

Aflatoxins can develop rapidly in fungal infected maize. Studies done indicate that they can develop within 24 hours in mold and fungi infected maize stored under conditions of high moisture (above 14%) and higher temperatures (26.6 degrees centigrade) (Summer *et al*., 2009). The recommended moisture content for safe storage of cereals including maize is 13.5% or less (Hosney, 2015). The foregoing factors are important for consideration when exploring better farm, harvest and storage practices of susceptible food crops.

2.2 Effects of aflatoxins in human health

Exposure to myctoxins, particularly aflatoxins, may result in numerous profound health effects in humans. Studies indicate that aflatoxins, mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Patten, 1981), are of particular public health importance because of their effects on human health. The human gastrointestinal tract rapidly absorbs aflatoxins after consumption of contaminated food, which is then tranported to the liver by circulatory system (Fung *et al*., 2004; Eduardo *et al*., 2005). Studies have shown that when 1 to 3 % of aflatoxins are ingested they irreversibly bind to proteins and DNA bases to form adducts such as aflatoxin B1-lysine in albumin (Skipper *et al.,* 1990).

Previous studies done in Makueni found an association between aflatoxin concentrations in maize and aflatoxin B1-lysine adducts concentrations in serum of case patients (Eduardo *et al*., 2005). The same study showed that having aflatoxin B1-lysine adducts

concentrations at or above 0.25 ng/mg was a risk factor for aflatoxicosis, although it was unclear why some people with higher B1-lysine adducts concentrations did not manifest symptoms of aflatoxicosis. Previous investigations have demonstrated that the 249^{ser} TP53 mutation is a biomarker of the aflatoxi-associated mutational effect $P^{53\ g}$ gene (Kuniholm *et al*., 2008; Mace *at al*., 1997). This gene can be used as biomarker for determination of exposure of aflatoxin risk in humans.

Exposure to aflatoxins can either be in form of chronic or acute toxicity (Emitati *et al.*, 2013). Chronic toxicity due to long time exposure can have both carcinogenic and hepatoxic effects, depending on the duration and level of exposure. Chronic dietary exposure to aflatoxins is a major risk factor for hepatocellular carcinoma, particularly in areas where hepatitis B virus infection is endemic (Kuniholm *et al.*, 2008). Hepatocellular carcinoma (HCC), among other etiologic factors, is also associated with aflatoxin exposure [International Agency for Research on Cancer (IARC), 1993)].

Acute toxicity caused by ingestion of higher doses (i.e. above 10ppb) of aflatoxin from heavily contaminated food can result in acute aflatoxicosis syndrome, which is characterized by vomiting, abdominal pain, pulmonary edema, convulsions, coma as well as hepatotoxicity, fulminant liver failure, cerebral edema, fatty involvement of kidneys and heart, and if not well managed could result in death (Cornell University, 2014; Emitati *et al*., 2013; Fung & Clark, 2004).

Although no human is immune to toxic effects of aflatoxins, adult humans have a high tolerance level for aflatoxin exposure and rarely succumb to acute aflatoxicosis (Williams *et al*., 2004). However, aflatoxin related diseases could be influenced by age, sex, nutritional status, and/or concurrent exposure to other causative agents such as hepatitis B virus (HBV) or parasite infection (Cornell University, 2014). Carriers of hepatitis B and hepatitis C virus have high risk of developing hepatocellular cancer when they are exposed to aflatoxin (William *et al.*, 2004).

Aflatoxins have been linked with higher prevalence of hepatocellular carcinoma (Hell *et al*., 2010; Strosnider *et al*., 2006). In addition, prolonged exposure to aflatoxin has been associated with stunted growth and underweight in children (Eduardo *et al*., 2005; Gong *et. al*., 2003). This is because aflatoxin lowers the nutrition value of food, and also negatively affects palatability of meal made from maize thus affecting the nutritional status of consumers (Mboya *et al.,* 2011). Aflatoxins are also known to suppress immune system thereby making people vulnerable to attack by other diseases such as malaria and HIV (Mboya *et al*., 2011). Moreover, aflatoxin could also put babies at risk through their mother's breast milk as aflatoxin B1 has been detected in breast milk (Emitati *et al.,* 2013). In young children aflatoxins can cause poor cognitive development and greater susceptibility to infectious diseases (Platform for Africa-Europeanship Partnership in Agricultural Research Development (PAEPARD), 2015).

Studies have also shown that people chronically exposed to elevated serum concentrations of aflatoxins (above 0.25ng/mg) are three times more likely to develop hepatocellular carcinoma than those with lower concentrations (Eduardo *et al*., 2005; Williams *et al*., 2004)). Further, study findings indicated that aflatoxicosis had high case fatality, about 39% in 2004 outbreak in eastern Kenya, and that males were more likely to die from it inspite of eating similar quantities of maize as females (Williams *et al*., 2004). Risk factors of developing aflatoxicosis include sharing of contaminated food and genetic polymorphisms of cytochrome P_{450} enzymes, which may place families at risk of aflatoxicosis (Williams *et al*., 2004; Chen *et al*., 2000).. However, the authors recommended further research to determine if the the high incidence of liver cancer in eastern Kenya is attributable to chronic asymptomatic exposure to aflatoxins.

Conditions which may enhance likelihood of aflatoxicosis include limited availability of food and environmental conditions that favor fungal development in crops, as well as inadequate regulatory system for aflatoxin monitoring and control (Cornell University, 2014).

Contamination of food supplies by aflatoxins is of particular concern in rural communities of developing countries (Bhat *et al*., 1997), as they cause aflatoxicosis. The food and Agriculture Organization estimates that mycotoxins contaminate 25% of agricultural crops worldwide (Smith *et al*., 1994).). Dietary exposure to aflatoxins through complementary foods to infants is also still of concern (Shabani *et al*., 2015). In addition, there is an association between aflatoxin concentrations in maize and aflatoxin B1-lysine adduct concentrations in serum (Eduardo *et.al.,* 2005). This association can be used to estimate the risk levels of exposure in humans. Furtherore, factors affecting individual variation in susceptibility to concentration of aflatoxin B1-lysine adducts in serum after having been exposed to aflatoxin contaminated food need also to be explored.

2.3 The burden of aflatoxin problem

It is estimated that about 155,000 people worldwide have been affected by liver cancer associated with consumption of aflatoxin contaminated products, and out of this affected population 40% occur in Africa (Emitati *et al*., 2013). In addition, a number of outbreaks of acute aflatoxicosis have been reported in some countries resulting from consumption of food contaminated with aflatoxins. These aflatoxicosis outbreaks have been documented in Kenya, India, and Thailand (Council for Agriculture Science and Technology (CAST), 2003).

The largest reported aflatoxin outbreak occurred in western India in 1974 from consumption of contaminated maize which resulted in 397 cases and 107 deaths (FSRIO, 2009). In Kenya, in April 2004, an outbreak of acute hepatotoxicity was

identified among people living in Kenya's eastern and central provinces. Epidemiologic investigations that were conducted found that the outbreak was as a result of aflatoxin poisoning from ingestion of contaminated maize. During this outbreak, a total of 317 cases and 125 deaths occurred, making this one of the largest and most severe fatal outbreak of acute aflatoxicocosis documented worldwide. The outbreak affected mainly four counties namely Makueni, Kitui, Machakos, and Kiambu. Of the four counties, Makueni and Kitui were mostly affected representing 47% and 32% of case-patients, respectively, followed by Machakos (6%of cases) and Kiambu (4% of cases) (CDC, 2004).

During the outbreak, samples collected by Public Health officials from affected areas found concentrations of aflatoxin B1 as high as 4,400 ppb,(Misore *et.al*., 2005; Nyikal *et al*.,2004). Furthermore, 40% of maize samples taken from farmers in Eastern and Western Kenya in 2011 had aflatoxin levels above 10 ppb (Emitati *et al*., 2013). The fungi causing aflatoxin was found to be dominant in semi-arid regions of Kenya (Claudia *et al.*,2007). Among the fungal strains that caused aflatoxicosis outbreak in Makueni was *S* strain morphotype of *A. flavus* (Claudia *et al.*, 2007). This S strain was associated with lethal aflatoxicoses that occurred in 2004 outbreak. Probst *et al*. (2010), found high incidence of of S strain of *Aspergillus flavus* to be highly correlated with acute aflatoxicosis in Eastern region of Kenya. Factors leading to dominance of this fungal strain have not been identified and therefore need to be explored for better control and prevetion of aflatoxin.

Since it has been realized that absolute aflatoxin safety cannot be achieved, many countries have attempted to limit exposure to aflatoxins by imposing regulatory limits on commodities intended to be used as food (Cornell University, 2014). Further, in an effort focused on reducing aflatoxin exposure to humans by keeping aflatoxin levels in food as low as reasonably possible and ensuring that those exceeding legal limits are not used as

food, many countries have set regulatory aflatoxin limits to guide their action points (Arther Yau, 2012). In Kenya, the country has adopted aflatoxin limit of 10 parts per billion (ppb) for humans (KEBS, 1988; Mwihia *et al.,* 2008), but enforcing these regulations is challenging especially for home grown maize which are grown and consumed locally.

This is further compounded by the fact that information about risk factors associated with aflatoxin was found to be limited (Unger *et.al.,* 2007). Besides, it has been observed that in developing countries aflatoxin exposure is more common because regulations are non-existent or not stringent and/or food shortage exists. This is aggravated further by the fact that food produced in developing countries are usually stored, prepared, and consumed by the families without consideration for the risks of aflatoxin exposure. Another aspect of consideration is that although heavily contaminated foods are not permitted for consumption through regulatory limits, concern still remains for possible adverse effects resulting from long term exposure to low levels of aflatoxins (Cornell University, 2014). As aflaoxin contamination cannot be completely eradicated from foods, exposure through foods should therefore be kept as low as possible (Akowuah *et al*., 2015).

Despite decades of research progress, aflatoxin has been found to be a challenging problem (Garry, 2003). William *et al*. (2009) observed that complete elimination of aflatoxins is not possible since it is a common contaminant in agricultural products. The *Aspegillus flavus* which produce aflatoxin is a common fungus found in soil and debris and occur frequently in nature particularly as airborne spores, and can be found on most grains in the field and in storage (Summer *et al.,* 2009). However, despite the difficulties of eliminating aflatoxins, sound agronomic and post harvest practices when followed keenly will reduce or prevent contamination or build up of contamination once the crop

is harvested and stored. Besides, aflatoxin can further be minimized through proper pre-harvest, harvest, drying and storage practices at household level.

Maize, which is the most important cereal in Kenya and stable food for 90 percent of the population (Wambugu *et al.,* 2009), was the primary dietary staple food in Makueni and also the main crop produced in the areas which were affected with aflatoxin outbreaks. At harvest, farmers stored most of their maize for household consumption and sold the rest to meet other household needs (Lewis *et al*., 2005). This local home grown maize was found to be the primary source of aflatoxin in Makueni (Mwihia *et al*., 2008). As majority of people subsist on maize, there is need to focus on proper harvesting, drying and storage in order to prevent aflatoxin contamination. Moreover, compared to other cereals, maize is more susceptible to moulding and aflatoxin development because of its relatively high moisture and starch content (Weinberg *et al.,* 2008).

Besides causing ill health, aflatoxin and pests cause considerable post-harvest losses. These post-harvest losses attributed to pests and other hazards account for about 30% (Likhayo *et al*., 2004), and maize post-harvest losses in the tropics, mainly due to pests, have been estimated at about 20% (Bett *et al*., 2007). In particular, Bett *et al*.,(2007) in their study found out that pests, poor storage facilities and diseases including aflatoxin cause losses of 10-20%, 5-10% and 5% respectively. Inappropriate drying, poor storage and hygiene are some of the factors contributing to post-harvest losses. Given the prevailing perennial food shortages, these losses are enormous and aggravate the food security problem. Poor farmers are mostly affected as they are more vulnerable (Mboya *et al*., 2011). Hell *et al*. (2010) observed that the socio-economic and food security status of majority of inhabitants of sub-Saharan Africa gives them little option of choosing good quality products.

Aflatoxin-producing fungi also cause direct economic losses by spoiling grain (Hell *et al*., 2007). Animals which are fed on grains contaminated with aflatoxins also have

lower growth and productivity. Further, aflatoxin affects marketing of agricultural products internationally. Exported maize has to comply with food safety and quality requirements of importing countries. The market value of maize which is contaminated with aflatoxin is low, and countries with widespread aflatoxin occurrence export best quality and retain poor quality to be consumed locally thereby harming the health of local people (AATF, 2014). Since maize is increasingly becoming a stable food in many countries, and is one of the cereals mostly susceptible to aflatoxin, high consumption of maize coupled with frequent and elevated aflatoxin levels, leads to high aflatoxin risk (Wilson *et al*., 2006).

The losses resulting from aflatoxicosis incase of an outbreak can be enormous if replacements of contaminated maize is considered. According to Akowuah *et al.*, (2015), the quantity of safe food required to replace contaminated food during acute aflatoxicosis in Kenya in 2004 was 166,000 tonnes for 1.8 million people over six months period. When these losses are considered along side with other losses attributed to adverse effects on human health, the burden due to aflatoxin-contaminated maize is enormous.

2.4 Methods and techniques of aflatoxin detoxification

Aflatoxin detoxification measures can be undertaken to reduce aflatoxin in maize before consumption or after consumption. Detoxification of aflatoxins can be achieved either physically by sorting or segregation, or chemically by use of calcium hydroxide or ammonia, or microbiologically by incorporating pro-biotics or lactic acid bacteria into the diet (Hell *et al*., 2010). The technique of sorting out of physically damaged and infected grains can reduce about 40-80% of aflatoxins (Afolabi *et al*., 2006). Levels of aflatoxins in contaminated commodities prior to consumption may also be reduced through food processing methods such as wet and dry milling, dehulling, grain cleaning, canning (autoclaving), roasting, baking, frying, alkali cooking (nixtamalization),

extrusion cooking etc (Hell *et al.,* 2007). These are some of the diverse food processing methods that could significantly reduce the amount of aflatoxin in food prepared from maize. These measures may go along way in reducing aflatoxin exposure to consumers. Dehulling of maize which is practiced by many communities can reduce aflatoxin contamination by 92% (Siwela *et al*., 2005). However, further evaluation of other methods is also required to identify the ones which are effective in exposing consumers to less amount of aflatoxin.

A number of techniques have also been applied to reduce or limit the toxic effects of aflatoxins in consumers by natural or synthetic agents with varying degrees of success (Hell *et al*., 2007). Some of these agents are anti-oxidants such as selenium, vitamins, provitamins, phenolic compounds, coumarin, chlorophyll and its derivatives, fructose and aspartame, medicinal herbs and plant extracts. Others are mineral and biological binding agents e.g. hygrated sodium calcium aluminosilicate, bentonites, zeolites, activated carbons, bacteria, and yeast (Farombi, 2006). Chemoprevention can block, retard or even reverse the carcinogenic effect resulting from aflatoxin exposure. A drug such as oltipraz is a potent inducer of enzymes that detoxify carcinogens including aflatoxins. Natural components in fruits and vegetables such as chlorophyll also form another group of potential chemopreventive agents (Farombi, 2006). However, detoxification methods used should be cost- effective, efficient and safe.

Another innovative technique of reducing human exposure to aflatoxin is through the use of Novasil clay. Novasil clay works by binding aflatoxins with high affinity and high capacity in the gastrointestinal tract thereby reducing bioavailability of toxins without interfering with utilization of vitamins and other micronutrients (Philips *et al.,* 2008). This strategy is being evaluated as a potential sustainable remedy for acute aflatoxicosis intervention by including novasil clay in the diet. A recent study in Ghana found that novasil clay at the dose of 0.25 % is effective in decreasing biomarkers of aflatoxin

exposure without interfering with vitamins A and E, as well as iron and zinc (Philips *et al.*, 2008). However, this technique needs to be evaluated further to determine its safety and efficacy before being adopted for large scale use.

Another approach of reducing aflatoxin exposure is by reducing consumption of high risk foods such maize and groundnuts by consuming a more varied diet through diversifying into less risk foods such as sorghum and millet (Hell *et al.,* 2010). In this regard, efforts to diversify food production will go along way in enhancing implementation of this approach.

2.5 Different altitudes maize seasons and aflatoxin development

Maize grown in different altitudes and harvested in different seasons could have an effect on aflatoxin development. Fungal development and aflatoxin contamination in foods occur as a consequence of interactions among the fungus, the host and the environment (Milani, 2013). Production of aflatoxins is thus affected by environmental factors which are in turn linked to climatic conditions which are also affected altitudes and seasons (Cornell University, 2014). The environmental factors that favour *Aspergillus flavus* development and aflatoxin production include high soil and/or air temperature, high relative humidity, high rates of evapotranspiration, water availability, drought stress, crowding of plants and conditions aiding dispersal of fungi during silking (Hell *et al.,* 2010).

Moreover, among the many factors involved in mycotoxin production, namely biological, harvesting and storage, climate is one of the important factors particularly temperature and moisture (Milani, 2013). Mycotoxin production, besides being affected by pre and/or post harvest problems, is also affected by changes in weather and climatic conditions. The prevailing conducive weather in sub-saharan Africa due to wet and humid climates, characterized by high temperatures and high relative humidity coupled with dryness, promote fungal growth and aflatoxin production (Abbas *et al*., 2009). In

addition, studies have found significant correlation in aflatoxin levels in maize after long storage in agro-ecological zones with wet and humidy climates in dry regions (Hell *et al*., 2000). Moreover, mycotoxins besides dependent on climate and environment are also affected by availability of micronutrients as well as attack by insect and other pests which are also determined by climatic conditions (Milan, 2013).

Thus, climatic variability attributed to different altitudes and seasons could have diffrent effects on fungal growth and aflatoxin occurrence. Wetness and high humidity that characterize climatic condition of an area create conditions that favour the growth of fungi in stored maize (Mboya *et al*., 2011; Arora, 2008). In addition, wet and moist weather conditions at harvest and after harvest and during storage also increase mould growth and aflatoxin production (AATF, 2014).

Susceptibility of maize to fungal infections has further been found to be influenced by favourable conditions such as high humidity and high temperature (Mboya *et al*., 2011). Equilibrium of temperature and humidity is also crucial for fugal growth. Channaiah *et al*., (2014) observed that safe storage of maize is the equilibrium of moisture content concept relating air temperature and relative humidity to grain temperature and moisture content. Further, high ambient humidity make the control of maize moisture content difficult (Chulze, 2010), whereas high temperature aggravated by climate change accentuates aflatoxin contamination (AATF, 2014).

Previous studies have also shown that climate change (global warming) which affect temperature and humidity have effect on aflatoxin producing fungi and aflatoxin occurrence in maize and other crops (Cotty *et al*.,2007; Patterson *et al.,* 2010). Since aflatoxin producers are favoured by warm conditions, global warming particularly in temperate climates, poses potential aflatoxin problem (Milani, 2013). Furthermore, warm and humid tropical and sub-tropical conditions are ideal for growth of *A. flavus* and *A. parasiticus* species resulting in formation of aflatoxins. Thus, as the world

continue to experience climate change as the result of global warming it should brace for numerous health challenges including those posed by aflatoxin.

Besides seasonal climate change, the extent of aflatoxin contamination among other factors varies with geographic location (Hell *et al*., 2007). Hence, aflatoxin contamination will vary with geographic location (Cornell University, 2014). In addition, types of maize storage structures and their placement also vary across agroecological zones. Studies done in in West Africa, Nigeria and Benin, showed that the incidence of aflatoxin contamination and the level of aflatoxin present in maize varied in different agroecological zones and seasons (Hell *et al*., 2007). Thus, strategies to control aflatoxin should take into account variability of different agroecological zones and seasons.

2.6 Control of aflatoxin

2.6.1 Pre-storage practices of maize

Aflatoxin occurrence in maize is minimized by sound agricultural, pre-harvest, harvest, drying and storage practices, since studies indicate that aflatoxin contamination is likely to increase along the maize production chain starting from the farm to the household or market (Akowuah *et al*., 2015). At farm level, it has been found that irrigated maize generally has fewer problems with *Aspergillus* infection due to better growing conditions leading to less drought and heat stress (Summer *et al*., 2009). Moreover, some varieties of maize seeds are more resistant to mould infestation, a predisposer to aflatoxin development (Emitati *et al.*, 2013). Some high yielding yellow maize varieties which include AO901-25 with grain yield of 7115 kg/hectare and low aflatoxin level have been found to have good resistance to *Aspergillu*s species, but most people in Africa have preference to white maize (Emitati *et al.*, 2013).

Adherence to proper pre-harvesting practices is not only important in reducing mould growth, insect pests' infestation and aflatoxin contamination in maize; but it can also go along way in increasing maize yields. Hell *et al*., (2010) observed that pre-harvest measures that are efficient in reducing aflatoxin contamination in maize are the same as those that will enhance yields. These pre-harvest practices include timely planting, ensuring optimal plant densities, proper plant nutrition, avoiding drought stress, controlling other plant pathogens, weeds and insect pests, and proper harvesting (Bruns, 2003).

Pre-harvest interventions such as proper agricultural and pest management practices can reduce the presence and growth of *Aspergillus* fungi on pre-harvested crops and consequently reduce aflatoxin levels. Insect infestation, which could result from improper agricultural and poor pest management, has been found to be related to aflatoxin contamination (Hell *et al*., 2007). It is observed that in most communities maize is traditionally left to mature and dry in the field prior to harvesting (Thamanga-chtja *et. al*., 2004). Although this could be a good practice, harvesting of maize should be done in such a way as to prevent damage to the seed coat since damaged seed permits easy entrance of molds and fungi, thus promoting rapid development of aflatoxins. Harvesting during heavy rains should be avoided as this could have serious effects on the quality of maize including rotting (Hosney, 2015). High levels of moisture could enhance development of mould leading to aflatoxin contamination.

When maize reaches maturity, that's at moisture content of about 28 to 30 percent, it should be harvested immediately and dried to reduce field exposure to *Aspergillu*s fungi. Kaaya *et al*. (2006) recommended harvesting maize immediately after physiological maturity to combat aflatoxin problems, since aflatoxin contamination increases when maize stays longer in the field after attaining maturity. Further, extended field drying

besides likelihood of contributing to pests, mould and aflatoxin problems could also result in serious grain losses (Kaaya *et al*., 2006).

After early harvesting, maize has to be dried to safe levels inorder to stop fungal growth, as leaving harvested crop in the field prior to storage promotes fungal infection and insect infestation (Kaaya *et al*., 2006). It has also been noted that drying temperature and drying time have an effect on the development of aflatoxin in grain to be stored. Studies indicate that slow drying with low heat over long periods of time promote aflatoxin development. It is also important that during handling of grain, physical damage should be minimized and high moisture maize should not be held longer than six hours in transportation wagons or trucks (Summer *et al.,* 2009).

Clearing remains of previous harvest and destroying infested crop residues are important measures that contribute to reduction of deterioration of maize in the field prior to storage (Hell *et al*., 2007). In addition, drying crops properly before storage as well as sorting and disposing of visibly moldy or damaged kernels before storage can prevent or reduce the development of aflatoxins during post harvest, drying and storage, although not eliminating them (Heather *et al.,* 2006). Drying maize in a cob prior to shelling is a good practice and is recommended (Chulze, 2010). Storing maize when not completely dry has been found to be associated with aflatoxicosis (Eduardo *et al*., 2005).

Furthermore to ensure proper quality of maize for storage, rapid drying and cooling followed by treating with insecticides is recommended for preventing pests and development of fungi in maize (Reed *et al.,* 2007). Other studies have demonstrated that simple technologies such as use of platforms or mats during drying of maize increases efficacy and reduces risk of aflatoxin contamination (Hell *et al*., 2010). This underscores the importance of proper drying of maize crops before storage after harvest.

In addition to proper drying of maize, handling of harvested and/or dried maize particularly during transportation is equally important for consideration as improper transportation or handling could damage maize grains and promote insect pest infestation which could in turn promote mould growth and aflatoxin development. As it has been noted in other studies, handling of maize grain should be done is such a way as to minimize physical damage of grains, and maize should not be held for more than six hours in transportation wagon or trucks (Summer *et.al*., 2009). Furthermore, maize requires proper handling and transportation at all levels from farm upto storage point.

2.6.2 Storage practices of maize

Maize storage practices and methods should ensure that proper storage conditions are maintained to prevent development of aflatoxins. Fandohan *et. al. (*2006) in their study found that storage systems such as type of storage structure, hygiene and insect infestation interact and influence fungal infection and mycotoxins contamination. Insect infestation in maize stores was found to be correlated with aflatoxins (Hell *et al.,* 2000). Hell *et al*. (2007) also observed that the type of storage structure and placement, which varies across different zones, also influences aflatoxin levels in maize. Moisture content which is also linked to storage affects aflatoxin development in maize. It is therefore recommended that moisture in stored grain should be kept below 12-13 percent and insect activity should be kept to a minimum. When temperatures are below 18.3 degrees centigrade and moisture of the grain is below 12 to 13 percent, development of the fugus usually stops (Summer *et al.,* 2009).

Aflatoxin contamination problems are also minimized with good management practices such as thorough grain cleaning to remove trash, broken and damaged kernels before storage which help to curtail aflatoxin development. Besides, Channaiah *et al*., (2014) observed that the best management for successful storage of maize incorporates sanitation, loading, aeration, and monitoring. Further, preventing fungal infection in

maize through proper drying and storage is a better management practice compared to treating mouldy maize (Channaiah *et al*., 2014).

Maize is stored in various ways among the different communities. In most Sub-Sahara African countries, maize is generally stored in cob form either in wooden granaries, under the roofs of the farmers' houses, or on floor in houses (Fandohan *et al.*, 2006). Traditional methods which are common in many communities consist of temporary storage, used primarily for drying, and long term storage structures which are made fron plant materials mainly wood, bamboo, thatch, clay or bags (Hell *et al*., 2007).

The storage form (cobs or shelled grain) of maize influences contamination by toxigenic fungi, and maize stored as grain has higher levels of *A.flavus* than maize stored on cob with the husk (Hell *et al*., 2007). Storage in grain form may be in clay containers, mud silos, or in bags or sacks. However, these conditions have been found to be unfavorable for good drying of maize, particularly in humid and semi-humid regions (Mboya *et al.*, 2011). Storage in bags (in particular gunny bags), clay containers or silos does not allow air circulation in maize, thus making maize vulnerable to *Aspergillus* growth and subsequent aflatoxin contamination (Fandohan *et al.*, 2006). These types of storage facilities promote fungal infection and subsequent production of mycotoxins. However, sisal bags (sacks) may provide better maize storage than gunny bags because they allow air circulation so long as they are placed in raised platforms and kept in well ventilated building.

Regarding proper storage of maize, it has been observed that any storage structure or method should ensure good drying of maize while in storage (Fandohan *et al.*, 2006). This may be airtight storage or non -airtight storage, but airtight storage has been found to be superior to non-airtight storage owing to their viability and vigour in preventing damage of maize by insects (Wambugu *et al.*, 2009).

It has been observed that infrastructure and grain storage practices in developed countries can prevent post harvest development of mycotoxins, but this aspect remains a threat in developing countries, especially in tropical areas (Garry, 2003). For instance, methods of drying and storing maize in elevated improved granaries were found to be protective against aflatoxin development, but are not used by many (Eduardo *et. al.*, 2005). The traditional improved granaries are raised structures that are well ventilated and promote proper drying of maize. Since they are elevated platforms they isolate the maize from spores and insects on the ground. As a result of the effectiveness of granaries in prevention of aflatoxin, a study needs to be carried out to determine the extent and challenges of adoption of this mode of storage at households.

Although a study has been done in Makueni to assess the effect of storage practices on aflatoxin, varying sources of maize, inadequate households with homegrown maize as well as different storage practices of maize was a challenge during the study (Mwihia *et al.,* 2008). Further, this study indicated that most maize found in household was purchased and not home grown thus making it difficult to determine relationship between drying practice and aflatoxin levels.

Since traditional methods of maize storage are culture sensitive, there is need for finding ways of improving these methods. This view is supported by Thamaga-chtja *et.al*. (2004) who in their study observed that the wide spread and continued use of traditional practices by small scale and subsistence farmers despite considerable aflatoxin development risk and considerable loses, warrants investigation with respect to improved storage. They also recommended further studies geared towards finding of appropriate and inexpensive post harvest technologies for small scale farmers.

Besides, it has been found that storing maize inside the home rather than in a granary was associated with aflatoxicosis (Eduardo *et al*., 2005). However, more studies are needed to

address the various existing gaps in traditional storage practices, and in particular exploring whether there are any correlations/associations between different storage practices and aflatoxin occurrence in maize.

Maize storage is sometimes combined with other methods for better control of insect pests and fungal infestations which are precursors to afltoxin development. Several methods are available for use to control insect pests and fungal infestation in stored maize albeit with varying levels of success. These methods include use of insecticides, fungicides, smoking, natural local plant products. However, the use of insecticides and fungicides is limited by availability and cost (Hell *et al*., 2007). Some farmers have been known to use smoking (Udoh *et al*., 2000) while others have been known to use natural plant products to preserve grain and reduce aflatoxin levels by reducing moisture content and preventing insect damage (Nguefack *et al*., 2004).

Besides having proper storage of maize, to reduce aflatoxin contamination maize should be sorted and stored in well ventilated store. It is also advisable that grain quality should be checked regularly while in storage and if there is any insect or mould infestation it should be controlled immediately (Hell *et al*., 2007). If high insect or mould infestation levels are found, maize should be shelled and bad grains removed, and good grains put in jute bags (Hell *et al.*, 2007). This practice is important because distribution of aflatoxin is heterogeneous and higher concentrations of aflatoxins are usually found on heavily molded or damaged kernels.

Furthermore, cleaning storage areas prior to filling them with the new harvest has been found to reduce aflaoxin levels (Summer *et al*., 2009). The practice of regular cleaning, checking and sorting will reduce further contamination of good grains and when coupled with other measures will go along way in preventing afaltoxin contamination. Although it has been found that in many communities' farmers prefer to store maize in

polypropylene bags, the poor aeration in these bags encourage fungal growth and aflatoxin production, if grains are not dried properly to safe level (Udoh *et al*., 2000). It's also important for storage facility surroundings to be kept clean. S'etamou *et al*., (1997) observed that keeping the area surrounding the storage facility clean reduces infestation with insects that take refuge in host plants near the storage facility.

2.6.3 Recent innovations in aflatoxin control at pre-harvest and post-harvest level

Owing to numerous challenges encountered in aflatoxin control, various innovations and strategies are being tried to eliminate or reduce aflatoxin contamination at stages of pre-harvest and post-harvest. One such innovation being explored involves a bio-pesticide consisting of a non aflatoxigenic strain of *Aspergillus* which may competitively exclude toxic strains from infecting the crop. However, the allergenic and human health aspects of the atoxigenic strain need to be evaluated (Heather *et. al.,* 2006). Another innovative approach is bio-control "aflasafe" which has been successfully field tested in Nigeria and found to be effective in control of aflatoxins during crop development and post-harvest storage (AATF, 2014).

This technology addresses the fungus in the soil which is the source of aflatoxin before it can contaminate the crop prior to harvest, and the atoxigenic strains are carried over from field to stores and thus continue providing protection. Its working principle is tthat the harmless strains of aflasafe fungus compete with the poisonous strains, blocking them from multiplying thus reducing aflatoxin production in grains during crop development and post-harvest storage (Hosney, 2015). Besides, the bio-control products also persist in environment for several years, thus continuing to provide protection (AATF, 2014). Nevertheless, while further studies are on going to discover better technology and methods of controlling aflatoxin, there is need to continue exploring proper pre-storage and storage methods of maize crops.

A new storage triple-layered bag that is hoped to prevent post-harvest losses is on field trial in Makueni (ICIPE Kenya, 2014). The triple-layered bags, consisting of tough plastic bags, was designed at U.S Purdue University, and are already being used to protect cowpeas crops in West Africa from insects. The airtight (hermetic) Purdue Improved Crop Storage (PICS) bags could prevent the growth of moulds and insects in the humid areas across Kenya since being airtight they deprive the mould and insects of oxygen and moisture which they need for survival (ICIPE Kenya, 2014). These bags are better than ordinary bags since, among other advantages, they reduce post-harvest losses to less than 2% thus increasing availability of maize. The triple storage bags have been adopted for use in Rwanda after being successful piloted, and a campaign has been initiated to promote their use for storage of maize and beans (Katengwa *et al*., 2013). Grains including maize can be stored in the bags safely for upto one year, and the bags can be re-used up to 4 years (Katengwa *et al*., 2013). The adoption of these bags for wide scale use for household maize storage could go a long way in reducing insect and mould infestation as well as aflatoxin contamination.

Another technology of controlling aflatoxin is through effectively dealing with pest problem in stored maize, by use of diatomite, a fine powder made up of fossilized microscopic plant called diatoms containing millions of small particles with very sharp edges (Hosney, 2015). This technology is not new as some farmers in other countries have used it for decades to control pests. Diatomite is an excellent natural pesticide safe for both humans and animals, and cereals preserved with diatomite can stay for up to 4 years or longer without damage by pests (Hosney, 2015). However, though effective, their use is limted by their unavailability and inaccessibility locally.

Some studies have evaluated cost-effectiveness of aflatoxin control strategies. Strategies which have been found to be cost-effective include pre-harvest control using atoxigenic strains of *Aspergillus flavus* to completely exclude *toxigenic* strains in maize, and post-

harvest interventions for reducing aflatoxin contamination which include proper harvesting, drying and storage of maize (Emitati *et al.*, 2013; Wu *et al*., 2010). In addition, a strategy for improvement of quality management systems for hazard analysis critical control point (HACCP) have been found to be effective for management of mycotoxins including aflatoxins (Schmale *et al*., 2011). These strategies when implemented alongside with others will go along way in reducing aflatoxin contamination in maize.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study design

This was comparative analytical study to determine influence of maize storage and pre-storage practices on aflatoxin occurrence among households in areas of different altitudes, in Kibwezi and Kilome Sub-counties of Makueni County. Makueni County was purposefully chosen because it had previously experienced most severe aflatoxicosis outbreaks compared to other counties.

The study design entailed enrollment of equal number of households with home grown maize from both high and low altitude areas. Random samples of homegrown maize were then collected from maize harvested in first season for analysis for aflatoxin. The same households were followed up in second season of maize harvest and samples collected for aflatoxin analysis (Figure 3.2).

