

**Isolation and Enumeration of Fungi and Determination of
Contributing Factors to Fungal Spoilage in Maize (*Zea mays* L.)
Originated from Selected Woredas of Oromia, Ethiopia**

By:

Temesgen Assefa Gelaw



**A Thesis Submitted to The Department of Applied Biology in Partial
Fulfillment of the Requirement of the Award of degree of
Master of Science in Applied Biology (Biotechnology)**

School of Applied Natural Science

Office of Graduate Studies

Adama Science and Technology University

Adama, Ethiopia

June, 2019

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Advisor: Dr. Teshome Geremew (PhD)

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APPROVAL SHEET

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This is to certify that the thesis prepared by **Temesgen Assefa Gelaw** entitled “**Isolation and Enumeration of fungi and Determination of Contributing Factors to Fungal Spoilage in Maize (*Zea mays* L.) Originated from selected woredas of Oromia, Ethiopia**” Submitted in Partial Fulfillment of the Requirement of the Award of Degree of Master of Science in Applied Biology (Biotechnology) complies with the regulation of the university.

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DEDICATION

This thesis work is dedicated to my beloved parents and the rural community.

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Tables of Contents

Contents	Page
ACKNOWLEDGMENTS.....	i
LIST OF TABLES	iv
LIST OF FIGURES.....	v
LIST OF ACRONYMS.....	vi
ABSTRACT	viii
1. INTRODUCTION.....	1
1.1 Background of the study	1
1.2 Statement of Problem.....	2
2. OBJECTIVES OF THE STUDY	3
2.1 General Objective.....	3
2.2 Specific objectives.....	3
2.3 Significance of the study.....	3
3. LITERATURE REVIEW.....	4
3.1 Biology of Fungi.....	4
3.1 The Role of Fungi in Postharvest Losses of Maize.....	5
3.2. Traditional Grain Storage in Ethiopia.....	6
3.3 <i>Fusarium</i> and its importance in maize.....	7
3.3.1 <i>Fumonisin</i> s	7
3.3.2 <i>Trichothecenes</i>	8
3.3.3 <i>Zearalenone</i>	9
3.4 <i>Aspergillus</i> and its importance in maize.....	9
3.4.1 <i>Aflatoxin and its Exposure</i>	10
3.5 Factors influencing fungal infection of maize.....	12
3.6 Control of Fungal contamination.....	13
3.6.1 <i>Pre-harvest control</i>	13
3.5.2 <i>Post-harvest control</i>	14
4. MATERIALS AND METHODS	15
4.1 Description of sampling area.....	15
4.2 Sample type, size and sampling technique.....	16
4.3 Fungal isolation and morphological characterization.....	17
4.4 Data analysis.....	18
5. RESULTS AND DISCUSSIONS	19

5.1 Fungal isolation from maize.....	19
6. CONCLUSION AND RECOMMENDATIONS.....	31
6.1. CONCLUSION.....	31
6.2. RECOMMENDATIONS.....	32
REFERENCES.....	33
APPENDICES.....	43

LIST OF TABLES

Table 1: Mean fungal percent of infection on maize sampels obtained from 12 districts.....	21
Table 2: Correlation between moisture content, storage duration and fungal infection.....	22
Table 3: Moisture content and percent of infection record of the maize samples.....	24
Table 4: Fungal genera isolated from maize samples.....	28

LIST OF FIGURES

Figure 1: Map of the study area.....	16
Figure 2: Incidence of fungal genera and fungal percent of infection on maize	20
Figure 3: Maize storage type versus percent of infection	23
Figure 4: Sample source versus fungal percent of infection.	23
Figure 5: Mean comparison of percentage of storage duration with fungal percent of infection.	27
Figure 6: Isolation of fungi from maize samples.....	29
Figure 7: Five day's old cultural characteristics of fungal pure cultures.	29
Figure 8: Microscopic examination of isolates.....	28

LIST OF ACRONYMS

AF	Aflatoxin
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CSA	Central statistical agency
DON	Deoxynivalenol
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
IARC	International Agency for Research on Cancer
Masl	meters above sea level
PDA	Potato Dextrose Agar
SPSS	Statistical Package for the Social Sciences

