MYCOFLORA OF LAYER FEED IN NAIROBI COUNTY AND THEIR TOXIGENIC POTENTIAL

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DECLARATION

I hereby declare that this thesis is my original work and has not been submitted elsewhere for examination or award of degree. Where other people's work has been used, this has been properly acknowledged and referenced in according with the University of Nairobi's requirements

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DEDICATION

I dedicate this work to my parents Mr. and Mrs. Paul Polo for granting me with the gift of education, supporting me all the way through and enabling me to achieve my dreams. I also dedicate my work to my siblings, Jessica Akeyo, Jessica Ogindo and Clifford Polo for always encouraging me to journey on and above all I give all the glory to God.

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LIST OF ABBREVIATIONS

AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFM1	Aflatoxin M1
AFM2	Aflatoxin M2
CAM	Coconut Agar Medium
CPA	Cyclopiazonic acid
CTAB	Cetyl Trimethyl Ammonium Bromide
DCPA	Dichloran chloramphenicol peptone agar
DG18	Dichloran 18% glycerol agar
DRBC	Dichloran rose bengal chloramphenicol agar
EC	Esophageal Cancer
EU	European Union
ELEM	Equine leukoencephalomalacia
ELISA	Enzyme Linked Immunosorbent Assay
FB	Fumonisin B series
FB1	Fumonisin B1
FB2	Fumonisin B2
FB3	Fumonisin B3
FB4	Fumonisin B4
KEBS	Kenya Bureau of Standards
NTD	Neural Tube Defects
PPE	Porcine Pulmonary Edema
Sa	Sphinganine
So	Sphingosine
VCGs	Vegetative compatibility groups

ABSTRACT

Layer feeds consist mostly of grains such as maize which are susceptible to fungal growth and subsequent mycotoxin production. Under favorable environmental conditions, fungi are able to colonize the grain at any stage from processing to storage. Mycotoxins in feed are a health risk to both animals and humans. Mycotoxins negatively impact the feed intake, animal performance, reproductive rate, growth efficiency and immunity. This study aimed at investigating the mycoflora associated with layer feeds and quantify the aflatoxin and fumonisin content. One hundred and fifty feed samples comprising chick mash, grower mash and finisher mash were collected from five regions in Nairobi County where small-scale poultry farming is practiced. Dilution plate technique was used to isolate fungi in PDA and fungal colonies identified using both morphological and molecular methods. Toxigenic potential of *Aspergillus flavus* isolates were assessed using Coconut Agar Medium (CAM). Total aflatoxins (AF) and total fumonisins (FUM) levels in the samples were screened using the ELISA technique. The following genera of fungi were isolated from the feeds; *Aspergillus* (91%), *Penicillium* (77%), *Fusarium* (13%), *Mucor* (11%), *Rhizopus* (9%), *Cladosporium* (4%) and *Cochliobolus* (1%). Three strains of *Aspergillus flavus* were found to be toxigenic, among the *Aspergillus flavus* isolates, as confirmed by their blue fluorescence on CAM under UV light. AF contamination was detected in 65% of feed samples having a range of 0.22 µg/kg to 157.89 µg/kg while FUM contamination was detected in 91% of feed samples with a range of 210 µg/ kg to 15173 µg/kg. Sixty-two samples (42%) had AF levels greater than the Kenya Bureau of standards (KEBS) recommended limit of 10 µg/kg in finished poultry feed while no sample contained total FUM levels higher than the recommended European Union (EU) maximum permitted limit of 20000 µg/kg. This study provides insights on the quality of layer feeds in Nairobi County and will play a role in formulating food and feed safety policies.

CHAPTER ONE: INTRODUCTION

1.1 Background of study

Poultry farming is defined as rearing of domesticated birds such as chickens, ostriches and turkeys for the purpose of farming meat and eggs. The poultry industry in Kenya has been on the rise over the years due to increase in human population which has created demand for chicken by-products (Magothe *et al*., 2012). As of 2006, Kenya had an approximated poultry population of 37.3 million birds where 31.4 million were free-ranging indigenous birds, 3.1 million were layers, 2.1 million were broilers while other poultry species were 0.7 million (Omiti and Okuthe, 2009). The sector contributes roughly 55% to the livestock sector and 30% of the agricultural gross domestic product (GDP), or 7.8% of the total national GDP offering employment to roughly two million people either directly or indirectly (Justus *et al*., 2013; Omiti and Okuthe, 2009).

Layer feeds contain six classes of nutrients that are essential to life, growth, production and reproduction of the layer hens. These are carbohydrates, protein sources, fats, vitamins, minerals and water. The choice of feed depends on the nature, purpose, season, weight and age of the birds (Mariana *et al*., 2014). Starter feeds are given to the newly hatched chicks until they are about 6 weeks of age. Starter feeds have the highest protein content (18-20%) and are formulated to give proper nutrition to fast-growing baby chickens (Hermes, 2016). As the chick develops, it requires a smaller percentage of dietary protein and a higher fraction of energy. Once birds are 6 weeks of age, they are fed on grower feeds. Grower feeds contain approximately 15% or 16% protein and are formulated to sustain good growth to maturity (Hermes, 2016). Once the birds are 14 weeks of age, grower feed can be substituted with developer feeds which contains approximately 14% or 15% protein. Layer feeds are given to birds once they are 20 weeks of age or when the first egg is laid. Layer feeds contain 16% protein with extra calcium (3-4%) so that chickens will lay eggs with strong shells and not deplete the calcium in their bodies (Hermes, 2016).

Layer feeds largely comprise of grains such as corn and barley which are highly susceptible to fungal growth and subsequent mycotoxin production (Sivakumar *et al*., 2014). Contamination with fungi can occur at any stage from grain production, processing to storage (Jean *et al*., 2013). Factors such as geographical locations, storage conditions, time of harvest, moisture levels and temperature affect the rate of fungal growth with water being the most critical factor (Seyed *et al*., 2016; Murugesan *et al*., 2015). Mycotoxins are low molecular weight compounds produced during secondary metabolism of various toxigenic fungi mainly of the genera *Aspergillus, Fusarium* and *Penicillium* (Iheshiulor *et al*., 2014). Their presence in feed poses a health and production hazard in the poultry industry (Alberto *et al*., 2017). All poultry species are sensitive to mycotoxins with their sensitivity depending on the type, age and category of the poultry species, nutritive status, duration and quantity of mycotoxin ingestion (Iheshiulor *et al*., 2014; Resanovic *et al*., 2009). Major mycotoxins include: aflatoxins, citrinin, ergot alkaloids, fumonisins, ochratoxin, patulin, trichothecenes and zearalenone (Adeyeye, 2016).

Aflatoxins and fumonisins are of great concern in Africa since they are reported to be prevalent in major dietary food products (Darwish *et al*., 2014). They commonly occur in tropical climates where environmental conditions favor fungal infection by *Aspergillus* and *Fusarium* (Adeyeye, 2016; Kagot *et al*., 2019). Aflatoxins are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* and its discovery and isolation can be traced back to 1960 where more than 100,000 turkeys died in England after consumption of toxic groundnut meal imported from Brazil (Hossein and Gürbüz, 2016; Murugesan *et al*., 2018). Aflatoxins are divided into B and G groups depending on their green and blue fluorescence under UV light when absorbed to solid particles (Ahmad and Gurbuz, 2015). Alternatively, fumonisins are primarily produced by *Fusarium verticilloides* and *F. proliferatum* and are divided into four series A, B, C and P with the B series, mainly, Fumonisn B1 (FB1), Fumonisin B2 (FB2) and Fumonisin B3 (FB3) being the most abundant naturally occurring fumonisins (Kamle *et al*., 2019; Kouzi *et al*., 2018).

Aflatoxins in layer feeds results in decreased egg production, poor quality of eggs and increased mortality in layer hens (Hossein and Gürbüz, 2016). Aflatoxin residues can be transferred from feed to eggs and meat products meant for human comsumption thus posing a threat to the health of consumer as they are known carcinogens (Salwa and Anwer, 2009; Saqer, 2013). Conversely, fumonisin contaminated feed results diarrhoea, reduction in food intake, egg production and body weight and death in layer hens (Murugesan *et al*., 2018). In Kenya, little information is available regarding the quality of layer feeds with respect to fungal counts and mycotoxin contamination. This study investigated the mycoflora, aflatoxin and fumonisin levels present in layer feeds collected from five regions in Nairobi County where urban poultry farming is carried out.

1.2 Statement of the Research Problem

Cereals and cereal based products form a major part of the daily diet of animals and are chief ingredients in animal compound feeds (Pereira *et al*., 2019; Pinotti *et al*., 2016). These cereals include maize, wheat and barley which are highly susceptible to fungal growth and subsequent mycotoxin production (Sivakumar *et al*., 2014). Feed manufacturing industries compete with humans for feed materials therefore low-quality raw materials are often used for poultry feed production (Thuita *et al.*, 2019). In Kenya, to avoid total loss, these off-color cereals rendered unfit for human consumption are often utilized as feed ingredients resulting in production of poor quality feeds (Thuita *et al.*, 2019). These grains are frequently contaminated with mycotoxin and there are dangers that contamination may surpass the regulated standards. Mycotoxin in feed is of great concern due to its negative effects in animal health and the possible carry over effect of each toxin to humans through their by-products (Pinotti *et al.,* 2016). In farm animals, mycotoxins negatively impact the feed intake, animal performance, reproductive rate, growth efficiency, immunological defense as well as being carcinogenic, mutagenic, teratogenic, tremorgenic, heamorrhagic and causing damage to the liver and kidney (Akande *et al*., 2006).

Aflatoxins and fumonisins are the two major mycotoxins detected in cereals in Kenya (Kang'ethe *et al*., 2017). Various studies have detected their presence in feed samples in Kenya (Atherstone *et al*.,

2016; Marijani *et al*., 2017; Thuita *et al*., 2019). The poultry industry as a result faces the risk of suffering from the negative impacts of these mycotoxins. In layer hens, aflatoxins results in decreased weight gain, decreased growth rate, decreased feed conversion efficiency, decreased egg production and egg quality, reduced hatchability, reduced reproductive performance, changes in organ weights, kidney disorders, leg and bone problems, pigmentation problems, immortality and immune suppression (Hossein and Gürbüz, 2016; Iheshiulor *et al*., 2014; Lizárraga-Paulín *et al*., 2006; Murugesan *et al*., 2016; Sharma *et al*., 2018). Fumonisins causes changes in body weight gain, feed consumption and feed conversion ratio, immune functioning and histological changes in the liver and kidney (Iheshiulor *et al*., 2014; Murugesan *et al*., 2018; Patil *et al*., 2014).

1.3 Research Objectives

1.3.1 General objective

To assess the quality of layer starter, grower and finisher feeds sold in Nairobi County.

1.3.2 Specific Objectives

i. To identify the mycoflora present in the layer feeds.

ii. To determine the toxigenic potential of *Aspergillus flavus* strains isolated from the layer feeds.

iii. To quantify the aflatoxin and fumonisin content of the layer feeds.

1.4 Research questions

i. What is the mycoflora of the layer feeds?

ii. What is the toxigenic potential of *Aspergillus flavus* strains isolated from the layer feeds?

iii. Are the feeds contaminated with aflatoxins and fumonisins?

1.5 Justification of the study

Nairobi County houses Nairobi which is the capital city of Kenya. Nairobi serves as the economic hub of the country and has since attracted many migrants from the rural parts of Kenya. Over the years, population in the county has been increasing. According to the 2019 census, Nairobi County was the most populous region with a population of 4.3 million residents (KNBS, 2019). The increase in urban population caused by rapid urbanization in the city has created a high demand for urban food. This presented an opportunity to carry out urban farming to enhance food security and act as a source of income.

The rise in poultry urban farming has been attributed to its small space requirement, less investment, faster returns and dietary shift towards white meat (Omondi, 2018). Urban poultry farming rely on feed manufacturing industry for its' continued success. Nutrient composition and presence or absence of substances that may be harmful to human and animal health are the parameters used in assessing feed quality. Good quality poultry feeds are expensive and, in a bid, to save money, some farmers prefer making their own feeds or end up purchasing low quality feeds and add additional constituents such as maize at home. Feeds account for 60-80% of the total production cost (Oyedeji *et al*., 2013). Poor quality feeds results in high mortalities, low productivity, product condemnation and low rate of investment returns (Oyedeji *et al*., 2013). These feeds are rarely screened for mycotoxins thus stand the risk of being contaminated with mycotoxins beyond the regulated limit.

Animal feeds play a crucial role in farm animal to human food chain especially in urban farming, therefore, infectious and non-infectious hazards existent in feed pose a threat to human health (Kokic *et al*., 2009). Aflatoxins not only affect the productivity of layer hens but may also become concentrated in meat and eggs meant for human consumption (Akande *et al*., 2006). Aflatoxins are potent carcinogens and have been associated with liver cancer in humans under chronic exposure. The population at large may therefore be exposed to the health risks associated with aflatoxins and fumonisins.

CHAPTER TWO: LITERATURE REVIEW

2.1 Popular layer breeds in Kenya

Layer hens are classified into white and brown egg laying hens based on the nature and color of eggs. White egg laying hens are comparatively smaller in size, eat less food, and have white as the egg shell color. Alternatively, brown egg laying hens are relatively larger in size, consume more foods as compared to white egg layers, lay bigger eggs than other laying breeds and have brown as the egg shell color. In Kenya, the most popular layer breeds available are Isa brown, Hy-line Brown and Lohmman brown while improved dual purpose breeds such as Improved KARI Kienyeji, Kenbro, Kuroiler and Rainbow Rooster are also used as layers.

Isa brown is classified as the most popular and efficient layer hens in the poultry industry due to their low cost of maintenance, adaptability to changing climatic conditions and prolificacy that can attain 300 eggs per hen in the first year of laying (Tadesse *et al*., 2013; Kolawole and Folake, 2019). They are friendly, non-aggressive and make a good hen for families. With proper feeding management, the hen should weigh 360g in 5weeks and 1.425kg in 17 weeks (ISA, 2017). Additionally, it has an excellent food to egg performance ratio therefore making it a very profitable breed.

Hy-line Brown is categorized as the world's most balanced brown layer hen. At 100 weeks, the breed can lay between 468 and 483 eggs per hen (Hy-line, 2018). Its' high productivity combined with excellent livability, superior interior egg quality, superb feed efficiency and mild temperament give the Hy-Line Brown the perfect balance (Hy-line, 2018). The breed is ideally suited for free range production, has low maintenance cost and can adapt easily to various climates and surroundings.

Lohmann Browns is selectively bred from lines of Rhode Island breed and White Rock breeds. The hens can be raised in both free-range and caged chicken farming systems. High yield, good egg quality, and high level of adaptability as well as their production efficiency are attributes

that make them preferred (Lohmann, 2013). The breed has a long life expectancy of 10 years and can lay up to 360 eggs per hen at 80 weeks (Lohmann, 2013). Lohmann Browns are docile, friendly, and easy to keep.

The KARI breed can produce more eggs and meat as compared to local indigenous chickens. The breed is quiet temperament, has excellent feathering and is able to adapt fast to the conditions under which it is kept compared to other breeds. It can be reared in free range conditions particularly for farmers who want to produce chickens organically. When managed properly, the KARI Improved hens can lay between 220 to 280 eggs a year (Angweny, 2016). A hen from this breed can attain 1.5kg in 5 months while a cock weighs 2kg over the same period when they are well nourished (Angweny, 2016).

Kenbro breed is a dual-purpose breed precisely developed to serve the Western Kenya market. Kenbro is more resistant to diseases when compared to other hybrid birds and can survive on free range. The Kenbro breed are able to put on weight quickly since they are heavy feeders and farmers confirm it has tastier meat compared to small indigenous and exotic broilers (Angweny, 2016). With proper nourishment, the bird matures faster and starts laying eggs at 5 months as well as lay 200 eggs annually (Angweny, 2016; Mulwa, 2017). Full-grown Kenbro cock can weigh between 2.2 and 2.5kg while hens can weigh between 1.9 and 2.2kg (Angweny, 2016).

Kuroiler is also a dual purpose breed originally introduced in Uganda from Keggs farm in India. The breed can survive on free range, but need to feed regularly. Kuroiler cocks are tall, and majestic and weigh above 4kg at full maturity while hens are large and full bodied and weigh over 2kg at maturity. Kuroiler chickens lay bigger eggs as compared to indigenous chicken and have the deep yellow yolk color. A Kuroiler hen can lay between 140 and 150 eggs in a year, however, Kuroilers' egg quality decreases when cross bred with indigenous chickens (Angweny, 2016). Additionally, Kuroiler hens cannot sit on their eggs to hatch so as to maximize their egg production.

Rainbow Rooster is a dual purpose breed from Indbro Research Breeding farm in Hyderabad. It is a multicolored disease resistant cross breed suitable for backyard rearing and organic chicken production. The breed is a heavy feeder and is able to put on weight fast attaining 3kg to 4kg in a period of 6 months (Angweny, 2016). When managed as a layer, they start laying at 5 months and lay 160 to 180 eggs in 72 weeks. Like Kuroiler, Rainbow Rooster cannot sit on their eggs to hatch.

2.2 Feeding of layers

Feed formulation comprises different ratios of raw materials that make up the diet which supplies the necessary nutrients to an animal. Feed ingredients are chosen on a number of factors including the nutrients they provide, their palatability, the absence of toxic compounds and their cost. The nutrient requirements and feed formulation depends on animal species, age and stage of production and composition of raw materials present (Guerre, 2016). Feeding alone accounts for at least 60-80% of the entire production cost (MOLD, 2008). The key to maximum egg production of layers is by providing them with appropriate feed right from the hatch to their productive period.

Starter feeds are usually the chick or duck mash fed to baby chicks until they are about 6 weeks of age and contain between 18-20% proteins (Hermes, 2016). The high protein and vitamin content help in building up flesh and bones. Additionally, they have a relatively smaller particle size which gives room for proper digestion and feed utilization. Grower feeds are given to birds once they are 6 weeks of age. They have less protein content (16%) as compared to the starter feeds and are formulated to sustain good growth to maturity (Hermes, 2016). In the growers' stage, the reproductive organs which would affect the rate of egg production are developing. Developer feeds serve as a good substitute for grower feeds once the birds are 14 weeks of age. As compared to grower feeds, they contain much lower protein content (14-15%) with their sole purpose being to prepare the birds for egg production (Hermes, 2016). They also contain higher calcium content which in turn helps with egg shell formation of first batch of laid eggs. Once

birds are between 18-20 weeks of age or when they have laid their first egg, they are fed on layer feeds until the end of production. They contain 16% protein with extra calcium (3-4%) to ensure the laid eggs have strong shells which are clean and crispy and to ensure calcium is not depleted in the birds bodies (Hermes, 2016). However, they have less carbohydrate content since the birds are not very active and to prevent the birds from getting overweight. If the laid eggs have thin shells that easily crack or shells that are rough with almost a sandpaper feel, layer hens may be given oyster shell to help increase shell strength and quality.

2.3 Factors favoring fungal growth and toxin formation

Fungal contamination has negative impacts on the nutritional and organoleptic properties of the feeds (Shareef, 2010). These fungi may invade, colonize and produce mycotoxins in food at pre-harvest period, harvest time, post-harvest handling, during processing and storage (Rajeev *et al*., 2010). Factors such as geographic locations, storage conditions, processing of various feeds and moisture content influence the spread of fungal infections (Seyed *et al*., 2016). Among the different animal feeds, poultry feeds are the most contaminated with mycotoxigenic fungi (Sivakumar *et al*., 2014).

