SURVEY ON FUNGAL AND AFLATOXIN CONTAMINATION OF STORED MAIZE (*Zea mays*) GRAINS IN SOUTHERN ETHIOPIA

MSc THESIS

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HAWASSA UNIVERSITY, HAWASSA, ETHIOPIA

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SURVEY ON FUNGAL AND AFLATOXIN CONTAMINATION OF STORED MAIZE (*Zea mays*) GRAINS IN SOUTHERN ETHIOPIA

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A THESIS SUBMITTED TO DEPARTMENT OF PLANT SCIENCES,

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SCHOOL OF GRADUATE STUDIES

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This is to certify that the thesis entitled **"Survey on fungal and aflatoxin contamination of stored maize (*zea mays*) grains in southern Ethiopia"** submitted in partial fulfillment of the requirements for the degree of Master of Science with specialization in **Crop protection**, the Graduate Program of the School of Plant and Horticultural Sciences and has been carried out by Memhiru Mekiso Madebo ID. No. GpcrPr/R 0003/11 under my/our supervision. Therefore I/we recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to the department.

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DEDICATION

I dedicate this thesis manuscript to MY FAMILIES for their dedicated partnership in the success of my life.

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STATEMENT OF THE AUTHOR

I declare that this thesis is my genuine work and that all sources of materials used for this Thesis have been duly acknowledged. This Thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree at Hawassa University and is reserved at the University Library to be made available to borrowers under rules and regulations of the Library. I solemnly declare that this Thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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Place: Hawassa University, Hawassa

Date of Submission: February, 2022.

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LIST OF ABBERVATIONS AND ACRONYMS

ANOVA	Analysis of Variance
CSA	Central Statics Agency
CV	Coefficient of Variation
ECEA	Ethiopia Commodity Exchange Authority
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
GPS	Global Positioning System
FDA	Food and Drugs Authority
LSD	Least Significant Difference
masl	Meter above sea level
MATI	Ministry of Agriculture Training Institute
MOA	Ministry of Agriculture
MTL	Maximum Tolerable Limits
PDA	Potato Dextrose Agar
ppb	Parts per billion
SNA	Spezieller Nahrstoffer Agar
SNNPR	Southern Nations Nationalities and Peoples' Region
WFP	World Food Program
SSA	Sub-Saharan Africa (SSA)
SAS	Statistical Analysis System
SE	Standard Error

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SURVEY ON FUNGAL AND AFLATOXIN CONTAMINATION OF STORED MAIZE (*Zea mays*) GRAINS IN SOUTHERN ETHIOPIA

Memhiru Mekiso (BSc, in Plant Science)

Major advisor: - Elfinesh Shikur (PhD)

ABSTRACT

Maize (Zea mays L.) is attacked by more than sixty diseases and a number of species of insect pests and microorganisms in the field as well as in the storage. Fungi are among the principal causes of deterioration and yield loss on farmers' maize during the storage period. Among the storage fungal pathogens Fusarium, Aspergillus, and Penicillium are the most predominant species attacked maize grain and resulting in production of harmful products of Mycotoxins. The study was conducted at the Hawassa University College of Agriculture in plant pathology laboratory. The study was aimed to study effect of storage duration and storage type on fungal contamination and assessments of aflatoxin contamination of maize grains in southern Ethiopia. A total of 165 Maize grains samples were collected from different storage type and storage duration in southern Ethiopia, in 2019/2020. A total of seven fungal genera consisting of twenty one species of fungi were isolated from maize grain by using morphological characterstics; Fusarium spp. were most frequently isolated, followed by Aspergillus spp.The fungal contaminations of maize grains were significantly different ($p<0.05$) increased with storage periods. At the last six months of storage, the contamination of the Fungal isolates revealed that Fusarium spp had the highest contamination of 43.6% followed by Aspergillus spp, 28.6%, then Penicillium spp 5.27%, Alternaria spp 4.12%, Rhizopus spp 3.08%,Tricoderma spp 1.65% while the least contamination of 1.22%, was Cladosporium spp. The highest contamination of Fusarium spp (44.79%) and Aspergillus spp (24.98) were recorded in storage basket whereas the minimum contamination of Fusarium spp (35 %) and Aspergillus spp (15.5%) were obtained from plastic storage. As a result of this research, the Plastic container storage was determined to be more appropriate for protecting the stored maize grains from fungal attack during the storage periods and the stored grains have low fungal contamination until initial to six months. Therefore, storage Basket, storage Crips, storage Sacks and Gombisa *storages were inadequate for protecting stored maize from fungal attacks. Total aflatoxins has quantified from the maize samples collected from different maize growing areas in southern Ethiopia by using Enzyme Linked Immune Assay (ELISA) Kit. The result reveal that the mean 32(66.7%) of 48 maize grain sample were positive for aflatoxin contamination with levels of aflatoxin concentration ranging from 0.21-18.06µg/kg. The average aflatoxin concentrations detected in the present study were below the US Food and Drug Administration (FDA), the World Health Organization (WHO) maximum limit of total aflatoxin 20µg/kg.* The EU has maximum limit of 4 µg kg^{-1} total aflatoxin in cereal for direct human consumption. Thus, most of the samples contaminated with aflatoxins in this study are not suitable for human consumption by the EU standards.

Key words: Maize**,** Aflatoxins, ELISA Kit, fungal frequency, Storage time, Storage type

1. Introduction

1.1. Background and justification

Maize (*Zea mays* L) is one of the most important grains in the world (Olawuyi *et al*., 2014). It is the third most important cereal grain after wheat and rice global rank, which is also called the "Queen of Cereals" because of its highest genetic yield potential (Jeyaraman, 2017).

In the Eastern and Southern parts of Africa, where maize is the most important staple and the main source of calorie intake, agricultural households receive up to 20% of their income from maize production and spend more than 15% of their total household expenditure on maize alone (Chauvin *et al.*, 2017). From 2007 to 2017, the area coverage on which maize is grown in Sub-Saharan Africa has increased by almost 60 % (FAO STAT, 2019).

Maize is the second most widely cultivated crop in Ethiopia and is grown under diverse agro-ecologies and socioeconomic conditions (Tsedeke *et al*., 2017). Because of its multiple advantages, it ranks second in production area, next to teff, but first in its productivity among major cereal crops (Tsedeke *et al*., 2015). Due to its widespread significance in the country, maize is one of the strategic field crops targeted to ensure food security in Ethiopia (CSA, 2019). Despite the importance of maize as a principal food crop, its average yield in Ethiopia (3.92 t/ha) (CSA, 2019) is still lower than that of the world's average (5.6 t/ha in 2016) (FAO, 2019).

Maize cultivation in the world is limited by contamination of maize grain by different microorganisms. Fungi are among the principal causes of deterioration and loss of maize grain (Ominski *et al*., 1994). Kossou and Aho (1993) reported that fungi could cause about 50-80 % of damage on farmers' maize during the storage period if conditions are favorable for their development. During storage, several kinds of fungi can remain associated to corn seeds either

causing their deterioration or simply remain viable to infect germinating seedling. It has been reported by several researchers that fungal infestation in maize results in color change, decreases in nutritional values, and reduction of overall quality and quantity of the maize as well as contamination of maize grain with mycotoxin. The major fungi genera typically found in stored grains are *Aspergillus*, *Penicillium*, *Fusarium* (Orsi *et al*., 2000; Castellari *et al*., 2010). According to Campbell *et al*., (2004), the estimates of the cost of grain loss due to insect and microorganism damage of grain stored in developing countries each year ranged from $500 million to $1 billion.

The development of these fungi can be affected by moisture content of the product (Gtorni *et al*., 2009). In addition, temperature, storage time and degree of fungal contamination prior to storage, insect and mite activity facilitate fungi contamination (Suleiman and Omafe, 2013). Subsequently, the maize is stored while still relatively moist and warm; both warmth and high moisture contents can result in rapid deterioration of the grains and promote the growth of microorganisms (e.g. fungi and bacteria) and insects in the grains (Ekechukwua and Norton, 1999). To maintain high quality maize during storage, maize should be protected from weather (including relative humidity and temperature), growth of microorganisms, and insects (Oyekale *et al*., 2012).

In tropical and subtropical countries, a large proportion of the grain (such as maize) is harvested and stored under hot and humid conditions, and most farmers lack proper knowledge, equipment and methods of drying grains (Weinberg *et al*., 2008). In Ethiopia, maize grains produced are stored under poor storage conditions for considerable period of time. Traditional storage of maize in Ethiopia are made up of mud, bamboo strips, and pits .In comparison of these storage conditions, better storage technologies in maize involve storage of maize in polyethylene bags

and gunny bags (Anjum *et al*., 2012). Extended storage of maize under unacceptable storage conditions enhances fungal growth which promotes the production of respective mycotoxins (Chauhan *et al*., 2008).

Fungal growth in maize presents a major risk for humans and animals, through production of mycotoxins (especially Aflatoxins). According to Manoch *et al*., (1988), aflatoxin production by the fungi in the grain depends on the storage conditions, including relative humidity, temperature and storage period. Mycotoxins are toxic secondary metabolites of fungi that frequently contaminate maize in the field and/or during storage (Smith *et al*., 2012). The most important mycotoxin in maize are the aflatoxins, fumonisins, deoxynivalenol, and ochrotoxin (Kimanya *et al.,* 2012). Aflatoxins are naturally occurring mycotoxin produced by some strains of moulds such as *Aspergillus flavus* and *Aspergillus parasiticus* (Pasone *et al*., 2010 and Sardin *et al*., 2011). Aflatoxins cause serious problems in many foods and are most abundant in maize and maize products since maize can be infected while in the field under specific environmental conditions (Krnjaja *et al*., 2013). Contamination of maize with mycotoxin depends on the co-existence of susceptibility of hybrids and environmental conditions favorable for proliferation of mycotoxigenic fungi (Blandino *et al*., 2009). In Ethiopia,maize is vulnerable to mycotoxins contamination due to poor harvesting practices, improper storage practices, different geographical and climatic conditions and poor handling of the crop and storage (Alemu, 2008).

Mycotoxin contamination of crops and their negative effects has been a worldwide problem for thousands of years (Miller and Trenholm 1994). The mycotoxins may have negative impact on the economy and health status of the world population. Owing to these factors, toxicogenic fungi attract huge interest among scientists and policymakers. However, much of the work on these

fungi and the associated mycotoxins is concerning the developed countries, while sub-Saharan Africa, except South Africa, is lagging behind (Ayalew 2010; Chala *et al*. 2014). Various surveys revealed that the problems with mycotoxins and their contamination are more pronounced under tropical environment and more often in the developing countries (Wu 2004). Limited surveys have been reported in relation to aflatoxin contamination of maize grain and ways to protect the food from contamination in Ethiopia (Alemu, 2008). Therefore the aim of the present study was to survey on fungal and aflatoxin contamination of stored maize grains southern Ethiopia by using ELISA technique.

1.2. Objectives

1.2.1. General Objectives

The general objective of the study was to survey on fungal and aflatoxin contamination of stored maize grains southern Ethiopia.

