KNOWLEDGE OF YOUNG CHILDREN CAREGIVERS ABOUT MYCOTOXINS, THEIR POSTHARVEST HANDLING PRACTICES OF SORGHUM AND EFFECTS OF FERMENTATION ON AFLATOXIN AND FUMONISIN LEVELS IN SORGHUM GRAINS AND FLOUR IN KERIO VALLEY, KENYA

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A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Master of Science Degree in Nutritional Sciences of Egerton University

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DECLARATION AND RECOMMENDATION

Declaration

I declare that this thesis is my original work and has not been previously submitted for the award of a degree or diploma in any other University or institution. To the best of my knowledge and belief, it contains no material previously published or written by another person except where due reference is made.

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DEDICATION

I dedicate this work to my late father Ltantarian Lesuuda.

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ABSTRACT

Undernutrition among children under five years old is still a problem in many developing countries including Kenya. Kerio Valley of Elgeyo Marakwet County is among the regions with high stunting rates in Kenya. Mycotoxin contamination of complementary foods has been suggested to cause undernutrition. Sorghum is one of the basic ingredients used to prepare complementary foods. This study, therefore, aimed to; document children under five years caregivers' knowledge of aflatoxin and fumonisin contamination in sorghum alongside their postharvest handling and storage practices of sorghum and determine the effects of fermentation on aflatoxin and fumonisin levels in sorghum grain and flour. A cross-sectional study was conducted to obtain both qualitative and quantitative data from 374 caregivers. Multistage sampling procedures were used to select study participants. Overall, majority of the caregivers had poor knowledge (61.8%) about mycotoxin contamination and poor postharvest handling and storage practices of sorghum (74.5%). The caregiver's knowledge about mycotoxins was significantly associated with age [(AOR=4.629, p <.001], education level [(AOR=0.275, p =.001], marital status [(AOR=15.187, p =.012] and household monthly income [(AOR=2.623, p <.001]. Furthermore, the caregiver's age [(AOR=3.845, p =.003], education level [(AOR=0.196, p <.001)], monthly income [(AOR=3.291, p =.002] and knowledge of mycotoxin contamination of sorghum [AOR, 5.428, p <.001] were significantly associated with postharvest handling and storage practices. The mean value for total aflatoxin level in sorghum grains (33.85±26.00) and flour (36.72±19.50) were above 10ppb the regulation limits for aflatoxins. Additionally, fumonisin mean values for the sorghum grains (12.90±8.07) and flour (10.04±7.21) samples were above the (1 ppm) regulation limit. Type of fermentation (natural and use of *Lactobacillus Plantarum*) (F=24.287, p=0.001), type of sample (flour or grain) (F=9.706, p=0.004) and fermentation duration (F= 3.690, p=0.037) contributed significantly to the reduction of aflatoxin and fumonisin levels. In conclusion, poor caregivers' knowledge coupled with suboptimal post-harvest handling and storage practices may have contributed to high aflatoxin and fumonisin recorded in sorghum grains and flour. This increases the risk of mycotoxin exposure to young children, which could be one of the contributing factors to high stunting rates in Kerio valley. This thus necessitates mitigation measures including sensitization campaigns and social behaviour change communication such as the adoption of fermentation by caregivers of children under five years as one of the complementary foods processing techniques.

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LIST OF ABBREVIATIONS

AFB_1	Aflatoxin B_1
ANOVA	Analysis of Variance
ASAL	Arid and Semi-Arid Land
AVCD-DTC	Accelerated Value Chain Development-Drought Tolerant Crop
DHS	Demographic and Health Survey
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food and Agriculture Organization of United Nations
FGD	Focus Group Discussion
g	grams
Ha	Hectare
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IYCN	Infant and Young Child Nutrition
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KDHS	Kenya Demographic and Health Survey
KIRDI	Kenya Industrial Research and Development Institute
KEBS	Kenya Bureau of Standards
LAB	Lactic Acid Bacteria
MoALF	Ministry of Agriculture, Livestock and Fisheries
MeOH	Methanol
mg	Milligrams
ml	Milliliter
mm	Millimeter
NRF	National Research Fund
SPSS	Statistical Package for Social Sciences
UNICEF	United Nations Children's Fund
WHO	World Health Organization

CHAPTER ONE
INTRODUCTION

1.1 Background

Mycotoxins are harmful substances produced by some species of fungi that contaminate food material when stored with high moisture content, darkness, poorly ventilated rooms and a temperature range of 12 °C to 48 °C, which favour fungal growth (Obonyo & Salano, 2018). Due to favourable environmental conditions coupled with the traditional methods of crop cultivation, harvesting, handling, and storage, 25% of the worldwide food crops are contaminated by mycotoxins (Zain, 2011). Among food-borne mycotoxins, aflatoxin and fumonisin are of the greatest significance in Africa due to their ubiquitous nature and acute and/or chronic health effects (Gnonlonfin *et al*., 2013; Wu *et al*., 2014). Kenya is not exempted from the occurrence of aflatoxin and fumonisin, as they have been detected and found to be the major fungal toxins that contaminate Kenyan cereals (Mutiga *et al*., 2015). Sorghum is susceptible to aflatoxin and fumonisin contamination (Ssepuuya *et al*., 2018) and levels reported in sorghum from Kenya were above the critical limits set by the Kenya Bureau of Standards (KEBS) and World Health Organization (WHO) (Kang'ethe *et al*., 2017; Sirma *et al*., 2015). Despite sorghum's susceptibility to mycotoxins contamination, its grains are grounded or milled to flour to make the local bread (*ugali*), local brew *busaa*, porridge, cookies, pancakes and other beverages (Obuoyo, 2017). Sorghum is a good source of vitamins (fat-soluble and B-complex vitamins), minerals, and antioxidants and has a high content of dietary fibre, protein oil and fats (Daneil *et al*., 2016).

Sorghum is a staple food crop in the drier parts of India, China, and Africa (Mrema *et al*., 2020) including the arid and semi-arid lands of Kenya (ASALs) (Orr *et al*., 2016). This makes sorghum a critically important food security crop for millions of people living in harsh and dry environments. Owing to sorghum's drought-hardy characteristics and being a good source of vitamins (fat-soluble and B-complex vitamins), minerals, and antioxidants (Hadebe *et al*., 2017), its production and utilization have been promoted by the Accelerated Value Chain Development-Drought Tolerant Crops project (AVCD-DTC) in Elgeyo Marakwet County to address food insecurity and malnutrition (Kiome *et al*., 2016). Currently, sorghum is one of the basic ingredients in the preparation of infant and young children's food in Kerio Valley (Kiome *et al*., 2016) and other parts of Kenya (Kiarie *et al*., 2016; Okoth *et al*., 2017). Thus, ensuring that sorghum is safe from aflatoxin and fumonisin contamination is a major concern, as dietary exposure to mycotoxins can result in acute and chronic effects causing death (Probst *et al*., 2007) or stunting (Knipstein *et al*., 2015) respectively. Despite numerous interventions (such as nutrition-specific and sensitive) by the government and other non-governmental organizations to address malnutrition, undernutrition rates among children under five years in Kerio Valley are still high. A recent study Kipyego and Mugalavai (2019) reported a prevalence of

underweight (27%), stunting (67%), and wasting (9%) among children under five years from smallholder households that were targeted by the AVCD-DTC program. These stunting rates were way above the Elgeyo Marakwet county and national levels of 29.9% and 26% respectively (KDHS, 2015). In addition to other casual factors, consumption of aflatoxin and fumonisin-contaminated complementary foods could be among the main contributors to these undernutrition rates in Kerio Valley, possibly through environmental enteropathy that reduces the absorptive capacity and barrier function in the small intestine or impaired immune system function (Leroy *et al*., 2015; Smith *et al*., 2012). Noteworthy, children are more sensitive to the adverse effects of mycotoxins than adults because of lower body mass, higher metabolic rate, underdeveloped organ functions and detoxication mechanisms (Kimanya *et al*., 2014) which could explain why dietary exposure to mycotoxins is considered one of the major causes of undernutrition, morbidity and mortality (Paudyal *et al*., 2017).

Factors such as climatic conditions, susceptibility of food commodities to fungal invasion, farmer's knowledge about mycotoxins and agronomic practices influence aflatoxin and fumonisin production in susceptible food commodities (Makun, 2013). As such, sorghum which is susceptible to aflatoxin and fumonisin contamination (Ssepuuya *et al*., 2018), can easily be contaminated when hot and dry climatic conditions in Kerio Valley are coupled with poor farmers' knowledge about mycotoxins and suboptimal postharvest handling and storage practices. Limited information exists regarding farmers' knowledge about aflatoxin and fumonisin, postharvest handling practices of sorghum, aflatoxin and fumonisin occurrence in sorghum in Kerio Valley. In this regard, this study was designed to assess young children caregivers' knowledge of mycotoxins, aflatoxin and fumonisin contamination of sorghum alongside their postharvest handling practices and quantify aflatoxin and fumonisin levels in sorghum samples. Additionally, this study documented the effects of fermentation on aflatoxin and fumonisins levels in sorghum as preliminary studies have shown that fermentation had a significant effect on mycotoxins load in maize-based foods (Ademola *et al*., 2018; Mukandungutse *et al*., 2019).

1.2 Statement of the problem

Undernutrition among children under five years has remained a major challenge in Elgeyo Marakwet County, particularly along the Kerio Valley, despite the government and non-governmental organizations' interventions (agricultural and nutritional) to address this problem. Undernutrition puts children at greater risk of dying from common infections, increases the frequency and severity of infections and delays recovery. Causes of undernutrition go far beyond simply lacking adequate nutrients from the diet. Other factors including dietary exposure to mycotoxins lead to poor child growth and development by targeting the intestinal tract and inducing environmental enteropathy (EE) that is characterized by altered intestine barrier integrity, mucosal inflammation and reduced nutrient absorption. The use of aflatoxin and fumonisin-

contaminated sorghum-based complementary foods could be one of the main contributors to the high malnutrition rates reported in Kerio Valley. Poor mycotoxins knowledge among children's caregivers could increase the risk of mycotoxin exposure due to suboptimal post-harvest handling and storage of sorghum grains and flour. Scarce information exists on children caregivers' knowledge about mycotoxin, aflatoxin and fumonisin contamination in sorghum-based foods, their post-harvest handling practices of sorghum and aflatoxin and fumonisin contamination of sorghum in Kerio valley thus the need for this study. To fill this gap, the study documented caregivers' knowledge of aflatoxin and fumonisin contamination in sorghum alongside their post-harvest handling and storage practices and quantified aflatoxin and fumonisin levels in sorghum. The study also documented the effects of fermentation on aflatoxin and fumonisin levels in sorghum as one of the interventions to decontaminate it.

1.3 Objectives

1.3.1 General objective

Determine children under five years caregivers' knowledge of mycotoxins contamination, postharvest handling practices of sorghum and effects of fermentation on aflatoxin and fumonisin levels in sorghum grains and flour in Kerio Valley.

1.3.2 Specific objectives

i. Document knowledge about aflatoxin and fumonisin contamination in sorghum among caregivers of children 6-59 months in Kerio Valley, Elgeyo Marakwet County.
ii. Assess postharvest handling and storage practices of sorghum grains and flour among sorghum-growing households with children 6-59 months in Kerio Valley, Elgeyo Marakwet County.
iii. Quantify aflatoxin and fumonisin levels in sorghum grains and flour in Kerio Valley, Elgeyo Marakwet County.
iv. Determine the effects of fermentation on aflatoxin and fumonisin levels in sorghum flour in Kerio Valley, Elgeyo Marakwet County.

1.4 Research questions

i. Do caregivers of children aged 6-59 months have adequate knowledge about mycotoxins contamination of foods in Kerio Valley, Elgeyo Marakwet County?
ii. How do households with children 6-59 months handle and store sorghum grains and flour in Kerio Valley, Elgeyo Marakwet County?
iii. Is there a significant difference in the levels of aflatoxin and fumonisin between sorghum grains and flour in Kerio Valley, Elgeyo Marakwet County?
iv. What are the effects of fermentation on the aflatoxin and fumonisin levels in sorghum grains and flour in Kerio Valley, Elgeyo Marakwet County?

1.5 Justification of the study

Human exposure to mycotoxins is a worldwide concern due to their negative effect on health. Aflatoxins and fumonisins contamination of food has been associated with increased incidence of deaths (Probst *et al*., 2007), cancer (Wild *et al*., 2015) and stunting (Knisptein *et al*., 2015). Their health effects however are more noticeable in young children than in other populations due to their lower body weight, less acidic stomachs and underdeveloped immune systems (Kimanya *et al*., 2014). Hot and dry climatic conditions (like those in Kerio Valley) are likely to favour mycotoxins colonization of susceptible food crops, especially when coupled with poor mycotoxins awareness and suboptimal postharvest practices among food handlers (Nyangi *et al*., 2016). Thus, it is critical to understand if children under five years caregivers' have any knowledge about aflatoxin and fumonisin contamination in sorghum and assess their post-harvest handling and storage practices of sorghum. Fermentation was employed to evaluate its effects on aflatoxin and fumonisin levels in sorghum samples as studies have shown that it could reduce mycotoxins load in maize-based foods (Ademola *et al*., 2018; Mukandungutse *et al*., 2019).

1.6 Significance of the study

The findings from the study are useful in planning and implementing interventions aimed at creating awareness of aflatoxin and fumonisin among all stakeholders, notably smallholder farmers and children under five years caregivers'. The study recommendations inform the ministry of health, ministry of agriculture and non-governmental organizations on the need for mycotoxins mitigation sensitization campaigns and behaviour change communication including utilization of the fermentation process as a way of decontaminating sorghum-based food products including complementary foods.

1.7 The scope of the study

This study was carried out in Kerio valley, targeting sorghum-growing households with children aged 6-59 months in Emsoo (Keiyo South), Arror (Marakwet West) and Endo (Marakwet East) wards in Elgeyo Marakwet County.

1.8 Limitations of the study

This study focused on assessing aflatoxin and fumonisin levels in sorghum grains and flour although there are other foods used as complementary foods in Kerio Valley. The study also did not correlate the aflatoxin and fumonisin levels reported in sorghum samples and children's nutrition status.

1.9 Assumption of the study

Conventional food preparation with temperatures up to 100°C has little effect on most mycotoxins (Karlovsky *et al*., 2016). The study assumed that aflatoxin and fumonisin levels in sorghum samples are the same as those in plate-ready food.

1.10 Operational definition of key terms and concepts

Aflatoxins refer to toxic secondary metabolites produced by moulds; mainly those of the species *Aspergillus flavus* and *Aspergillus parasiticus.*

A caregiver is a mother, another family member, or a helper who regularly looks after a child.

Complementary food refers to the food**,** which is introduced to an infant at the age of 6 months to provide more nutrients in addition to breast milk.

Dehulling is the process of removing the pericarp from the grain.

Fermentation refers to any process in which the activity of microorganisms brings about a desirable change to a foodstuff or beverage.

Fumonisins refer to a group of naturally occurring mycotoxins produced by fungi *Fusarium proliferatum* and *Fusarium perticilloides.*

Malnutrition is a condition that results from imbalances in a person's intake of nutrients.

Mycotoxins are toxic metabolites produced by fungi that normally contaminate agricultural cereals, either in the field during harvest, or storage.

Smallholder farmers- are those farmers owning small-based plots of land (usually less than 5 acres) on which they grow subsistence crops and one or two cash crops relying almost exclusively on family labour.

Stunting is a condition where a child fails to attain its potential height in relation to its age.

Undernutrition- is a physical condition resulting from insufficient food intake and/or poor absorption of nutrients consumed because of infectious disease.

Young child- in this study means a child from the age of six months up to the age of five years.

CHAPTER TWO
LITERATURE REVIEW

2.1 Introduction

This chapter reviews relevant literature on sorghum production in Kenya, post-harvest handling and storage practices of grains, mycotoxin contamination of sorghum and complementary foods, health effects of mycotoxins consumption, trends in malnutrition, fermented foods/beverages, and human health, reduction of mycotoxins in foods through fermentation. It also provides a critical evaluation of these research works to identify knowledge gaps.

2.2 Overview of sorghum production in Kenya

Sorghum is a native and well-adapted cereal crop to the African agro ecosystems as it is tolerant to both droughts and extended periods of waterlogging (Kumar *et al*., 2017). Sorghum grain is gluten-free, rich in B-complex vitamins and the minerals magnesium, potassium, phosphorus, iron, and zinc, and an excellent source of fiber, antioxidants, and protein (Shen *et al*., 2018). In Kenya, sorghum is majorly grown in the arid and semi-arid regions (ASALs) which are frequently affected by drought and crop failure. The main varieties cultivated are *Serena, Seredo, Gadam, Mtama* 1 and 2 and local varieties (Ogeto *et al*., 2012). To address frequent food and nutrition securities in these regions several interventions have been made towards increasing sorghum production. Such interventions include the release of improved sorghum varieties (ISVs) by research institutions like the International Crop Research Institute for Semi-Arid Tropics (ICRISAT) and by the year 2018, about 43 improved sorghum varieties had been released (Government of Kenya (GoK), 2018). Sorghum is consumed in several forms depending on the part of the world concerned. Generally, whole grain is processed into flour, which is used to make the unleavened bread prepared from fermented or unfermented dough in Asia and parts of Africa; thin or thick fermented or unfermented porridge mainly consumed in Africa; and boiled products similar to those prepared from rice or maize grits. In India, sorghum is utilized in the preparation of many traditional foods and bakery products such as bread, cakes, and biscuits (Hariprasanna & Rakshit, 2016). In Kenya, sorghum is mainly consumed in form of *ugali* or porridge (Mwadalu & Mwangi, 2013). As well, porridge from composite flour of sorghum and maize is commonly used as a complementary food (Okoth *et al*., 2017).

Sorghum contains more abundant and diverse phenolic compounds compared to other major cereal crops (Shen *et al*., 2018). The unique phenolic profile confers sorghum with some human health benefits such as reducing oxidative stress and cancer prevention (González-Montilla *et al*., 2012). Additionally, proximate analyses show that sorghum grain has a higher protein, fats and oil and crude fibre compared to maize grain (Table 1). With a shift in consumers' demand toward healthy and plant-based food, sorghum has enormous potential for exploitation and development into healthy and functional foods. Attempts have been made to

use sorghum whole grain or ingredients to make novel foods such as sorghum grain tea, as well as incorporating sorghum into foods, such as bread (Mariera *et al*., 2017) and meat products, to improve food quality and health benefits (Wu *et al*., 2018). To address rural poverty, malnutrition and food insecurity, ICRISAT in collaboration with local communities in Kenya have developed recipes from drought-tolerant crops including sorghum as part of the Smart Food component of Accelerated Value Chain Development (AVCD) project (ICRISAT, 2019). It is equally important to note that, despite sorghum's diverse uses and nutritional value, its susceptibility to aflatoxin and fumonisin contamination is a point of concern.

Table 1: Proximate analyses of maize and sorghum grains

Parameter %	**Sample**	**Sample**
	Maize	Sorghum
Moisture content	7.16	6.36
Protein	8.75	9.10
Fats and oil	2.40	3.10
Crude fibre	2.40	2.86
Ash content	7.19	2.07
Carbohydrate	77.46	76.51

Source: Daneil *et al*. (2016).

2.3 Postharvest handling and storage of grains and mycotoxins contamination

Poor post-harvest handling and storage practices in warm humid areas could lead to the rapid growth of fungi and hence higher levels of toxins (Hell & Mutegi, 2011). This particularly occurs in developing countries where appropriate measures for preventive actions are often overlooked (ICRISAT & NASFAM, 2009). Harvesting is regarded as the first step in the grain supply chain and is a critical process in deciding the overall food quality. A large amount of losses is encountered if harvesting is not done when crops are fully mature. Harvesting too early increases the cost of drying and insect infestation making grains susceptible to mould growth (Khan, 2010). Furthermore, leaving the matured crop un-harvested results in high shattering losses and exposure to birds and rodent attacks (Baloch, 2010).