Mycotoxin contamination has been proven to be a generally climate-dependent, plant and storage-associated problem (Liu *et al*., 2016). Factors favoring mycotoxin production have been divided into physical, biological and chemical factors. Physical factors include environmental conditions such as temperature, relative humidity and insect infestation while chemical factors comprise the use of fungicides or fertilizers along with biological factors which depend on the interactions between the colonizing toxigenic fungi and the substrate (Assefa and Geremew, 2018).

Tropical and subtropical regions favor the colonization and dominance of *A. flavus* and *A. parasiticus* resulting in the production of aflatoxin (Bhat *et al.*, 2010; Milani, 2013). Optimum conditions for aflatoxin production by these two species are at 33°C and 0.99 a_w while that of their growth is 35°C and 0.95 a_w (Milani, 2013). Aflatoxin contamination is also enhanced by stress or damage to the crop

because of drought before the harvest period, insect damage, poor harvest timing, high temperature stress, heavy rains present during or after harvest period and insufficient drying of the crop before storage (Dhanasekaran *et al*., 2011; Lizárraga-Paulín *et al*., 2006). Cotty and Jaime (2007) reported higher aflatoxin contamination in crops receiving over 50mm of rain during boll opening while cotton seed harvested in Arizona between 1995 and 1996 had high aflatoxin levels which was attributed to warm and humid conditions (Bock and Cotty, 1999). Peanuts subjected to high temperatures during pod maturation and rain on windrows also act as susceptibility factors (Milani, 2013).

Fumonisins are produced either in the pre-harvest period or early stage of storage and its' concentration during storage does not increase unless under adverse storage conditions (Assefa and Geremew, 2018; Kamle *et al*., 2019). Temperatures in the range of 15°C to 30°C coupled with a_w of 0.97 favor fumonisin production with optimum growth temperatures for both *F. verticilloides* and *F. proliferatum* being recorded at 30°C. (Marina *et al*., 1999; Samapundo *et al*., 2005). Fumonisin contamination increases when kernels are physically damaged, especially by insect feeding which was evident with field surveys done on European corn borer and thrips (WHO, 2000).

2.4 Structure and types of aflatoxins

The major types of aflatoxins are B1, B2, G1 and G2 based on their blue or green fluorescence under ultraviolet light and relative chromatographic mobility during silica gel thin layer chromatography (Bennett *et al*., 2008). However, there are over 18 different aflatoxin types presently known but more emphasis has been placed on the four types due to their high incidence and toxicity (Benkerroum, 2020). *A. flavus* produces the AFB1 and AFB2 while *A.parasticus* produces AFB1, AFB2, AFG1 and AFG2 (Ajani *et al*., 2014). Of the four, G2 occurs in high quantities although less toxic while B1 is the most toxic of all aflatoxins (Bbosa *et al*., 2013; Dhanasekaran *et al*., 2011). AFB2 and AFG2 are dihydroxy derivatives of AFB1 and AFG1 respectively (Ajani *et al*., 2014; Bbosa *et al*., 2013). AFM1 is a metabolite of AFB1 in humans and animals while AFM2 is a metabolite AFB2 in

milk of cattle fed on contaminated feeds (Ajani *et al*., 2014; Bbosa *et al*., 2013). Aflatoxins are classified as difuranocoumarins and are divided into two groups based on their chemical structure (Bbosa *et al*., 2013). Difurocoumarocyclopentenone which consist of a bifuran ring fused to a coumarin nucleus with a pentenone ring present in B and M aflatoxins and difurocoumarolactone which have a six membered lactone ring present in G aflatoxins, (Figure 2.1) (Bbosa *et al*., 2013; Dhanasekaran *et al*., 2011).

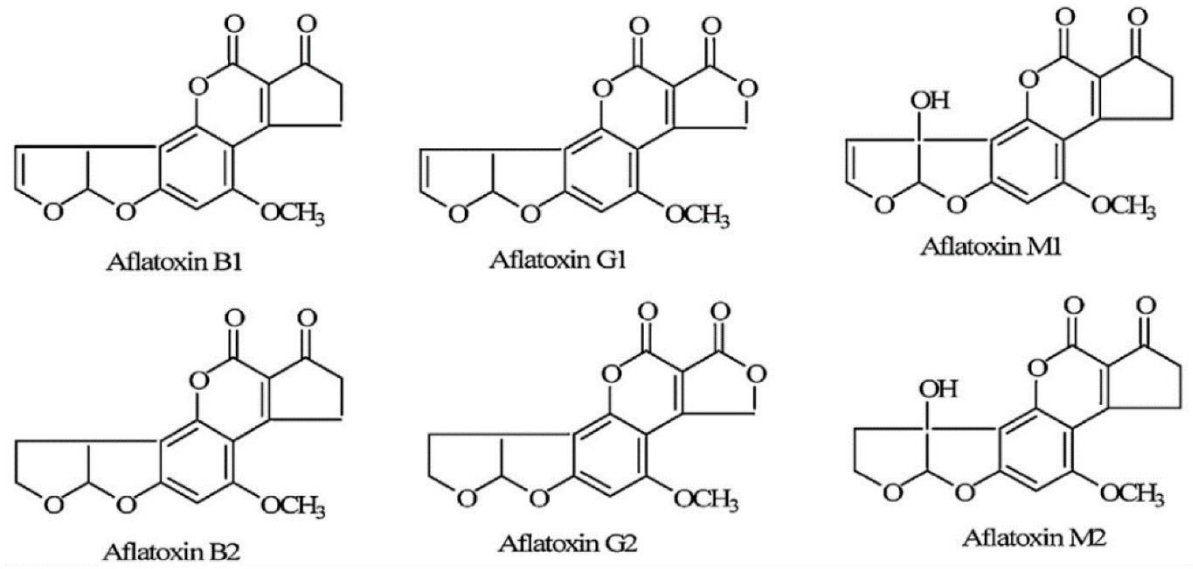

Figure 2.1: Chemical structures of Aflatoxins B1, B2, G1, G2, M1 and M2 (Zain, 2011)

2.4.1 Aflatoxins and animal health

Aflatoxicoses is the disease that arises after consumption of feeds and foods containing aflatoxins. It may either be acute causing death or chronic causing cancer or even immune suppression. The severity of the disease depends on the breed, species, age, dose, length of exposure and nutritional status of the animal (John, 2007). AFBI is highest in concentration in contaminated feed and food (Sweeney and Dobson, 1998). The primary target organ for aflatoxin B1 is usually the liver with liver damage being observed in poultry, fish, rodents and non-human primates fed on aflatoxin B1 (Zain, 2010).

Beef and dairy cattle are more predisposed to aflatoxicosis than sheep or horses although all species are affected (Cassel and Campbell, 2001). Aflatoxins negatively impact production, immune system and rumen metabolism in cattle (Zain, 2011). Bodine *et al*., (1984) reported that bovine lymphocyte blastogenesis was inhibited by aflatoxins in cattle. Later on Cook *et al*., (1986) used radio telemetry to measure rumen motility and established a dose dependent relationship between aflatoxin and rumen motility. Aflatoxin B1 is metabolized to AFM1 which is excreted into cows' milk. Veldman *et al*., (1992) demonstrated that the carry over effect of AFB1 to AFM1 in cattle milk was higher in early lactation as compared to late lactation. Levels of 1.44µg/kg of AFM1 with a mean of 0.30 µg/kg were detected in milk samples in Serbia (Škrbić *et al*., 2014).

Lambs fed on 2.5 mg of aflatoxin for 21 days resulted in hepatic and nephritic lesions, altered mineral metabolism and increased the size and mass of liver and kidney (Fernendez *et al*., 1997). Sows fed on 1 and 3 ppm of aflatoxin depicted clinical signs of anorexia, jaundice and loss of body weight with histopathological findings revealing hypertrophy of the bile duct epithelium, dissociation of the liver cords, karyomegaly and adenoma in the liver and depletion of lymphocytes in germinal epithelium in the spleen (Yalagod *et al*., 2013). Changes in some aspects of humoral and cellular immunity coupled with a decrease in growth rate was observed in piglets fed on 140 and 280 ppb of aflatoxin (D. Marin *et al*., 2002). Mature horses fed on 58.4 µg/kg of AFB1 contaminated feed were jaundiced and anorexic before death with post-mortem results showing enlarged livers, kidney damage and lesions of bile-duct hyperplasia (Eva *et al*., 2011; Zain, 2011).

The order of aflatoxin sensitivity in poultry species is first the ducks which are closely followed by turkeys then broilers and finally layers (Okoli *et al*., 2011). In poultry species, aflatoxins impairs production parameters such as weight gain, feed intake, feed conversion efficiency, pigmentation, processing yield, egg production, male and female reproductive system (Dhanasekaran *et al*., 2011). Decrease in the weight of thymus and bursa of fabricius, impairment

of the humoral and cellular immune function and an increase in number of apoptotic cells were observed in broilers fed with corn naturally contaminated with AFB1 (82.4 μg/kg and 134 μg/kg) and AFB2 (14.2 μg/kg and 23.6 μg/kg) in the starting and growing period respectively (Peng *et al*., 2015). High incidence of mycotoxins was correlated to outbreaks of Newcastle disease and Infectious Bronchitis in layers and broilers (Yunus *et al*., 2009). Aflatoxins caused a delay in growth of cherry calley meat male ducks and growth of the skeletal muscle (Chang *et al*., 2016). Rauber *et al*., (2007) established a dose dependent relationship between aflatoxin and the effect of these toxins on feed consumption, relative weight of gizzard and liver, mortality and total protein and cholesterol levels in turkey.

2.4.2 Aflatoxins and Layers.

Protein synthesis is inhibited by aflatoxins thus destroying cells and organs such as liver, gut epithelium, and immune system which have a high protein turn over (Ahmad and Gurbuz, 2015). Pandey and Chauhan (2007) reported feeding of aflatoxin B1 at the dose rate of 2.50, 3.13, 3.91mg/kg to the White Leghorn layers from first week to 40 weeks of age resulted in decrease in weight gain, feed intake, increased age of sexual maturity, reduction in the color of the yolk, affected the ability of the chicks to retain dry matter, crude protein, ether extract, calcium and metabolisable energy. Adverse effects were also observed in liver, kidney, heart, ovaries and bursa of fabricius with aflatoxin residues detected in eggs and breast muscles of the hen (Pandey and Chauhan, 2007). Jia *et al*., (2016) demonstrated a decrease in egg production, egg shell quality, feed intake, feed conversion ratio and were able to detect aflatoxin residues in eggs. Wolzak *et al*., (1986) illustrated that aflatoxin contamination resulted in pale, hemorrhagic and enlarged livers and caused a reduction in the size of ovaries as compared to the hens fed on aflatoxin free diet. They also detected aflatoxin residues in tissues with the highest levels being detected in gizzards, kidneys and liver and the lowest levels present in breast, blood serum and leg (Wolzak *et al*., 1986).

Aflatoxins inhibit thymus development and affect the weight of bursa of fabricius resulting in cellular and antibody irresponsiveness in the chicken immune system leading to immune

suppression and vaccination failures (Hossein and Gürbüz, 2016). Azzam and Gabal (1998) demonstrated that aflatoxin contaminated feeds lowered antibody titres in chickens immunized against Newcastle disease, Infectious Bronchitis and Infectious Bursa disease thus impairing the immune system and an increase in mortality of layer chickens. A similar study conducted also showed a decrease in egg weight and egg production of layers fed with high levels of aflatoxins for six weeks (Azzam and Gabal, 1998).

2.4.3 Aflatoxins and Human Health

Aflatoxins enter the human food chain either directly after ingestion of contaminated crops or processed food products or indirectly from tissues, eggs, milk and dairy products of animals fed with aflatoxin contaminated feeds (Pereira *et al*., 2019). It is estimated that 4.5 billion people residing in developing countries are regularly exposed to largely uncontrolled amounts of the toxin (Williams *et al*., 2004). Acute aflatoxicosis in humans is characterized by symptoms such as abdominal pain, vomiting, low appetite, malaise, slight fever, hepatitis and fatal liver failure while chronic aflatoxicosis is characterized by increased risk of developing liver cancer, impaired immune function and malnutrition. Of the two, chronic aflatoxicosis is more prevalent than acute aflatoxicosis (YunYun *et al*., 2016). Toxicity hangs on factors such as age, gender, health and nutritional status and presence or absence of underlying conditions (Benkerroum, 2020).

Gong *et al*., (2003) in their study in Benin and Togo were able to show a correlation between aflatoxin exposure and the extent of stunting and being underweight in children. Children fed on mycotoxin contaminated flour were shown to be malnourished and wasting away in Kenya (Okoth and Ohingo, 2004). Epidemiological studies have revealed that aflatoxins play a role in development of kwashiorkor, a type of protein energy malnutrition, in malnourished children (Dhanasekaran *et al*., 2011; Shephard, 2008). Aflatoxin accumulated in body fluids and tissues from children suffering from kwashiorkor and severe marasmic kwashiorkor (De Vries HRI *et al*., 1990). Autopsy results disclosed that aflatoxin amassed in lungs of children who had

kwashiorkor in Nigeria (Oyelami *et al*., 1997). Aflatoxins also serves as etiological agents of Reye syndrome in children in Thailand and New Zealand (Arvin and Mansoul, 2013; Dhanasekaran *et al*.., 2011; Bressac *et al*., (1991). Glucose 6 phosphate deficiency and the presence of aflatoxins in serum proteins acted as risk factors in neonatal jaundice in a study conducted in Nigeria (Sodeinde *et al*., 1995). Acute jaundice was also observed in an outbreak of aflatoxicosis in Kenya and Tanzania (Barrett, 2005; Kamal *et al*., 2018).

Aflatoxin serves as a risk factor for hepatocellular carcinoma, which is a leading cause of death worldwide, especially when hepatitis B is present (Arvin and Mansdoul, 2013; Bennett and Klich, 2003). It has been described by WHO as the third foremost cause of cancer death worldwide with 83% of these deaths occurring in East Asia and sub-Saharan Africa (Salisu & and Almajir, 2020). Approximately 50% to 100% of liver cancer cases are estimated to be connected with persistent hepatitis B or C virus infections (Henry *et al*., 2002). Potency of aflatoxin in hepatitis B positive individuals is 30% higher than in individuals without the virus (Henry *et al*., 2002). Liver cancer differs from one country to another but is one of the most frequent cancers reported in China, Philippines, Thailand and many African countries with the population cancer risk being greater in developing countries (Bennett and Klich, 2003; Shephard, 2008).

Aflatoxins interfere with the immunological mechanisms such as motility, phagocytes and antigen recognition, production of antibodies, complement and interferon thus causing a decrease in the performance of the immune system (Magda and Pavel, 2009). Low levels of secretory IgA in saliva was correlated with aflatoxin exposure in children in Gambia (Turner *et al*., 2003). Negative correlation was observed between AFB1 and the production of CD3+ and CD19+ that showed the CD69+ activation marker in a study carried out in Ghana (Jiang *et al*., 2005). HIV patients with high AFB1 albumin adduct levels had lesser percentages of CD4+ T regulatory cells and naïve CD4+ as well as B cells compared to HIV patients with low AFB1 albumin adduct levels (Iiang *et al*., 2008). Traces of aflatoxins have also been found in blood of expectant women, in neonatal umbilical cord blood and breast milk with some levels in umbilical cord

blood being the highest ever recorded in human tissue and fluids (Peraica *et al*., 1999; Bbosa *et al*., 2013).

2.4.4 Incidences of aflatoxicosis

The first major outbreak was reported in West India in 1974 from October to November where 106 fatalities were recorded in households that consumed contaminated corn (Ajani *et al*., 2014; Krishnamachari *et al*., 1975; Rom *et al*., 2017). Examination of the contaminated maize revealed that the affected population had consumed between 2 and 6 mg of aflatoxin daily for a period of one month (Krishnamachari *et al*., 1975). Twenty patients from two family groups from Makueni district were admitted to three hospitals in Machakos district with jaundice and other symptoms suspecting viral hepatitis where 12 of them eventually succumbed due to liver failure in Kenya (Augustine *et al*., 1982). The two families were found to be eating maize contaminated with AFB1 with levels as high as 12000 µg/kg (Augustine *et al*., 1982). Thirteen Chinese children died of acute hepatic encephalopathy in northwestern state of Perak in peninsular Malaysia after consumption of Chinese noodle suspected to have up to 3mg/kg of aflatoxins (Ajani *et al*., 2014; LyeMS *et al*., 1995).

In January 2004 to June 2004, 317 people sought hospital care for symptoms of liver cancer where 125 people died from Makueni and Kitui districts in Eastern province in Kenya (Barrett, 2005; Lewis *et al*., 2005; Rom *et al*., 2017). Maize sampled from the shaken area had high levels of AFB1 with 55% of the samples exceeding the Kenyan legal limit 20 µg/kg, 35% had levels above 100 µg/kg and 7% above 1000 µg/kg with the maximum level found being 8000 µg/kg (Shephard, 2008). A similar outbreak occurred in the eastern province of Kenya in June 2005 where 32 deaths occurred out of the 75 cases admitted in hospital (Shephard, 2008). The maize samples collected revealed that 42% had AFB1 levels above 20 µg/kg, 24% had levels above 100 µg/kg and 7% above 1000 µg/kg (Shephard, 2008). A total of 68 cases were recorded between May 2016 and November 2016 from two regions in the central part of Tanzania where 20 people died as a result of consuming maize contaminated with aflatoxins (Kamal *et al*., 2018). The maize samples were contaminated with high levels of aflatoxins ranging from 10-51,100 µg/kg and 2.4-285 µg/kg for case and control households respectively (Kamal *et al*., 2018).

2.5 Structure and types of fumonisins

Fumonisins are produced by *Fusarium verticilloides, F. proliferatum* and other *Fusarium* species (Antonissen *et al*., 2015). These fungi are ubiquitous in nature and have been shown to be major contaminants of maize and maize based animal feeds (Braun and Wink, 2018; Voss *et al*., 2007). *Fusarium* species have been illustrated to cause a substantial reduction in quality and yield of many food and feed crops (Shi *et al*., 2017). More than 28 homologues of fumonisins have been found and are divided into four groups that is A, B, C and P series based on their structure (Antonissen *et al*., 2015; Dang *et al*., 2016). Of the FB analogues, FB1 is the most predominant accounting for almost 70% of total FB contamination (Eskola *et al*., 2019). FB1 is also the most studied, toxic and has been grouped as group 2B possible human carcinogen (Kamle *et al*., 2019; Voss *et al*., 2007). FB2 and FB3 co-occur with FB1 although in lower concentrations and all together comprise the main food contaminants (Kamle *et al*., 2019). Traces of fumonisins have also been found in rice, sorghum, wheat bran, soybean meal and poultry feed (Kouzi *et al*., 2018).

FB comprise of two methyls (-CH3), one amine (-NH2), one to four hydroxyl (-OH-), and two tricarboxylic ester groups situated at different positions along with the linear polyketide-derived backbone, (Figure 2.2). FB2, FB3 and FB4 differ structurally from FB1 in the number and placement of hydroxyl group on the molecule's hydrocarbon backbone (Voss *et al*., 2007). FB have similar chemical structure to sphingoid bases, sphinganine and sphingosine therefore the proposed mode of action is inhibiting ceramide synthase enzymes thus interfering with sphingoid turn over (Rauber *et al*., 2013). This leads to accumulation of sphingoid bases commonly sphinganine which exert proapoptotic, cytotoxic and growth inhibitory effects (Rauber *et al*., 2013).