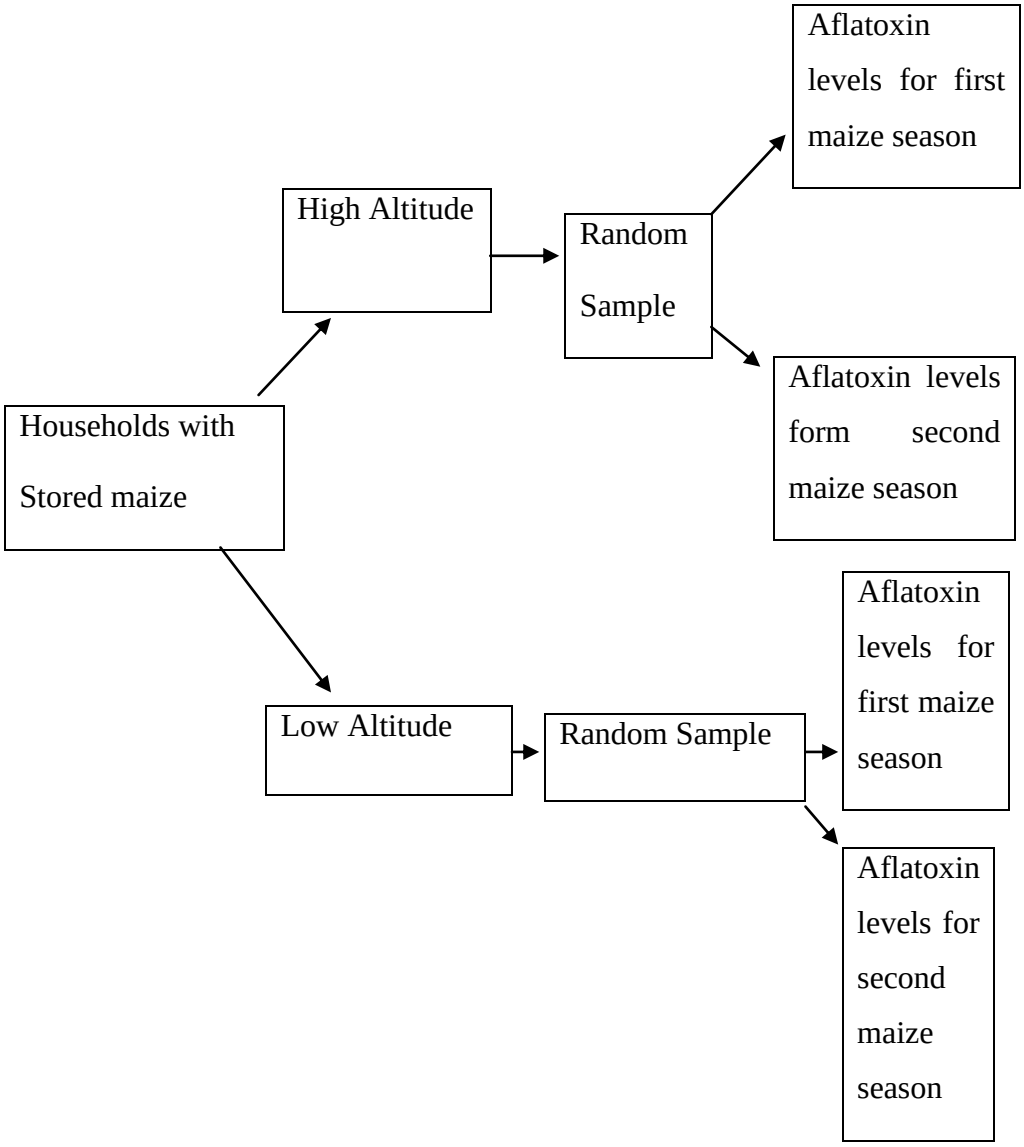


Figure 3.2 Study design

3.2. Study sites

This study was conducted in Kibwezi and Kilome sub-conuties of Makueni County. Mikuyuni sub-location which was randomly sampled from Kibwezi sub-county, is a

lower altitude area, and it's estimated mid point is located at S02.40157, E037.95143 SW, at an altitude of 916 M above sea level. The Maiani sub-location which was also randomly sampled from Kilome sub-county, is a higher altitude area, and it's estimated mid point is located at S01.84098, E037.31536NE, at an altitude of 1750 M above sea level. Makueni county covers an area of 8,034.7 square Km and according to 2009 population census it had a population of 884,527(GOK-MPND, 2009), which in 2012 was projected to 922,183 with estimated annual population growth of 1.4% (MDP, 2013). Kibwezi sub-county had population of 155,560 people while Kilome sub-county had population of 83,771 people (GOK-MPND, 2009). Mikuyuni and Maiani subloations had population of 5,329 and 5,598 people, respectively. Administratively, the county comprises six sub-counties with sixty-six locations and a hundred and eighty-seven sub-locations. For Political representation it is divided into 6 constituencies and 30 wards (MDP, 2013).

The land rises from 600 m above sea level at the southern parts of the county which include Kibwezi and Makindu which are low-lying areas, to 1900 m above sea level in northern highest parts of the county which include the hilly areas of Kilome and Kilungu (GOK-MPND, 2009). Due to change in altitude, the county has climatic variations and pronounced differences in temperatures. The northern hilly part with high-lying areas is usually cool while the southern part with low-lying areas is usually hot. The mean temperatures in Makueni County range from 20.2 to 24.6 degrees centigrade. The County experiences two rainy seasons, namely: the long rains occurring in March/April and the short rains occurring in November/December. Main food crops produced are maize, beans, cow peas and pigeon peas in that order, with maize being the predominant staple food. Figure 3.3 shows map of study sites in Makueni County.

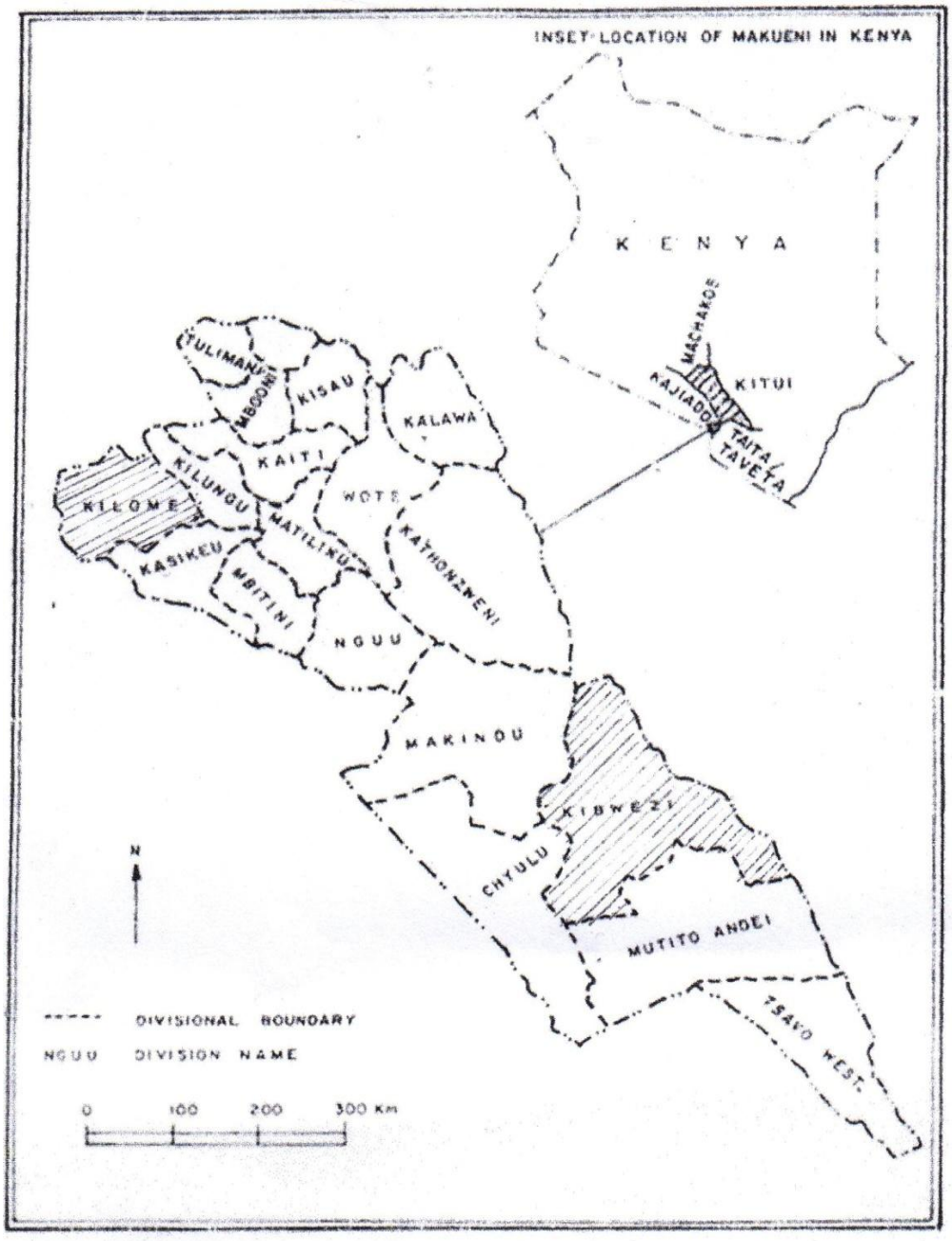


Figure 3.3 Map showing study sites in Makueni County

3.3 Study population

The study population comprised 239,331 people residing in 47,866 households in study areas who store home grown maize in their households. Target population comprised 884,527 residents of Makueni County since the county has largely similar socio-cultural and economic characteristics as well as environmental/ecological conditions.

3.4 Inclusion criteria

The study included all adults (above 18 years of age) who are household owners within the study area and store homegrown maize. Using this criterion, the study included 9,803 households, within the two study areas, which stored homegrown maize, having an estimated population of 58,820 (KNBS, 2009). The household heads were included as respondents representing the households. The study also included, 6 agricultural workers and 6 public health workers working in the area as well as 24 community leaders who consented to participate in the study.

3.5 Exclusion criteria

The study excluded people below 18 years of age and household which dif not store homegrown maize. Using this criterion the study excluded 6,382 households which did not store homegrown maize. These households had an estimated population of 31,910 people. Within the households, non-household heads were excluded from interview. About 143,599 people, including those below 18 years and those above 18 years with no households residing in the study area, were excluded from study (KNBS, 2009).

3.6 Sample size determination and sampling

3.6.1 Sample size determination

For objectives 1, 3 and 4, the sample size for households selected was determined using a formula as used by Fisher *et al.* (1998):

$$n=\frac{z^2 pqD}{d^2}$$

Where n= desired minimal sample size (Where population>10000)

Z= Standard normal deviate which is 1.96 at 95% confidence level

P= Proportion of the target population estimated to have a particular characteristic being measured. In this case it is estimated to be 0.25(FSRIO, 2009)

q=1-p and p=0.25, hence q=1-0.25=0.75

d= degree of accuracy = 0.05

D= desired effect and in this case it is 2 since this study has two different study sites

Thus

$$n=\frac{1.96^2 \text{ x}0.25 \text{ x } 0.75\text{x}2}{0.05^2}=576.24, \text{ approximately } =576$$

Since the entire population of households in the two study areas was estimated to be 9,803 (KNBS, 2009) which was less than 10,000 (ie Population<10,000) the following second Fisher's formula was used with the value of n obtained in the formula above:

$$n=\frac{n}{1+n/N}=\frac{576}{1+576/2000}=576/1.288=447.2, \text{ approximately}=448$$

where N was the preceding estimated total population of households (n).

Therefore the minimum total sample size for study was approximated to 448, giving a sample size of 224 households for each study site.

However, the sample size in this study was increased to 240 for each study site, thus giving a combined total of 480 for the two sites.

For objective 2, a sub-sample of 10% of the 450 households with stored homegrown maize were selected and maize samples were collected for analysis of aflatoxin and moisture content. The sub-sampling of households for maize samples collection was due to limited resources for carrying out analysis for the entire sample. The purpose and justification for sub-sampling is supported by Smith (2006).

3.6.2 Sampling and study sites selection

Makueni County was purposefully selected from 47 counties in Kenya because of having experienced severe outbreaks of aflatoxicosis in the past compared to other counties. Within the county, two geographically and ecologically different sub-counties were randomly selected for this study for comparison purposes. Kibwezi sub-county was randomly selected to represent the low altitude area which had 3 sub-counties namely Makindu, Wote and Kibwezi. Kilome sub-county was randomly selected to represent the high altitude area which also had 3 sub-counties namely Kilungu,Mbooni and Kilome. In each sub-county, one sub-location was randomly selected. For Kibwezi Sub-county Mikuyuni sub-location was randomly selected from twenty sub-locations while in Kilome Sub-county Maiani sub-location was randomly selected from fifteen sub-locations. To prevent bias, a table of random numbers was used for selection of sub-counties and sub-locations by assigning numbers at sub-counties and sub-locations, and picking the numbers at random. Representative sample of households was then selected from clusters/villages in sub-locations based on method of probability proportional to size (pps) (Sundar Rao *et al*.2003).

Thus, using this proportional determination, households were allocated in villages as follows: In Mikuyuni sub-location which had 4 villages with a total of 715 households, Kyanzili village with 166 households allocated 56 households, Mikuyuni village with 204 households allocated 68, Kalembwani village with 165 villages allocated 55 and Kibuauni village with 180 households allocated 61. In Maiani sub-location which had 7 villages with a total of 519 households, Mboni village with 65 households allocated 30 households, Matumbini village with 64 households allocated 30, Mbole village with 80 households allocated 37, Lyuki with 80 households allocated 37, Ikomoa with 70 households allocated 32, Sakini with 80 households allocated 37 and Katumini with 80 households allocated 37. The allocated households were then randomly selected as described in sub-section 3.6.2.1 below.

3.6.2.1 Households selection and recruitment of study participants

At each of the sub-locations sampled in the two sub-counties selected, households storing maize, which formed sampling units, were selected at random through systematic random sampling methods using a table of random numbers. The household owners/heads (or their representatives in case they could not be found) of households who consented to participate in the study had their households randomly selected for study. The household heads were consequently recruited as study participants. The random selection of households was done as follows: A Village in each study site was mapped out and all households in that village were numbered sequentially to create a list of households (sampling frame). Households were then selected using systematic random sampling method by selecting households at intervals determined by dividing total number of households in the village by its proportional sample size to obtain household to be sampled. This gave n^{th} interval, thus every n^{th} household was recruited in the study after randomly determining starting household.

To get the random starting household, random numbers from listed households were used to get the household to start with. After getting the starting household, the remaining households were then selected at fixed n^{th} intervals determined by dividing total number of households by sample size. The direction of sampling after obtaining the first household was determined at random by spinning a bottle and the direction it pointed was the diection for sampling.

After selecting the household, the purpose, nature, procedure and expected benefits of study were explained to each household head after which consent was sought. Upon consenting, the household head was requested to sign the consent form and was then recruited to participate in the study. Particulars of household head such as household number, name, contact address, phone number, sex, age, education level, religion etc. were recorded in the register for the follow up visit in the next phase/season of data collection. After registration, the household head of selected households with homegrown maize were then administered a face-to-face interview using structured interview schedule.

Owing to limitation in resources which could not enable analysis of 480 household samples, maize samples were obtained from sub-sampled 10% of 240 households with maize which were interviewed from each study site using structured interview schedules/questionnaires (Smith, 2006). A representative sub-sample was drawn through systematic random sampling method. The samples were thus obtained from every 10^{th} household. The sub-sampled households were requested to provide one Kg samples of their stored homegrown maize for aflatoxin analysis. Maize sampling was done as stated in section 3.6.2.2. The sub-sampled households which were recruited were later followed up for collection of samples in the following season's maize harvest in order to take into account seasonal climatic variations. Recruitment of households for study was done in October 2013.

3.6.2.2 Maize sampling

Maize sampling procedure was as described by Pichler (2006) and Latimer (2012). A one kg of maize sample was taken from maize found in the sub-sampled household. The sample was taken in such a way that it was a representative of the lot. Samples were collected from homegrown maize intended for human consumption found in the household. In case of maize packed in small volumes in different bags, multiple samples were taken from different parts of one bag or several bags belonging to one household and combined to produce a one kg sample for analysis.

The maize samples were collected using scoops/probes and put in paper bags, and carried and stored in paper bags while awaiting analysis. Each sample had a sampling form filled with specific identification information pertaining to the sample. The maize sample collection was done in two consequent seasons of harvested maize, August/September 2013 and February/March 2014, respectively.

During sampling the amount (quantity) of maize found in storage was estimated in Kilograms and recorded.

3.7 Data collection

3.7.1 Data collection tools

Questionnaires/interview schedules, checklists, in-depth interviews schedules and focus group discussion guides were used to collect data from the study sites. Questionnaires/Interview schedules were used to obtain data from respondents either in Kiswahili, Kikamba, or English, which was then translated (if interview was in Kikamba or Kiswahili) and recorded in English and some answers recorded as given. Information collected using questionnaires/interview schedules included 1) socio-demographic information including sex, age, marital status, religion, level of education, occupation, economic status, household size, 2) knowledge and awareness on aflatoxin, 3)

perception on health problems associated with aflatoxin, 4) pre-storage practices, and 5) storage practices. The questionnaire also collected information on which altitude/climate (high or low) increased aflatoxin contamination, and which maize harvest season (long or short) increased aflatoxin contamination. The questionnaire further collected information on quantity of maize or maize products consumed in Household by a person per day so as to get a clue on the likely exposure risk of aflatoxicosis.

A checklist was used as a guide for collection of information on quantity of maize in storage in Kilograms by estimation, and visual assessment of condition of maize as observed during storage as well as the condition of the storage structures.

Focus group discussions (FGDs) with key community opinion leaders/informants using FGD guides and in-depth interviews with 6 agricultural workers and 6 public health workers were conducted to corroborate and support information collected using questionnares/interview schedules. Thus, two FGDs, one in each study site, were conducted each comprising 12 people. One FGD was conducted in Kibwezi area while the other was conducted in Kilome area. Each FGD comprised selected local opinion leaders that included women group leaders, youth group leaders, village leaders and other key stakeholders in the study area.

Data on temperature and humidity of the two study sites was collected at the two study sites during the short rains season (November/December 2013) and long rains season (April/May 2014) of harvested maize. Digital Hygrothermometer instrument was used to obtain data on temperature and humidity simultaneously at sampled households. After each reading was noted, the reading was cleared from memory before doing a new test, by reseting the instrument.

Collection of data using questionnaires and taking of maize samples were done at the end of two seasons of maize harvest each year. These were the long rain season where

maize was harvested in August/September (first season harvest), and the short rain season where maize was harvested in February/March (second season harvest).

The questionnaires/interview schedules, checklists, in-depth interviews schedules and focus group discussion guides were pretested in Nguu division, an area situated between high altitude zone (Kilome) and low altitude zone (Kibwezi). The purpose of pretesting was to improve the tools by identifying errors and inconsistencies, and rectifying them before they were used for actual data collection.

3.7.2 Maize sample analysis

Moisture content and temperature were taken at the field during collection of samples. Moisture content was determined as described by AOAC (Latimer, 2012) using Portable Grain Moisture Tester. Before laboratory analysis, the maize samples were visually inspected for insect/pest infestation, mould or discolouration. The analysis was done in two stages. The first stage entailed analysis of the samples to determine presence and levels of total aflatoxin using ELISA test for total aflatoxin (Latimer, 2012). The second stage involved analyzing the samples which tested positive on ELISA aflatoxin test with HPLC test, which determined sub-types of aflatoxin and their quantities (Latimer, 2012). The maize samples were analysed for aflatoxin and other parameters at Bora Tech Laboratories, and University of Nairobi Laboratories.

The procedures for moisture content determination, ELISA test and HPLC test were as described by Latimer, 2012.

3.7.2.1 Moisture content determination

Moisture content was measured in the field using Portable Grain Moisture Tester/Metre model GMK303 powered by 9volts battery, calibrated for maize, beans, peas, green grams and millet and had accuracy of +or -0.5% with measuring range of 5-35% moisture content.

Seventy (70) g of maize sample was then taken, well shaken and filled into Portable Grain Moisture Tester to flash level and corked tightly. The moisture Tester device was then powered on. Appropriate scale applicable to maize (1-16) was then chosen. The sample was then allowed to run in the device for one minute and the moisture content was then read.

A new test for a different sub-sample from the same sample was repeated then an average of the two readings for the sample was determined. After each reading was noted, the reading was cleared from memory before doing a new test.

3.7.2.2 Aflatoxin determination using Enzyme Linked Immunoassay (ELISA) test

Aflatoxin determination using ELISA was done according to the method described by AOAC International Codex Official Methods of Analysis (Method R5 Mendez ELISA method) (Latimer, 2012). The ELISA kit used was Improved R-Biopharm AG manufactured by R-Bioharm Rhone Ltd, Germany (www.r-biopharm.com).

3.7.2.2.1 The principle of the test

The basis of this test is the antigen-antibody reaction. The wells in the microtiter strips are coated with capture antibodies directed against anti-aflatoxin antibodies. Standards or the sample solutions, aflatoxin-enzyme conjugate and anti-aflatoxin are added. Free and enzyme conjugated aflatoxin compete for the aflatoxin antibody binding sites (competetive enzyme immunoassay). At the same time, the anti-aflatoxin antibodies are

also bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. Substrate/chromogen solution is added to the wells and incubated. Bound enzyme conjugate converts the chromogen into a blue product. The addition of the stop solution leads to colour change from blue to yellow. The measurement is made photometrically at 450nm; the adsorption is inversely proportional to the aflatoxin concentration in the sample.

3.7.2.2.2 Enzyme Linked Immunoassay (ELISA) test procedure

3.7.2.2.2.1 Extraction of Sample

The mill was thoroughly washed with clean water mixed with 3.5% sodium hypochloride and rinsed with distilled water then dried using clean cloth before grinding each maize sample. One Kilogram (Kg) of maize sample was ground into flour with a mill and then homogenized. Then 20 grams (g) of homogenized sample was weighed and 20 ml of 70% Methanol was added into the sample.

This was mixed for 2 hours (hr) and filtered using Buchner funnel. The extraction jar was then rinsed with 20 mililitres (mls) of extraction solution. The total volume of the extract was then measured and recorded.

3.7.2.2.2.2 Column Preparation

Five (5) g in 25 mls (70% methanol) of extract was taken and 10 % of methanol in Phosphate Buffered Saline (PBS) was prepared. Then 5 ml of 10% methanol PBS was passed through without letting it dry. A sample (sample with 1ml extract and 6ml water) was then applied and let to run slowly at the rate of 1 drop in 3 seconds. Distilled water (15ml) was then applied and passed slowly at a rate of I drop per second. Then air was passed to dry and the column was put to a receptacle for eluent. One (1) ml methanol (100 %) was then applied and passed slowly into receptacle.

3.7.2.2.2.3 Cleaning up with Acetonitrile

Nine (9) mls of sample extract was taken and evaporated to dryness with nitrogen/rotavapour. It was then diluted with PBS buffer to 10mls (the amount of organic solvent did not exceed 5% of solution). The extract solution was then filtered and dropped off into the immumo-affinity column at the rate of 1-3ml/min. The Immuno-affinity column was washed with 20ml water and the water dropped through the column with gravity. The column was dried to ensure total Aflatoxins recovery.

3.7.2.2.2.4 Derivatisation

All samples were evaporated to dryness and then 200µl Trifluoroacetic acid (TFA) was added and incubated at room temperature for 40 minutes, after which 800µl Acetonitrile: water (30:70) was added and dissolved using a sonicator. This was then filtered through a 0.2µm membrane filter into a vial.

3.7.2.2.2.5 Enzme Linked Immunoassay (ELISA) analysis

A sufficient number of micro-titer wells were inserted into the microwell holder for all standards and samples run in duplicate. Standard and sample positions were recorded. Fifty microlitres of the standard solutions or prepared sample was added to separate duplicate wells, and 50 µl of the enzyme conjugate was then added to each well. Subsequently 50 µl of the antibody solution were added to each well and mixed gently by shaking the plate manually and incubating for 30 minutes (min) at room temperature (20-25°C). The liquid was then poured out of the wells and the microwell holder tapped upside down vigorously (three times in a row) against absorbent paper to ensure the liquid from the wells was removed completely.

All the wells were filled with 250 µl washing buffer and the liquid poured out again. The washing procedure was repeated two times. After which 100 µl of substrate/chromogen

(brown cap) were added to each well and mixed gently by shaking the plate manually and incubated for 15 min at room temperature (20-25°C) in the dark. Then 100 µl of the stop solution were added to each well and mixed gently by shaking the plate manually and the absorbance measured at 450 nm. Reading was done within 30 minutes after additing stop solution.

Maize samples which tested positive in ELISA test were further subjected to confirmation quantitative HPLC test which was done as described in Latimer, 2012.

3.7.2.3 Determination of aflatoxin using high performance liquid chromatography

Determination of aflatoxin sub-types B1, B2, G1 and G2 and their quantities using High Performance Liquid Chromatography **(**HPLC) was done according to method described by AOAC International Codex Official Methods of Analysis (Latimer, 2012). The immunoaffinity columns Kit used was Zonrhone Wide Product Code P118/100 manufactured by R-Biopharm Rhone LTD, Germany (www.r-biopharm.com).

3.7.2.3.1 The Principle of the test

The procedure was based on monoclonal antibody technology, which makes the test highly specific, sensitive, rapid and simple to perform. The columns contain a gel suspension of monoclonal antibody specific to the toxin of interest. Following extraction of the toxin the sample extract was filtered, diluted and passed through the immunoaffinity column. Any toxin which was present in the sample is retained by the antibody within the gel suspension.

The column is washed with methanol to remove unbound material and the toxin was then released by the antibody following elusion with solvent. The eluate was collected prior to analysis by HPLC.

The total extraction and clean-up time took approximately 20 minutes. The result was improved clean-up and concentration of the toxin from food and feed samples giving a much cleaner chromatogram and therefore providing more accurate and sensitive detection.

The columns also have the added advantage in that they can be automated for large scale analysis of samples.

3.7.2.3.2 HPLC Test Procedure

3.7.2.3.2.1 Extraction of sample

The mill was thoroughly washed with clean water mixed with 3.5% sodium hypochloride and rinsed with distilled water and dried using clean cloth before grinding each maize sample. One Kg of maize sample was ground into flour with a mill and then homogenized. Then 20g homogenized sample were taken and weighed. After which 20 ml of 70% Methanol were added into the sample. It was then mixed for 2 hours and filtered using Buchner funnel. The extraction jar was then rinsed with 20 mls of extraction solution. Then the total volume of the extract was measured and recorded.

3.7.2.3.2.2 Column preparation

Five (5) g of solid extract were added to 25 mls of 70% methanol. Then 10mls of 10 % of Phosphate Buffered Saline (PBS) were added, and then 5ml of 10% methanol PBS passed through the column without letting the column to dry. A sample (sample with 1ml extract and 6 ml water) was applied, and let Let run slowly at the rate of 1 drop in 3 seconds. Distilled water (15 ml) was then applied and passed slowly at rate of I drop per second, and then air was passed to dry the column. After which a column was put to a receptacle for eluent and then 1 ml methanol (100 %) was applied and passed slowly into receptacle.

3.7.2.3.2.3 Cleaning up with acetonitrile

Nine (9) mls of sample extract were taken and evaporated to dryness with nitrogen/rotavapour. It was then diluted with PBS buffer to 10mls (the amount of organic solvent did not exceed 5% of solution). The extract solution was then filtered and dropped off onto the immumo-affinity column at the rate of 1-3 ml/min. The Immuno-affinity column was washed with 2*10ml water and the water dropped through the column with gravity. The column was dried to ensure total Aflatoxins recovery.

3.7.2.3.2.4 Derivatisation

All samples were evaporated to dryness and 200 µl Trifluoroacetic acid (TFA) were added and incubated at room temperature for 40min. Then 800 µl acetonitrile: water (30:70) was added and dissolved using a sonicator. They were then filtered through a 0.2 µm membrane filter into a vial.

3.7.2.3.2.5 High performance liquid chromatography (HPLC) analysis

Twenty (20) ul of the filtrate were added into the HPLC. Then a calibration curve of aflatoxin B1, B2, G1, G2 was run and results quantified as µg/kg (ng/g) using the formula below:

$$\text{Ug/kg (Ppb)} = \frac{C \times V_{total} \times V_f}{VIA \times m}$$

Where:

C=Concentration from HPLC run (ng/g)

Vtot=Total volume of extract (ml)

VIA=volume of extract for IA clean up

V f=final volume for measurement

M=Sample weight extracted (g)

3.7.2.3.2.5 High performance liquid chromatograph analysis conditions

Mobile phase: A. HPLC grade water and B. Acetonitrile

Column: Waters Novapak 3.9×150mmx4.6um

Flow rate= 1ml/min, Injection volume: 20ul and Run time: 35min

Detector: fluorescence detector was set at: Gain X1, Excitation λ=363nm, Emission λ=440nm, Column oven temperature: 35˚

3.8 Determination of environmental temperature and relative humidity

Determination of environmental temperature and relative humidity of sampled households was done using Portable Digital Hygrothermometer of Brannan model 290512 made in England. This device has temperature range of 10°C to 85°C and humidity range of 11% to 97%. The temperature and relative humidity were taken simultaneously as described by Arregui (2003), and as in manufacturer's instruction manual. The procedure for taking temperature and relative humidity was as follows: the instrument was placed at sampled household for 3 minutes after which the reading was taken. Before doing a new reading the instrument was reset and previous reading erased completely. There were 3 repetitions, that's for each measurement, readings were taken 3 times and the mean was calculated and recorded.

3.9 Data management and analysis

Data were cleaned, coded and entered in computer MS Windows Excel software and then transferred to SPSS for Window Version 17.0 (SPSS Inc., Chicago, Illinois) for Statistical analyses. Analyzed data (results) are presented using percentages, frequency

tables and bar charts. Descriptive statistics such as frequencies and means were applied in order to group and summarize data to facilitate presentation. Chi-square test for independence was used to determine association of categorical variables such as age, education, occupation, knowledge of aflatoxin, maize storage practices, pre-storage practices and presence of fungi/mould and insects/pests.

Student t-test for independent samples was used to compare means of aflatoxin content and other quantifiable variables between Kibwezi and Kilome study sites located at altitudes of 916 M and 1750 M above sea level, respectively. Pearson Correlation coefficient was used to analyze relationships of quantitative variables among different storage and pre-storgae practices, and Aflatoxin levels. Tests of significance were set at α =0.05 level of significance, and confidence levels at 95%. Focus group discussion results were recorded, summarized and presented in tables.

Quality of data was ensured by proper sampling, data collection and analysis at all stages of research including analysis of samples in duplicate, use of appropriate standards, training of research assistants and their supervision.

3.10 Limitations of study

The study limitations included;

1) The study of aflatoxin exposure in humans by taking and analyzing blood samples in study areas which was not done owing to resource limitation. Thus, aflatoxin levels in maize were used as a surrogate for exposure to aflatoxins. Aflatoxin levels in maize provide a good indication of aflatoxin exposure in humans relying on maize as their staple food (Lewis *et al*., 2005).

2) The analysis of aflatoxin causing fungi species among the sampled maize which was also not done owing to limitation of resources.

3) The study was limited two sub-counties and was not extended in many different regions that are affected by aflatoxin contamination owing to inadequate resource availability. The study also did not monitor seasonal changes on aflatoxin contamination over a long period of time.

3.11 Ethical considerations

The study adhered to the ethical principles of research, which included obtaining permission from relevant authorities as well as informed consent from respondents. Ethical approval was obtained from KEMRI Ethical Review Committee. Further Clearance was also obtained from local administration prior to conducting the study. Informed consent was obtained from respondents and a consent form was signed prior to the interview.

Maize samples were purchased from households. Confidentiality of the information provided by informants was ensured. The results of the study were to be disseminated to the community by government and agencies through seminars and meetings.

CHAPTER FOUR`
RESULTS

4.1 Descriptive results for socio-economic and demographic characteristics and maize pre- storage and storage practices

4:1:1Socio-economic and demographic characteristics of respondents

4.1.1.1 Age and sex of study respondents

The mean age of the respondents (household heads or their representatives) was 47.4 years (95% CI= 45.7 to 49.2) in Kibwezi, and 46.5 (95% CI =46.5 to 48.4) in Kilome (n=240). Although there was a difference in mean age of respondents in the two study sites, the difference was not statistically significant (t_{238} =0.693, P>0.5).

The sex of respondents who were heads of households or their principal representatives/assistants found in households at time of interview, were 102 (42.5%) male and 138 (57.5%) female in Kibwezi, while in Kilome male accounted for 112 (46.7%) and female 128 (53.3%). In both study sites there were more female respondents than male.

4.1.1.2 Marital status of respondents

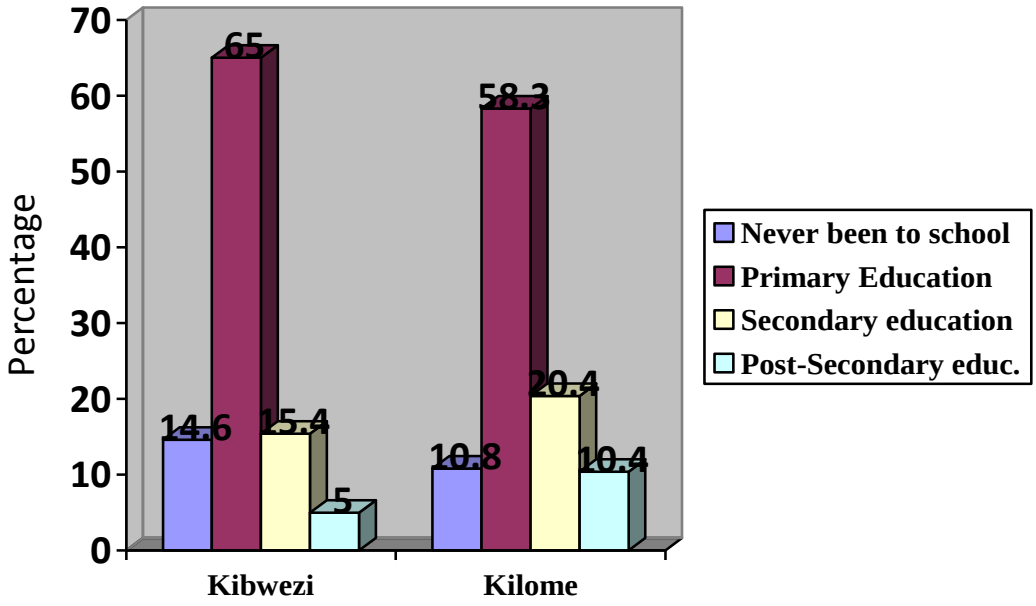
In Kibwezi married respondents accounted for 68.8%, while in Kilome the married respondents accounted for 79.6% (Table 4.1).