The threshing process is done after harvesting to detach the grain from the panicles. In the rural setting, threshing involves beating the dried sorghum panicles with sticks on the ground or in sacks or using a mortar and pestle (Khan, 2010). Manual threshing may increase grain’s vulnerability to moulds infestation and growth through grain breakage due to excessive striking. Also, delayed threshing after sorghum harvesting leads to high moisture accumulations, rodents, birds and insect attacks leading to mould growth (Alavi, 2011). Therefore, appropriate threshing using threshing machines is recommended.

Grain drying is another important aspect of maintaining grain quality. In developing countries, sorghum is sun-dried on the panicle and/or after threshing (Abass *et al*., 2014). In many semi-arid areas, sorghum is stacked in bundles of panicles in the field and allowed to dry in the sun. However, unseasonal rains or cloudier weather may restrict the proper sun drying and the crop may be stored at high moisture (above 13%), which leads to mould growth (Abass *et al*., 2014). In addition, the surfaces used to dry the grains are important in controlling mycotoxins contamination. Achaglinkame *et al*. (2017) reported that drying groundnuts on clean tarpaulins reduced aflatoxin levels by about 50% compared to drying on bare ground. Moreover, pre-storage handlings such as winnowing and/or sorting of grains at harvest or later are recommended to remove shrivelled small grains, which may contain more infection than healthy normal grains (Atanda *et al*., 2013).

Furthermore, paramount among postharvest operations are methods and length of storage (Issah *et al*., 2015; Okello *et al*., 2010). Methods and duration of storage of food commodities differ from one agro-ecological area or ethnic group to another as dictated by the cost and availability of storage units (Issah *et al*., 2015). In developing countries, about 50%–60% of the grains are stored in traditional structures, at the household and farm levels (Grover *et al*., 2012). These structures may not provide the right internal atmosphere, and maximum protection from water, insects and rodents and are not easy to clean (Okello *et al*., 2010). Poor storage conditions such as the use of nylon bags allow the accumulation of moisture contents in cereals and the invasion of storage insects and pests thus facilitating fungal growth and the production of toxins (Monda & Alakonya, 2016; Nyangi, 2014). As well, stores with high temperatures and moisture contents surrounding them without air circulation influence the production of aflatoxins and fumonisins (Taye *et al*., 2018).

2.4 Aflatoxin and fumonisin contamination of sorghum

Food products especially cereals and cereal products can be easily contaminated with mycotoxins. Conditions such as poor hygienic practices, high temperatures and moisture content, are often experienced in Africa and have been linked to mycotoxins contamination of foods particularly cereals (Darwish *et al*., 2014). Though sorghum has intrinsic defence systems to prevent pest and microbe infestations, the innate systems are unable to prevent contamination by fungi making the food crop unsafe for human consumption (Ediage *et al*., 2015). Sorghum grains suffer from infection and colonization by several fungi during panicle and grain developmental stages (Waliyar *et al*., 2008) and fumonisin and aflatoxin are the most prevalent fungal contaminants (Ssepuuya *et al*., 2018).

Sorghum is more prone to aflatoxin contamination and levels exceeding acceptable limits (maximum level of 210.1ppb) have been reported in Nandi County (Sirma *et al*., 2015). In addition, a study in low-income

areas of Nairobi found that all sorghum samples analysed were aflatoxin-positive and had the highest concentrations (194 ppb) compared to maize (Kiarie *et al*., 2016). This indicates that children fed with sorghum-based complementary foods had greater aflatoxin exposure considering the high levels detected in the dietary sorghum. The rising importance of sorghum as a staple food especially in ASALs and being an important ingredient in preparing complementary foods makes it necessary to come up with effective ways to decontaminate it.

2.5 Complementary foods and mycotoxins contamination

Poor agricultural inputs, poverty, heavy reliance on home-grown cereals (not subjected to regulatory control) and a low level of awareness of the mycotoxin problem are threats to food safety (Wild *et al*., 2015). It is known that a single crop can be prone to several mixtures of mycotoxins (Ediage *et al*., 2015; Okeke *et al*., 2015) and most times, foods consumed at the household level are a combination of diverse mycotoxin-prone crops. The consumption of diverse grains, nuts and their products leads to mycotoxin co-exposure (Ojuri *et al*., 2018). Studies have reported the co-occurrence of aflatoxin and fumonisin in complementary foods (Kamala *et al*., 2017; Kimanya *et al*., 2014; Ojuri *et al*., 2018) and have been correlated with childhood stunting (Gleason *et al*., 2016; Kumar *et al*., 2017; Magoha *et al*., 2016). Cereals such as maize, millet, sorghum, or rice, which are mainly used to prepare complementary foods in Kenya, were reported to contain aflatoxin and fumonisin levels exceeding critical limits set by the KEBS and WHO (Kang'ethe *et al*., 2017; Sirma *et al*., 2015). Hence, it is important to establish mycotoxins levels in sorghum, one of the basic ingredients in the preparation of complementary foods in Kerio Valley and develop possible ways of reducing mycotoxins levels in it.

2.6 Fumonisin and child growth

Fumonisins are naturally occurring toxins produced by several species of *Fusarium* fungi (moulds). Fumonisin B1 is considered the most prevalent and toxic derivative within the group of fumonisins (B2 and B3) (Wild & Gong, 2010). High concentrations of fumonisins are associated with hot and dry weather, followed by periods of high humidity. *Fusarium* toxins are known to be produced during cereal harvesting under high moisture conditions whereas pre-harvest aflatoxin contamination of crops is associated with high temperatures, insect-mediated damage and prolonged drought (Pleadin, 2015). According to Miller (2008), fumonisins are found mainly in maize and sorghum as *Fusarium verticillioides* and *Fusarium proliferatum* (the fungi that produce fumonisins).

Dietary fumonisin intake is high among young children in rural areas of sub-Saharan African countries and has been shown to interfere with children's growth. For example, infants in Tanzania with relatively higher

fumonisins intakes (exceeding the JECFA's PMTDI of 2 mg/kg bw/day, estimated from caregivers' dietary recall questionnaires) were significantly shorter and lighter than those whose fumonisin intakes were below the JECFA's guidance value (Kimanya *et al*., 2010). Arthur *et al*. (2015) also reported that there was a negative relationship between fumonisin exposure and child growth in Tanzania based on validated urinary biomarker levels of fumonisin exposure. The major causal pathways suggested were decreased food intake and inhibition of sphingolipid metabolism, which could lead to the degradation of the epithelial barrier and stimulation of inflammatory immune response within the gut (Bulder *et al*., 2012; Kimanya *et al*., 2010; Marin *et al*., 2013). These may additionally potentiate or prolong infection within a damaged epithelium (Masching *et al*., 2016). Depletion of sphingolipids, in turn, inhibits maternal uptake of folate in different cells causing neural tube defects in unborn babies (Gheysens, 2015; Zain, 2011). Moreover, stunted children were found to have significantly lower serum concentrations of sphingomyelins compared with non-stunted children in rural areas of Malawi (Semba *et al*., 2016). By contrast, aflatoxin exposure does not have a significant impact on child growth in these cohorts although co-exposure to aflatoxin and fumonisin was associated with impaired growth. Multitoxin contamination of food commodities results in more severe effects when compared to single toxin contamination due to synergism (WHO, 2018). Fumonisin exposure, therefore, is a significant risk factor in the length and weight of young children. However, other risk factors such as micronutrient status or exposure to infectious agents may have contributed to these effects and were not taken into account in these studies.

2.7 Aflatoxin and child malnutrition

Aflatoxins are produced by the fungi *Aspergillus parasiticus* and *Aspergillus flavus* as secondary metabolites when the temperatures are between 24˚C and 35˚C (Ephrem & Guchi, 2015) and they grow in many commodities under excess moisture during harvest and storage. Children in sub-Saharan Africa are exposed to aflatoxins very early in life including in utero through maternal food intake, during breastfeeding and during complementary feeding periods (Khlangwiset *et al*., 2011). Studies have shown that consumption of aflatoxin-contaminated foods during pregnancy increases the risk of low birth weight babies as aflatoxins can cross the maternal placental barrier exposing the unborn child to biochemical toxicities whose effects such as stunting become apparent in early infancy (Shuaib *et al*., 2010). Majority of young children particularly in developing countries are exposed to high aflatoxin levels throughout their lives. This is because most communities rely on subsistence farming with unavailable alternative food options and lack awareness of the presence of mycotoxins in foods. A large proportion of the subsistence farmers have limited dietary diversity and little or no interventions to control aflatoxin contamination. Evidence from West Africa showed that chronic exposure to aflatoxins in children under five years was linked to poor growth

(underweight and stunted) and poor immune status (Westhuizen *et al*., 2011). In this region, complementary foods had high levels of aflatoxin, which led to growth faltering, particularly stunting.

Aflatoxins and fumonisins play a big role in the development of oedema in malnourished populations and they aggravate the pathogenesis of kwashiorkor (a severe protein-energy malnutrition (PEM) disease) in children (Kimanya, 2015; Wild *et al*., 2015). Studies have shown higher aflatoxin levels in the blood and urine of children with kwashiorkor compared to healthy children or children with other forms of PEM (such as marasmus) (Khlangwiset *et al*., 2011). AFB1 was also detected in the urine and blood of malnourished children with kwashiorkor and marasmic kwashiorkor in a four-year study conducted in Cameroon (Tchana *et al*., 2010). A link was established ($p<.05$) between kwashiorkor and the presence of AFB1 in urine when comparing malnourished children and the control group. In Nigeria, aflatoxins were detected more frequently and at higher concentrations in children with kwashiorkor and marasmic kwashiorkor compared to control groups (Onyemelukwe *et al*., 2012). An Egyptian study found that aflatoxins and their metabolites were more prevalent with a significantly higher serum concentration in kwashiorkor patients than in marasmic patients while no aflatoxins were detected in control patients (Hatem *et al*., 2005). Aflatoxins are believed to inhibit protein synthesis and immune factors and their exposure may delay recovery from kwashiorkor even if they did not cause the condition.

Although it is not yet clear how aflatoxin exposure results in malnutrition (stunting, growth faltering and kwashiorkor), several mechanisms have been proposed. For instance, aflatoxins have been shown to damage the intestinal tract leading to impaired intestinal barrier function leading to nutrient malabsorption, which causes growth faltering and immune dysfunction (Smith *et al*., 2012). Additionally, aflatoxin inhibits the synthesis of proteins, enzymes, and clotting factors and impairs glucose metabolism, phospholipid synthesis and fatty acid synthesis (Wild *et al*., 2015). This mediated immune system dysfunction increases the risk of infections (such as gastroenteritis) in children, which may lead to impaired growth from loss of energy caused by the infection or energy expended recovering from illness (Wild *et al*., 2015).

2.8 Trends of undernutrition and the possible link with mycotoxin contamination in food

Malnutrition among children is one of the most important causes of morbidity and mortality in the world, particularly in developing countries (Schwinger *et al*., 2019). In 2020, globally, 149.2 (22.0%) million children under the age of 5 years of age were stunted, 45.4 (6.7%) million were wasted and 38.9 (5.7%) million were overweight. The number of children with stunting is declining in all regions except Africa and around 45% of deaths among children under 5 years of age globally are linked to undernutrition (WHO, 2020). Although Kenya has made substantive strides in reducing the prevalence of stunting nationally, falling from 35% in 2008 to 26% in 2014 (KNBS *et al*., 2015; KNBS & ICF Macro, 2010), stunting is still

high (above the national levels) in the Coast, Eastern and Rift Valley regions. For instance, the stunting rate in Elgeyo Marakwet county among children under five years was 29.9% (KNBS *et al*., 2015) with the Kerio Valley region having the highest stunting rates (67%) in the county (Kipyego & Mugalavai, 2019). Stunting is most prevalent among children 18–23 months, indicating that poor complementary feeding; hygiene and sanitation practices are likely contributors to stunting in this age group (KNBS *et al*., 2015).

Table 2: Undernutrition rates in Kenya

Region	Child stunting %	Child wasting %	Child mortality %
Coast	30.8	4.5	5.7
North Eastern	24.7	13.3	4.4
Eastern	30.1	4.4	4.5
Central	18.4	2.3	4.2
Rift Valley	29.8	5.7	4.5
Western	25.2	1.9	6.4
Nyanza	22.7	2.0	8.2
Nairobi	17.2	2.5	7.2
Total	**26.0**	**4.0**	**5.2**

Source; KNBS *et al*. (2015).

Malnourished children are more likely to encounter long-lasting problems related to cognitive function and vulnerability to infectious disease and have lower adulthood achievement (Lomborg, 2013). While child growth impairment has in the past been linked to undernutrition, infectious disease and sanitary and hygienic factors (Schmidt, 2014) greater interest has emerged in examining whether dietary toxins play a role in compromising children's growth. Mycotoxins in complementary foods have been suggested to be among the major causes of undernutrition, morbidity and mortality among children (Paudyal *et al*., 2017). Children are generally more sensitive to mycotoxins exposures than other populations due to lower body weight, less acidic stomachs and underdeveloped immune systems (Magoha *et al*., 2016). As a result, daily consumption of foods contaminated with low levels of aflatoxin and fumonisin can result in chronic exposure associated with impaired growth and kwashiorkor in children, immune suppression, cancer and reduced life expectancy (Gnonlonfin *et al*., 2013).

2.9 Caregivers' knowledge and practices toward mycotoxin contamination in foods

Contamination and exposure to mycotoxins are often unavoidable because their levels of awareness and knowledge are low among majority of people in rural areas who rely on their own grown food that is not subjected to regulatory control (Cotty & Jaime-Garcia, 2007). Literature shows an association between a lack of awareness and inadequate knowledge about aflatoxin contamination with the high rate of exposure

to aflatoxins (Monyo *et al*., 2010). Majority of farmers have limited knowledge about the causes of mycotoxin contamination and in turn, they are not willing to incur the costs of controlling the occurrence of these toxins (Sirma *et al*., 2014). For example, farmers from Eastern Kenya and the Democratic Republic of Congo mentioned high costs, unavailability of technology and low awareness of potential benefits as the main reasons for not applying postharvest management techniques in controlling mycotoxins contamination in foods (Marechera & Ndwiga, 2014; Udomkun *et al*., 2018)**.** Households in Eastern Kenya, where aflatoxicosis outbreaks occurred in 2004, had a higher perception of risk, as expected, but low knowledge of the actions needed to minimize exposure to aflatoxin (Marechera & Ndwiga, 2014). These observations suggest that a lack of understanding of the problem contributes to poor control of aflatoxin and other mycotoxins. This knowledge gap stands as a great threat to the acquisition and utilization of safe food for humans. To reduce the risk for vulnerable communities in the absence of market regulation, there is a need for innovative, safe, and economically viable interventions such as fermentation to be promoted in combination with other programs to raise mycotoxin awareness.

2.10 Fermentation and mycotoxin contamination

Fermentation is the process of converting carbohydrates to alcohol or organic acids using microorganisms such as yeast or bacteria under anaerobic conditions. Traditional fermented foods are produced using largely uncontrolled spontaneous inoculation methods in which microorganisms associated with the raw food material and the processing environment serve as inoculants (FAO, 2010). An indigenous fermented sorghum-based porridge known as *ting* is a commonly complementary food as well consumed by adults in Southern African countries (Adebo *et al*., 2018). In Kenya, *Kirario* a non-alcoholic lactic-fermented porridge, produced from mixtures of green maize and millet/sorghum flour, is a refreshing drink for all segments of the population (Kunyanga *et al*., 2009). Fermentation has been shown to retain food's shelf-life time as well enhance the bioavailability of nutrients; enrich the sensory quality of food (Kabak & Dobson, 2011; Tamang *et al*., 2016). Specifically, it has been shown to improve the texture, nutritional and digestive properties of sorghum (Adebo *et al*., 2017).

Maize and sorghum are often contaminated by different mycotoxins (Ezekiel *et al*., 2018). Fermentation has been suggested as a means of reducing occurring mycotoxins in cereal-based foods (Nyamete *et al*., 2016). For instance, different studies have reported a reduction in aflatoxin content in maize after fermentation (Mukandungutse *et al*., 2019; Okeke *et al*., 2015). Reduction of aflatoxins by spontaneous natural fermentation has been linked to the non-covalent binding of mutagens by fractions of the cell wall skeleton of the lactic acid bacteria (initially present in the food material) (Dalie & Deschamps, 2010). Additionally, the application of defined starters with lactic acid bacteria (LAB) may confer preservative and detoxifying

effects on food and feeds (Nyamete *et al*., 2016). Lactic acid bacteria can absorb mycotoxins either by attaching them to their cell wall components or by active internalization and accumulation (Gobbetti *et al*., 2005). Therefore, mycotoxin reduction in fermented food products could be attributed to the ability of certain LAB strains to bind, degrade, or inhibit aflatoxin biosynthesis in food matrices (Nyamete *et al*., 2016). Adebo *et al*. (2019) reported that fermentation could significantly reduce the levels of mycotoxins in *ting* (sorghum-fermented product) after 72hrs of fermentation, especially with the use of lactic acid bacteria strains. Therefore, our study aimed at documenting the effects of both natural (spontaneous) and use of bacterial strains on fermentation aflatoxin and fumonisin levels in sorghum grains and flour.

2.11 Research conceptual framework

Suboptimal postharvest handling and storage practices and poor mycotoxins knowledge has been shown to contribute to the production of mycotoxins in already susceptible crops (Makun, 2013). Therefore, when poor postharvest practices and lack of knowledge on mycotoxins contamination are accompanied by dry spells and prolonged drought periods along the Kerio Valley, sorghum is more likely to be infested by toxigenic fungi. This study used the conceptual framework shown in Figure 1 to explore the effects of independent and intervening variables on the outcome variable. Each arrow starts from the variable that has the causal influence and points to the variable that is affected.

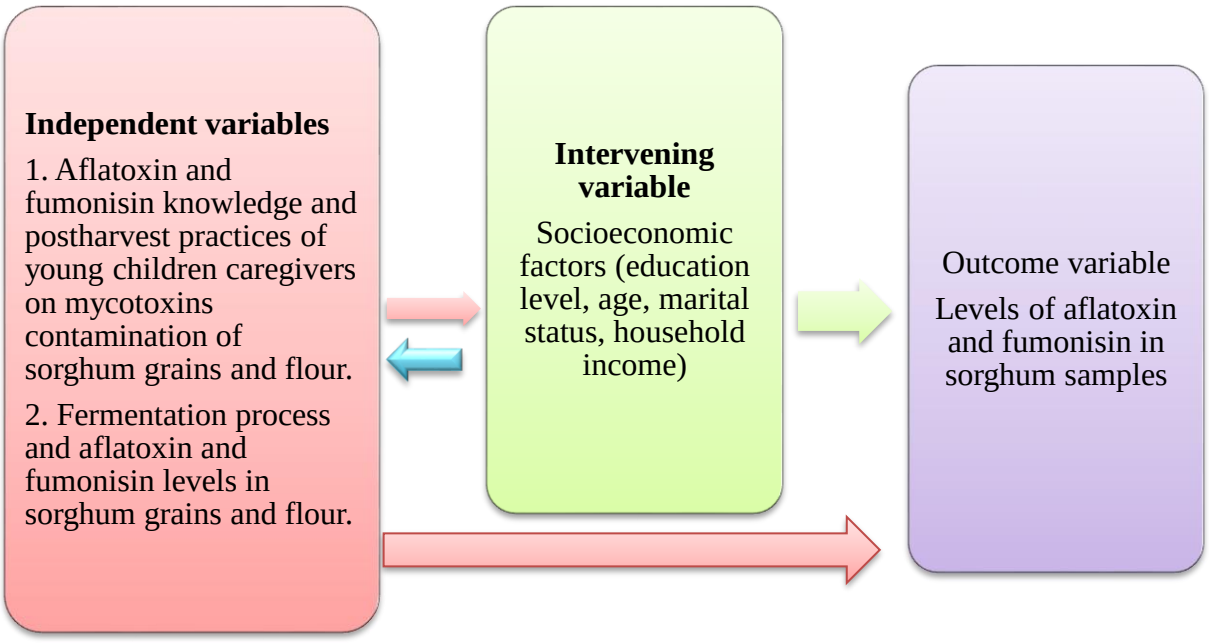

Figure 1: Research conceptual framework

CHAPTER THREE
METHODOLOGY

3.1 Introduction

This chapter describes the methodology that was used in the study. It comprises the research design, study area, sample size, study population, sampling procedure, instrumentation, data collection procedures, ethical approval, and permission to carry out the study and data analysis.