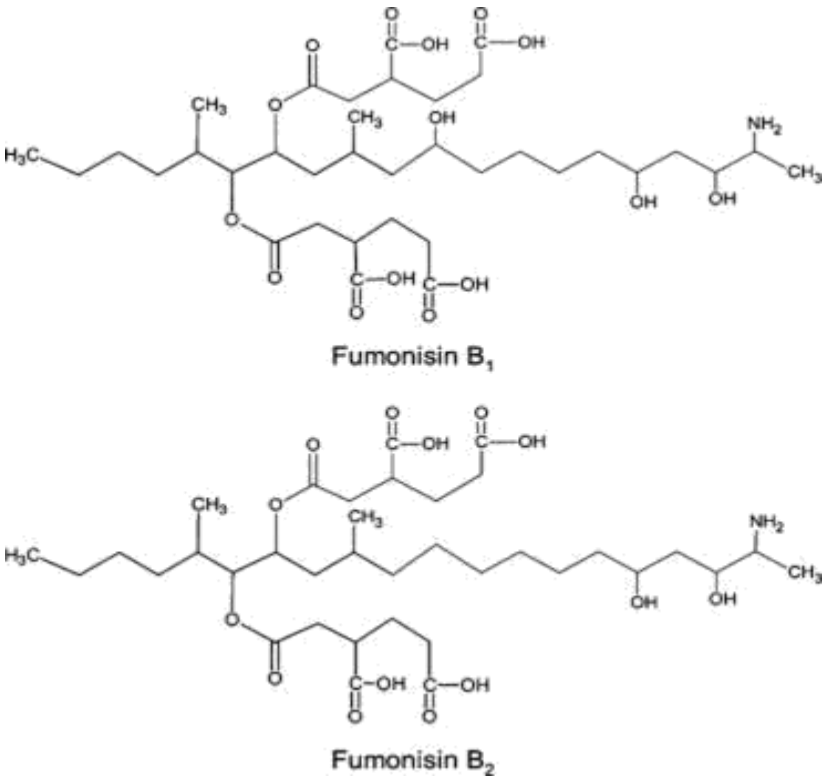

Figure 1.2: Chemical structures of Fumonisin B1 and B2 (Zain, 2011)

2.5.1 Fumonisins and Animal health

Ruminants are generally less sensitive to fumonisins as compared to horses and pigs with horses being the most vulnerable species (Knutsen *et al*., 2018; Voss *et al*., 2007). Fumonisins have been shown to cause equine leukoencephalomalacia (ELEM) in horses and porcine pulmonary edema (PPE) in pigs (Voss *et al*., 2007). The primary target organs of ELEM are heart, central nervous system and lungs manifesting clinical symptoms such as lethargy, blindness, decreased feed intake, convulsions and eventually death (Pereira *et al*., 2019; Voss *et al*., 2007). Ross *et al*., (1993) reported a reduction in feed consumption, hepatic and histopathological lesions with mortality being observed in ponies fed on 88 ppm of FB1. PPE in swine occurs as a result of hepatic injury and/or myocardial failure with clinical symptoms including reduced feed intake, dyspnea, weakness, cyanosis and death (Knutsen *et al*., 2018; Kouzi *et al*., 2018; Pereira *et al*., 2019). Smith *et al*., (2000) demonstrated that FB1 altered cardiovascular function and that fumonisin induced pulmonary edema is caused by left sided heart failure and not by altered endothelial permeability.

Similar results were also obtained by Constable *et al*., (2000) after short term ingestion of FB1 containing culture material to pigs which resulted in negative inotropic and chronotropic effects along with a decrease in mechanical efficacy of the left ventricle.

Hepatic and renal lesions were observed in rats consuming *F. monliforme* infected corn (Voss *et al*., 1993). These lesions were portrayed by simultaneous cell loss (apoptosis and necrosis) and propagation where an imbalance observed between cell death and replacement facilitates carcinogenicity (Voss *et al*., 2001). Study with B6C3F1 mice revealed that FB1 was hepatocarcinogenic to females at 50 mg/kg in the diet while primary hepatocellular carcinomas and cholangial carcinomas were induced in male BD IX rats fed on 50 mg/kg of FB1 for 26 months (WHO, 2000). Male F344/N Nctr rats fed 50 mg/kg FB1 had renal tubule adenomas and carcinomas (WHO, 2000). Nephrotoxic and hepatotoxic effects were observed in adult rabbits administered with FB1 intravenously (Gumprecht *et al*., 1995). Rabbits fed on 7.5 and 10 mg/kg FB1 delayed the onset of puberty, weakened semen quality and spermatogenesis and induced embryo mortality without adversely affecting the fertility rates of male rabbits (Ogunlade *et al*., 2013). Lambs intra-ruminally dosed with 0, 11.1, 22.2, and 45.5 mg/kg of total fumonisins had reduced feed intake, diarrhea, lethargy, liver damage with mortality occurring in lambs fed with high doses (Edrington *et al*., 1995). Milk production was reduced in Holstein and Jersey cows fed on 100 mg/kg of fumonisins from 7 days before calving to 70 days after parturition (Diaz *et al*., 2000). Calves treated with 1 mg/kg of FB1 for 7 days were lethargic and had reduced appetite as from day 4, had severe liver and bile duct injury, renal injury and an increase in sphinganine and sphingosine (Sa/So) concentrations in the liver, kidney, lung, heart and skeletal muscle (Mathur *et al*., 2001).

2.5.2 Fumonisins and poultry

Poultry species are generally resistant to fumonisins (Voss *et al*., 2007). Biliary hyperplasia, hepatocellular hyperplasia and increase in absolute organ weights of liver, pancreas, kidney, heart and proventriculus were observed in white peckin ducklings fed on FB for 21 days (Bermudez *et al*., 1995). Mallard ducks force fed on naturally contaminated maize containing 20 mg/kg FB1 had 8% mortality rate, decrease in feed conversion ratio and increase in Sa/So ratio in the liver and plasma at doses of 10 mg/kg or greater in feed (Tardieu *et al*., 2004). Similar results were also obtained by Bailly *et al*., (2001) on ducks fed on 0, 5, 15 and 45 mg/kg FB1 for 12 days with liver alterations obtained with only 5mg/kg of FB1. Ducks and turkeys fed on 10 mg/FB1 and FB2/kg had increased Sa concentrations in the liver and serum with hepatic toxicity being more pronounced in ducks than in turkeys and reduction in body weight and increase in serum biochemistry being observed only in ducks (Benlasher *et al*., 2012).

Turkeys fed on fumonisin concentrations of 0, 5, 10, and 20 mg of FB1 and FB2/kg of feed experienced no significant change in body weight, feed consumption, feed conversion ratio and average daily weight gain but had a slight increase in mean daily consumption in turkeys fed on 20 mg/kg of fumonisins (Tardieu *et al*., 2007). The Sa/So ratio increased in the liver and kidney of the turkeys but remained constant in the serum (Tardieu *et al*., 2007). Rauber *et al*., (2013) observed hyperplasia of biliary ducts, lymphoid hyperplasia, and hepatocellular degeneration, proliferation of bile ducts and necrosis of the tubular epithelium in broilers fed on FB1. Broilers fed on 18.6 mg/kg of FB1 and FB2 altered sphignolipid metabolism, modified the composition of intestinal micro-biota, negatively affected small intestinal length, ileal villus height and crypt length and increased the susceptibility for *Clostridium perfringens* induced necrotic enteritis (Antonissen *et al*., 2015). Both broiler chicks and turkeys fed on 0, 25 and 50 mg/kg FB1 for 7 and 14 weeks respectively had higher liver Sa/So ratios (Broomhead *et al*., 2002).

Feed intake, body weight gain and feed conversion ratio of chicks was not affected while a reduction in feed intake was noticed in turkeys (Broomhead *et al*., 2002). FB1 decreased cell viability and mitogenic response in splenic cells but not on thymocytes or blood lymphocytes (Keck and Bodine, 2006). FB1 at doses of 0, 2, 4, 8, 16, 32, and 64 µg per egg resulted in embryonic mortality of 5, 12.5, 17.5, 20.0, 52.5, 77.5 and 100%, respectively after injection into air cell of chicken eggs with severe hemorrhages of the head, neck and thoracic area being evident in dead embryos (Henry and Wyatt, 2001). Japanese quail fed on 50 and 250 mg/kg of FB1 had reduced feed intake, body weight gain and egg shell weight as compared to the controls while feed conversion ratio, mean egg production and egg weight were reduced in birds fed on 250 mg/kg of FB1 (Butkeraitis *et al*., 2004). Laying birds fed up to 15.2 ppm of FB1 experienced no significant change in feed intake, daily weight gain, feed conversion ratio, egg cholesterol and triglycerides but had a significant change in the color of the yolk (Ogunlade *et al*., 2013). Fumonisins caused a decrease in the length of small intestine and an increase in abdominal fat deposition in hisex brown layer hens (Siloto *et al*., 2013).

2.5.3 Fumonisins and human health

Sadler *et al*., (2002) reported that fumonisin exposure hindered sphingolipid synthesis, reduced growth, and caused cranial neural tube defects in a dose dependent manner. Neural tube defects (NTD) are embryonic defects of the spinal cord and brain arising from failure of the neural tube to close during the first few weeks of development (Kouzi *et al*., 2018). Marasas *et al*., (2004) also proposed that fumonisins acted as risk factors for NTD, craniofacial abnormalities and birth defects arising from neural crest disorders due to their interference in foliate utilization. In South Texas, Missmer *et al*., (2006) reported that fumonisin exposure resulted in increased risk of NTD occurrences in a dose dependent relationship and a dose above threshold level may cause fetal death. NTD defects doubled between 1990 and 1991 in Mexican-American women due to consumption of corn suspected to be contaminated with fumonisin in form of tortillas (Missmer *et al*., 2006). Ncayiyana (1986) reported high incidence of NTD (6, 13 NTDs/1 000 births) among

rural blacks in Transkei district in South Africa between 1980 and 1984 while the NTD prevalence rate at birth was found to be higher in northern region of china than in the southern region (Chen *et al*., 2009).

Fumonisins have been linked to esophageal cancer (EC) in South Africa and China (Voss *et al*., 2001). High incidence of EC were reported in four districts in Trankei in South Africa between 1985 and 1995 (Makaula *et al.*, 1995; Somdyala *et al*., 2003). Incidences of EC were higher in southern part of Transkei than the northern part and this was correlated with FB contamination in maize (Marasas, 2001). Studies done in China suggest that FB1 play a role in human esophageal and hepatocarcinogenesis (Sun *et al*., 2007). High levels of FB1 were found in corn sampled from counties of Cixian and Linxian which are areas with high incidence of EC in china (Chu and Li, 1994). However, Yoshizawa *et al*., (1994) found no correlation between levels of FB1 and FB2 in corn with the incidence of EC in high risk and low risk areas in China. Further studies in the same regions revealed the same results but correlated the incidence of EC with the natural occurrence of tricothecenes and zearalenone (Gao and Yoshizawa, 1997). Ueno *et al*., (1997) reported that FB in high risk area of primary liver cancer, Haimen, was 10 to 50 fold higher than those in low risk areas, Penlai, suggesting that FB act as a risk factor for promotion of primary liver cancer in China. In contrast, Persson *et al*., (2013) did not find any correlation between FB1 exposure and hepatocellular cancer in their study in two cohorts in China.

Studies conducted in Tanzania have linked fumonisin exposure to growth impairments in infants (Kimanya *et al*., 2010). Urinary FB1 concentrations were negatively correlated with length for age Z scores in children in Tanzania (Shirima *et al*., 2015). Breast feeding and weaning practices were thought to be associated with high growth impairments in children as a result of FB1 exposure (Chen *et al*., 2018).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study setting

This study was carried out in Nairobi County which houses the capital city of Kenya and covers a land area of 695 square kilometers (Otiso, 2017; KNBS, 2019). The county receives a mean annual rainfall of 879 mm with a mean maximum temperature of 23.6° and lies between 1,600 and 1,850 meters above sea level on the southeastern edge of Kenya's agricultural heartland at 1016'S latitude and 36048'E longitude (Obiero and Onyando, 2013; Otiso, 2017).

Poultry farming in Nairobi County serves as one of the lucrative businesses carried out by farmers to meet the increasing food demand of the growing population in the urban centers. An estimated 3,432 households are reported to carry out layer farming in the county (KNBS, 2019). Five regions, selected as sample sites (Dagoretti South, Kasarani, Langata, Roysambu and Ruai) were identified by the Ministry of Agriculture, Livestock and Fisheries as the areas where active small scale poultry production is carried out in Nairobi County, (Figure 3.1).

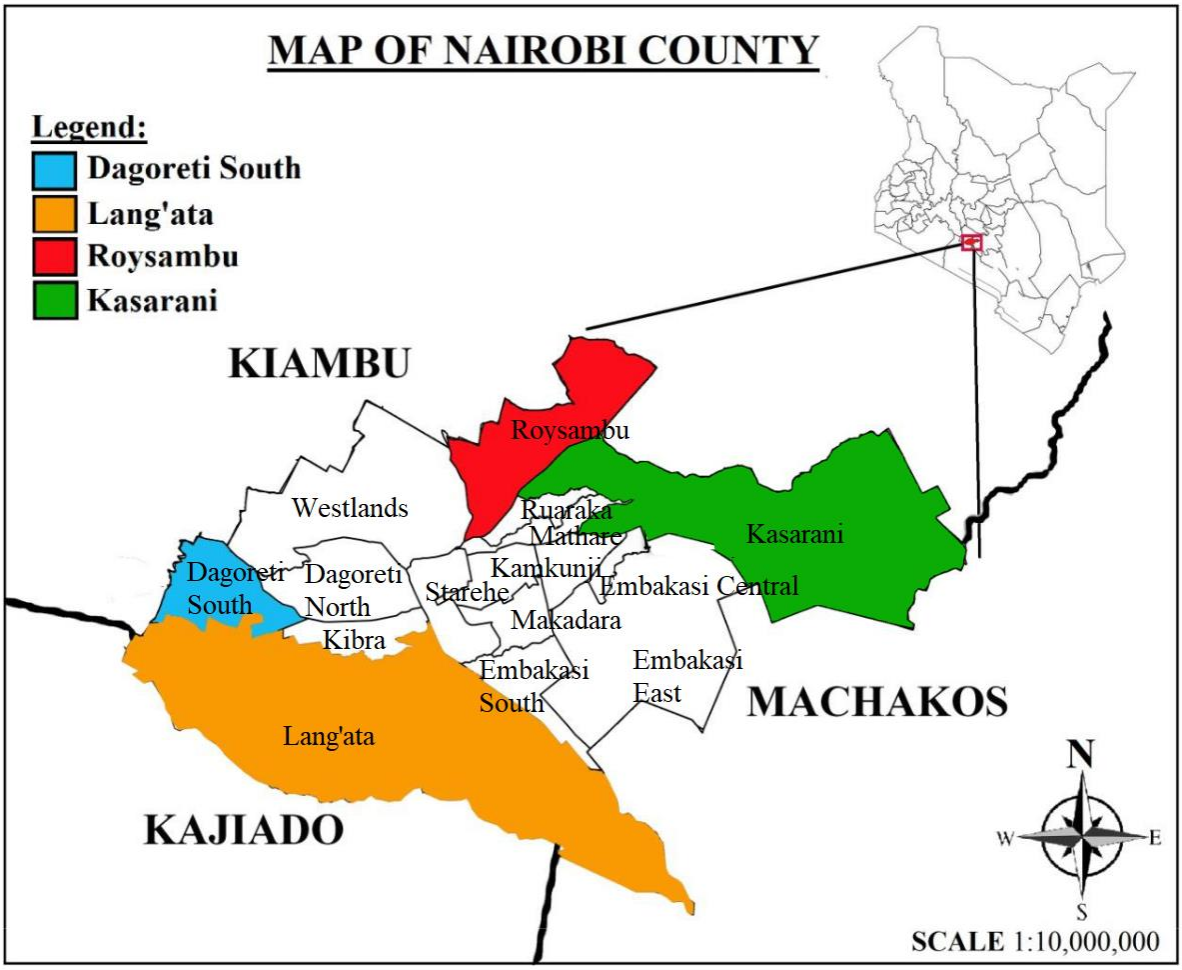

Figure 3.1: Map of Nairobi County showing the sampling sites generated via QGIS V.3.1 (Nairobi City Council, 2014)

3.2 Sample collection

Thirty farmers from each of the five regions were randomly selected with the help of Ministry of Agriculture, Livestock and Fisheries extension officers. The selected farmers were then informed about the purpose of the study. Information on poultry practices with respect to feed acquisition was obtained from participating farmers using a questionnaire after a verbal consent to take part in the survey. A hundred grams of feed was sampled from farmers after administration of the questionnaire. The samples were collected using a closed spear driven through the top and bottom sides of each sack and placed in sterile bags, sealed and stored at 4°C. A total of 150 feed samples (50 chick mash, 50 grower mash and 50-layer mash) were collected between January 2019 and February 2019 from the five regions.

3.3 Determination of fungal contamination of feeds using serial dilution method

For each feed sample, three serial dilutions, 10^{-1}, 10^{-2}, 10^{-3} were made. From each dilution, 1 ml was plated on Potato Dextrose Agar (PDA) in triplicates using the spread plate method. The plates were incubated at 29°C for 5-7 days and examined daily for fungal growth and sporulation. On day 7, the colonies were counted on the plate and expressed in colony forming units per gram of the unit sample (CFU/g). CFU was calculated using the formula:

$$CFU = \frac{\text{Number of colonies}}{\text{Volume used}} \times \text{dilution factor}$$

Pure cultures of the fungal growth were obtained by sub-culturing onto freshly prepared PDA and incubated at 29°C for 5-7 days.

3.4 Identification of fungal isolates using morphological methods

Filamentous fungi were identified at genus level according to macro and microscopic features in accordance with Pitt and Hocking (2009). Fungal isolates identified to species level were *Penicillium spp* according to Pitt and Hocking (2009), *Aspergillus spp.* according to Klich (2002), *Fusarium spp*. according to Booth (1971). The isolation frequency (Fr) and Relative density (Rd) of each fungal genera isolated was calculated using the formula:

$$Fr\ (\%) = \frac{\text{Number of samples with a genus or species}}{\text{Total number of samples}} \times 100,$$

$$Rd\ (\%) = \frac{\text{Number of isolates of a genus or species}}{\text{Total number of fungi isolated}} \times 100,$$

3.5 Molecular Characterization of fungal isolates

Molecular analysis was done to confirm morphological identification of fungi isolated from the feed samples.

3.5.1 DNA Extraction from fungal isolates.

The DNA extraction was done by modifying Rapid procedure for the extraction of DNA from fungal spores and mycelia by John J. Weiland of USDA-ARS, Northern Crop Science Laboratory, Sugarbeet and Potato Research Unit Fargo, N.D. 58105-5677 USA.

Mycelium (0.1-0.2g) was scraped using sterile scalpel from Potato Dextrose Agar (PDA), put into 1.5 ml eppendorf tubes, weighed and then placed in a mortar. Liquid Nitrogen or white quartz sand was added and ground using a pestle until the tissue was fine. Using a micropipette, 500µl of extraction buffer [100 mM Tris-HCl (pH 8.0), 20 mM Na2 EDTA, 0.5 M NaCl and 1% sodium dodecylsulfate] was added to the mortar so that the mixture became saturated. An equal amount of phenolchloroform isoamyl alcohol (24: 24: 1) was added and grounding continued vigorously with a pestle until a thick paste was formed. From the paste, 1ml was transferred into new labeled 1.5 ml Eppendorf tube, capped and centrifuged at 16,000 rpm for 10 minutes at 4°C. Tissue debris and the sand pelleted to the bottom of the tube. Using a micropipette, 500µl of the supernatant was transferred into a new Eppendorf tube.