Table 4.1 Marital status of respondents

Marital status	Kibwezi		Kilome	
	No.of respondents (n=240)	Percent (%)	No.of respondents (n=240)	Percent (%)
Married	165	68.8	191	79.6
Single	17	7.1	11	4.6
Divorced	13	5.4	11	4.6
Seperated	15	6.2	7	2.9
Widowed	30	12.5	20	8.3

4.1.1.3 Highest education levels of respondents

Regarding highest level of education attained by respondents, results indicated that majority of respondents, 156 (65.0%) in Kibwezi and 140 (58.3%), in Kilome had primary level of education**.** Those who had secondary education accounted for 37 (15.4%) in Kibwezi and 49 (20.4%) in Kilome, while those who had post-secondary education accounted for 12 (5.0%) in Kibwezi and 25 (10.4%) in Kilome (n=240). Figure 4.4 shows highest education levels of respondents.



.

Figure 4.4 Highest Education Levels of Respondents

4.1.1.4 Religious affiliation of respondents

Majority of respondents, 81.7% in Kibwezi and 78.3% in Kilome, were Protestants. This was followed by Catholics, 6.7% in Kibwezi and 17.5% in Kilome (Table 4.2).

Table 4.2 Religious affiliations of respondents

Category of religious affiliation	Kibwezi		Kilome	
	No.of respondents (n=240)	Percent	No.of respondents (n=240)	Percent
Non-religious	21	8.8	8	3.3
Catholic	16	6.7	42	17.5
Protestant	196	81.7	188	78.4
Other Christians	1	0.4	0	0
Traditional religion	1	0.4	1	0.4
SDA	0	0	1	0.4
Muslim	5	2.1	0	0

4.1.1.5 Main occupations of respondents

The main occupation of respondents was farming which accounted for 196 (81.7%) in Kibwezi (n=240) and 184 (76.7%) in Kilome (n=240) (Figure 4.5).

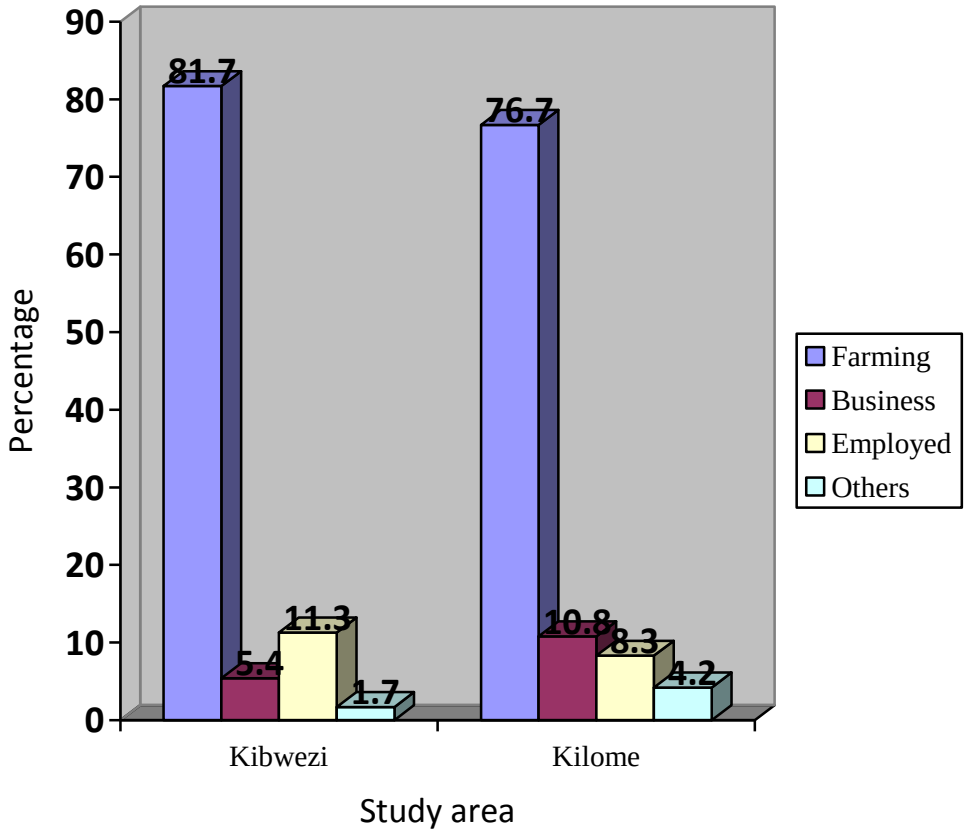


Figure 4.5 Main occupations of repondents

4.1.1.6 Income of households

Majority of the householdss, 72.9% in Kibwezi and 63.8% in Kilome, had income in the range of between Ksh 0 and 5000. This was followed by those who had income range of between Ksh 5001 and 10000 who accounted for 17.9% in Kibwezi and 23.8% in Kilome (Table 4.3).

Table 4.3 Income of households

Income Range	Kibwezi		Kilome	
	No.of households(n=240)	Percent	No.of households (n=240)	Percent
Ksh 0 to 5000	175	72.9	153	63.8
Ksh 5001 to 10000	43	17.9	57	23.8
Ksh 10001 to 15000	13	5.4	16	6.7
Ksh 15001 to 20000	6	2.5	10	4.2
Ksh 20001 to 25000	0	0	0	0
Ksh 25001 to 30000	3	1.3	4	1.7

The mean income of households was found to be Ksh.4862.9 (95% CI=4283.3 to 5442.6) in Kibwezi (n=240) and 4731.3 (95% CI=4192.3 to 5270.2) in Kilome (n=240). There was no significant difference in mean income of households between the two study sites (p>0.5).

4.1.2 Quantity of maize stored and utilized in households

4.1.2.1 Quantity of maize found in household store during sampling

During sample collection in the first phase (November/December 2013) the quantity of maize found in storage was estimated in Kilograms. This could give an indication of maize yield and level of poverty in the household, since maize is a staple food in the community hence an important indicator of food security. Besides, availability of food supplements income in the household.

The average (mean) quantity of maize found in household store during sampling was 31.95 Kg (95% CI=28.03 to 35.87) in Kibwezi and 78.4 Kg (95% CI=64.85 to 91.85) in Kilome. The mean quantity of maize indicates that there was more maize in storage in Kilome than in Kibwezi. It also implied that maize yield for first season was low.

4.1.2.2 Quantity of maize consumed in household

During sampling the amount (quantity) of maize found in storage was estimated in Kilograms and recorded. The mean quantity of maize (e.g. muthokoi, ugali, Githeri, uji etc.) consumed per person per day in household was 1.12 Kg (95% CI=1.083 to 1.174) in Kibwezi and 0.82 Kg (95% CI=0.739 to 0.891 in Kilome.

4.1.3 Knowledge and awareness of aflatoxin

4.1.3.1 Awareness levels

Most respondents were aware of aflatoxin. In Kibwezi 224 (93.3%) of respondents were aware of aflatoxin while in Kilome 221 (92.5%) of respondents were aware of aflatoxin. This indicates that aflatoxin awareness level was slightly higher in Kibwezi than in Kilome.

4.1.3.2 Source of information for knowledge/awareness of aflatoxin

The main source of information in both study sites was electronic media at 48.3% in Kibwezi and 65.0% in Kilome.This was followed by Print media at 5.0% in Kibwezi and 18.8% in Kilome. Other sources of information included schools, Churches, Mosques and Public meetings (Table 4.4).

Table 4.4 Sources of information for knowledge/awareness of aflatoxin

Source of information	Kibwezi		Kilome	
	No.of respondents(=240)	Percent	No.of respondents (n=240)	Percent
Print Media	12	5.0	45	18.8
Electronic Media (Radio,TVetc.)	116	48.3	156	65.0
School	7	2.9	19	7.9
Church	79	35.9	12	6.5
Mosque	8	3.3	0	0
Public meeting	11	5.6	6	2.8
Others	7	2.9	2	0

4.1.3.3 Awareness of health problems associated with improperly stored maize

About 95.8% of respondents in Kibwezi were aware of health problems resulting from consumption of maize not dried and stored in a proper manner while in Kilome it was 90.4%. Those who did not know accounted for 4.2% in Kibwezi and 9.6 % in Kilome.

The highest proportion of respondents, 45.0% in Kibwezi and 30.4% in Kilome, cited abdominal pains as the main health problem resulting from consumption of aflatoxin contaminated maize. This was followed by diarrhea at 15.0% and 13.3 % in Kibwezi and Kilome, respectively. Other health problems cited were fever, cancer, and death (Table 4.8). Majority of respondents, 69.1% in Kibwezi and 59.9% in Kilome, identified correct health problems which are abnominal pains, fever, headache, diarrhea and cancer (Table 4.5).

Table 4.5 Awareness of perceived health problems associated with improperly stored maize

Health Problem	Kibwezi		Kilome	
	No.of respondents(n=240)	Percent	No.of respondents (n=240)	Percent
Abnominal pains	108	45.0	73	30.4
Fever	8	8.3	17	7.1
Headache	2	0.8	8	3.3
Diarrhoea	36	15.0	32	13.3
Cancer	0	0	14	5.8
Death (%)	76	31.7	91	37.8
Others	10	4.2	5	2.1

4.1.3.4 Knowledge on identification of maize suspected to be contaminated with aflatoxin

About 156 (65.0%) of respondents in Kibwezi said they could identify maize contaminated with aflatoxin while in Kilome 160 (66.7%) said they could do so. Further, 209 (87.1%) of respondents in Kibwezi and 229 (95.4%) in Kilome said they knew signs of aflatoxin in maize (n=240).

4.1.3.5 Knowledge of of signs of aflatoxin contamination in maize

For the respondents who knew signs of maize suspected to be aflatoxin contaminated, majority of them (66.9 % in Kibwezi and 77.2 % in kilome) identified green colour as sign of aflatoxin contamination in maize, followed by rotting which was 33.8%in Kibwezi and 26.9% in Kilome. Majority of respondents, 91.4% in Kibwezi and 92.4% in Kilome, identified correct signs of aflatoxin contamination in maize which are green colour and rotting of maize (Table 4.6).

Table 4.6 Knowledge on signs of aflatoxin contamination of maize

Signs of aflatoxin	Kibwezi	Kilome		
	No.of respondents(n=240)	Percent	No.of respondents(n=240)	Percent
Green colour	120	57.4	177	77.2
Germination	14	6.7	11	5.1
Rotting	71	34.0	35	15.2
Others	0	0	5	2.1
Don’t know	4	1.9	1	0.4

4.1.3.6 Knowledge of causes of aflatoxin contamination of maize

Inadequate drying was viewed as the main cause of aflatoxin by the highest proportion of respondents, 46.3% of in Kibwezi and 38.8% in Kilome. This was followed by improper storage at 35.0% in Kibwezi and 28.8% in Kilome. Other causes of aflatoxin fonntamination included harvesting maize when not dry, early harvesting and fungi/mould growth on maize. Majority of respondents, 96.7% in Kibwezi and 97.2 % Kilome, identified correct causes of aflatoxin contamination of maize which are inadequate drying, improper/poor storage, harvested not properly dry and fungi/mouldy (Table 4.7).

Table 4.7 Knowledge of causes of aflatoxin contamination of maize

Causes of aflatoxin	Kibwezi		Kilome	
	No.of repondents(n=240)	Percent	No.of respondents(n=240)	Percent
Inadequate drying	111	46.3	81	38.8
Improper/poor storage	84	35.0	69	28.8
Harvested not properly dry	30	12.5	66	27.5
Early harvest	3	1.3	17	9.1
Fungi/mouldy	7	2.9	5	2.1
Others	5	2.1	2	0.8

4.1.3.7 Awareness on effect of altitude/climate variation of aflatoxin contamination

Data was collected on perception of respondents on whether altitude/climate affects aflatoxin contamination of maize. Respodents were asked which altitude/climate (high or low) increased aflatoxin contamination of maize. In Kibwezi 131 (54.6%) of respondents thought low altitude/climate increased aflatoxin contamination while 109 (45.4%) thought it was high altitude/climate (n=240). In Kilome 149 (62.1%) of respondents thought high altitude/climate increased aflatoxin contamination while 91 (37.9%) thought it was low altitude/climate (n=240). The 91 (37.9%) of respondents who said low altitude increased aflatoxin contamination were correct according to aflatoxin analysis results (n=240).

4.1.3.8 Awareness on effect of different maize harvest seasons on aflatoxin contamination

Data was collected on perception of respondents on whether they thought different maize harvest seasons affected aflatoxin contamination of maize. Respondents were asked which maize harvest season (Long or short season of maize harvest) they thought increased aflatoxin contamination of maize. In Kibwezi 121 (50.4%) of respondents thought short season increased aflatoxin contamination of maize while 119 (49.6%) thought it was the long season (n=240). In Kilome 147 (61.3 %) of respondents thought short season increased aflatoxin contamination while 93 (38.8%) thought it was long season (n=240).

4.1.3.9 Knowledge on prevention of aflatoxin

Majority of respondents, in Kibwezi 63.3% and in Kilome 73.3%, cited proper drying of maize after harvest as a way of prevention of aflatoxin, followed by proper storage at 20.8% in Kibwezi and 16.2 % in Kilome. Harvesting of mature maize was cited by 11.7% in Kibwezi and 7.5% in Kilome.Those who did not know accounted for 4.2% in Kibwezi and 1.7% in Kilome. Majority of respondents, 95.8% in Kibwezi and 97.0% in

Kilome, mentioned proper drying of maize after harvest, proper storage and harvesting of mature dry maize which all contribute to prevention of aflatoxin (Table 4.8).

Table 4.8 Knowledge on prevention of aflatoxin

Views on prevention aflatoxin	Kibwezi		Kilome	
	No.of respondents(n=240)	Percent	No.of respondents(n=240)	Percent
Proper drying of maize after harvest	152	63.3	176	73.3
Proper storage	50	20.8	39	16.2
Harvesting of mature dry maize	28	11.7	18	7.5
Others	0	0	3	1.3
Don't know	10	4.2	4	1.7

4.1.4 Maize pre-storage practices

4.1.4.1 Removal of outer cover of maize cob during harvest and duration in days of maize in field before harvest

Regarding removal of outer covering of maize cob during harvesting**,** in Kibwezi 218 (90.8%) households removed the outer cover (n=240) while 22 (9.2%) did not (n=240). In Kilome 238 (99.2%) removed outer cover while 2 (0.8%) did not (n=240).

On how long mature maize had been left in the field before harvesting, the mean duration was 24.2 days (95% CI=23.55 to 26.75) in Kibwezi and 45.7 days (95% CI= 41.95 to 49.53) in Kilome (n=240).

Regarding length of exposure of maize to dry immediately after harvest and before storing in their households' storage facilities for later use, the mean duration of drying was 14.2 days (95% CI=13.08 to 15.33) in Kibwezi and 16.6 days (95% CI=15.45 to 17.69) in Kilome (n=240).

4.1.4.2 Methods used by households for drying of maize after harvest

Most respondents, 231 (96.3%) for both Kibwezi and Kilome, dried their maize in open sun while 4 (1.6%) of respondents in Kilome and 0% (none) in Kibwezi dried their maize in the shade. Those who used other methods such as placing maize above fire place, kitchen granary were 9 (3.8%) in Kibwezi (n=240) and 5 (2.1%) in Kilome (n=240). Figure 4.6 below indicates methods used by households to dry maize after harvest.

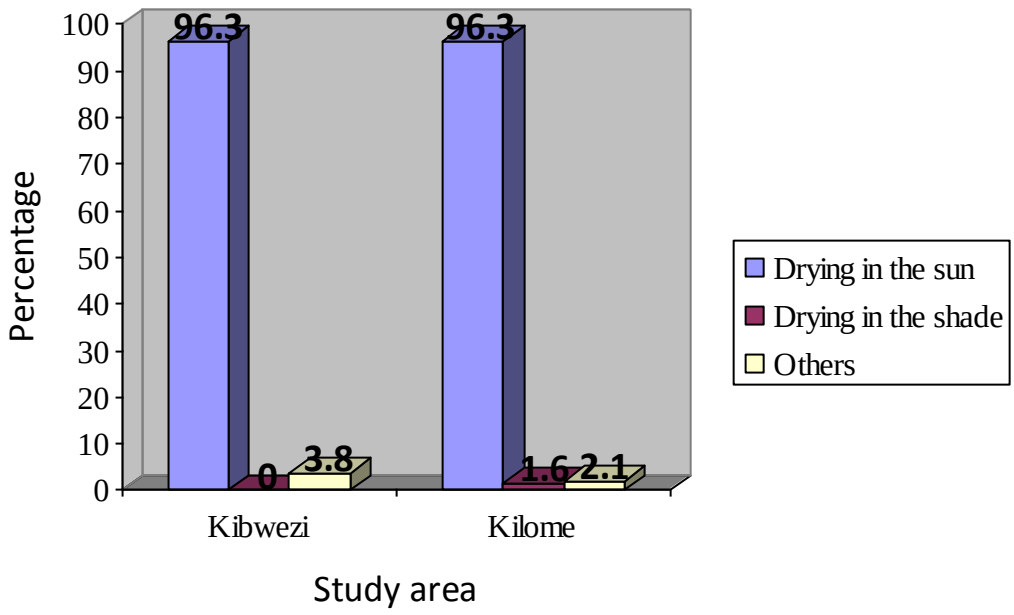


Figure 4.6 Methods used by households to dry maize after harvest

4.1.4.3 Placing of Maize on top of any material underneath during drying

Majority of households, 210 (87.5%) in Kibwezi and 184 (76.7%) in Kilome, placed their maize on top of some material (polythene sheet, iron sheet etc.) on the ground during drying to prevent maize being in direct contact with the ground surface (n=240).

Among the reasons given for drying maize on top of some materials were to prevent contamination 35.4% and 45.6 %, prevent rotting 24.6% and 21.7%, to dry faster 6.1% and 1.1%, and prevent pests 6.1 and 1.1% for Kibwezi and Kilome respectively. Other reasons given include improving maize quality which accounted for 6.0% and 4.9% for Kibwezi and Kilome Kilome respectively (Table 4.9).

Table 4.9 Reasons for Placing Material underneath during Drying of Maize

Reason for placing material underneath during drying of maize	Kibwezi		Kilome	
	No.of respondents *(n=210)	Percent	No.of respondents (n=184)	Percent
To Prevent contamination	74	35.4	84	45.6
To prevent rotting	52	24.6	40	21.7
To dry faster	58	27.6	49	26.6
To prevent pests	16	6.1	2	1.1
Others	10	6.0	9	4.9
Total	210	100.0	184	100.0

*****n is the number of respondents who placed material underneath during drying maize

4.1.4.4 Reasons for drying Maize Properly

Among the various reasons given for drying maize properly were to prevent aflatoxin contamination 55.4% and 56.3 %, prevent rotting 34.6% and 28.3%, avoid germination 10.8% and 12.5%, and avoid insect pests infestation 4.2% and 6.3% for Kibwezi and Kilome respectively (Table 4.10).

Table 4.10 Reasons for drying maize properly

Reasons for drying maize properly to attain moisture content of less than 14%	Kibwezi		Kilome	
	No.of respondents (n=240)	Percent	No.of respondents (n=240)	Percent
To prevent from rotting	83	34.6	68	28.3
To avoid germination	14	5.8	30	12.5
To prevent aflatoxin contamination	133	55.4	135	56.3
To Avoid insect/pest infestation	10	4.2	15	6.3
Others	0	0	4	1.7

4.1.4.5 Cleaning of Maize Prior to Storage

In Kibwezi majority of respondents 222 (92.5%), cleaned their maize to remove broken grains, fine materials, among other undesirable contaminants, prior to storage (n=240), while in Kilome 204 (85.0%) did so (n=240).

4.1.5 Maize storage practices

4.1.5.1 Storage of maize in households

In Kibwezi 73.3% of households stored their maize in bags but on raised platform, 3.3% stored their maize in bags directly on the floor, 15.8% on traditional crib, 6.3% in cobs directly on the floor, 0.8% stored their maize under or top of roof of building (attic) and 0.4% stored stored their maize on improved maize crib (n=240). In Kilome 82.5% of households stored their maize in bags, but on raised platform, 14.6% stored their maize in bags directly on the floor, 1.3% on traditional maize crib, 0.8% stored their maize on improved maize crib, 0.4 % stored their maize under or on top of the roof of building (attic) and another 0.4% stored in cobs directly on the floor (n=240). None of the households in both study sites shelled and stored maize in sealed containers (Table 4.11).

Table 4.11 Types of maize storage practices of maize in households

Storage practices	Kibwezi		Kilome	
	No.of households (n=240)	Percent	No. of households (n=240)	Percent
In bags directly on the floor	8	3.3	35	14.6
In bags, but on raised platform	176	73.3	194	80.8
Under or on top of the roof of building(arctic)	2	0.8	1	0.4
In cobs, directly on the floor	15	6.3	1	0.4
On traditional maize cribs	38	12.8	5	2.1
On improved maize cribs	1	0.4	4	1.7
Shelled and stored in sealed containetrs	0	0.0		0.0

4.1.5.2 Proportion of households which stored maize properly

Storage of maize practices was further categorized into proper storage and improper storage. Proper maize storage was maize which was stored either in raised platform, granary, traditional cribs (those which are raised from ground) or improved cribs. Improper maize storage was maize which was stored directly on the floor regardless of whether in bags or in cobs form. In Kibwezi 209 (87.3 %) households had their maize stored properly while in Kilome 204 (85.0 %) households stored their maize properly.Storage methods were the same for maize harvested in first season and second season.

4.1.5. 3 Arrangement of maize stored in bags

Observations were made on the maize which was stored in bags and their pattern of arrangement. Majority of households 125 (67.9%) and 140 (61.1%) stored their maize in bags arranged in stacks without spaces between them, 36 (19.6%) and 84 (36.8%) arranged their bags of maize in stacks with space between them, and 21 (11.5%) and 3 (1.3%) arranged their bags haphazardly (without any order) in Kibwezi and Kilome respectively. Other arrangements which included bags arranged with some order but no interspaces in between were 2 (1.0%) in Kibwezi (n=240) and 2 (1.0%) in Kilome (n=240). The 19.6% of respondents in Kibwezi and 61.1 % respondents in Kilome who arranged their bags of maize in stacks with space between them had proper arrangements (Figure 4.7).

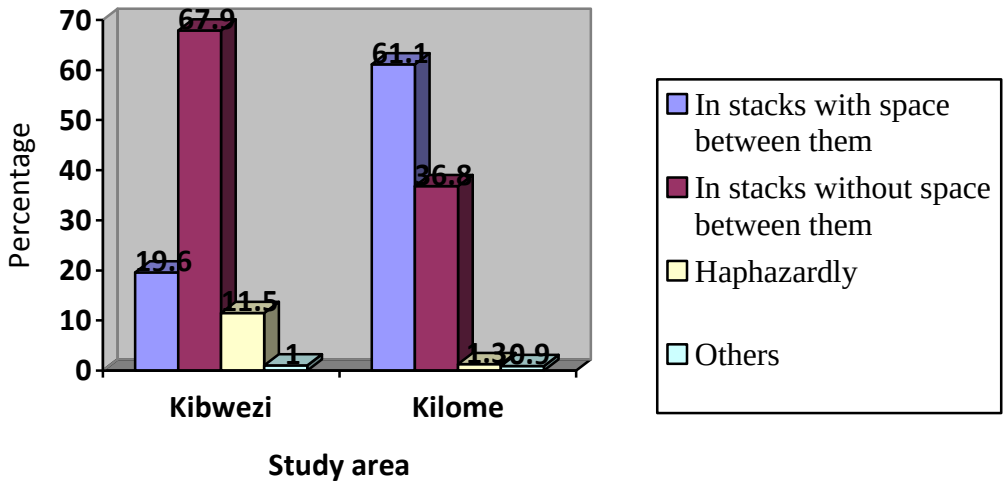


Figure 4.7 Arrangement of bags of maize in store

4.1.5.4 Form in which maize is stored in household

The form in which maize was stored was observed and recorded in the checklist. Majority of households stored their maize in grains form 75.2% and 92.1%, in cob form 18.1% and 3.8%, and 3.4%, in cob with outer covering form 3.4% and 1.7% for Kibwezi and Kilome respectively. Other forms of storage included storage in de-husked form, accounting for 3.4% in Kibwezi and 2.5% in Kilome (n=240) (Table 4.12).

Table 4.12 Form in which maize was stored in households

Form of maize storage	Kibwezi		Kilome	
	N.of hopuseholds (n=240)	Percent	No.of households (n=240)	Percent
In cob form	43	18.1	9	3.8
In grains form	179	75.2	221	92.1
In cob with outer covering form	8	3.4	4	1.7
Others	8	3.4	6	2.5

4.1.5.5 Duration of maize in store and frequency of store cleaning

Kilome had slightly higher mean storage time, 64.9 days (95% CI=58.9 to 70.8) than Kibwezi which had 63.5 days (95% CI=54.7 to 72.4) (n=240).

The respondents were further asked how often they cleaned their maize stores in the last three month. This was then calculated to mean frequency of store cleaning per three month period. The mean frequency was 2.5 times (95% CI=2.27 to 2.68) in Kibwezi while in Kilome it was 2.8 times (95% CI=2.62 to 3.04) (n=240). Kilome households cleaned their stores slightly more frequent than Kibwezi households.

4.1.5.6 Insect pests affecting maize in storage

For maize harvested in first season, 60 (25.0%) households in Kibwezi had their maize infested with insect pests while in Kilome 50 (20.8%) households had their maize infested. For maize harvested in second season, 110 (45.8%) households in Kibwezi had their maize infested with insect pests while in Kilome 100 (41.7%) households had their maize infested.

The 68 household owners in Kibwezi and 98 household owners in kilome whose maize was infested with insect pests took various actions to try to get rid of insect pests. In Kibwezi majority of respondents 75.6% sprayed with pesticides, 4.9% applied herbs, 12.2% did nothing while 3.7% did not know what to do (n=240). In Kilome majority of respondents 63.7% sprayed with pesticides, 11.9% applied herbs, 14.0% did nothing while 5.2 % did not know what to do. Other actions which were taken included sieving and smoking (n=240). Those who sprayed with pesticides, 75.6% in Kibwezi and 63.7% in Kilome, took proper action as this could be more effective in getting rid of insect pests (Table 4.13).

Table 4.13 Action taken to remove insect pests affecting maize in household

Action taken to remove pests affecting maize	Kibwezi		Kilome	
	No.of households *(n=68)	Percent	No.of households *(n=98)	Percent
Sprayed with pesticides	51	75.6	62	63.7
Applied herbs	3	4.9	12	11.9
Others	3	3.7	6	5.2
Did nothing	8	12.2	13	14.0
Don’t know	3	3.7	5	5.2

***** n is number of respondents who took action to remove insect pests affecting maize in households

4.1.5.7 Conditions of maize stores

Regarding cleaniness, in Kibwezi 54.2% of maize stores were maintained clean while in Kilome 76.2% of maize stores were maintained clean. On ventilation, in Kibwezi 37.1% of maize stores were properly ventilated naturally while in Kilome it was 69.6%. On natural lighting, 40.0% of stores in Kibwezi had adequate natural lighting while in Kilome it was 72.9%. Regarding stocking of the stores, in Kibwezi 67.9% of stores were overstocked with other non-food items while in Kibwezi it was 36.3% (n=240) (Table 4.14).

Table 4.14 Conditions of maize stores

Conditions observed	Kibwezi		Kilome	
	No.of maize stores (n=240)	Percent	No. of maize stores (n=240)	Percent
Maize store was Clean	130	54.2	183	76.2
Maize store was properly ventilated naturally	89	37.1	167	69.6
The maize store had adequate natural lighting	96	40.0	174	72.9
Maize store was over stocked	163	67.9	87	36.3

4.1.5.8 Reasons for storing maize properly

Various reasons were given as to why maize should be stored properly (i.e. in well ventilated clean store and not directly on the floor.A higher proportion 43.8% and 47.5 % did so to avoid dirt, while 40.0% and 19.2% did so to avoid rotting/spoilage, for Kibwezi and Kilome respectively. Other reasons given were to avoid/reduce moisture, avoid aflatoxin contamination and to let maize dry (n=240) (Table 4.15).

Table 4.15 Reasons for storing maize in well ventilated clean store

Reasons for storing maize in well ventilated clean and not directly on the floor	Kibwezi		Kilome	
	No.of respondents (n=240)	Percent	No.of respondents (n=240)	Percent
To avoid aflatoxin contamination	21	8.8	20	8.3
To avoid dirt	105	43.8	114	47.5
To avoid/reduce moisture content	7	2.9	47	19.6
To avoid rotting/spoilage	96	40.0	46	19.2
To let maize dry	4	1.7	11	4.6
Others	1	0.4	1	0.4
Don’t know	1	2.5	1	0.4

4.2 Maize sample analysis results for aflatoxin and physical parameters

4.2.1 Maize physical parameters and aflatoxin levels for first season maize harvest

Regarding maize physical parameters for first season maize (August/September) for Kibwezi, discolouration of maize was observed in 20.8 % of maize samples, mouldy condition was observed in 20.8 % of maize samples and insect/pest infestation was observed in 25.0% of maize samples. The mean moisture content was 12.78% (95% CI=12.68 to 12.88) (n=24). Regarding physical parameters for first season maize for Kilome, discolouration of maize was observed in 12.5% of samples, mouldy condition was observed in 12.5 % and insect/pest infestation was observed in 20.8 %. The mean moisture content was 12.85% (95% CI=12.75 to 12.95) (n=24). The ELISA test results of 24 random maize samples gave 25.0% and 4.2 % aflatoxin positivity for Kibwezi and Kilome respectively at 1.75 µg/Kg cut –off detection point (Table 4.16).

Table 4.16 Physical parameters and aflatoxin level for first season maize

Study sites	Maize Samples with Discoloration		Maize Samples with Insect pests		Maize Samples with Mould		Maize Samples with Moisture content above 13%		Maize Samples with Aflatoxin detected cutt-off point 1.75ug/Kg		Maize Samples with Aflatoxin levels above 10 ug/Kg	
n=24	No	%	No	%	No	%	No	%	No	%	No	%
KBZ	5	20.8	6	25.0	5	20.8	4	16.7	6	25.0	1	4.2
KLM	3	12.5	5	20.8	3	12.5	4	16.7	1	4.2	0	0

4.2.3 Aflatoxin sub-types and quantities for maize harvested in first season

The maize samples that were positive for aflatoxin when analyzed using HPLC test showed presence of AFB1, AFB2, AFG1 and AFG2 at mean 30.08 µg/Kg, 0.88 µ/Kg, 0.48 µg/Kg, and 0.25 µg/Kg respectively, for Kibwezi (n=6). AFB1 was most abundant (30.08 µg/Kg) while AFG2 was the least abundant (0.25 µg/Kg). For Kilome, maize samples which were positive for aflatoxin when analysed using HPLC test showed presence of AFB1, AFB2, AFG1 and AFG2 at mean 1.55µg/Kg, 0.1 µ/Kg, 0.1 µg/Kg, and 0.05 µg/Kg respectively (n=1). AFB1 was also most abundant at 1.55µg/Kg while AFG2 was the least at 0.05µg/Kg. The high aflatoxin B1 in one of the samples could be attributed to high level of contamination due to poor stoarage conditions as the lot sampled was placed directly on the floor (Table 4.17).

Table 4.17 Aflatoxin sub-types and quantities for first season

S/No	AF B1(µg/Kg)		AF B2(µg/Kg)		AF G1(µg/Kg)		AF G2(µg/Kg)	
	KBZ *(n=6)	KLM (n=1)	KBZ (n=6)	KLM n=1	KBZ (n=6)	KLM (n=1)	KBZ (n=6)	KLM (n=1)
01	6.0	1.55	0.8	0.1	0.5	0.1	0.5	0.05
02	3.6	-	0.2	-	0.3	-	0.1	-
03	6.3	-	0.7	-	0.5	-	0.3	-
04	159.5	-	3.2	-	1.2	-	0.3	-
05	2.8	-	0.5	-	0.3	-	0.2	-
06	2.3	-	0.15	-	0.05	-	0.1	-
Mean	30.08	1.55	0.88	0.1	0.48	0.1	0.25	0.05

*****n is the number of maize samples which tested positive on ELISA test

4.2.4 Maize physical parameters and aflatoxin levels for second season maize

Regarding maize physical parameters second season maize (February/March) for Kibwezi, discolouration of maize was observed in 45.8% of samples, mouldy condition was observed in 37.5% of maize samples, and insect pest infestation was observed in 45.8% of maize samples. The mean moisture content was 13.63% (95% CI=13.23 to 14.03) (n=24).

Regarding physical parameters of maize for Kilome, discolouration of maize was observed in 33.3% of samples, mouldy condition was observed in 25.0 %, and insect pest infestation was observed in 41.7%. The mean moisture content in was 13.48% (95% CI=13.17 to 13.79) (n=24).

The ELISA tests results of the 24 random maize sub-samples for second maize harvest season gave 33.3% and 12.5% aflatoxin positivity for Kibwezi and Kilome respectively at 1.75µg/Kg cut-off detection point (n=24) (Table 4.18).

Table 4.18 Physical parameters and aflatoxin level for second season maize

Study sites	Maize Samples with Discoloration		Maize Samples with Insect pests		Maize Samples with Mould		Maize Samples with Moisture content above 13%		Maize Samples with Aflatoxin (detection cut-off point 1.75ug/Kg)		Maize Samples with Aflatoxin levels above 10 ug/Kg	
n=24	No	%	No	%	No	%	No	%	No	%	No	%
KBZ	11	45.8	11	45.8	9	37.5	15	62.5	8	33.3	5	20.8
KLM	8	33.3	10	41.7	6	25.0	14	58.3	3	12.5	2	8.3

4.2.5 Aflatoxin Sub-types and quantities for maize harvested in second season

The samples that were positive for aflatoxin when analysed using HPLC test, showed presence of AFB1, AFB2, AFG1 and AFG2 at mean 26.64 µg/Kg, 9.47 µg/Kg, 0.55 µg/Kg, and 1.05 µg/Kg respectively, for Kibwezi (n=8). AFB1 was most abundant (26.64 µg/Kg) while AFG2 was the least abundant (1.05 µg/Kg). For Kilome, maize samples which were positive on ELISA test when analyzed using HPLC test showed presence of AFB1, AFB2, AFG1 and AFG2 at mean 26.7 µg/Kg, 5.5 µg/Kg, 1.06 µg/Kg, and 1.93 µg/Kg respectively (n=3). AFB1 was also most abundant at 26.7µg/Kg while AFG2 at 1.93 µg/Kg, was the least (Table 4.19).