LIST OF APPENDICES

Appendix 1: Sample collection photos.....	41
Appendix 2: Fungal culturing and incubation.....	42
Appendix 3: Media preparation.....	43
Appendix 4: Microscopic examination and slide culture technique.....	44
Appendix 5: Pure culture preparation.....	45
Appendix 6: Mean moisture content and percent of infection.....	46
Appendix 7: Chemical composition of Potato Dextrose Agar	47

ABSTRACT

Maize (Zea mays L.) is one of the most important staple crops in developing countries and it is the most widely cultivated plants in East Africa. Fungal spoilage on maize resulted in grain deterioration and mycotoxin contamination. In Ethiopia, maize production is prone to losses due to mold contamination. The purpose of this study was to isolate and characterize mycoflora and determine the Contributing factors to fungal spoilage in Maize. A total of 72 maize samples were collected based on incremental sampling method from east and west shewa zones of Oromia, Ethiopia. Four top maize producing districts consisting of two rural kebeles and one town were selected and from each site 6 maize samples of 1 kg were collected. Samples were properly labeled with sample history and the moisture content was measured upon collection. From each sample, 15 maize grains were taken randomly and disinfected with 70 % ethanol for 2 minute, rinsed with distilled water and triplicates of five grains were cultured in Potato Dextrose Agar (PDA) amended with 0.01% chloramphenicol for 5-7 days at 25°C. Fungal colonies were purified by sub-cultured on PDA. Morphological and microscopic characterization of the isolates was done based on fungal identification manual. Statistical data was analyzed using SPSS version 20. From the total 1080 maize grains analyzed, 613 fungal isolates were recorded with 54.1% mean percent of infection. 448 isolates belongs to the genus Fusarium followed by 52 Penicillium, 46 Muchor and Rhizopus, 33 Aspergillus and 34 others. Samples obtained from Dano district showed higher mean percent of fungal infection (68.15%) followed by Adami Tulu (60.37%), Bako Tibe (50%) and Arsi Negelle (37.78%). Maize stored in plastic bag showed higher fungal percent of infection (62.5%) followed by gotera (51.1%) and fertilizer bag (49.63%). The grain mean moisture content was 14.16% with 11.0% minimum and 17% maximum value. Moisture content and storage duration were negatively correlated with fungal prevalence and were found statistically insignificant ($p > 0.05$). Grains collected from warehouse were more infected (58.88%) followed by store (55%), open market (54.28%) and household (52.78%). No significant difference was obtained between the use of fertilizer and compost with fungal infection. Storage duration and percent of fungal infection showed weak negative correlation with statistical insignificance where one month storage showed higher prevalence (63.7%). Overall, the prevalence of fungal infection in the study sites was higher. Awareness creation to farmers, and capacity building training to experts and agricultural extension workers about pre and postharvest handling, maize breed type and mycoflora association studies and use of holistic, cumulative integrated management, monitoring, and precautionary measures are recommended.

Keywords: Fungal Spoilage, Maize, Mold, Mycoflora, Mycotoxin

1. INTRODUCTION

1.1 Background of the study

Cereals represent a substrate that facilitates mold growth and contamination. Maize is the most commonly contaminated cereal, followed by small grain cereals (wheat, sorghum, oat, barley and rice) and oilseeds (peanut and cotton seed). Contamination may occur as early as in the cultivation period, but also during the crop storage period (Jelka *et al.*, 2017). Maize (*Zea mays* L.) was originated from tropical zones of America and has now become the highest cereal worldwide production. It has become particularly important in developing countries and it is one of the most widely cultivated gramineous plants in East Africa. It is believed to have been introduced to Ethiopia in the 1600s to 1700s and has become an important cereal grain widely cultivated under a wide range of environmental conditions, between 500 to 2400 meters above sea level. Accordingly, maize is being grown in different parts of the country; Oromia and Amhara regional states are the major producers (Yibrah and Dereje, 2015).