3.2 Research design

The study used both cross-sectional and experimental research designs. Using a cross-sectional study design, the study targeted 374 households. Out of this, 353 young children caregivers participated in the household survey (Emsoo, = 118; Endo, = 117 and Arror, =118). Of 353 participants, 159 (Emsoo, = 46; Endo, = 57 and Arror, =56) of them had children aged 6-23 months and sorghum grains and flour samples for experimental design were collected from 120 randomly selected households from 159 households. Children aged 6-23 months of age are nutritionally vulnerable and the introduction of complementary foods that are contaminated with mycotoxins could further impair their growth and development (Alamu *et al*., 2018). Therefore, it was important to determine aflatoxin and fumonisin levels in the ingredients used to prepare complementary food for children 6-23 months of age. Additionally, another 21 children aged 6-23 months caregivers' who did not participate in the household survey were purposively selected for FGDs. Therefore, 374-targeted households included those who participated in the household survey (353) and those who participated in FGDs (21).

3.3 Study area

The study was conducted in Kerio Valley, Elgeyo Marakwet County, in the Great Rift Valley that lies between the Tugen hills and the Elgeyo escarpment and is accessible from Eldoret town by road (110 km). Specifically, the study focused on three administrative wards; Emsoo in Keiyo North, Arror in Marakwet West and Endo in Marakwet East. Elgeyo Marakwet County is located between longitude 35° 20′ and 35° 45′ East Longitude and 0° 10′ and 0° 20′ North Latitude. It Borders, West Pokot County to the North, Baringo to the East, Uasin-Gishu to the West and Trans Nzoia to the North West (Appendix I). It covers a total land area of 3,030 Km^2 and a population of 369,998 (KNBS, 2009) with an altitude ranging from 1,000 meters in the Kerio Valley to 3,350 meters above sea level in the highlands. Temperatures in the Kerio Valley Basin vary from as low as 10°C in the highland areas of the Cherangany and Tugen Hills, with higher temperatures in the lower altitude areas of the Valley floor that reach a maximum of 40°C.

Agriculture generates revenue for more than half (78%) of the households in Elgeyo Marakwet mostly through engagement in crop and/or livestock husbandry activities. In the Lowlands, farmers produce drought-tolerant crops like sorghum, millet, groundnuts, cowpeas and fruits and rear livestock including

Zebu cattle, poultry, sheep and goats. Most of the farmers in the county are smallholders with an average of 1.36 hectares (ha); the few large-scale farmers have an average of 17.3 ha (MoALF, 2017). Food and nutritional insecurity in the County are at 73.3%, which are worsened by the high poverty levels that stand at 57% with high rates (67%) in Kerio Valley compared to the national average of 46% (MoALF, 2017).

3.4 Target population

The study targeted all sorghum-growing households with children aged 6-59 months in the three selected wards of Kerio Valley, Elgeyo Marakwet County.

3.5 Sample size determination

The sample size was calculated using a statistical formula of Fisher *et al*. (1998): $n = \{Z^2 \times P\ (1 - p)/d^2$. A proportion of 67% was used based on the sorghum samples that tested positive for aflatoxin in Nandi County (Kang'ethe *et al*., 2017). Then ninety-five percent confidence level with a 5% desired level of precision and a non-response rate of 10% based on previous survey reports in the area was applied in this study. Where, n = desired sample size; z = standard normal deviation which is 1.96 (95%); p = proportion of sorghum samples expected to have toxins. Sixty-seven percent (0.67%) was used as the expected positive proportion. q = population without the characteristics measured (1-0.67) d = degree required for accuracy which is 0.05. $n=1.96^2$ (0.67) (1-0.67) ÷ (0.05^2) =340, attrition rate of 10% of n=10/100×340=34
n=(340 + 34) =374.

A sample size of 30 often increases the confidence interval of the population data set enough to warrant assertions against the study findings (Chang *et al*., 2006). To make this study's findings more representative, 60 sorghum grains and flour samples each were analysed for total aflatoxin and fumonisin levels. For those samples that had detectable levels of aflatoxin and fumonisins, 3 grain and flour samples respectively were randomly selected for fermentation. This is due to financial constrains otherwise more samples would have been fermented.

3.6 Sampling techniques

A multi-stage sampling technique was adopted in this study. First, Elgeyo Marakwet County was purposively selected because it is one of the counties where drought-tolerant crops including sorghum were promoted by the Accelerated Value Chain Development-Drought Tolerant Crop (AVCD) project. Additionally, it is one of the counties with high rates of undernutrition (stunting rate 29.9%) above the national levels (26%) among children under five years (KDHS, 2015). Kerio Valley was also purposively selected, because of high undernutrition rates among children under five years (stunting rates of 67%) even after numerous interventions by the government and non-governmental organizations (Kipyego & Mugalavai, 2019). Emsoo, Arror, and Endo wards were selected purposively because they are the main sorghum growing areas in Kerio Valley. From these wards, a list of villages growing sorghum was obtained

from the Ward Agriculture Office. From the list of villages obtained, a second sampling frame was generated, with the help of the Ward Agriculture Officer, village elders and lead farmers, by enlisting all sorghum-growing households with children 6-59 months old. Based on logistical factors such as access, security and the number of households available per village, thirty-one villages were selected in the three wards for the study. Simple random sampling was used to select 353 households (Emsoo, = 118; Endo, = 117 and Arror, =118) from the households list using Microsoft excel for the household survey. Further, from the selected 353 households, 120 households with children 6-23 months of age (40 from each ward) were selected using simple random sampling. Grains and flour samples were collected from these (120) households.

For qualitative data, 21 caregivers of children 6-23 months were selected from the three wards to participate in focus group discussions. Caregivers who participated in FGD were selected from the villages that were not sampled for the household survey. Understanding how caregivers handle ingredients for preparing complementary foods is of great importance as exposure to aflatoxin and fumonisin during the first 1,000 days of life is known to exacerbate child malnutrition and cause negative health impacts (Gong *et al*., 2016).

3.7 Recruitment and training of enumerators

Data was collected by the researcher with the help of a trained team of enumerators with at least undergraduate university education and basic nutrition and biochemistry background. The enumerators were fluent in English, Kiswahili and the vernacular language (Marakwet). Before enumeration, the enumerators were trained on the household questionnaire and focus group discussion guide.

3.8 Data and sample collection procedures

The tools that were used to collect data in this study included; a household structured questionnaire (Appendix II) and a focus group discussion guide (Appendix III).

3.8.1 Household questionnaire

A structured interview guide was used to gather basic socio-demographic information, household characteristics, aflatoxin and fumonisin awareness and postharvest handling and storage practices of sorghum among young children caregivers'.

3.8.2 Focus group discussion

A focus group discussion guide was used to capture information on how mothers/caregivers of children aged 6-23 months handle sorghum after harvest and its processing. Three FGDs that lasted about 60-90 minutes were conducted in the three wards (one FGD per ward), which consisted of seven caregivers of children aged 6-23 months.

3.8.3 Data collection procedures

Before administering the questionnaire, the researcher obtained verbal inform consent from the child's mother/caregiver. Verbal informed consent was sought over written informed consent to minimize personal contacts to curb the spread of COVID-19. To ensure the accuracy and completeness of the questionnaire, the questionnaire was administered at the participant's home in an open area through a face-to-face interview. For focus group discussions, the facilitator guided the discussion while another person took notes.

3.8.4 Sorghum sample collection procedures

Since conventional food cooking has little effect on most mycotoxins in this study sorghum grains (1 kg) and flour (1 kg) were collected for aflatoxin and fumonisin quantification. Sorghum samples were collected from 40 households selected randomly with children aged 6-23 months of age in each ward. The samples were collected according to Obade *et al*. (2015) procedure to ensure sample representativeness. The samples were scooped at different points of all available bags/containers (if less than 10 bags are available in the household) and combined to produce a representative sample for analysis. However, for those households that had more than 10 bags, the square root of the extra bags was calculated and added to the 10 bags. In addition, samples were collected preferably from bags at the front, centre and areas close to the walls of the store according to Sadhasivam *et al*. (2017) to ensure uniformity. From every sampling point, sorghum samples were kept in khaki bags that were labelled and dated and sources were recorded, stored in a cool box, and then transported for mycotoxins analyses at Mycotoxin Research Laboratory at Egerton University. In the laboratory, the samples were kept at 4°C before further analysis.

3.9 Aflatoxin and fumonisin analysis and quantification

3.9.1 Isolation and identification of fungi associated with sorghum grain and flour

Fungi isolation was carried out in 120 sorghum grains and 120 flour according to the procedure described by Owuor *et al*. (2018) and Wagacha *et al*. (2016). Sorghum grains were surface sterilised in 1.3% sodium hypochlorite and subsequently rinsed three times for two minutes in sterile distilled water. Then, fifteen grains were plated on Petri dishes (90 × 15 mm) containing potatoes dextrose agar (PDA) amended with 50 mg of streptomycin sulphate per litre of medium. The setup was replicated thrice for each sample. As for sorghum flour samples, the dilution plate technique was used. Briefly, 1 gram of flour was suspended in 9 ml sterile distilled water and serially diluted to 1 x 10^{-3}. One ml of 10^{-3} was uniformly spread in duplicate plates that contained PDA (amended as above). The plates were incubated for 5-7 days at 25°C under 12 hours of daylight and 12 hours of darkness cycles. Counts of the total number of infected grains and flour samples and fungal colonies per plate were done.

To enable cultural characterization and microscopic identification, the resultant fungal colonies from the above-mentioned procedures were individually sub-cultured. To facilitate the sporulation of *Fusarium*

isolates, its colonies were sub-cultured on synthetic nutrient agar (SNA) medium (Nirenberg, 1981): (KH2PO4 1.0g, KNO3 1.0g, MgSO4 0.5g, KCl 0.5g, Glucose 0.2g, Agar 20g) at 25±2°C in 12 hours light and 12 hours darkness condition for 7 to 14 days. *Fusarium* cultures on PDA were used for cultural characterization while cultures on SNA were used for microscopic identification. *Fusarium* species were identified using manuals by Jenkinson and Parry (1994), features used for microscopic identification included morphology of macroconidia and microconidia, type of conidiophores, phiallides and chlamydospores. Other fungal genera were individually sub-cultured on PDA amended with 50 mg each of streptomycin sulphate per litre and identified based on cultural and morphological features like mycelia colour, spore shape, pigmentation, septation and sporophore characteristics using the pictorial Atlas of fungi (Appendix V) (Klich, 2002). The frequency of fungi and relative percentage of particular species within a genus of fungi was calculated using the formula of Ghiasian *et al*. (2004).

$$Frequency = \frac{Number\ of\ samples\ infected\ with\ fungal}{Total\ number\ of\ samples\ analysed} \times 100$$

$$Relative\ percentage = \frac{Number\ of\ fungal\ species\ isolated}{Total\ number\ of\ fungal\ isolated} \times 100$$

3.9.2 Qualitative method for screening of Aspergillus isolates for aflatoxin production Dichlorvos-Ammonia vapour test

Three hundred and twenty-seven species identified belonging to *Aspergillus* were grown on PDA at 28°C for 7 days amended with 2 ml of Dichlorvos solution per litre of the medium. After incubation, Petri-dishes were turned upside down and 2 ml of concentrated ammonia solution (28%) (SRL Extra pure AR Grade) was poured into the lid of the inverted culture plate and kept for 10±15 minutes to release ammonia vapour (Kumar *et al*., 2007). On exposure of the *Aspergillus* culture to ammonia vapour, colour development was recorded. Toxin production (positive test) was evidenced by the formation of a pink to plum-red colour on the underside of the fungal colony while negative tests with no observable colour changes (Zrari, 2013).

3.9.3 Quantification of total aflatoxin and fumonisin in sorghum grains and flour

Since qualitative methods such as visual observation and dichlorvos-ammonia test can detect toxins-producing fungi and are easy to conduct and less expensive than analytical methods (Shekhar *et al*., 2017), this study used these qualitative methods to screen and select sorghum samples for quantitative analysis using Enzyme-Linked Immuno- Sorbent Assay (ELISA) method. Visual observations were used to identify samples (in 2.3 above) that are contaminated by *Aspergillus* and *Fusarium* species based on the colony top and reverse colour and other microscopic features. While the dichlorvos-ammonia test (in 3.9.2 above) was used to determine the ability of single *Aspergillus* colonies to produce aflatoxin through the colour change from yellow to purple-red/pink (Appendix V) (Shekhar *et al*., 2017). Thereafter, a list of samples that were colonised with *Aspergillus* and *Fusarium* genera based on cultural and microscopic characterization and

those that tested positive for toxigenic fungi using the dichlorvos-ammonia test was generated per ward. From this list, 20 sorghum grain and 20 flour samples were randomly selected from each ward for aflatoxin and fumonisin quantification.

For ELISA analysis, sorghum grains were ground to obtain 200 grams of fine flour from each sample. To obtain a homogenous sub-sample for analysis each sorghum flour, collected from the households and the one obtained by grinding the grains in the laboratory, was thoroughly mixed. Then toxins quantification was carried out according to kit manufacturer instructions. For the Eurofins *Celer AFLA* ELISA kit (total aflatoxin quantification) manufacturer's procedure, fifty grams of each flour were weighed in a clean conical flask (500mL) and diluted with 250 ml of 70% methanol (MeOH) and 4% sodium chloride (NaCl) solution. The mixture was thoroughly mixed by shaking for 3 minutes, filtered using Whatman 1 paper and the filtrate was collected in falcon tubes. Two millilitres of the filtrate/supernatant were transferred to *Eppendorf* tubes, mixed vigorously for 30 seconds and the gel was allowed to settle for 10 minutes, after which the clear filtrate was used for the analysis. For fumonisin quantification, toxins were extracted from 20 grams of homogenized sub-samples of each sorghum sample using 40 mL of extraction solvent (90% methanol; 4mL of distilled water added to 36mL of methanol) and analysed according to the ELISA kit manufacturer's protocol (Helica Biosystems, Inc., Fullerton, CA). The optical density (OD) was read at 450 nm in a microplate reader (ThermoScientific) and the standard curves were generated using the absorbance and the known concentrations of the kits' standards.

3.10 Fermentation of sorghum grains and flour

Six samples (3 grains and 3 flour) were selected for fermentation. The fermentation process was carried out in two ways; through the action of native microflora (natural fermentation) and *Lactobacillus Plantarum*. Each sorghum sample was divided into two portions and each portion was fermented separately by mixing sterile distilled water (167 ml) and 250 grams of sorghum grains and flour (1:1.5, w/v) according to Mukandungutse *et al*. (2019). The first portion of the samples was allowed to spontaneously ferment under room temperatures without any bacteria or microorganisms added. The second portion of the samples was fermented after the addition of *Lactobacillus Plantarum*. The *Lb. Plantarum* strain was cultivated according to Ntsamo *et al*. (2020) protocol. Where, the stock culture of *Lb. plantarum* was pre-cultured in Man Rogosa Sharp (MRS) broth at 37°C for 24 h. The resulting culture broth was centrifuged at 6500 rpm at 4°C for 20 minutes. The bottom was collected, washed thrice with sterile saline water (8.5 g/L NaCl) and used to prepare the inoculum. Sorghum samples were inoculated with 3mL of suspension of *Lb. plantarum* to achieve a final concentration of 10^{-7} CFU/g. Then homogenized aseptically with a sterile glass rod and incubated at 37°C. Samples from each portion were withdrawn at 24, 48 and 72 hours according to Ntsamo

et al. (2020). Immediately, the withdrawn samples were dried in the dryer cabinet at 60°C for 16 hours (Kurniadi *et al*., 2019). The dried samples were then ground into a fine powder and analysed for total aflatoxin and fumonisin levels using the ELISA method as mentioned above.

3.11 Pretesting of the questionnaire

Data collection tools were pre-tested in 37 randomly selected households (10 percent of the sample size) in Kwenoi village in Endo ward. This village was out of the selected study sites, but they grow sorghum and have similar farming activities. Pretesting was necessary to ensure that questions were clearly articulated and the response options are relevant and comprehensive. This helped to assess response latency and the amount of time it takes to complete one questionnaire. The responses from pretesting helped in the modification of the research questionnaire accordingly.

3.12 Validity

Validation is the process of verifying the degree to which research tools measure what it is intended to measure (Wong *et al*., 2012) and there are two measures of validity; external and internal. To ensure external validity, the study participants were selected randomly so the findings can be generalized across the study population. Internal validity was achieved through content and face validity. Content validity of the study tools such as household questionnaires and FGD guide was reviewed by the experts (study supervisors and other examiners) in the area of study to ensure they meet the study objectives. In addition, face validity was done among caregivers of children 6-59 months through pretesting to assess whether the questionnaire is legible, clear, simple and understandable. Necessary changes were made after every verification stage.

3.13 Reliability

Reliability refers to the degree to which the results obtained by a measuring tool and procedure can be replicated (Wong *et al*., 2012). After the pretesting, the reliability of the study tools was measured using Cronbach's alpha. Using SPSS software, the Cronbach alpha value for the study tools used was 0.79, which indicates the questionnaire was reliable (Heale & Twycross, 2015).

3.14 Ethical considerations

Ethical clearance (Appendix VI) and research permits (Appendix VII) were obtained from the Egerton university Research Ethics committee (ethical code, EUREC/APP/130/2021) and National Commission for Science, Technology and Innovation (NACOSTI) respectively. Permission was also obtained from relevant authorities at Sub-County, Location and Sub-location levels. Upon arrival at the household level, the purpose of the study was explained to the respondents (Appendix VIII) and those who consented verbally were interviewed. To ensure the confidentiality of the study participants, no information was recoded in a way that links subject responses with identifying information such as their names or other methods of identification including identity card numbers. In addition, the information collected was kept in strict confidence and exclusively used for the study.

3.15 Data management and analysis

The filled household questionnaires were checked daily for accuracy and completeness in capturing responses. Questionnaires coding and cleaning were done before data entry. Data were entered and analysed using the Statistical Package for Social Sciences (SPSS) version 26 and summarized using tables and graphs. Data on caregivers' socio-demographic characteristics, their knowledge of mycotoxins and post-harvest handling and storage practices of sorghum were analysed using the Chi-Square (χ^2) test. To assess caregivers' mycotoxin knowledge and post-harvest handling and storage practices, every correct answer to the questions was assigned a score of 1 while a score of 0 was given to a wrong answer and where the respondent did not know. Scores on knowledge and practices for each respondent were calculated by summing up the scores attained on each question and the overall score ranked as good or poor. Twelve knowledge questions were asked and thus the scores ranged from 0 to 12. Individuals scoring 7 and below (score below 60%) were categorized as having poor knowledge and scores above 7 as good knowledge (ul Haq *et al*., 2012; Wang *et al*., 2015). Regarding the caregivers' post-harvest practice, 18 questions were asked and a total score per individual of less than or equal to 10 (score below 60%) was categorized as poor practice and scores above 10 as good practices (Papagiannis *et al*., 2020; Wang *et al*., 2015). Binary logistic regression was used to determine the association between household socio-economic factors and their mycotoxins' knowledge and post-harvest practices of sorghum and $p<.05$ was considered statistically significant.

The qualitative data from the focus group discussion were analysed using the thematic content analysis method by identifying similarities, differences and trends between the individual and group responses (Busetto *et al*., 2020). Common themes that emerged from each individual and throughout the interviews were identified as well as any differences in responses and observed practices based on the demographics. The findings from the FGDs guide were triangulated onto the household survey data to provide an in-depth understanding of the concepts studied. Mean and percentages were used to describe data from laboratory experiments. The mean differences among sorghum samples on their aflatoxin and fumonisin levels were computed using analysis of variance (ANOVA) and $p<.05$ was considered statistically significant. The least significant difference test was used to separate samples with significantly different means. The summary of objectives, study variables and statistical tests used are shown in Table 3.