Afterwards, 3M sodium acetate and 600 µl of ice-cold isopropanol was added and incubated at -20°C overnight to precipitate the DNA. The tubes were then centrifuged at a speed of 12,000 rpm for 10 minutes to pellet the DNA at room temperature. The isopropanol was discarded taking care not to pour out the pellet. The pellet was washed by adding 500µl of 70% ethanol and spinning it at a speed of 12,000 rpm for 10 minutes at room temperature. Final wash of the pellet was done by adding 500µl absolute ethanol and spinning at 12,000 rpm for 10 minutes at room temperature. The absolute ethanol was discarded taking care not to dislodge the pellet. The pellet was then air dried by inverting the tubes on a clean paper towel for one hour at room temperature.

The pellet was re-suspended in 100µl of TE buffer (10 mM Tris-HCl, 1 mM Na2EDTA, pH 8.0) and 20µl of RNAse (20 ug/ml) enzyme was added to remove any RNA contamination. Quality and quantity of DNA was done by subjecting 7 µl of the preparation to 1% agarose gel electrophoresis.

3.5.2 PCR amplification of DNA extracted from fungal isolates.

Polymerase chain reaction (PCR) analysis of the internal transcribed spacers (ITS1 and ITS2) and 5.8S gene were amplified using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') regions of the ribosomal DNA gene cluster. PCR amplifications was performed on 25 µL of a reaction mixture containing MgCl2-free reaction buffer, 50 mM MgCl2, 10 mM dNTP mix, 10 µM of each primer, 5 U/µL Taq DNA polymerase and 5 ng/µL of template DNA. PCR was carried out as follows: (1) one step at 94 °C for 3 minutes; (2) 30 cycles of the following three steps: 1 minute at 94°C, 1 minute at 57°C, 1 minute at 72°C and (3) one final 10-minute step at 72°C.

The PCR products were separated by 1.2% agarose gel electrophoresis in a Tris-base, acetic acid and EDTA buffer, and stained with Ethidium Bromide. PCR product purification was done using QIAquick PCR Purification Kit (Qiagen) and sequencing performed at Inqaba Ltd (Cape Town, Republic of South Africa). The obtained DNA sequences were trimmed using CLC Genomics Workbench version 5.5.2 and then compared with the sequences deposited in the NCBI GenBank via nucleotide Blast (http://www.ncbi.nlm.nih.gov/). Phylogenetic trees were constructed using maximum likelihood with 1000 bootstraps replicates using Seaview version 5.0.4.

3.5.3 Determination of toxigenic potential of Aspergillus flavus isolates using Coconut Agar Media.

Toxigenic potential of *Aspergillus flavus* isolates was done according to Lin (1976). Readily available brands of shredded coconut were obtained from locally available supermarket. A quantity of 100 grams of shredded coconut was homogenized for 5 minutes with 200 ml of hot

distilled water. The homogenate was filtered through gauze, and the pH of the clear filtrate was adjusted to pH 7.0 with NaOH. Agar was added (20 g/liter), and the mixture was heated to boiling and cooled to about 50°C. The pH was measured and adjusted to 7.0 when necessary. The mixture was autoclaved for 18 minutes, cooled to about 40°C to 45°C and poured while being stirred into sterile petri dishes. Inoculations were done by mass conidial transfer from stock cultures maintained on PDA plates to the center of inverted plates, which were then be incubated upside down at 28°C. The reverse side of each plate was periodically observed under long-wave (365 nm) UV light for blue fluorescence. An un-inoculated plate was used as point of reference.

3.6 Aflatoxin extraction and quantification using ELISA technique

By measurement, 2g of the ground feed sample was transferred to 200 ml of 80% acetonitrile in a beaker and mixed thoroughly for 10 minutes. The sample was centrifuged at 3,500 rpm to pellet the particulate matter and the supernatant obtained. The aliquot of the extract was reconstituted in wash buffer and a ration of 1:10. Using a micropipette, 200µl of the sample diluent was dispensed into each mixing well. With the use of a new pipette tip for each, 100 µl of each standard and prepared sample was added to the appropriate mixing well containing the diluent and mixed by priming the pipette at least three times. Thereafter, 100 µl of the contents from each mixing well was transferred to a corresponding antibody coated microtiter well and incubated at room temperature for 30 minutes. The microwells were washed 5 times by filling each with PBS-Tween wash buffer and decanting the wash into a discard basin. The microwells were tapped faced down on a layer of absorbent paper to remove residual wash. Afterwards, 100 µl of HRP conjugate was added to each antibody coated well and incubated at room temperature for 30 minutes. The microwells were washed once again and 100 µl of substrate solution was added to each microwell and incubated at room temperature for 10 minutes. Finally, 100 µl of the stop solution was added to each microwell and the optical density for each microwell read with a microtitre plate reader using a 450nm filter and the results recorded.

3.7 Fumonisin extraction and quantification using ELISA technique

Exactly 5g of the ground feed sample was placed in a clean beaker and 25ml of 70% methanol was added and mixed thoroughly for 3 minutes. The sample was allowed to settle and filtered using Whatman filter paper to obtain the filtrate. Using a micropipette, 200 µl of the conjugate was dispensed into the green bordered dilution well. Using a fresh pipette tip for each, 100 µl of each standard or sample was dispensed into the dilution well containing the 200 µl conjugate and mixed well by priming the pipette at least three times. Afterwards, 100 µl of the contents from each dilution well was transferred into a corresponding antibody coated microwell and incubated at room temperature for 10 minutes. Contents of each microwell were emptied into a waste container and washed by filling each well with distilled water five times. The microwells were tapped faced down on a layer of absorbent paper to remove residual wash. Using a micropipette, 100 µl of the substrate was added into each microwell and incubated at room temperature for 5 minutes. Finally, 100 µl of the stop solution was then added and the optical density for each microwell read with a microtitre plate reader using a 450nm filter and the results record

3.8 Statistical analysis

Statistical analyses were performed using R version 3.5.2 with 95% accuracy. Poisson negative binomial distribution was run to predict the number of fungal counts based on region and feed type in the five regions while two-way Analysis of Variance (ANOVA) with interaction effect was used to compare aflatoxin and fumonisin concentration between the feed types and the five regions

CHAPTER FOUR: RESULTS

4.1 Poultry practices carried out by farmers in Nairobi County.

4.1.1 Farmer preference on poultry layer breeds

All the farmers from the 150 identified poultry farms gave their consent to take part in the survey. Farmers from Dagoretti south, Ruai, Kasarani and Langata preferred the layer breed Isa brown while farmers from Roysambu preferred the layer breed Kenbro, (Table 4.1). Other layer breeds identified were Kuroiler, Hy-line brown, Leghorn, Brown leghorn and Rhode Island. Farmers located in Dagoretti South, Ruai, Roysambu and Langata acknowledged high egg production as the main reason for choosing their layer breed while farmers from Kasarani stated the criteria for breed selection is the resultant brown egg shell color, (Table 4.2). Additional criteria cited included good feed conversion ratio, availability of the breed from the hatcheries, consistency in production, good quality of eggs and meat, adaptability to the environment, market preference and cost of production.

Table 4.1: Farmer preference of poultry layer breeds in Nairobi County

Breed / Region	Isa brown n (%)	Rhode Island n (%)	Kenbro n (%)	Kuroiler n (%)	Brown leghorn n (%)	Others n (%)
Dagoretti south (n=30)	19 (63%)	5 (17%)	4 (13%)	2 (7%)	0 (0%)	0 (0%)
Kasarani (n=30)	17 (57%)	8 (27%)	4 (13%)	1 (3%)	0 (0%)	0 (0%)
Langata (n=30)	21 (70%)	0 (0%)	1 (3%)	1 (3%)	2 (7%)	5 (17%)
Roysambu (n=30)	4 (13%)	2 (7%)	13 (43%)	0 (0%)	0 (0%)	11 (37%)
Ruai (n=30)	20 (67%)	0 (0%)	0 (0%)	9 (30%)	0 (0%)	1 (3%)

***n = number of farmers with the breed; %percentage**

Table 4.2: Factors considered by farmers during selection of poultry layer breed in Nairobi County

Region / Reason for preference	Dagoretti south n (%)	Kasarani n (%)	Langata n (%)	Roysambu n (%)	Ruai n (%)
High egg Production	17 (57%)	0 (0%)	16 (53%)	10 (33%)	10 (33%)
Disease resistance	1 (3%)	0 (0%)	1 (3%)	5 (16.67%)	1 (3.33%)
Longer laying Period	0 (0%)	1 (3%)	0 (0%)	5 (16.67%)	1 (3.33%)
Fast maturity rate	0 (0%)	0 (0%)	0 (0%)	2 (7%)	0 (0%)
Brown egg shell Colour	0 (0%)	15 (50%)	0 (0%)	2 (7%)	0 (0%)
(others)	12 (40%)	14 (47%)	13 (43%)	6 (20%)	18(60%)

4.1.2 Source of layer feeds to farmers in Nairobi County

Over 80% of farmers from the five regions preferred buying their feeds from agrovets rather than formulating their own feeds, (Table 4.3). Agrovets were popular due to their proximity to farmers and ability to sell small quantities of feeds to farmers (Table 4.3).

Table 4.3: Source of poultry layer feeds to farmers in Nairobi County

Region / Feed source	Dagoretti South n (%)	Kasarani n (%)	Langata n (%)	Roysambu n (%)	Ruai n (%)
Buy	27 (90%)	30 (100%)	30 (100%)	30 (100%)	26 (87%)
Formulate	3 (10%)	0 (0%)	0 (0%)	0 (0%)	4 (13%)
Region / Feed supply					
Agrovet	25 (83%)	30 (100%)	30 (100%)	25 (83%)	23 (77%)
Manufacturer	2 (7%)	0 (0%)	0 (0%)	5 (17%)	3 (10%)
N/A	3 (10%)	0 (0%)	0 (0%)	0 (0%)	4 (13%)

4.1.3 Farmer preference on poultry layer feed brands in Nairobi County

Farmers pay close attention not only to the availability of poultry feed but also to the quality and the proper usage of the feeds as they account for 60-70% of total production costs. Presently, there are a large number of feed millers producing poultry feeds in Kenya. Some of the most popular poultry feed millers in Nairobi County are Unga feeds, Sigma feeds, Pembe feeds, Care vet feeds, Farm feeds, Premium feeds, Farmers choice and Bellfast millers. Brand A type of feed was preferred in Ruai, Roysambu and Langata while Brand B was favored in Kasarani. Both brand A and B were preferred in Dagoretti South, (Table 4.4). Consistency in quality and steady market prices were the main factors considered when choosing a particular feed brand.

Table 4.3: **Farmers preference on poultry layer feed brand in Nairobi County**

Region / Feed brand	Dagoretti South n (%)	Kasarani n (%)	Langata n (%)	Roysambu n (%)	Ruai n (%)
Brand A	7 (23%)	4 (13%)	11 (37%)	12 (40%)	12 (40%)
Brand B	7 (23%)	8 (27%)	6 (20%)	4 (13%)	4 (13%)
Brand C	3 (10%)	5 (17%)	2 (67%)	0 (0%)	0 (0%)
Brand D	5 (17%)	0 (0%)	5(17%)	0 (0%)	0 (0%)
Others	8 (27%)	13 (43%)	6 (20%)	14 (47%)	14 (47%)

4.1.5 Poultry feed storage methods used by farmers in Nairobi County

Sacks were the ideal storage item for feed used by farmers in all the five regions, (Table 4.5). Over 50% of farmers from Dagoretti south, Ruai, Roysambu and Langata regions examined their feeds daily for any signs of contamination except for farmers in Kasarani, (Table 4.6).

Table 4.4: Farmers preference on feed storage item in Nairobi County

Region / Storage item	Dagoretti south n (%)	Kasarani n (%)	Langata n (%)	Roysambu n (%)	Ruai n (%)
Sacks	16 (53%)	30 (100%)	29 (97%)	27 (90%)	30 (100%)
Plastic containers	6 (20%)	0 (0%)	1 (3%)	3 (10%)	0 (0%)
Metal containers	8 (27%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 4.5: Frequency of examination of layer feeds for signs of contamination by farmers

Region / Feed check	Dagoretti South n (%)	Kasarani n (%)	Langata n (%)	Roysambu n (%)	Ruai n (%)
Daily	28 (93%)	3 (10%)	23 (77%)	16 (53%)	25 (83%)
Weekly	2 (7%)	0 (0%)	1 (3%)	6 (20%)	2 (7%)
Monthly	0 (0%)	1 (3%)	0 (0%)	8 (27%)	0 (0%)
Not at all	0 (0%)	26 (27%)	6 (20%)	0 (0%)	3 (10%)

4.1.6 Incidences of aflatoxicosis in layer flock by farmers in Nairobi County, Kenya

More than 80% of farmers from the five regions have not encountered any case of aflatoxicosis in their flock, Table (4.7). Shifting from one brand of feed to the next was the preferred solution for farmers who had encountered aflatoxicosis in their flock.

Table 4.6: Incidences of aflatoxicosis in layer flock by farmers

Region / Aflatoxicosis cases	Dagoretti South n (%)	Kasarani n (%)	Langata n (%)	Roysambu n (%)	Ruai n (%)
Yes	2 (7%)	0 (0%)	3 (10%)	1 (3%)	5 (17%)
No	28 (93%)	30 (100%)	27 (90%)	29 (97%)	25 (83%)

4.2 Fungal contamination of feeds

All the feed samples had positive growth for at least one type of fungus. Chick mash had a CFU of $6.11\times10^{-2}\pm4.51$ while growers mash had a mean total CFU of $5.80\times10^{-2}\pm4.69$ and layers mash had a mean total CFU of $4.73\times10^{-2}\pm3.866$. Feeds from Kasarani had the highest mean total CFU of $7.16\times10^{-2}\pm4.68$ whereas feeds from Roysambu recorded the lowest mean CFU of $4.20\times10^{-2}\pm3.22$. The mean total CFU of feeds from Langata, Ruai and Dagoretti South were $5.76\times10^{-2}\pm4.43$, $5.13\times10^{-2}\pm3.62$ and $5.49\times10^{-2}\pm5.28$ respectively. Mycological quality of the feeds were determined according to Gimeno where samples can be qualified as good (count range $<3.10^{-4}$ CFU/g), regular (count range 3.10^{-4}–7.10^{-4} CFU/g), and bad ($>7.10^{-4}$ CFU/g) (Greco *et al*., 2014). According to this criterion, all the feed samples regardless of the feed type were qualified as good. A negative binomial Poisson regression was run to predict the number of fungal counts based on region and feed type. Region and feed type had a significant effect on the number of fungal counts in Kasarani, Langata and Dagoretti south ($P < 0.05$) while in Roysambu and Ruiru there was no significant effect on the number of fungal counts ($P > 0.05$).

4.3 Mycoflora of poultry feeds sampled from Nairobi County

Seven fungal genera were isolated from layer feeds, three of which, *Aspergillus, Fusarium* and *Penicillium,* are known to be mycotoxigenic, (Table 4.8).

Table 4.7: Incidence of fungi on poultry layer feeds in Nairobi County (n =150)

Fungal genera	No of isolates*	Isolation frequency (%)	Relative density (%)
Aspergillus spp	136	90.67	44.01
Penicillium spp	116	77.33	37.54
Fusarium spp	20	13.33	6.47
Mucor spp	16	10.67	5.18
Rhizopus spp	14	9.33	4.53
Cladosporium spp	6	4	1.94
Cochliobolus spp	1	0.007	0.32

* Total number of samples was 150

4.3.1 Microscopic and macroscopic features of *Aspergillus spp.*

Aspergillus flavus Complex

Colony diameter at 7 days: 30mm-40mm

Colony colors and textures: Colonies varied from yellow, yellowish green, plain green to dark green with white mycelia at the edges and had pale yellow as the reverse color. Some strains produced dark brown to black sclerotia and brown exudates with glass like appearance.

Microscopic characteristics as illustrated in Figure 4.1: Conidial heads were radiate which later split into loose columns with age, mostly uniserate. Conidiophore stipes were smooth walled, hyaline and coarsely roughened, often noticeable near the vesicle. Vesicle shape varied from globose to subglobose with the phialides covering up to three quarter of the vesicle. Conidia were globose to subglobose, pale green with smooth or finely roughened walls.

Diagnostic feature: Colonies were yellow green with coarsely roughened stipes near the vesicle and conidia that were smooth to finely roughen.

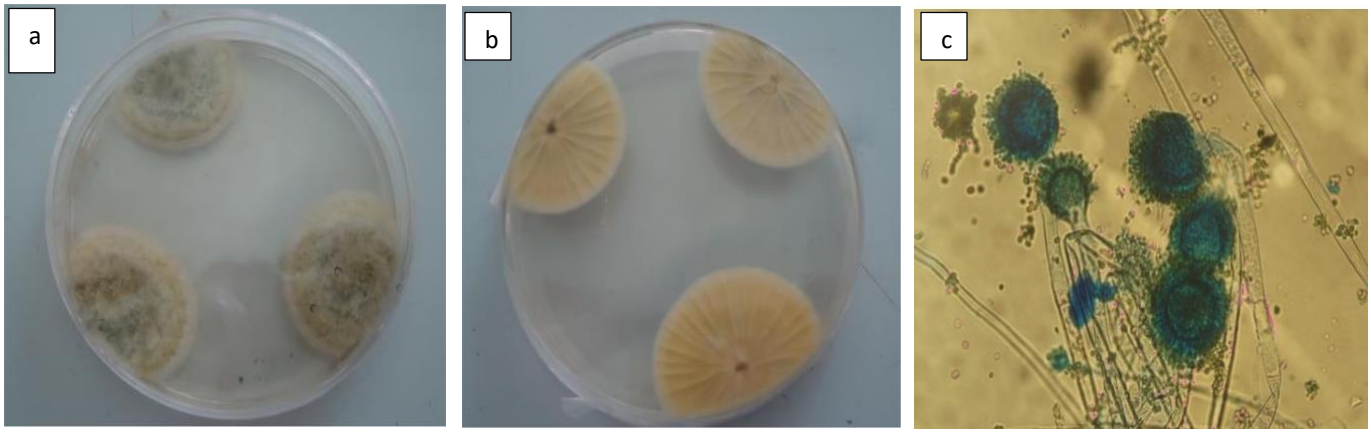

Figure 4.1: Macroscopic and Microscopic features of *Aspergillus flavus* grown on PDA. (a) Colonies showing the yellowish green color encircled with a white border; (b) The reverse pale yellow colour of the colonies; (c) Biserate conidiophores bearing conidia on globose vesicles (40X)

Aspergillus fumigatus

Colony diameter at 7 days: 40mm

Colony colors and texture: Colonies were greyish green with white mycelia at the edges and had greyish ash as the reverse color.

Microscopic characteristics as illustrated in Figure 4.2: Conidial heads were typically columnar and uniserate. Conidiophore stipes were short smooth walled with conical or pyriform shaped vesicles. They were also smooth walled, uncolored or greyish near the apices. Vesicles had compact phialide covering confined to the upper two thirds and curving to be roughly parallel towards each other. Conidia were globose to subglobose, smooth to finely roughened and green in color.

Diagnostic features: Colonies were greyish green, columnar with phialides constricted to the upper two thirds of the vesicle and curving to be roughly parallel towards each other and the axis of the stipe.