Table 4.19 Aflatoxin sub-types and quantities for second season maize in Kibwezi

S/No	AFB1(µg/Kg)		AFB2(µg/Kg)		AFG1(µg/Kg)		AFG2(µg/Kg)	
	KBZ *(n=8)	KLM (n=3)	KBZ (n=8)	KLM (n=3)	KBZ (n=8)	KLM (n=3)	KBZ (n=8)	KLM (n=3)
01	6.8	12.9	1.0	1.9	0.2	0.0	0.2	0.0
02	1.4	5.4	0.3	0.0	0.0	0.0	0.0	0.0
03	1.6	61.8	0.0	14.6	0.0	3.2	0.0	5.8
04	44.9	-	6.6	-	0.0	-	0.5	-
05	99.5	-	14.8	-	3.4	-	6.1	-
06	13.8	-	1.1	-	0.0	-	0.0	-
07	35.5	-	3.3	-	0.8	-	1.6	-
08	9.6	-	1.3	-	0.0	-	0.0	-
Mean	26.64	26.7	9.47	5.5	0.55	1.06	1.05	1.93

*n is the number of maize samples which tested positive on ELISA test

4.2.6 Temperature and Humidity of Kibwezi and Kilome study sites

Temperature and Humidity of the two study sites were taken and recorded in households sampled where maize sample was collected for analysis using hygrothermometer instrument. The recordings were then computed into means for each study site. In the first phase (first season of maize harvest) of maize sample collection in October/November the mean temperature was 30.7^{o}C (95% CI=30.47 to 30.91) in Kibwezi, and 23.6 ^{o}C (95% CI=23.24 to 23.81) in Kilome, while humidity was 45.8% (95% CI=45.14 to 46.55) in Kibwezi and 32.3% (95% CI=31.84 to 32.78) in Kilome. In the second phase in April/May of maize sample collection the mean temperature was 31.6^{o}C (95% CI=30.26 to 31.18) in Kibwezi and 25.4% ^{o}C (95% CI=25.02 to 25.71) in Kilome, while humidity was 49.9% (95% CI=48.85 to 50.99) in Kibwezi, and 42.4% (95% CI=40.02 to 44.83) in Kilome.

4.3 Comparison of maize pre-storage and storage practices, physical parameters aflatoxin levels and moisture content

4.3.1 Comparison of maize storage and pre-storage practices

For maize harvested in first season, the mean duration of maize stay in the field before harvest was found to be 24.2 (95% CI=23.55 to 26.75) in Kibwezi and 45.7 (95% CI= 41.95 to 49.53) in Kilome. The difference of the mean duration of maize stay in field before harvest between the two sites was significant at P<0.001. Mean duration of drying was found to be 14.21 days (95% CI=13.08 to 15.33) in Kibwezi and 16.57 days (95% CI=15.45 to 17.69) in kilome. The difference of the mean duration of maize drying between the two sites was significant at P<0.001.

In Kibwezi the mean storage duration of maize in storage was 63.5 days (95% CI=54.7 to 72.4) while in Kilome it was 64.9 days (95% CI=58.9 to 70.8). The difference of the mean duration of maize storage between the two sites was not significant at P>0.05.

For maize harvested in second season, the mean duration of maize stay in the field before harvest was found to be 42.7 (95% CI=31.83 to 53.67) in Kibwezi and 41.38 (95% CI= 35.28 to 47.47) in Kilome. The difference of the mean duration of maize stay in field before harvest between the two sites was not significant (P>0.05).The mean duration of drying was found to be 17.08 days (95% CI=12.79 to 21.38) in Kibwezi and 19.71 days (95% CI=15.81 to 23.61) in kilome. The difference of the mean duration of maize of maize drying between the two sites was significant at P<0.01

In Kibwezi the mean duration of maize in storage was 64.35 days (95% CI=57.98 to 70.71) while in Kilome it was 50.21 days (95% CI=46.15 to 54.27). The difference of the mean duration of maize storage between the two sites was significant at P<0.001. Table 4.20 shows comparison of results of maize storage and pre-storage practices between Kibwezi and Kilome for maize harvested in first and second season.

Table 4.20 Comparison of maize storage and pre-storage practices between Kibwezi and Kilome for first and second season maize

Pre-storage Practices	First season maize			Second season maize		
Variable/parameter	Kibwezi	Kilome	*Sig t test	Kibwezi	Kilome	Sig t test
Mean duration of maize in field before harvest (days)	25.2	45.7	$t_{24=}$ 6.114 P<0.001	42.7	41.4	$t_{24=}$ 0.816 P>0.05
Mean duration of drying of maize harvest and before storage (days)	14.2	16.8	$t_{24=}$ 3.90 P<0.01	17.1	19.7	$t_{24=}$ 3.252 P<0.01
Storage	First Season Maize			Second Season Maize		
Variable/parameter	Kibwezi	*Sig t test	kilome	Kibwezi	Kilome	Sig t test
Mean duration of maize in storage (days)	63.5	$t_{24=}$ 2.39 P<0.05	64.9	64.35	50.21	$t_{24=}$ 13.953 P<0.001
% of proper maize storage	87.3	No difference	85.0	87.3	85.0	No difference

*Sig. stands for significance of t-test

4.3.2 Comparison of physical parameters of stored maize

Comparison of Physical parameters of stored maize for maize harvested in first season showed that in Kibwezi discolouration of maize was in 20.8 % of samples while in Kilome discoloration was in 12.5% of samples. Mouldy condition was in 20.8 % of maize samples of Kibwezi and 12.5 % in Kilome. Insect/pest infestation was in 25.0% of maize samples of kibwezi and 20.8 % of Kilome. For maize harvested in second season, in Kibwezi discolouration of maize was in 45.8% of samples while in Kilome discoloration was in 33.3% of samples. Mouldy condition was in 37.5% of maize samples of Kibwezi and 25.0 % of Kilome. Insect pest infestation was in 41.7% of maize samples of kibwezi and 45.8% of Kilome. Table 4.21 shows comparison of the physical parameters of maize.

Table 4.21 Comparison of physical parameters of maize

Conditions Observed		First season maize				Second season maize			
		Kibwezi		Kilome		Kibwezi		Kilome	
	Observation	No	%	No	%	No.	%	No.	%
Discolouration condition of maize (n=24)	Discoloured	5	20.8	3	12.5	11	45.8	8	33.3
	Not discoloured	19	79.2	21	87.5	13	54.2	16	66.7
Mouldy Condition (n=24)	Mouldy	5	20.8	3	12.5	9	37.5	6	25.0
	Not mouldy	19	79.2	21	87.5	15	62.5	18	75.0
Insect/pest infestation (n=24)	Infested	6	25.0	5	20.8	10	41.7	11	45.8
	Not infested	18	75.0	19	79.2	14	58.3	13	54.2

4.3.3 Comparison of aflatoxin and moisture content of maize

For maize harvested in first season, the mean moisture content was 12.78 (95% CI=12.68 to 12.88) in Kibwezi and 12.85 (95% CI=12.75 to 12.95) in Kilome. The sample positivity was 25.0 % in Kibwezi and 4.2 % in Kilome.

For maize harvested in second season, the mean moisture content was 13.6 in Kibwezi and 13.5 in Kilome. The mean total aflatoxin content was 31.8 (µ/Kg) in Kibwezi and 28.4 (µ/Kg) in Kilome and sample positivity was 33.3 % in Kibwezi and 12.5 % in Kilome. The difference of mean moisture content in Kibwezi and Kilome for maize harvested in first season was significant (t_{24} =2.203,P<0.05) whereas for maize harvested in second season it was not significant (t_{24} =0.615,P>0.05).

There was a significant difference on aflatoxin sample Positivity, i.e % of samples with >or=1.75ppm (µ/Kg) aflatoxin content, beween Kibwezi and Kilome for first and second season maize (P<0.05) (Table 4.22).

Table 4.22 Comparison of aflatoxin levels between Kibwezi and Kilome for first and second season maize harvest

	First season maize			Second season maize		
Parameter of Sample	KBZ n=24	KLM n=24	Significance of difference	KBZ n=24	KLM n=24	Significance of difference
Mean Moisture content (%)	12.78	12.85	t_{24} =2.203 P<0.05	13.6	13.5	t_{24} =0.615 P>0.05
Sample Positivity i.e % of samples with >or=1.75µg/Kg aflatoxin content	25.0	4.2	P<0.05	33.3	12.5	P<0.05

4.3.4 Comparison of maize storage practices and aflatoxin positivity

Among the storage practices, for aflatoxin positive samples in Kibwezi storage of maize in bags directly on the floor accounted for 33.3% and37.5% positivity, storage in bags but on platform 16.7% and 33.3%, in cobs directly on the floor 33.3% and 33.3% and on traditional cribs 16.7% and 16.7% , for first and second season respectively. For Kilome the only positive sample in first season was from maize stored in bags directly on the floor (100%) while in second season storage in bags accounted for 66.7% in bags directly on the floor and 33.3% in cobs directly on the floor (Table 4.23)

Table 4.23 Maize storage practice and aflatoxin positivity

Type of maize storage practice	First season				Second season(=24)			
	Kibwezi *(n=6)		Kilome (n=1)		Kibwezi (n=8)		Kilome (n=3)	
	No	%	No	%	No	%	No	%
In bags, directly on the floor	2	33.3	1	100	3	37.5	1	66.7
In bags, but on platform	1	16.7	-	-	2	33.3	0	0
In cobs, directly on the floor	2	33.3	-	-	2	33.3	2	33.3
On traditional cribs	1	16.7	-	-	1	16.7	-	-

*n is the number of maize samples which tested positive for aflatoxin on ELISA test

4:4 Analytical results for associations and correlations among maize pre-storage and storage practices and aflatoxin occurrence

4.4.1 Associations of maize pre-storage practices with aflatoxin occurrence for first season maize

Pre-storage practices were analysed with aflatoxin occurrence variable to determine their associations/correlations. Duration of drying of maize after harvest and before storage had negative correlation with aflatoxin occurrence in maize although not statistically significant in Kibwezi (Correlation r, $_{22}$ = -0.325, P>0.05), and Kilome (Correlation r,$_{22}$ = -0.341, P>0.05).

4.4.2 Associations of maize storage practices with aflatoxin occurrence for first season maize

Maize pre-storage practices were analysed with aflatoxin occurrence variable to determine their associations/correlations. Duration of maize in storage had significant positive correlation with aflatoxin content in maize in Kibwezi (Correlation r,$_{22}$=0.411, P<0.05) but not in Kilome (Correlationr,$_{22}$=0.060), P>0.05). Frequency of store cleaning had significant negative correlation with aflatoxin occurrence in Kibwezi (Correlation r,$_{22}$=-0.405, P<0.05), while in Kilome the correlation was not significant (Correlation r,$_{22}$=0.0.105, P>0.05).

Mouldy condition of maize in Kibwezi had significant association with aflatoxin occurrence ($\chi 2_{,1}$ =4.047, P<0.05) but in Kilome association was not significant ($\chi 2_{,1}$ =0.049, P>0.05).

Maize affected by pests/insects in storage had significant association with aflatoxin occurrence in Kilome (Correlation r$_{,22=}$ 0.407, P<0.05), but in Kibwezi association was not significant(Correlation r $_{22}$=0.149, P>0.05) (Table 4.24).

Table 4.24 Association of maize storage practices with aflatoxin occurrence for first season

Independent variable	**Dependent variable**	**Kibwezi**		**Kilome**	
Duration of maize in storage	Aflatoxin occurrence in maize	Correlation $r_{,22}=0.411$	P <0.050	Correlation $r_{,7}=0.105$	Pvalue=0.060 *NS, P>0.05
Frequency of store cleaning	Aflatoxin occurrence in maize	Correlation $r_{,22}=0.405$	P=0.049 **Sig (P<0.05)	$\chi 2_{,1}=1.043$	Pvalue =0.307 NS, P>0.05
Proper maize storage	Aflatoxin occurrence in maize	$\chi 2_{,1}=3.865$	Pvalue=0.049 Sig P<0.05	$\chi 2_{,1}=1.0403$	Pvalue=0.307 NS, P>0.05
Mouldy condition of maize	Aflatoxin occurrence in maize	$\chi 2_{,1}=4.047$	Pvalue=0.044 Sig P<0.05	$\chi 2_{,1}=0.049$	Pvalue=0.825 NS, P>0.05
Maize affected by pests/insects in storage	Aflatoxin occurrence in maize	Correlation $r_{22}=0.149$	Pvalue=0.487,NS P>0.05	Correlation $r_{,22=}$ 0.407	Pvalue=0.049 Sig P<0.05

* NS stands for Not significant, **Sig stands for significant

4.4.3 Association of maize pre-storage practices with aflatoxin occurrence for second season maize

In Kibwezi duration of maize in field after attaining maturity (ready for roasting) and before harvesting for drying and storage was significantly negatively correlated with aflatoxin occurrence in maize (Correlation r_{22}=-0.409, P<0.05), while in Kilome the correlation was not significant (Correlation r, 22=-0.385, P>0.05). Duration of drying of maize after harvest before storage was found to be significantly negatively correlated with aflatoxin occurrence in Kilome (Correlation r, $_{22}$=-0.404, P<0.05), but in Kibwezi the correlation was not significant (Correlation r,$_{22}$=-0.141, P>0.05).

Placing of maize on top of material during drying had significant association with aflatoxin occurrence in maize in Kibwezi ($\chi 2_{,1}$ =4.689, P<0.05), but in Kilome the association was not significant ($\chi 2_{,1}$ =3.158,P>0.05) (Table 4.25).

Table 4.25 Association of maize pre-storage practices with aflatoxin occurrence for second season maize

Independent variable	Dependent variable	Kibwezi		Kilome	
Duration of maize in field before harvest after maturity(ready for roasting)	Aflatoxin occurrence in maize	Correlation r_{22}=-0.409	P=0.047 Sig P<0.05	Correlation r,22=-0.385	Pvalue=0.063 *NS, P>0.05
Duration of drying of maize after harvest before storage	Aflatoxin occurrence in maize	Correlation $r_{,22}$=-0.141	P=0.203 NS,P>0.05	Correlation r, $_{22}$=-0.404	Pvalue=0.05 Significant at P<0.05
Placing of maize on top of material during drying	Aflatoxin occurrence in maize	$\chi 2_{,1}$ =4.689	Pvalue=0.030 Significant P<0.05	$\chi 2_{,1}$ =3.158	Pvalue =0.076 NS, P>0.05

* NS =Not significant

4.4.4 Association of maize storage practices with aflatoxin occurrence for second season maize

In Kibwezi proper maize storage was associated with aflatoxin occurrence ($\chi 2_{,1}$ =3.856, P<0.05), and in Kilome proper maize storage was significantly associated with aflatoxin occurrence in maize ($\chi 2_{,1}$ =3.854, P<0.05). Proper ventilation of store had significant association with aflatoxin occurrence in Kibwezi ($\chi 2_{,\ 1}$ =4.200, P<0.05) and in Kilome ($\chi 2_{,1}$ =4.643, P<0.05). Similarly infestation of stored maize with pests/insects had significant association with aflatoxin occurrence in Kibwezi ($\chi 2_{,1}$ =4.000,P<0.05) and Kilome ($\chi 2_{,1}$ =3.845,P<0.05) (Table 4.26).

Table 4.26 Association of maize storage practices with aflatoxin occurrence for second season

Independent variable	Dependent variable	Kibwezi		Kilome	
Infestation of stored maize with pests/insects	Aflatoxin occurrence in maize	$\chi 2_{,1}$ =4.000	Pvalue=0.046 Sig P<0.05	$\chi 2_{,1}$ =3.845	Pvalue=0.050 Sig P<0.05
Proper ventilation of store	Aflatoxin occurrence in m,aize	$\chi 2_{,1}$ =4.200	Pvalue=0.040 Sig P<0.05	$\chi 2_{,1}$ =4.643	Pvalue=0.031 Sig P<0.05
Proper maize storage	Aflatoxin occurrence in maize	$\chi 2_{,1}$ =3.856	Pvalue=0.049 Sig at P<0.05	$\chi 2_{,1}$ =3.854	Pvalue=0.049 Sig,P<0.05
Maize storage practices	Aflatoxin occurrence in maize	$\chi 2,3=10.4$	Pvalue=0.015	-	-

4.4.5 Association of knowledge/ awareness and moisture content with aflatoxin occurrence in maize

Knowledge/awareness of aflatoxin was significantly associated with aflatoxin occurrence in maize in Kibwezi ($\chi 2_{,1}$ =3.98,P=0.0485), but in Kilome knowledge/awareness of aflatoxin problem was not significantly associated with aflatoxin occurrence ($\chi 2_{,1}$ =0.149, P=0.69).

Moisture content was negatively correlated with afaltoxin occurrence in maize in Kibwezi (Correlationr, $_{22}$=-0.725, Pvalue=0.000), and in Kilome (Correlation r, $_{22}$=-0.634, Pvalue=0.01).

4.4.6 Association of pre-storage and storage practices with intermediate variables linked to Aflatoxin Occurrence in Maize

Duration of drying of maize after harvest before storage had negative correlation with moisture content in Kibwezi (Correlation r, $_{22}$=-0.355, P<0.05), and Kilome although not statistically significant (Correlation r, $_{22}$=-0.221, P>0.05).

Proper maize storage had significant association with mouldy condition of stored maize in Kibwezi ($\chi 2_{,1}$ =7.969,P=0.05) but the association was not significant in Kilome ($\chi 2_{,1}$ =0.434, P=0.51). Proper maize storage had significant association with infestation of maize with insect pests in Kibwezi ($\chi 2_{,1}$ =6.706, Pvalue=0.010),and in Kilome ($\chi 2_{,1}$ =4.938, Pvalue=0.026).

Furthermore, Proper maize storage had significant association with discoloration of maize in KIbwezi ($\chi 2_{,1}$ =28.202, Pvalue =0.000), and Kilome ($\chi 2_{,1}$ =0.098, Pvalue=0.754).

4.4.7 Focus group discussion results

Results for focus group discussion for Kibwezi and Kilome showed that aflatoxin is a problem and is caused by mould locally known as “mbuka” which infests improperly dried and stored maize. Discussion demonstrated high levels of awareness on proper maize storage and pre-storage practices. Harvesting maize at right stage of maturity, proper drying and storage were identified as main measures of preventing aflatoxin. Many participants believed that altitude/climate did not affect aflatoxin contamination of maize, but on the other hand they believed different harvest seasons affected aflatoxin contamination with February/March harvest season having higher contamination.

Many participants mentioned poverty and theft of maize at farm leading to harvest of immature/undry maize, and lack of drying facilities as main constraints/challenges affecting control of aflatoxin. They recommended households to be assisted in construction of storage granaries, and government to research and develop appropriate policies for control and prevention of aflatoxin. There was no much variation in FGD results in the two study sites. Summary of FGD results for Kibwezi and Kilome are shown in Tables 4.27 and 4.28, respectively.

Table 4.27 Kibwezi focus group discussion results

Item	Item discussed	Results
1	Problems associated with improper maize storage	1)Insect pests eg weevil infestation in maize 2)May cause aflatoxin and damage leading to loses
2	Aflatoxin and their causes	1)Maize problem caused by mould known as ‘mbuka ’in Kikamba 2)Caused by harvested wet maize not dried properly 3)Badly stored maize eg directly on floor
3	Seriousness of aflatoxin problem	1)Causes suffering and death, no known cure 2)Difficult to diagnose,most cases die before diagnosis
4	Signs of aflatoxin in suspected maize	1)Maize turns blackish, appears ash like 2)Maize smells bad, and has mildew on maize
5	Action taken to prevent Aflatoxin	1)Proper maize drying, preservation/storage 2)Frequent testing of maize,and harvesting dry maize
6	Pre-harvest and pre-storage practices	1)Harvest when stalks are dry and drooping 2)Sun dry maize, place material below when drying
7	Importance of harvesting mature maize and drying them properly before storage	1)To minimize spoilage due to pests 2) To minimize spoilage and Prevent aflatoxin

Table 4.27 Kibwezi focus group discussion results (Cont…)

Item	Item Discussed	Results
8	Main storage practices/methods	1)Stored in bags placed on the floor 2)Stored in bags placed on pallets/platform 3)Stored on granary inside house 4)Stored in maize crib outside house
9	Altitude/climate and aflatoxin contamination	1)Altitude/climate does not affect aflatoxin 2)Depends on drying and storage of maize
10	Different maize growing and harvest season and aflatoxin contamination	1)Maize planted in short rain (November/December) and harvested in February/March has a lot of Aflatoxin, because of higher temperatures 2)Maize planted in long rain (March/April) and harvested in August/September has less of aflatoxin because of lower temperatures
11	Prevention of Aflatoxin at household level	1)Plant approved seeds 2)Proper drying and storage
12	Constraints/challenges in prevention of aflatoxin	1)Lack of knowledge/ignorance, and poverty 2)Theft of maize at farm leading to harvest of immature/undry maize 3)Lack of drying facilities
13	Recommendations on prevention of aflatoxin	1)Households to be assisted to construct storage granaries

Table 4.28 Kilome focus group results

Item	Item Discussed	Results
1	Problems associated with improper maize storage	1)Insects, weevils infestation, aflatoxin in maize 2)Spoilage of maize leading to loses
2	Aflatoxin and their causes	1)Maize problem known as sumu or " kivuti 'in Kikamba 2)Caused by keeping/storing maize improperly 3)Placing maize directly on the floor
3	Seriousness of aflatoxin problem	1)Not a big problem and not serious 2)Causes suffering and death, No known treatment/cure 3)Diagnose difficult, most cases die before diagnosis
4	Signs of Aflatoxin in suspected maize	1)Maize turns blackish, indication of mould growing, if no black colouration, no aflatoxin
5	Action taken to prevent aflatoxin	1)Proper drying of maize before storage 2)Storing maize in areas with good ventilation
6	Pre-harvest and pre-storage practices	1)Removal of cob cover during harvesting 2)Sun drying of maize after harvest
7	Importance of harvesting mature maize and drying them properly before storage	1)Maize will not be infested with weevils 2)Flour from mature maize is good 3)Good seeds for planting from dried maize

Table 4.28 Kilome focus group discussion results (Cont…)

Item	Item discussed	Results
8	Main storage practices/methods	1)Maize dried in the sun prior to storage 2)Storing maize with covers removed and shredded 3)Stored in bags placed on pallets/platform 4)Stored in granary or in maize crib outside house
9	Altitude/climate and Aflatoxin contamination	1)Higher altitude areas like Kilome have higher chances of aflatoxin occurrence
10	Different maize growing and harvest season and aflatoxin contamination	1)Maize planted in short rain (November/December) and harvested in February/March has more aflatoxin, because of high humidity 2)Maize planted in long rain (March/April) and harvested in August/September has less of aflatoxin because of low humidity
11	Prevention of aflatoxin at household level	1)Maize should be stored when properly dry 2)Storage places to be clean and properly ventilated
12	Constraints/challenges in prevention of aflatoxin	1)Poverty and lack of good storage facilities
13	Recommendations on prevention of aflatoxin	1)Construction of proper storage facilities 2)Government to research and develop appropriate policies for control and prevention of aflatoxin

CHAPTER FIVE
DISCUSSION

5:1 Household socio-demographic and economic characteristics

In this study respondents in both high altitude and low altitude areas were mature adults with mean age of about 47 years. The slight mean age difference was not statistically significant (t_{240} =0.693, P>0.5), implying that there was no much variation in terms of age characteristics of respondents. It also implies that any differences which might be noted in respondents' views between the two areas could not be solely attributed to age disparities.

In this study there were more female respondents than male respondents in both study areas. This could be attributed to women being traditionally more concerned with household roles compared to their male counterparts who traditionally have beeen concerned with outdoor activities of seeking for livelihood to sustain their families. This is consistent with Kenya 2009 census report which showed that females were more than males in the population in the ratio of 100:105(KBS census report, 2009). However, although there were more females than males in both study groups, there was no significant disparity in gender composition among the two study communities.

Majority of respondents were married, but high altitude area of Kilome had more married respondents than low altitude area of Kibwezi, the difference between the two study sites being about 10%. Marriage as the primary family institution is crucial to management of family affairs at household level necessary for survival and prosperity. Besides, parents have joint responsibility for giving care to their children. They can also pool their resources and efforts together for the good upbringing of their children.

On education level, majority of respondents (over 50%) in both low and high altitude areas had attained primary education which is considered to be basic level of education

in Kenya. This implied that most respondents were sufficiently literate to respond to interview questions particularly when translated into Kiswahili language. Although in low altitude area there were more respondents with primary education than high altitude area, the high altitude area had more respondents with secondary and post secondary education. The moderate literacy level could be attributed to free primary education policy of 2003 (KNBS, 2014).

Education is important since low education level can limit or slow understanding of particular issues. Onemolease *et al.* (2005) and Akowuah *et al*. (2015), observed that low education level impeded understanding of aflatoxin causes, implications and measures to minimize its contamination. Thus, education is crucial to understanding various concepts as well as their contextual application. Education is also important as it increases general knowledge and awareness and is believed to influence positive health behaviour of a person.

In this study, over 75% of respondents in both high and low altitude areas practiced small scale farming. The main crops farmed included cereal maize, legumes and variety of fruits. Maize is a dietry staple food crop food in Makueni community, and is also staple food for about 90% of Kenya population (Wambugu *et. al*., 2009). Other occupations were practiced by few respondents, implying that the two communities are predominantly agrarian. Small scale farming was also the main source of income for the majority of households in both low and high altitude areas accounting for about 75%. Thus, the two communities derive their income mainly from farming which appeared to be the main occupation probably because the area is predominantly rural.

In this study majority (over 60%) of households were poor as they earned a montly income of less than Kshs 5000, implying that they survive on less than a dollar per day.This is corroboratd by Socio-economic Atlas Report of Kenya 2014 based on 2009 census which estimated pobverty rate for Makueni County at 60.6% (Wiensmann *et,al*,

2014). This poverty rate was higher than the national poverty rate for 2009 of 45.2% (Wiensmann *et,al*, 2014). As storage practices have cost implications, level of income in a household could have a bearing on pre-storage and storage practices as well as other positive health and social practices for well living and prosperity.

That there was a slight difference on mean income between high and low altitude areas (variance 2.8%) indicates that the two communities were almost similar economically. The high level of poverty found in this study is consistent with other findings from government departments/agencies (MPND, 2008; CBS, 2009) as well as Makueni County Demographic Profile (Ministry of Devolution and Planning (MDP, 2013). This finding is also consistent with the finding that the area is poverty stricken with majority of the people being absolute poor (MOPND-ROK, 2008; MDP, 2013). Agriculture is the main economic activity of the community and also main source of income accounting for 78% (MDP, 2013). Drought is considered to be the main cause of poverty in the area as it reduces yields from farming.

During sample collection in the first maize harvest season households were found to have little maize in storage further confirming the poverty situation. Maize being a staple food in the community is an important indicator of food security and by extention poverty.

5.2 Knowledge and awareness of aflatoxin

The level of awareness of aflatoxin was found to be substantially high in both areas as nearly everyone was aware of aflatoxin. This was consistent with a previous study which found out that nearly 100% of farmers in Makueni were aware of aflatoxin (Walker *et al.,* 2013). This could be attributed to sensitisation campaigns which had been conducted in the area following previous outbreaks of aflatoxicosis (Mwihia *et al*., 2005; CDC, 2004). It could also have been triggered by previous aflatoxicosis outbreaks which occurred in the area thus raising awareness and sensitisation on potential hazards of

aflatoxin, a view supported by FGDs conducted in the area as well as by other studies (Walter *et.al*.,2013). Results indicate that information on awareness was obtained from various sources, the main source of information in both areas being electronic media at 48.3% in low altitude areas and 65.0% in high altitude areas. This means electronic media was convenient and preferred source of information for disemination. A Previous study also found that media such as radio, TV, and newspapers were the key sources of aflatoxin awareness (Walker *et al*., 2013).

Overwhelming majority (over 90%) of respondents was aware of problems associated with improper drying and storage of maize. This finding is supported by other findings which found farmers were aware of health problems associated with aflatoxin contaminated maize (Walker *et al*., 2013). Besides, majority of repondents knew signs of aflatoxin and could also physically identify aflatoxin contaminated maize. This practice tends to confirm the prevailing high level of awareness of aflatoxin in the households.

In this study significant proportion of respondents (about 40%) from both high and low altitude areas cited improper drying of maize as the main cause of aflatoxin contamination, which was followed by improper maize storage. Few respondents both in low and high altitude mentioned mould/fungi as the cause of aflatoxin indicating that the level of awareness linking mould/fungi infestation in maize and aflatoxin causation was low. Early harvest and harvest of maize not properly dry was also metioned by less than 30% of resondents despite being a potential contributer to causation of aflatoxin. This is contrary to prevailing high level of awareness on aflatoxin (Walker *et al*., 2013). Thus, these aspects call for increased awareness on causes and prevention of aflatoxin particularly focusing on behavior change.

Perception on severity of aflatoxin problem which was evidenced by 116 aflatoxin cases perceived to have occurred in households since 2004 could be viewed as an indicator of

the severity of aflatoxin problem. This perception on severity of aflatoxin problem was further underscored by the fact that besides causing illness and suffering, nearly half of respondents believed that aflatoxin was incurable/causes death. This was consistent with the views expressed during focus group discussions (FGDs) with community and indepth interviews with health workers, where aflatoxin was considered to be the main serious health problem afflicting households and the community in general. Household perception on severity of aflatoxin problem could trigger adoption and practice of its prevention and control measures.

Indeed studies have reported that aflatoxin poisoning can cause serious health effects to humans. They can cause both chronic and acute effects, depending on the duration and level of exposure (Kuniholm *et. al*., 2008). Chronic dietary exposure to aflatoxins has been found to be a major risk factor for hepatocellular carcinoma, particularly in areas where hepatitis B virus infection is endemic (Kuniholm *et al*, 2008). Ingestion of higher doses (i.e. above 10ppb) of aflatoxin can result in acute aflatoxicosis, which manifests as hepatotoxicity or, in severe cases, fulminant liver failure (Fung and Clark, 2004). In addition, prolonged exposure to aflatoxin has been associated with stunted growth and underweight in children (Gong *et al*., 2003).

Overwhelming majority of respondents in both areas cited proper drying and proper storage of maize after harvest as the way of preventing aflatoxin. This finding was also corroborated in FGDs, as well as in an earlier study conducted in Kenya (Walker *et al.*, 2013; Mwihia *et al*., 2008). Harvesting of mature maize, though cited by few respondents, is one of the important ways of preventing mould growth and aflatoxin contamination. However, there were few respondents (about 5%) who did not know of any ways of preventing aflatoxin, pointing to the need for further dissemination of awareness. Knowledge and awareness on various aspects of aflatoxin prevention is vital

and could be a motivation to undertaking of preventive and control measures such as proper maize harvesting, handling, drying and storage practices.

About half of respondents' said altitude increased aflatoxin occurrence with higherlands having a higher proportion of respondents than lowlands who thought so. This was consistent with ecological conditions affecting mycotoxin production in cereals (Milani, 2013, Kaaya *et al.,* 2006). Ironically, majority of respondents in low altitude believed that low altitude increased aflatoxin occurrence while those in high altitude believed that high altitude increased aflatoxin occurrence. This was contrary to ecological conditions affecting mycotoxin production in cereals (Milani, 2013, Kaaya *et al*., 2006). Nevertheless, this underscores the need for more awareness on causes of aflatoxin occurrence as well as the importance of undertaking preventive measures irrespective of whether one is in low or high altitude. Moreover, knowledge on perception of which altitude increases aflatoxin occurrence could also be crucial in upscaling preventive interventions in altitude perceived to have higher risk of aflatoxin occurrence.

Further, about half of respondents'believed second maize harvest season after short rains increased aflatoxin occurrence with more respondents in highlands than lowlands believing so. Moreover majority thought aflatoxin contamination increased in maize harvested in second which occurs around the months of February and March than first maize harvest season which occurs in August/September every year. This could be attributed to prevailing higher temperatures and humidity during months of February/March compared to August/September. This was consistent with other findings which showed that production of aflatoxins is highly dependent on environmental factors such as temperature and humidity (Cotty *et al.,* 2007). Knowledge on perception of which maize harvest season increased aflatoxin occurrence could be crucial in upscaling preventive interventions during that particular season perceived to have higher risk of aflatoxin occurrence.

5.3 Maize pre-storage and storage practices

5.3.1 Pre-storage practices

The pre-storage practices included maize pre-harvesting, harvesting and and drying of maize before storage.

5.3.1.1 Pre-harvesting and harvesting practices

In this study, farmers in both high and low altitude areas were found to undertake various practices just before harvesting and after harvesting their maize. The harvest practices included allowing mature maize in the field for a period of time inorder to dry before harvest and removal of outer cover of maize cob during harvest (de-husking).

The vast majority of farmers left their mature maize to dry before harvesting. Most communities in Africa traditionally left their maize to mature and dry in the field prior to harvesting (Thamanga-chtja *et al*., 2004). Its good harvest practice that maize should be left to attain maturity and dry, that's at moisture content of about 20 to 30 %, before it is harvested immediately and dried to reduce field exposure to Aspergillus fungi (Summer *et al*., 2009). Duration of leaving maize in the field to attain maturity/dry prior to harvest is therefore important in reducing moisture content which will go along way in preventing development of aflatoxin (Summer *et al*., 2009). This is corroborated by studies done elsewhere which found that maize cobs may be left in the field for several weeks after attaining maturity in order to reduce moisture content (ACP-FAO, 2014). It has been found that most framers in Africa do not harvest maize based on physiological maturity period, but employ traditional practices to determine readiness of maize for harvesting by observing the dried tassels of cobs and drooping of cobs as a sign of maturity before harvesting (Akowuah *et al.,* 2015).