Table 3: Summary of objectives, variables, and statistical test

Objective	Variables	Statistical test
Children under five years caregivers' knowledge of mycotoxins, aflatoxin and fumonisin contamination	Caregivers' Knowledge	Descriptive: Mean, standard deviations, frequencies and range. Association: chi-square test, binary regression
Post-harvest practices sorghum grains and flour among mothers/caregivers of children 6-59 months in Kerio Valley, Elgeyo Marakwet County.	post-harvest handling and storage practices	Descriptive: Mean, standard deviations, frequencies and range. Association: chi-square test, binary regression
Quantify aflatoxin and fumonisin levels in sorghum grains and flour in Kerio Valley, Elgeyo Marakwet County.	Aflatoxin and fumonisin levels	Descriptive (Mean and standard deviations) Analysis of variance (ANOVA)
Determine the effect of natural fermentation on aflatoxin and fumonisin levels in sorghum flour in Kerio Valley, Elgeyo Marakwet County.	Aflatoxin and fumonisin levels in non-fermented and fermented sorghum porridge	-ANOVA, t-test

CHAPTER FOUR
RESULTS

4.1 Introduction

This study aimed to determine aflatoxin and fumonisin awareness levels alongside post-harvest handling and storage practice of sorghum among caregivers of children 6-59 months and establish aflatoxin and fumonisin levels in sorghum grain and flour in Kerio Valley, Kenya. The study also determined the effects of natural and bacterial fermentation on the aflatoxin and fumonisin levels in the sorghum samples.

4.2 Socio-demographic characteristics of the study population

A total of 353 caregivers participated in the household survey and their socio-demographic characteristics are shown in Table 4. The household heads for majority of the farmer household were male (93%). More than half (52.1%) of the caregivers interviewed were aged 31-45 years and were in monogamous marriages (87.3%). About 45% of caregivers had at least primary school education, indicating some level of literacy while more than a third (38%) had a monthly income of less than Ksh. 5,000. All socio-demographic characteristics (sex of the household head (p=0.034), age of the respondent (p=0.025), an education level (p=0.001) and monthly income were significantly different (p=0.001) in the three administrative wards except for marital status (p=0.232). The difference in the socio-demographic characteristics could influence the caregivers' knowledge of aflatoxin and fumonisins as well as their postharvest handling and storage practices of sorghum.

Table 4: Socio-demographic characteristics of caregivers of children aged 6-59 months in Kerio Valley

	Administrative wards									
Socio-demographic factors	**Total (n=353)**		**Emsoo (n=118)**		**Endo (n=117)**		**Arror (n=118)**		**χ^2 value**	***p*-value***
	n	%	n	%	n	%	n	%		
HH head sex									6.760	0.034*
Male	328	92.9	104	88.1	110	94.9	114	96.6		
Female	25	7.1	14	11.9	7	6.0	4	3.4		
Marital status									10.501	0.232
Married monogamous	308	87.3	100	84.7	101	86.3	107	90.7		
Married polygamous	20	5.7	4	3.4	9	7.7	7	5.9		
windowed	9	2.5	5	4.2	2	1.7	2	1.7		
separated	12	3.4	7	5.9	4	3.5	1	0.8		
single	4	1.1	2	1.9	1	0.9	1	0.8		
Age in years									14.445	0.025*
18-30	110	31.2	51	43.2	31	26.5	28	23.7		
31-45	184	52.1	51	43.2	62	53.0	71	60.2		
46-60	39	11.0	12	10.2	16	13.7	11	9.3		
Above 60	20	5.7	4	3.4	8	6.8	8	6.8		
Education level									30.198	0.001*
None	47	13.3	1	0.8	26	22.2	20	16.9		
Primary	159	45.0	67	56.8	44	37.6	48	40.7		
Secondary	113	32.0	37	31.4	40	34.2	36	30.5		
Tertiary	34	9.6	13	11.0	7	6.0	14	11.9		
Monthly income									91.990	0.001*
Less than 5,000	134	38.0	67	56.8	4	3.4	63	53.4		
5,001-10, 000	107	30.3	31	26.3	52	44.4	24	20.3		
Above 10, 000	112	31.7	20	16.9	61	52.1	31	26.3		

HH, Household head; *P <0 .05 significant by χ^2 test

4.3 Children under five years caregivers' knowledge about mycotoxin contamination in foods

According to methods described in the data management and analysis section (3.15), the average score for knowledge about mycotoxins among the caregivers was 6.48± 2.94, out of a possible 12 scores, indicating a low level of knowledge (Fig. 2). In addition, the average practice score among caregivers was 9.15±1.90, out of 18 total scores, which was suboptimal.

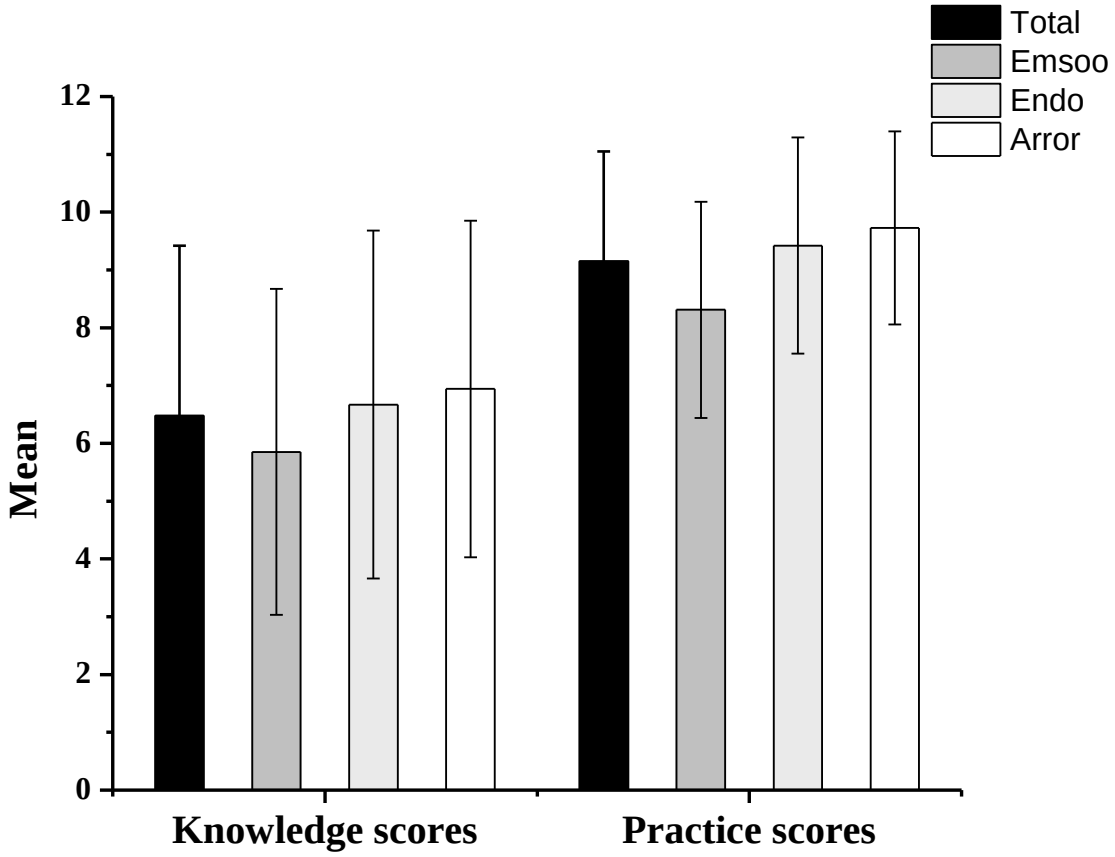

Figure 2: Mean scores of young children caregivers' knowledge about aflatoxin and fumonisin and post-harvest handling and storage practices of sorghum in Kerio Valley, Elgeyo Marakwet County. Data are means ± standard deviations; P <0.05 significant by Analysis of variance.

Although more than half (60.9%) of the caregivers heard about aflatoxins, only 25.2% and 19.8% were familiar with the terms fumonisin and mycotoxins respectively (Table 5). Furthermore, out of the caregivers who heard about aflatoxin and fumonisin, 49 (13.9%) and 27(33.8%) knew that sorghum can be contaminated by aflatoxin and fumonisin respectively. Majority (67.4 %) of them knew that consumption of mycotoxin-contaminated food could lead to adverse health effects such as abdominal pain (55.5%) and diarrhoea (22.0%), while 40.8% and 45.9% thought it could also lead to childhood stunting and immune suppression respectively. Compared to caregivers from Endo (63.2%) and Emsoo (57.6%) wards, more than

three-quarters of caregivers from Arror (81.4%) were knowledgeable of the health effects that consumption of mycotoxins in foods can cause.

Table 5: Knowledge of caregivers of children under five years old about mycotoxins contamination in foods in Kerio Valley

	Administrative wards									
Questions regarding mycotoxins knowledge	**Total (n=353)**		**Emsoo (n=118)**		**Endo (n=117)**		**Arror (n=118)**		**χ^2 value**	***p*-value**
	n	%	n	%	n	%	n	%		
Heard of mycotoxins? **Yes**	70	19.8	23	19.5	30	25.6	17	14.4	4.677	0.096
Heard of aflatoxin? **Yes**	215	60.9	66	55.9	80	68.4	69	58.5	4.261	0.119
Heard of fumonisin? **Yes**	89	25.2	24	20.3	36	30.8	29	24.6	3.428	0.180
Can you mention foods that can be contaminated by aflatoxin? **Yes**	195	90.7	60	90.9	72	90.0	63	91.3	0.080	0.961
Can you mention foods that can be contaminated by fumonisin? **Yes**	57	64.0	17	70.8	20	69.0	20	55.6	1.912	0.384
Able to identify spoilt sorghum grains? **Yes**	327	92.6	108	91.5	111	94.9	108	91.5	1.284	0.526
Do you know the causes of mould growth in foods? **Yes**	321	90.9	109	92.4	103	88.0	109	92.4	1.786	0.409
Does consumption of mycotoxins contaminated food have any negative health effects? **Yes**	238	67.4	68	57.6	74	63.2	96	81.4	28.939	0.001*
Can aflatoxins and fumonisins in complementary foods cause:										
Stunting? **Yes**	144	40.8	38	32.2	43	36.8	63	53.4	15.207	0.004*
Impaired immunity? **Yes**	162	45.9	40	33.9	51	43.6	71	60.7	22.974	0.001*
Abdominal pain? **Yes**	230	65.2	68	57.6	74	63.2	88	74.6	11.756	0.019*
Diarrhoea? **Yes**	239	67.7	70	59.3	84	71.8	85	72.0	7.657	0.105

*P <0.05 significant by χ^2 test

The source of information about aflatoxin, fumonisin, or mycotoxins was not significantly different (χ^2, =16.308, p=0.178) across the three wards. However, mass media (39.4%) was reported as the main source of information. Out of the 353 respondents, 218 (61.8%) were within the poor knowledge score, with more caregivers from Emsoo (71.2%) having poor knowledge scores compared to those from Arror (57.6%) and Endo (56.4%) (Table 6).

Table 6: Source of knowledge on mycotoxins contamination and knowledge scores for children under five years caregivers' in Kerio Valley

	Administrative wards									
	Total (n=353)		**Emsoo (n=118)**		**Endo (n=117)**		**Arror (n=118)**		**χ2 value**	**p-value**
	n	%	n	%	n	%	n	%		
Source of information about mycotoxins									16.308	0.178
Mass media (e.g., radio)	86	39.4	24	36.9	34	42.0	28	38.9		
Agricultural extension officers	41	18.8	11	16.9	17	21.0	13	18.1		
Friends/neighbours	41	18.8	10	15.4	17	21.0	14	19.4		
Reading	35	16.1	18	27.7	8	9.9	9	12.5		
Seminars/experts	12	5.5	2	3.1	5	6.2	5	6.9		
Women group	1	0.5	-	-	-	-	1	1.4		
AVCD-DT Project	2	0.9	-	-	-	-	2	2.8		
Knowledge score									6.711	0.035*
Poor	218	61.8	84	71.2	67	57.3	67	56.1		
Good	135	38.2	34	28.8	50	42.7	51	43.2		

*P <0.05 significant by χ^2 test

4.3.1 Caregivers' knowledge of factors associated with moulds growth in foods and their prevention strategies

The main causes of moulds growth and spoilage in foods mentioned, although not significantly different across the three wards, by the caregivers were poor drying (35.1%), poor storage (31.2%) and heavy rains during harvesting (19.5%) while 15.2% were not aware of any causes (Fig 3). In this regard, a good number of them (25.4% and 16.4%) stated proper drying and good storage practices as the best strategies to prevent mould food spoilage.

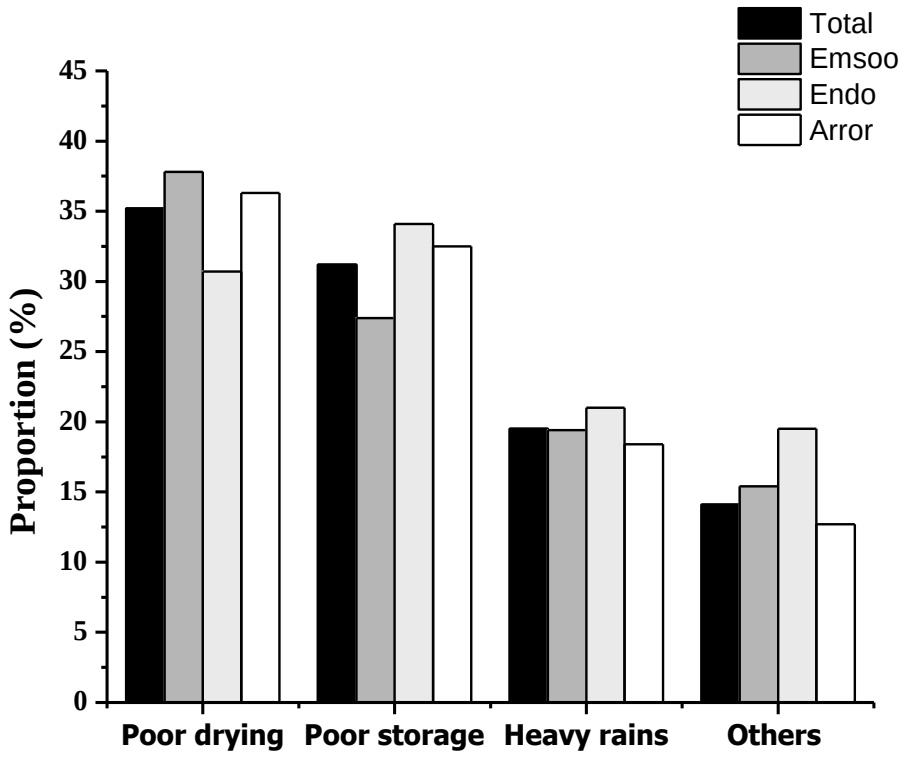

Figure 3: Responses on causes of mould spoilage in sorghum among caregivers of young children in Kerio Valley, Elgeyo Marakwet County. P <0.05 significant by χ^2 test

4.3.2 Food crops mostly affected by aflatoxin and fumonisin

Figure 4 shows caregivers' responses regarding their knowledge of food crops that are likely to be contaminated by aflatoxin and fumonisin. The four most frequently identified food crops that are thought to be susceptible to aflatoxin contamination were maize (27.1%), groundnuts (11.2%), sorghum (8.8%), and millet (6.5%) while fumonisin include maize (22.6%), sorghum (19.7%), millet (8.8%) and beans (7.3%). Most of the caregivers did not know the crops that are susceptible to aflatoxin (35.2%) and/or fumonisin (41.6%) contamination. However, these findings were not significantly different across the three wards.

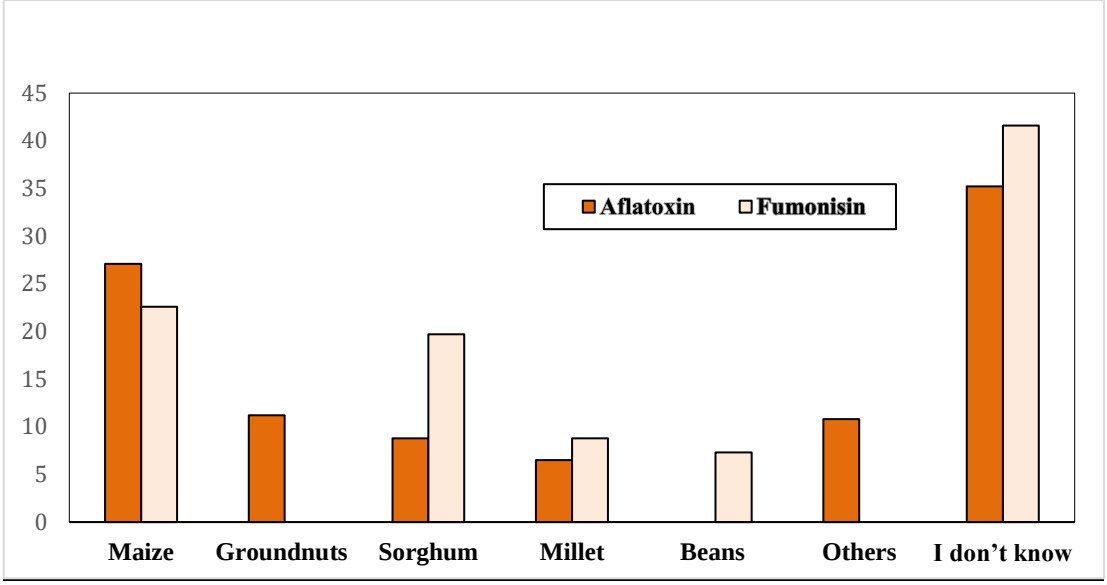

Figure 4: Caregivers' responses on the food crops susceptible to aflatoxin and fumonisin contamination. *P* <0.05 significant by χ^2 test

4.4 Caregivers' post-harvest handling and management practices of sorghum

The post-harvest practices for majority (75.4%) of the caregivers were poor and diverse (χ^2, =12.237, *p*=0.002) across the three wards. Caregivers from Emsoo ward recorded the highest number of respondents (86.4%) with poor post-harvest practices followed by Endo (71.8%) and Arror (70.8%) respectively (Table 7).

Table 7: Caregivers' postharvest handling and storage practices score

	Administrative wards									
Caregivers post-harvest practices	**Total (n=353)**		**Emsoo (n=118)**		**Endo (n=117)**		**Arror (n=118)**		**χ^2**	**P-value**
	n	%	n	%	n	%	n	%		
Practices score									12.237	0.002
Poor	266	75.4	102	86.4	84	71.8	80	70.8		
Good	87	24.6	16	13.6	33	28.2	38	32.2		

P <0.05 significant by χ2 test

4.4.1 Post-harvest management of sorghum grains before storage

Majority of caregivers dried sorghum after harvest; however, 12.2% left their crops to dry while standing in the field. Among those who dried their grains after harvest (87.8%), the duration of drying (χ^2, =20.807, *p*=0.001) and materials used for drying (χ^2, =15.157, *p*=0.004) were different across the wards (Table 8).