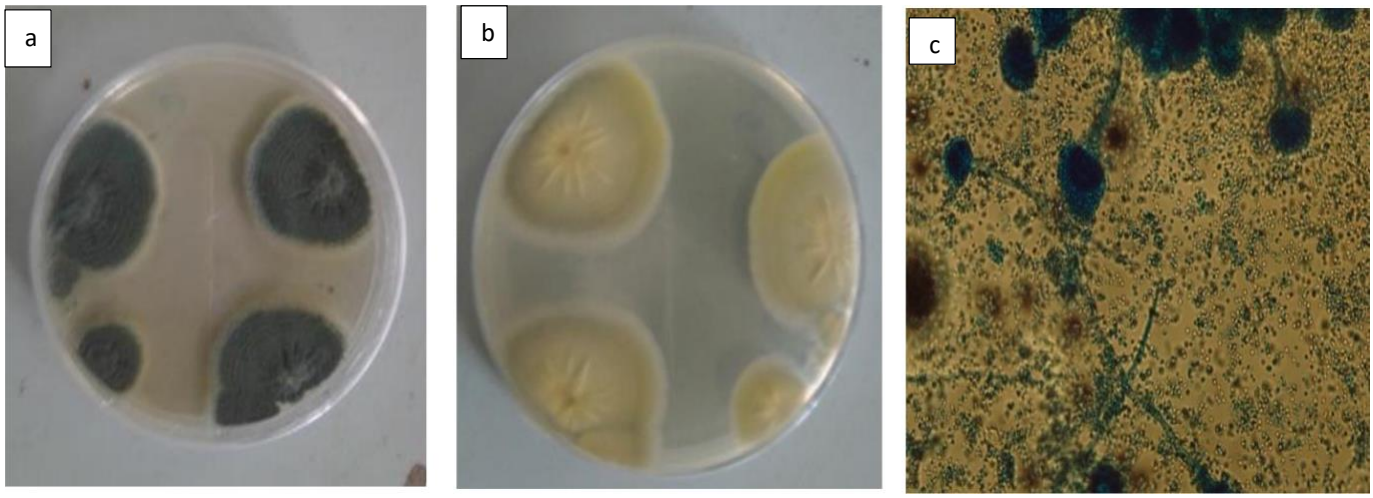

Figure 4.2: Macroscopic and Microscopic features of *Aspergillus fumigatus* grown on PDA. (a) Colonies showing the greyish green color encircled with a white border; (b) The reverse ash grey color of the colonies; (c) Uniserate conidiophores bearing pyriform vesicles (40X*)*

Aspergillus niger

Colony diameter at 7 days: 30-45mm

Colony colors and textures: Colonies were black with white or yellow mycelium at the edges and had pale yellow as the reverse color.

Microscopic characteristics as illustrated in Figure 4.3: Conidial heads were radiate and biserate bearing globose vesicles. Conidiophore stipes were smooth walled, hyaline and turning dark or slightly brown towards the vesicle. They had an entire metulae covering bearing conidia that were dark brown to black, globose and rough walled.

Diagnostic features: Colonies were black, biserate and had large vesicles with dark brown or black conidia that were rough walled.

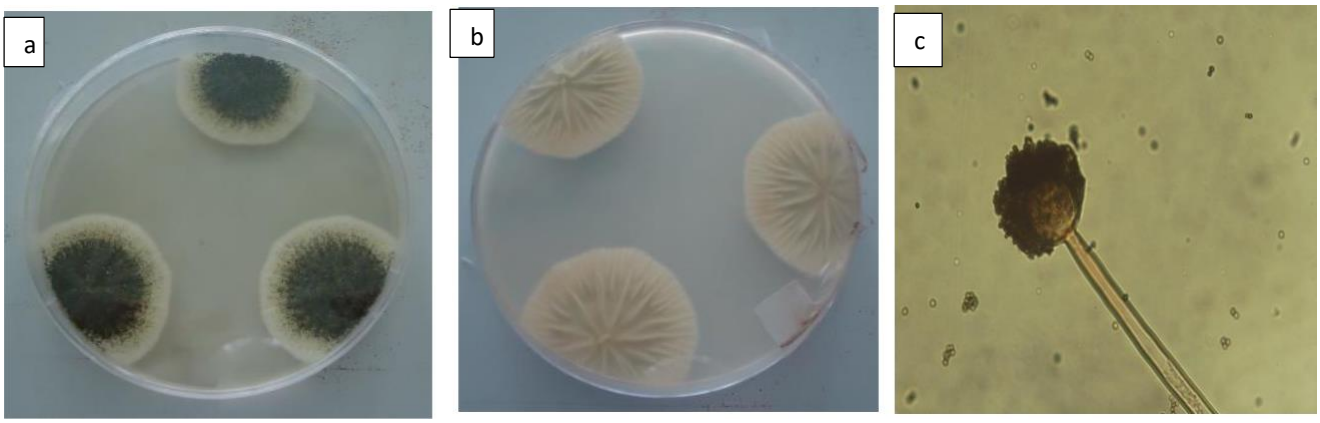

Figure 4.3: **Macroscopic and Microscopic features of *Aspergillus niger* grown on PDA. (a) Colonies showing the black color encircled with a white border; (b) The reverse pale yellow color of the colonies; (c) Biserate conidiophore bearing rough walled conidia on globose vesicle (40X)**

Aspergillus clavatus

Colony diameter at 7 days: 20mm-25mm

Colony colors and textures: Colonies were greenish grey with white mycelia at the edges. The reverse color was white to dull yellow.

Microscopic characteristics as illustrated in Figure 4.4: Conidial heads were radiate and uniserate with clavate vesicles where the phialide covering was entire. Conidiophore stipes were smooth walled, colorless to slightly brown near the apices. Conidia were green in color, smooth and ellipsoidal.

Diagnostic features: Formation of large club shaped vesicles, uniserate, with closely packed phialides bearing green ellipsoidal conidia.

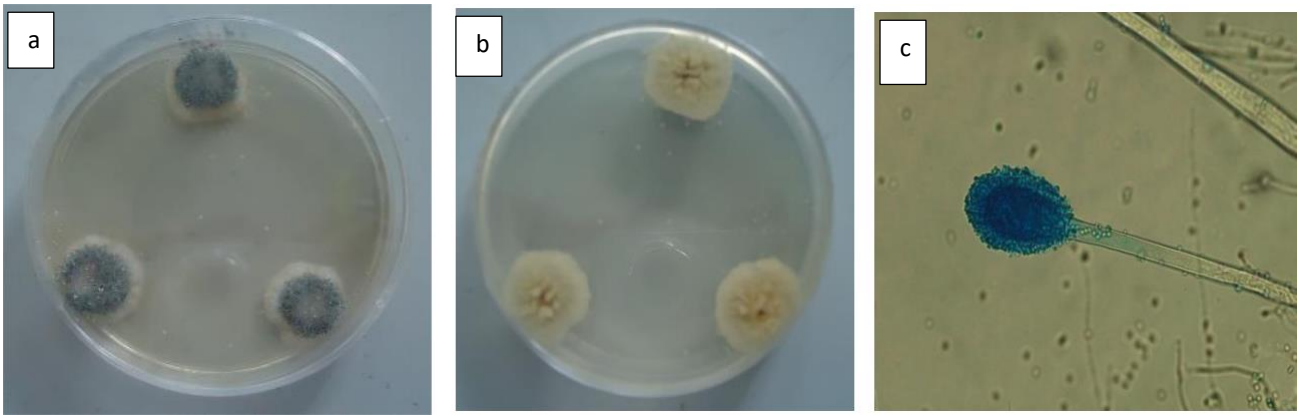

Figure 4.4: Macroscopic and Microscopic features of *Aspergillus clavatus* grown on PDA. (a) Colonies showing the greenish grey color encircled with a white border; (b) The reverse white color of the colonies; (c) Uniserate conidiophore bearing clavate vesicle with an entire phialide covering (40X)

Aspergillus novoparasiticus

Colony diameter at 7 days: 30mm-40mm

Colony colors and textures: Colonies were dark green with white mycelia at the edges which roughened with age after splitting into columns and had brown as the reverse color.

Microscopic characteristics as illustrated in Figure 4.5: Conidial heads were radiate and uniserate where the philaide covering was at least half of the vesicle. Conidiophore stipes were short, smooth and uncolored. Their vesicles ranged from pyriform to globose in shape. Conidia were green, globose to sub globose and distinctly rough walled.

Diagnostic feature: Colonies were dark green in color, uniserate, bearing conidia that were distinctly roughened.

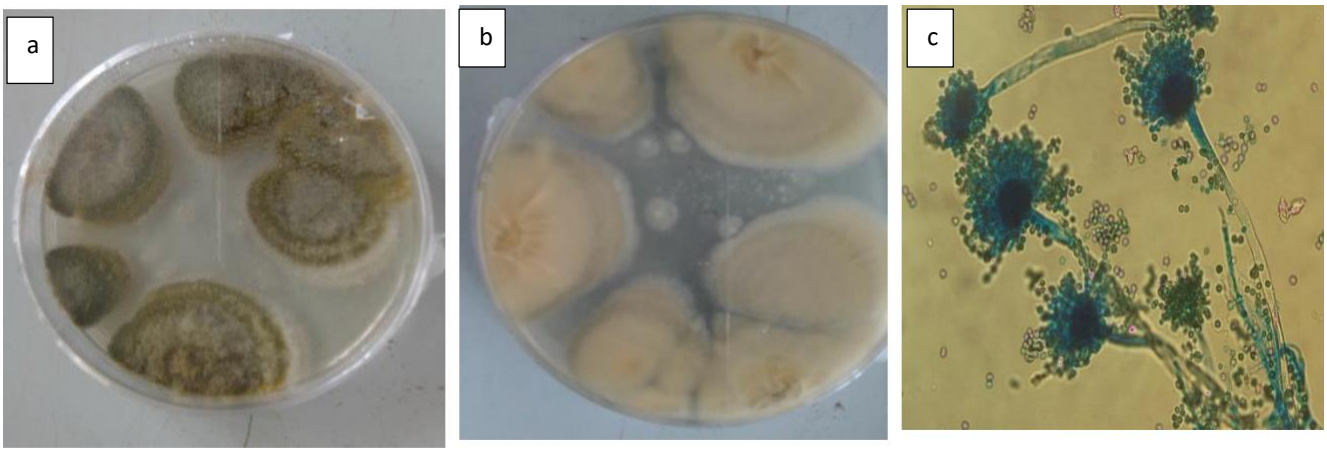

Figure 4.5: Macroscopic and Microscopic features of *Aspergillus novoparasiticus* grown on PDA. (a) Colonies showing the dark green color encircled with a white border; (b) The reverse brown color of the colonies; (c) Uniserate conidiophores bearing globose vesicles which bear distinctly rough walled conidia (40X)

Aspergillus ruber

Colony colors and textures: Colonies were small, yellow green surrounded by white mycelia at the edges and had pale brown as the reverse color.

Microscopic characteristics as illustrated in Figure 4.6: Conidial heads were loosely radiate and uniserate. Conidiophore stipes were smooth walled, hyaline and coarsely roughened near the vesicle. Vesicle shape varied from globose to pyriform with the phialides covering half of the vesicle, there were few phialides per conidia. Conidia were ovate and were distinctly echinulate.

Diagnostic feature: Colonies were small, yellow green in color with elliptical conidia that were distinctly echinulate.

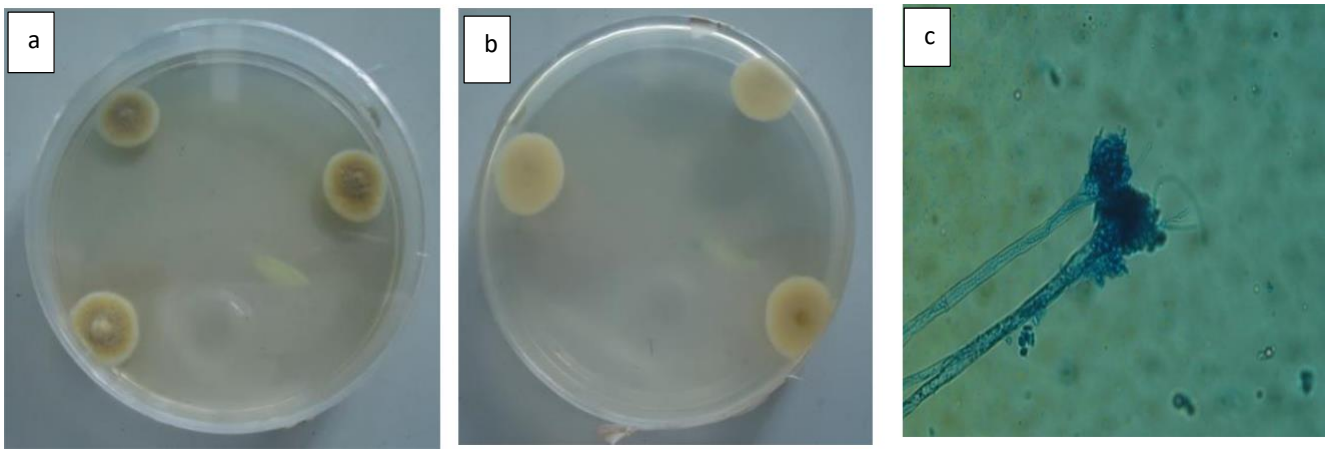

Figure 4.6: Macroscopic and Microscopic features of *Aspergillus ruber* grown on PDA. (a) Colonies showing the yellow green color encircled with a white border; (b) The reverse brown color of the colonies; (c) Uniserate conidiophores bearing globose vesicles which bear distinctly rough walled oval conidia (40X)

Aspergillus tamarii

Colony diameter: 30mm-35mm

Colony colors and textures: Colonies were circular, olive brown to deep brown, surrounded by white mycelia at the margin and had dull white as the reverse color

Microscopic characteristics as illustrated in Figure 4.7: Conidial heads were radiate and uniserate. Conidiophore stipes were rough walled and uncolored. Vesicle shape varied from globose to subglobose with the phialides covering the entire vesicle. Conidia were globose to subglobose and conspicuously echinulate.

Diagnostic feature: Colonies were deep brown bearing rough walled conidia that were globose to subglobose in shape

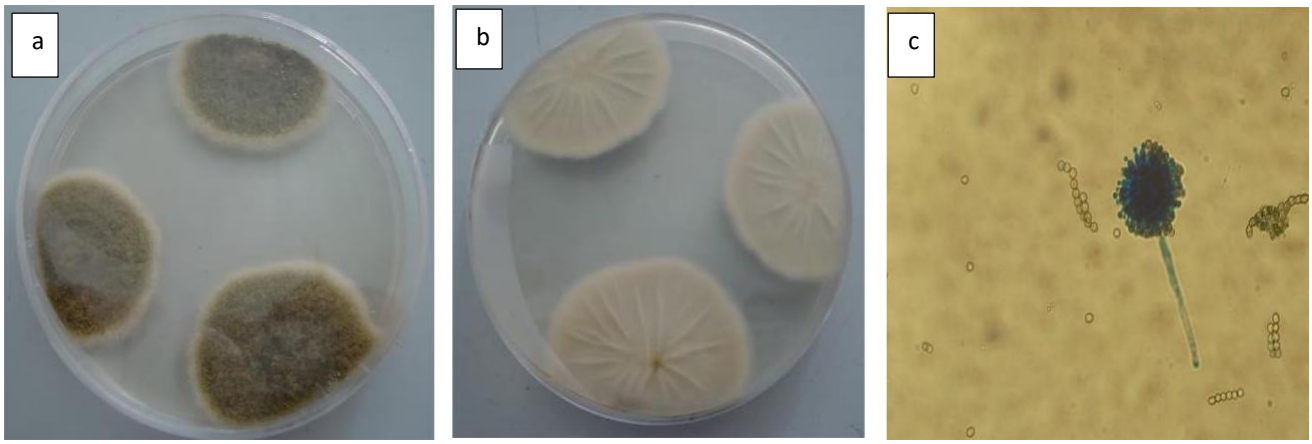

Figure 4.7: Macroscopic and Microscopic features of *Aspergillus tamarii* grown on PDA. (a) Colonies showing the olive brown color encircled with a white border; (b) The reverse dull white color of the colonies; (c) Uniserate conidiophores bearing globose vesicles with an entire phialide covering (40X)

4.3.2 Macroscopic and microscopic features of *Fusarium species*

Fusarium verticilloides

Colony diameter: 45mm

Colony colors and textures: Colonies had delicate white or peach mycelium but with a purple tinge, floccose like growth and had deep violet as the reverse color.

Microscopic characteristics as illustrated in Figure 4.8: Micro conidiophores were simple, lateral with subulate phialides formed on aerial hyphae**.** Microconidia were formed in chains, abundant, fusiform to clavate with a slightly flattened base and occasionally became one septate. Macroconidia were sparse, thin walled, fusoid, with an elongated sharply curved apical cell, mostly three septate although some were four septate. Swollen stromatic cells that were somewhat globose in shape were present in the hyphae.

Diagnostic feature: Colonies had delicate floccose white to peach mycelium with a sparse tinge of purple and deep violet as the reverse color. Microconidia were produced in chains from relatively long phialides.

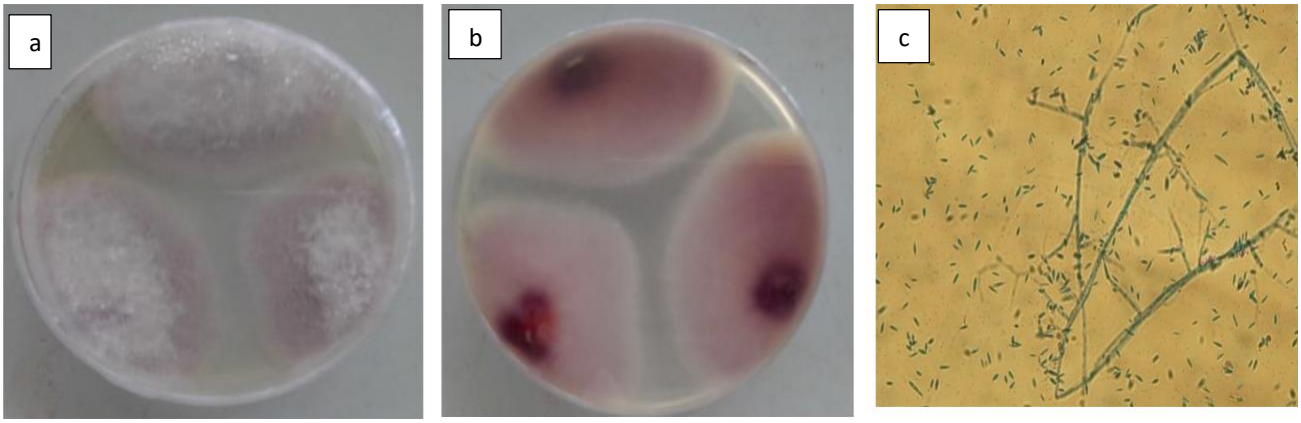

Figure 4.8: Macroscopic and Microscopic features of *Fusarium verticilloides* grown on PDA. (a) Colonies showing the delicate peach mycelium with a purple tinge; (b) The reverse violet color of the colonies; (c) Numerous microconidia of *Fusarium verticilloides* that are fusiform in shape (40X)

4.3.3 Macroscopic and microscopic features of *Penicillium spp*

Penicillium aethiopicum

Colony diameter: 25mm-35mm

Colony colors and textures: Colonies were blue green in color surrounded by white mycelia at the margin, radially sulcate, circular and had maize yellow as the reverse color. They had a suede like texture with clear liquid exudates.

Microscopic characteristics as illustrated in Figure 4.9: Had septate hyphae, conidiophores were simple, smooth walled and commonly borne in fascicles. Metulae that bore flask shaped phialides had terverticillate branching bearing conidia in chains that were ellipsoidal in shape and pale green in color.

Diagnostic feature: Colonies were fast growing producing blue green conidia with conidiophores that had terverticillate branching and yellow as the reverse color.