In this study farmers in higher altitude area left their maize in the field for a mean duration of 45 days which was nearly two times longer than farmers in lower altitude

area where the mean duration was 25 days. This could be attributed to prevailing cooler climate in higher altitude area than in lower altitude area. It has been observed that leaving maize in the field for unnecessary long periods prior to drying and storage promotes fungal infection and insect infestation (Akowuah *et al*., 2015).

Kaaya *et al*. (2006) recommended harvesting maize immediately after physiological maturity to combat aflatoxin problems, since when maize stays longer in the field after maturity aflatoxin contamination increases. The author noted that aflatoxin contamination increased four-fold by third week and seven-fold by seventh week.

In addition, leaving maize longer in the field prior to storage promotes fungal growth and insect infestation (Kaaya *et al*., 2006), although this has been found to be a common practice in some communities owing to labour constraints and need to let crop dry completely prior to harvest (Udoh *et al*., 2000). During FGD it was reported that theft of maize while in field discouraged farmers from leaving their maize for longer period in the field prior to harvest. Another concern from farmers was damage of maize by wild animals thereby causing damage and loses. As a result of this finding, improving general security in the community as well as controlling wild life will therefore go along way in improving length of stay of maize in the field after maturity prior to harvest.

The practice of removing outer covering of maize cob was a done by vast majority of households in both high and low altitude areas. It was observed that overwhelming majority of respondents (over 90%) both in low altitude and high altitude areas removed outer covering of maize cob during harvest. However, there were more farmers in higher altitude area removing removing outer coverings of maize cob during harvesting than those in low altitude area. This indicates removal of outer covering of maize during harvest is a common practice among households, but at varying levels. This practice of removing outer covering of maize cob removes pests/insects which could have been embedded in coverings and thus contribute to reduction of infestation and possibly

aflatoxin contamination (Sumer *et al*., 2009; Hell *et al*. 2007). However, maize with outer covering removed should be handled in such a way that damage to seed coat which could permit ease entrance of moulds and fungi is minimized.

5.3.1.2 Drying of maize

Farmers exposed their maize to dry at varying length of time (duration) before storage. They exposed their maize to dry immediately after harvest for mean duration of 14 days in higher altitude area and 17 days in lower altitude area, before storing them for later use. These results indicate that farmers in lower altitude area dried their maize for significantly shorter time than those in higher altitude area. This variation in drying time could be attributed to higher altitude farmers harvesting their maize earlier than their counterparts in lower altitude, hence the need for them to dry their maize for a longer period of time. This could be attributed to maize taking longer to mature and the need to prepare their shamba for next planting season before the onset of rains. It has been noted that proper drying of maize in order to attain moisture content of less than 14% before storage as well as sorting and disposing of mouldy or damaged grains can prevent or minimize development of aflatoxins (Heather *et al*., 2006). In addition, properly dried grains keep longer, are rarely attacked by insects and do not support mould growth, owing to unavailability of free water required for their development (Hell *et al.*, 2007).

This study revealed two main methods/options used by households to dry their maize after harvest before storage. These were 1) drying in open sun which entailed placing maize in the open directly exposed to sunlight and 2) drying in the shade in which maize is placed in a shade shielded from direct sunlight and left to dry naturally. However vast majority (over 95%) of households in both high and low altitude areas placed their maize in the open exposed directly to sunshine to dry while only a few in high altitude area placed their maize in the shade to dry. Other studies have also found sun drying of maize to be preferred by majority of households compared to other methods of drying

(Heather *et al*., 2006; Mwihia *et al.,* 2005)). This method is preferred in order minimize the risk of theft of maize in the field since if left in the field for a longer period to dry fully before harvesting they can be stolen (Mwihia *et al*., 2005).

Sun drying which entails spreading maize under the sunshine for several days to attain low moisture content of 12-14 % is an effective method recommended by Kenya National Ceareals and Produce Board (Heather *et al*., 2006).

Nevertheless, other methods such as placing maize above fire place or kitchen granary or dried by hunging to house rafters (tied by tassels into small bundles and hung) are preferred by few households, implying that these methods are inconvenient and less effective (Heather *et al*., 2006). Although maize can be dried using solar or artificially, these methods were not found in use in this study. In addition, although drying in the field may also be carried out after harvest with the harvested plants laid in stacks with the grains, maize cobs or panicles raised above the ground and exposed directly to the sun (ACP-FAO,2014), this practice was not observed or reported during the undertaking of this study.

Relying predominantly on sun drying could be attributed to the fact that this being a tropical region with plenty of sunshine, sun drying of maize could be the most appropriate and convenient way of drying maize. However, when temperatures are extremely as high as 60°C under clear skies the rate of drying can be extremely high and grains may crack and result in losses (ACP-FAO, 2014). Thus, covering grains or drying under shade has an advantage of preventing Kernel cracking. It has been noted that, drying temperature has an effect on the development of aflatoxin on stored grain. A study done elsewhere showed that slow drying with low heat over long periods of time promotes aflatoxin development (Summer *et al*., 2009). Hell *et al*. (2010) also observed that harvested maize should be dried as quickly as possible to safe moisture level of 10-13%.

Thus, ideally maize should be dried with moderate heat over short period of time to discourage aflatoxin development, although it could be difficult to achieve this through simple sun-drying under high humidy conditions in some regions. However, even though maize is properly sun-dried after harvest, there is need to maintain it in dry condition during storage as exposure to high relative humidity during storage may result in resumption of moisture uptake by the stored grain (Weinberg *et al.*, 2008).

This study has found that over three-quarters of respondents dried their maize by placing them on top of sheet material such as polythene, not directly on the ground. Besides, there was no significant difference between high and low altitude areas on this practice. .Maize should ideally not be placed directly on the ground during drying to avoid being contaminated with dirt, debries, germs, fungi spores and other contaminants. The *Aspergillus flavus* which produce aflatoxin is a common fungus/mould found in soil and debris (Summer *et. al*., 2009), and can easily contaminate maize if placed directly on the ground. It has been noted that drying on flat exposed surfaces has been the most convenient way of drying grains after threshing, but contamination with dirt cannot be avoided with this method and thus cleaner grains can be obtained by drying grains on plastic sheets, preferably black (ACP-FAO,2014).

Maize should therefore be dried on raised platform as this often reduces contamination by toxigenic fungi (Hell *et al*., 2007). Plastic sheets acts as platforms in that they provide suitable intervening impervious layer that will prevent maize from being in direct contact with the ground surface, hence avoiding contamination of grains. This is proper and hygienic way of drying maize since apart from mould and aflatoxin prevention, it also prevents against other health hazards (summer *et al*., 2009). Majority of respondents who were small scale or peasant (subsistence) farmers in both high and low altitude areas cited prevention of contamination as the main reason for placing impervious layer material underneath of maize during drying. Further a sizable proportion of respondents

said they did so to make maize dry faster and to prevent maize from rotting. Besides, a few respondents did so to improve maize quality in general. Similar views were expressed during community focus group discussions as well as indepth interviews with technical officers in the field.

This was an indication that previous and ongoing awareness campaigns appeared to have had an impact on positive behavior change towards proper maize drying (Walter *et al*., 2013).

Other studies have also found that use of drying platforms or mats increases efficacy of maize drying and reduce risk of aflatoxin contamination (Hell *et al*., 2010). Thus, sustaining the practice of using polythene sheets during drying will go along not only on improvement of maize drying but also on reduction of aflatoxin contamination as well as prevention of other contaminants from getting into contact with maize.

5.3.2 Maize storage practices

This study has revealed various maize storage practices used by the households in both high and low altitude areas, but only few households stored their maize in improved maize cribs which was recommended by government authorities (Songa *et al*., 2010), as well as by FAO (1998). This is an indication that acceptability and uptake of this method was low despite public education on the importance of the same. As noted, any maize storage practice or method should ensure that proper storage conditions are maintained in order to prevent development of aflatoxins (Fandohanet *et. al*., 2006). These storage practices were not much different from storage practices in other communities as Fandohan *et. al*.,(2006) observed that in most Sub-saharan African countries maize is generally stored in cob form either in wooden granaries, under the roofs of farmers houses, or on floor in houses.

Majority of households in both study areas stored their maize in bags placed on wooden platform. However, observations made to the arrangements of stored bags revealed that majority of households in high altitude areas stored their bagged maize in stacks with space between them than in low altitude areas which majority of households stored their bagged maize in stacks without space between them. This could be an indication of enhanced level of awareness in higher altitude area than in lower altitude area.

However there was a small proportion in both areas, who stored their bagged maize haphazardly (without any order), with a higher proportion of these being in low altitude area than in higher altitude area.

Maize stored in bag form either on platform or directly on the floor, bags should be arranged in such a way that there are spaces in between the bags to allow air circulation for aeration (Summer *et al*., 2009; Akowuah *et al.,* 2015). This air circulation or ventilation improves the quality of stored maize and minimize mouldy growth (Eduardo *et al*., 2005). Besides, storage of maize in sisal bags is better than in plastic bags since sisal bags provide more aeration in stored maize than plastic bags, thus further minmising mouldy growth (Turner *et al.,* 2005). Improper arrangement of stored maize in bags is an indication on the need for more awareness on the importance of arranging bags of maize properly in store.

Although maize may also be stored in grain form in clay containers or mud silos, this storage methods were not found in Makueni, although they are considered unfavourable for proper drying of maize, particularly in humid and semi humid conditions since they do not allow air circulation (Summer *et. al*., 2009)). This could be due to prevailing humid climate in Makueni especially in low altitude areas, or perhaps these methods were not culturally acceptable, feasible or convenient to household owners.

Although different maize storage structures were found in use, proper storage strucurure or method should ensure good drying of maize while in storage (Fandohan *et al.*, 2006). This may be either airtight storage or non-airtight storage, but airtight storage has been found to be superior to non-airtight storage owing to its effectiveness in preventing infestation by insect pests (Wambugu *et al*., 2009). Proper storage of maize is vital to prevention of mould growth and aflatoxin contamination as previous studies have shown that infrastructure and proper grain storage practices in develoing countries can prevent post harvest development of mycotoxins (Garry, 2003). For instance methods of drying and storing maize in elevated granaries were found to be protective against aflatoxin development (Eduaedo *et. al.,* 2005). Granaries being elevated platforms isolate maize from spores and insects on the ground. For this reason, among others, traditional improved granaries which are raised strucures that are well ventilated thus promoting proper drying of maize are more appropriate maize storage facilities compared to others mentioned. It has also been observed that the improved maize crib in its many forms acts as both a dryer and storage structure (ACP-FAO, 2014).Thus, owing to its dual purpose, there use therefore needs to be further promoted for adoption and use by communities.

This study revealed that majority of households stored their maize in grain form. However there were few households which stored their maize in cob form and few more other households which stored their maize with outer covering unremoved. Form of maize storage is important because certain forms of storage such as storage in cob form and/or with outer covering could facilitate infestation of pests/insects and other contaminants. Some of these pests/insects could be carried from the field into the store and become reservoir for contamination, especially if bad maize has not been sorted and removed in the field (Summer *et al*., 2009). These can contribute to mould growth with attendant production of aflatoxin. For this reason storage of maize in grain form should be encouraged owing to prevention of contaminants which could be carried from the field during harvest if outer coverings were not removed.

In this study household maize was found to have been stored for average period of two months and there was no significant difference in storage time between high and low altitude areas as well as between first and second maize harvest seasons. Duration of maize storage is important as study done in Uganda found out that aflatoxin levels increased with storage time (Kaaya *et al.,* 2006). This study also showed that maize stored for more than six months had higher aflatoxin contamination levels than maize stored less than six months. This implies that the longer maize stays in storage the more likely for it to be contaminated with aflatoxin. However, this applies when maize is stored for unnecessary long period without proper drying and storage (Hell *et al*., 2007).

Results of this study have shown that over half of household owners maintained their stores clean with more households in higher altitude area maintaining their stores clean than households in lower altitude area. Maintaining stores clean is important considering that clean maize stores have been found to have fewer aflatoxins levels (Hell *et al*., 2000).

On frequency of store cleaning, household owners cleaned their maize stores about three times in each maize storage season in both high and low altitude areas, and there was no significant difference in frequency of cleaning between the two areas. Given that the estimated storage time for maize was two and half months, this implied that cleaning of maize store was done once per month. It is important for maize stores to be maintained clean in order to prevent maize contamination (Summer *et al*., 2009; Hell *et al*., 2007). Maintaining them clean is one way of reducing dirt and other contamiants getting to maize as well as making storage environment unfavorable for mould growth and aflatoxin contamination.

About a third of stored maize was found to be infested with pests/insects in varying levels, but infestation was slightly higher in higher altitude than in lower altitude areas. The kind of insect pests infesting maize were mainly *Sitophilus zeamais* (common weevils), the more destructive pest Prostephanus truncates (larger grain borer) was

however not detected. This showed that some insects' pests may affect maize either in field or in storage if proper precautions are not taken to prevent their infestation (Hell *et al.*, 2007). Studies in other regions have found insect pests and rodents to be most important maize storage problems (Shaban *et al*., 2015).

Household owners whose maize was infested with pests/insects took various measures to try to get rid of them. About three-quarters in Kibwezi and nearly two-thirds of households which had their maize infested with insect pests reported that they sprayed with pesticides to get rid of insect pests while a few said they applied herbs. This implied that majority of households were concerned with pest infestation of maize and at least did something to get rid of insect pests. However, there were few household owners who did nothing to get rid of insect pests, while few others did not know what to do to get rid of insect pests. This finding was consistent with other studies which found that some farmers used smoking (Udoh *et al*., 2000) while others used natural plant products to preserve grain and reduce aflatoxin levels by reducing moisture content and preventing insect damage (Nguefack *et al.*, 2004). Other studies have found that use of insectricides, smoking or plants as storage protectants is related to lower aflatoxin levels in maize (Hell *et al*., 2000). Insect infestation in stored maize, which is a predisposing factor to aflatoxin, should be absolutely minimal inorder to avoid fungal and aflatoxin contamination (Chulze, 2010). According to a recent study, insect damage of host (maize) is a major determing factor in mold infestation (Cornell University, 2014).

Damged grain is more prone to fungal invasion and subsequent aflatoxn contamination. Study has shown that when grain is attacked by insects, moisture accumulates from insect activities, thus providing ideal conditions for fungal activity (Chulze, 2010). Besides, insect infestation also increases temperature and moisture which are pre-disposing factors to fungal growth (Machangi, 2005). In order to avoid moisture and fungal contamination, insect infestation should be kept to a minimum or eliminated completely. This

underscores the need for public education for adoption of insect control measures to avoid damage before and during drying, and storage in order to prevent fungal invasion.

Considerable number of maize stores had proper ventilation with about two thirds and a third of maize stores in higher and low altitude areas respectively having proper ventilation. Ventilation is important because it has been found that the rate of uniformity of drying when maize is in storage is controlled by movement of air through the loaded store (ACP-FAO, 2014). Ventilation also reduces relative humidity in maize stores (Machangi, 2005). Furthermore, maize which is thoroughly dry can still take up moisture from storage environment if not properly ventilated (Mboya *et al*., 2011). Therefore, apart from ensuring that maize is properly dry, there is need also to ensure that storage facility is properly ventilated.

A significant proportion of stores also had proper lighting. Lighting particularly natural lighting from the sun is important because of its germicidal effect and aiding in keeping the store clean. Good lighting also assists in keeping cleanliness (Rukunga, 2001).

Overstocking of maize stores with non food items, leading to congestion, was observed in some stores, and is not a healthy practice. About two thirds of stores in low altitude area were overstocked with other non-food items while only a third in low altitude area was overstocked implying that there was more overstocking in low altitude than higher altitude area. As a result of these observations, there is need for advocacy on proper contruction of maize stores/granaries and maintaining them in a good and clean condition and with adequate ventilation and lighting as well as not overstocking with other non food items. Maize cribs, in particular, should be properly designed, contructed and operated/maintained for proper storage of maize.

Although substantial proportion of households knew the reasons why maize should be stored properly such as avoidance of contamination and reduction of moisture content as well as prevention of maize spoilage, there were few households which did not know.

This therefore underscores the need for public education particularly on the proper maintenance of maize stores, and storing maize in well ventilated, clean and not directly on the floor. Knowledge/awareness on the importance of storing maize properly (i.e. in a well ventilated clean store and not directly on the floor) is crucial for adoption of improvement of proper storage practices (Walker *et al*., 2013).

Extraneous factor particularly theft was reported to have discouraged storage of maize in granaries situated outside houses. During FGDs it was reported that theft of maize while in storage in granaries located outside houses discouraged people from storing their maize in granary or maize cribs. This observation is supported by a previous study in Makueni which found that households preferred storing maize inside their homes to ensure it was not stolen during food shortage (Eduardo *et al*., 2005).

Improving general security in the community will therefore go along way in enhancing adoption of maize storage in households.

5.4. Prevalence of aflatoxin occurrence in maize and maize physical conditions

5.4.1 Prevalence of aflatoxin occurrence in maize

The study results have revealed existence of significant levels of contamination of household maize with aflatoxin with some exceeding permissible lvels. A considerable proportion of contaminated household maize exceeded permissible levels of 10 µg/Kg set by authorities (KEBS, 1988, FDA, 1997; EU-EFSA, 2014). Previous studies done in the area also found high levels of aflatoxin contamination in maize (Mwihia *et al*., 2008; Claudia *et al*., 2007; Lewis *et al.,* 2005). There was significant variation in aflatoxin

contaminated maize between low and high altitude areas of Makueni County, with low altitude areas having higher contamination. In addition, there was significant variation in aflatoxin contamination among different seasons of maize harvest. Maize harvest in first season of August/September had more contamination than maize harvested in second season of February/March in both high and low altitude areas, with low attitude area having relatively higher contamination. The difference in aflatoxin contaminated maize positivity between these two areas of different attitudes was significant ($P<0.05$). This could be attributed to the fact that February/March season is more warm and humid than August/September season. This is consistent with other studies which showed that aflatoxin is affected by changes in weather conditions in that maize harvested in more warm and humid seasons had more aflatoxin contamination than maize harvested less warm and humid conditions (Milani *et al*., 2013; Kaaya *et al*., 2006).

Further, lower altitude maize harvested in second season had higher aflatoxin positivity than maize harvested in higher altitude area, and the variation between these two different areas in altitude was significant ($P<0.05$). The prevailing cooler climate in higher altitude areas, which their altitude is about two times higher the altitude of lower areas, could have likely contributed to low aflatoxin contamination of maize since conditions of cooler areas are comparatively unfavorable for fungal growth and aflatoxin development. This is consistent with a study by Kaaya *et al*., (2006), in Uganda which found higher aflatoxin contamination in mid-altitude maize than highland maize.

In terms of comparison of maize harvested in first season and those harvested in second season, results revealed significant variation in aflatoxin contamination between the two seasons ($P<0.05$), implying a likelihood of higher risk of consuming maize harvested in second season, and particularly those harvested in higher altitude areas. This is consistent with likely effects of changes in weather conditions on mould growth and aflatoxin

production (Milani, 2013). Thus, this point to a more likely exposure to people consuming maize harvested in second season.

The existing levels of aflatoxin contamination of household stored maize found in this study were almost consistent with the 25% estimate of aflatoxin contamination of food by Food and agriculture Organisation (FDA, 1997), thus indicating continued existence of considerable risk of exposure and likelihood of occurrence of aflatoxicosis in humans.

Analysis of first maize season maize harvest showed that 50% of positive aflatoxin contaminated maize samples in lowlands had aflatoxin levels exceeding 10µg/Kg, while in highlands none of the maize had aflatoxin contamination exceeded 10 µg/Kg.

Further analysis of positive samples on ELISA test for maize harvested in second season using quantitative HPLC method showed varying aflatoxin levels. The low altitude samples that showed samples with aflatoxin content exceeding 10 µg/Kg increased from 1(16.7%) to 5 (62.5 %) , but overall aflatoxin contamination increased with more maize testing positive for aflatoxin in second maize harvest season (33.3%) than first maize harvest season (25.0%). In high altitude area, maize with aflatoxin level exceeding 10 µg/Kg increased from 0% to 2 (66.7) %. The overall sample positivity also increased from 4.2% to 12.5%. These results indicate that there was higher aflatoxin contamination of maize harvested in second season than maize harvested in first season, in both lowland and highland areas of Makueni.

However, lowlands had higher levels of aflatoxin contamination than highlands, and this is supported by other other studies done elsewhere (Kaaya *et al*., 2006, Hell *et al*., 2007).

Overall the results showed that in both maize harvest seasons, low altitude areas had more aflatoxin contaminated maize than high altitude areas. This could be because lower attitude areas are warmer /hotter, characterized by higher temperatures and humidity, while higher altitude areas are cooler, characterized by low temperatures and humidity.

Similarly maize harvested in second season had more aflatoxin contaminated maize than maize harvested in first season. This could be because second maize harvest season which occurs around February/March has higher temperatures and humidity than first maize harvest season which occurs in August/September. Indeed the mean temperature for first season was 30.7°C in low altitude and 23.6°C in high altitude while in second season the mean temperature was 31.6°C in low altitude and 25.4°C in high altitude. The relative humidity for first season was 45.8% in low altitude and 32.3% in high altitude while in second season the mean relative humidity was 49.9% in low altitude and 42.2% in high altitude. Warm and humid conditions are ideal for growth of Aspergillus species resulting in the formation of aflatoxins (Milan, 2013; Summer *et al*., 2009).

Regarding aflatoxin sub-types, results further indicated that the most common strain/type of aflatoxin in both study sites and in both maize harvest seasons was AFB1 followed by AFB2. However lowlands had higher AF B1 mean aflatoxin contamination than highlands in first maize harvest, but in second maize season harvest the mean contamination of AFB1 was virtually the same with no significant difference, except for total aflatoxin contamination. Second maize season harvest had slightly higher mean AFB1 as well as total aflatoxin contamination than maize of first harvest season which was slightly lower. The AFB1 sub-type was implicated with previous occurrences of aflatoxicosis (Claudia *et al.*, 2007).

Similarly, in both areas, the mean aflatoxin concentrations of other sub-types of AFB2, AFG1 and AFG2 were higher in maize harvested in second season than maize harvested in first season indicating increased aflatoxin contamination in maize harvested in second season. The levels of these aflatoxin sub-types were also higher in lowlands than in highlands, indicating higher contamination of maize in lowlands.

Predominance of AFB1 sub-type is consistent with findings from other studies done elsewhere (Cornnel University, 2014; Kaaya *et al*., 2006). The presence of AFB1 and

AFB2 types could probably be attributed to sporadic occurrences of aflatoxicosis cases in the area as these aflatoxin sub-types have been implicated as the cause of aflatoxin poisoning and they are portent carcinogenic substances (Cornnel University, 2014). Indeed previous occurrences of aflatoxicosis in Kenyan eastern region were associated with AFB1 sub-type (Claudia *et al*., 2007).

Regarding moisture content of maize, maize harvested in lowland area had slightly higher moisture content than maize harvested in highland area in both seasons of maize harvest. This finding is consistent with the results of a study which found out that mean moisture content was significantly lower in highland maize kernels than in mid-altitude (Kaaya, *et.al.,*2006). The moisture content was also higher in maize harvested in second season than in first season in both areas. The increased moisture content could have contributed to increased aflatoxin contamination of maize in second maize harvest season. Similarly the higher moisture content in lowlands than highlands could more likely have contributed to higher aflatoxin contamination in lowlands.

Furthermore, results of this study have revealed that some maize had moisture content above recommended level of 13%. This could have been attributed to inadequate drying and improper storage conditions. Maize of moisture content above 13 % is likely to be attacked by pests and molds which are predisposing factors to aflatoxin development (Akowuah *et al.,* 2015).

These study findings have demonstrated that levels of aflatoxin contamination were quite high especially in lowland areas and in second maize harvest season, thus exceeding permissible limits of 10 µg/kg for humans adopted in Kenya and many other countries for guiding intervention action points (KEBS, 1988; Arther Yau, 2012; EU-EFSA, 2014). Maize or foods exceeding permissible limits is not supposed to be used as food or animal feed (Arther Yau, 2012). As a result of this limit, various efforts have been ongoing focusing on reducing aflatoxin exposure to humans by keeping aflatoxin levels in food as

low as reasonably possible and removing those exceeding legal limits not to be used as food. However, enforcing these regulations has been quite challenging especially for home grown maize which are consumed locally. This therefore underscores the need for proper maize harvesting, drying, handling and storage.

5.4.2 Maize physical conditions

This study showed that some maize grains in both high and lowland areas had notable discoloration. However lowland areas at 20.8% had more discolored maize than highland area which had 12.5% of maize discolored. The difference in discolored maize between the two areas was quite significant ($P<0.05$). Discoloration of maize is an important sign of spoilage and a pointer to mould infestation and exention improper storage (Hosney, 2015; Hoffman *et al*., 2013). This discoloration sign is important as it is commonly used during visual inspection for screening of maize to separate suspected contaminated/unsuitable maize for further analysis to determine suitability for human consumption. Besides, discoloration of maize could be attributed to poor storage conditions.

Discoloration of maize was higher in second season maize harvest than first season maize harvest with lowlands which had 45.8% discolored maize having a higher increase than highlands which had 33.3% of maize discolored. This implied that maize harvested in second season had more discoloration than maize harvested in first season.

Mould infestation was observed in 20.8% of maize samples in lowlands and 12.5% of maize samples in highlands indicating that it was a problem. Moreover mould intestation increased in second maize harvest season as it was observed in 37.5% of maize in lowlands and 25.0% in highlands indicating an increase of mould problem. This is an indication that maize harvested in second season had more moldy infestation than maize harvested in first season. Mould infestation is a predisposing condition to aflatoxin contamination (Summer *et al*., 2009).

Insect pest infestation was also a problem as it was observed in 25.0% of maize in lowlands and 20.8% in highlands. In addition second maize harvest season showed an increase in insect pest infestation as it was observed in 37.5% of maize in lowlands and 45.8% of maize in highlands indicating that insect pest infestation problem escalated in second maize harvest season. This is also an indication that maize harvested in second season had more insect pest infestation than maize harvested in first season. Insect pest infestation creates favourable conditions for mould growth and aflatoxin contamination owing to the damage they cause to kernel coat (Hell *et al*., 2007).

Since maize storage methods were the same in first and second season maize, climate variation between the two seasons could be a factor to increased discoloration, mould and insect pest infestation in stored maize. These unfavorable physical conditions observed in maize which could be as result of unsatisfactory storage conditions pose a likely potential problem for occurrence of aflatoxin contamination in maize.

However, visual inspection to determine discoloration, mould or insect infestation cannot be solely relied upon to indicate presence of aflatoxin contamination since good looking grains can still have aflatoxins. Thus, chemical analysis is the most reliable way to determine alatoxin contamination in foods (AATF, 2014).

5.5 Comparison of aflatoxin prevalence and maize pre-storage and storage practices in different altitudes and maize harvest seasons

As discussed in sub-section 5.4.1, there were significant variations in aflatoxin prevalence for maize harvested in low and high altitude zone as well as in maize harvested in first and second season. Moreover, aflatoxin positivity of maize harvested in first season was significantly higher by 20.8% in lower altitude than in higher altitude area. In second maize harvest season aflatoxin positivity also increased in both lower and higher altitude areas, by 8.3 %. This finding implies that low altitude area which is usually warm as well as second maize harvest season of February/March which is also usually warm have an

influence in increasing aflatoxin occurrence. Other studies done elsewhere had also found low altitudes with warm climates to increase mould growth and aflatoxin production (Milani, 2013; Hell *et al*., 2007; Kaaya *et al*., 2006).

The study findings have also shown that moisture content of maize was significantly higher in lower altitude area than in higher altitude area, and was linked to aflatoxin contamination of maize in that higher moisture content had corresponding higher aflatoxin content in maize. However, the mean moisture content of maize in both areas was within the range of 12-14% as recommended for safe storage (Summer *et.al*., 2009).

There were also significant differences in maize pre-storage and storage practices between low and high altitude areas. There was significant difference in duration of leaving of maize in field before harvest between low and high altitude areas, in that higher altitude maize is left for longer period in the field than maize in lower altitude area. This could be attributed to prevailing cooler temperatures in higher altitude compared to low altitude thus making farmers keep their maize longer to dry.

Similarly, there was a significant difference in duration of drying of maize after harvest between high and low altitude areas, with farmers in high altitude drying their maize for longer period than farmers in lower altitude areas. The same trend was observed during second maize harvest season where drying period was longer in higher altitude area than in lower altitude area. This could also be attributed to existence of cooler temperatures in higher altitude compared to low altitude thus making farmers dry their maize for longer period.

Other studies elsewhere have shown that maize grown in higher altitude zone take longer to dry in the field before harvest compared to maize in low altitude (Hell *et al*., 2007).

It has been observed that most household owners keep maize at least for some period in storage as they continue to use. Households in both high and low altitude stored their maize for an average period of two months. However there was slight difference between lower and higher altitudes in terms of duration of keeping maize in storage, with higher altitude households storing maize slightly longer than lower altitude households, although the difference was not significant. This finding was corroborated in second maize harvest season where maize also stayed for about two months in storage period, although it stayed for average longer period in storage in lower altitude area than in higher altitude area. Length of maize storage is important as it could have an effect on aflatoxin contamination. Hell *et al.,* (2007), in their study found that maize stored from 8-10 months had higher aflatoxin contamination than maize stored from 3-5 months.

5.6 Maize pre-storage and storage factors associated with aflatoxin occurrence

In this study various variables/factors were analyzed to determine their correlations/associations with aflatoxin contamination of maize. These factors included pre-storage and storage practices of maize.

5.6.1 Maize pre-storage practices associated aflatoxin occurrence

The study findings showed that duration of maize in the field before harvest in higher altitude area was significantly positively correlated with maize affected by pests/insects while in storage, implying that the longer maize is left in field the more likely for it to be infested with insect pests. Legnth of stay of maize in field was also found to be significantly negatively correlated with aflatoxin occurrence in maize, implying that the longer the duration of maize in field the likely less the aflatoxin occurrence in maize.

Results further indicated that duration of maize in field before harvest and before drying/storage had significant negative correlation with moisture content in maize both in lower altitude and higher altitude. This finding implied that when maize is kept longer

in the field to dry it had lower moisture content than maize left in the field for a shorter period. This is consistent with other findings which showed that moisture content was reduced when harvest was delayed (Kaaya *et. al.*, 2005). Thus, keeping maize longer in the field prior to harvest is likely to have beneficial effect in reducing moisture content.

The practice of placing of maize on top of impervious material/layer during drying was associated with proper maize storage. This meant that those households which placed their maize on top of impervious material during drying were more likely to store their maize properly than those household which did not place any material underneath during drying of maize. This practice is important as it prevents contaminants from gaining access to maize during drying. It should therefore be promoted for adoption in households owing to its useful effects.

Duration of drying of maize after harvest especially in higher altitude area had negative correlation with aflatoxin occurrence in maize, implying that the longer the drying of maize after harvest the lower the aflatoxin contamination. Duration of drying of maize was also negatively correlated with moisture content in maize, implying that the longer the drying of maize after harvest the lower the moisture content in maize. This finding was consistent with a previous study done in Makueni which found incomplete drying of maize to be associated with aflatoxin contamination (Eduardo *et al*., 2005).

5.6.2 Maize storage practices associated with aflatoxin occurrence

This study has shown that duration of maize while in storage (storage time) had significant positive correlation with aflatoxin occurrence in maize particularly in lower altitude areas of maize harvest. This finding indicates that the longer the maize is stored the higher the aflatoxin contamination. This was demonstrated by increased aflatoxin content for maize that stayed longer in storage. This consistent with a study done elsewhere which found that aflatoxin levels increased with storage time (Kaaya *et.al*., 2006).

Frequency of store cleaning had significant negative correlation with aflatoxin occurrence. This implied that the more frequent the store is cleaned the less the likelihood of aflatoxin contamination of maize. This finding is supported by a previous study which showed that storage of maize on the dirt floor may promote fungal growth in maize kernels (Eduardo *et al*., 2005). The implication of this is that if the store is not cleaned frequently dirt will accumulate thereby creating favourable condition for mould growth and subsequent likelihood of aflatoxin production. Other studies have also found that cleaning of the structure lowers aflatoxin contamination (Hell *et al.,* 2000; Hell *et. al*., 2007). As a result of this finding there is need for maize stores to be maintained clean by cleaning them more frequently. This will go along way in minimizing mould growth and subsequent aflatoxin contamination of maize while in storage.

Ventilation of maize store had significant association with aflatoxin content in maize especially in lower altitude areas. Proper controlled ventilation reduces relative humidity in maize stores (Machangi, J., 2005). Other studies have also found that maize stored in aerated stores had lower aflatoxin levels (Hell *et. al*., 2007), while storing maize in poorly ventilated windowless homes promote fungal growth and aflatoxin production (Eduardo *et al.,* 2005), thus underscoring the importance of proper ventilation of maize stores.

Results further indicated significant association between proper maize storage in households and discoloration of maize (ie. maize loosing its original colour). This means that when maize is stored properly it is less likely to be discoloured, and thus lower its quality. This finding which is supported by Hell *et al*. (2007) and Hoffman (2013), underscores the need for proper maize storage to minimize discoloration which leads to mouldy infestation, besides lowering quality, particuarly in lower warm humidy areas.

Proper maize storage was also associated with pests/insects infestation in stored maize in both high and low altitude areas, indicating that maize stored properly are liklely to have

less insect pests infestation. Moreover proper storage was also significantly associated with mouldy infestation and aflatoxin occurrence in maize in lower altitude arrea, implying that properly stored maize was less likely to develop aflatoxin. This finding was supported by previous study conducted in Makueni which also associated proper maize storage with less aflatoxin occurrence (Eduardo *et al*., 2005).

Among the household storage practices, storage of maize in bags directly on the floor had higher aflatoxin occurrence compared to other storage practices, implying that this practice of maize storage had more risk to aflatoxin occurrence. On the other hand storage on raised maize cribs had lower aflatoxin occurrence, implying that this storage practice had less risk to aflatoxin occurrence. This finding was consistent with other studies (Mboya *et al*., 2011; Thamanga-Chitja *et al*., 2004; Udoh *et al*., 2000).Thus adoption of improved maize cribs for household maize storage will more likely reduce aflatoxin contamination. Besides, maize storage practices and aflatoxin were associated, implying that there is a link between storage practice and aflatoxin occurrence in maize. This was consistent with finding from another study done in Makueni which found storing maize inside the home granary to be associated with aflatoxin contamination (Eduardo *et al*., 2005).