Table 8: Caregiver's responses on post-harvest practices of sorghum before storage in Kerio Valley

Post-harvest practices	Administrative wards								χ^2	P-value
	Total (n=353)		Emsoo (n=118)		Endo (n=117)		Arror (n=118)			
	n	%	n	%	n	%	n	%		
Dried sorghum after harvest? Yes	310	87.8	100	84.7	102	87.2	108	91.5	2.602	0.272
Drying duration										
<7 days	36	11.6	4	4.0	8	7.8	24	22.2	20.807	0.001*
7 days	243	78.4	82	82.0	86	84.3	75	69.4		
>7days	31	10.0	14	14.0	8	7.8	9	8.3		
Dried on;										
Bare grounds	199	64.0	67	67.0	57	55.9	75	68.8	15.157	0.004*
Canvas	103	33.1	33	33.0	37	36.3	33	30.3		
Others (Concrete asphalt, rock surface)	9	2.9	0	-	8	7.8	1	0.9		
Methods of checking grains dryness									3.053	0.802
Traditional methods										
Looking at it	87	24.6	27	22.9	27	23.1	33	28.0		
Squeezing/touching	85	24.1	29	24.6	29	24.8	27	22.9		
Biting	180	51.0	62	52.5	60	51.3	58	49.2		
Listening to the sound	1	0.3	0	0.0	1	0.9	0	0.0		
Modern method										
Moisture meter	0	-	-	-	-	-	-	-		
Threshing method used										
Hand threshing	340	96.3	116	98.3	114	97.4	110	93.0	4.918	0.086
Use machine/thresher	13	3.7	2	1.7	3	2.6	8	6.8		
Removed spoilt panicles before threshing? Yes	353	100	118	100	117	100	118	100	-	-

P <0.05 significant by $\chi 2$ test

Drying sorghum for more than a week was popular in Emsoo (14%) while drying grains on bare ground (68.8%) was mainly practiced in Arror ward. To confirm grain dryness, none of the caregivers used a

moisture meter but instead, they used traditional methods such as biting (51.0%), looking (24.6%) and touching/squeezing (24.1%). Nearly all the caregivers (96.3%) used manual threshing methods to shell sorghum and before shelling, all caregivers (100%) separated spoilt sorghum panicles from the clean ones. Although more than a third (32.3%) of the caregivers threw away the spoilt grains, more than half (51.6%) used them as animal feed and prepared local brews (13.6%) while some from Emsoo ward (5.1%) reported using such grains for human consumption(Table 9). Before grain storage, less than half of the caregivers sorted (41%) their grains, cleaned (21.8%), or fumigated the storage area (7%).

Table 9: Caregiver's post-harvest practices of sorghum before storage in Kerio Valley

	Administrative wards									
Post-harvest practices	**Total (n=353)**		**Emsoo (n=118)**		**Endo (n=117)**		**Arror (n=118)**		χ^2	**P-value**
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**		
Suspect fungal contamination in grains based on;										
Grains discoloration	265	81.8	85	79.4	80	73.4	100	92.6		
Off-smell	152	46.	56	52.3	38	34.9	58	53.7		
Bitter taste	3	0.9	3	2.8	1	0.9	0	0		
I do not know	4	1.2	2	1.9	1	0.9	0	0		
Use of spoilt grains									18.357	0.019*
Thrown away	114	32.3	32	27.1	47	40.2	35	29.7		
Feed livestock	183	51.8	62	51.7	45	47.0	67	56.8		
Making local brew	48	13.6	19	16.1	14	12.0	15	12.7		
Re-dry and consume	6	1.7	6	5.1	0	0	0	0		
Sell	2	0.6	0	0.0	1	0.9	1	0.9		
Other practices before storage of sorghum grains?										
Grains sorting	111	41.0	33	33.3	37	40.2	42	50.6		
Winnowing after threshing	59	21.8	16	16.7	20	21.7	23	27.7		
Cleaning of storage area	227	83.8	74	77.1	78	84.8	75	90.4		
Fumigation of storage area	19	7	6	6.3	6	6.5	7	8.4		

P <0.05 significant by χ2 test

4.4.2 Type of storage structures used and storage practices of sorghum

More than half (52.4%) of the caregivers stored their grains in traditional granaries (Table 10). Hermetic bags were mainly used as the storage material for the sorghum grains mostly by caregivers from Arror (81.4%) as compared (χ^2, =40.828, p=0.001) to those from Emsoo (45.2%) and Endo (68.4%). Additionally, a large proportion of the caregivers from Arror (84.7%) and Endo (84.6%) wards used plastic buckets to store sorghum flour for preparing children's porridge with majority of them storing the flour for less than a week (χ^2, =11.393, p=0.003).

Table 10: Type of storage structures used and storage practices of sorghum in Kerio Valley

HH post-harvest practices	Administrative wards									
	Total (n=353)		Emsoo (n=118)		Endo (n=117)		Arror (n=118)		χ^2	*p*-value
	n	%	n	%	n	%	n	%		
Type of store used									11.267	0.080
Traditional store	285	52.4	53	44.9	61	52.1	71	60.2		
Modern store	18	5.1	6	5.1	3	2.6	9	7.6		
Living room with improved structure	37	10.5	17	14.4	12	10.3	8	6.8		
Living room without improved structure	131	32.0	42	35.6	41	35.0	30	25.4		
Type of storage bags used									40.828	0.001*
Gunny bags	94	26.6	47	39.8	28	23.9	19	16.1		
Hermetic bags	227	64.3	51	43.2	80	68.4	96	81.41		
Sisal bags	23	6.5	14	11.9	7	6.0	2	.7		
Placed bundle on the floor	9	2.5	6	5.1	2	1.7	1	0.8		
Stacking of storage bags									16.396	0.012*
On wood pallets	207	58.6	59	50.0	64	54.7	84	71.2		
On the floor	131	37.1	52	44.1	48	41.0	31	26.3		
On the stones	6	1.7	1	0.8	3	2.6	2	1.7		
Duration of grains storage									3.316	0.314
0-6 months	235	66.6	71	65.3	84	71.8	74	62.7		
More than 6 months	118	33.4	41	34.7	32	28.2	44	37.3		
Sorghum flour storage material									36.830	0.001*
Plastic bucket	265	75.1	66	55.9	99	84.6	100	84.7		
Gunny bag	63	17.8	39	33.1	10	11.9	14	11.9		
Others (Animal skin bags)	25	7.1	13	11.0	8	6.8	4	3.4		
Duration of flour storage									11.393	0.003*
Less than 7 days	319	90.4	100	84.7	104	88.9	115	97.5		
More than 7 days	34	9.6	18	15.3	13	11.6	3	2.5		
Ferment sorghum flour	140	39.7	52	44.1	41	35.0	47	39.8	2.002	0.368

HH, Households; $P < 0.05$ significant by $\chi 2$ test

4.5 Young children caregivers' trained on Infant and Young Child Feeding (IYCF)

Among the small proportion of caregivers (10.2%) who attended training on Infant and Young Child Feeding (IYCF), only nine (9) of them were trained on mycotoxin contamination of complementary foods (Table 11). Most of the caregivers from Endo ward (χ^2, =17.752, p=0.001) were trained about mycotoxins during IYCF training while none of the IYCF training content included mycotoxin contamination of foods in Emsoo ward.

Table 11: Infant and Young Child Feeding (IYCF) trainings attended by the caregivers in Kerio Valley

	Administrative wards									
	Total (n=353)		**Emsoo (n=118)**		**Endo (n=117)**		**Arror (n=118)**		**χ^2**	***p*-value**
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**		
Attended IYCF training (Yes)	36	10.2	12	10.2	7	6.0	17	14.4	4.552	0.103
Mycotoxins contamination was part of the training (Yes)	9	25.7	-	-	6	85.7	3	18.8	17.752	0.001*

*P <0.05 significant by χ2 test

4.6 Factors associated with caregivers' knowledge about mycotoxin contamination in sorghum and their post-harvest practices

4.6.1 Association of socio-demographic factors with caregivers' knowledge about mycotoxin contamination in sorghum

Using binary logistic regression, the caregivers' knowledge scores about mycotoxins contamination in sorghum were associated with age [(AOR=4.629, (95% Cl: 2.530-8.472), p<0.001], education level [(AOR=0.275, (95% Cl: 0.088-0.434), p=0.001], marital status [(AOR=15.187, (95% Cl: 1.830-126.007), p=0.12] and household monthly income [(AOR=2.623, (95% Cl: 1.550-4.439), p<0.001] (Table 12).

Table 12: Association of socio-demographic factors with caregivers' knowledge about mycotoxin contamination of sorghum in Kerio Valley

Demographic variables	Knowledge scores (≤7 and >7)		*P*-value
	AOR	95% Cl	
Age group (years)			
18-30 (reference)	1		0.001*
>30	4.629	2.530-8.472	
Level of education			
No formal education (reference)	1		0.001*
Formal education	0.275	0.129-0.588	
Marital status			
Not married (reference)	1		0.012*
Married	15.187	1.830-126.007	
Monthly average income (in Ksh)			
Less than 5, 000 (reference)	1		0.001*
More than 5, 000	2.623	1.550-4.439	

AOR, adjusted odds ratio; *P <0.05 significant using binary logistic regression

4.6.2 Association of socio-demographic factors with caregivers' post-harvest handling and storage practices of sorghum

With the use of binary logistic regression, the post-harvest handling and practices of sorghum (Table 13) were significantly associated with caregivers' age [(AOR=3.845, (95% Cl: 1.558-9.490), p=0.003], education level [(AOR= 0.196, (95% Cl: 0.088-0.434), p<0.001], monthly income [(AOR=3.291, (95% Cl: 1.550-6.986), p=0.002] and knowledge about mycotoxins [(AOR= 5.428, (95% Cl: 2.855-10.319), p<0.001]. However, marital status [(AOR= 3.579, (95% Cl: 0.403-31.775, p=0.252)] was not associated with caregivers' post-harvest handling practices.

Table 13: Association of socio-demographic factors with post-harvesting handling and storage practices of sorghum among caregivers of children aged 6-59 years old in Kerio Valley

Factors	Practices scores (≤10 and >10)		*P*-value
	AOR	95% Cl	
Age group (years)			
18-30 (reference)	1		0.003*
>30	3.845	1.558-9.490	
Level of education			
No formal education (reference)	1		0.001*
Formal education	0.196	0.088-0.435	
Marital status			
Not married (Reference)	1		0.252
Married	3.579	0.403-31.775	
Monthly average income (Ksh.)			
Less than 5, 000 (Reference)	1		0.002*
More than 5, 000	3.291	1.550-6.986	
Mycotoxins knowledge			
Poor (Reference)	1		0.001*
Good	5.428	2.855-10.319	

AOR, adjusted odds ratio; *P <0.05 significant using binary logistic regression

4.7 Total aflatoxin and fumonisin concentration in sorghum grain and flour

4.7.1 Characterization of fungal isolates from sorghum grain and flour

In the present study, mycological examination of 240 sorghum samples revealed the occurrence of 14 fungal genera such as *Fusarium, Aspergillus, Curvularia, Alternaria, Penicillium, Chaetomium, Phoma, Cladosporium, Nigrospora, Epicoccum, Verticillium, Trichoderma, Fusidium* and *Mucor*. Among the isolated genera, the most dominant were *Fusarium* (frequency,75.2% and relative percentage, 32.7%) and *Aspergillus* (frequency, 69.6% and relative percentage, 36.82%), with variation in their occurrence and abundance in sorghum grains and flour (Fig. 5). In the sorghum grains samples, *Fusarium* was the most common genera with a frequency of 90.8% and a relative percentage of 43.3%, followed by *Aspergillus* genera with a frequency of 51.7% and a relative percentage of 17.6%. While in sorghum flour, *Aspergillus* was the most dominant fungal genera with 87.5% frequency and 54.4% relative percentage, followed by *Fusarium* genera with 60.8% frequency and 23.1% relative percentage. The other fungal genera such as

Trichoderma, *Alternaria*, *Curvularia*, *Penicillium* and others were also isolated with different levels of frequency and relative percentages.

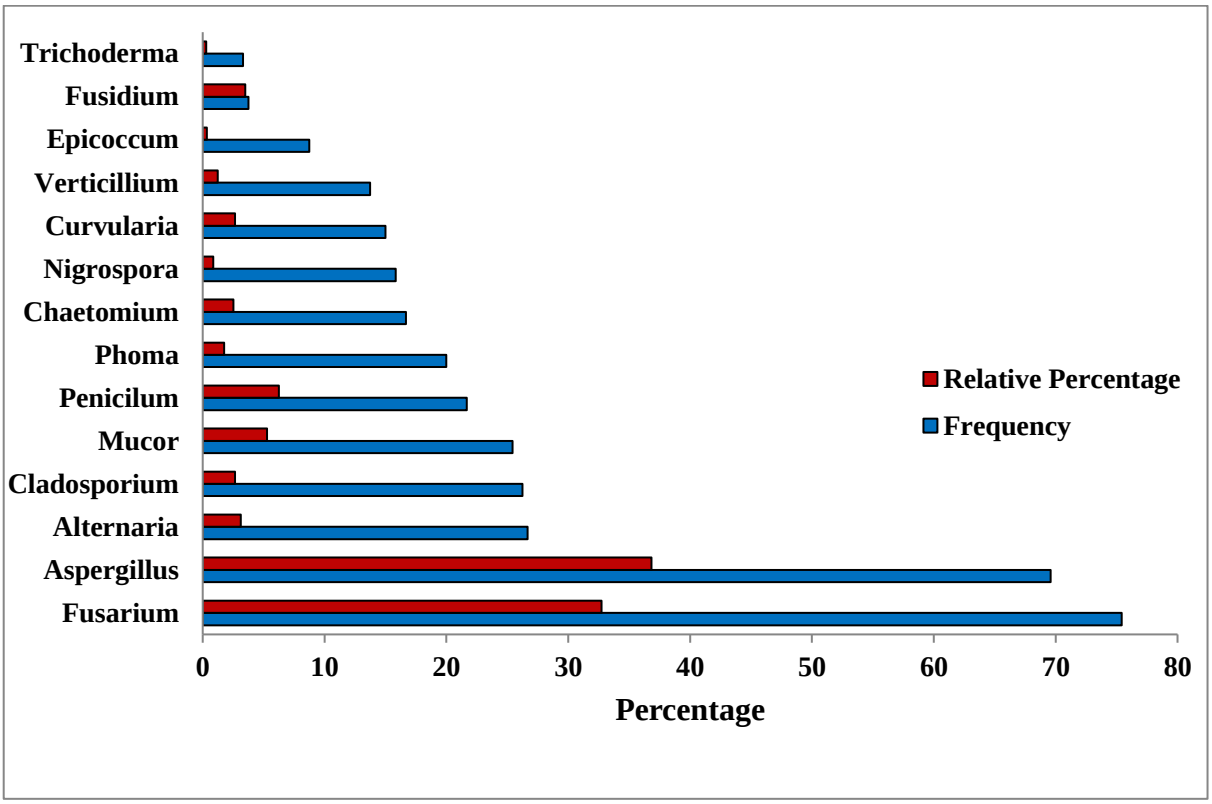

Figure 5: Fungal genera isolated from sorghum samples collected from Kerio Valley of Elgeyo Marakwet County, Kenya.

Considering the abundance of *Fusarium* and *Aspergillus* genera in the samples analysed and their association with aflatoxin and fumonisin production (Machio, 2016), their isolates were identified up to the species level. High frequencies of *A. flavus* (28.3%) and *A.parasiticus* (23.3%) were recorded from sorghum grains samples while *A. fumigatus* (56.7%) and *A. flavus* (41.7%) in sorghum flour. For *Fusarium* species, *F. proliferatum* and *F.verticilloide* were the most dominant with occurrence frequencies of 69.2%, 34.2% in grains and 38.3% and 24.2% in flour (Table 14 and 15) respectively.

Table 14: *Aspergillus* and *Fusarium* species isolated from sorghum grain samples

No	Name of the fungus	Total number of isolates	Frequency (%)	Relative percentage
1.	*Aspergillus niger*	39	14.2	4.61
2.	*Aspergillus flavus*	74	28.3	8.74
3.	*Aspergillus parasiticus*	82	23.3	9.69
4.	*Aspergillus fumigatus*	8	4.2	0.95
5.	*Fusarium proliferatum*	292	69.2	34.52
6.	*Fusarium verticilloides*	91	34.2	10.76
7.	*Fusarium solani*	51	33.3	6.03
8.	*Fusarium oxysporum*	58	20.0	6.86
9.	*Fusarium keratiti*	9	0.8	0.01

Table 15: *Aspergillus* and *Fusarium* species isolated from sorghum flour samples

No	Name of the fungus	Total number of isolates	Frequency (%)	Relative percentage
1.	*Aspergillus parasiticus*	106	32.5	10.2
2.	*Aspergillus flavus*	153	41.7	14.7
3.	*Aspergillus niger*	55	15	5.3
4.	*Aspergillus fumigatus*	281	56.7	27.0
5.	*Aspergillus lentulu*	6	4.2	0.6
6.	*Aspergillus tereus*	46	15.8	4.4
7.	*Aspergillus tamari*	19	8.3	1.8
8.	*Aspergillus candidus*	25	5.8	2.4
9.	*Fusarium proliferatum*	144	38.3	13.8
10.	*Fusarium verticilloides*	47	24.2	2.6
11.	*Fusarium solani*	68	11.7	6.5
12.	*Fusarium oxysporum*	3	16.7	5.0
13.	*Fusarium keratitis*	52	2.5	0.3

4.7.2 Distribution of aflatoxin and fumonisin contamination in sorghum grains and flour across the three wards in Kerio valley

4.7.2.1 Occurrence of aflatoxin in sorghum grains and flour

For the sorghum grain and flour samples analysed, 98.3% and 100% had detectable levels of aflatoxin respectively. The aflatoxin levels ranged from 0-119.91 ppb and 2.70- 89.36 ppb in sorghum grains and flour respectively. Across the three wards, a significant difference in total aflatoxin levels in sorghum grain was observed ($F=7.28$, $p=0.002$). Endo ward recorded the highest aflatoxin mean concentration of 42.21±21.26 ppb followed by Emsoo (41.95±32.77 ppb) and Arror (17.42±12.11 ppb). Further, there was a difference in mean total aflatoxin levels in flour samples among the wards ($F=6.25$, $p=0.004$), with Emsoo ward having the highest mean levels of 43.45±20.00 (Table 16). Generally, flour samples had the highest aflatoxin mean value (36.72±19.50) compared with sorghum grains (33.85±26.00).

Table 16: Total aflatoxin levels in sorghum grains and flour across the three wards of Kerio Valley

Ward	Sorghum samples (n)	Positive samples (%)	Range (ppb)	Mean ± SD (ppb)#	F	*p*-value
Arror	Grains (20)	31.7	0-38.65	17.42±12.11[a]	7.28	0.002[*]
Endo	Grains (20)	33.3	5.87-84.35	42.21±21.26 [b]		
Emsoo	Grains (20)	33.3	3.83-119.91	41.95±32.77[b]		
Total	**60**	**59 (98.3)**	**0-119.91**	**33.85±26.00**		
Arror	Flour (20)	33.3	2.70-55.28	25.17±10.24[a]	6.25	0.004[*]
Endo	Flour (20)	33.3	17.87-89.36	41.54±21.55[b]		
Emsoo	Flour (20)	33.3	8.46-87.43	43.45±20.00[b]		
Total	**60**	**100**	**2.70-89.36**	**36.72±19.50**		

[#]Data are mean ± standard deviation, means followed by the same letters are not significantly different $P<0.05$, significant by analysis of variance (ANOVA).

4.7.2.2 Occurrence of fumonisin in sorghum grains and flour

All sorghum flours had detectable levels of fumonisin with a contamination range of 0.22-27.27 ppm while 96.7% of sorghum grains had detectable levels with a maximum level of 30.65 ppm. Grains (12.90±8.07) had the highest mean value compared to sorghum flour (10.04±7.21) although the difference is not significant. Moreover, no significant difference was observed in the total fumonisin mean levels in sorghum samples across the three wards (Table 17). Although, Endo ward recorded the highest mean concentration for fumonisin levels in sorghum grains (14.00±6.74 ppm) and flour (11.68±7.62 ppm) compared with the other two wards. Overall, all wards had mean values for the aflatoxin and fumonisin above regulatory limits.

Table 17: Total fumonisin levels in sorghum grains and flour across the three wards of Kerio Valley

Ward	Sorghum samples (n)	Positive samples (%)	Range (ppm)	Mean ± SD (ppm)[#]	F	*p*-value
Arror	Grains (20)	30.1	0-25.06	11.84±8.25	0.34	0.707
Endo	Grains (20)	33.3	7.88-25.48	14.00±6.74		
Emsoo	Grains (20)	33.3	3.02-30.65	12.87±9.29		
Total	**60**	**58 (96.7)**	**0-30.65**	**12.90±8.07**		
Arror	Flour (20)	33.3	2.13-20.39	10.90±6.91	0.125	0.883
Endo	Flour (20)	33.3	2.41-27.27	11.68±7.62		
Emsoo	Flour (20)	33.3	0.22-25.02	10.55±7.42		
Total	**60**	**100%**	**0.22-27.27**	**10.04±7.21**		

[#]Data are mean ± standard deviation, $P<0.05$, significant by analysis of variance (ANOVA)

4.7.2.3 Mycotoxin knowledge and postharvest handling practices of caregivers

More than half (55%) of the caregivers from households where the sorghum grains and flour were collected had poor knowledge regarding aflatoxin and fumonisin occurrence in foods and their health effects. Regarding their postharvest and storage practices, more than three-quarters (66.7%) of the caregivers had poor practices. There was no difference ($p>0.05$) observed in caregivers' knowledge regarding aflatoxin and fumonisin contamination in foods and their postharvest practices (Table 18). Although the overall mean scores for the mycotoxins knowledge (6.7±3.1) and postharvest practices (9.40±1.9) were poor, a significant difference was observed in postharvest practices ($F=24.30$, $p=0.027$), with Arror ward having the highest means score (10.30±1.4).