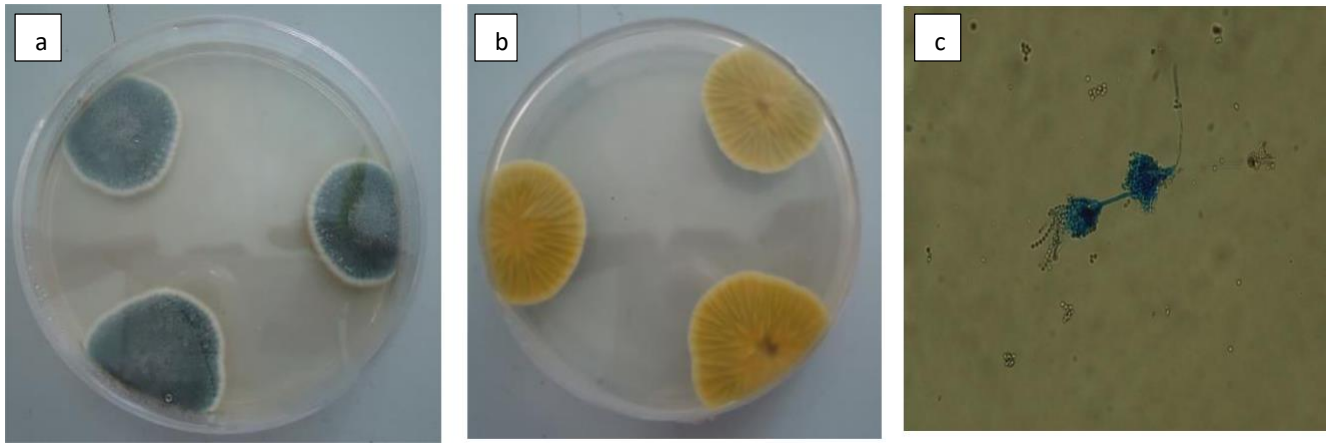

Figure 4.9: Macroscopic and Microscopic features of *Penicillium aethiopicum* grown on PDA. (a) Colonies showing the blue green color encircled with a white border; (b) The reverse maize yellow color of the colonies; (c) Conidiophores bearing ellipsoidal conidia in chains (40X).

Penicillium crustosum

Colony diameters: 26mm-30mm

Colony colors and textures: Colonies were low with veluntious or granular texture, became blue green as conidia develop often greyish turquoise at the marginal areas. The colonies had bright yellow as the reverse color.

Microscopic characteristics as illustrated in Figure 4.10: Hyphae were septate with branched conidiophores that were rough walled, long, bearing terminal penicilli with either terverticillate or quaterverticillate branching bearing flask shaped phialides and globose conidia in chains.

Diagnostic feature: Colonies often displayed blue green margins, but mature conidia were grey green having bright yellow as the reverse color. They had long conidiophores that were rough walled.

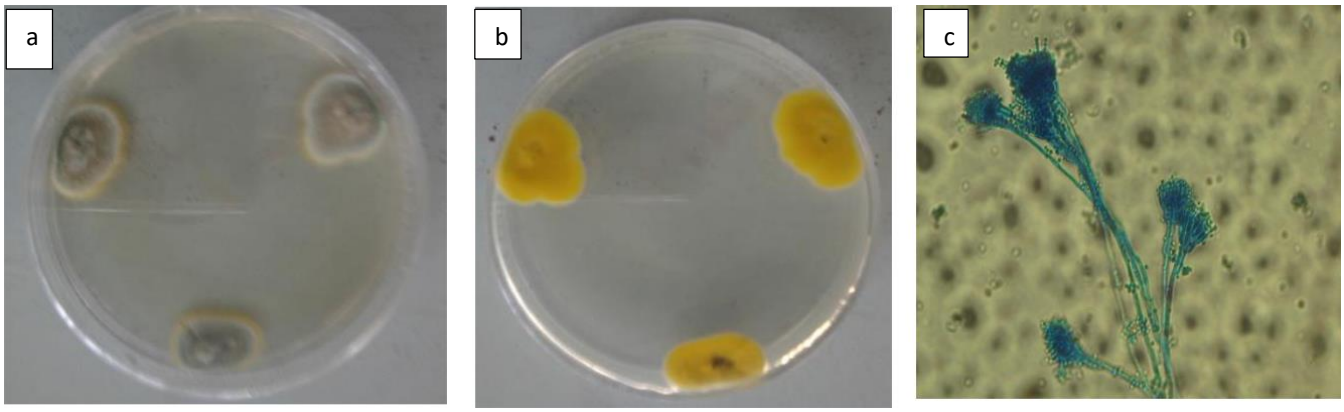

Figure 4.10: Macroscopic and Microscopic features of *Penicillium crustosum* grown on PDA. (a) Colonies showing the greyish turquoise color encircled with a white border; (b) The reverse bright yellow color of the colonies; (c) Long conidiophores bearing flask shaped phialides (40X)

4.3.4 Macroscopic and microscopic features of other fungal isolates

Mucor circinelloides

Colony color and textures: Colonies were fast growing, had cotton like appearance, greyish brown in color and had dull white as the reverse color.

Microscopic characteristics as illustrated in Figure 4.11: Had aseptate hypha with hyaline sporangiophore that were mostly sympodially branched with long branches erect and shorter branches becoming circinate. Sporangiophore bore brown sporangium which were globose in shape and enclosed ovoid sporangiospores inside. Sporangiospores were hyaline, brownish, smooth walled and ovoid in shape. Collarettes, which were remnants of the sporangial wall, were visible at the base of the columella and were spherical to ellipsoidal in shape after sporangiospore dispersal.

Diagnostic feature: Greyish brown colonies that had cotton candy appearance with globose sporangia and ovoid sporangiospores.

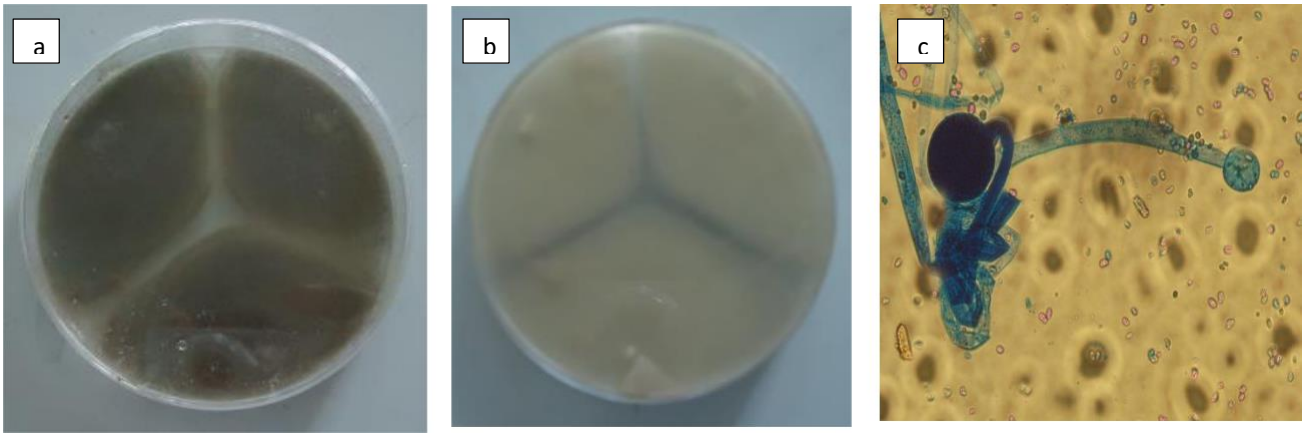

Figure 4.11: Macroscopic and Microscopic features of *Mucor circinelloides* grown on PDA. (a) Colonies showing the greyish brown color; (b) The reverse dull white color of the colonies; (c) Sympodially branched sporangiophores bearing brown sporangia (40X).

Mucor fragilis

Colony colors and textures: Colonies were fast growing covering the whole petridish, had cotton like growth with creamy white appearance and pale yellow as the reverse color. Colonies were slightly aromatic.

Microscopic characteristics as illustrated in Figure 4.12: Had aseptate hypha with hyaline sporangiophores that were mostly sympodially branched. Sporangiophore bore brownish yellow sporangium which was globose to subglobose in shape and enclosed sporangiospores that were globose to ellipsoidal in shape inside. Columellae were ellipsoidal to subglobose with a collarette.

Diagnostic feature: Colonies were fast growing, slightly aromatic with creamy white appearance and pale yellow as the reverse color.

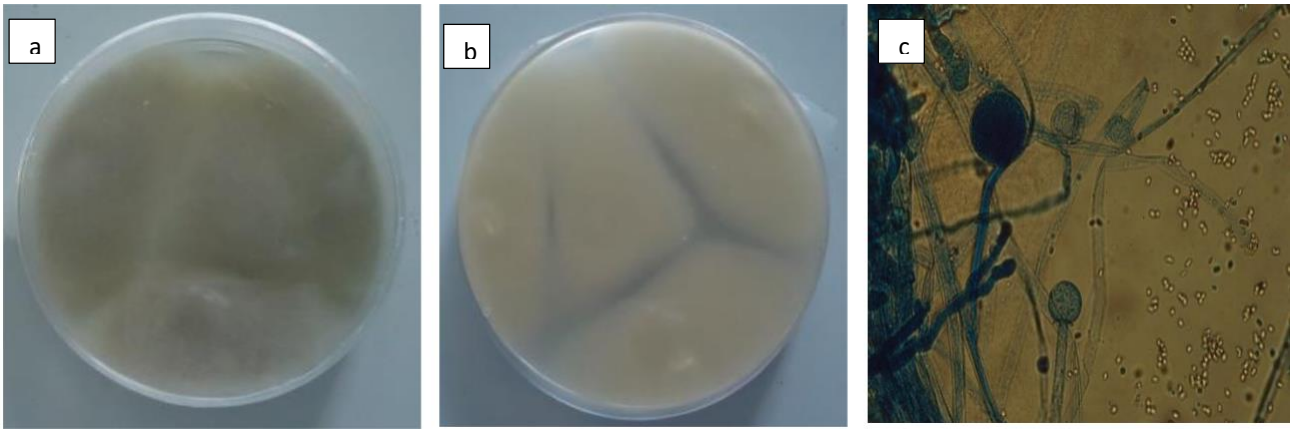

Figure 4.12: Macroscopic and Microscopic features of *Mucor fragilis* grown on PDA. (a) Colonies showing the creamy white color; (b) The reverse pale yellow color of the colonies; (c) Sympodially branched sporangiophores bearing brown sporangia (40X).

Rhizopus microsporus

Colony colors and textures: Colonies were fast growing and had cotton candy growth. Colonies were initially white then turned to grey then black with the formation of black sporangiospores and had pale white as the reverse color.

Microscopic characteristics as illustrated in Figure 4.13: Had aseptate hyphae that gave rise to sporangiophores that were brown in color and hyaline. Sporangiosphores were borne in groups of three to five from clusters of rhizoids. At the tip of the sporangiophore was the sporangium that was round with flattened bases. After releasing sporangiospores, the apophyses and columella collapsed to form an umbrella-like structure. Sporangiospores were unicellular, one celled, ovoid in shape, brown to black in color with striate walls.

Diagnostic feature: Colonies are fast growing, initially white becoming grey to black in time with pale white as the reverse color.

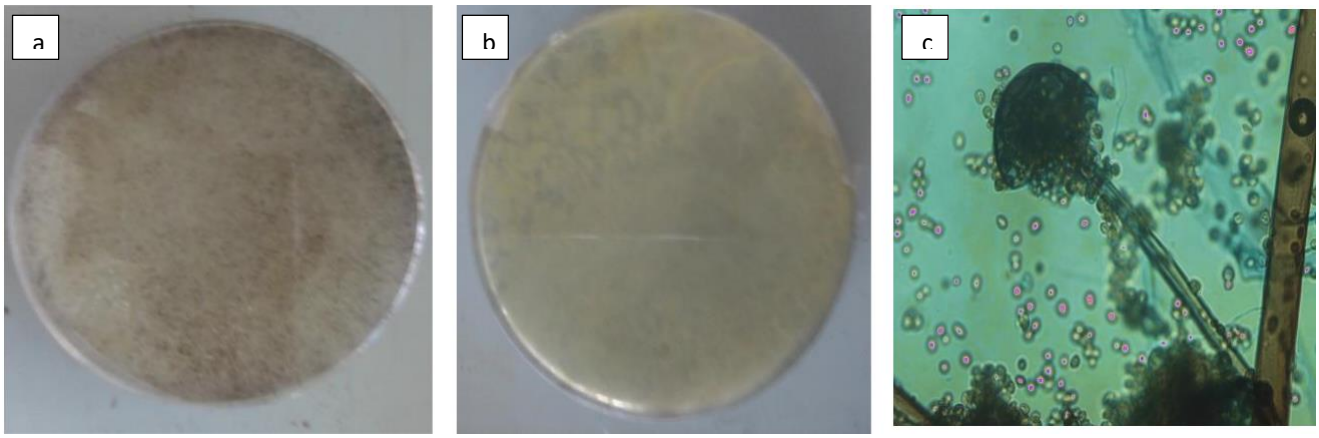

Figure 4.13: Macroscopic and Microscopic features of *Mucor circinelloides* grown on PDA. (a) Colonies showing the black color; (b) The reverse pale white color of the colonies; (c) Sporangium with a flattened base releasing sporangiophores (40X).

Cladosporium spp

Colony diameters: 35mm

Colony colors and textures: Colonies are olivaceous grey, greyish blue towards margins, fluffy, suede like to floccose texture, slightly wrinkled having iron-grey as the reverse color

Microscopic characteristics as illustrated in Figure 4.14: Conidiophores are pale brown and mostly arise terminally from hyphae. Conidiogenous cells are mainly integrated terminally with distal conidiogenous loci, crowded at or towards the apex, sometimes slightly geniculate due to sympodial proliferation. Have numerous conidia that are, brown, formed in branched chains, branching in all directions and ovoid or ellipsoid in shape.

Diagnostic feature: Colonies are olivaceous grey having iron grey as the reverse color and macronematous conidiophores bearing conidia in branched chains that are brown in color and ellipsoidal in shape.

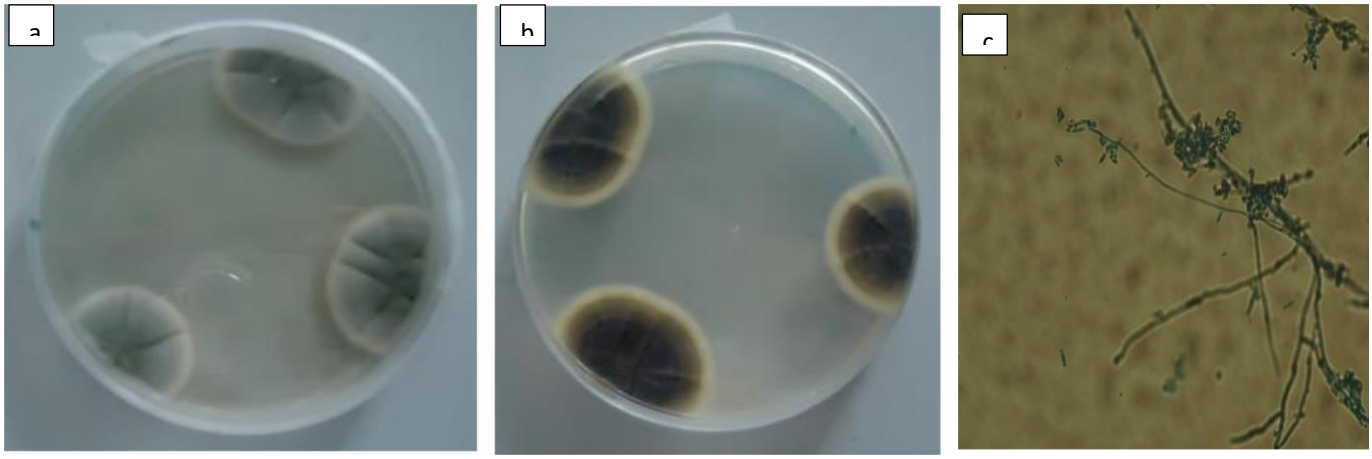

Figure 4.14: Macroscopic and Microscopic features of *Cladosporium sp* grown on PDA. (a) Colonies showing the olivaceous grey color; (b) The reverse iron grey color of the colonies; (c) Numerous conidia of *Cladosporium sp* that are ellipsoidal in shape (40X)

Cladosporium pseudocladosporoides

Colony colors and textures: Colonies are black-greyish in color, slightly wrinkled; appear raised at the center, circular with suede-like texture becoming powdery as the culture ages due to production of conidia and bluish black as the reverse color.

Microscopic characteristics as illustrated in Figure 4.15: Have dark brown septate hyphae, erect and pigmented. They have tree like conidiophores which branch irregularly by budding from youngest cells and disintegrate partially or totally in wet mounts, leaving masses of conidia, which may show buds or bud scars. Conidia are elliptical to cylindrical in shape.

Diagnostic feature: Colonies are black grayish in color have bluish black as the reverse color with elliptical to cylindrical conidia,

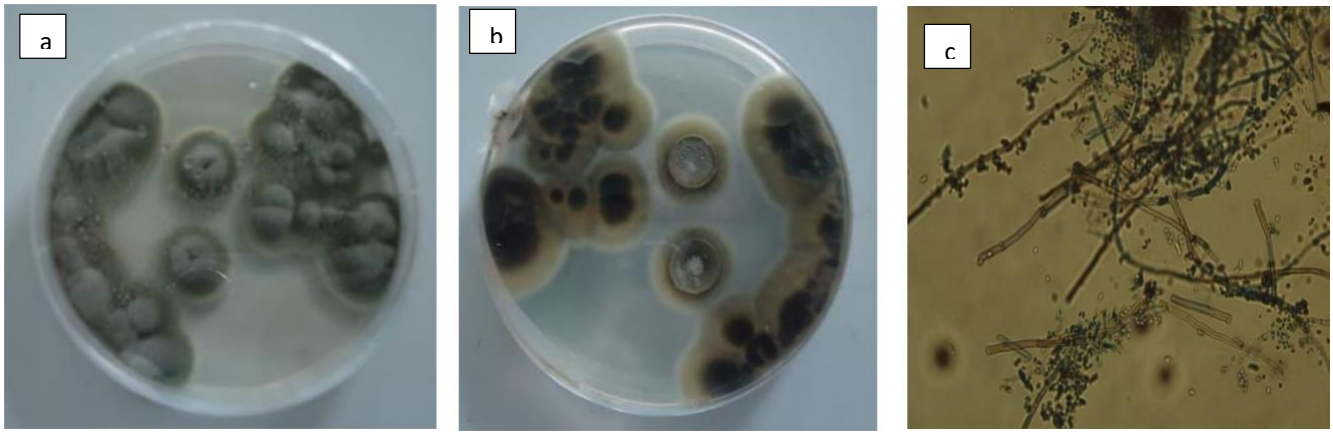

Figure 4.15: Macroscopic and Microscopic features of *Cladosporium pseudocladosporoides* grown on PDA. (a) Colonies showing the black-greyish color; (b) The reverse bluish black color of the colonies; (c) Tree like conidiophores of *Cladosporium pseudocladosporoides* that are ellipsoidal in shape (40X).

Cochliobolus heterostrophus

Colony diameters: 35mm-40mm

Colony colors and textures: Colonies are dark grey, floccose, raised with an undulate margin having bluish black as the reverse color.

Microscopic characteristics as illustrated in Figure 4.16: Conidiophores may be single or arranged in small groups, straight to flexuous or geniculate, dark brown, branched, thick-walled and septate. Conidia are slightly curved, fusoid or canoe shaped, dark brown, smooth walled, six to nine septate and mostly borne singly from nodes on knobby or geniculate mid to dark brown hyphae.