Maize is an agricultural crop of worldwide importance grown both for the food industry and for other purposes (Jan *et al.*, 2012). Fungal contamination becomes an issue of special concern during rainy seasons characterized by substantial temperature variations, since these conditions favor mold contamination and therefore also mycotoxin production (Pleadin *et al.*, 2013). In substantial proportions of maize-producing areas worldwide, the crops are subject to contamination from mycotoxins (Tomoko *et al.*, 2007).

In most parts of Africa, the mycotoxin hazard is very high because the limited food supply has forced people not to reject any material that can be used as food, even if the organoleptic quality of the food has been changed by molds while the prevailing malnutrition enhances people's susceptibility to even very low levels of mycotoxins. This situation is further aggravated by the warm humid tropical conditions and improper drying and storage practices (Dejene *et al.*, 2004) which provide optimal conditions for mold growth and subsequent build-up of mycotoxins within a short spell of time (Samuel *et al.*, 2011).

In Ethiopia, fungal contamination is causing maize production to be prone to losses via yield reduction as well as kernel contamination by mycotoxins that poses a health risk to humans and animals. Members of the fungal genera *Aspergillus*, *Fusarium*, and *Penicillium* cause

frequent and problematic contamination of foods and feeds. They are ubiquitous and contaminate various feedstuffs and agricultural crops and induce a range of harmful effects (Jolly *et al.*, 2011). These are found in many feeds and foodstuffs especially in plants during pre and post-harvest, transportation, processing and storage and are detected in cereal crops (Ezekiel *et al.*, 2014).

Maize became increasingly important in the food security of Ethiopia following the major drought and famine that occurred in 1984. More than 9 million smallholder households grow maize in Ethiopia at present (Tsedeke *et al.*, 2015). In Ethiopia, maize production is higher and is the second highest in Sub-Saharan Africa. According to World data atlas, maize production of Ethiopia increased from 2.34 million tons in 1998 to 8.12 million tons in 2017 growing at an average annual rate of 7.57 % (Hadush *et al.*, 2017).

The important contribution of maize to smallholders' food security makes it a commodity of national interest. In terms of internal distribution of maize production, Oromia (61%), Amhara (20%) and Southern Nations Nationalities and Peoples Region (12%) are the dominant areas of maize cultivation (CSA, 2013). Accordingly, 80% of the maize produced by smallholders is used for household consumption without being processed, 10% for sale and the remaining 10% for seed and other purposes (Rashid *et al.*, 2013).

1.2 Statement of Problem

In Ethiopia, even if higher production and utilization, maize production is prone to various factors such as insect infestation, fungal invasion and mycotoxin contamination. Molds such as *Aspergillus*, *Fusarium* and *Penicillium* species and their mycotoxins are significantly retarding crop production where the occurrence of maize fungal contamination in developing countries gets global attention (Maria *et al.*, 2013).

In Ethiopia the majority of farmers (more than 93.3%) use traditional maize storage containers that expose their stored grains to attack by storage pests, molds and or other factors. The average actual loss per household is about 12 % of the average total grain production (Dubale *et al.*, 2018). The major contributing problems for high postharvest losses relates with poor postharvest infrastructure and marketing systems, poor research and improvement capability, and insufficiencies in guidelines and information sharing (Dubale, 2018). Fungal

contamination jeopardizes both human and animal health (Pleadin *et al.*, 2013). Even if it has an impact on economic, public health and animal productivity across several countries; in Ethiopia, it is narrow or least researched and not resolved. Therefore, isolation and enumeration of fungi and determination of factors for fungal spoilage is crucial to adopt better preventive and control strategies.

2. OBJECTIVES OF THE STUDY

2.1 General Objective

The general objective of this research was to isolate and enumerate fungi and determine the contributing factors to fungal spoilage in maize Originated from selected woredas of Oromia, Ethiopia.

2.2 Specific objectives

- ✓ To isolate and characterize major fungi associated with maize samples
- ✓ To evaluate prevalence of fungi associated with maize samples.
- ✓ To determine environmental factors contributing to fungal contamination of maize.