Results have shown that mouldy condition, insect infestation and moisture content in maize had an association with aflatoxin occuurence. Mouldy condition of maize had significant association with aflatoxin occurrence, further confirming that certain type of fungi (mould) when it infests foods/maize produces aflatoxin (Cornell Univesity, 2014). Maize affected by insect pests had significant association with aflatoxin content, implying that the more maize is affected by insect pests the more likely for it to develop aflatoxin.

Other studies have also found that insect infestation was positively corelated to aflatoxin contamination of maize (S'etamou *et al*., 1997; Hell *et..al.*, 2000; Udoh *et.al*. 2000; Mboya *et al*., 2011). Damage to maize grains by pests/insects render them susceptible to mouldy infestation resulting in aflatoxin contamination.

Moisture content in maize had positive correlation with aflatoxin occurrence implying that the increase in moisture content is likely to increase aflatoxin content. Conversely thus drying maize to safe low moisture level reduces aflatoxin production. This consistent with the finding in another study done in Makueni which found association between completely dried maize with less aflatoxin contamination (Eduardo *et al*., 2005).

5.7 Effects of temperature and humidity on aflatoxin occurrence in maize

These results indicate that there was higher aflatoxin contamination of maize harvested in first season of February/March than maize harvested in second season of August/September, in both lowland and highland areas of Makueni. The overall results also showed that low altitude area had more aflatoxin contaminated maize than high altitude area. This could be because lower attitude areas are usually warmer /hotter characterized by higher temperatures and humidity while higher altitude areas are usually cooler characterized by low temperatures and humidity. Similarly maize harvested in first season had more aflatoxin contaminated maize than maize harvested in second season. This could be because first maize harvest season has higher temperatures and humidity than second maize harvest season. Higher temperatures and humidity are associated with increase in mould growth and aflatoxin production (Mialni, 2013; Summer *et al.,* 2009).

As found out in this study, temperatures and humidity were variable depending on altitude and season. The mean temperature for first season was 30.7°C in low altitude and 23.6°C in high altitude while in second season the mean temperature was 31.6°C in

low altitude and 25.4°C in high altitude. The relative humidity for first season was 45.8% in low altitude and 32.3% in high altitude while in second season the mean relative humidity was 49.9% in low altitude and 42.4% in high altitude. Although these variable temperatures and humidity are slightly below optimum favourable for growth of *Aspergillus species* and production of aflatoxin, they might have had an influence on aflatoxin occurrence in maize as observed in other studies (Milani, 2013). In Nigeria and Uganda, aflatoxin levels in maize samples were found to be higher in more humid areas compared to the drier areas (Atehnkeng *et al*., 2008; Kaaya *et al*., 2006).

On aflatoxin sub-types the mean aflatoxin of AFB1, AF B2, AF G1 and AF G2 were higher in maize harvested in second season than maize harvested in first season indicating increased aflatoxin contamination in maize harvested in second season. The levels of these aflatoxin sub-types were also higher in lowlands than in highlands, indicating higher contamination of maize in lowlands. These higher levels of aflatoxin sub-types could have been influenced by higher temperatures and humidity during second maize harvest season. The presence of AFB1 and AFB2 can probably be attributed to sporadic occurrences of aflatoxicosis cases in the area as these aflatoxin sub-types have been implicated as the cause of aflatoxin poisoning and they are portent carcinogenic substances (Cornnel University, 2014).

It was also observed in this study that moisture content of maize, which has an effect on mold growth and aflatoxin contamination (Summer *et al*., 2009), was slightly higher in maize harvested in lowland area than in highland area in both seasons of maize harvest. This finding is consistent with the results of a study which found out that mean moisture content was significantly lower in highland maize kernels than in mid-altitude (Kaaya, *et.al.*,2006). The moisture content was also higher in maize harvested in second season than in first season in both areas. The increased moisture content could have contributed to increased aflatoxin contamination of maize in second maize harvest season.

Similarly the higher moisture content in lowlands than highlands could more likely have contributed to higher aflatoxin contamination in lowlands. This implies that variation in temperature and humidity as a result of different altitudes and different seasons has an influence on moisture content and aflatoxin occurrence in maize.

Previous studies have also shown that climate change (global warming) which affect temperature and humidity has an influence on aflatoxin producing fungi and aflatoxin occurrence in maize and other crops (Milani, 2013; Patterson *et al*., 2010; Cotty *et al*.,2007). Aflatoxin occurrence is more prevalent in warm humid climates since such climates create favourable conditions for proliferation of insect infestation and aflatoxin producing fungi. Hell *et al*. (2010) in their study found that high humidity and temperature favour fungal proliferation. Thus, besides maize storage and pre-storage practices having an influence on afltatoxin occurrence in maize, temperature and humidity also has an influence.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study findings have showed that majority of households practiced good pre-storage practices and stored their maize properly, mostly in grain form and in bags which were kept on raised platform. Proper household storage of maize was attributed to high level of awareness and was associated with less aflatoxin occurrence in maize. Thus enhancing level of awareness could lead to improvement of maize storage which could likely lead to reduction in aflatoxin occurrence in maize.

Storage of maize directly on the floor had higher risk of aflatoxin occurrence while storage on raised cribs had lower risk compared to other practices of storage. Thus, storage of maize directly on the floor should be discouraged while storage of maize on raised cribs should be encouraged.

Levels of aflatoxin contamination of maize in Makueni were significantly high, with some exceeding permissible levels indicating a likelihood risk of occurrence of aflatoxicosis, thus putting maize consumers at risk of ill health. The most abundant strain/sub-type of aflatoxin was AF B1 further indicating a likely risk of aflatoxicosis since this strain had previously been associated with occurrence of aflatoxicosis.

Low altitude had influence on aflatoxin occurrence as moisture content and aflatoxin positivity in contaminated maize was higher in low altitude area than in high altitude area. Thus, low altitude maize had higher risk of aflatoxin than high altitude maize. Maize harvest seasons have influence on aflatoxin occurrence as moisture content and aflatoxin positivity in maize was also higher in second season maize than first season maize. Thus, second season maize had higher risk of aflatoxin than first season maize.

Maize pre-storage practices particularly length of stay of maize in field before harvest and duration of drying do influence aflatoxin occurrence in maize. Maize storage practices particularly proper storage, storage time and frequency of store cleaning do influence aflatoxin occurrence in maize. In addition, mould and insect pests' infestation, as well as moisture content do also influence aflatoxin occurrence in maize. Moreover, different altitudes and harvest seasons with variable temperature and humidity also have an influence on aflatoxin occurrence.

6.2 Recommendations

The following are recommendations derived from this study.

For policy and implementation

1. There is need for households to adopt proper maize storage practices, and in particular properly constructed, ventilated and well maintained stores such as improved maize cribs. Households should also be encouraged to adopt and use purdue improved crop storage bags which have been found to be effective in storage of maize.

2. Policy makers and stakeholders should also assist by promoting household positive maize storage practices which include use of properly constructed, ventilated and maintained stores such as improved maize cribs, as well as purdue improved crop storage bags which have been found to be effective in storage of maize.

3. There is urgent need for continued public education on the likely risk posed by consumption of aflatoxin contaminated maize so that households can adopt preventive measures to prevent aflatoxicosis and/or minimize long-term exposure to aflatoxins .

4. There is need for focused interventions targeting aflatoxin prevention in general and focusing specifically in high risk low land areas as well as high risk maize harvest seasons so as to minimize or eliminate aflatoxin contamination in maize.

5. There is urgent need for regulatory authorities to constantly monitor aflatoxin contamination levels or concentrations in maize and other foods particularly focusing on regulatory limits, for timely intervention should they exceed permissible levels. This could prevent occurrences of sporadic cases or outbreaks of acute aflatoxicosis as well as reducing long-term exposure to aflatoxins.

6. Further, there is need for adoption of bio-control methods such as use of aflasafe which have been known to be effective in control of aflatoxin especially at farm, pre- harvest and harvest stages, when they become available and accessible in the area.

For Further Research

1. There need for further experimental or intervetional study on non-household storage practices such as traders storage facilities to determine their influence on aflatoxin occurrence in maize.

2. There is need for further study on maize farming practices and seed varieties used for planting to determine their influence on aflatoxin occurrence as well.

3. There is need for further comparative studies on other different altitude areas and different maize harvest seasons for further determination of effect of climatic variations on aflatoxin occurrence.

4. As this study has established that some maize pre-storage and storage practices are associated with aflatoxin occurrence, there is need for a further study on these aflatoxin- associated practices and other factors with a view of developing a predictive model on aflatoxin contamination in maize which can serve as an early warning indicator for possible occurrence of aflatoxicosis.

5. Since improper maize storage besides being associated with aflatoxin result in food losses which could contribute to food insecurity problem, there is need for further study to determine influence of maize storage methods and aflatoxin on household food security.

6. There is need for study to determine aflatoxin concentration in human blood serum in these studied areas so as to establish association with continued consumption of aflatoxin contaminated maize since study findings have shown existence of significant levels of aflatoxin contamination in maize. This, when further studied, could lead to indentification of factors affecting individual susceptibility to aflatoxin accumulation and concentration in blood serum following continued exposure to aflatoxin contaminated maize. Such a study could further lead to identification of specific biomarkers of aflatoxin exposure in humans.

REFERENCES

AATF (African Agricultural Technology Foundation), (2014). Aflatoxin Mitigation in Africa. Retrieved from http://www.aflasafe.com/aflatoxins.

Abbas, H.K., Wilkinson, J.R., Zablotowicz, R.M., Accinelli, C., Bruns, H.A. and Weaver, M.A. (2009). Ecology of ASpergillus flavus regulation of aflatoxin production and management strategies to reduce afltoxin contamination of corn. *Toxin Reviews 28*, 142-153.

ACP and FAO,(2014). Grain Storage Techniques-Evolution and trends in developing countries. Drying Methods: Natural and solar drying. Retrieved from http://www.fao.org/docrep/t1838eEOv.htm.

Afolabi, C.G., Bandyoadhyay, R., Leslie, J.F.and Ekpo, E.J.A (2006). Effect of sorting on incidence and occurrence of fumosin and fumosin-producing Fusarium species on maize (Zea mays L.)in Nigeria. *Journal of Food Protection 69,* 2019-2023.

Akowuah, J.O., Mensah, L. D., Chan, C. and Roskilly, A. (2015). Effects of practices of maize farmers and traders in Ghana on contamination of maize by aflatoxins: case study of Ejura-Sekyeredumase Municipality. *African Journal of Microbiology Research 9*(25), 1658-1666.

Arora, D.K. (2004). Fungal Biotechnology in Agriculture. *Food and Environment Applications 21,* 5-20.

Arregui, F.J. (2003). Simultaneous measurement of humidity and temperature by combining a reflective intensity-based optical fiber sensor and a fiber Bragg grating. *Sensors Journal, IEEE. 2*(5), 482-487.

Arthur, Y. (2012) Food safety focus (73rd issue)-Food safety platform- Aflatoxins in Food. Hong kongo: centre for food safety.

Atehnkeng, J., Ojiambo, P.S., Donner, M., Ikotun, T., Sankora, R.A., Cotty, P.J.and Bandyopadhyay, R. (2008). Distribution and toxigenicity of Apergillus species isolated from maize kernels from three agro-ecological zones in Nigeria. *International Journal of food microbiology 122,* 2019-2023.

Bett, C. and Nguyo, R.(2007). Post harvest storage practices and techniques by farmers in semi-Arid Eastern and Central Kenya. African Crop Science Conference Proceedings El-Minia, Egypt, 27-31 October.

Bhat, R.V., Shetty, P.H., Amruth, R.P. and Sudersham, R.V. (1997). A food-borne disease outbreak due to consumption of moldy sorghum and maize containing fumonisin Mycotoxins. *J Toxicol Clin Toxicol 35*, 249-255.

Bruns, R.L. (2003). Controlling aflatoxin and fumonisin in maize by crop management. *Journal of Toxicology, Toxin Reviews 22,* 153-174.

CAST, (2003). Mycotoxins: Risks in plant, Animal, and Human System No. 139. *Ames, IA*, 109, 5-17.

CDC, (2004). Outbreak of aflatoxin poisoning-eastern and central provinces, January-July, 2004. *MMWR Morbidity/Mortal Weekly Report, 53(34), 790-793.*

CDC. (2014). Understanding chemical exposures: Saving Lives, Prorecting People, Retrieved from http://www.cdc.gov/nceh/hsb/chemicals/aflatoxin.htm.

Channaiah, L.H. and Maiyer, D.E. (2014). Best stored maize management practices for the prevention of mycotoxin contamination, in mycotoxin reduction in grain chains (eds J.F Leslie and A.F. Logrieco, Chichester, UK: John Wiley & sons, Ltd.

Chen, S.Y., Chen, C.J., Tsai, W.Y., Ahsan, H., Liu, T.Y. and Lin, J.T. (2000). Associations of plasma aflatoxin B1-albumin adduct concentration with plasma selenium concentration and genetic polymorphisms of glutathione S-transferase M1 and T1. *Nutrition Cancer 38*, 179-185.

Chulze, S.N., (2010). Strategies to reduce mycotoxin levels in maize during storage.Food Addit Contam Part A Chem Anal Control Expo Risk. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20349375.

Claudia, P., Nyapau, H. and Peter, J. (2007). Outbreak of Acute Aflatoxicosis in Kenya in 2004: Identification of the Causal Agent. *Applied and Environmental Microbiology,* 73(8), 2762-2764.

Cornell University, (2014). Department of Animal Science-Plants Poisonous to Livestock. Aflatoxins: Occurrence and Health Risks. Retrieved from http://www.ansci.cornell.edu/plants/toxicagents/aflatoxin.html.

Cotty, P.J. and Jaime-Garcia, R. (2007) Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *International Journal of Food Microbiology, 119*, 109-115.

Decock, K. and Rubin, C. (2005). Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in Eastern and central Kenya. *Health perspective, 113*, 1763-1767.

Delmulle, B.S., DeSaeger, S.M., Sibanda, L., Barma-Vetro, I. and Van Peteghem, C.H. (2005). Development of an ImmunoAssay-based lateral flow dipstick for the rapid detection of aflatoxin B1 in pig feed. *Journal of Agri Food Chemistry,53*(9), 3364-8.

Egal, S., Housa, A., Gong, Y., Turner, P.C., Wild, C.P., Hall, A.J., Hell, K. and Cardwell, K.F.(2005) Dietary exposure to aflatoxin from maize and groundnuts in young children from Benin and Togo, West Africa. *International Journal of Food Microbiology 104*, 215-224.

EU-EFSA Press centre, (2014). Aflatoxins in Food. Retrieved from http://www.efsa.europa.eu/en/topics/topic/aflatoxins.htm.

Emitati, A.A., Monda, E.O. (2013). A new approach in Aflatoxin management in Africa: Targetting aflatoxin sterigmatocystin biosynthesis in Aspergillus species by RNA silencing technique 1-10. Retrieved from http://dx.doi.org/10.5772/51440

Fandohan, P., Gnonlonfin, B., Hell, K., Marasas, W.F.O. and Wingfield, M.J. (2006).Impact of indigenous storage systems and insect infestation on the contamination of maize with fumosins. *African Journal of Biotechnology,5* (7), 546-552.

Eduardo, A.B., Kimberly, L., Karen, G., Hellen, S.R., Stephanie, K.,Henry, N., … and Laurence, S.(2004). Case control study of an Acute Aflatoxicosis Outbreak, Kenya. *Environmental Health Perspectives .113*(12), 1-5.

FAO, (1998). African Experience in the Improvement of post –harvest techniques. Rome: Retrieved from http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/W1544E00.htlm .

FAO.(2005) Food outlook:Global information and early warning system on food and agriculture (GIEWS) No. 4 December 2005.

Farombi, E.O. (2006). Aflatoxin contamination of foods in developing countries: implications for hepatocellular carcinoma and chemopreventive strategies. *African Journal of Biotechnology 5,* 1-14.

Fisher, A. A., Laing, J.E and Strocker, J.E. (1998). Handbook for family planning,operation research design in sampling.Population Council. N.Y: Population council.

Food and Drug Administration (FDA), (1997). Adulterated Food. Federal Food Drug and Cosmetic Act, Chapter IV: Definitions and Standards for Food, *402(a)(1)*US: Food and Drug administration.

Food Safety Research Information Office (FSRIO). (2009). A focus on Aflatoxin contamination.USDA.National Agricultural Library. US: FSRIO.

Fung, F. and Clark, R.F. (2004). Health effect of mycotoxins: a toxicological overview. *Journal of clinical Toxicology 42*, 217-234.

Garry, P.M. (2003). Cultural and genetic approaches to managing mycotoxin in maize. *Annual Review of Phytopathology, 41,* 99-116.

GOK-MPND. (2009). Makueni District Development Plan (2008-2012). Nairobi Government printers.

Gong,Y., Egal, S.,Hounsa, A.,Turner, P., Hall, A.,Cardwell, K. and Wild, C. (2003). Determinants of Aflatoxin exposure in young children from Benin and Togo, West Africa:the croitical role of weaning. *International of Epidemiol 32*, 556-562.

Groopman, J.D., Kensler, T.W. and Wild, C.P. (2008). Protective interventions to prevent aflatoxin induced carcinogenesis in developing countries.*Annual Rev.Public Health 29,* 187-2003.

Heather, S., Eduardo, A., Marianne, B., Ramesh, V., Robert, B., Marie-Znoel, B., … and Mensah M. (2006). Workgroup Re[port: Public Health Strategies for Reducing Aflatoxin Exposure in Developing Countries: *Environmental Health Perspective,114,* (12).

Hell, K., Udoh, J., Setamou, M., Cardwell, K.F. and Visconti, A. (1995). Fungal infection and mycotoxins in maize in the different agroecological zones of Benin and Nigeria, West Africa in Food in Africa.*Journal of stored products research 2,* 66-74.

Hell, K., Cardwell, K.F., Setamou, M. andSchulthess, F. (2000). Influence of insect infestation on Aflatoxin contamination of stored maize in four agriecological regions in Benin.*African Entomology 8*, 169-177.

Hell, K., Fandohan, P., Ranajit, B., Kiewnick, ? ., Sikora, R. and Cotty, P.J.(2007) Pre- and post harvest management of aflatoxin in maize:An African perspective. A research project. Arizona: University of Arizona. Retrieved from ag.arizona.edu/research/cottylab/.../hell%20et%20a...

Hell, K., Mutegi, C., and Fandohan, P. (2010). Aflatoxin control and prevention strategies in maize for Sub-Saharan Africa. Publication of 10 International Working Conference on Stored Product Protection. Pp. 534-540

Hoffman, V., Mutiga, S., Harvey, J., Nelson, R. and Milgroom, M.(2013). Afaltoxin contamination of maize in Kenya: observability and Mitigation Behaviour. Selected paper prepared for presentation at Joint Annual Meeting, Washington, DC, 4-6.

Holcomb, M., Wilson, D.M., Trucksess, M.W. and Thompson, H.C. (1998). Determination of aflatoxins in food products by chromatography. *Journal of Chromatography, 624,* 341-352.

Hosney, R. (2015). The Organic Farmer. Principles of Cereal Science and Technology.21-24 Retrieved from http://dx.doi.org/10.1002/jsfa.2288.

International Agency for Research on Cancer (IARC). (1993). Aflatoxins IARC Monogr Eval Risks Hum 56, 245-395.

International Centre for Insect Physiology and Ecology (ICIPE), (2014). New maize storage bags to protect against insects and deadly fungi poisoning:1-2. Rtreived from http://www.icipe.org/index.php/news/842-aflatoxin-bags.html.

Kaaya, A.N.,Warren, H.L., Kyamanywa, S. and Kyamuhan, W.(2005) The effect of delayed harvest on Moisture content,insect damage,moulds and aflatoxin contamination of maize in Mayuge district of Uganda. *Journal of the science of Food and Agriculture 85*, 2595-2599.

Kaaya, A.N. and Kyamuhangire, W. (2006). The effect of storage time and agroecological zone on mould incidence and aflatoxin contamination of maize from traders in Uganda.*International Journal of food microbiology, 110,* 217-223.

Katengwa, S., Rwanda, F.L.O. and Baijukya, F.(2013).Tripple Layer hermetic bags is a potential solution to storage pests in Rwanda. N2Africa Putting nitrogen fixation to work for smallholder farmer. Retrieved from http://www.n2africa.org/content/triple-layer-hermitic-bags-potential-solution-storage-pest.

Kenya Bureau of Standards (KEBS) (1988). Dry-Milled Maize Products. Kenyan Standard No.05158. Nairobi: Kenya Bureau of Standards.

Kenya National Bureau of Statistics (KNBS). (2009). Kenya:2009 Population Census. Nairobi: Central Bureau of Statistics, Ministry of Planning and National Development.

Kenya National Bureau of Statistics (KNBS) (2014). Socio-Economic Atlas of Kenya. Nairobi: Ministry of Devolution and Planning.

Imtiaz, H. (2011). Aflatoxin measurement and Analysis, Aflatoxins-Detection, Measurement and Control Dr. Irineo Torres-Pacheco (Ed).Retrieved from http://www.intechopen.com/books/aflatoxins-detection-measurement-and-control/aflatoxin-measurement-and-analysis.

Kuniholm, M.H., Lesi, O.A., Mendy, M., Akano, A.O. and Hall,A.J.(2008).Aflatoxin exposure and viral Hepatitis in the Etiology of Liver Cirrhosis in the Gambia,West Africa.*Environ Perspect 116,* 1553-1557.

Klich, M.A. (2007). Aspergillus flavus*:* the major producer of aflatoxin. *Molecular plant pathology.8*(6), 713-722.

Latimer, G.W. (2012). Official methods of analysis. Association of Officail Analytical Chemists (AOAC) International, (19th ed.) Canada:AOAC.

Lee, J.E., Kwak, B.M., Ahn, J.H. and Jeon, T.H. (2009). Occurrrence of aflatoxin M1 in raw milk in South Korea using an immunoaffinity column and liquid chromatography. *Food Control, 20*(2), 136-138.

Lewis, L.,Onsongo, M.,Njpau, H.,Schurz, R.,Luber, G., Kieszak, L., … and Marasas W.F.O. (1995). Fumonisins. Their implications for human and animal health. *Natural Toxicology.3,* 193–198.

Likhayo, P.W., Mbugua, J., Ngatia, C. and Mugho, S. (2004). Review of maize post-harvest research in Kenya.In: Post harvest research in maize: practical approaches to reduce maize storage losses in Africa. Workshop held at KARI-Kiboko, Kenya, June 15-17, 2004, CD publication, CIDA.

Machangi, J. (2000) Guideines on post harvest and grain storage management. Nairobi: Ministry of Agriculture.

Mace, K., Aguilar, F.Wang, J.S., Vautravers, P., Gomez-Lechon, M. and Gonzalez, F.J. (1997). Aflatoxin B1 –induced DNA adduct formation and P53 mutations in CYP450-expressing human liver cell line. *Carcinogenesis 18*(7), 1291-1297.

Mboya, R., Pangirai, T., Kwasi, S.Y., Derera, J., Maxwell, M. and Langyintuo, A.(2011). The quality of maize stored using roof and sack storage methods in Katumba Ward, Rungwe District, Tanzania: Implications on household food security. *Journal of stored food products and postharvest Research.2*(9), 189-199.

MDP, (2013). Makueni County Development Profile. Nairobi: MDP.

Milan, J. M. (2013). Ecological conditions affecting mycotoxin production in cereals: a review. *Veterinarni Medicina. 58*(8), 405-411.

Misore, A., DeCock, K., Carol, R. and Laurence, S. (2005). Case control study of an Acute Aflatoxicosis outbreak, Kenya. *Environmental Health Perspective. 113*(12).

Montes, G.N., Reyes, M.C.A., Montes, R.N. and Cantu, A.M.A. (2009). Incidence of potentially toxigenic fungi in maize (*Zea mays L*.) grain used as food and animal feed . *Journal of Food 7(*2), 119-125.

Mwihia, J.T., Straetmans, M., Ibrahim, A., Njau, J., Muhenje, O., Guracha, A. and Gikundi, S. (2008). Aflatoxin Levels in Locally Grown Maize fro Makueni District. *East African Medical Journal.85(7),* 311-317.

Nguefack, J., Leth, V., Amvam Zollo, P.H. and Mathur, S.B. (2004). Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi. *International Journal of Food Microbiology 94,*329-334.

Ngindu, A., Kenya, P.R., Ocheng, D.M., Omondi, T.M., Ngare, W. and Gatei, D.(1982). Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. *Lancet 1,* 1346-1348.

Nyikal, J., Lewis, L., Onsongo, M., Njpau, H., Schurz, R., Luber, G., … and Rubin C. (2004). Outbreak of Aflatoxin poisoning-Eastern province,Kenya,January-July 2004.Morb,Mortal.Wkly Rep.53:790-793.(Medline)contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. *Health perspective 113,* 1763-1767.

Onemolease, E.A., Aghanenu, A.S. and Adisa, T. (2005). Effect of formal school education on farmers output and adoption of innovations: A case study of rubber farmers in Ikpoba-Okha local government area of Edo State. *J. Teach. Educ teach. 5*(1), 114-118.

Platform for Africa-European Partnership in Agricultural Research for Development (PAEPARD) (2015). Reducing maize-based aflatoxin contamination and exposure in Zmibabwe. Retrieved from http://paepard.blogspot.co,ke/2015/10/reducing-maize-based-aflatoxin.html .

Patten R.C. (1981). Aflatoxin and disease. *American Journal of Tropical Medicine Hygiene 30,* 422-425.

Patterson, R.M. and Lima, N. (2010). How will climate change affect mycotoxins in food?.*Food Research International.4*(7), 1902-19143.

Philips, T.D., Afriyie-Gyawu, E., Williams, J., Huebner, H., Ankrah, N.A., Ofori-Adjei, D., … and Wang, J.S. (2008). Reducing human exposure to aflatoxin through the use of clay: a review. Food Additive Contamination Part A Chemical Analysis Control Exposure Risk Assessment. *PubMed. 25*(2), 134-145.

Pichler, E. (2006). Sampling for mycotoxins. Do we care enough? Romer Lab s Diagnostic GmbH, Tulln,Austria May-June 2013.Retrieved from www.romerlabs.com/.../user.../Press.../CFW-58-3-0117hr.pd.

Probst, C., Schulthess, H. and Cotty, P. (2010). Impact of *Aspergillus flavus* sect. Flavi community structure on the development of lethal levels of aflatoxins in Kenyan maize (Zea mays). *Journal of Applied Microbiology 108*, 600-610.

Reddy, S.V and Farid, W. (2000). Properties of Aflatoxin and ITS Producung Fungi. International Crop Research Institute for the Semi-Arid Tropics. Retrieved from http://www.icrist.org/aflatoxin/aflatoxin.asp.

Reed, C., Doyungan, S., Loerger, B. and Getchell, A. (2007). Response of storage molds to different initial moisture content of maize stored at 250C, and effect on respiration rate and nutrient composition. *Journal of stored prod. Res.43,* 443-448

Rukunga, G.K. (2001). Environmental health for East Africa. African Medical and Research Foundation Rural Health Series No.16. Nairobi: AMREF.

Schmale, D.and Munkvod, G. (2011). Mycotoxins in crops: A threat to human and demostic animal. health Retrieved from http://www.apsnet.org/edcenter/intropp /topics/pages/managementstrategies.aspx.

Skipper, P.L. and Tannenbaun, S.R. (1990). Protein adducts in the molecular dosimmetry of chemical carcinogens. *Carcinogenesis 11,* 507-518.

Setamou, M.,Cardwell, K.F., Schulthess, F. and Hell, K. (1997). *Aspergillus flavus* infection and aflatoxin contamination of pre-harvest maize in Benin. *Plant Disease 81,* 1323-1327.

Shaban, I., Kimanya, M., Gichuhi, P., Bonsi, C., Bovell-Benjamin, A. (2015). Maize storage and consumption practices of farmers in Handem district ,Tanzania: Collaries for Mycotoxin Contamination. *Open Journal of Preventive Medicine. 5,* 330-339.

Siwela, A.H., Siwela, M., Matindi, G., Dube, S. and Nziramasanga, N. (2005). Decontamination of aflatoxin contaminated maize by dehulling. *Journal of Science of the Food and agriculture 85,* 2535-2538.

Sundar Rao, P.S.S and Richard, J. (2003). An Introduction to Biostatistics. A manual for students in health sciences,3rd ed. New Delhi: Prentice/Hall.

Smith, J.E., Solomons, G.L., Lewis, C.W. and Anderson, J.G. (1994). Mycotoxins in Human Nutrition and Health. Brussels: European Commission CG XII. Luxembourg, EU: Agro-Industrial Division.

Smith, T.W. (2006). The sub-sampling of non-respondents on the 2004 general social survey. National Opinion Research Centre/University of Chicago. GSS Methodology Report No.106.

Songa, W. and Irungu, J. (2010) Post harvest challenges to food security in Kenya. Ministry of Agriculture report. Nairobi: Ministry of Agriculture.

Strosnider, H., Azziz-Baumgartner, E., Banziger, M., Bhat, R.V. Breiman, R. and Brune, M.N. (2006). Workgroup report: public health strategies for reducing aflatoxin exposure in developing countries. *Environmental Health Perspectives 114*, 1898-1903.

Summer, E.P. and Lee, D. (2009) .Reducing Aflatoxin in Corn During Harvest and Storage. *Learning for Life Bulletin 1231*, 4-12.

Thamanga-Chitja, J.M.,Sheryl, L.H., Gerald, F.O. and Maryan, G.(2004). Impact of maize storage on rural household food security in Northern Kwazulu-Natal. *Journal of Family Ecology and Consumer Sciences, 32,* 8-15.

Trucksess, M.W., Stack ,M.E., Nesheim, S., Page, S.W., Albert, R.H. and Hansen, T.J. (1991). Immunoaffinity column coupled with solution fluorometry or liquid Chromatography post-columnn derivatization for determination of aflatoxins in corn, peanuts, peanut butter: collaborative study. *Assoc off Anal Chem 74*(1), 81-88.

Turner, P.C., Sylla, A., Gong, Y.Y. and Diallo, C. (2005). Reduction in exposure to carcinogenic aflatoxins by post-harvest intervention measures in West Africa. A community based intervention study. *Lancet.365*, 1950-1956

Udoh, J.M., Cardwell, K.F. and Ikotun, T. (2000). Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria. *Journal of Stored Products Research*.36, 187-201.

Unger, P.D., Mehendale, H.M. and Hayes, A.W. (1997). Hepatic uptake and disposition of aflatoxin B1 in isolated perfused rat liver. *Toxicol Applied Pharmacol 41,* 523-534.

Walker, S. and Davies, B. (2013). Afaltoxins: Finding Solutions for Improved Food Safety. Farmers Perceptions of Aflatoxins: Implications for Intervention in Kenya.*Food Agriculture and Environment.20*(7), 2-3.

Wambugu, P.W., Mathenge, P.W., Auma, E.O., and HA Van, R.(2009). Efficacy of traditional maize (*Zea mays L.)* seed storage methods in Western Kenya. *African Journal of Food Agriculture Nutrition and Development. 9*(4), 1110-1118.

Weinberg, Z.G., Yan, Y., Chen, Y., Finkelman, S., Ashbell, G. and Navarro, S. (2008). The effect of moisture level on high-moisture maize (Zea mays L.) under hermetic conditions-*in vitro* studies. *Journal of Stored Products Research 44,* 136-144.

Wiesmann, U., Kiteme, B. and Mwangi, Z.(2014). Socio-Econominc Atlas of Kenya: Depicting the National Population Census by County and Sub-Location. Nairobi: KNBS.

Williams, J.H., Philips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M. and Aggarwal, D. (2004). Aflatoxicosis in developing countries: A Review of Toxicology, Exposure and potential Health consequences, and Interventions. *American Society of Clinical Nutrition.80,* 1106-1122.

Wilson, J.P., Jurjevic, Z.,Hana, W.W.,Wilson, D.M., Potter, T.L. and Coy, A.E. (2006) Host specific variation in infection by toxigenic fungi and contamination by mycotoxins in pearl millet and Corn. *Mycopathologia 161,* 101-107.

Wilson, H. J., Philips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M. and Aggarwal, D. (2006). Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences and interventions. *American Journal of Clinical Nutrition 82,* 1106-1122.

Wilson, D.M. and Abraham, M. (1992). Mycotoxins. In: D.B. Saucer (Ed). Storage of cereal grains and their products: *American Association of Cereal Chemists, Inc; 5,* 341-391.

Wu, F. and Khlangwiset, M. (2010). Health economic impact and cost-effectiveness of aflatoxin reduction strategies in Africa: case studies in bio-control and post-harvest interventions. *Food Additives and Contaminants, 27,* 496-509

APPENDICES

APPENDIX 1: INFORMED CONSENT FORM IN ENGLISH LANGUAGE

INTRODUCTION

The researcher, James Malusha, is a student at Jomo Kenyatta University of Agriculture and Technology, the supervisors Professor Mohammed Karama from Kenya Medical Research Institute and Professor Anselimo Makokha from Jomo Kenyatta University of Agriculture and Technology, are carrying out a study in your community to assess maize storage and pre-storage practices in your household. The proposed study focuses on improving maize storage and pre-storage practices so as to prevent aflatoxin contamination of maize and hence prevent ill health resulting from consumption of contaminated maize as well as reducing post-harvest loses. Although the study is for academic purposes, the findings will inform planning for improvement of maize storage and pre-storage practices with the aim of preventing aflatoxin contamination.

Before you decide if you should participate in this study, you need to know about any good or bad things that may arise if you join. This form tells you about the study. You can ask any questions you have at any time.

BEING IN THE STUDY IS YOUR CHOICE

Participating in this study is your choice. The consent form gives you information about the study and the risks will be explained to you. Ounce you understand the study, and if you agree to take part, you will be asked to sign your name or make your mark on this form.

Before you learn about the study, it is important that you know the following:

- Your participation in this study is entirely voluntary

- You may decide not to answer questions, give any samples or even withdraw from the study at any time.

PARTICIPATION IN THE STUDY

In this study we are trying to learn more about storage and pre-storage practices of maize and their relationship with aflatoxin contamination which adversely affects people health.

If you agree to participate in this study by signing at the end of this form, you will participate in the study. You will be asked questions about yourself and socio-demography characteristics of household, and how you harvested, dried and stored your maize of the previous season. You will also be asked to provide one kilogram of maize sample for analysis for aflatoxin contamination.

Once you agree to participate you will be followed up again in the next season of maize harvest.