1.2.2. Specific objectives

- To identify the major storage fungal pathogens of maize grains under different storage conditions .
- To determine the levels of fungal contamination under different storage type and duration of maize storage conditions in southern Ethiopia.
- To asses/quantify levels of Aflatoxin contamination of maize grains samples collected at harvest from southern Ethiopia**.**

2. Literature Review

2.1. Climatic requirements of maize

Maize is a versatile crop grown over a range of agro climatic zones. It is grown from 58^{o}N to 40^{o}S, from below sea level to altitudes higher than 3000 m, and in areas with 250 mm to more than 5000 mm of rainfall per year (Nadzger, 2010). The major reason maize has spread so widely is its ability to produce high yield of grain under a wide variety of climate conditions (Nadzger, 2010). Maize is the second most widely cultivated crop in Ethiopia and is grown under diverse agro-ecologies and socioeconomic conditions typically under rain-fed production. The maize agro-ecologies in Ethiopia can be broadly divided into six major categories, including Moist and Semi-moist mid-altitudes (1700–2000 m above sea level; 1000–1200 mm rainfall), Moist upper mid-altitudes (2000– 2400 masl; >1200 mm), Dry mid-altitudes (1000–1600 masl; 650–900 mm), Moist lower mid-altitudes (900–1500 masl; 900–1200 mm), Moist lowlands (<900 m, 900–1200 mm), and Dry lowlands (<1000 m, <700 mm) (MOA, 2005). The moist and semi-moist mid-altitude zones comprise the bulk of the national maize area in Ethiopia. These are mostly located in the SW and W Oromia, W and NW Amhara, parts of the Southern Nations Nationalities and Peoples Region (SNNPR), and Ben Shangul-Gumuz (BSG). Taken together, the Semi-moist and Moist ecologies cover about 75 % of the national maize production area whereas the dry ecologies cover the remaining 25 %.

2.2. Importance of maize in Ethiopia

Maize is one of the most important staple food crops in sub-Saharan Africa (SSA), predominantly produced and consumed directly by the smallholder farmers (Shiferaw *et al*., 2011). Maize is everything for the Ethiopian maize farmers. Three forth of the maize produced is consumed at the house hold level by the small-scale producers themselves (CSA, 2017). The

grain is consumed in different forms of food; the Stover is used as feed, fuel and construction material. Maize is also used for making local beverages. Besides, it serves as a major source of income and means of employment for tens of millions of farming and business communities in the country. A small quantity of the grain produced is currently used in livestock and poultry feed, and this is expected to increase with the development of the livestock and poultry enterprises in the country. The green fodder from thinning and topping is an important source of animal feed and the dry fodder is used during the dry season. Moreover, the crop has potential uses for industrial purposes, serving as a starch, a sweetener for soft drinks, an input for ethanol fuel production and oil extraction, etc. Ethiopia is already a significant maize producer in Africa, and this role could be further enhanced. Currently, maize is the cheapest source of calorie intake in Ethiopia, providing 20.6 % of per capita calorie intake nationally (Diriba *et al*., 2011; EATA, 2012). Due to its widespread significance in the country, maize is one of the strategic field crops targeted to ensure food security in Ethiopia.

2.3. Production constraints of maize in Ethiopia

A significant portion of this yield gap is attributable to biotic and abiotic stresses, slow turnover of varieties tolerant or resistant to these stresses and low level use of improved management and other inputs (Worku *et al*., 2012; Abate *et al*., 2017). Some of the main abiotic factors affecting maize production and productivity are drought, heat, soil acidity, frost and poor soil fertility mainly in N and P. Biotic stresses hindering maize production in Ethiopia are diseases (e.g., Grey Leaf Spot, *Turcicum* Leaf Blight, Common Leaf Rust, Maize Streak Virus, Maize Lethal Necrosis), parasitic weeds (mainly *Striga hermontica*), and insect pests (such as the maize stem borer, maize weevils and the newly emerged fall armyworm). The spread of insect pests, plant diseases and invasive alien plant species to new regions, as the world's climate changes, is a

threat to farmers globally, especially in Africa where climate change effects are projected to be the most severe in the world (Swaminathan and Kesavan, 2012; Biber-Freudenberger *et al*., 2016; Early *et al*., 2016). It is anticipated that the spread of diseases, insect pests and weeds will potentially cause the loss of more than 40% of the world's food supply. Of the many countries in SSA, Ethiopia is one of the climate change vulnerable countries due to its agriculture led economy (Conway and Schipper, 2011).

2.5. Methods of maize storage

The grain needs to be protected from unfavorable conditions and pests during storage. This is usually accomplished to a certain extent by storing them in structures made from different materials or by mixing them with natural or chemical products. According to Nukenine (2010), "Storage is a way or process by which agricultural products or produce are kept for future use". Common storage methods used by small scale farmers in Africa are Sack storage, Storage cribs, Storage baskets, Air tight storage , Concrete silos, Metal silos, Air tight plastic bags, Underground storage pits, Roof storage, Suspension of crops on a tree or above the fireplace (Obetta and Daniel, 2007). Plastic bags are widely used for storage in Ethiopia (Tilahun, 2007). Maize and grain storage systems are classified into three main type; crib, bags, and bulk storage (Yakubu, 2009; Montross *et al*., 1999).

2.6. Factors Affecting Storage

Both abiotic and biotic factors affecting storage. Temperature and moisture content of the cereal grains are the two key abiotic factors affecting the resulting quality of the grain, biochemical reactions, dry matter losses, allowable storage times and overall storage management of the grain (Gonzales *et al*., 2009; Lawrence and Maier, 2010). The main biotic agents causing deterioration

of stored grains are microorganisms (fungi, bacteria, and yeast/mold), insects and mites, rodents, birds, and metabolic activities (Fekadu, 2007). Moisture content plays a significant role in the storage of grain; when grain has more moisture, it heats up and can have mold spoilage (Brewbaker, 2003). As a general expression, the higher the moisture content, the more susceptible the maize grain is to mold and insect deterioration. If the moisture content in a product going in to store is too low, microorganisms will be unable to grow provided that the moisture in the store is also kept low. Moisture should therefore be prevented from entering the store. The moisture content below which micro-organisms cannot grow is referred to as the safe moisture content. The safe moisture content and temperature for maize is13.5 and 27^{o}C, respectively (Tilahun, 2007). In general, it is essential that all food stuffs are below their safe moisture content .The temperature within a store is affected by sun, the cooling effect of radiation from the store, outside air temperatures, heat generated by the respiration of both the grain in the store and any insects present (Tilahun, 2007). The safe moisture content is to some extent related to the storage time.

2.7. Major molds and fungi

Mold and fungal species can develop on grains, in the field as well as in storage. Contamination of maize grain with mold and fungi is regarded as one of the most serious safety problems in the tropical countries and throughout the world (Kaaya and Kyamuhangire, 2006). Toxigenic fungi invading maize are divided into two distinct groups, field fungi and storage fungi (Barney *et al*., 1995).Field fungi invade maize and produce toxins before harvest or before the grains are threshed, and can develop under high relative humidity of over 80 %, with moisture content of 22 % to 33 % and wide range of temperature (10 ± 35 °C) (Williams and Macdonald 1983; Montross *et al*., 1999). These usually die out in storage, but some can live under storage

conditions (Sanchis *et al.*, 1982), cause significant damage reducing the yield and quality, especially in warm humid climates (Moturi, 2008). Conversely, storage fungi invade grain primarily during storage and require moisture content in equilibrium with relative humidity of 70 % to 90 %. In both circumstances, fungi originated from the field. Storage molds replace field molds that invade/ contaminate the maize before harvest (Reed *et al*., 2007).

There are several key fungal spp. associated with stored grains, including *Fusarium* spp., *Penicillium* spp., *Rhizopus* spp., *Aspergillus* spp and *Tilletia* spp. (Williams and MacDonald, 1983; Barney *et al*., 1995). Infection of maize grain by storage fungus results in discoloration, dry matter loss, chemical and nutritional changes and overall reduction of maize grain quality (Chuck-Hernández *et al*., 2012). It has been reported by Fandohan *et al*., (2003) that storage fungi contributes to loss of more than 50 % of maize grain in tropical countries, and ranks second after insects as the major cause of deterioration and loss of maize. According to Williams and McDonald (1983), when storage molds invade maize grain they cause rot, kernel discoloration, loss of viability, vivipary, mycotoxin contamination, and subsequent seedling blights. It was revealed by Sone (2001), that broken maize and foreign materials promote development of storage molds, because fungi more easily penetrate broken kernels than intact kernels. Similarly, Dharmaputra *et al*., (1994) reported that mechanical damage during or after harvesting on maize grains can provide entry points to fungal spores. Likewise, Fandohan *et al*., (2006) reported that increases in grain damage and cracking create an opportunity for fungi to grow and penetrate the maize grain.

Moisture content and temperature are the two key environmental factors that influence growth of molds and fungi (Alborch *et al*., 2011). Maize grain is generally harvested with moisture content

of around 18 % to 20 % and then dried. If inadequately dried the conditions are favorable for molds and fungi to grow, which can result in a significant decreases in grain quality and quantity (Marín *et al*., 1998). Barney *et al*., (1995) and Rees (2004), report that fungal growth in stored grain in the tropical countries is mainly associated with increases in grain moisture contents, and fluctuation in temperatures, resulting in unsafe storage of high-moisture grain and moisture migration and condensation.

Furthermore, a study conducted by Reed *et al*., (2007) on the effect of moisture contents and temperature on storage molds, found that the higher the initial moisture contents the greater the infection of maize kernels. According to Miller (1995), the growth and development of storage fungi in grain are governed by three main factors, crop (nutrients), physical (temperature, moisture) and biotic (insects, interference competition) factors.

2.8. Mycotoxins

Molds growing on maize grains present a great threat, especially through production of secondary metabolites (mycotoxins) (Weinberg *et al*., 2008). Mycotoxins are a chronic problem for maize grown in warm, humid, tropical, and sub-tropical regions (Kaaya and Kyamuhangire, 2006). Molds and fungal infections can result in mycotoxin contamination in all stage from growing, harvesting, storage to processing (Chulze, 2010). The most important mycotoxins that frequently occur in cereal grains are aflatoxins, ochratoxins, fumonisins, trichothecenes, and zearalenone (Pitt, 2000). The two most common and toxic mycotoxin compounds encountered on maize in tropical and subtropical regions are aflatoxins and fumonisins (Krska, 2008).

According to Miller (1995), aflatoxin is predominantly a problem in cereal grains, particularly in maize; it is produced by three main species of fungi, *Aspergillus flavus*, *Aspergillus parasiticus*,

and *Aspergillus nomius*. These fungi tolerate and resist a wide range of conditions, and can be found everywhere such as in soil, in plant and animal remains, milk, and in grains and seeds such as peanuts and maize (Pitt, 2002). World Health Organization (WHO) categorizes aflatoxins as class1 carcinogens, as they are highly poisonous, toxic substances (Martinez *et al*., 2011).

Several researchers report that aflatoxin contamination in grain increases with storage period and environmental conditions. Aflatoxins contamination is facilitated by long-term storage under unhygienic and unventilated conditions (Egal *et al*., 2005). Research conducted Liu *et al*., (2006) in China showed a significant increase in aflatoxins with storage length (i.e. 0.84 µg/kg in twelve months to 1.17 µg/kg in twenty four months). Aflatoxin contamination and *A. flavus* infection are often associated with high temperature and drought conditions.

Many researches consistently found high temperature to be a major factor influencing aflatoxin contamination and fungal growth (Widstrom, 1996; Kaaya and Kyamuhangire, 2006; Tubajika and Damann, 2001). Alborch, *et al*., (2011) revealed that temperature and water activity (aw) influence not only rate of fungal spoilage, but also the production of mycotoxins.