2.3 Significance of the study

The finding of this study will benefit maize farmers by raising awareness to minimize fungal invasion in maize. It also benefits researchers in the area of mycology and plant pathology by providing scientific data on the prevalence of fungal contamination in maize in the study area. Moreover, it will provide valuable information to the direct policy makers to design appropriate fungal invasion preventive and control strategies.

3. LITERATURE REVIEW

3.1 Biology of Fungi

Fungi are distinctive organisms that digest their food externally by secreting enzymes into the environment and absorbing the dissolved organic matter back into their cells. Most have cell walls composed primarily of chitin (a substance that is also found in the animal kingdom, for example in the exoskeletons of insects and shells of crabs and lobsters). They also store food reserves as glycogen and lipids (not starch as in plants). Thus, despite the superficial resemblance of some fungi to plants (e.g. having rooted, stalked structures), their non-photosynthetic, absorptive method of feeding and their different cell walls, cell membrane chemistry, methods of food storage and DNA indicate that they form an independent kingdom (Wainright, 1993).

Some fungi exist as microscopic, single-celled yeasts (e.g. the bloom on the skin of a plum or grape), while the most complex forms have a far more elaborate multicellular body comprising an interconnected network, or mycelium, of minute, protoplasm-filled tubes called hyphae. The individual threadlike tubes extend at their tips and form branches that explore their environment, fight with other fungi to occupy territory, or interact with other organisms. These activities can occur inside a few cells of a leaf, in a column of decay extending for several metres inside a tree trunk, or in the soil, for example forming a giant 'fairy ring' of mushrooms in ancient grassland (Woese et al., 1990).

Fungi are associated with the roots of almost all plants, including forest trees and most food crops – the fungi act as living intermediaries between the plant and the surrounding soil. This type of root–fungal interaction is known as a mycorrhiza and, like lichens, the partners engage in a mutualistic relationship. The plant benefits from the greater capacity of the fungus to absorb water and nutrients and to mobilize minerals that would otherwise be unavailable, and the fungus benefits from a steady source of carbohydrates from the plant. Fungi are also the most significant organisms that break down cellulose, hemicellulose and lignin. These are the tough polymers in plant cell walls that give wood its great strength and durability. Their decomposition by wood-decaying fungi releases key plant nutrients back into the soil, thereby allowing the next generation of seedlings to grow. Without nutrient cycling, life on Earth as

we know it would not exist; nutrients would be in such short supply that biological growth would be severely limited right across the globe (ShalchianTabrizi, 2008).

Fungi have diverse, complex life cycles and can reproduce sexually, asexually and/or parasexually (which involves combining genes from different individuals without forming sexual cells and structures). They can do this through the production of different kinds of spores and/or through fragmenting hyphae. For most of their life cycles, the majority of animals and plants are built of diploid cells (i.e. combining one genome from each parent) and form bodies with determinate growth. In contrast, many fungal lineages are more complex, and for much of their life cycle their cells may be haploid (with just one genome), diploid, dikaryotic (two nuclei per cell) or multikaryotic (multiple nuclei per cell). In addition, many fungi have indeterminate growth, which means they can continue to grow as long as resources and conditions are suitable and enables them to take the shape of their environment (e.g. a leaf, a cheese, a lung) (Kew, 2018).

3.1 The Role of Fungi in Postharvest Losses of Maize

Maize production undergoes a number of procedures such as harvesting, drying, threshing, winnowing, processing, bagging, storage, transportation, and exchange beforehand then finally to the consumer (Dubale, 2018). Efficient post-harvest system is used to ensure that the food commodities fulfill the needs of the customer in terms of quality, quantity, and safety. Post-harvest losses in the developed countries normally are lower than in the developing countries. Due to lack of more efficient farming systems, better transportation infrastructure, better farm management and efficient storage and processing facilities that ensure a higher percentage of the harvested and processed foods, developing countries have high post-harvest loss (Abramson *et al.*, 2015).