Table 18: Mycotoxin knowledge and postharvest handling practices of the households

	Administrative wards									
HH knowledge and post-harvest practices	**Total (n=60)**		**Emsoo (n=20)**		**Endo (n=20)**		**Arror (n=20)**		χ^2	**P-value**
	n	%	n	%	n	%	n	%		
Knowledge									5.253	0.072
Poor	33	55.0	14	23.3	12	20.0	7	11.7		
Good	27	45.0	6	10.0	8	13.3	13	21.7		
Practices									2.850	0.241
Poor	40	66.7	13	21.7	16	26.7	11	18.3		
Good	20	33.3	7	11.7	4	6.7	9	15.0		
Knowledge means score#	6.7±3.1		5.75±2.9		6.70±2.7		7.65±3.4		36.100	0.149
Practices mean score#	9.40±1.9		8.95±2.2		8.95±1.6		10.30±1.4		24.30	0.027*

#Data are mean ± standard deviation, *P<0.05, significant by analysis of variance (ANOVA)

4.8 Effects of fermentation on aflatoxin and fumonisin levels

Aflatoxin and fumonisin levels in sorghum grain and flour were significantly ($p \leq 0.05$) reduced after fermentation. The average reduction of total aflatoxin after 72 hours of fermentation was 54.82% while fumonisin was at 48.19% (Fig 6). Although the samples were fermented under different conditions, fermentation using *Lactobacillus Plantarum* bacteria was observed to be more effective at reducing both aflatoxin (59.38%) and fumonisin (49.71%) than without (natural fermentation).

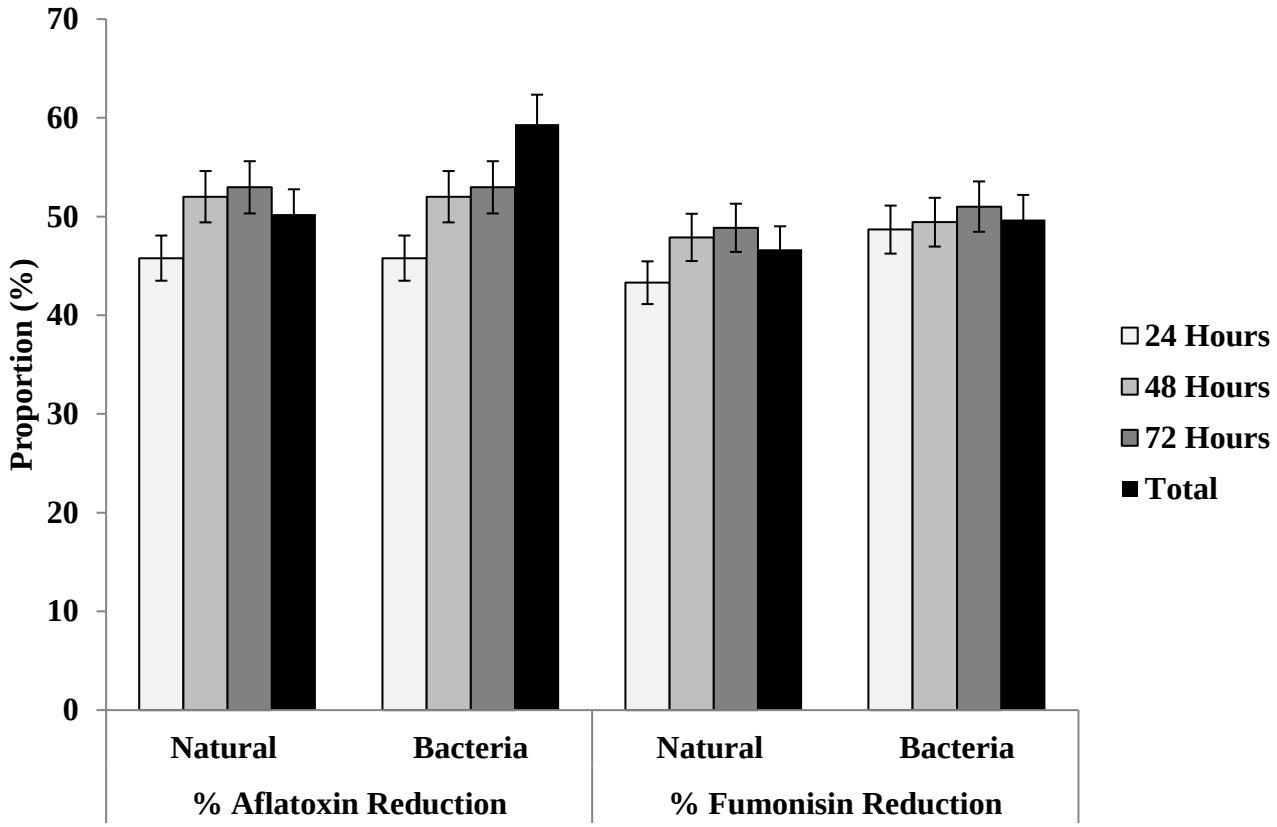

Figure 6: Effect of fermentation time with and without *Lactobacillus Plantarum* on total aflatoxin and fumonisin in sorghum

Additionally, a significant reduction in aflatoxin levels occurred in flour samples at 57.70% compared with grain samples (51.93%) (F=9.706, *p*=0.004). Fermentation with *Lactobacillus Plantarum* contributed to a significant (F=24.287, p=0.001) reduction in aflatoxin in sorghum samples compared with natural fermentation. Largely, fermentation time (duration) influenced the percentage of aflatoxin reduction with 72 hrs of fermentation resulting in a high reduction, which was significantly different from 24 and 48hrs respectively (F= 3.690, *p*=0.037) (Table 19).

Table 19: Percent reduction of total aflatoxins after 24-72 hours of fermentation

	% Aflatoxin reduction in flour		% Aflatoxin reduction in grains	
Duration of fermentation	**Natural fermentation[#]**	**Lactobacillus fermentation[#]**	**Natural fermentation[#]**	**Lactobacillus fermentation[#]**
24 hours	45.79±4.18	61.06±7.65	45.80±4.18	52.88±3.86
48 hours	53.67±2.64	64.61±7.26	50.34±9.25	54.31±2.54
72 hours	54.80±3.01	66.26±7.33	51.13±8.77	57.14±2.55
Total	51.42±5.14	63.98±6.82	49.09±7.16	54.78±3.24

[#]Data are mean ± standard deviation, *P<0.05, significant by analysis of variance (ANOVA).

On the hand, a higher reduction in fumonisin levels was observed in grain (55.78%) samples than in flour samples (40.61%) (F=364.029, p<0.001). An increase in fermentation time was also associated (F=8.542, p=0.001) with a greater reduction in fumonisin levels in sorghum grain and flour samples. Just like on the aflatoxins levels, fermentation with *Lactobacillus Plantarum* resulted in a greater reduction in fumonisins levels in both grains and flour samples (F=14.472, p=0.001) (Table 20).

Table 20: Percent reduction of total fumonisin after 24-72 hours of fermentation

	% Fumonisin reduction in flour		% Fumonisin reduction in grains	
Duration of fermentation	**Natural fermentation#**	**Lactobacillus fermentation#**	**Natural fermentation#**	**Lactobacillus fermentation#**
24 hours	35.00±3.89	40.55±2.54	51.59±2.29	56.81±0.81
48 hours	40.19±2.40	41.79±2.46	55.60±2.30	57.07±0.56
72 hours	41.70±2.29	44.40±3.86	56.02±2.04	56.62±0.69
Total	38.96±3.97	42.25±3.12	54.40±2.86	57.17±0.69

[#]Data are mean ± standard deviation, *P<0.05, significant by analysis of variance (ANOVA)

4.9 Focus group discussion themes and sub-themes

Table 21 shows the themes and sub-themes that emerged during focus group discussions. Majority of the respondents, caregivers of children aged 6-23 months, mill sorghum grains, and mix sorghum flour with other flour. Although the respondents could relate aflatoxin, fumonisin, or other toxins exposure to some health effects such as death, diarrhoea, etc., their contamination was not their concern during complementary foods preparation. This is because they never knew that toxins-producing fungi could also infect sorghum. Only 3 out of 21 caregivers interviewed attended IYCF training.

Table 21: Focus group discussion themes and sub-themes

Theme	Sub-theme	Notes
Grains processing into flour	Grains Milling	• A good number of caregivers take grains to *posho* mills for milling whereas some still use traditional stones and/or tree trunks for grain milling. • These traditional mortar and pestle are not frequently cleaned.
	Mixing flour with other flours	• Ninety-five percent of the FGD participants mix sorghum flour with other flour such as millet, maize,*Omena*, groundnuts, and cassava. This was to increase the flour quantity and make it soft and more nutritious.
	Flour storage	• Flour was mainly stored using tins, plastic buckets,and sacks. It was believed that leaving flour in an open tin or bucket cause toxins contamination. • Majority of the FGD participants store flour for less than a week before it is depleted. It loses taste if it stays for a long.
	Sorghum flour fermentation	• Was not practiced because the community believes it is not good for children under 2 years. Porridge from fermented flour causes stomach upset.
Aflatoxin and/or fumonisin contamination	Particularly in sorghum	• A few of the reports heard about aflatoxin contamination in Maize. They thought sorghum could not be contaminated by either aflatoxin or fumonisin. Thus, toxins contamination was not their concern when preparing child porridge.
	Aflatoxin and /or fumonisin health effects	• Causes death, growth reduction, vomiting, stomach upset and diarrhoea
IYCF trainings	If mycotoxins contamination was part of these trainings	• Three caregivers from Endo ward attended a training where they were trained on food preparation and aflatoxin contamination was mentioned particularly in maize.

CHAPTER FIVE
DISCUSSION

5.1 Socio-demographic and economic status of the study participants

Socio-demographic and economic factors such as age, education level, marital status and income can influence the adoption of better post-harvest handling and storage practices and mycotoxin awareness (Ayo *et al*., 2018). In the present study, the education level, age and monthly income of the farmers had a significant impact on awareness of aflatoxin and fumonisin and postharvest handling and storage practices of sorghum (Tables 12 and 13). Women play a key role in post-harvest storage of crops, food processing and preparation as well as caring for and feeding infants and young children, who are most biologically vulnerable to mycotoxins (Waithanji & Grace, 2014). They also contribute between 42-65% of the agricultural labour force in Kenya (Ahearn & Tempelman, 2010); therefore, since women can influence the household diet quality and children's nutritional status, nearly all respondents in this study were women. In this study, more than two-thirds of the participants were above the age of 30 years. Notably, caregivers with ages greater than 30 years were more knowledgeable about aflatoxin and other toxins and had better post-harvest management compared to younger farmers (below 30 years). This demonstrates the use and value of information on the management of mycotoxins gained by farmers as they age (Seetha *et al*., 2017).

The education level of the caregivers was different across the three wards with majority of them (45%) having attended at least a primary level of education, which is considered a basic level of education in Kenya. Endo ward had the highest number of caregivers who did not attend school at all, which could be due to rampant insecurity in the area. Education levels observed in this study, however, differ from the Elgeyo Marakwet county education statistics where 28.6% of the women of reproductive age had primary level (KDHS, 2015). The difference could be due to the method of categorizing education levels. In the current study, the education level was classified into four categories; none, primary, secondary and tertiary while in the Kenya Demographic and Health Survey (KDHS) it was grouped into six groups; no formal education, some primary education, completed primary education, some secondary education, completed secondary education and more than secondary education (KDHS, 2015). Education level is crucial as it increases individual general knowledge and awareness and is believed to influence the positive health-seeking behaviour of a person and community in general (Malusha *et al*., 2015). For example, according to Apuleni and colleagues (2021), mothers who attended at least a primary level of education had better health-seeking behaviours and sought appropriate treatment for their children when sick compared to those that did not have primary education.

Mycotoxin control and prevention have cost implications in terms of the adoption of modern postharvest aflatoxin control technologies such as shelling machines, drying materials, hermetic bags, etc hence household income is an important factor to be considered. In this study, more than a third of the households earned a monthly income of less than Ksh. 5,000 implying that most of them survived on less than a dollar per day (Table 4). Overall, Elgeyo Marakwet County has 57% of residents living below the poverty line compared to the national poverty level of 46% (Elgeyo Marakwet County, 2018). While at the Escarpment and Kerio Valley, 67% of the population was living below the poverty line. Low household income could contribute to high food insecurity, food scarcity and undiversified diets (Leroy *et al*., 2015), thus influencing the quality of food consumed in the household. Further, high poverty levels especially along Kerio Valley might have exacerbated malnutrition rates (stunting, 67% and wasting, 9%) among children under five years in food-insecure households as reported by Kipyego and Mugalavai (2019) by predisposing them to consumption of mycotoxin-contaminated foods. For instance, Emsoo ward recorded the highest number of caregivers earning less than Ksh 5000 per month and some of them highlighted food shortage as a reason for consuming mouldy foodstuffs (Table 9).

5.2 Aflatoxin and fumonisin awareness among young children's caregivers

The current study demonstrated that majority of children's caregivers had poor knowledge about aflatoxin and fumonisin contamination of sorghum-based foods. This knowledge gap stands as a great threat to the acquisition and utilization of safe food. For instance, during focus group discussions (FGDs), caregivers revealed that mycotoxin contamination was not their concern when preparing sorghum-based complementary foods.

''*We have not heard of aflatoxin nor fumonisin in sorghum and it is not our concern when we prepare a child's food. We prepare these foods as usual because we never thought sorghum could contain such poisonous substances. Some of us have only heard of aflatoxin contamination in maize.''* (Female FGD participant, Endo Ward)

A larger proportion of the caregivers thought that aflatoxin and fumonisin solely affect maize, another major staple crop in Kenya. These findings corroborate several other studies that have reported that large rural populations in Kenya are only knowledgeable about the aflatoxin contamination of maize-based foods (Njeru *et al*., 2019; Obonyo & Salano, 2018; Walker & Davies, 2013). Aflatoxin in maize may have received considerable attention in Kenya because of severe aflatoxin contamination of maize that has caused human deaths repeatedly over the years (Probst *et al*., 2011). Comparable to the findings reported by Njeru *et al*. (2019), majority of caregivers in this study were not familiar with fumonisins. This could be explained by the current national efforts that are solely focused on aflatoxins, a situation that is similar in most African countries (Matumba *et al*., 2016). This indicates that low awareness campaigns on other mycotoxins and

susceptible food crops may continue to pose health and nutrition threats. For instance, oesophageal cancer a condition that is a major health problem in Western Kenya (Parker *et al*., 2010) has been associated with fumonisin consumption. Njeru *et al*. (2019) and Sirma *et al*. (2015) have reported a high prevalence of fumonisin contamination in staple food in this region, which might have contributed to high cases of oesophageal cancer.

Sorting and removing visibly defective panicles and grains is an important critical control point for reducing mycotoxin contamination in farm produce. However, visible grain mould may indicate mycotoxins presence, but still very high levels are possible without any noticeable effect on appearance or smell (Ayo *et al*., 2018). In the current study, caregivers suspected the presence of fungal toxins in sorghum if the grains are discoloured, have off smell, or bitter taste and they used these features during panicles or grains sorting (Table 9). The spoilt sorghum however still ends up in the food chain for majority of caregivers because they mainly used it as animal feed and/or for human consumption, a practice that was notable in Emsoo ward. Lack of knowledge on the health effects associated with mycotoxins (Mboya & Kolanisi, 2014) and food shortage are among the contributing factors to the use of spoilt grains for human consumption and as animals feed (Anitha *et al*., 2019; Matumba *et al*., 2016). The study participants showed a general lack of awareness of the health effects associated with mycotoxins on humans (Table 5). Notably, like their counterparts from Tanzania (Ngoma, 2016), a large number of children's caregivers in the present study associated mycotoxins consumption mainly with acute health effects (abdominal pain and diarrhoea). As such, majority of them could not link chronic health effects such as stunting with aflatoxins or any other mycotoxin possibly because these are long-term effects.

Awareness of mycotoxin is critical to its management because such information forms a basis for initiating and sustaining measures to control their exposure and associated health and economic implications (Falade, 2019). Educating farmers on the causes of mycotoxins contamination in foods and their associated risks using available channels of communication is necessary for behavioural changes. In the present study, majority of the caregivers who had heard about aflatoxin and/or fumonisin obtained information from mass media such as local radio (KASS FM) (Table 6). This means that mass media was convenient and a preferred source of disseminating information. Previous studies in Kenya also reported media as a key source of information about mycotoxins (Malusha *et al*., 2015; Walker & Davies, 2013). Extension officers also played a key role in creating awareness about mycotoxins despite their shortage in the field, as their ratio to the farmers in the country is about 1:5000. Unfortunately, events like the spread of the COVID-19- global pandemic greatly reduced the ability of these officers to reach out to many farmers. This is due to the suspension of social gatherings and the government's recommendation that people work from their homes. It was revealed during the survey that the limitation in movement and social gathering significantly reduced

the services of agriculture extension in the area. Across the country, the pandemic has greatly reduced the services of agricultural officers, the interaction among farmers, and the visits to government offices to seek agricultural information (Munyua, 2020). Cattle rustling is another impediment to the role of the extension workers and other community development partners in raising food safety and security in Kerio Valley, especially in Endo ward. The resurgence of cattle rustling has led to the loss of lives and property, displacement of people, closing of businesses, poverty and defying interventions from the local community, civil society and the government (Murkomen, 2011).

In addition, poor knowledge about aflatoxin and fumonisin observed in this study could be attributed to the low number of mycotoxins awareness campaigns in Kerio valley. Because a very small proportion of the participants have been trained on issues concerning mycotoxins contamination of complementary foods (Table 11). Furthermore, more than three-quarters of caregivers in the current study had not attended any training on infant and young children complementary feeding (IYCF); this proportion is larger compared to their counterparts from Tanzania (Katengesya, 2018). A lower number of such trainings among caregivers in this study could have contributed to their low knowledge of mycotoxins and eventually increase the risk of producing and feeding young children with contaminated complementary foods. For instance, mixing sorghum flour for preparing a child's food with more than two other flours such as maize, millet, groundnuts, or cassava was a common practice in the study area (Table 21). Apart from this practice interfering with the absorption of important nutrients, it could also increase children's exposure to several mycotoxins (Ojuri *et al*., 2018). Likewise, it was revealed during FGDs that traditional tools that were used for grain threshing and grinding were not cleaned between each batch of grains. One FGD participant for instance explained that the hollow tree trunk (*wero konee*) which is used as mortar for threshing is not washed, as it would crack. This indicates that caregivers of young children do not have adequate knowledge of the risks of cross-contamination of foods.

''We do not clean mortar which is made of the tree trunk as it will crack'. In addition, we do not clean the drying materials, threshing and grinding stones as our mothers and grandmothers never used to clean them.'' (Female FGD participant, Endoo Ward)

5.3 Post-harvest handling and storage practices of sorghum

Poor harvesting and post-harvest handling practices are the major factors that promote mycotoxin contamination (Magembe *et al*., 2016). In the current study, the caregivers had inadequate knowledge regarding the contribution of these practices to mycotoxin production in grains (Fig 3). For example, most of the caregivers both in FGDs and household surveys pointed out inadequate drying and poor storage as the main factors for the development of moulds and production of mycotoxins in foods although other critical factors like length of storage contribute significantly (Gnonlonfin *et al*., 2013). While a few of the

farmers allowed their products to dry in the field, majority of them sun-dried sorghum on the bare ground due to the cost of drying materials.