2.9. Strategies for mycotoxin control and prevention

Clearly the pre- or post-harvest prevention of mycotoxin contamination is the preferred strategy for minimizing mycotoxins in foods and feeds. Failure to prevent fungal invasion and toxin formation in the field or in storage will inevitably lead to an increased risk of adverse health effects and economic loss. However, if chemical monitoring is successful, maize consumption should not be a significant source of increased health risk from mycotoxins (Ronald et al., 1999). Several technologies have been tested in Africa to reduce aflatoxin risk. Field management practices that increase yields can reduce the risk of aflatoxin development. They include use of

resistant varieties, crop rotation, well-timed planting, weed control, pest control especially control of insect pests and avoiding drought and nutritional stress through fertilization and irrigation. Measures to stop the infection process by controlling the aflatoxin causing fungi in the field are achieved through use of pesticides and aflatoxigenic fungi to competitively displace toxigenic fungi, and timely harvest. Postharvest interventions that reduce aflatoxin include rapid and proper drying, proper transportation and packaging, sorting, cleaning, drying, smoking. Post-harvest insect control, and the use of botanicals or synthetic pesticides as storage protectants. Another approach is to reduce the frequent consumption of „high risk" foods (especially maize and groundnut) by consuming a more varied diet, and diversifying the diet into less risky staples. Chemo-preventive measures that can reduce aflatoxin effect include daily consumption of chlorophyllin or oltipraz and incorporating hydrated sodium calcium alumino-silicates into the diet. Reduction and detoxification of aflatoxin is often achieved physically (sorting, physical segregation, flotation etc.), chemically (e.g. calcium 18 hydroxide, ammonia) and microbiologically by incorporating pro-biotics or lactic acid bacteria into the diet. Millers can use blending of less and more contaminated products to reduce the overall risk. Efficient monitoring and surveillance with cost-effective sampling and analytical methods also reduces the risk. Public education and awareness can sensitize the population on aflatoxin risk and its management (Kerstin et al., 2011; Bankole et al., 2003).

3. Materials and Methods

3.1 Description of the study area

The sample collection was made from four zones (Gammo, Wolayita, Alaba and Gurage) in SNNPR and one region (Sidama) in Southern of Ethiopia. The eleven districts that were covered during sample collection were Mirab Abaya, Boreda, Hubbo, Bolosso Sorre, Damota Gale, Dore Bafana, Boricha, Wera, Atoti Uli, Meskan and Sodo. These areas were selected for this studies based on their maize production potential and available storage systems. These areas alititude,annual rainfall and temperature range from 1100 –2200m, 800 – 1000 mm and 15°C – 29°C, respectively. The laboratory study was conducted at Hawassa University College of Agriculture Plant Pathology laboratory in 2019/2020.

3.2. Experimental plan and design

The experiment was arranged in a 3×5 factorial combination with two factors, storage types and storage period in complete randomized design with three (3) replications. Storage types have five levels, that is storage Basket, Crips, Sack, Gombisa and Plastic container, while storage period have three levels that is 0, 3 and 6 months of storage periods. Data were collected at every three months interval, including at the start of the study making up four levels for the factor storage period.

3.3. Sampling methods

A total of 165 samples of maize grains were collected from each of storage structures periodically starting from the beginning of the storage (0, 3 and 6) months of the storage periods. The samples were taken from the top, middle and bottom of the storage structures. The initial maize samples from each storage structures were taken as a control at the beginning of the storage. Each sample was taken by inserting the spear into the grain mass straight to the

maximum depth from the top, middle and the bottom of the storage structures and composite working sample (1 Kg for each sample) was made by mixing the grains from the different levels of the storage structures (top, middle and bottom). The samples collected were properly labelled with the relevant information and transported to the laboratory for further analysis.

3.3. Laboratory studies

3.3.1. Measurement of grain moisture content

Once the samples were brought to the laboratory**,** grain moisture content of each sample was measured using a digital moisture tester.

3.3.2. Assessment of fungal infestation of maize grains

The maize grain samples were first surface sterilized with 70% ethanol for 2 minutes and rinsed three times in sterile distilled water. In order to assess fungal infestation, surface-sterilized maize grains (100 maize seed for each sample) were placed on moist blotter paper in metallic trays which were covered with transparent plastic sheets and kept in microbiological incubator at 28 ± 2° C for 7–14 days. Emerging fungal colonies were transferred to potato dextrose agar (PDA) and kept at in an incubator at a temperature of 25$\pm$1 for five to seven days. Sub culturing to new PDA was carried out to get fungal pure culture for identification. Those fungal cultures showing typical *Fusarium* mycelium characteristics (white, orange, pink mycelial color) were transferred to SNA for further identification based on conidial structure. Identification of Fungi was carried using appropriate manual depending on the fungal genera suspected. The identification manuals used include Leslie and Summerell (2006), Barnett and Hunter (1987) and Domsch *et al*. (1993). The level of fungal infestation of maize grain samples on blotter paper in metallic tray was assessed in terms of incidence of fungi and isolation frequency based on the following formula.

Incidence of fungi (INF):

Incidence of fungal infections on each sample were calculated by using the following formula:

$$\textbf{INF (\%)} = \frac{\text{Number of maize grain infected by a given fungi}}{\text{Total number of grain assessed}} \times 100$$

Isolation Frequency (IF):

The proportions of samples, for each fungus, yielded its isolates were determined and expressed as percent by using the following formula (Marasas *et al*., 1988).

$$\textbf{IF (\%)} = \frac{\text{Number of sample of occurrence of fungal species}}{\text{Total number of samples assessed}} \times 100$$

3.3.3. Aflatoxin analysis

A total of 500 g of maize grain was arbitrarily taken from each sample, and it was ground, sieved through a 1 mm sieve and stored at -20ºC until aflatoxin analysis was made. This sampling method was based on Maringe *et al*., (2017). Two gram of previously ground maize sample was weighed and added into plastic tubes and 10 ml of 70 % methanol was added and mixed for 10 minute at room temperature using a shaker and the mixture was centrifuged for 3 minutes at a speed of 3000rpm. Then 100 µl of filtrate was diluted with 600 µl distilled water. A sufficient number of microliter wells was inserted in to the micro well holder for all standards and samples. After The standard and sample positions was recorded 50 µl of the standard or prepared sample was added to separate duplicate wells and 50 µl of the conjugate was added to each well. Then 50 µl of the antibody was added to each well and was mixed gently by shaking the plate manually and incubated for 30 minute at room temperature (20-25 ºC). The liquid was poured out of the well and the micro well holder was taped upside down vigorously against absorbent paper (3 times in row) and all well were filled with 250 µl wash

buffer and the well were empty again. This procedure was repeated two times then 100 µl of substrate or chromogen was added to each well. Mixed gently by shaking the plate manually and incubated for 15 min at room temperature (20-25). Finaly 100 µl of stop solution was added to each well. This was mixed gently by shaking the plate manually and the absorbance was measured at 450nm. The reading was made within 30 minutes after addition of stop solution . .

3.4. Data analysis

All the data collected were subjected to analysis of variance (ANOVA) by using the PROC GLM procedure (SAS institute, 2004) and difference among means was compared by the Least Significant Difference at 5% level of significance.

4. Results and Discussion

4.1. Storage fungi infestation across location

Mycotoxigic fungal occurrence was assessed in the major maize growing areas of southern Ethiopia, namely Mirab Abaya, Boreda, Hubbo, Bolosso Sorre, Damota Gale, Halaba, Dore Bafana, Boricha, Wera, Atoti Uli, Meskan and Sodo which are the major maize-producing districts in Southern Ethiopia. There was difference in fungal contamination percentage of maize grain in the different districts visited and at different storage durations. At zero month storage (at harvest), higher fungal contamination (72.92%) was observed in maize grains collected from Dore bafana district followed by Hunibo district (72.66%) (Table1). The higher fungal contamination at Dorebafana might be associated with the lack of improper preharvest treatment and cultural practices like, using local maize varaity, lack of crop rotation, type of crop intercroped as well as the moist eniviromental contions prevailing in the area. The lower fungal contamination (66.67%) was observed in maize grain sampled from Mirab Abaya district followed by Wera (66.1%) (Table1). This might be due to the optimal environmental condtions in thee area for maize growth. In addition majority of farmers in these areas use disease resistant maize varieties, rotate their maize crop with different crops, as well as they practice proper fertilization and good agronomic pratices. At three month storage, higher fungal contamination (80.87%) was observed in maize grains sampled from Bolosso Sore district followed by Atoti Uli (79.89%) (Table1). This might be due to lack of adequate knowledge by the farmer about the fact that maize should be dried to moisture contents below 14% immediately after harvest. Besides this farmers in thesea areas do not use improved storage method chemicals during storage. On the otherhand, the lower fungal contamination (74.83%) was observed in maize grains sampled from Mirab Abaya district followed by Wera (75.27%) (Table 1) because farmers in these areas follow

good post harvest practices such as drying maize to adequate moisture content, use of funcides like DDT during storage, use of appropriate material. At six month storage, higher fungal contamination (89.55%) was observed in maize grains sampled from Bolosso Sore district followed by Boreda (89.47%) and lower fungal contamination (84.67%) was observed in maize grains sampled from Boricha district followed by Meskan (85.35%) (Table 1). The populations of all the fungi were higher in samples collected from 6 month storage than in the samples collected from 0 and 3 month storage duration, with contamination percentages of 87.54, 77.63 and 69.64 respectively. Among the fungal isolated from current 165 maize grain samples, collected from (0, 3 and 6 months) storage interval, *Fusarium* spp. was the dominant fungal spp. in each district followed by *Aspergillus* spp., (Table 1). Similarly, Camila *et al*. (2015) reports the *Fusarium* genera was most frequently found followed by Aspergillus spp. Similarly, Asela *et al*., (2019) carried out fungal contamination analysis of 180 wheat grain samples collected from three major wheat growing zones (Arsi, West Arsi and Bale) of South East Ethiopia. Their result revealed that the contamination of wheat grains by fungal species at different locations and storage time with different frequencies. According to their finding, fungal contamination varied with storage period with the highest incidence of (98.62%) followed by (89.78%) and (86.77%) being observed after six months, upon harvest and three months of storage, respectively. The result of the present study were in agreement with (Girma *et al.* 2009), which reported various grain mold fungi including *Fusarium*, *Penicillim*, *Aspergillus*, and *Nigropora* spp., from maize samples collected from Hawassa, Areka, Billito, Shallo and Arsi-Negel. Their finding indicated that the populations of all the fungi were higher in samples collected from 6 month storage than in the samples collected from 0 and 3 month storage duration.

Table 1. Fungal contamination (%) in 0, 3 and 6 month stored maize grains.

Name of district	***Zone/region***	**Storage time**	*Fungal Genera*							
			Fusariu mspp	*Aspergillus spp*	*Penicillium spp*	*Alternaria spp*	*Rhizopus spp*	*Trichoderma spp*	*Cladosporium spp*	Total
Boreda	Gammo	0	39.13	22.13	4.66	2.20	1.40	1.00	0.73	69.55
		3	41.73	24.53	5.00	2.60	2.26	1.33	1.13	79.67
		6	44.06	30.40	5.47	1.73	2.06	0.80	0.60	89.47
Mirab Abaya	Gammo	0	38.73	20.66	4.53	1.80	1.26	0.86	0.53	66.67
		3	39.33	23.00	4.67	2.47	2.20	1.40	0.87	74.83
		6	42.67	29.33	5.26	1.47	2.00	0.73	0.67	87.55
Hunibo	Wolayita	0	41.66	22.13	5.00	2.31	1.53	1.00	0.73	72.66
		3	42.53	23.80	4.67	2.33	2.0	1.80	0.93	78.95
		6	44.00	30.00	5.27	1.40	2.20	0.93	0.80	88.95
Bolosso Sorre	Wolayita	0	38.60	23.00	4.93	2.60	1.46	1.06	0.66	70.61
		3	43.13	25.60	4.80	2.26	2.00	1.13	1.06	80.87
		6	44.20	30.53	5.07	1.53	2.20	1.00	0.67	89.55
Damota Gale	Wolayita	0	38.66	24.00	4.65	2.27	1.40	0.87	0.70	70.85
		3	40.66	23.93	5.00	2.40	2.27	1.30	1.13	77.58
		6	44.40	28.47	5.40	1.67	2.13	0.87	0.80	88.09