Diagnostic feature: Colonies are dark grey with bluish black as the reverse color and conidia that are six to nine septate and fusoid in shape.

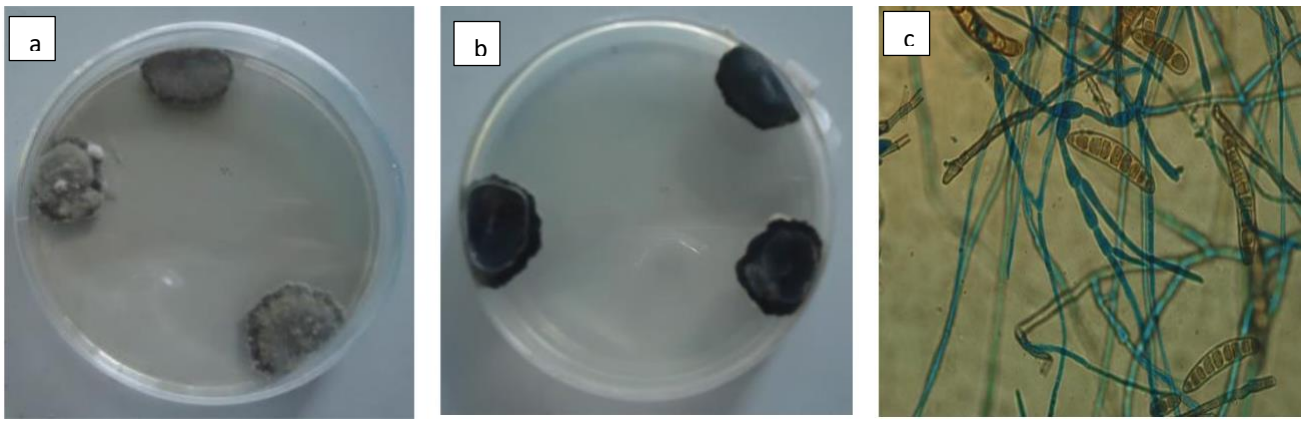

Figure 4.16: Macroscopic and Microscopic features of *Cochliobolus heterostrophus* grown on PDA. (a) Colonies showing the dark-grey color; (b) The reverse bluish black color of the colonies; (c) Dark brown six to nine septate conidia that are slightly curved, fusoid or canoe shaped

4.3 Molecular characterization of fungal isolates

4.3.1 DNA Quality and Concentration

The quality and quantity of DNA isolated from 36 fungal isolates were subjected to 1.2% agarose gel electrophoresis and considered to be good. PCR amplication using ITS1 and ITS4 yielded fragments of 500bp which were sequenced, (Figure 4.17). The obtained sequences were compared with the sequences that are deposited in the NCBI GenBank via nucleotide blast after being trimmed using CLC Genomics Workbench version 5.5.2. This confirmed the initial identification of fungal isolates using morphological methods and relevant taxonomic keys. Molecular identification had 100% similarity with the morphological identification of fungal isolates. Phylogenetic trees were constructed using Seaview version 5.0, (Figure 4.18).

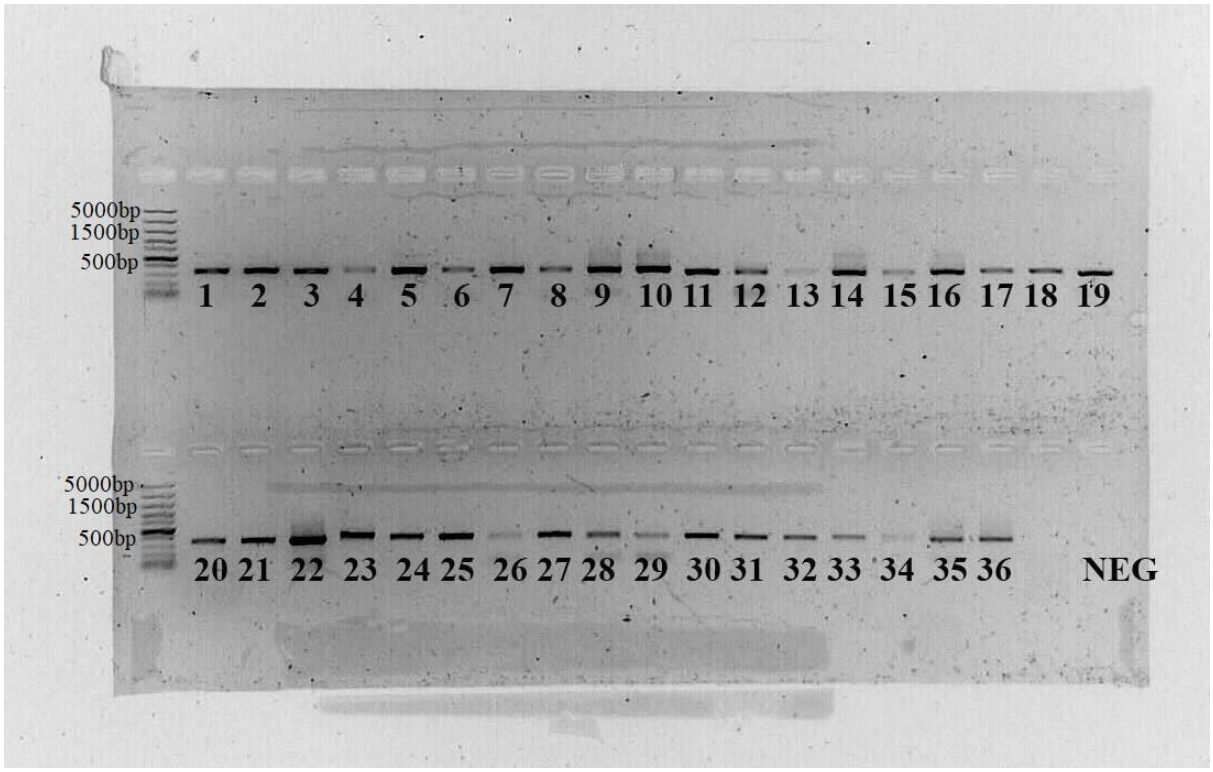

Figure 4.17: Agarose gel electrophoresis (1.2% agarose) of PCR amplified products obtained with primer pair ITS1 and ITS4, 1 kb DNA size marker.

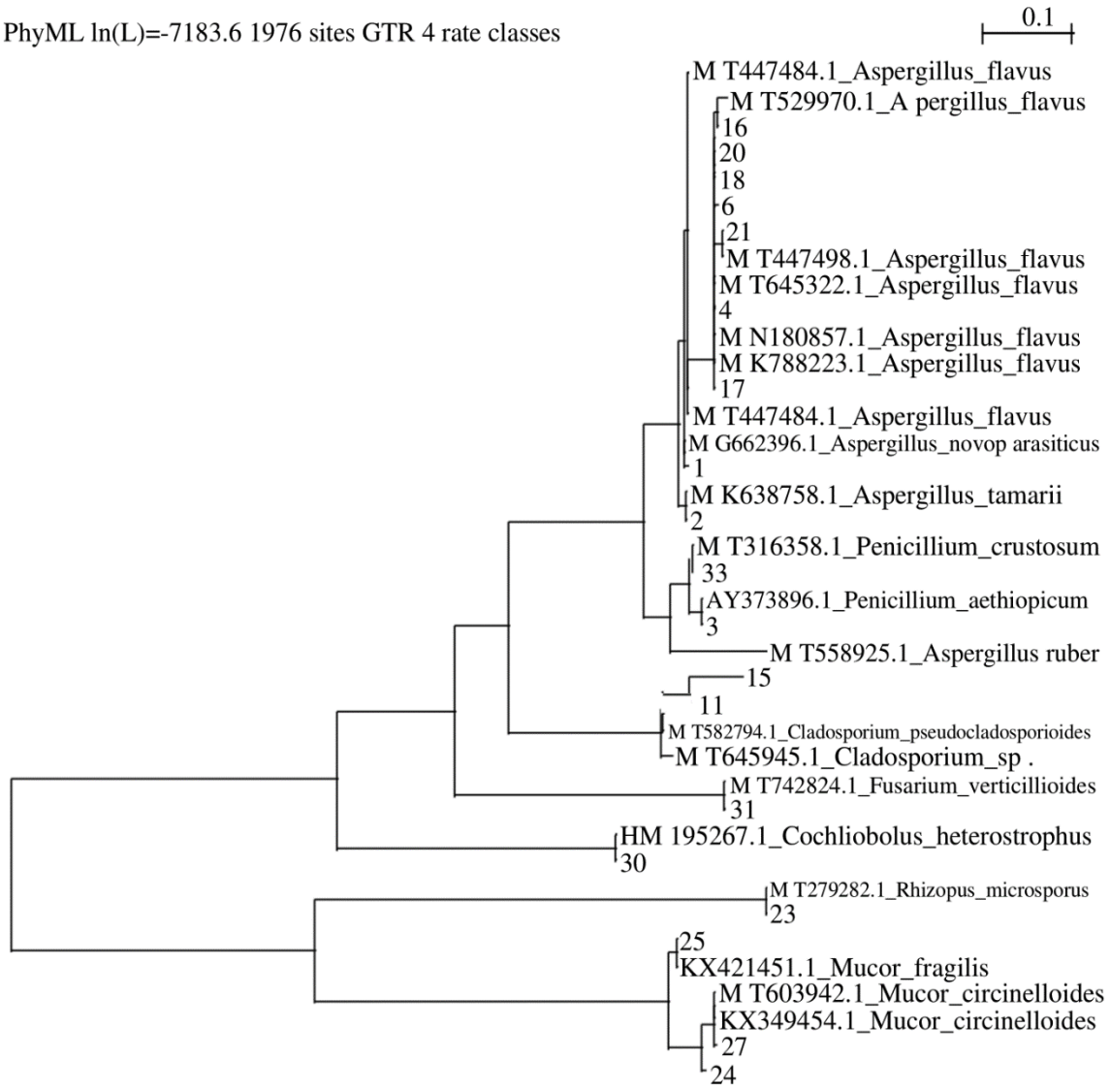

Figure 4.18: Phylogenetic analysis of ITS1-5.8S-ITS4 rDNA sequences from fungal species isolated from layer feeds in Nairobi County. The tree was constructed via maximum likelihood method and the bootstrap values were calculated from 1000 replications. GenBank access numbers are listed before the species names. Scale bar represent a genetic distance of 0.1

4.4 Toxigenic potential of *Aspergillus flavus* strains using coconut agar media.

Six different strains isolated from feed samples were plated in Coconut Agar Medium and examined for blue fluorescence under ultraviolet light at 365nm. Three strains of *Aspergillus flavus* emitted the blue fluorescence confirming the production of aflatoxins, (Figure 4.19).

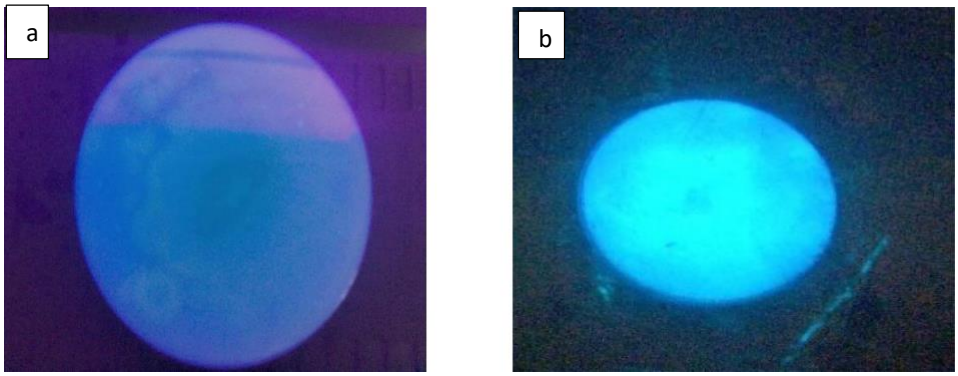

Figure 4.19: Fluorescence under UV light at 365 nm in coconut agar medium on the fourth day of incubation at 28°C. (a) Colony of non-aflatoxigenic *Aspergillus flavus* strain without fluorescence; (b) Colony of aflatoxigenic *Aspergillus flavus* strain exhibiting the characteristic blue fluorescence.

4.5 Aflatoxin and fumonisin contamination of layer feeds sampled from Nairobi County, Kenya

4.5.1 Aflatoxin contamination of poultry feeds sampled from Nairobi County, Kenya

Ninety-eight samples (65%) had quantifiable levels of AF while fifty-two samples (35%) had no detectable levels of AF. Sixty-three samples (42%) exceeded the Kenya Bureau of Standards (KEBS) of 10 µg/kg maximum permitted limit of AF in finished poultry feed (Kibugu *et al*., 2019). Total AF contamination of all the feed types ranged from 0.22 µg/kg to 157.89 µg/kg. The mean of total AF content in chick mash, grower mash and layers mash were 56.00±50.1 µg/kg, 49.10±46.0 µg/kg and 35.30±42.6 µg/kg respectively, (Table 4.9). Ruai recorded the highest AF mean of 53.3±50.8 µg/kg whereas the lowest AF mean of 40.2±41.8 µg/kg was observed in Langata, (Table 4.10).

Two-way Analysis of Variance revealed that total AF levels were not significantly different among the feed types (F = 1.813, df = 2, *P* =0.170) and regions (F = 0.350, df = 4, *P* =0.843) in Nairobi County. However, a significant interaction effect (F = 2.116, df = 8, *P* =0.043) was observed between the region and the feed type on the total AF levels in the feed samples.

Table 4.8: Aflatoxin contamination of poultry layer feeds in Nairobi County

Feed type	Occurrence of Total Aflatoxins (n) %	>KEBS limit* (n) %	Range of Total Aflatoxin (ug/kg)	Mean ± Standard deviation of Total Aflatoxin (ug/kg)
Chick mash (n=50)	32 (64)	21 (42)	0.74-157.89	56.00±50.1
Grower mash (n=50)	33 (66)	24 (48)	0.8-154.30	49.10±46.0
Layers mash (n=50)	33 (66)	18 (36)	0.22-145.74	35.30±42.6

* KEBS maximum permitted limit of 10 µg/kg of finished poultry feeds

Table 4.9: Aflatoxin contamination of poultry layer feeds collected from different regions in Nairobi County

Region	Occurrence of Total Aflatoxins (n) %	>KEBS limit* (n) %	Range of Total Aflatoxin (ug/kg)	Mean ± SD of Total Aflatoxin (ug/kg)
Dagoretti south (n=30)	20 (67)	16 (53)	2.2-157.89	44.3±42.4
Kasarani (n=30)	15 (50)	9 (30)	1.18-145.74	52.4±55.8
Langata (n=30)	20 (67)	12 (40)	1.18-145.74	40.2±41.8
(Roysambu n=30)	24 (80)	13 (43)	0.22-129.0	45.3±47.2
Ruai (n=30)	19 (63)	13 (43)	0.8-154.30	53.3±50.8

* KEBS maximum permitted limit of 10 μg/kg of finished poultry feeds

4.5.2 Fumonisin contamination of poultry layer feed sampled from Nairobi County

One hundred and thirty-six samples (91%) had detectable levels of FUM while fourteen samples (9%) had no detectable levels of FUM. No sample had FUM levels higher than the recommended EU guidance limit of 20000 µg/kg (FAO, 2003). Total FUM contamination ranged from 210 µg/kg to 15173 µg/kg with a mean of 1694 µg/kg. The mean of total FUM contamination in chick mash, grower mash and layers mash were 2020 µg/kg, 1620 µg/kg 1460 µg/kg respectively, (Table 4.11). Ruai recorded the highest total FUM mean of 2450 µg/kg while Kasarani had the lowest total FUM mean of 1110 µg/kg, (Table 4.12). Two-way Analysis of Variance revealed that total FUM levels were not significantly different among the feed types (F = 0.169, df = 2, *P* =0.844) but were significantly different among the regions (F = 5.524, df = 4, *P* =0.000) in Nairobi County. There was no significant interaction effect observed between the region and the feed type on the total FUM levels in the feed samples. (F = 1.427, df = 8, *P* = 0.193).

Table 4.10: Fumonisin contamination of poultry layer feeds sampled from Nairobi County

Feed type	Occurrence of Fumonisins (n) %	Range of Total Fumonisins (mg/kg)	Mean ± SD of Total Fumonisins (mg/kg)
Chick mash (n=50)	44 (88)	0.21-15.2	2.02±15.2
Grower mash (n=50)	48 (96)	0.22-13.0	1.62±13.0
Layer mash (n=50)	44 (88)	0.24-12.3	1.46±12.3

Table 4.11: Fumonisin contamination of poultry layer feeds in different regions in Nairobi County

Region	Occurrence of Fumonisins (n) %	Range of total Fumonisins (mg/Kg)	Mean ± SD of Total Fumonisins (mg/Kg
Dagoretti South (n=30)	30 (100)	0.26-4.25	1.40±0.868
Kasarani (n=30)	26 (87)	0.32-4.85	1.11±1.11
Langata (n=30)	22 (73)	0.21-13.0	1.45±2.79
Roysambu (n=30)	29 (97)	0.22-12.3	1.94±2.49
Ruai (n=30)	29 (97)	0.24-15.2	2.45±3.21

CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Poultry practices carried out by layer farmers in Nairobi County, Kenya

Poultry production has become the choice of investment to many households due to low start-up capital, low maintenance costs, low space requirements, quick returns to investment and simple management practices coupled with numerous market outlets for products (Kingori *et al*., 2010). Egg production, egg size and egg quality are some of the key factors considered by farmers before choosing a particular layer breed. Isa brown was the preferred breed citing high egg production as the main reason which ultimately results to higher profits.

Majority of farmers preferred buying their feeds as opposed to formulating their own. However, poor quality of commercial feeds was pointed out by farmers as a major factor affecting poultry production. ABS (2013) attributed poor quality of feeds to lack of standardization and high cost of ingredients, poor training of the feed manufacturers, failure to use laboratories for nutritional analysis in addition to weak legal and institutional framework to enforce quality assurance. The production of good quality and affordable feeds rest on the availability of the ingredients for human use and for feed manufacture (Macharia *et al*., 2020).

High feed price was also a major concern to the farmers. Feeds account for almost 70% of total cost of production therefore higher feed prices results in lower profits. Altahat *et al*., (2012) reported feed price as the factor that had the highest negative impact on profitability in layer enterprises. High production cost negatively impacts the small-scale farmers causing some farmers to abandon the venture. Farmers preferred buying their feeds from agrovets as opposed to manufacturers. This was attributed to their proximity and the fact that they can buy feeds in small quantities as many were small scale farmers.

Although majority of the farmers examined their feed daily for signs of contamination, quite a number of farmers did not do this particularly in Kasarani. Regular inspection of feeds allows one to properly examine the color, odor, physical form, presence of molds and insect damage. These are

some of the physical approaches adopted in combating mycotoxins (Tola and Kebede, 2016). Majority of the farmers preferred sacks as their ideal storage item. Others preferred to use the same original sack in which the feed was bought in. However, sacks are not safe from attack by insect species especially if the sacks are not raised from the ground out of insect reach and may also accumulate moisture thus providing ideal conditions for infection of fungal organisms. Insects primarily feed on feed ingredients and contaminate them with faeces, webbing, body parts, foul odors, and microorganisms resulting in loss in weight and quality of the feed. Moisture acts as a crucial factor in eliminating mold growth and its presence in the feed arises from three sources, that is, feed ingredients, feed manufacturing processes and the environment in which the feed is stored in (Chukwuka *et al*., 2010). Recycling of sacks can also serve as route in introducing fungal microorganisms through carry over inoculums. The ideal storage item for feeds would be the hermetic bags. Hermetic bags are air tight bags which prevent air or water from getting into the cereals stored in them. Additionally, they limit the presence of cereal pests by depleting oxygen supply levels and producing carbon dioxide therefore arresting aflatoxin accumulation.