"*Well, for the white sorghum variety (KARI Mtama 1) we usually harvest before it is fully dried as it is more prone to attack by birds than the red variety. So, we must dry it after harvest.*" (Female household survey participant, Arror ward).

Interestingly, to confirm grain dryness before storage, all farmers used traditional methods such as biting, touch/squeezing and a combination of visualization and sound tests (Table 8). Such traditional practices are not accurate and might lead to the storage of grains that have high residual moisture content (Hell *et al*., 2008). In the current study, nearly all caregivers threshed/shelled sorghum using manual methods (beating sorghum panicles in a sack or using a traditional pestle (stone) and mortar (hollow tree trunk) (Appendix VIII), with some of them threshing sorghum on bare ground. Manual threshing exposes grains to fungi through kernel breakage and eventually, mycotoxin production (Taye *et al*., 2018).

The proper storage of dried grains in a clean and dry environment as well as controlling pests by use of insecticides and hermetic bags can reduce the build-up of mycotoxins in the store (Abass *et al*., 2018). In the present study, more than half of the caregivers from Endo and Arror wards used hermetic bags to store sorghum grains attributable to the intervention by different community development programmes such as the Accelerated Value Chain Development-Drought Tolerant Crops project (AVCD-DT). Some caregivers, however, highlighted placing sorghum bundles directly on the floor. Worse still, a good number of them placed the storage bags directly on the floor, which may increase fungal growth in stored food commodities due to moisture absorption (Suleiman *et al*., 2018). Storage structures also play an important role in determining the quality of stored food. Traditional storage structures, such as traditional granaries and living rooms increase the colonization of grains by mycotoxins due to rodent attacks, insect damage, high temperatures and humidity (Maina *et al*., 2016). In this study, over two-thirds of caregivers stored sorghum grains in traditional granaries and dwelling houses (Table 10). Noteworthy, the material for storing flour is of critical importance to maintain the quality of the flour (Hemery *et al*., 2020). In the current study, most of the caregivers stored the flour for preparing children's porridge in plastic buckets, which are known to retain heat and moisture and easily promote fungal growth and mycotoxin contamination (Mutegi *et al*., 2013). Sacco *et al*. (2020) and Selemani (2018) suggested the use of paper bags as a suitable packaging material for flour as they found lower fungal count in flour stored in these materials compared with flour stored in plastic buckets and low-density polyethylene bags.

5.4 Determinants of young children caregivers' knowledge about mycotoxin contamination and their post-harvest practices

A remarkable finding from the study is that the caregivers above 30 years were four times more likely to be aware of aflatoxin and/or fumonisin and had better post-harvest practices compared to those below the age of 30 years. (Table 12). It is possible that caregivers above the age of 30 years have traditional knowledge or may have learned about these toxins through local mass media during aflatoxin outbreaks in Eastern Kenya. For instance, one of the FGD respondents mentioned having heard about aflatoxin from "the Machakos outbreak" a part of the Eastern Kenya hotspot (Obonyo & Salano, 2018).

''*I have not heard of fumonisin but I have heard about aflatoxin in maize from radio during the Machakos outbreak (2004-2005).*'' (Female FGD participant, Emsoo Ward)

The experience caregivers gained over time could also improve their knowledge of mycotoxins and their management practices (Udomkun *et al*., 2018). Remarkably, caregivers who attended a formal system of education were less likely to know about mycotoxins or to have good post-harvest handling practices. This may imply that knowledge about mycotoxins and management practices is more of a transferrable skill (Gichohi-Wainaina *et al*., 2021) than what is learned in school or formal trainings. In the study, very few caregivers obtained information about mycotoxins through reading despite some level of literacy reported which probably indicates the scarcity of written resources, low reading motivation on the side of farmers, or else the materials are too technical for them to understand (Logrieco *et al*., 2018). Hence, the findings suggest the need to possibly include topics on mycotoxin and food safety in the available infant and young child feeding guidelines as well as in the primary and secondary school curricula as mycotoxin issues are not explicitly covered in the Kenyan curricula, primary and secondary school levels. However, the current study finding on the education level contradicts previous studies that reported that the literate population had more knowledge on aflatoxin and other mycotoxins than those who have not attended school (Magembe *et al*., 2016; Matumba *et al*., 2016; Udomkun *et al*., 2018). Therefore, more studies are needed to better inform social behaviour change communication strategies targeted at mitigating mycotoxin contamination of complementary foods.

Another important determinant of knowledge about mycotoxin contamination of foods was the marital status of the caregiver. Similar to findings reported by Magembe *et al*. (2016), the current study clearly showed that caregivers who are married and living together with their spouse were more likely to be aware of aflatoxin and/or fumonisin than those who are not (single, separated, or widowed). Living together with a spouse might help in sharing information on mycotoxin issues and their management thus contributing to better mycotoxin knowledge and management. Income is also one of the most salient factors that influence farmers' perception and awareness of mycotoxins (Redzwan *et al*., 2012). In this study, a significant

relationship was observed between household monthly income and knowledge about mycotoxins and post-harvest handling practices of sorghum. This study's findings revealed that caregivers with better monthly incomes were more knowledgeable about mycotoxins and had better post-harvest practices than those with lower incomes. The discussions with the caregivers suggested the reluctance of sorghum farmers to invest their limited income to learn about or pay for aflatoxin or fumonisin control. This implies that caregivers with less monthly income are less likely to purchase non-food items such as drying materials, hermetic bags, or radios that will enhance good post-harvest practices and mycotoxins awareness. Additionally, people with high incomes are more likely to be careful about food and willing to pay for food safety than those with lower incomes (Redzwan *et al*., 2012). This suggests that poverty and lack of sufficient income might contribute to low levels of awareness and knowledge about mycotoxin, leading to high mycotoxin exposure (Leroy *et al*., 2015) through poor food handling and preparation.

5.5 Aflatoxin and fumonisin contamination of sorghum grains and flour

The current study findings showed that sorghum grains and flour allocated for household consumption were contaminated by high levels of aflatoxin and fumonisin. Aflatoxin and fumonisin-producing fungi may occur in certain food products in form of spores and thus when conditions are favourable, the fungi germinate and may produce these toxins in varying amounts (Ukwuru & Ohaegbu, 2017). Among different fungi isolated from sorghum samples in this study, *Aspergillus* and *Fusarium* were the most dominant genera with *Aspergillus* being the most dominant in flour samples and *Fusarium* in grains samples. The difference in their occurrence could be attributed to variations in the post-harvest handling and storage practices of sorghum grains and flour by the caregivers. Grain milling facilities, for instance, could redistribute fungal spores that are initially concentrated in the outer layer of the grain into the final product (flour) (Schaarschmidt & Fauhl-Hassek, 2018) and/or introduce other new different fungal species into the flours due to a lack of cleaning or sterilization after every batch of grains milled. Similar to EL-Sisy *et al*. (2019), in this study, other new *Aspergillus* species were isolated in sorghum flour (Table 15) compared with those isolated in sorghum grains (Table 14). The difference in storage material and conditions might have also favoured the growth of a certain type of fungi while suppressing the growth of others (Birgen *et al*., 2020). Current study mycological examination results agree with those of Machio (2016) and Ackerman *et al*. (2021), who reported dominancy of *Aspergillus* and *Fusarium* genera especially *A. flavus*, *A. parasiticus* and *F. proliferatum* and *F.verticilloide* species in sorghum. It is of interest to note that not all the species within a given genus are capable of producing toxins. However, the abundance of aflatoxin and fumonisin-producing species such as *A. flavus, A. paraciticus*, *F. proliferatum* and *verticilloides* respectively (Machio, 2016), in this study implies the risk of aflatoxin and fumonisin contamination. Data on the frequency and relative percentage of fungi are of great significance in predicting the extent of postharvest infection,

colonization and subsequent mycotoxins contamination in foods (Osman *et al*., 2017). Therefore, for food safety purposes, correct fungal species identification is of high importance (Probst *et al*., 2014), as different species may have different mycotoxin profiles and physiology.

Furthermore, this study has shown the co-occurrence of aflatoxin and fumonisin in sorghum grain and flour. The co-occurrence of these toxins was observed almost in all sorghum samples analysed; however, flour samples had the highest mean concentration levels for aflatoxin than sorghum grains (Tables 16 and 17). The low mean value for total aflatoxin levels in sorghum grain can be attributed to post-harvest handling and storage practices. For example, to obtain flour from grains many farmers in rural areas use local *posho* mills or traditional milling tools that are likely to contribute to cross-contamination of the final products due to the residual flour in the mills or traditional milling tools (mortar and pestle or stones) (Ntuli *et al*., 2013). This type of cross-contamination is likely to occur in Kerio Valley as a large number of caregivers reported during FDGs that they use a traditional pestle and mortar (not cleaned) to grind sorghum grains to obtain flour. A similar scenario was also observed in Ethiopia where majority of young children's caregivers/mothers do not clean grain-milling facilities between each batch of grains (Beyene *et al*., 2016). As such, in addition to storing flour in plastic buckets, the lack of proper cleaning of shelling and grinding/milling facilities could be another reason for the high levels of aflatoxin recorded in sorghum flour in this study. Comparable to our study finding, other studies reported high aflatoxin levels above regulation limits in sorghum flour related to poor postharvest handling and storage practices (Kihara, 2015; Makori *et al*., 2019). Nonetheless, aflatoxin levels recorded in sorghum grains in the current study were comparable to levels reported in Nandi County. In 2015, Sirma et al. reported high aflatoxin levels in a good number of sorghum samples from Nandi County although their total aflatoxin overall means (26.0 ppb) were lower compared with the aflatoxin mean levels reported in this study.

Fumonisin levels varied between sorghum grains and flour. Sorghum flour recorded a low mean value for total fumonisin compared with grains. This observation could be associated with grain sorting as a large proportion of children's caregivers in the study reported sorting sorghum grains before milling (Table 9), a practice that was shown to reduce significantly fumonisin levels compared to aflatoxin (Mutegi *et al*., 2013). There was a low correlation between apparent mouldiness and aflatoxin contamination and consequently, visual sorting may not be sufficient in reducing it as with fumonisin (Westhuizen *et al*., 2011). This kind of observation is important in identifying critical control points when developing strategies for controlling mycotoxins contamination and exposure in cereal-based foods. Overall, based on the result of the current study, the mean concentrations of total aflatoxin and fumonisin in both sorghum grains and flour are higher

than the Kenya limits of 10 (ppb) and European Union legislation regulation limit of 1 (ppm) for aflatoxins and fumonisin respectively.

High aflatoxin and fumonisin levels in sorghum samples from Kerio valley might be attributed to poor mycotoxins knowledge and poor postharvest handling and storage practices among caregivers that were observed during sample collections. This finding implies that poor mycotoxin knowledge/awareness leads to poor post-harvest practices, which in turn contribute to mycotoxin production in susceptible food commodities (Makun, 2013). Although mycotoxin awareness and postharvest handling and storage practices scores varied across the wards, however, Arror ward had better mycotoxins awareness levels and post-harvest harvest practices compared to Endo and Emsoo wards (Fig. 2). This might have contributed to the low mean total aflatoxin and fumonisin levels recorded in sorghum grains and flour collected from Arror ward. Overall, in this study, all caregivers used traditional methods such as biting, touch/squeezing, and a combination of visualization and sound tests to confirm grains' dryness before storage. Moreover, drying and shelling of the sorghum grains on bare grounds were common practices observed in the study (Table 8). These practices are associated with grains' fungal contamination (Kamala *et al*., 2016). To complicate the situation, majority of the caregivers in the study area store sorghum grains in traditional granaries or living rooms that lack proper airflow capacity or with leaking roofs and most of them place their storage bags directly on the floor. Such poor storage practices contribute to increased mycotoxins contamination in foods, especially when coupled with hot and dry climatic conditions like in Kerio Valley (Taye *et al*., 2018; Walker *et al*., 2018). Additionally, low household income could be another important contributor to the high mycotoxins levels recorded in this study. It was evident that caregivers with low resources chose not to invest their time, energy and resources in post-harvest technologies such as drying materials, hermetic bags and other items that would minimize mycotoxins contamination in food. Some of the caregivers reported using spoilt (mouldy) grains for human consumption due to food shortages (Table 9). This implies that the poorest families are at the greatest health risk from food-borne toxins due to food shortage and/ or the cost associated with mycotoxins prevention and control methods. Therefore, the need for other cheap and practical alternative approaches such as the use of fermentation to reduce the risk of mycotoxins exposure.

5.6 Effects of fermentation on aflatoxin and fumonisin levels in sorghum grain and flour

Processing of cereals by fermentation has been shown to reduce aflatoxin and fumonisin levels significantly (Adebo *et al*., 2018; Mukandungutse *et al*., 2019), however, few caregivers in Kerio Valley (Table 10) adopted this practice. It was revealed during FGDs that caregivers do not give fermented foods to children below the age of 2 years, as they believe it will cause stomach upset (Table 21). The use of *Lactobacillus Plantarum* and natural fermentation in this study showed promising potential as both methods resulted in the reduction of aflatoxin and fumonisin levels in sorghum grains and flour to acceptable levels (Fig. 6).

Overall, a greater reduction in aflatoxin levels was recorded compared to fumonisin. This observation may due to the difference in the chemical structures between aflatoxin and fumonisin, which could have influenced their interaction mechanisms with the metabolites produced during fermentation. A comparison of toxins reduction between sorghum samples showed lesser aflatoxin reduction levels in sorghum grains but a higher reduction in fumonisin concentration compared to flour samples (Tables 19 and 20). The initial toxins concentration and microbial composition of the sample might have influenced the extent of mycotoxin reduction. The microbial composition of the sample influences the type of microbial metabolites to be produced during fermentation and their interactions with the toxins (Adebo *et al*., 2019). In this study, a greater reduction in aflatoxin and fumonisin levels was observed with an increase in fermentation time, which corroborates other studies' findings (Mukandungutse *et al*., 2019). This trend can be attributed to an increase in the production of lactic acid and other metabolites that are associated with mycotoxins reduction as fermentation time prolongs (Adebo *et al*., 2019).

Comparable to findings from other studies' (Adebo *et al*., 2019; Mukandungutse *et al*., 2019), this study recorded the highest mycotoxins reduction with the use of bacteria strains, which might be related to accelerated fermentation due to fermentation conditions and increased *Lactobacillus Plantarum* action compared to natural fermentation. Natural fermentation, which was carried out at room temperature to mimic conditions in an ordinary household setting, was equally able to reduce close to half levels of both aflatoxin and fumonisin in sorghum (Fig. 6). Natural microflora present in the samples and high concentrations of bioactive compounds (such as polyphenols, flavonoids and tannins) in sorghum (Adebo *et al*., 2018; 2019) may have facilitated aflatoxin and fumonisin reduction recorded in this study under natural fermentation. Generally, fermentation has been identified as an effective process to reduce aflatoxin, fumonisin and other mycotoxins due to their breakdown by endogenous enzymes and compounds secreted and released into the food matrix by the fermenting organisms (Adebo *et al*., 2018; Okeke *et al*., 2018). LAB produced during fermentation reduces levels of mycotoxins in food through; inhibition of mycotoxins production (inhibition of the mycelial growth) (Gomaa *et al*., 2019), denaturing of the cell wall proteins of mycotoxins due to acidic conditions (Guimarães *et al*., 2018), and mycotoxin elimination through cell wall binding. Therefore, fermentation being an old food processing technology, simple and affordable, farmers and caregivers of young children can use it to transform unsafe cereal-based foods into safe food for human consumption.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

This chapter presents conclusions and recommendations made from the study findings. It also gives suggestions for further research in this subject area.

6.2 Conclusions

From the study findings, the following conclusions were made:

i. The study demonstrated that caregivers of young children in Kerio valley had poor knowledge of aflatoxin and fumonisin contamination of food. Majority of them were not aware that sorghum can be contaminated by aflatoxins and/or fumonisin.

ii. A large number of caregivers in this study had suboptimal post-harvest handling and storage practices of sorghum, which are dependent on the caregiver's age, education level, monthly income and knowledge about mycotoxin contamination.

iii. Sorghum grains and flour intended for the preparation of complementary foods for infants and young children in Kerio Valley were contaminated with high levels of aflatoxin and fumonisin.

iv. Both spontaneous and use of *L. Plantarum* fermentation significantly reduced the levels of aflatoxin and fumonisin in sorghum grains and flour samples, especially after 72 hours of fermentation.

6.3 Recommendations

The recommendations from this study are:

i. Education campaigns to enlighten smallholder farmers and young caregivers of children under five years on the existence of aflatoxin, fumonisin and other toxins, their causes and negative health effects and foods that are susceptible to mycotoxins contamination including sorghum.

ii. It is practical to obtain toxin-free products from contaminated sorghum after 72 days of natural fermentation; therefore, farmers and young children caregivers' can use natural fermentation to decontaminate contaminated sorghum-based food products.

Suggestions for further research:

a. Further research is still needed to better understand which sorghum varieties (brown or white) are more vulnerable to aflatoxin and fumonisin contamination and identify stages in the sorghum value chain where greater mycotoxin contaminations occur.

b. Compare the levels of aflatoxin and fumonisin and those present in young children biochemical parameters including urine and blood.

c. Further studies may be done on the screening of mycotoxin-degrading microorganisms, especially in natural fermentation and this might lead to the detection of efficient and

applicable bacteria and/or compounds, which may be engineered to improve the quality and safety of foods, thereby protecting consumer's health.

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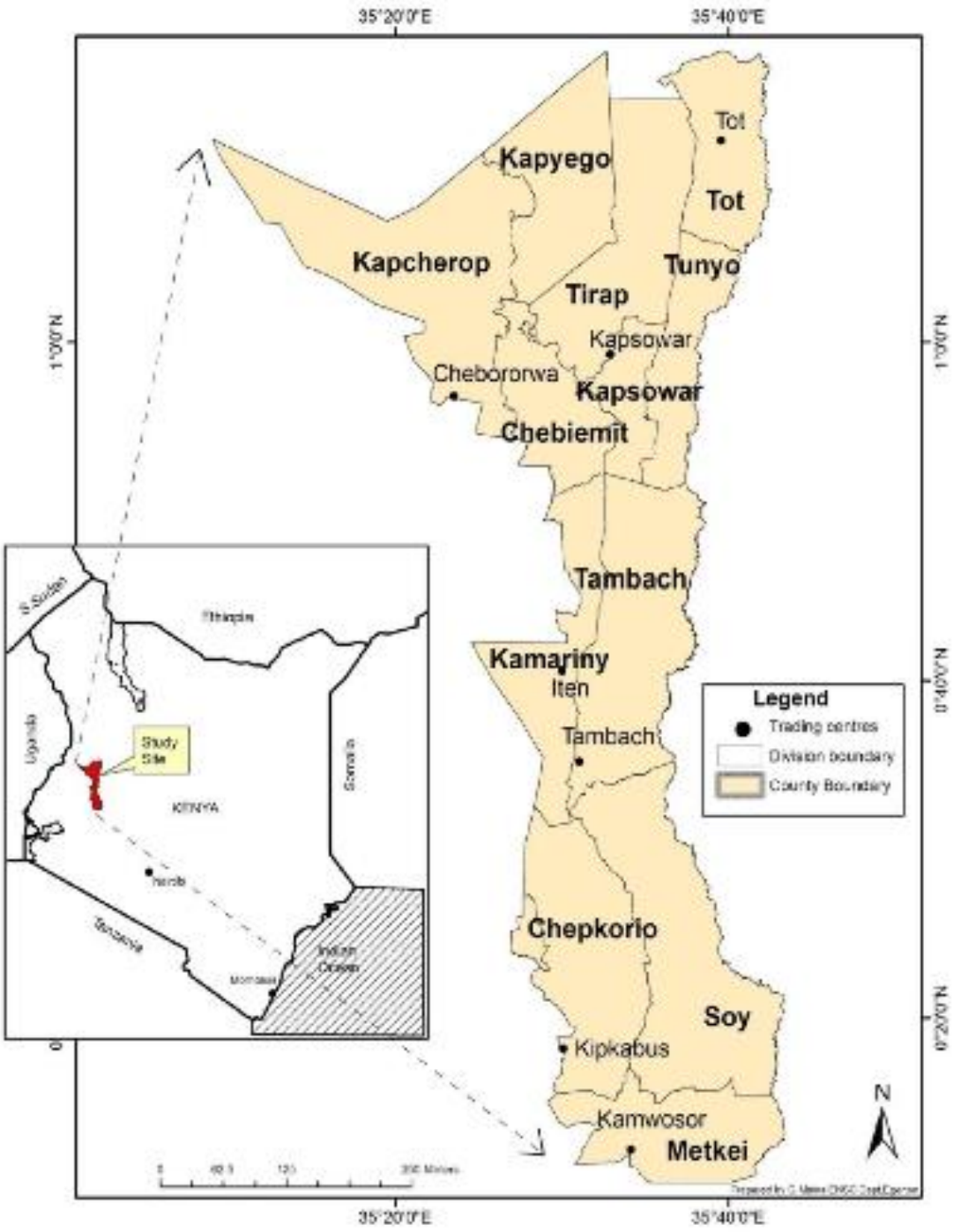
APPENDICES

Appendix I: Map of the study area



Source: World Resource Institute (2013).