Fungal contamination of grains is often an additive process, which starts in the field and potentially increases during harvest, drying and storage conditions. In addition to grain yield losses, the fungal infection of maize has been determined to reduce the processing and nutritional quality of grains (Miller, 2008). The extent of reduction in grain quality is logically related to the degree of fungal development. The losses incurred due to mycoflora growth are not only of economic importance but also of significant public and animal health concern due to mycotoxin production (Golob, 2007).

Fungal spoilage can happen during storage or in the field. Field fungi are those which predominate in the field and are assumed to have insignificant consequences in the post-harvest period while storage fungi dominate the mycoflora during storage and may also be present on the crop during the pre-harvest period. The *Fusarium* spp. is considered as storage fungi, whereas the *Penicillium* and *Aspergillus* are considered as field fungi. In a number of cases worldwide, poor postharvest practices enable typical field fungi to become important during the storage period (Bryden, 2009).

Fungi contaminate and produce toxins called mycotoxins either before harvest or under post-harvest conditions. The most important fungal species implicated in the production of mycotoxins belongs to the genera *Aspergillus*, *Fusarium*, and *Penicillium*. The most important mycotoxins produced include aflatoxin (AF), ochratoxins (OT), deoxynivalenol (DON), zearalenone (ZEA), fumonisin (FUM) and trichothecenes. Furthermore, DON, ZEA, FUM, and trichothecenes are all produced by the *Fusarium* species (Golob, 2007).

3.2. Traditional Grain Storage in Ethiopia

In Ethiopia, grain storage facilities used by farmers are invariably traditional. The type of storage structure in a particular region of the country usually varies depending on the type of building materials available in the area. Nowadays bags storage system is being adapted by farmers in rural areas of Ethiopia. Farmers use either indoor and/or outdoor structures or underground pits to store their grain (Amare, 2002).

The outdoor grain storage facility known locally as *Gotera* (Amharic) is probably the most common type of storage, especially in the highlands. It is a sort of basketwork woven from bamboo splits or slender wooden slicks where the inside and sometimes the outside walls are plastered with freshly cow dung and mud. The top of the *Gotera* is covered with thatched conical roofing. The duration of grain storage in *Gotera* is on the average about 8 - 10 months. Often a *Gotera* is neither rodent nor moisture proof. On the other hand as long as adequate pest control methods are not available to farmers, *Gotera* ought to be preferred to corrugated iron bins; the problem of heating and cooling of the grain next to the walls of the iron bin, and subsequent moisture migration which might lead to more insect and mold growth is avoided by using a well- constructed *Gotera* (Amare, 2002). Dibignit in Amharic (*Gota*) is another

cylindrical structure which is constructed from mud mixed with grass or tef straw. It is kept inside the house. It usually used to keep grains for short time storage (Amare, 2002).

The use of underground pits is probably the oldest form of storage of grain which has been practiced for thousands of years in the Middle East, Africa and India (Lynch *et al.*, 1986). In Ethiopia, pit storage is reported to be used by farmers in Hararghe, Wollo, Shewa, Gojam and Illubabor regions. But it is of particular importance in the Hararghe and Wollo regions. Sorghum, maize, wheat, barley and to less extent bean are main crops stored in pits. This method is widely used because of the ease of construction and allows safer storage of grain for a long time (Lynch *et al.*, 1986).

3.3 *Fusarium* and its importance in maize

Fusarium species are ubiquitous in soils. Several phytopathogenic species of *Fusarium* are found to be associated with maize including *F. verticillioides* (Sacc.) Nirenberg, *Fusarium proliferatum* (Matsushina) Nirenberg, *F. graminearum* Schwabe and *F. anthophilum* (A. Braun) Wollenweber. Among them, *F. verticillioides* is likely to be the most common species isolated worldwide from diseased maize (Fandohan *et al.*, 2003).

Fusarium verticillioides infects maize at all stages of plant development, either via infected seeds, the silk channel or wounds, causing grain rot during both the pre- and postharvest periods. Symptomless infection can exist throughout the plant in leaves, stems, roots, grains, and the presence of the fungus is ignored in many cases because it does not cause visible damage to the plant (Fandohan *et al.*, 2003).