		0	41.73	23.33	5.13	1.80	1.33	0.80	0.50	72.92
Dore Bafana	Sidama	3	40.73	25.53	4.87	2.26	2.00	1.20	1.13	78.61
		6	42.40	29.73	5.00	1.46	2.06	0.87	0.93	86.8
		0	38.26	22.13	4.53	2.13	1.26	0.80	0.60	68.01
Boricha	Sidama	3	39.40	24.73	4.80	2.30	2.00	1.13	0.93	76.18
		6	41.86	27.66	5.27	1.47	2.13	1.06	0.87	84.32
		0	37.86	20.80	4.86	1.73	1.26	0.80	0.53	66.1
Wera	Halaba	3	37.06	25.40	4.93	2.53	2.13	1.26	1.06	75.26
		6	42.67	29.47	5.07	1.53	2.20	0.93	0.66	86.88
		0	39.50	21.53	4.73	2.47	1.40	0.80	0.53	69.26
Atotiuil		3	38.93	25.80	4.93	2.40	2.06	1.20	1.13	77.34
		6	42.73	30.67	5.20	1.60	2.13	1.00	0.67	88.35
		0	39.80	21.80	4.53	1.93	1.33	0.87	0.60	69.16
Meskan	Gurage	3	38.13	25.26	4.80	2.33	2.27	1.06	0.87	75.61
		6	41.53	28.73	5.13	1.66	2.40	0.80	0.60	85.2
		0	39.40	21.60	4.73	2.40	1.60	1.06	0.60	69.69
Sodo	Gurage	3	42.07	24.20	4.70	2.47	2.20	1.33	1.07	78.93
		6	42.73	29.80	5.27	1.40	2.20	0.93	0.67	87.35

4.2. Effect of storage periods on fungal contamination of stored maize grains

Effects of storage period on fungal contamination of stored maize grains are presented in Table 2. Fungal contamination for all the fungi genera detected increased with storage period. *Fusarium* was the most contaminant fungal genera with 37.1% fungal contamination at harvest and this increased significantly ($p<0.05$) to 43.6% at six months of storage period. On the other hand the least contaminant genera were *Cladosporium* which resulted in fungal contamination with a range of 0.65 to 1.22% from harvest to six months storage.

Similarly, Negasa *et al. (*2019) also reported that the storage period increased, the contamination of *Fusarium, Aspergillus* and *Penicillium* spp. increased significantly ($p<0.05$) in (0, 2, 4 and 6) months. Similarly Angeline W *et al*. (2016) reported, the population of *Aspergillus* spp. in maize increased from harvest to sampling after three months of storage in Plastic bags. This study also agrees with reports by Domenico *et al*. (2016) who observed an increase in the population of *Aspergillus* spp. in maize after three months of storage with a progressive increase until nine months. Similarly Chauhan *et al*., (2008) reported, extended storage of maize under unacceptable storage conditions enhances fungal growth which promotes the production of respective mycotoxins. Research conducted Liu et al., (2006) in China showed a significant increase in fungal contamination with storage length. Suleiman, and Omafe, 2013 also reported the development of fungi that contaminate maize grain increase storage time.

Table 2. Effect of storage period on fungal contamination in stored maize grains.

	Frequency of fungal species (%)							
Storage Period	***Fusarium***	***Aspergillus***	***Penicillium***	***Alternaria***	***Rhizopus***	***Trichoderma***	***Cladosporium***	Total
0	37.10±0.34[c]	22.9±0.52[c]	4.01± 0.13[c]	2.43±0.15[c]	1.55±0.24[c]	1.00±0.08[c]	0.65±0.05[c]	69.64
3	40.01±1.09[b]	25.52±0.41[b]	4.52±0.04[b]	3.15±0.08[b]	2.23±0.29[b]	1.32±0.05[b]	0.88±0.07[b]	77.63
6	43.60±1.09[a]	28.60±1.27[a]	5.27±0.21[a]	4.12±0.11[a]	3.08±0.29[a]	1.65±0.07[a]	1.22±0.04[a]	87.54
LSD (5%)	2.80	2.55	0.46	0.36	0.38	0.2	0.16	3.26
CV(%)	4.98	7.30	6.41	12.97	14.75	13.61	14.90	14.36

Mean values ± standard deviation of three replicates within each column sharing similar letters were not significantly different by LSD test at $p \leq 0.05$. CV: Coefficient of variation, LSD: least significant different.

4.3. Effect of storage type on fungal contaminations of stored maize grain

Fungal contaminations were assessed in all maize samples collected from the five of storage types; namely storage Basket, Crips, Sack, Gombisa and Plastic container (Fig.1). The highest contaminations of fungi were observed in Basket storage type which has been accounted about 45.09 % *Fusarium* spp., 28.47% *Aspergillus* spp., 6.74% *Penicillium* spp., 3.06 % *Alternaria* spp., 2.99% *Rhizopus* spp., 1.43% *Trichoderma* spp. and 1.36% *Cladosporium* spp. On the other hand the lowest fungal contamination were observed in Plastic container storage type which has accounted for 37.02% *Fusarium* spp., 20.22% *Aspergillus* spp., 3.26% *Penicillium* spp., 1.25% *Rhizopus* spp., 1.45% *Alternaria*, 0.83% *Trichoderma* and 0.83% *Cladosporium*..*Fusarium verticillioides, Fusarium proliferatum, Fusarium gramminearum, Aspergillus flavus, Aspergillus parsiticus, Penicillium verrucoseum* and *Penicillium viridicatum* were the most prevalent mycotoxigenic fungi detected in all storage types.

Thus, from above five storage types the use of plastic container storage structures reduced fungal contamination, this might be because it protects insect infestation which damages maize grains and increases fungal development. The other probable reason may be the low moisture content of maize grain stored in plastic container (Appendix Table 1). The finding of this study indicated that Basket, Crips, Gombisa and Sack storages were inadequate for protecting stored maize from insect pests and fungal attacks. The increased fungal contamination in these storage types might be attributed to the high moisture content recorded in the storage types.

Similarly, Negasa *et al*. *(*2019) also reported the Hermetic bag was determined to be more appropriate than Gombisa and Sack for protecting the stored maize grains from fungal attack during the storage periods.

Similarly, Ng'ang'a al., 2016, reported in the lowest contamination of *Fusarium, Asperigillus* and *Penicillium* was recorded in Plastic bags. Thus Plastic bag protects maize grains better than conventional storage bags (Baoua *et al*., 2014) and can therefore be used for effective grain storage. In a study in West Africa, Hell *et al*. (2000) reported that some types of farmers' storage structures (storage basket, Crips and Sacks provided conditions that were more conducive to fungal infection and aflatoxin contamination than others (Prude Improved Cowpea Storage (PICS) bags. Similarly, Chauhan *et al.,* 2008 reported storage of maize grains under poor storage methods enhances the growth of fungi and promotes the production of mycotoxins. Similar studies done by Yilma *et.al.*,mycotoxigenic fungal occurrence were assessed in all maize samples collected from the six of storage types; namely open above ground(OAG), sack in open air (SOA), sack in house(SH), open sorghum stalk (OSS), improved gottera (IG), and in house ground (IHG) storages from West Showa and East Wallega Zones of Oromia regional state from 3 district namely Ilu Gelan, Gobu Sayo and Bako Accordingly, his finding the highest occurrence of mycotoxigenic fungi were seen in OAG storage type which has been accounted about 44(29.7%) followed by SOA 27(18.2%) and few mycotoxigenic fungal 5(3.4%) was isolated from IG. Similarly Kaaya *et al.* (2006) also reported that storage of maize in improved granaries in Uganda was related to reduced fungal and aflatoxin contamination.

Many studies have proven PICS to be effective storage systems for a variety of crops, including cowpeas, maize, peanuts, sorghum, wheat and common beans against insect infestation, fungal growth and aflatoxin accumulation (Zorya *et al*., 2011: William et al., 2014). Similarly Mahmoudi *et al*., 2013 reported, poor harvesting practices, improper storage type can also contribute to fungal growth and increase the risk of mycotoxins production.

Table 3. Effect of storage type on fungal contamination in stored maize grains.

	Incidence of fungal spp. (%)							
Storage Type	***Fusarium***	***Aspergillus***	***Penicillium***	***Alternaria***	***Rhizopus***	***Trichoderma***	***Cladosporium***	Total
Basket	44.79±1.86^{e}	24.98±2.53^{e}	5.54±0.33^{e}	5.02±0.29^{a}	4.09±0.41^{a}	2.13±0.08^{e}	2.12±0.11^{e}	88.67
Crips	42.03±1.49^{d}	22.49±1.68^{d}	4.35±0.33^{d}	3.76±0.27^{b}	2.65±0.28^{ab}	1.52±0.05^{d}	1.50±0.5^{d}	78.3
Sacks	39.54±1.40^{c}	20.15±1.53^{c}	3.15±0.22^{c}	2.54±0.22^{b}	1.85±0.16^{bc}	1.02±0.09^{c}	0.96±0.06^{c}	69.21
Gombisa	37.12±1.16^{b}	17.89±1.78^{b}	2.05±0.33^{b}	1.62±0.20^{b}	1.16±0.15^{bc}	0.61±0.15^{b}	0.52±0.16^{b}	60.97
Plastic container	35.00±1.84^{a}	15.75±1.5 ^{a}	1.06±0.32^{a}	0.75±0.15^{b}	0.55±0.15^{c}	0.23±0.15^{c}	0.13±0.15^{a}	53.47
LSD	2.12	1.82	0.97	0.77	0.55	0.35	0.32	6.24
CV	6.57	13.04	10.88	20.19	23.34	17.34	18.20	13.54

Mean values ± standard deviation of five replicates within each column sharing similar letters were not significantly different by LSD Test at $p \leq 0.05$. CV: Coefficient of variation, LSD: least significant different.

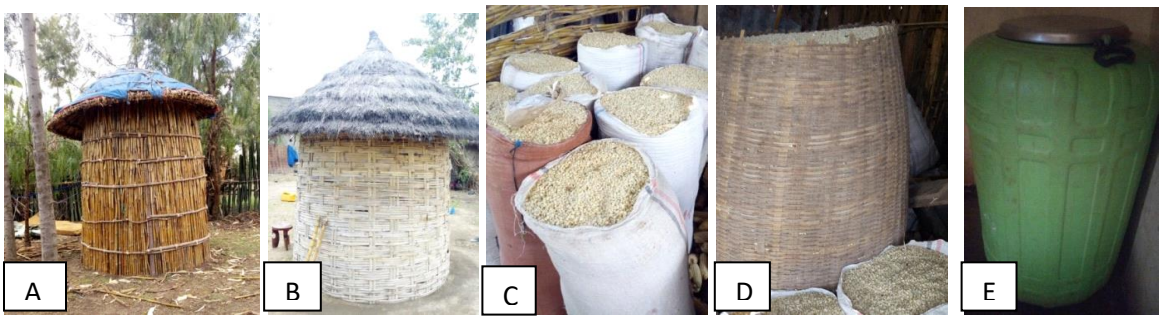

Figure 1. Tradtional storage type in southern Ethiopia.

Basket (A), Crips (B), Sacks (C), Gombisa (D), Plastic container (E).