STUDY PRTICIPANTS

The study participants will comprise adult heads of selected households. All participants selected are important to this study.

RISKS AND BENEFITS OF THE STUDY

The study has no serious risks to participants. However we shall require one kilogram of maize for aflatoxin analysis to determine afltatoxin levels. By agreeing to participate you will receive free advice on improvement of maize storage and pre-storage at the end of the study, where necessary

COSTS TO YOU

There is no cost to you for your participation in the study.

CONFIDENTIALITY

All information provided to us throughout the study will remain confidential and will only be used to provide for the objective it is intended to. Only the study team will have access to this information and will not be relayed to any other person. Data will be securely managed.

YOUR RIGHTS AS A PARTICIPANT

This research has been reviewed and approved by the Ethical Review Committee of Kenyan Medical Research Institute (KEMRI), if you have any questions about your rights as a research participant you may contact the secretary of the KEMRI Ethical Review Committee (a group of people who review the research to protect your rights) at 020-272-2541, or 020-272-6781.

WITHDRAWAL FROM THE STUDY

Participation to the study is voluntary. No one will be upset if you do not participate, or if you change your mind later and want to stop. You can also skip any of the questions you do not want to answer.

If there are any questions you have about the study, please fill free to ask them to the investigator prior to signing your consent form. You may contact James Malusha (0734533343) or Professor Mohammed Karama of KEMRI (0722885366) or Professor Anselimo Makokha of Jomo Kenyatta University of Agriculture and technology (0713817436), or the secretary National/KEMRI Ethical Review Committee (ERC) on Tel: 2722541/2713349.

YOUR STATEMENT OF CONSENT AND SIGNATURE

If you have ready the informed consent, or had it read and explained to you, and you understand the information and voluntarily agree to join this study, please carefully read the statements below and think about your choice before signing your name or making your mark below. No matter what you decide. It will not affect your rights in anyway:

- I have been given the chance to ask any questions I have and I am content with the answers to all my questions.
- I know that my records will be kept confidential and that I may leave this study any time
- The name, phone number and address of whom to contact in case of an emergency has been told to me , and has also been given to me in writing
- I agree to take part in this study as a volunteer, and I will be given a copy of this informed consent form to keep.

---------- ----------

Name of Participant Signature and date

……. ……

Name of Research staff Signature and date

APPENDIX 2: INFORMED CONSENT TRANSLATED INTO KIKAMBA LANGUAGE

KWITHIMA NTHINI WA UKUNIKILI /KWIYENDEA KWA MWENE

UKUNIKILI IULU WA WII WA MBEMBA NA UWAU WA AFLOTOXIN MISYINI YA COUNTY YA MAKUENI – KENYA

KWIYIELESYA

Ula ukwika ukunikili uu James Malusha ni musomi kuma university (kana sukulu mun ene) ya Jomo Kenyatta uimi na uvundi ; atongoesye ni asomi anene (professors) Mohammed Karama wa Sukulu ya kusomethya/kuvundisya Matakitali (MKTC) na Anselimo Makokha wa university ya Jomo Kenyatta ya uimi na uvundi.

Kielelo kya. Ukunikili uu ni kwongela umanyi iulu wa wii wa mbemba (ngetha) na uwau wa aflotoxin nikenda tutonye kwivetana /kusiia uwau usu (aflatoxin) ula uetawe ni kuya mbemba (isyo) ila syina aflatoxin; na twivetana na mawasyo ala mokaa itina wa ngetha.

Ona kutwika ukunikili uu ni wa masomo ukatumika kuvanga mawalanio ma undu wa kwivetangana na afatoxin kwisila wii museo wa ngetha.

Mbee wa kusuania kwithiwa nthini wa ukunikili vu, ni useo kumanya useo na uthuku ula wisa kuetwe kwisila kwithiwa nthini wa ukunikili uu. Ithangu/form yii yieleetye iulu wa ukunikili uu na wimwitikilye ukulya makulyuo ivinda yonthe.

KWITHIWA NTHINI WA UKUNIKILI IN NGENDA YAKU (YAMWENE)

Kwitikila kwithiwa nthini wa ukunikili uu ni kwa kwiyendea na form/ithangu ya kwitikila kuendeea na ukunikili nikueleetye miisyo/mbanga ya ukunikili uu.

Waelewa na kwitikila kwithiwa nthini wa ukunikiliuu, wikulwa uandike isyitwa yaku kana alama yaku.

Mbee wa kumanya/kusoma iulu wa ukunikili/uthuimi uu in useo amanye maundu aa:

- Kuendeea na uthuimi/ikunikili uu ni kwa kwiyumia/kwiyendea
- Nutonya kulea kusungia makulyo, kunengane mbemba(sample) nini kana kueka uthuimi/ukunikili uu ivinda yonthe.

KWITHIWA NTHINI WA UTHUIMI/UKUNIKILI

Nthini wa ukunikili uu twienda kumanya muno iulu wa nzia na usuvii wa ngetha/mbemba na undu itaenie na uwau wa aflatoxin. Weetikila kwithiwa nthini wa ukunikili uu kwa kwikia saii itina wa form/ithangu yii nukwitikilwa kuendeea na ukunikili uu. Ukakulwa makulyo ala maukonetye na ala makimaendeeo nthini wa musyi waku; undu wakethie, kwa nika na kwia mbemba /ngetha mbua

Ukakulwa unengane kilo kimwe kya mbemba ikekwe ukunikili/kuthimwa iulu wa aflatoxin. Weetikila kwithiwa nthini wa ukunikili uu ukaatiiwa nginya mbua ila yukite, ya ngetha ya mbemba.

ALA MAKETHIWA NTHINI WA UKUNIKILI

Ala makethiwa nthini wa ukunikili in ene misyi ila ikasakuwa. On the ala makethiwa nthini wa ukunikili ni mavata.

MIISYO NA VATA WA UKUNIKILI/UTHOIMII UU

Uthoimi uu ndwina miisyso/mbanga kwa ala makethiwa nthini. Ateo nukakulwa kumya kilo kimwe (mukeve umwe) wa mbemba ikathuimwe/ikathimwe maundu ma aflotoxin.

Weetikila kwithiwa nthini wa ukunikili, ukakwata motao vate ndivi iulu wa undu wa kusuvia/kwia ngetha.

NGALAMA YAKU

Vai ngalama kwa kwithiwa nthini wa ukunikili uu

KIMBITHI

Uvoo wonthe twikwata ukunikilini uu ukethiwa wi wa sili/kimbithi na ukatumiwa kwoondu wa keileelo kya ukunikili na no ala me nthini wa ukunikili makethwa matonya ukwata uvoo usu. Uvoo usu ukasuviwa muno.

Ukunikili uu nuthuimitwe na uketikilithwa ni nzama yaKenya ya Utakitali na uthuimi/ukunikili (KEMRI). Ni aki yaku wethiwa na makulyo ukulye muandiki wa nzama isu (KEMRI) kwisila nambani ino ya simu – 020 272-2541 kana 020-272-6781

KWIVETA UKUNIKILINI UU

Kwithiwa nthini wa ukunikili ni kwiyendea, vayi mundu euthatya kulea kwithiwa nthini waukunikili uu kana kwisilya kuma nthini wawo. Nutonya kulea kusungia makulyo ala utekwenda kusungia.

Ketha wina makulyo iulu wa ukunikili uu, wina uthasyo wa ukulya uthoimi/ukunikili mbee wa kusaini form/ithangu ya kwitikila kwithiwa nthini wa ukunikili uu. Etha wina thina nutonya ukulya mundu ula niwe ukusisya maundu menyu nake niwe :-
Malusha kwisila nambani ya simu – 0734 533 343, kana Mohammed Karama wa KEMRI – 0722 885 363, Anselimoi Makokha wa JKUAT – 0713817436 kana muandiki wa nzama ya KEMRI (ERC) Tel 2722541/2713349

UKUSI WA KWIKIA SAII

Ketha niwasoma kana wasomewa na kueleswa na waelewa na kwitikila kwa ngenda (vate kulasimithya) kwika ukunikili uu, kwa ndaia soma aa utanekia saii, isyitwa yake kana alama yaku.

- Ninanewa mwanya kukulya makulyo naneaniwa ni mausungio ala nanewa.
- Ninisi kama uvoo wakwa ukeethiwa wiwa kimbithi na nindonya kueka ukunikili ivinda yonthe.
    - Isyitwa yakwa, namba yakwa ya simu, isanduku ya valua ya mundu ula ndonya kwona ivinda yonthe ninatavwa kwa kuandikwa ithanguni.
    - Ningwitikila kwithiwa nthini wa ukunikili uu kwa ngenda, na nganewa ithangu/form ino ya kwitikila.

…… ……..……..

Isyitwa ya ula wi nthini Wa ukunikili Saii Matuku

…… ……. ……

Isyitwa ya ula eendesya ukunikili Saii Matuku

APPENDIX 3: QUESTIONAIRE FOR HOUSE HOLDS IN ENGLISH LANGUAGE

District: Household No.

Division: Household owner:

Location: Enumerator name:

Sub location: Date of interview:

Village:

PART ONE: SOCIO-DEMOGRAPHIC DATA OF RESPONDENTS

1. Sex of respondent: 1. Male ☐ 2. Female ☐

2. Age of respondent in years……..

3. Highest level of education of respondent.

Level	Completed	
	Yes	No
1. Never been to school (no education)	☐	☐
2. Primary education	☐	☐
3. Secondary education	☐	☐
4. Post secondary/tertiary education (Degree, Diploma, Certificate)	☐	☐

5. Marital status of respondent.

1. Married ☐ 4. Separated ☐

2. Single/never married ☐ 5. Widowed ☐

3. Divorced ☐

6. What is your religious affiliation?

1. Non- religious (Atheists) ☐ 5. Other Christians ☐

2. Catholic ☐ 6. Traditional religion ☐

3. Protestant ☐ 7. Muslim ☐

4. SDA ☐ 8. Other specify ….

7. What is your main occupation?

1. Farming ☐

2. Business (Shop, hotel, bar, grocery/vegetable kiosk etc)…… ☐

3. Employed (Teacher, Health worker, Agricultural worker etc) …… ☐

4. Others specify…….

8 .What is the main source of income in the household?

1. Farming ☐ 3. Salary ☐

2. Business ☐ 4. Casual/temporary jobs ☐

5. Others specify……..

9. What is your estimated income per month? Kshs……

Indicate range of respondents income by ticking appropriate category below.

1. Kshs 0 to 5,000 ☐
2. Kshs 5,001 to 10,000 ☐
3. Kshs 10,001 to 15,000 ☐
4. Khs 15,001 to 20,000 ☐
4. Khs 20,001 to 25,000 ☐
5. Khs 25,001 to 30,000 ☐
5. Above Kshs 30,000 ☐

10. How many people live and eat in this household?.....

PART TWO: KNOWLEDGE/AWARENESS ON AFLATOXIN

Have you heard of health problems known as aflatoxin associated with consumption of Maize?

1. Yes ☐ 2. No ☐

11. If yes, what is the source of information?

1. Print media (newspapers, posters etc) ☐
2. Electronic media (Radio, TV etc) ☐
3. School ☐
4. Mosque ☐
5. Public meeting ☐
6. Church ☐

7. Other specify……

12.Which main health problem may result from consumption of maize which is not dried and stored in a proper manner?

1. Abdominal problems ☐
2. Headache ☐
3. Fever ☐
4. Diarrhoea ☐
5.Others specify……
6. Don't know ☐

13.Are there any problems which result from improper maize storage (i.e.maize not stored in well ventilated clean store and directly on the floor)?

1. Yes ☐ 2.No ☐

14.If yes, which ones?

1. Maize turns greenish ☐ 4. Insects /pests infestation ☐

2. Maize change color ☐ 6. Not Applicable ☐

3. Maize rot/decompose ☐

5. Others specify……...

15. Has anybody in this household suffered from disease suspected to result from consumption of maize contaminated with aflatoxin (ie aflatoxicosis) since 2004?

1. Yes ☐ 2.No ☐

16. If yes, how many people?..... and did any one die ? 1. Yes ☐ 2. No ☐

17. Can you identify maize which has been contaminated with aflatoxin?

1. Yes ☐ 2 No ☐

18. Do you know of any signs which indicate possibility of maize being contaminated with aflatoxin?

1) Yes ☐ 2. No ☐

19. If yes, what are these signs? (Start with main ones).

1. Green colour ☐ 2. Germinating ☐ 3. Rotting ☐

4. Others specify……...

5. Don't know ☐ 6. N/A ☐

20. What do you think is the main cause of aflatoxin contamination in maize?.....

1. Improper/poor storage ☐

2. Inadequate drying ☐

3. Harvest of maize when not properly/readily dry ☐

4. Fungi/mould ☐

5. Others specify……

6. Don't know……. ☐

21. What main health problem may result from consuming maize contaminated with aflatoxin?

1. Abdominal pain ☐ 2. Fever ☐ 3.Headache ☐

4. Diarrhoea ☐ 5. Death ☐ 6. Cancer ☐

7. Others specify…… 8. Don't know ☐

22. What do you do to prevent maize from being contaminated with aflatoxin?

1. Drying before storage ☐ 5. Don't know ☐

2. Proper storage ☐

3. Harvesting mature maize ☐

4. Others specify……

23. What is the approximate quantity of maize or maize products you as an individual consume per day? (eg. Muthokoi, Githeri, Ugali, Uji etc) ……Kg.

PART THREE: MAIZE PRE-STORAGE AND STORAGE PRACTICES

PRE- STORAGE PRACTICES

24. Where did you obtain your maize seeds for planting during the last season?

1. Shop/market . ☐ 4. Free issue from GOK/Donor ☐

2. From previous harvest ☐ 5. Others specify……

3. From neighbor ☐

25. What type/variety of maize seeds did you plant last season?

1. katumani ☐ 2. 512 ☐ 3. 612 ☐ 4. 614 ☐

5. Pwani hybrid ☐ 6. Dume 43 ☐ 7. Traditional maize ☐

8. Others specify……

26. Were they treated with any chemical preservative?

1. Yes ☐ 2. No ☐

27. Do you suspect the seeds can contribute to aflatoxin contamination?

1. Yes ☐ 2. No ☐

28. If yes, which type/variety of seeds?

1. Katumaini ☐ 2. 512 ☐ 3. 612 ☐ 4. 614 ☐

5. Pwani hybrid ☐ 6. Pioneer hybrid ☐ 7. Others specify……

8. N/A ☐

29. Did you irrigate your maize while growing in the shamba?

1. Yes ☐ 2. No ☐

30. Did you apply insecticides to your maize while growing in the shamba/Garden?

1. Yes ☐ 2. No ☐

31.If yes how many times…….. N/A ☐

32. What is the duration of maize in the field before harvest after maturity?.....

33. Do you de-husk (remove cob cover) maize during harvest? 1. Yes ☐ 2. No ☐

34. How long do you expose maize (immediately after harvest) to dry before storage?.....days

35. Which method do you use for drying maize after harvest?

1. Drying in the open sun ☐ 3. Kitchen granary above fire place ☐

2. Drying in the shade ☐ 5. Don't dry ☐

4. Others specify……

36. Do you place maize on top of any material (e.g. polythene sheet, iron sheet etc) on the ground during drying to prevent maize contact with the ground?

1. Yes ☐ 2. No ☐

37.If yes, why? 1. To prevent contamination ☐. Prevent maize rotting ☐

3. To dry faster ☐ .Others specify……5. N/A ☐

38. Why should maize be dried properly (ie to attain required moisture content of <14 %?)

1. To prevent it from rotting ☐ 2. To avoid germination ☐

3. To prevent aflatoxin contamination ☐ 4.To avoid pest/insect infestation ☐

5. Others specify…… 6.Don't know ☐

39. Do you clean maize to remove broken grains, fine materials, dirt etc. prior to storage?

1. Yes ☐ 2. No ☐

STORAGE PRACTICES

42. Where do you store your maize?

1. In bags directly on the floor ☐ 6. On traditional cribs ☐

2. In bags, on the platform /trestles ☐ 7. On improved cribs ☐

3. In cob form, directly on the floor ☐

4. Under or on top of the ceiling/granary ☐ 8. Others specify……..

5. Shelled and stored in sealed containers ☐

43. If stored in bags, how are they arranged?

1) In stacks with space in between ☐ 5. Not applicable ☐

2) In stacks without space in between ☐

3) Haphazardly ☐

4) Other specify…….

44. Where did you obtain your maize from?

1) My own farm/homegrown ☐

2) Directly from other farmers within the locality ☐

3) Middlemen/women ☐

4) Fromshops/markets ☐

5) Other specify……..

45. What is the quantity of maize in storage (estimate in Kilograms) ? …….

46. In what form are maize stored? 1. Cob ☐ 2. Grains ☐ 3. Husks ☐

4. Others specify……

47. For how long has this maize been in the store.....? (Take samples only from Maize of this season harvest).

48. Are there any pests'/insects affecting maize in storage?

1) Yes ☐ 2) No ☐

49. If yes, what have you done to remove them?

1. Sprayed with pesticides ☐ 2.Applied herbs ☐ 3. Others……

4. Done nothing ☐ 5. N/A ☐

50. How often do you clean the store? 1. Once per week ☐ 2. Once per two weeks ☐

☐

3. Once per month 4. Never ☐ 5. 0thers specify……..

51. What do you do to prevent pest/insect infestation of stored maize?

1. Proper drying ☐ 4. Others specify……

2. Spraying with pesticides ☐ 5. Don't know ☐

6. Maintaining store clean ☐

52. Why should maize be stored properly (ie. in well ventilated clean store and not directly on the floor)?

1. To avoid dirt ☐ 4. To prevent rotting/spoilage ☐

2. To avoid aflatoxin contamination ☐ 5. To let them dry ☐

3. To avoid/reduce moisture ☐ 7. Don't know ☐

6. Others specify……

ALTITUDE/CLIMATE VARIATION

Which climate/altitude (high or low) increased aflatoxin contamination of maize?

1. High ☐ 2. Low ☐

MAIZE HARVEST SEASONS

54. Which maize harvest season (Long or Short season) increased aflatoxin contamination of maize?

1. Long season ☐ 2. Short season ☐

Any other comments from respondent and observation……..

APPENDIX 4: QUESTIONAIRE FOR HOUSE HOLDS IN KIKAMBA LANGUAGE

MAKULYO MA NYUMBA

Wilaya…….

Tarafa…….

Jina la mwenye kuhoji……..

Kata…….

Muthenya wa makulyo …….

Kata ndogo……

Utui…….

No. ya nyumba……..

PART ONE: SOCIO-DEMOGRAPHIC DATA

1. Wi mundu muka kana munduume? 1. Munduume ☐ 2. Mundumuka ☐

2. Wina miaka yiana?……

3. Usomete ukavika va**?**

	Kiwango	Kumina	
	☐	Yii	Aie
1. Ndyaasoma		☐	☐
2. Primary	☐		
3. Secondary	☐		

4. Post secondary/tertiary education (Degree, Diploma, Certificate etc)

☐ ☐ ☐

5. Wina kiveti/Muume?.

1**.**Nimutwae ☐ 2. Ni mataanisye ☐

3. Ndimutwae ☐ 4. Ndiwa ☐

5. Nimatianie ☐

6. Wi mwitikili kana mulei ?

1. Ndyithaa na muikiio ☐ 5. Miikiio ingi ☐
2. Mukatholiki ☐ 6. Mizimo ☐
3. Muprotestant ☐ 7. Muislam ☐
4. Savato ☐ 8. Makanisa angi……..

7. Uthukuma wia viya?

1. Muimi …….. ☐

2. Viasala (nduka, uteli,uaa, mboka, kiosiki na kuendeea) ☐
3. Muandikwa (mwalimu, ndakitali, muimi na kuendeea) ☐
4. Angi…….

8. Nyumba ikwataa mbesa kumana na wia mwau?

1.Uimi ☐
2. Viasala ☐
2. Musaala ☐
3. Kivalua ☐5. Weta angi…….

9.Ukwati waku kwa nita mbesa syiana kwa mwei?

1.Kshs 0 to 2500 ☐

(Kuma silingi umwe muvaka silingi ngili ili na maana atano)

2. Kshs 2501 to 5000 ☐

(Kuma ngili ili na maana atano muvaka ngili itano)

3.Kshs 5001 to 10000 ☐

(Kuma ngili itano na silingi umwe muvaka ngili ikumi)

4. Khs 10001 to 20000 ☐

5. Zaidi shilingi Kshs 20000 ☐

10. Ni andu meana mekalaa na kuya nyumb ani ino?.....

PART TWO: KNOWLEDGE/AWARENESS ON AFLATOXIN/UMANYI KUMANA NA AFLATOXIN

11.Waaiwa uwau uetawa ni kuya mbemba syina sumu?

a) Yii… ☐ b) Aiee…… ☐

12.Ketha niwo weewie va?

a)Mathanguni ☐

b) Redioni, television ☐

c)sukulu ☐

d) Makanisani ☐

e) Musikitini ☐

f) Valasa ☐

g) Kundu kungi weta……

13. Ni thina meva utonya kukwatikana na kuya mbemba itenyau na itembie nesa? ……

14. Vena thina utonya kumana na kwia mbemba nai, kwangelekonyo kuia vandu vatekulikya nzeve nesa kana kwia simitini? 1.Yii ☐ 2.Aiee ☐

15. Ketuiwo niwo, nituma mwau……..

16. Vena mundu kuma nyumba ino waithiwa e muwau nundu wa kuya mbemba syina aflotoxin kuma 2004? a)Yii…. …… b) Aiee…….. ☐ ☐

17. Ketha mevo ni andu meana?..... a)Yii…… ☐ b) Aiee…. ☐

18.. Nutonya kuvathukania mbemba syina uwau wa afloxaxin

a) Yii…. ☐ **b)**Aiyee…….. ☐

19. Niwitha wisi ndalili itonya kwonanya mbemba syina aflotoxin

a) Yii……. ☐ b) Aiee …… ☐

20. KETHA NIWISI NI NDALILI SYIKU? ……

11. .Ni Ni kyau kitumaa mbemba ithuka?..... ☐

a) Kuia nai

b) kulea kwanika mbemba nesa ☐

d) Kuketha itanamba kuma

c) Ingi…… ☐

d) Ndyisi……. ☐

21. Ni mathina meva ma kimwii maumanaa na kutumia mbemba syina uwau wa aflotoxin

22.Utonya kwika ata kusiia mbemba siikakwatwe ni uwau wa afatoxin…….

.

23. Ni kiwango kiana ata kya mbemba kana liu useuvitwe na mbemba mundu utonya uya kwa muthenya e.g. (muthokoi, issyo, ngima usuu na kuendeea? (eg. Muzokoi, Githeri, Ugali, Uji etc) ……Kg.

PART THREE: MAIZE PRE-STORAGE AND STORAGE PRACTICES/ USUVII WA MBEMBA/ MWILE WA MBEMBA

PRE- STORAGE PRACTICES/USUVII WA MBEMBA

24. Waumisye mbeu ya kuvanda va mbuani ila yiila kua?

1. Ndukani ☐ 2. Ngethani ila nguu 3☐ Kwa mutui 4☐ Kwa silikali ☐

5. Ingi……

25.Ni muthemba wiva wa mbeu wavandie mbua ila yiila kua?

(katumaini, 512, 612, pwani hybrid nk)?.....

26. Ni syeekiitwe ndawa yakusiia siikanangike? 1.Yii ☐ 2. Aie ☐

27. Nukwona mbeu ta itonya kuete uwau wa aflotoxin? 1. Yii 2☐ . Aie ☐

28. Ketha niwo ni mbeu syiva?.....

29.Niwitikiasya mbemba kiwu siendee kumea 1. Yii ☐ 2. Aie ☐

30. Nukunaa mbemba ndawa yila syi muundani? 1. Yii…… ☐ Aie……. ☐

31. Ketha niwo mala meana?……..

32.Mbemba syikalaa muundani ivinda yiana ata syeewa?.....

33. Nusungaa mbemba ivinda ya ngetha? 1. Yii… ☐ 2. Aie… ☐

34. Wanikaa mbemba ivinda yiana ata unatanamba kwikia ikukumbini?.....

35. Utumiaa nzia syiva uyanika mbemba? a). Wanikaa suani ☐

b) wanikaa muunyini ☐

. c). Ingi……

36. Niwalanasya mbemba ta ithanguni kana ivatini ivinda ya kwanika inyae nikana usiie siikakwate nthi?

a). Yii…….. ☐ b). Aie……. ☐

37. Niki mbemba syaile kunyaa nesa (ikwate unyau ula waile ute itheo wa 14%……

38. Nunyuvaa mbemba kumya ila ndilikangu na kiko?

a) Yii… ☐ b) Aie… ☐

STORAGE PRACTICES/MWILE WA MBEMBA

39. Wiaa mbemba syaku va?

a) Simitini ☐

b) Mbwauni ☐

c) Kialani ☐

d) Kiango/laa ☐

e) Sitoo ☐

f) Ingi……..

40. Ketha wiaa makuniani umavangaa ata?

a) Makunia mena myanya katikati (Makunia ma makonge) ☐

b) Makunia matena myanya katikati (Mavasania) ☐

c) Vate muvango ☐

d) Ingi……..

41. Wumasya mbeu syaku va?

a) Muundani wakwa ☐

b) Kuma kwi aimi ala angi atui ☐

c) Blocka ☐

d) Ingi……..

42. Wina kilo syiana ata sya mbemba ila uiite.....

43. Mbemba siiawa ata? a). .misakwa ☐ b) Syimbale ☐

c). Ingi……

44. Mbemba ii syikalite ivinda yiana atsa nthini wa sitoo

45. Vena tusamu ta ngulu ikwatie kwananga mbemba syi sitoo?

a) Yii…….. ☐ b) Aie ☐

46. Etha syivo wikanaa ata nasyo

47. Utheasya sitoo yaku ivinda yiana ata? 1. imwe kwa kyumwa…. ☐

2. imwe kwa syumwa ili.. ☐

3. imwe kwa mwei….. ☐ 4. nditheasya 5. ingi…….. ☐

48. Wikaa ata nikenda usiie ngulu kusyaana mbembani yila syi sitoo?

49. Niki mbemba syaile kwiwa nesa (ta store ntheu, ikulikya nzeve nesa na ti simitini nundu wa uthithu

MAVINDA ME KIVATHUKANIO

50. Nukwisilya kana nzeve ya vandu vaa nitonya kwananga mbemba na uwau uu wa aflatoxin?

a) ii ☐ b) Aiee ☐

53. Etha nitonya kwananga ni ata?

a)Itonya kwongeleela uwau wa aflatoxin ☐

b)Itonya kunyivya uwau wa aflatoxin ☐

c)Nditonya kwithiwa na uthuku ☐

d)Nzia ingi……

MAVINDA MA NGETHA YA MBEMBA

54. Niwisilasya kana mauvandi ma mbemba na ngetha ya mbemba syi kivathukanio nitonya kutuma aflatoxin yananga mbemba?

a) ii ☐ b) Aiee ☐

APPENDIX 5: OBSERVATION CHECKLIST FOR MAIZE STORAGE

1. Quantity of maize in storage in Kilograms…….

2. Physical condition of maize a) Discoloration……Yes… ☐ No… ☐

b) Mouldy…… Yes… ☐ No….. ☐

c) Insect/pests infestation…Yes… ☐ No….. ☐

3. Condition of the storage facility (maize store)

	Clean	Dirty	Score (1 poor -5 excellent)	other observations
a) Floor	…… ☐	…….. ☐		…….
b) Walls	…… ☐	……. ☐		…. …….
c) Roof/ceiling	…… ☐	…… ☐		…….
d) Platform/trestles	…….. ☐	…….. ☐	☐	……

e) Others (specify) ……..

4. Is the store properly ventilated? That's it has openable windows or air inlets.

Yes..... ☐ No. ☐ Score (1 poor-5satisfactory)…….. ☐

5. If yes, what type of ventilation?

a) Cross ventilation ☐

b) Side ventilation ☐

c) Through ventilation ☐

d) Permanent Air vents (P.Vs) ☐

e) Other specify…….

6. Is there adequate natural lighting? Yes…☐ No….. ☐ Score (1 poor -5 satisfactory)

7. Is the store overstocked? Yes… ☐ No…. ☐

APPENDIX 6: FORM FOR COLLECTION OF MAIZE SAMPLE FOR AFLATOXIN ANALYSIS

Maize sample collection for analysis

1. Particulars of sample: Sample no……

Head of house hold ……

Household no……

Date collected……

Amount collected (kg)……..

2. Date sample submitted…….

3. Sample analysiss:

Moisture content (%)……

Aflatoxin content (B1…….B2…….....G1…….G2…….)

Total Aflatoxin content (B1+B2+G1+G2)……..…….

APPENDIX 7 FORM FOR COLLECTION OF DATA ON TEMPERATURE AND HUMIDITY

HOUSEHOLD NO.

STUDY SITES	**SEASONS**			
	SEASON 1		**SEASON 2**	
	Temperature	**Humidity**	**Temperature**	**Humidity**
KIBWEZI				
KILOME				

APPENDIX 8: FOCUS GROUP DISCUSSION GUIDE FOR COMMNITY INFORMANTS IN ENGLISH LANGUAGE

1. What problems are associated with improper storage of maize?
2. What is your understanding on aflatoxin/Aflatoxicosis?
3. How serious has been aflatoxin problem in this area?
4. What causes Aflatoxin contamination in maize?
5. What signs lead to suspicion that maize is contaminated with Aflatoxin**?**
6. Are there any community cultural beliefs regarding Aflatoxin
7. What happens when a person consumes maize contaminated with Aflatoxin?
8. What actions do you take to prevent Aflatoxin**?**
9. What are the pre-harvest/pre-storage practices?
10. Why it important to harvest mature/dry maize?
11. Why is it important to properly dry maize prior to storage?
12. What are the main storage practices of maize after harvest?
13. What are Government authorities i.e. Ministry of Agriculture, Ministry of Health doing to prevent Aflatoxin contamination of maize?
14. What can be done at farm and household level to prevent contamination of maize with Aflatoxin?
15. What challenges/constraints and recommendations, if any, in implementing Aflatoxin prevention measures in maize?

APPENDIX 9: FOCUS GROUP DISCUSSION GUIDE TRANSLATED INTO KIKAMBA LANGUAGE

(MAWONI MA UNENANISIA MBAI)

1. Ni mathina mepa maitawa ni kuya mbemba nai?
2. Ni kyau kitawa Afrotoxin?
3. Aflotoxin yietae mathina meku kuu kwenyu?
4. Ni kyau kitumaa wona kana mbemba syina aflotoxin?
5. Ni kyau kitumaa wona kana mbemba sina Aflotoxin?
6. Pe syithio kumana na Aflotoxin?
7. Mundu aya mbemba syina aflotoxin ethaa ailyi ata?
8. Ni kyau utonya kwika usiiye Aflotoxin?
9. Mwasua na mwaketha mbemba mutwaa va?
10. Ni Mauseo mepa maumanaa na kusua mbemba syi mbumu?
11. Useo wa mbemba syithiwe syi mbumu mbee wa kwikia store
12. Musupia ngetha ata?
13. Serikali mwondo wa Agriculture na heath mekaa ata kusiiya Aflotoxin mbembani?
14. Ni kyau kitonya kwikiwa kusiia Aflotoxin muundani na musyi?
15. Ni mathina mepa matumaa Aflotoxin itathela?
16. Utonya kwika ata kusiia Aflotoxin mbembani?

APPENDIX 10: IN-DEPTH INTERVIEW SCHEDULE FOR AGRICULTURAL AND PUBLIC HEALTH OFFICIALS

1. What are your comments regarding aflatoxin problem in this area?
2. What is the role of your organization/ministry in aflatoxin control and prevention in maize?
3. What activities does your organization carryout to control and prevent aflatoxin in maize?
4. What, in your view, are causes aflatoxin contamination of maize in your area?
5. How frequent do you collect maize samples for aflatoxin analysis?
6. How were the results of analysis for past two years or so?
7. What levels of aflatoxins contamination are considered permissible/tolerable for human maize consumption?
8. What actions do you or your organization take when results of analysis exceed permissible levels?
9. What storage and pre-storage practices do you advise the community to undertake to prevent aflatoxin contamination of maize?
10. What challenges do you or your organization face in efforts to control and prevent aflatoxin contamination of maize, and what are your recommendations?