Among the most toxic and prevalent *Fusarium* toxins are the fumonisins, zearalenone, moniliformin and trichothecenes (T-2/HT-2 toxin, deoxynivalenol, diacetoxyscirpenol, nivalenol) (Nesic *et al.*, 2014).

3.3.1 *Fumonisins*

Fumonisins are *fusarium* toxins first discovered in 1988 (Gelderblom, 1988) and constitutes the large family of compounds (Antonio *et al.*, 2018) which are produced by various fungi most dominantly *Fusarium verticillioides* and *Fusarium proliferatum*. They are a group of potentially carcinogenic mycotoxins. Other fungal species, including *F. dlamini*, *F. nygamai*

and *F. napiforme* also produce fumonisins (EFSA, 2005). Fumonisins have strong structural similarity to sphinganine which are the precursor of sphingolipids.

A large number of fumonisins have been identified so far, but the B group (including FUM B1 to FB4) is the dominant one in food and feed commodities, with FB1 the most toxic. Related diseases vary in different animals, ranging from neurological to pulmonary and esophageal diseases (Desjardins, 2006). Ecological needs of *F. verticillioides* are deeply studied and they agree on maximum fumonisins production around 30 °C, strongly influenced by water dynamics (Battilani *et al.*, 2011).

Undesirable effects of FUM B in animal and human organisms result from the similar chemical structure of FUM B1 compared to sphingosine and sphinganine-substrates of ceramide synthetase, being a key enzyme in the biosynthesis of sphingolipids. FUM B1 as a specific inhibitor of this enzyme also inhibits the formation of sphingolipids, leading to a reduction of their contents in eukaryotic cells as well as in serum, kidneys, the liver and urine (Direito *et al.*, 2009). Inhibition of sphingosine biosynthesis and increase in sphinganine concentration is the most sensitive indicator of exposure to fumonisins. Disruption of sphingolipid metabolism probably also explains the important biological effects caused by these toxins, e.g. disorders in the cell cycle, an increase in oxidative stress, and cell apoptosis followed by necrosis (Domijan *et al.*, 2008). Based on numerous data concerning contamination of agricultural products with fumonisins and the diseases they cause, the International Agency for Research on Cancer (IARC) in 2002 classified fumonisin B1 among substances probably carcinogenic to humans (class 2B) (IARC 2002).

3.3.2 Trichothecenes

Trichothecenes, mainly produced by various species of *Fusarium*, include deoxynivalenol (DON), nivalenol, 3-and 15-acetyldeoxynivalenol, T-2 toxin, HT-2 toxin and diacetoxyscirpenol (Miller, 2002). Other genera such as *Trichoderma*, *Trichothecium*, *Myrothecium* and *Stachybotrys* are also known to produce these compounds. Trichothecenes are mainly found in cereals in almost all their growing areas around the world. The optimal temperature for DON production ranges from 26 to 30 °C (Paterson and Lima, 2010). DON in food and feed can influence human and animal health with symptoms such as vomiting, anorexia, growth reduction, reproductive disorders and immunosuppression. The most

frequent contaminants are deoxynivalenol (DON), also known as vomitoxin, nivalenol (NIV), diacetoxyscirpenol (DAS), while T-2 toxin is rare. Common manifestations of trichothecenes toxicity are depression of immune responses and nausea, sometimes vomiting. The first recognized trichothecene mycotoxicosis was alimentary toxic aleukia (Peraica *et al.*, 1999). Trichothecenes inhibit protein, DNA and RNA synthesis, and have immunosuppressive and haemorrhagic effects. Acute and sub-acute toxicity of trichothecenes in animals is characterized by vomiting, feed refusal, decreased weight gain and increased susceptibility to infectious diseases (Samuel *et al.*, 2011).

3.3.3 Zearalenone

Zearalenone (ZEA) is mainly produced by *Fusarium graminearum* and related species such as *Fusarium culmorum* in cereals (Desjardins, 2006). Therefore, its presence is commonly related to DON production (Van der Fels-Klerx *et al.*, 2012). The contamination of food and animal feeds with ZEA is worldwide. It has estrogenic and anabolic activity, and can cause infertility, abortion or other breeding problems, especially in swine and occasionally hyper estrogenic syndromes in man (Zinedine *et al.*, 2007).