4.4. Detection of total aflatoxin in maize grain samples collected from Southern Ethiopia

Total aflatoxins has quantified from the maize samples collected from different maize growing areas in southern Ethiopia by using Enzyme Linked Immune Assay (ELISA) Kit. The result reveal that the mean 32(66.7%) of 48 maize grain sample were positive for aflatoxin contamination with levels of aflatoxin concentration ranging from 0.21-18.06µg/kg. Highest total aflatoxin concentration (18.06µg/kg) was observed in maize grains sampled from Dore Bafana district followed by Boricha (11.13 µg/kg) while the lowest total aflatoxin concentration (0.21 µg/kg) was observed in Wera (Table 3). The determination of total aflatoxin concentration was done by developing a curve from the supplied aflatoxin standard which has ranged from 0-4.5 µg/kg (Fig. 2). Although aflatoxin was detected in the maize samples tested, the aflatoxin concentrations detected in the present study were below the US Food and Drug Administration (FDA), the World Health Organization (WHO) maximum limit of total aflatoxin 20µg/kg, which is the maximum limit allowed in human food in the US, except for milk (FAO 1996). The EU has maximum limit of 4 µg kg−1 total aflatoxin in cereal for direct human consumption. Thus, most of the samples contaminated with aflatoxins in this study are not suitable for human consumption by the EU standards. Similarly Getachew A, *et al*., 2018 observed Five types of

aflatoxins (B1, B2, G1, G2, and M1) by collecting 100 maize samples from southern and south western ethiopia at different levels with Up to 8% of the samples were contaminated with aflatoxins, with level of aflatoxin B1 of 24_513 μg kg−1. Similarly Mahamudu Mohamed (2017). Results obtained from farmers storage structures during the storage period of 180 days, aflatoxins contaminated samples were 18(56%), 12(36%) and 14(53%) of Mlanga, Kongwa and Manungu of maize samples, respectively were contaminated with aflatoxins. Similarly, Yilma *et.al* (2019), observe aflatoxin B1 concentration in the majority of the sample are below the recommended level however, in few of the sample its level is much higher than the EU standard.

In contrast Chauhan *et al*. Springer Plus (2016) reported our data demonstrate a total number of 150 different maize samples were collected from different Gedeo zones that all the samples reveal that mean aflatoxins concentration for all samples was observed as 53 ppb tested were beyond the safety level of aflatoxins as determined by Food and Drug. Giray *et al*., 2007 studied aflatoxins level in wheat samples consumed in some regions of Turkey from 41 wheat sample concentrations of total AFs in the wheat samples were determined to be ranging from 10.4 to 643.5 ng/kg. Fifty nine percent of the samples were found to be positive for total AFs and he detected levels are under the permitted levels for AFs in cereals, these amounts should be considered in regard to overall daily exposure to mycotoxins. Fu *et al*., 2008 determined aflatoxins by using UHPLC–UV in corn and peanuts collected from China. From 16 peanut samples, 2 (12.5% incidence) were contaminated with aflatoxin; among 18 corn samples, 4 (22% incidence) were contaminated with aflatoxins B 1, B 2, G1 and G2 at concentrations from 0.22 to 5 g/kg were between 83.4% and 94.7%.

Liu *et al*., 2006 started work on the determination of aflatoxins 110 samples collected from stored maize and rice grains by using by high-performance liquid chromatography with

fluorescence detection. The results showed that almost all samples collected contaminated aflatoxins in maize and rice were found to with concentration of 0.99, and 0.88 mgkg -1, respectively. Similarly Masresha Ahmed 2015 reported 30 samples were collected from farmers‟ fields of west Gojam and analyzed Samples for AFG2, AFG1, AFB2 and AFB1 by HPLC-FLD and the result showed that 33.3 % of pre harvest and 73.3 % of postharvest maize samples were exceeded the US Food and Drug Administration (FDA), the World Health Organization (tolerance limit of 20 µg/kg).Similarly Angeline W *et al*. (2016) also reported there were varying levels of aflatoxin contamination in maize grains sampled at harvest and three months after storage. Sixty three percent of maize grains collected at harvest had total aflatoxin levels above the acceptable limits set by the European Commission (≤ 4 ppb) while only 3.3% exceeded the limits set by the Kenya Bureau of Standards (≤ 10 ppb) and the US Food and Drug Administration (≤ 20 ppb).

Table 4. Prevalence of aflatoxin total in southern region across all maize grain samples.

Name of District	No. of maize grain sample	No. of aflatoxin positive samples	average concentration aflatoxin(µg/kg)
Boreda	4	3 (75%)	2.59-8.85
Mirab Abaya	6	4 (66.7%)	0.43-6.79
Bolloso Sorre	4	3 (75%)	0.49-10.97
Dore Bafana	5	3 (60%)	1.15-18.06
Damota Gale	5	5 (100%)	0.68-9.32
Boricha	5	5 (100%)	0.23-11.13
Meskan	6	2 (33.3%)	3.77-9.01
Sodo	4	2 (50%)	2.67-5.52
Wera	5	3 (60%)	0.21-9.01
Atotiuil	4	2 (50%)	2.04-8.14
Total	48	32(66.7%)	0.21-18.06

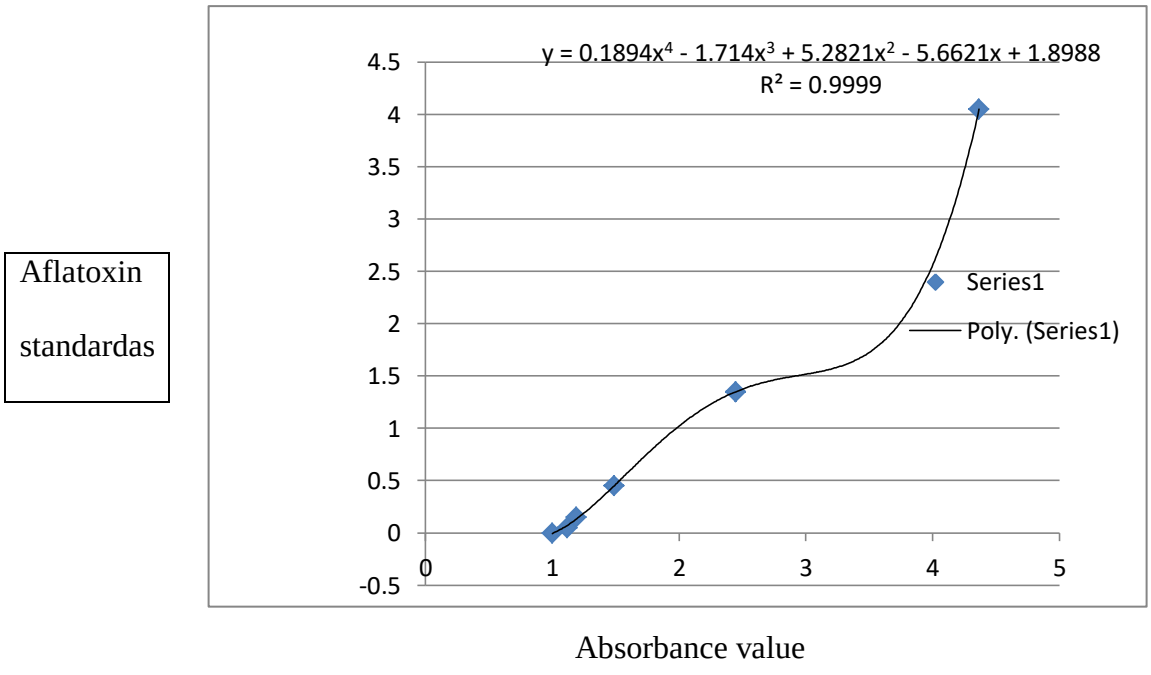

Figure 2. Aflatoxin determination curve

4.5. Identification of fungal microflora associated with maize grains

In present study a total of seven fungal genera consisting of twenty one species of fungi were isolated and identified using morphological characteristics. These are: *Fusarium verticillioides*, *Fusarium proliferatum, Fusarium graminearum, Fusarium oxysporum, Fusarium culmorum, Fusarium armeniacum,Fusarium equiseti*, *Fusarium pseudograminearum,Fusarium avenaceum, Aspergillus flavus, Aspergillus parasiticus, Aspergilus niger, Aspergilus terreus, Penicillium verrucosum, Penicillium viridicatum,Penicillium citrinum, Penicillium chrysogenum , Alternaria infectoria, Rhizopus stolonifer, Tricoderma harzianum and Cladosporium macrocarpum.* From this study *Fusarium, Aspergillus* and *Penicillium* were the most common genera isolated from maize samples collected **.**Similarly the results of this study were in agreement with the works of (Getachew, *et al*., 2018, maize grains samples collected from south and southwestern Ethiopia in 2018 were contaminated by *Fusarium, Aspergillus* and *Penicillium* species. This study's is also line with the works of Amadi and Adeniyi (2009) who isolated *Rhizopus* , *Penicillium* , *Fusarium* and *Aspergillus* spp. from maize grain in Kenya. Fungal species within the genera *Fusarium, Aspergillus* and *Penicillium* were commonly identified on maize in tropical regions (Samson, 1991; Orsietal, 2000), in agreement with the present study. The results of this study were in agreement with (Girma *et al*., 2009), which is in Ethiopia various grain mold fungi including *Fusarium, Penicillim, Aspergillus*, and *Nigropora* spp., have been detected on maize samples collected from from Jimma Zone . Similarly Tsehaye et al. (2017) identified 11Fusarium species associated with maize kernels, the dominant species was *F. verticillioides* (42%), followed by *F. graminearum* species complex (22.5%) and *F. pseudoanthophilum* (13.4%).

4.5.1 Morphological identification of fungal spp.

4.5.1.1. *Fusarium* spp.

Fusarium verticillioids

Fusarium verticillioids isolate grew rapidly on PDA and had growth rates ranging from 32 to 35mm after 3 days of incubation at 25˚C (Table 5). Initially, cultures have white mycelia but developed pink to violet pigments with age (Fig. 3A and B). Microconidia are with a flattened base and 0-septate (Fig. 3c). Microconidia may form in long chains and clusters (Figure 3D). Macroconidia were relatively long and slender and slightly straight with 3 to 5 septate (Figure 3E).

Fusarium proliferatum

The species grow with growth rates ranging from 31 to 34mm after 3 days of incubation at 25˚Con PDA (Table 5). Initially, cultures have white abundant aerial mycelium which may become purple-violet with age and produced violet pigments on PDA media (Fig.3F). Microconidia were produced in chains and were 0-septate and abundant in the aerial mycelia (Fig.3H–I). It produced lender, thin-walled, relatively straight macroconidia which were 3-5 septate (Fig.3J).

Fusarium graminearum

Fusarium graminearum isolate grew rapidly on PDA and had growth rate of 44 to 47mm after 3 days of incubation at 25˚C (Table 5). Aerial mycelia of *Fusarium graminearum* were white, to pale orange, to pink on PDA (Figure 3K). It formed pink to red pigmentation on PDA (Fig. 3L). Microconidia were absent. Macroconidia were relatively slender, thick walled, moderately curved, with 5 to 6 septa (Fig.3M).

Fusarium oxysporum

Fusarium oxysporum isolates showed widely varying colony characteristics on PDA. They were slow to moderately growing with growth rates ranging from 35 to 38mm after 3 days of incubation at 25˚C on PDA (Table 5). *Fusarium oxysporum* exhibited flat to floccose mycelia that varied from white to pale purple to dark purple, with a pale purple to dark purple pigmentation produced on PDA (Fig. 3N-O). Microconidia were ovoid- or kidney-shaped with 0 to 1 septa (Fig. 3P). Macroconidia were slender and curved, with 3- septa (Fig. 3Q).

Fusarium culmorum

Almost all *F. culmorum* isolates showed similar colony characteristics on PDA. They were fast growing with growth rates ranging from 45 to 46mm after 3 days of incubation at 25˚C on PDA Table 5). *Fusarium culmorum* formed abundant white mycelia which completely covered the Petr dish in one week (Fig. 3R), and produced carmine red pigment on PDA (Fig. 3S). Microconidi was absent. Macroconidia were robust, relatively short, thick walled, widest at the midpoint of the macroconidium and were with usually 3- or 4-septa (Fig. 3T).

Fusarium armeniacum

This species is relatively fast growing with growth rates ranging from 36 to 40 mm after 3 days of incubation at 25˚C on PDA (Table 5), and produced abundant white mycelium (Fig 4A). *Fusarium armeniacum* produced various pigments ranging from bright orange to red and reddish-brown in color in the agar (Fig. 4B). Microcondia was absent. Macroconidia were usually 5-septate, but 3- and 4septate were also found (Fig.4C).