Appendix 11: Kibwezi ELISA Test Results and Physical Conditions for Maize harvested in First Season

S/No	Discoloration	Mouldy	Pest/insect infestation	Moisture Content (%)	Total Aflatoxin ppb/µ/Kg
01	No	No	No	12.9	7.8
02	No	No	Yes	12.9	<1.75
03	No	Yes	Yes	13.0	<`1.75
04	No	No	No	13.0	4.2
05	Yes	Yes	No	12.8	7.7
06	No	No	Yes	12.8	<1.75
07	No	No	No	12.6	164.2
08	Yes	Yes	Yes	12.8	3.8
09	Yes	Yes	No	12.4	<1.75
10	Yes	No	No	24.0	<1.75
11	No	No	No	12.9	<1.75
12	No	No	No	12.5	<1.75
13	No	No	No	12.4	<1.75
14	No	No	No	12.4	<1.75

15	**No**	**No**	**No**	**12.1**	**<1.75**
16	**No**	**No**	**No**	**13.0**	**<1.75**
17	**No**	**No**	**No**	**13.1**	**2.6**
18	**No**	**No**	**No**	**24.7**	**<1.75**
19	**No**	**No**	**Yes**	**12.4**	**<1.75**
20	**No**	**No**	**No**	**24.0**	**<1.75**
21	**Yes**	**Yes**	**No**	**13.0**	**<1.75**
22	**N0**	**N0**	**No**	**12.9**	**<1.75**
23	**No**	**No**	**No**	**13.0**	**<1.75**
24	**No**	**No**	**Yes**	**12.9**	**<1.75**
Summarry	**Yes=5 (20.8)** **No=19(79.2%)**	**Yes=5(20.8)** **No=19(79.2)**	**Yes=6(25.0%)** **No=18 (75.0%)**	**Mean=12.9**	**Above 1.75= 6(25.0%)**

Appendix 12: Kilome ELISA Test Results and Physical Conditions for Maize harvested in First Season

S/No	Discolouration	Mouldy	Pest/insect infestation	Moisture Content (%)	Total Aflatoxin ppb/µ/Kg
01	Yes	No	No	12.9	<1.75
02	No	No	No	12.8	<1.75
03	No	No	No	13.0	<1.75
04	No	No	Yes	12.9	<1.75
05	No	Yes	No	13.1	1.8
06	No	No	Yes	12.7	<1.75
07	No	No	No	12.6	<1.75
08	No	No	No	12.5	<1.75
09	No	No	No	12.6	<1.75
10	No	No	Yes	12.8	<1.75
11	No	No	No	13.0	<1.75
12	No	No	No	12.4	<1.75
13	No	No	No	13.0	<1.75
14	No	No	No	12.9	<1.75

15	**Yes**	**No**	**Yes**	**13.9**	**<1.75**
16	**No**	**No**	**No**	**13.2**	**<1.75**
17	**No**	**No**	**No**	**12.8**	**<1.75**
18	**No**	**No**	**No**	**13.0**	**<1.75**
19	**No**	**Yes**	**No**	**13.3**	**<1.75**
20	**No**	**No**	**No**	**24.4**	**<1.75**
21	**Yes**	**Yes**	**Yes**	**12.5**	**<1.75**
22	**No**	**No**	**No**	**12.9**	**<1.75**
23	**No**	**No**	**No**	**12.8**	**<1.75**
24	**No**	**No**	**N0**	**12.7**	**<1.75**
Summary	**Yes=3 (%)** **No=21(87.5 %**	**Yes=3(12.5 %)** **No=21(87.5%)**	**Yes=5(20.8 %)** **No=19 (79.2%)**	**Mean=12.8**	**Above 1.75=1(4.2%)**

Appendix 13: Kibwezi ELISA Test Results and Physical conditions for Maize harvested in Second Season

S/No	Discoloration	Mouldy	Pest/insect infestation	Moisture Content (%)	Total Aflatoxin ppb/µ/Kg
01	No	No	Yes	13.2	8.2
02	Yes	Yes	No	13.0	<1.75
03	Yes	Yes	Yes	12.6	<1.75
04	Yes	No	No	13.4	1.7
05	No	No	Yes	13.6	1.8
06	No	Yes	Yes	13.1	<1.75
07	Yes	No	Yes	15.3	52.3
08	No	Yes	No	15.6	123.8
09	Yes	No	No	13.7	<1.75
10	No	No	Yes	12.9	<1.75
11	Yes	No	No	15.8	41.2
12	No	No	No	13.8	<1.75
13	Yes	Yes	No	13.3	<1.75
14	No	Yes	No	12.6	<1.75

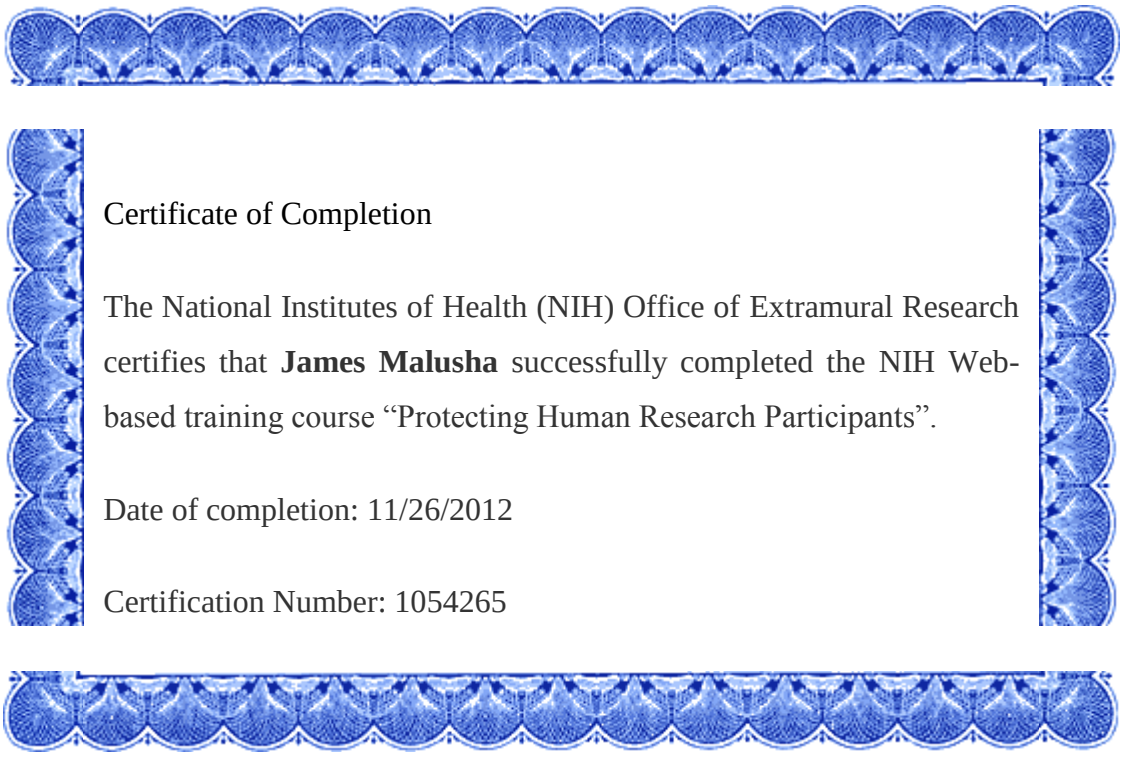
15	**Yes**	**No**	**No**	**13.0**	**<1.75**
16	**Yes**	**No**	**Yes**	**13.4**	**<1.75**
17	**No**	**No**	**No**	**14.9**	**14.9**
18	**Yes**	**No**	**Yes**	**13.3**	**<1.75**
19	**No**	**No**	**No**	**13.8**	**<1.75**
20	**Yes**	**Yes**	**Yes**	**13.3**	**<1.75**
21	**No**	**No**	**No**	**13.2**	**<1.75**
22	**Yes**	**Yes**	**Yes**	**12.6**	**<1.75**
23	**Yes**	**No**	**Yes**	**12.8**	**<1.75**
24	**Yes**	**Yes**	**Yes**	**14.9**	**10.9**
Analysis	**Yes=11(45.8%)** **No=13(54.2%)**	**Yes=9(37.5%)** **No=15(62.5%)**	**Yes=10(41.7%)** **No=14(58.3%)**	**Mean=13.6**	**Above 1.75=8(33.3%)**

Appendix 14: Kilome ELISA Test Results and Physical conditions for Maize harvested in Second Season

S/No	Discolouration	Mouldy	Pest/insect infestation	Moisture Content (%)	Total Aflatoxin ppb/µ/Kg
01	No	No	No	12.2	<1.75
02	No	No	No	13.3	<1.75
03	Yes	Yes	Yes	13.3	<1.75
04	Yes	Yes	Yes	13.2	5.4
05	Yes	Yes	No	15.6	14.8
06	No	No	No	13.7	<1.75
07	Yes	Yes	Yes	15.3	85.4
08	No	No	Yes	13.0	<1.75
09	Yes	No	No	13.6	<1.75
10	No	No	Yes	12.6	<1.75
11	No	No	No	12.4	<1.75
12	Yes	No	Yes	12.9	<1.75
13	No	Yes	No	13.4	<1.75
14	No	No	Yes\	12.8	<1.75

15	**No**	**No**	**Yes**	**13.9**	**<1.75**
16	**No**	**No**	**No**	**13.7**	**<1.75**
17	**No**	**No**	**Yes**	**13.1**	**<1.75**
18	**No**	**No**	**No**	**14.1**	**<1.75**
19	**No**	**No**	**No**	**13.3**	**<1.75**
20	**No**	**No**	**No**	**13.6**	**<1.75**
21	**Yes**	**No**	**Yes**	**13.4**	**<1.75**
22	**No**	**No**	**No**	**14.4**	**<1.75**
23	**Yes**	**Yes**	**No**	**13.7**	**<1.75**
24	**No**	**No**	**Yes**	**13.5**	**<1.75**
Summary	**Y=8(33.3)%** **N=16(66.7%)**	**Y=6(25.0%)** **N=18(75.0%)**	**Y=11(45.8%)** **N=13(54.2%)**	**Mean=13.5**	**Above 1.75=3 (12.5%)**

APPENDIX 15: CERTIFICATE OF TRAINING ON PROTECTING HUMAN RESEARCH SUBJECTS



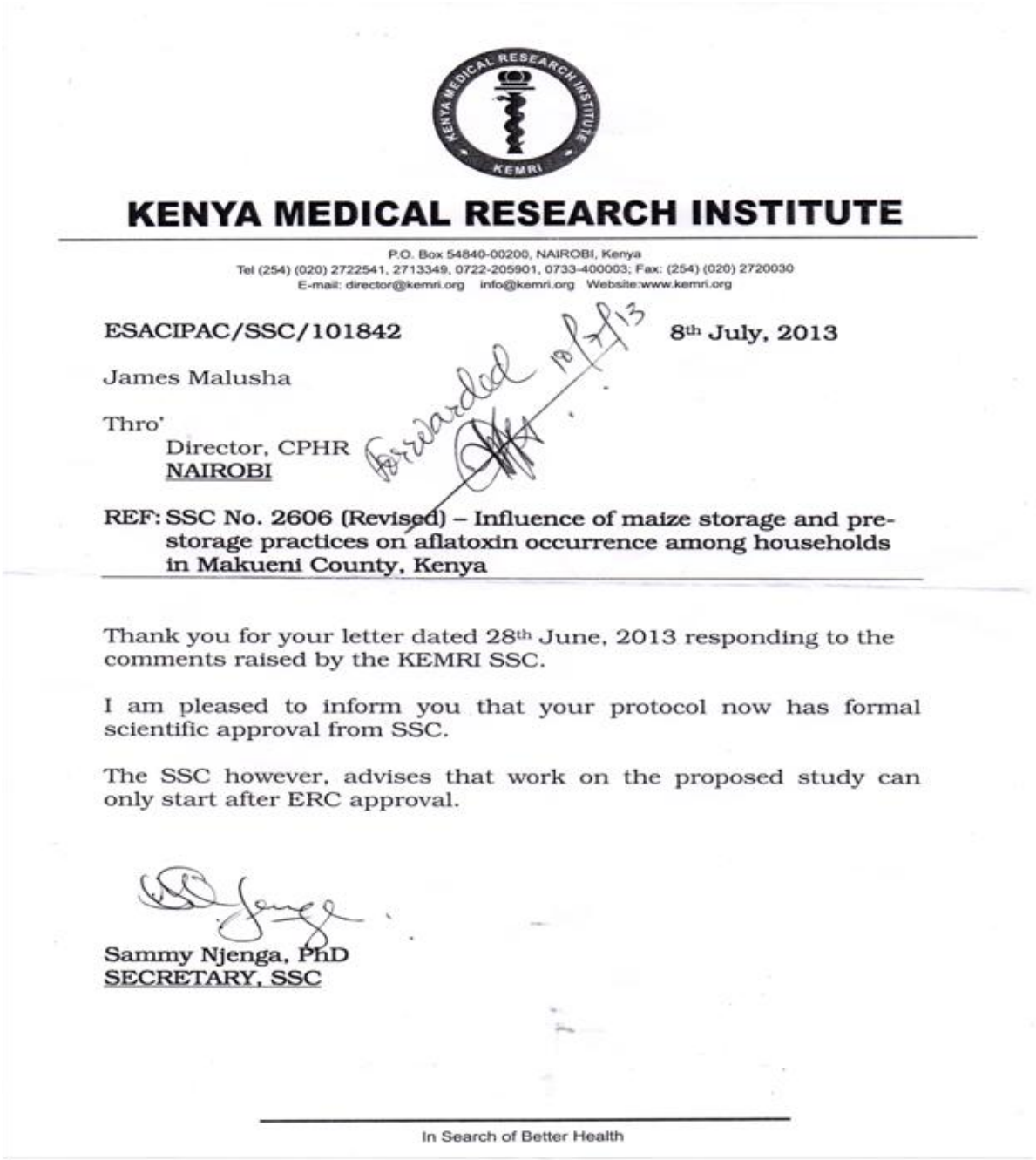
Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **James Malusha** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 11/26/2012

Certification Number: 1054265

APPENDIX 16: KEMRI SCIENTIFIC STEERING COMMITTEE STUDY APPROVAL



KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

ESACIPAC/SSC/101842 8th July, 2013

James Malusha

Thro'
Director, CPHR
NAIROBI

Forwarded 10/7/13

REF: SSC No. 2606 (Revised) – Influence of maize storage and pre-storage practices on aflatoxin occurrence among households in Makueni County, Kenya

Thank you for your letter dated 28th June, 2013 responding to the comments raised by the KEMRI SSC.

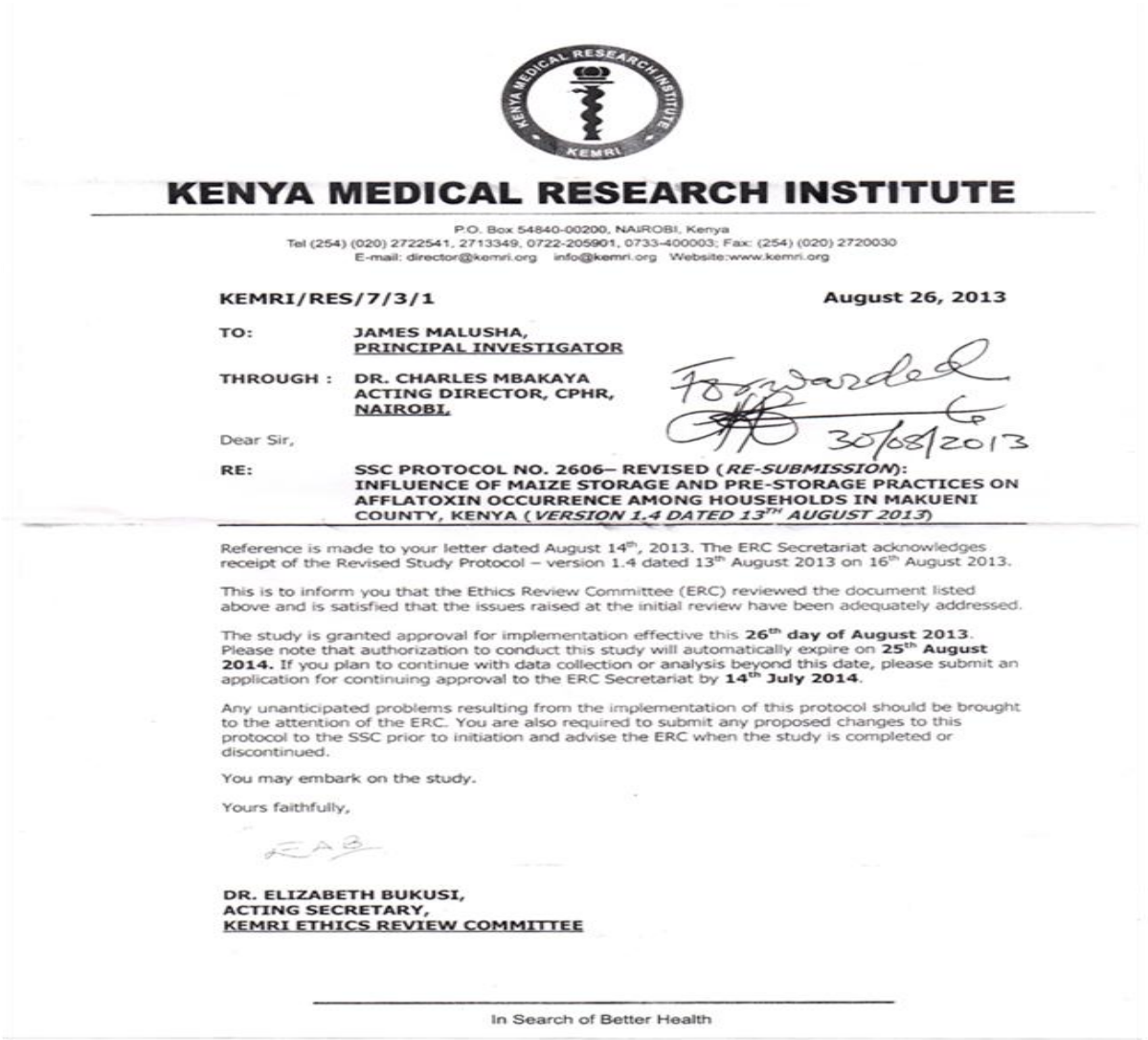
I am pleased to inform you that your protocol now has formal scientific approval from SSC.

The SSC however, advises that work on the proposed study can only start after ERC approval.

Sammy Njenga, PhD
SECRETARY, SSC

In Search of Better Health

APPENDIX 17: KEMRI ETHICAL REVIEW COMMITTEE STUDY APPROVAL



KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **August 26, 2013**

TO: **JAMES MALUSHA,**
PRINCIPAL INVESTIGATOR

THROUGH : **DR. CHARLES MBAKAYA**
ACTING DIRECTOR, CPHR,
NAIROBI.

Forwarded
30/08/2013

Dear Sir,

RE: **SSC PROTOCOL NO. 2606– REVISED (*RE-SUBMISSION*): INFLUENCE OF MAIZE STORAGE AND PRE-STORAGE PRACTICES ON AFFLATOXIN OCCURRENCE AMONG HOUSEHOLDS IN MAKUENI COUNTY, KENYA (*VERSION 1.4 DATED 13TH AUGUST 2013*)**

Reference is made to your letter dated August 14th, 2013. The ERC Secretariat acknowledges receipt of the Revised Study Protocol – version 1.4 dated 13th August 2013 on 16th August 2013.

This is to inform you that the Ethics Review Committee (ERC) reviewed the document listed above and is satisfied that the issues raised at the initial review have been adequately addressed.

The study is granted approval for implementation effective this **26th day of August 2013**. Please note that authorization to conduct this study will automatically expire on **25th August 2014.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **14th July 2014**.

Any unanticipated problems resulting from the implementation of this protocol should be brought to the attention of the ERC. You are also required to submit any proposed changes to this protocol to the SSC prior to initiation and advise the ERC when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

DR. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE

In Search of Better Health

APPENDIX 18: RESEARCH AUTHORIZATION FROM NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

REPUBLIC OF KENYA

NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Telephone: 254-020-2213471, 2241349, 254-020-2673550
Mobile: 0713 788 787 , 0735 404 245
Fax: 254-020-2213215
When replying please quote
secretary@ncst.go.ke

P.O. Box 30623-00100
NAIROBI-KENYA
Website: www.ncst.go.ke

Our Ref: **NCST/RCD/10/013/24** Date: **24th May 2013**

James Mwashembe Malusha
Jomo Kenyatta University
Of Agriculture and Technology
P.O Box 6200-00200
Nairobi.

RE: RESEARCH AUTHORIZATION

Following your application dated **15th May, 2013** for authority to carry out research on ***"Influence of Maize Storage and Pre-storage practices on aflatoxin occurrence among households in Makueni County, Kenya."*** I am pleased to inform you that you have been authorized to undertake research in **Makueni County** for a period ending **30th June, 2016.**

You are advised to report to **the District Commissioners, District Education Officers and District Agricultural Officers of Selected Districts, Makueni County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

DR. M. K. RUGUTT, PhD, HSC.
DEPUTY COUNCIL SECRETARY

Copy to:
The District Commissioner
The District Education Officer

"The National Council for Science and Technology is Committed to the Promotion of Science and Technology for National Development".

APPENDIX 19: APPROVAL OF Ph.D STUDY PROPOSAL AND SUPERVISORS

JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY

DIRECTOR, BOARD OF POSTGRADUATE STUDIES

P.O. BOX 62000
NAIROBI – 00200
KENYA
Email: director@bps.jkuat.ac.ke

TEL: 254-067-52711/52181-4
FAX: 254-067-52164/52030

OFFICE OF THE GRADUATE
RECEIVED
26 MAR 2015
PROGRAM COORDINATOR
ITROMID

REF: BPS/TM410-0454/2010 **12TH March 2015**

Malusha James Mwashembe
C/o COHES
JKUAT

Dear Mr. Mwashembe,

RE: APPROVAL OF Ph.D. RESEARCH PROPOSAL AND SUPERVISORS

Kindly note that your research proposal entitled: **"Influence of Maize Storage and Pre-storage Practices on Aflatoxin Occurrence among Households in Makueni County, Kenya"** has been approved. The following are your approved supervisors:-

1. Prof. Mohamed Karama
2. Prof. A.O. Makokha

Yours sincerely,

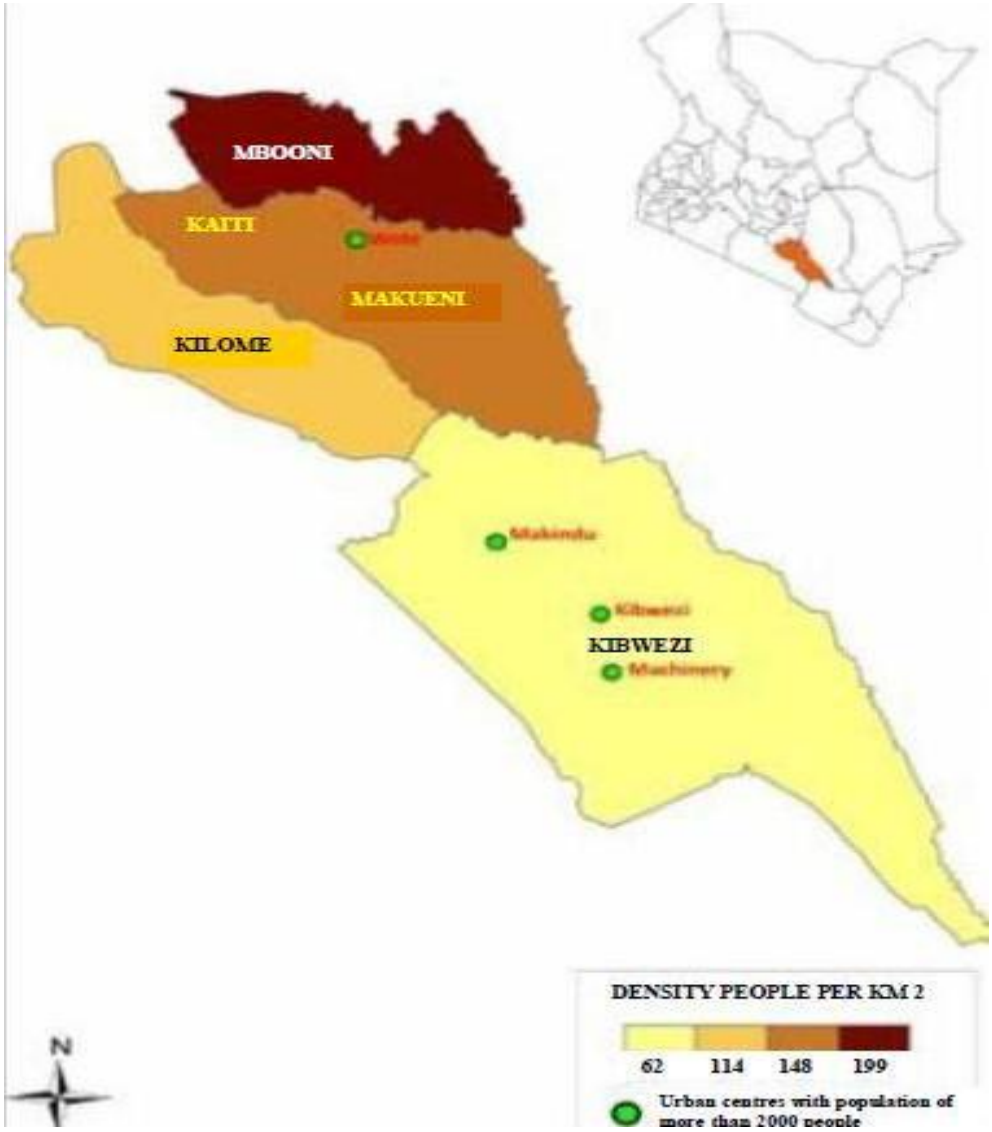
PROF. MATHEW KINYANJUI
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

/sn

APPENDIX 20: LOCAL HOUSEHOLD MAIZE CRIB

APPENDIX 21: IMPROVED HOUSEHOLD MAIZE CRIB

APPENDIX 22: MAP OF MAKUENI COUNTY



APPENDIX 23: COVER PAGE AND ABSTRACT OF PUBLISHED SCIENTIFIC PAPER ON EAST AFRICAN MEDICAL JOURNAL MARCH 2015 ISSUE

The East African Medical Journal

Established in 1923 ISSN 0012-835X

March 2015 Volume 92 No. 3

CONTENTS

Antimicrobial susceptibility patterns of urinary bacteria amongst paediatric patients at the Nairobi Hospital, Kenya
L. N. Njagi, S. Odera and F. Mutua 104

Association between Khat (*Catha edulis*) chewing and Infection with *Helicobacter Pylori*: A case control study in Nairobi County
M. A. Hassan, K. Mohamed, N. Zipporah and L. Hudson 112

Perception of Labour Pain among Rural Women Presenting to a Tertiary Hospital in Kenya
W. Waweru-Siika 120

Comparative analysis of aflatoxin contamination of maize in two different physiographic zones and maize seasons in Makueni County, Kenya
J. Malusha, M. Karama and A. N. Makokha 126

Evaluation of malaria infection in relation to age and residential area in Nandi County, Kenya
J. O. Nyamuni, E. Kokwaro, J. Ouma and T. Ambundo 136

Spontaneous rupture of the caesarean section scar in the first trimester: Case report
A. N. Bosire, O. Ogutu and P. O. R. Olang 147

Eye Munchausen's Syndrome: Case Report
P. W. Atipo-Tsiba 150

Tubo-ovarian presentation of Burkitt's Lymphoma: Case report
N. N. Sitati, Z. Thierry, W. Njau, F. Ogutu, F. X. Odawa, J. O. Ong'ech, E. A. Rogena, A. Medhat and R. J. Kosgei 152

Editorial Panel iii

www.eamj.org

East African Medical Journal Vol. 92 No. 5 May 2015
THE INFLUENCE OF HOUSEHOLD SOCIO-ECONOMIC CHARACTERISTICS AND AWARENESS ON AFLATOXIN CONTAMINATION OF MAIZE IN MAKUENI COUNTY, KENYA
J. M. Malusha, MSc, Post graduate, Jomo Kenyatta University of Agriculture and Technology (JKUAT), M. Karama, PhD, Professor, Kenya Medical Research Institute (KEMRI), Centre for Public Health Research and A. O. Makokha, PhD, Professor, Jomo Kenyatta University of Agriculture and Technology (JKUAT), Department of Food Science and Technology, P.O. Box 62000, Nairobi

THE INFLUENCE OF HOUSEHOLD SOCIO-ECONOMIC CHARACTERISTICS AND AWARENESS ON AFLATOXIN CONTAMINATION OF MAIZE IN MAKUENI COUNTY, KENYA

J. M. MALUSHA, M. KARAMA and A. O.MAKOKHA

ABSTRACT

***Background*: Aflatoxicosis resulting from consumption of contaminated maize poses a significant public health problem in many countries including Kenya, and many people living in developing countries could be chronically exposed to aflatoxin through their diet. It is caused by Aflatoxins produced by fungus of species *Aspergillusparasiticus* and *Aspergillus flavus* found mainly in cereals and other foodstuffs.**

***Objective*: To determine socio-economic and aflatoxin awareness factors associated with aflatoxin contamination in household maize in Makueni County, Kenya.**

***Design*: A comparative descriptive analytical study.**

***Setting*: Kibwezi and Kilome sub-counties of Makueni County, Kenya.**

***Subjects*: Four hundred and fifty household heads or their representatives with maize in their household stores, comprising 225 from each study site.**

***Results*: Majority of the households' main source of income was farming and most of them were poor, but level of awareness on aflatoxin was very high. The results further showed significant associations of some socio-economic characteristics and awareness with aflatoxin contamination of maize. Gender of household head was significantly associated with proper maize storage. Age of respondent was found to be significantly associated with knowledge/awareness of aflatoxin and knowledge on signs of suspected aflatoxin contaminated maize. There was significant association between age of respondent and perception on whether altitude/climate affected aflatoxin contamination, level of education and knowledge/awareness of aflatoxin. Level of education was significantly associated with Knowledge on identification of contaminated maize as well as on Knowledge on signs of aflatoxin. Besides, level of education was significantly associated with proper ventilation, discoloration of maize, and maize in storage affected by pests/insects, and cleaning of maize prior to storage. There was also significant association between occupation of respondent and proper maize storage. Income of house hold head was significantly associated with knowledge/awareness on aflatoxin. Knowledge/awareness of aflatoxin problem was also significantly associated with placing of material underneath of maize during drying, cleaning of maize prior to storage, moldy condition of maize, aflatoxin content in maize and proper maize storage.**

***Conclusion*: These study findings imply that efforts to control and prevent aflatoxin contamination of maize should take into consideration socio-economic characteristics as well as aflatoxin awareness. Thus reducing poverty levels by raising income, education levels and awareness of the community will most likely have a profound impact on control of aflatoxin. There is need, therefore, for policy makers and stakeholders to promote household positive socio-economic factors and aflatoxin awareness in households. This can greatly contribute to reduction of aflatoxin contamination in maize.**

APPENDIX 24: COVER PAGE AND ABSTRACT FOR PUBLISHED SCIENTIFIC PAPER ON EAST AFRICAN MEDICAL JOURNAL MAY 2015 ISSUE

The East African Medical Journal

Established in 1923 ISSN 0012-835X

May 2015 Volume 92 No. 5

CONTENTS

Prevalence and factors associated with depression among patients with epilepsy at Aga Khan University Teaching Hospital Nairobi
N. Kiko, N. Kitazi, G. Yonga and O. J. Jowi 208

Anti-malarial prescription practices for children with negative microscopy results for malaria parasites admitted at the Moi Teaching and Referral Hospital, Kenya
J. M. Simba, I. K. Marete, A. Mwangi and S. O. Ayaya 214

Prevalence of cutaneous manifestations of Diabetes Mellitus: A hospital- based cross-sectional study in Northern Tanzania
S. Kiprono, E. Minde, D. R Mavura and J. E. Masenga 221

The influence of household socio-economic characteristics and awareness on aflatoxin contamination of maize in Makueni county, Kenya
J. M. Malusha, M. Karama and A. O.Makokha 226

Comparison of reliability of rapid diagonostic test to microscopy in the diagnosis of malaria at Gulu Regional Referral Hospital
P. Kyambadde, H. Acidiri, H. Wabwire, D. Okello, L. Mbolwa, S. Asio, I. Byona, A. Oriekot, W. Kiganda, J. K Kuule and E. I. Odongo -Aginya 237

Retinoblastoma: Assessing the level of knowledge of tumor by midwives in Brazzaville
P. W. Atipo-Tsiba and C. Itoua 241

Histologic analysis of gynaecologic lesions in Nigerians
S. O. Keshinro, C. C. Nwafor and P. Oshun 245

Current microbial pattern of patients presenting with pre-labour rupture of membranes (prom) at Kenyatta National Hospital, Nairobi, Kenya
J. W. Gichuhi, O. Onyango and J. Ongech 253

Uterine rupture in a primigravida with mullerian anomaly at 27 weeks gestation
K. Kinuthia, Anne-Beatrice Kihara, R. J. Kosgei and A. N. Bosire 260

Editorial Panel iii

www.eamj.org

East African Medical Journal Vol. 92 No. 5 May 2015
THE INFLUENCE OF HOUSEHOLD SOCIO-ECONOMIC CHARACTERISTICS AND AWARENESS ON AFLATOXIN CONTAMINATION OF MAIZE IN MAKUENI COUNTY, KENYA
J. M. Malusha, MSc, Post graduate, Jomo Kenyatta University of Agriculture and Technology (JKUAT), M. Karama, PhD, Professor, Kenya Medical Research Institute (KEMRI), Centre for Public Health Research and A. O. Makokha, PhD, Professor, Jomo Kenyatta University of Agriculture and Technology (JKUAT), Department of Food Science and Technology, P.O. Box 62000, Nairobi

THE INFLUENCE OF HOUSEHOLD SOCIO-ECONOMIC CHARACTERISTICS AND AWARENESS ON AFLATOXIN CONTAMINATION OF MAIZE IN MAKUENI COUNTY, KENYA

J. M. MALUSHA, M. KARAMA and A. O.MAKOKHA

ABSTRACT

***Background*: Aflatoxicosis resulting from consumption of contaminated maize poses a significant public health problem in many countries including Kenya, and many people living in developing countries could be chronically exposed to aflatoxin through their diet. It is caused by Aflatoxins produced by fungus of species *Aspergillusparasiticus* and *Aspergillus flavus* found mainly in cereals and other foodstuffs.**

***Objective*: To determine socio-economic and aflatoxin awareness factors associated with aflatoxin contamination in household maize in Makueni County, Kenya.**

***Design*: A comparative descriptive analytical study.**

***Setting*: Kibwezi and Kilome sub-counties of Makueni County, Kenya.**

***Subjects*: Four hundred and fifty household heads or their representatives with maize in their household stores, comprising 225 from each study site.**

***Results*: Majority of the households' main source of income was farming and most of them were poor, but level of awareness on aflatoxin was very high. The results further showed significant associations of some socio-economic characteristics and awareness with aflatoxin contamination of maize. Gender of household head was significantly associated with proper maize storage. Age of respondent was found to be significantly associated with knowledge/awareness of aflatoxin and knowledge on signs of suspected aflatoxin contaminated maize. There was significant association between age of respondent and perception on whether altitude/climate affected aflatoxin contamination, level of education and knowledge/awareness of aflatoxin. Level of education was significantly associated with Knowledge on identification of contaminated maize as well as on Knowledge on signs of aflatoxin. Besides, level of education was significantly associated with proper ventilation, discoloration of maize, and maize in storage affected by pests/insects, and cleaning of maize prior to storage. There was also significant association between occupation of respondent and proper maize storage. Income of house hold head was significantly associated with knowledge/awareness on aflatoxin. Knowledge/awareness of aflatoxin problem was also significantly associated with placing of material underneath of maize during drying, cleaning of maize prior to storage, moldy condition of maize, aflatoxin content in maize and proper maize storage.**

***Conclusion*: These study findings imply that efforts to control and prevent aflatoxin contamination of maize should take into consideration socio-economic characteristics as well as aflatoxin awareness. Thus reducing poverty levels by raising income, education levels and awareness of the community will most likely have a profound impact on control of aflatoxin. There is need, therefore, for policy makers and stakeholders to promote household positive socio-economic factors and aflatoxin awareness in households. This can greatly contribute to reduction of aflatoxin contamination in maize.**