Shifting from one brand of feed to the next was the preferred solution of farmers who had previously encountered aflatoxicosis in their flock. This, however, may not be the solution as contamination can occur at any stage from production to storage (Agriopoulou *et al*., 2020). A better approach would be continued surveillance of the feed right from the feed manufacturer until it is supplied to the farmer and increased awareness on the effects of mycotoxins on poultry health.

5.2 Fungal contamination of layer feeds in Nairobi County, Kenya

The mean CFU of chick mash was 6.11×10^{-2}, grower mash 5.80×10^{-2} and layer mash $4.73\times10-^{2}$. CFU is used to estimate the population of microorganism that are present in a test sample. Fungal count act as indicators of the hygienic quality and safety of the feed (Krnjaja *et al*., 2008; Dalcero *et al*., 1998). A feed sample with a high fungal count signals high levels of contamination therefore unfit for use. Chick mash was the most contaminated as compared to grower and layers mash. This could be attributed to longer storage of chick mash by the agrovets and manufacturers due to less

demand by farmers. The longer the storage period of farm products, the higher the fungal load and toxin contamination of those products (Salisu and Almajir, 2020; Mokubedi *et al*., 2019). Feeds from Kasarani had the highest mean CFU and this may be partly attributed to the fact that majority of the farmers in this area did not regularly check their feeds for any signs of contamination. Poultry feeds can become contaminated either directly or indirectly through contact with soil, rodent, birds, dust, human carrier, sewage or water during processing and storage (Sultana *et al*., 2017). Fungal growth results in losses in volume, quality of feed ingredients and subsequently feeds made from them (Okoli *et al*., 2006).

Similar results were observed by Ghaemmaghami *et al*., (2016) who reported CFU between 1 and 1.2×10^{-3} with a mean of 0.302×10^{-3} in finished feed. Mean mold count of 7.0×10^{-2} was reported Cegielska-Radziejewska *et al*., (2013) with growers' mash reporting a higher mean CFU of 3.2×10^{-2}. The average CFU in mashed feeds was 15×10^{-3} while that of pelleted feeds was 11×10^{-2} with the fungal load being higher in the finisher feed as compared to the starter feed (Ghaemmaghami *et al*., 2018). Poultry feed mixtures from Slovakia were found to have a mean count of 1.8×10^{-3} (Labuda and Tančinová, 2006) while Shareef (2010) reported an average count of 7.2×10^{-5} CFU/g from finished feed in Nineveh. Studies regarding microbiological quality of feed materials used between 2009 and 2012 in Poland revealed mycological counts of up to 10^{-8} in cereal grains (Kukier *et al*., 2013). Counts of 2.18×10^{-3}, 5.13×10^{-3} and 3.27×10^{-3} were observed in dichloran rose bengal chloramphenicol agar (DRBC), dichloran 18% glycerol agar (DG18) and dichloran chloramphenicol peptone agar (DCPA) respectively by Oliveira *et al*., (2006). Queiroz *et al*., (2013) on the other hand reported fungal counts in the range 1.0×10^{-3} to 2.3×10^{-6} in DRBC and 1.0×10^{-2} to 2.9×10^{-6} in DG18 media. Fungal counts ranging from 6.6×10^{-3} to 6.3×10^{-5} CFU/g were seen in DRBC medium from poultry feeds collected from Rio Cuarto, Argentina (Dalcero *et al*., 1998) while counts of 1-2.41 x 10^{-5} were reported by Krnjaja *et al*., (2017). Mold counts higher than

1×10^{-5} were reported by Rosa *et al*., (2006) while counts between 10^{-4}-10^{-6} were seen by Heperkan and Alperden (1988). Mean fungal counts in the range of 3.0×10^{-4} to 9.6×10^{-5} were reported from four different brand of poultry feeds each constituting starter, grower, layer and finisher feed types (Ukaegbu-Obi *et al*., 2017).

5.3 Mycoflora of poultry layer feeds in Nairobi County, Kenya

Aspergillus spp, Penicillum spp, Fusarium spp, Mucor spp, Rhizopus spp, Cladosporium spp and Cochiliobulus spp were isolated from the collected feed samples. Similar results were obtained by Ariyo *et al*., (2013), Greco *et al*., (2014), Cegielska-Radziejewska *et al*., (2013), Ibrahim *et al*., (2017); Obi KM *et al*., (2017) and Ukaegbu-Obi *et al*., (2017). *Aspergillus* (90.67%), *Penicillum* (77.33%) and *Fusarium* (13.33%) were the most prevalent fungi isolated. These results are in accordance with Embaby *et al*., (2015), Shareef (2010), Saleemi *et al*., (2010), Sivakumar *et al*., (2014) and Okechukwu *et al*., (2019). Poultry feed have been found to be the more predominant animal feed infected with mycotoxigenic fungi (Sivakumar *et al*., 2014). *Aspergillus* and *Penicillium* are considered storage fungi while *Fusarium* species are often classified as field fungi (Kotinagu *et al*., 2015). An estimated 20-45% of world cereal production are contaminated with storage fungi (Harcarova *et al*., 2018). Field fungi require 70%–90% relative humidity, temperatures between 20°C and 25°C, a_w greater than 0.85 for active growth and 0.99a_w for optimal growth whereas most of *Aspergillus* and *Penicillium* species require a minimum a_w of 0.75–0.85 and grow well at 0.93–0.98 a_w (Agriopoulou *et al*., 2020). *Aspergillus spp* can adapt to temperatures of 30°C–40°C while *Penicillium spp* exhibits optimal growth at temperatures between 25°C –30°C (Agriopoulou *et al*., 2020).

Aspergillus spp has been found to be predominant in cereals and other ingredients used in poultry feed production (Ariyo *et al*., 2013). Maize alone accounts for 50-60% of the entire poultry feed (Krnjaja *et al*., 2017). Bankole *et al*., (2006) pointed out that maize and groundnuts were the most contaminated cereal crops with *Aspergillus* in Sub Saharan Africa. *Fusarium* and *Penicillium* have

also been shown to be major contaminants of corn (Kotinagu *et al*., 2015). Contrast to our results, Algabr *et al*., (2018), Ghaemmaghami *et al*., (2016) and Krnjaja *et al*., (2017) reported *Fusarium* as being the dominant fungal genera followed by *Aspergillus* and *Penicillium* while Greco *et al*., (2014) also found *Fusarium* to be the most dominant mycotoxigenic fungi but followed by *Eurotium*, *Penicillium* and finally *Aspergillus*. Dalcero *et al*., (1998) and Kubizna *et al*., (2011) identified *Aspergillus* and *Fusarium* as dominant genera isolated in poultry feeds. Many studies have depicted *Aspergillus* and *Penicillium* as the frequent fungal genera contaminating poultry feeds (Aliyu *et al*., 2012; Ariyo *et al*., 2013; Heperkan and Alperden, 1988; Labuda and Tančinová, 2006; Oliveira *et al*., 2006; Shareef, 2010). *Aspergillus, Cladosporium* and *Penicillium* were the frequently isolated from feeds intended for ornamental birds in Brazil (Queiroz *et al*., 2013). *Mucor, Rhizopus* and Yeast were found to be the most common fungi occurring in commercial poultry feed in Imo state, Nigeria by Okoli *et al*., (2006) whereas other studies reported *Rhizopus* as the most prevalent fungi in feed samples (Cegielska-Radziejewska *et al*., 2013; Osho *et al*., 2007; Uwaezuoke and Ogbulie, 2010). *Cochliobolus heterostrophus* which is prevalent in tropical and subtropical maize-growing areas and is the cause of Southern corn leaf blight was isolated from our study.

From the genus *Aspergillus*, *A.flavus, A.niger* and *A.fumigatus* were found to be the three most predominant species in poultry feed. These results are in accordance with Accensi *et al*., (2004); Ariyo *et al*., (2013); Azarakhsh *et al*., (2011); Ghaemmaghami *et al*., (2016); Heperkan and Alperden (1988); Oliveira *et al*., (2006), Queiroz *et al*., (2013), Sivakumar *et al*., (2014); Ukaegbu-Obi *et al*., (2017). Studies have reported *A. flavus* being the most frequently isolated from the genus *Aspergillus* (Dalcero *et al*., 1998; Ibrahim *et al*., 2017; Embaby *et al*., 2015; Habib *et al*., 2015; Labuda and Tančinová, 1995; Gherbawy *et a*l., 2019; Obi KM *et al*., 2017; Osho *et al*., 2007; Rosa *et al*., 2006). *A. flavus* has been shown to be adapt to different geographical locations particularly in the tropical and sub-tropical regions, habitats and substrates (Ibrahim *et al*., 2017; Scheidegger and Payne, 2003). Growth optimization studies revealed temperatures between 28°C and 30°C is optimal for the growth of *A.flavus, A.niger* and *A.fumigatus* while PH between 4.0 and 5.0 favors the growth of *A. flavus* and *A. fumigatus* while that of *A.niger* is 7.0-7.5 (Sivakumar *et al*.,

2014). Nairobi is located towards the south of the equator with the warmest average daily maximum temperature of 27.5°C occurring during January to March and coolest daily maximum temperature of 22.5°C occurring between June and August (UOC, 2017). It receives a mean annual rainfall of 879mm with rainfall occurring in two seasons, long rains occurring from March to May recording around 310mm and short rains occurring from November to December recording around 200mm (Obiero and Onyando, 2013; UOC, 2017). These conditions are ideal for the proliferation of *Aspergillus* species. *A. flavus* and *A.parasticus* were the predominant species of *Aspergillus* isolated from poultry feeds in Argentina (Magnoli *et al*., 1998) whereas *A. flavus* and *A.fumigatus* were frequently isolated from poultry feed in Zaria and Sokoto state, Nigeria (Aliyu *et al*., 2012; Ibrahim *et al*., 2017; Habib *et al*., 2015). Additional studies reveal *A. niger* as the most frequently isolated species from poultry feed (Saleemi *et al*., 2010). From the genus *Fusarium, Fusarium verticilloides* was isolated. These results are concurrent with Oliveira *et al*., (2006) and María *et al*., (2012). *Fusarium proliferatum, Fusarium subglutinans* and *Fusarium oxysporum* were isolated from poultry feed mixtures in Slovakia (Labuda *et al*., 2003). *F.verticilloides* is widespread in both humid and sub humid regions extending to sub-tropical and tropical regions in the world. *F. verticilloides* is the most dominant ear rot fungi in maize worldwide and in Kenya, it ranks highly as a maize production constraint (Alakonya *et al*, 2008).

5.4 Toxigenic potential of *Aspergillus flavus* strains isolated from layer feeds in Nairobi County, Kenya

Three strains of *Aspergillus flavus* were found to be potentially toxigenic confirmed by their blue fluorescence in CAM under UV light. This supports the levels of aflatoxins found in the sampled feeds. One of the major producers of aflatoxins in food and feed has been shown to be *A. flavus* (Fakruddin *et al*., 2015; Okoth *et al*., 2018). .*A. flavus* strains can vary in aflatoxin production from non-toxic to highly toxigenic and been grouped into aflatoxigenic and non aflatoxigenic strains using both culture and molecular methods (Okoth *et al*., 2018; Tola and Kebede, 2016). Aflatoxin biosynthetic pathway in *A. flavus* involves about 30 genes clustered together in a 75-kb DNA region including the two regulatory genes (*aflS* and *aflR*) and the structural genes such

as *aflD, aflM,* and *aflO* (Šimončicová *et al*., 2018; Tai *et al*., 2020). AF biosynthesis is also regulated by genes encoding the velvet proteins such as *veA* and *laeA,* along with developmental genes modulating morphology, conidiation, or sclerotia formation like *brlA* and *abaA*.

Differentiating between toxigenic and atoxigenic strains is key in determining the efficacy of bio-control strategies and understanding inter strain interactions (Mylroie *et al.*, 2016). Bio-control strategies aim at giving atoxigenic strains a competitive edge over their aflatoxin-producing relatives therefore decreasing the potential for contamination in crops and the environment (Okun *et al*., 2015). Products which employ bio-control mechanism such as aflasafe have already been registered for use in countries such as Nigeria, Kenya, Senegal and Gambia and whereas in other countries such as Burkina Faso, Burundi, Ghana, Malawi, Mozambique, Rwanda, Tanzania, Uganda, and Zambia research is underway (Bandyopadhyay *et al*., 2016).

5.4 Aflatoxin and Fumonisin contamination of poultry layer feeds in Nairobi County, Kenya

Fumonisins was detected in 91% of the samples while aflatoxin was detected in 65% of the feed samples. Mean AF level was 46.7 µg/kg with a maximum concentration of 157.9 µg/kg whereas mean FUM was 1694 µg/kg with a maximum concentration of 15173 µg/kg. 58% of the samples had AF levels within the KEBS recommended limit of 10 µg/kg while no sample had fumonisin levels greater than the EU guidance limit of 20000 µg/kg (FAO, 2003). Similar range of aflatoxin was reported in feeds collected from the southern, central, northern and east coast regions of Peninsular Malaysia (Wan *et al*., 2017). Mokubedi *et al*., (2019) reported FB1 as the most dominant mycotoxin recovered from feed samples from South Africa with a mean 1075.6 µg/kg with a maximum concentration of 7125.3 µg/kg and a mean AF 0.5 µg/kg with a maximum concentration of 3.7 µg/kg. Fumonisin was also the most frequently isolated mycotoxin from poultry feed and feed ingredients in Nigeria reporting a mean of 1014 µg/kg and a mean AF of 74 µg/kg (Akinmusire *et al.,* 2019). Fumonisin was detected in all feed samples from Argentina with a median of 1.750 µg/kg while aflatoxin was detected in 90% of the samples with a median of 2.685 µg/kg (Greco *et al.,* 2014).

Queiroz *et al*., (2013) reported fumonisin contamination in 95% of total samples with levels from 92 to 668 µg/kg and aflatoxins contamination in 40% of total samples with levels between 12 and 902 µg/kg.

Aflatoxins, however, were the most frequently isolated from grain and feed commodities from Kenya, Nigeria and Ghana and this was attributed to their warmer climates (Rodrigues *et al*., 2011). Ranges between 16 and 1930 µg/kg of FB1 with a mean of 468 µg/kg were observed in poultry feeds from Cameroon with a mean AF of 40 µg/kg (Abia *et al*., 2013). Kana *et al*., (2013) found 87% of both broiler and layer feeds were contaminated with aflatoxin recording a mean AF of 11.1 µg/kg and 6.6 µg/kg respectively. However, Shareef (2010) reported aflatoxins and ochratoxins as the most frequently occurring mycotoxins closely followed by fumonisins with means of 179.1 µg/kg, 159.4 µg/kg and 127 µg/kg respectively. Kehinde *et al*., (2014) reported ranges between 13.5 to 95.1 µg/kg in feeds in Abeokuta, Nigeria while Gherbawy *et al*., (2019) reported range between 0.90-60 µg/kg in feeds in the western region of Saudi Arabia

Mycotoxins are more widespread in developing countries due to improper agricultural, storage and processing practices (Velmurugu, 2009). Storage temperatures between 25°C and 30°C coupled with 97% relative humidity greatly favor the production of toxins (Sivakumar *et al*., 2014). Aflatoxins are the most common and toxic mycotoxins in poultry feed and majorly contaminate maize which is a chief component of the feed (Dandashire *et al*., 2020; Krnjaja *et al*., 2017). Stress caused by dry conditions, wounding by insects, rain prior to or during harvesting, delayed harvest and late irrigation are factors associated with increased aflatoxin levels in grains (Milani, 2013). FB1 and FB2 production in maize by *F.proliferatum* and *F. verticilloides* is greatly favored at 0.956a_w and 0.968a_w with temperatures between 25°C and 30°C (Marin *et al*., 1995). The detection of both aflatoxin and fumonisins in this study could be attributed to the isolation of toxigenic *A. flavus* and *F. verticilloides*. Among the three different types of feeds chick mash once again exhibited the highest levels of aflatoxins and fumonisins. This may still be attributed to the lower demand of feed as compared the other two thus leading to longer storage.

This study gives insight on the level of quality of layer feeds in Nairobi County. Additionally, it highlights the extent of contamination present in the feeds and emphasizes the need to create more awareness on the dangers of mycotoxin contamination in feeds. The results obtained would play a significant role in not only the coming up with a code of practice to be applied in the poultry industry but also aid in the formulation of feed and food safety policies in the country. Poultry is the fastest growing agricultural sub-sector, particularly in developing countries owing to growing populations, rising incomes and urbanization. Guaranteeing feed safety and quality would greatly impact the growth and production of the poultry industry.

5.5 Conclusion and Recommendations

Increase in demand for commodities used in production of animal feeds has been on the rise due to high demand of livestock production. This had led to increased awareness of animal feed safety. Animal feeds play a crucial role in farm animal to human food chain, therefore, infectious and non-infectious hazards existent in feed pose a threat to human health. One of the major hazards are mycotoxins which are commonly found in cereals used in the production of animal feeds. Mycotoxins are more prevalent in tropical and subtropical climates and pose a serious threat to the feed supply chain, animal and human health. The detection of both aflatoxin and fumonisin in the feed samples is of major concern in the poultry industry. Therefore, there is need for tougher implementation of regulations to ensure food and feed safety. Mitigation measures also need to be enforced to effectively manage the mycotoxin problem.

This study therefore recommends the following

- Enhance the facilitation of services of the agricultural extension officers to the farmers as they serve as a link between the government and the farmers.
- Increase in awareness of the dangers of mycotoxin through rallies and public participation activities.
- Educate the farmers on appropriate storage practices that minimize contamination and mycotoxin production in the feed.
- The government should put in place uniform standards, regulations and code of practice to be applied by manufacturers in the feed industry.
- Regular inspection of feed ingredients and finished feed being used to ensure they are of the right quality.
- Reduction of the costs of in testing for the presence of mycotoxins in poultry feeds

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APPENDICES

Appendix 1: structured questionnaire

LAYER FARMERS LOCATED IN DAGORETTI SOUTH, KASARANI, ROYSAMBU AND LANGATA.

A. BASIC INFORMATION

Questionnaire no

1 Date of interview ……

2 Constituency ……

3 Ward ……...

4 Estate/ Street ……

5 Time interview started ……

6 Time interview ended ……...

B STRUCTURED QUESTIONS

Tick where appropriate.

1. Which type of layer breed are you rearing?

   Rhode Island Red [ ]

   ISA Brown [ ]

   Leghorns [ ]

   California White [ ]

   Hyline Brown [ ]

2. Give a reason as to why you prefer that type of layer breed,

   ……

   ……

   ……

   ……

   ……

   ……

   ……..……

3. Do you buy or make your own feed?

Buy [ ]

Make [ ]

……

……..……

4. If you buy your feed where do you buy your feed?

Manufacturer [ ]

Agrovet [ ]

6. What storage item do you use to store the feed?

Plastic containers [ ]

Metal containers [ ]

Sacks [ ]

Other (specify) …….

7. How often do you check the feed for any signs of contamination?

Daily [ ]

Weekly [ ]

Monthly [ ]

Other (specify) ……..

8. Have you ever encountered any case of aflatoxicosis in layer chickens?

Yes [ ]

No [ ]

9. If yes, how many cases have you encountered so far?

1-5 []

50 [ ]

105 [ ]

15-20 [ ]

>20 (specify) …….

10. How the did you handle the outbreak of aflatoxicosis and what measures were put in place to prevent a future outbreak?

……

……

……

……

……

……