Fusarium equiseti

Fusarium equiseti isolate grew moderately on PDA and had growth rate of 42 to 45mm after 3 days of incubation at 25˚C; (Table 5). Abundant mycelia, ranging in color from white, to pale orange, to tan were seen in isolates of *Fusarium equiseti* (Fig.4D). Pigmentation in PDA fluctuated from a pale to dark orange (Fig.4E). Microcondia was absent. Macroconidia were abundant with 5 to 7 septa, and were slender (Fig.4F).

Fusarium pseudograminearum

Fusarium pseudograminearum was relatively fast growing with growth rates ranging from 38 to 43mm after 3 days of incubation at 25˚C; on PDA (Table 5) , and produced abundant white mycelia which were white in the periphery and yellowish at the center (Fig 4G). Red pigments were formed in the agar, (Fig.4H). Microconidia was absent. Macroconidia were relatively abundant, relatively slender and almost straight to moderately curved, with a range of 1- to 11-septa, but macroconidia with 5- to 6-septa were the most common ones (Fig. 4I-J).

Fusarium avenaceum

Fusarium avenaceum isolates showed slow to moderate growth 43 to 45mm after 3 days of incubation at 25˚C; on PDA (Table 5). It formed abundant mycelium which varied from white to light yellow to grayish rose (Fig.4K) and produced grayish rose to burgundy pigment in the agar (Fig.4L). It produced thin walled, long and slender macrocondia which were moderately abundant, usually with 5-septa, but 3 - 4 septate macroconidia were also observed (Fig.4 N). And it produced varying size and shaped microconidia/mesoconidia with 1- to 2-septate (Fig.4 M).

Table 5. Growth rate of *Fusarium* species isolated from maize grains.

Fusarium species	Growth rate (mm)*
Fusarium verticillioids	32–35
Fusarium proliferatum	30–34
Fusarium graminearum	43–47
Fusarium oxysporum	32–38
Fusarium culmorum	43–46
Fusarium equiseti	42–45
Fusarium avenaceum	40–45
Fusarium armeniacum	36–40
Fusarium pseudograminearum	36–43

*Measured after 3 days of incubation at 25˚C; on PDA

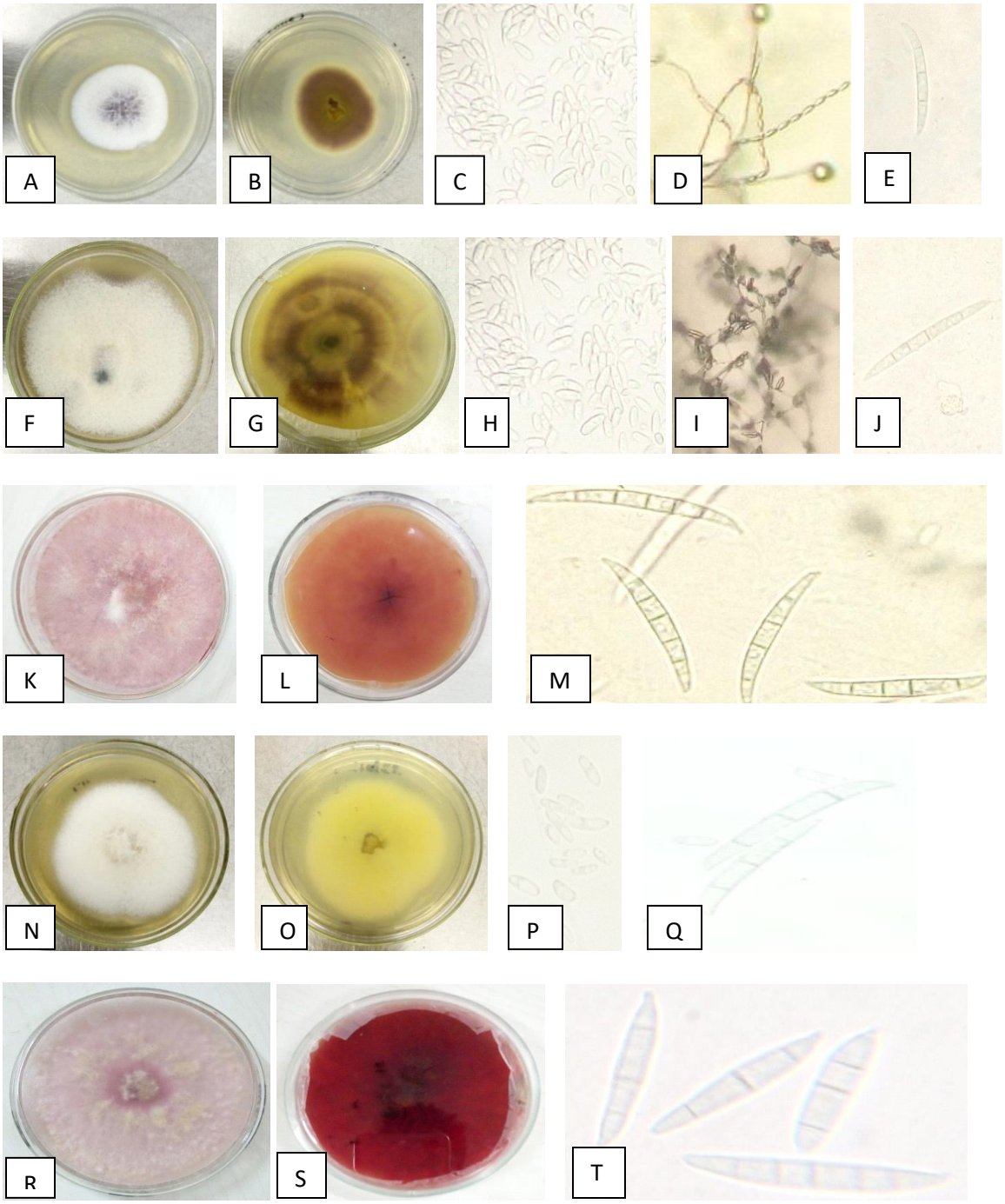

Figure 2. Morphological features of *Fusarium* spp. isolated from maize grain:

*F.verticilloids ;*surface (A), reverse (B),microconidia (C-D) and macroconidia (D) .*F. proliferatum ;*surface (F) ,reverse (G) and (H-I) macroconidia (J) . *F. graminearum;* surface (K)

reverse (L), macroconidia (M). *F.oxysporum* surface (N), reverse (O), microconidia (P) macroconidia (Q). *Fusarium culmorum*; surface (R), reverse (S) and macroconidia (T) at 40x

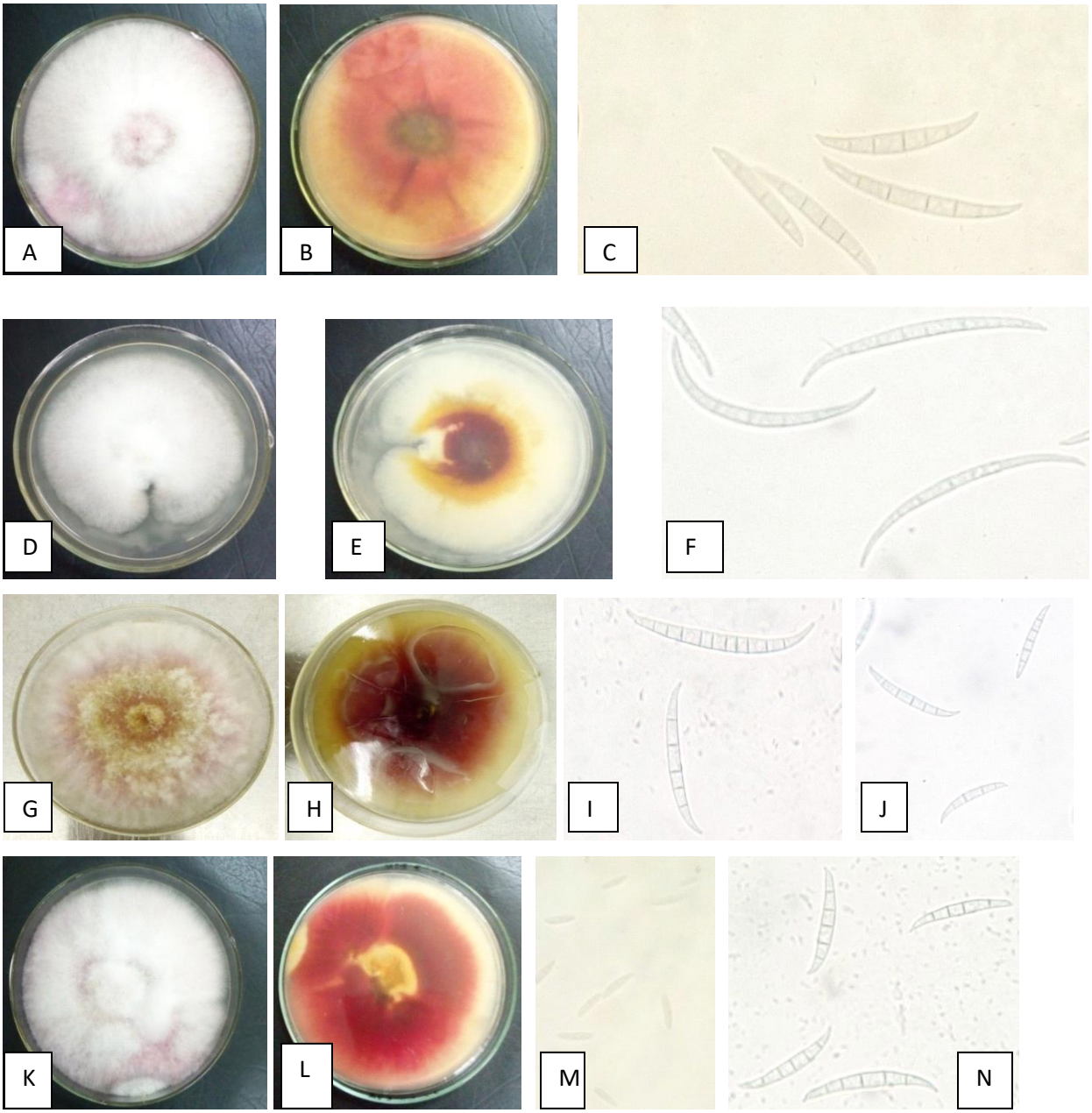

Figure 3. Morphological features of *Fusarium* spp. isolated from maize grains*:*

F. armeniacum; surface (A), reverse (B) and macroconidia (C). *Fusarium equiseti*; surface (D), reverse (E) and macroconidia (F). *Fusarium pseudograminearum;* surface (G), reverse (H) and macroconidia (I-J); *Fusarium avenaceum;* surface (K) and reverse (L) of culture on PDA; microconidia (M) macroconidia (N) at under 40x compound light microscope.

4.5.1.2. *Aspergillus* spp.

Aspergillus flavus

Aspergillus flavus was rapidly fast growing with growth rates ranging from 68mm to 76.5 after 7 days of incubation at 25˚C; on PDA (Table 6). It produced yellowish-green, colonies encircled by a white border and produced exudates on PDA media (Fig. 5A). The mycelium was commonly surface and plane. They produced soluble pigments with a brown color that was seen on the reverse of the colonies and heavy furrowed on reverse (Fig. 5B). It produced moderate condial production after 7 days of incubation period and they were predominantly uniseriate sterigmata but some were biseriate spp. were isolated; The uniseriate conidia heads had radiate vesicle while biseriates the vesicles were spherical to globose.The vesicle shape was globose and big in size; The conidia was globose and yellow green color as shown in, (Fig. 5C–D).