Appendix II: Household questionnaire

Choices, where provided, should not be read to the respondent and *multiple choices can be selected where appropriate. Circle the most appropriate choice(s) where applicable:*

1. IDENTIFICATION		
1.1 Date of Data Collection		1.2 Household Number
1.3 Sub County	1.4 Ward	1.5 Village
1. Keiyo South ☐	1. Soy south ☐	
2. Keiyo North ☐	3. Emsoo ☐	
4. Marakwet West ☐	5. Arror ☐	
6. Marakwet East ☐	4. Endo ☐	
2. HOUSEHOLD DEMOGRAPHIC CHARACTERISTICS		
2.1 Sex of the household head		1. Male ☐ 2. Female ☐
2.2 Age of the respondent (*in complete years*)		
2.3 Sex of the respondent		1. Male ☐ 2. Female ☐
2.4 Marital status of the respondent		1. Married monogamous ☐ 2. Married polygamous ☐ 3. Widowed ☐ 4. Single ☐ 5. Separated ☐
2.5 What is the highest level of education attained by the respondent?		1. None ☐ 2. Primary ☐ 3. Secondary ☐ 4. Tertiary ☐
2.6 How many people reside in this household?		
2.7 What is the average monthly income of your household? (***In Ksh***.)		1. Less than 5000 ☐ 2. 5,000-10,000 ☐ 3. 10,000-15,000 ☐ 4.15,000-20,0000 ☐ 5. More than 20,000 ☐

3. CAREGIVERS' KNOWLEDGE OF AFLATOXIN AND **FUMONISIN CONTAMINATION**	
3.1 Have you ever heard about mycotoxins contamination in food?	1. Yes ☐ 2. No ☐
3.2 Have you ever heard of the word; Aflatoxin? Fumonisin?	 1. Yes ☐ 2.No 1. Yes ☐ 2.No
3.3 If Yes, mention foodstuffs that are likely to be contaminated by; Aflatoxin; Fumonisin;	
3.4 What were the sources of information about aflatoxin/fumonisin?	1. Seminars/experts ☐ 2. Media ☐ 3. Agricultural Extension Agents ☐ 4. Friends/neighbours ☐ 5. Reading ☐ 6. AVCD-DTC project ☐ 7. Others (specify) ☐ _____
3.5 Can you identify spoilt sorghum grains?	1. Yes ☐ 2. No ☐
3.6 If Yes, which features will you ascertain about the presence of mycotoxin contamination in sorghum grains? (***Multiple responses applicable****)*	1. Colour/discoloration ☐ 2. Smell/rotten ☐ 3. Do not know any indicator ☐ 4. Others (*specify*) ☐ _____
3.7 What causes aflatoxin and/or fumonisin contamination? (***multiple responses applicable***)	1. Poor drying ☐ 2. Poor storage ☐ 3. Insect infestation ☐ 4. High levels of rain during harvesting ☐

	5. Delayed harvesting ☐ 6. Broken and bruised grains increase the chance of contamination ☐ 8. Poor soils ☐ 9. Contaminated seeds ☐ 10. Drought stress ☐ 11. Poor filed management ☐ 12. Dampness in the store ☐ 13. Untreated grains ☐ 14. Don't know ☐ 15. Other (*specify*) ☐ _____
3.8 (a) Do you think consuming mycotoxin-contaminated food can affect your health?	1. Yes ☐ 2.No ☐ 3. Don't know ☐
3.8 (b) If yes, which health problem (s)?	
3.8 (c) Do you think aflatoxin and fumonisin contamination in food contribute to; a) Growth failure/stunting? b) Impairs immunity? c) Abdominal pain? d) Diarrhoea?	 1. Yes ☐ 2. No ☐ 3. Don't Know ☐ 1. Yes ☐ 2. No ☐ 3. Don't Know ☐ 1. Yes ☐ 2. No ☐ 3. Don't Know ☐ 1. Yes ☐ 2. No ☐ 3. Don't Know ☐
4. Caregivers' postharvest hand**ling** and **storage practices of sorghum**	
4.1 Which month did you harvest sorghum?	1. January ☐ 2. February ☐ 3. March ☐ 4. April ☐ 5. May ☐ 6. June ☐ 7. July ☐ 8. August ☐ 9. September ☐ 10.October ☐ 11. November ☐ 12. December ☐
4.2 How were the climatic conditions when you last harvested sorghum?	1. Wet ☐ 2. Dry ☐

4.3 How was the condition of the harvested sorghum grains?	1. Clean ☐ 2. Spoiled ☐ 3. Don't know ☐
4.4 Did you dry sorghum grains after harvest?	1. Yes ☐ 2.No ☐
4.5 If yes, how did you dry your sorghum?	1. Standing-crop drying in the field ☐ 2. Drying in bundles of panicles ☐ 3. Drying grains ☐ 4. Others (*specify*) _____ _
4.6 How long did the drying process take (duration in days)?	1. 7 days ☐ 2. 14 days ☐ 3. More 14 days ☐
4.7 If the sorghum grains were sun-dried, where did you dry them?	1. On bare ground ☐ 2. On canvas ☐ 3. On concrete asphalt ☐ 4. On cow-dung smeared ground ☐ 5. Other (*specify*) ☐ _____
4.8 How do you check if your grain is sufficiently dry for storage?	1. By looking at it ☐ 2. By squeezing or touching ☐ 3. By using a moisture meter ☐ 4. By biting the grain and testing hardness ☐ 5. By listening to the sound it makes when handling it ☐ 6. I do not know how to check ☐ 7. Others (specify) ☐ _____
4.9 Which method do you use for threshing sorghum?	1. Hand threshing ☐

	2. Threshing with animals or vehicles ☐ 3. Threshing with hand-driven machines ☐ 4. Threshing or shelling with motorized equipment ☐ 5. Any other method, mention ☐ _____
4.10 If hand threshed, how do you thresh your sorghum grains?	1. On bare ground ☐ 2. On canvas ☐ 3. On concrete asphalt ☐ 4. On the cow-dung-painted ground ☐
4.11 Before threshing, do you separate rotten/spoiled sorghum panicles from the good ones?	1. Yes ☐ 2. No ☐
4.12 If no, why?	1. No need ☐ 2. We don't usually separate ☐ 3. It will reduce the amount of food that will be available for us ☐ 4. It requires much time ☐ 5. Others (specify) ☐ _____
4.13 Immediately after drying and before storage of sorghum grains, are there other processes carried out?	1. Yes, there are other processes ☐ 2. No, other processes ☐
4.14 If yes, which other process (s)? (***Multiple responses applicable, probe)***	1. Grains sorting ☐ 2. Winnowing ☐ 3. Cleaning of the storage structure ☐

	4. Spraying and dusting the storage structure/grains with fumigants /pesticides ☐ 5. Packaging ☐ 6. Other (specify) ☐ _____
4.15 What criteria do you use when sorting grains? (**If sorting was mentioned. Multiple responses applicable**)	1. Colour ☐ 2. Insect infested ☐ 3. Physically damaged ☐ 4. Mouldy ☐ 5. Bad odour (rotten/soil smell) ☐
4.16 What do you do with spoilt grains?	1. Throw them away ☐ 2. Feeding livestock/ Poultry ☐ 3. Making a local brew ☐ 4. Re-dry and consume ☐ 5. Sell to the market ☐ 6. Others (*specify*) ☐ _____
4.17 Where do you store your sorghum grains? (***Observe***)	1. Traditional granary ☐ 2. Living house without improved structure ☐ 3. Living house with improved structure ☐ 4. Modern store ☐ 5. Others (specify) ☐ _____
4.18 Which kind of bag (s) do you use to store your grains? (***Observe***)	1. Polypropylene bags ☐

	2. Purdue improved crop storage (PICS) ☐ 3. Sisal bags ☐ 4. Others specify ☐ _____
4.19 How do you store your storage bags in the store? (***Observe***)	1. On the wooden pallets ☐ 2. On the floor ☐ 3. Others specify ☐
4.20 How long do you usually store sorghum grains?	1. Less than three months ☐ 2. Three months ☐ 3. Six months ☐ 4. Over six months ☐
4.21 How often do you clean your storage area?	1. Once a month ☐ 2. Once every 3 months ☐ 3. Once every 6 months ☐ 4. Once per year ☐ 5. Whenever necessary ☐ 6. Never done ☐
4.22 Which strategies do you use to prevent aflatoxins and fumonisins from contaminating your crops?	1. Crop rotation ☐ 2. Seed treatment ☐ 3. Selection of healthy seeds for planting ☐ 4. Use of pest and disease control chemicals ☐ 5. Proper drying of grains before storage ☐ 6. Proper storage ☐ 7. Proper field management ☐

4.23 What do you use to store sorghum flour? ***(Observe)***	1. Plastic Bucket ☐ 2. Polythene bags ☐ 3. Tin ☐ 4. Sack ☐ 5. Kiondo bag ☐ 6. Basket ☐ 7. Other(specify) ☐ _____
4.24 On average, how long will you use sorghum flour before the next milling?	1. Less than a week ☐ 2. One week ☐ 3. Two weeks ☐ 4. More than two weeks ☐
4.25 Have you ever attended a seminar /workshop concerning the processing and handling of complementary foods?	1. Yes ☐ 2.No ☐
4.26 If Yes, who trained you?	1. Agriculture Extension agents ☐ 2. Ministry of Health ☐ 3. AVCD-DTC project ☐ 4. NGO ☐ 4. Others (*specify*) ☐ _____
4.27 Was aflatoxin and fumonisin contamination part of that training you attended?	1. Yes ☐ 2.No ☐
4.28 (a) Do you ferment sorghum flour used for preparing children's porridge?	1. Yes ☐ 2. No ☐
4.28 (b) If yes, why?	
4.28 (c) If no, why?	

4.29 (a) Do you think it is important to ferment sorghum flour for preparing children's porridge?	1. Yes ☐ 2. No ☐
4.29 (b) If yes, why do you think it is important?	
4.29 (c) If no, why do you think it is not important?	

End of survey

Thank you for your time and cooperation

Appendix III: Focus group discussion guide

Date		Sub-county		Ward	
Number of participants		Male		Female	
Facilitator			Documenter		

Mycotoxins contamination of complementary foods		
1.	**Questions guiding the discussion**	**Response**
2.	What are the procedures involved in the processing of sorghum grains into flour?	
3.	Do you mix sorghum flour used for preparing porridge for your child with other flour? If yes, mention those other flours you use. Why do you mix?	
4.	For how long will use the flour (for preparing the child's porridge) before the next milling?	
5.	Kindly, mention all the procedures you use in preparing your child's porridge.	
6.	What is aflatoxin or fumonisin?	
7.	In which way(s) can your child be infected with mycotoxins?	
8.	What measures did you put in place to ensure that your child's food is not contaminated by aflatoxin/fumonisin or any other mycotoxin?	
9.	How do you store flour that you use in the preparation of your child's porridge?	
10.	What will be the consequences of consuming porridge contaminated by aflatoxins and fumonisins to the child?	
11.	Have you ever attended any training on complementary foods preparations and handling or IYCF?	
12.	If yes, what did you learn from the training?	

End of the discussion

Thank you for your time* and *cooperation

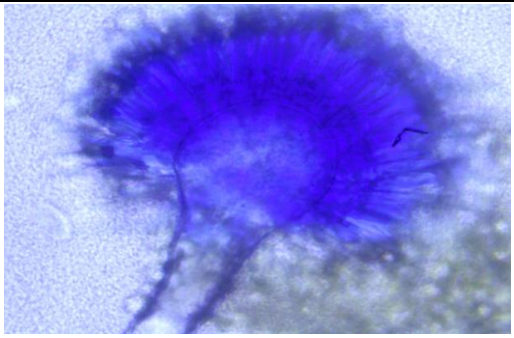
Appendix IV: Fungi isolation and microscopic identification

a) Fungus colonies isolated from sorghum grains (Front surface)

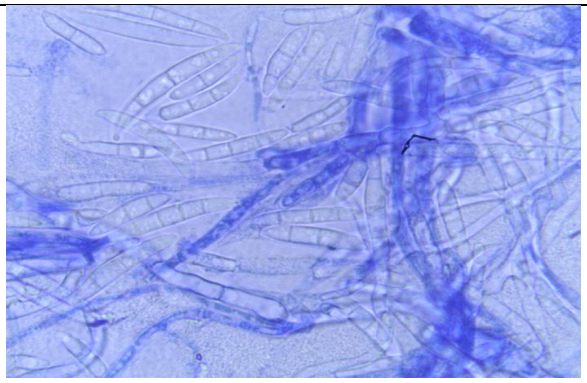
b) Fungus colonies isolated from sorghum grains (Reverse)

c) *Aspergillus parasiticus* colony. From the (a) above isolates

d) *Fusarium Oxsporum* colony. From the (a) above isolates



a) Biseriate conidial head of *Aspergillus paraciticus*. From colony (c) above.

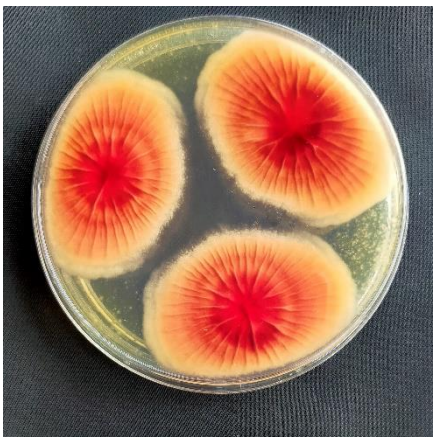


b) Micronidia of *Fusarium Oxysporum.* From colony (d) above.

Aflatoxigenic fungi identification using dichlorvos–ammonia (DV–AM) method

A top surface of *Aspergillus*

A reverse surface of *Aspergillus* before ammonia exposure



Change in color (Reverse surface after ammonia exposure)

Appendix V: EUREC- Ethical approval

EGERTON UNIVERSITY

TEL: (051) 2217808
FAX: 051-2217942

P. O. BOX 536
EGERTON

EGERTON UNIVERSITY RESEARCH ETHICS COMMITTEE

EU/RE/DVC/009
Approval No. EUREC/APP/130/2021 **30th June, 2021**

Lmeriai Lesmuda
P.O BOX 250-20600,
MARALAL
0715467299
E-mail: lesmuda.1457118@student.egerton.ac.ke

Dear Lesmuda,

RE: ETHICAL APPROVAL: ASSESMENT OF POSTHARVEST PRACTICES, MYCOTOXIN KNOWLEDGE AMONG CAREGIVERS OF CHILDREN AGED 6 59 MONTH AND AFFLATOXIN AND FUMONISIN LEVELS IN KERIO VALLEY OF ELGEYO MARAKWET COUNTY, KENYA

This is to inform you that ***Egerton University Research Ethics Committee*** has reviewed and approved your above research proposal. Your application approval number is ***EUREC/APP/130/2021***.The approval period is ***30th June, 2021 –1st July, 2022.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***Egerton University Research Ethics Committee.***
iii. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***Egerton University Research Ethics Committee*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***Egerton University Research Ethics Committee*** within 72 hours

v. Clearance for Material Transfer of biological specimens must be obtained from relevant institutions.

vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.

vii. Submission of an executive summary report within 90 days upon completion of the study to ***Egerton University Research Ethics Committee***.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely.

EGERTON UNIVERSITY
Date. 30/06/2021
RESEARCH ETHICS

Prof. R. Ngure
CHAIRMAN, EGERTON UNIVERSITY RESEARCH ETHICS COMMITTEE
RMNK/BK/

Appendix VI: Ethical clearance from NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **464213**

Date of Issue: **09/July/2021**

RESEARCH LICENSE

This is to Certify that Mr.. LMERIAI LESUUDA of Egerton University, has been licensed to conduct research in Elgeyo-Marakwet on the topic: ASSESSMENT OF POSTHARVEST PRACTICES, MYCOTOXINS KNOWLEDGE AMONG CAREGIVERS OF CHILDREN AGED 6-59 MONTHS, AND AFLATOXIN AND FUMONISIN LEVELS IN SORGHUM IN KERIO VALLEY OF ELGEYO MARAKWET COUNTY, KENYA for the period ending : 09/July/2022.

License No: **NACOSTI/P/21/11631**

464213

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix VII: Consent form

Good morning/afternoon

My name is Lmeriai Lesuuda, a master's student from Egerton University. I am carrying out a study on the mycotoxins safety of sorghum flour used for the preparation of young children's food in this area. I will therefore interview the primary caretaker of the child and collect 1 kg of sorghum grains and flour respectively for the selected households. The study finding will add information about the current status of aflatoxins and fumonisins in sorghum flour used to prepare complementary foods in Kerio Valley. This will help to strengthen standards and regulation mechanisms on consumer safety and provide a basis for the need to train farmers on better post-harvest practices.

Please note that the data collected will be treated with strict confidence and used only for academic purposes. Your participation in this survey is purely voluntary and you may choose to participate or decline.

If you have any questions, comments, or complaints about the study, please contact me at 0715467299.

Participant consent:

I have read and **understood the above information. I agree to participate in the research study.**

Participant

Name_____ Sign _____ Date_____

Confirmation of consent;

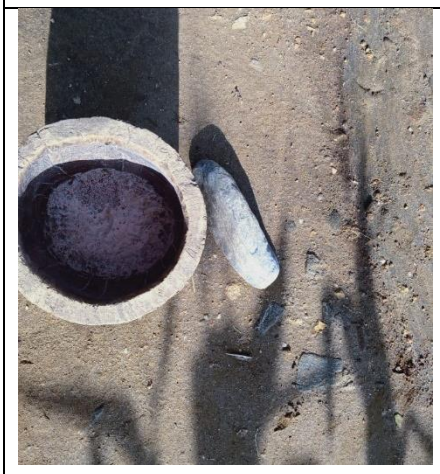
Researcher: Lmeriai Lesuuda

Name_____ Sign _____ Date_____

Appendix VIII: Some of the postharvest handling and storage practices in Kerio Valley

a) Use of traditional store

b) Placing storage bags on the floor



c) Mortar (tree trunk) and pestle (stone) for shelling

d) Caregiver grinding sorghum grains using stones

e) Other grain-grinding tools

Appendix IX: Research article abstract

Received: 1 April 2021 | Revised: 2 July 2021 | Accepted: 6 July 2021

DOI: 10.1002/fsn3.2502

ORIGINAL RESEARCH

Food Science & Nutrition Open Access WILEY

Determinants of knowledge about aflatoxin and fumonisin contamination in sorghum and postharvest practices among caregivers of children aged 6–59 months in Kerio Valley, Kenya

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Abstract

Stunting among children under five years old is still a problem in many developing countries including Kenya. However, there is little information linking stunting with mycotoxin contamination of complementary foods. The aim of this study was to assess knowledge about aflatoxin and fumonisin contamination in sorghum alongside postharvest handling and storage practices among caregivers of children under five years old in Kerio Valley, Kenya. A cross-sectional study was conducted to obtain data from 353 randomly selected caregivers of children aged 6–59 months. Qualitative data were obtained through Focus Group Discussions and Key Informant Interviews. Overall, majority of the caregivers of young children had poor knowledge (61.8%) about mycotoxin contamination of food, and poor postharvest handling and stor-