Aspergillus parasiticus

Aspergillus parasiticus was relatively fast growing with growth rates ranging from 55.5mm to 64.5 after 7 days of incubation at 25˚C on PDA (Table 6). On PDA the colonies were dark green with white mycelia and commonly produced surface and plane mycelium (Fig .5E). It produced soluble pigments with a brown color that was seen on the reverse of the colonies, (Fig. 5F). Predominantly they were uniserite sterigmata with radiate conidia heads; produce green condia, the vesicle shape was globose, and small in size, (Fig. 5G –H)

Aspergillus niger

Aspergillus niger was relatively fast growing with growth rates ranging from 60.5mm to 65.4 7 days of incubation at 25˚C; on PDA (Table 6). The myceliu was velvety which is plane and radial furrowed with typical black spores produced but lacked exudates and soluble pigments, (Fig. 5I). Reverse color was yellow and heavy furrowed, (Fig. 5J). It predominantly biseriates

sterigmata with radiate conidia heads; produce brown condia, the vesicle shape was globose, and big in size.They had large and wide stipe (Fig. 5K–L).

Aspergillus terreus

Aspergillus terreus was fast growing with growth rates ranging from 32.4mm to 34.3 after 7 days of incubation at 25˚C; on PDA (Table 6).The mycelium ,which is surface and submerged, yellow when young and turned to orange brown with age,(Fig. 5M). It produced biseriate sterigmata and smaller vesicle size, (Fig. 5P).

Table 6. Growth rate of *Aspergillus* spp. isolated from maize grains.

***Aspergillus* species**	**Growth rate (mm)**
A. flavus	24–35
A. parasiticus	17–21
A. niger	21–24
A. terrreus	12–14

*Measured after 7 days of incubation at 25˚C on PDA

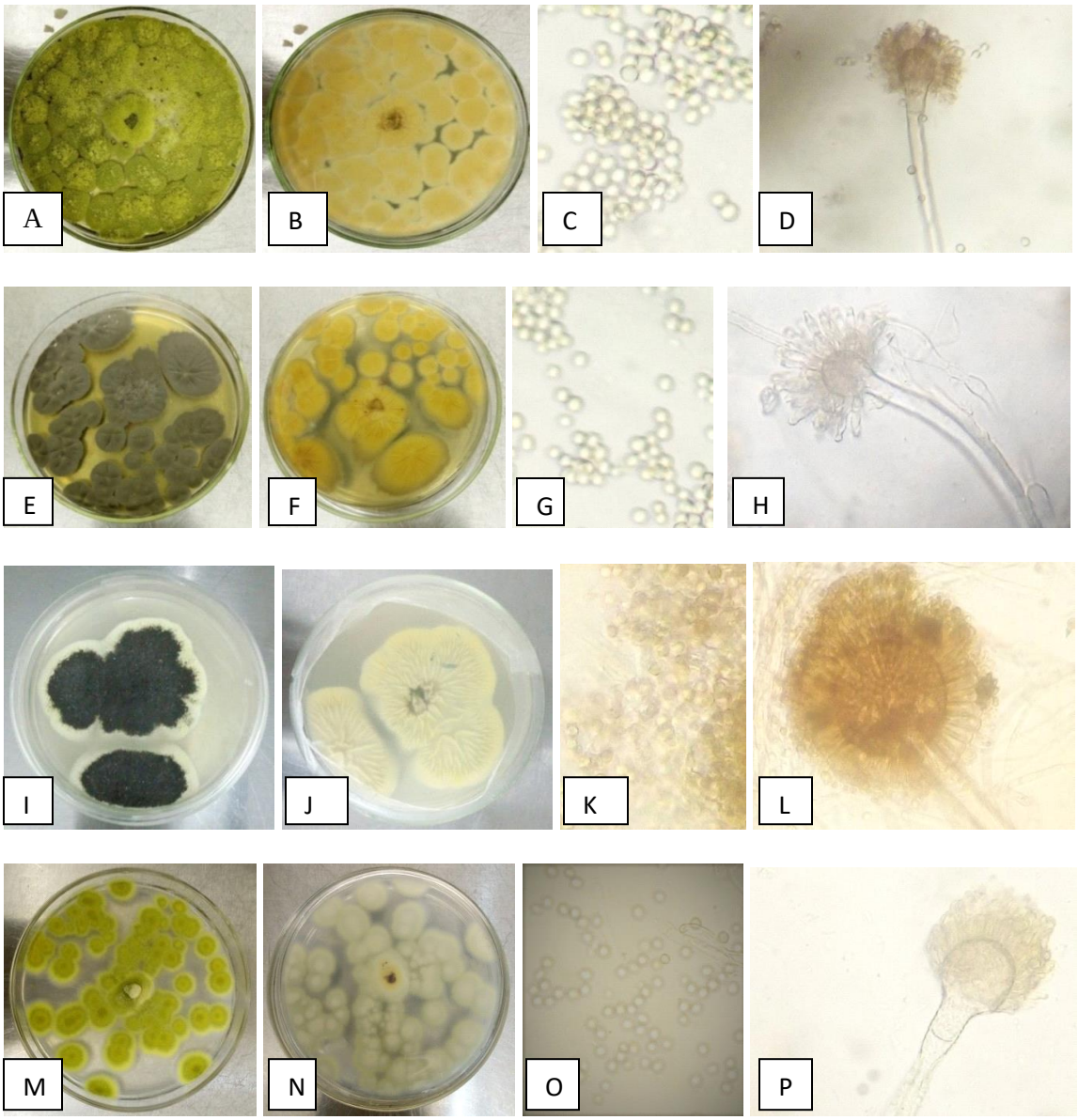

Figure 4. Morphological characteristics of *Aspergillus* spp. isolated from maize grains:

*Aspergillus flavus;*surface (A) and reverse (B) of culture on PDA; (7days old). Vesicle, sterigmata, conidiophore characters, size and shape of conidial heads (40x), (C–D). *Aspergillus parasiticus;* surface (E) and reverse (F) of culture on PDA; (7days old). Vesicle, sterigmata, conidiophore characters, size and shape of conidial heads (40x), (G–H). *Aspergillus niger;* surface (I) and reverse (J) of culture on PDA; (7days old). Vesicle, sterigmata, conidiophore characters, size and shape of Conidial heads (40x), (K–L). *Aspergillus terreus;* surface (M) and reverse (N) of culture on PDA; (7days old).Vesicle, sterigmata, conidiophore characters, size and shape of Conidial heads (40x), (O–P).

4 .5.1.3. *Penicillium* spp.

Penicillium verrucosum

Penicillium verrucosum was slow growing with growth rates ranging from 20.5mm to 24.7 after 7 days of incubation at 25˚C; on PDA (Table 7). It produced pure green conidia and granular to fasciculate colonies have a cream colored reverse. The colony of *Penicillium verrucosum* was velvet, (Fig. 6A). Conidiophores branching pattern was observed with tervert-cilliate phialides. The conidia shape was globose to sub globose. Flask shape phialides (Fig. 6C–D).

Penicillium viridicatum

Penicillium viridicatum was slow growing with growth rates ranging from 20.3mm to 22.4 after 7 days of incubation at 25˚C; on PDA (Table 7). It produced yellow green conidia and granular to fasciculate colonies, often with clear exudate droplets, (Fig. 6E). Reverse color of colony was yellow, (Fig. 6F). The colony of *Penicillium viridicatum* was velvet, (Fig. 6F). Conidiophores branching pattern was observed with tervert-cilliate phialides.The conidia shape was globose to sub globose. Flask shape phialides, (Fig. 6G).

Penicillium citrinum

Penicillium citrinum was fast growing with growth rates ranging from 68.6mm to 70.3 after 7 days of incubation at 25˚C; on PDA (Table 7). It produced blue grey green conidia and leathery colonies with moderate sporulation, often with no exudate droplets, (Fig. 6H). Reverse color of colony was Yellow, (Fig. 6I). Conidiophores branching pattern observed with tervert-cilliate phialides. The conidia shape was globose to sub globose. Cylindrical shape phialides (Fig. 6J–K).

Penicillium chrysogenum

Penicillium chrysogenum is fast growing with growth rates ranging from 34.5mm to 40.2 after 7 days of incubation at 25˚C; on PDA (Table 3). It producing yellow green to pale blue green conidia and velvety to somewhat floccose colonies, (Fig. 6 L). A typical yellow exudate produced. The colony reverse have citrine yellow color, (Fig. 19 M). Conidiophores branching pattern observed with tervert-cilliate phialides. The conidia shape was globose to sub globose cylindrical shape phialides (Fig. 6 O–P).

Table 7. Growth rate of *Penicillium* spp isolated from maize grains.

***Penicillium* species**	**Growth rate (mm)**
Penicillium verrucosum	11–13
Penicillium viridicatum	8–10
Penicillium citrinum	15–17
Penicillium chrysogenum	12–14

*Measured after 7 days of incubation at 25˚C; on PDA

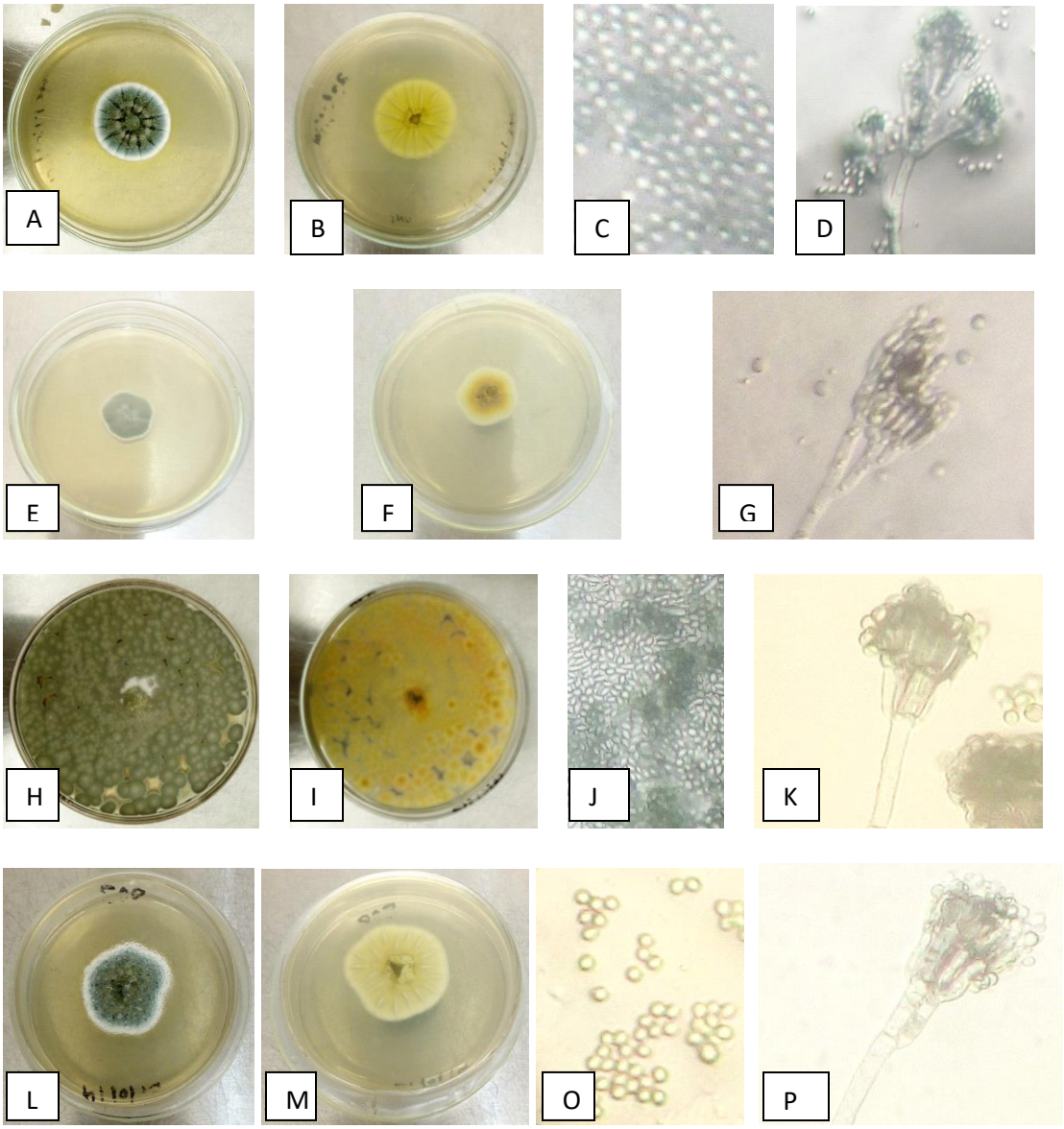

Figure 5. Morphological characteristics of *Penicillium* spp. isolated from maize:

Penicillium verrucosum; surface (A) and reverse (B) of culture on PDA; (7days old). Phialides, conidiophore characters, size and shape of conidial heads (40x), (C–D). *Penicillium viridicatum;* surface (E) and reverse (F) of culture on PDA; (7days old). Phialides, conidiophore characters, size and shape of conidial heads (40x), (G). *Penicillium citrinum;* surface (H) and reverse (I) of culture on PDA; (7days old). Phialides, conidiophore characters, size and shape of conidial heads (40x), (J–K). *Penicillium chrysogenum;* surface (L) and reverse (M) of culture on PDA; (7days old). Phialides, conidiophore characters, size and shape of conidial heads (40x), (O–P).

4.5.1.4. Other fungal spp.

Alternaria infectora

Alternaria infectoria was fast growing with growth rates ranging from 80.5mm to 81.3 after 7 days of incubation at 25˚C; on PDA (Table 8).The colony appeared flat and was covered by grayish, short, aerial hyphae in time white and hairy. Reverse side was reddish brown (Fig. 7A-B). Conidia were produced in chains that are branched. Both septate and none septate conidiophore presented (Fig. 7C).

Rhizopus stolonifer

Rhizopus stolonifer was fast growing with growth rates ranging from 83.5mm to 84.6 after 7 days of incubation at 25˚C; on PDA (Table 8). The colony was whitish,brownish sporangiophores and brown black sporangia. Large fluffy white milky colonies which later turns black as culture ages, (Fig. 7D). Sporagiophores were tall solitary in groups, branched rhizoids, (Fig. 7F-G).

Trichoderma harzianum

Trichoderma harzianum was fast growing with growth rates ranging from 80.5mm to 83.3 after 7 days of incubation at 25˚C on PDA (Table 8). The colony reverse side has grayish color (Fig. 7I). Conidiophores were branched in pyramidal structure, i.e. long repeatedly branched laterally branches. Conidia were sub globose to short ovoid in shape (Fig. 7J-K).

Cladosporium macrocarpum

Cladosporium macrocarpum was fast growing with growth rates ranging from 78.5mm to 80.7after 7 days of incubation at 25˚C; on PDA (Table 8). The colony appeared sulcate, velvety grayish white. Reverse is dark, 7L-M). The conidiophore was smooth, straight and sometime

branched and none septate conidia (Fig. 7 N). The conidia had ellipsoidal to sub cylindrical shape (Fig. 7 N).

Table 8. Growth rate of other fungal species isolated from maize grains.

Other fungal species	**Growth rate (mm)**
Alternaria infectoria	20–23
Rhizopus stolonifer	29–32
Trichoderma harzianum	22–24
Cladosporium macrocarpum	20–22

*Measured after 7 days of incubation at 25˚C on PDA

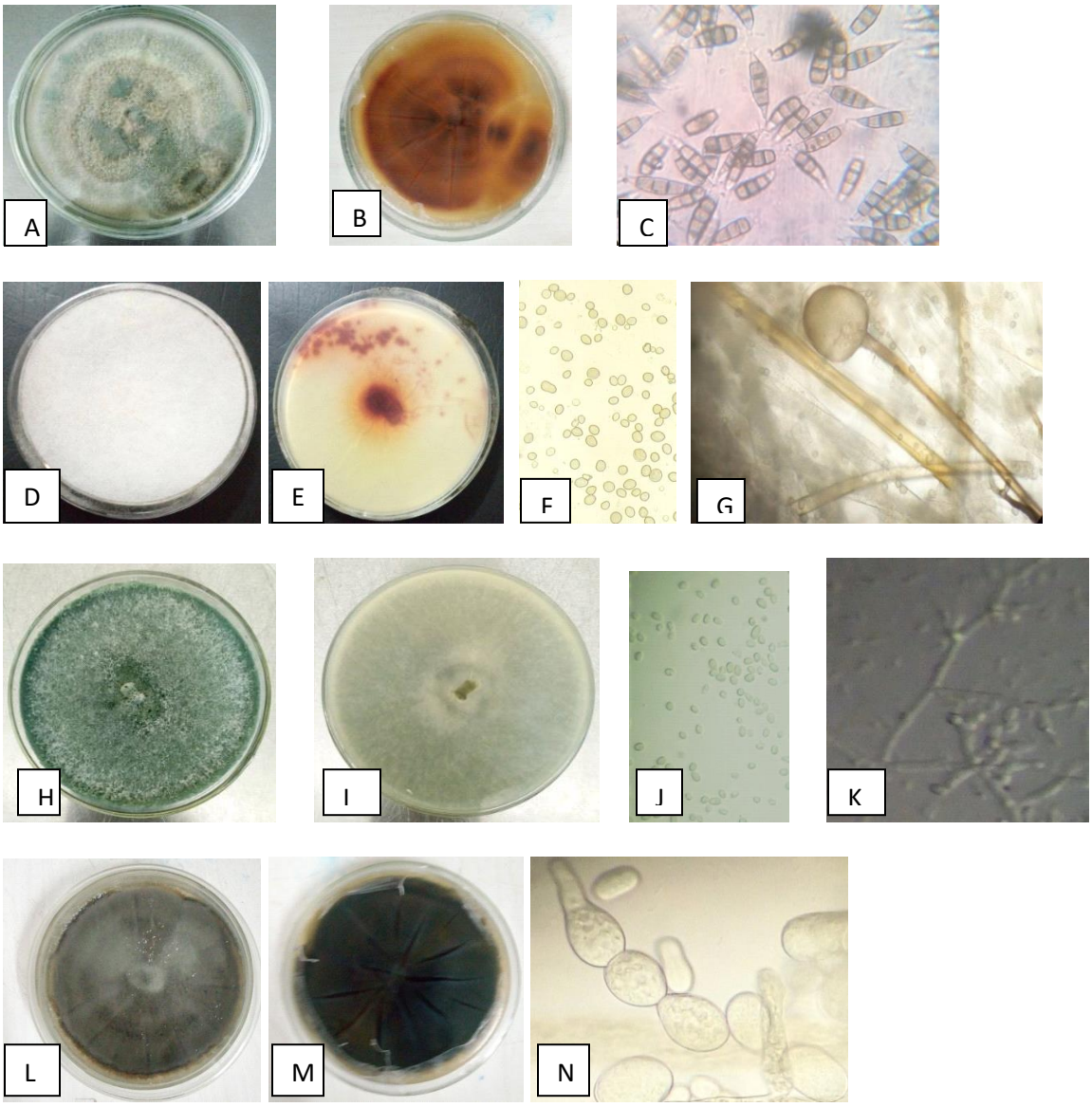

Figure 6. Morphological features of other fungal spp. isolated from maize grains:

Alternaria infectoria; surface (A) and reverse (B) of culture on PDA; (7days old). Conidiophore characters, size, septa and shape of conidial heads (40x), (C). *Rhizopus stolonifer;* surface (D) and reverse (E) of culture on PDA; (7days old).mycelia, sporangiophore, columela, rhizoids40x), (F–G). *Trichoderma harzianum;* surface (H) and reverse (I) of culture on PDA; (7days old). Mycelium, conidiophore characters, size and shape of conidial heads (40x), (J–K). *Cladosporium macrocarpum;* surface (A) and reverse (B) of culture on PDA; (7days old). , conidiophore characters, size and shape of conidial heads (40x), (C–D).

5. Summary, Conclusion and Recommendations

5.1. Summary and Conclusion

Maize is a key cereal crop that plays a tremendous role in increasing food security and household nutrition in Ethiopia. Maize production is constrained by biotic and abiotic factors. Among the biotic factors fungi are the major ones. During storage, several kinds of fungi can remain associated to maize seeds either causing their deterioration or simply remain viable to infect germinating seedling. The average moisture content data of grains stored in the five types of storages for six months are shown in Table 1 in appendix. As time passed by the moisture contents in all five storage types decreased. The reduction in moisture content of grains could be loss of moisture to the air in the storage through transpiration (Evaporation). Similarly, Negasa *et al*. (2019) reported as time passed by the moisture contents in all five storage types (Basket ,Crips,Gombisa, Sack and Plastic container) decreased.

The development of these fungi can be affected by moisture content of the grain, storage time and storage type. As time passed by the moisture contents in all five storage types decreased. The reduction in moisture content of grains could be due to loss of moisture to the air in the storage through transpiration (Evaporation). The fungi genera typically found in at harvest and stored grains in the present study were *Fusarium, Aspergillus, Penicillium, Alternaria, Rhizopus, Tricoderma* and *Cladosporium.*

The finding of this study revealed that, storage duration and storage types played important role and influenced fungal contamination in maize. Thus, the maize grains stored in different storage types for 6 month showed increase in fungal contamination levels with time. At the beginning of storage (0 month), the mean fungal contamination level was low ; however, after 3 and 6 months of storage the fungal contamination level increased proportionally with time. The *Fusariu*m spp.

were the most dominant storage fungi of maize followed by *Aspergillus* spp. These fungi are important in producing secondary metabolites which are carcinogenic to both humans and animals. Since these storage fungi are very important in causing postharvest yield losses and production of mycotoxins, giving attentions to these fungi may play a vital role in reduction of post-harvest loss of maize due to fungi and minimize the risk of mycotoxin exposure to human and animals. To avoid mycotoxin contamination, maize should be monitored regularly to assure safe moisture content and storage conditions. ,

5.2 Recommendations

To avoid contamination of maize by mold fungi, maize should be dried to moisture contents below 14% immediately after harvestand there is need for pre-harvest treatment of maize so as to reduce the rate of infection and spoilage by fungi. Since the practice of grain storage has direct effect on fungal contamination. The plastic and gombisa storage mechanisms in southern Ethiopia need to be improved so that they can protect stored maize from absorbing moisture from the storage areas. There is a need for increased awareness creation about the impacts of aflatoxins and extended storage period that enhance fungal contamination in maize grains and need for more research and technology for the prevention and control of Aflatoxins.

6. Referance

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7. Appendices

Table 1. Mean moisture content of stored maize grains over the storage periods, 2019/2020

Storage period (Months)	Mean of grain moisture content (% in dry base)				
	Storag Basket	Storage crips	Storage sacks	Storage gombisa	Plastic storage
0	18.5±0.30	16.60±0.20	15.47±0.12	14.10±0.12	13.51±0.16
3	16.3±0.44	15.17±0.06	14.13±0.08	13.23±0.09	12.73±0.19
6	15.1± 0.15	14.18± 0.20	13.40± 0.25	12.63±0.18	11.80±0.12
LSD (5%)	1.09	0.590	0.584	0.456	0.540
CV (%)	3.30	1.950	2.04	1.72	2.13

Biographical sketch

The author was born on April 1991 in Shinshicho town, Kambata Tambaro zone SNNPR of Ethiopia. He attended his elementary school at Shinshicho Metoma Zararo from 1989-1997 E.C. After successfully passing his grade eight qualifying exam he joined Shinshicho secondary and preparatory school and completed his preparatory education in 2001. Then he joined Hawassa University in 2002 and graduated with Bachelor of Science degree in plant science in July 2004. After his graduation he was employed in the Southern Region Kambata Tabaro Zone, Kachabirra woreda agricultural and natural resource office, as crop protection expert. After 5 years of service, he joined the Hawassa University in 2010 to study his MSc degree in Crop protection.