Generally; chronic dietary exposure to *Fusarium* toxins can cause a variety of toxic effects in both humans and animals. Among the identified FUMs, FB1 is the most prevalent with proven immunotoxic, hepatotoxic, neurotoxic and nephrotoxic effects. ZEA is an estrogenic toxin may lead to different changes in the reproductive system while DON is known to have an immunotoxic effect and may affect changes in brain neurochemicals. When T-2 and HT-2 toxins are ingested, they can influence the incidence of several effects: nausea, abdominal pain, dizziness, dermal necrosis, inhibition of protein synthesis (IARC, 2002).

3.4 *Aspergillus* and its importance in maize

The genus *Aspergillus* which belongs to the phylum Ascomycota includes over 185 known species. Several members of *Aspergillus* section *flavi* produce aflatoxin. These includes *Aspergillus flavus* and *Aspergillus parasiticus*, as well as several less common taxa including *Aspergillus nomius*, *Aspergillus tamarii*, *Aspergillus pseudotamarii*, *Aspergillus minisclerotigenes* and *Aspergillus bombycis* (Matthias, 2009). *Aspergillus* species other than section *flavi* such as *Aspergillus ochraceoroseus* can also produce aflatoxins. *Aspergillus* section *flavi* can survive temperatures ranging from 12 °C to 48 °C, with the optimal growth

from 28 °C to 37 °C, with a high humidity of above 80% (Matome *et al.*, 2017). The fungus mostly exists in the form of mycelium or asexual conidia spores (Matthias, 2009).

3.4.1 Aflatoxin and its Exposure

Dietary exposure to aflatoxin can be estimated by analysis of contamination level of food samples combined with dietary intake surveys, such as daily recalls and food frequency questionnaires. Although these methods can be effective in estimating aflatoxin exposure level in large samples, they have many limitations. For instance, aflatoxin contamination in food typically has a heterogeneous distribution that can confound accurate measurement of contamination levels due to uneven sampling. Dietary intake surveys are subject to recall bias and social desirability issues, which additionally could lead to an under- or over-estimation of actual exposure (Yun *et al.*, 2016).

Biomarkers are considered to be more accurate to measure the degree of individual exposure, as they are objective indicators and key determinants of internal dose and biologically effective dose. The biomarkers for aflatoxin exposure include the aflatoxin-N7-guanine adducts excreted in urine, which reflect the previous day's exposure; aflatoxin M1 (AFM1), the hydroxylated metabolite of AFB1, which is found in breast milk and reflects exposure over the previous 24 hours; and the aflatoxin-albumin adduct in plasma or serum, of a half-life of ~2 months, which permits the measurement of more chronic exposure to aflatoxin (Routledge and Gong, 2011).

Aflatoxin exposure is not major issues for developed countries, as there are strictly enforced regulatory limits in place and the diet are more diverse. Aflatoxin albumin is rarely detected in blood samples from populations in these regions. For instance, in a subset of 2051 individuals that participated in the 1999-2000 of National Health and Nutrition Examination Survey, which is a representative cross-sectional survey of the US population, only 1% had detectable levels ($\geq 0.02 \mu\text{g/L}$) of AFB1-lysine in their blood (Schleicher *et al.*, 2013).

There is high prevalence of aflatoxin exposure in Asian countries. For example in Malaysia, where 97% of 170 samples had detectable AFB1-lysine adduct levels ranging between 0.20 to 23.26 pg/mg (Leong *et al.*, 2012). Furthermore, a study examining aflatoxin exposure in pregnant women in South Asia using isotope dilution mass spectrometry to measure AFB1

lysine, found detectable levels of the biomarker in 94% of blood samples collected from Nepalese pregnant women (n = 141), with levels ranging between 0.45 to 2939.30 pg/mg (Groopman *et al.*, 2014).

Aflatoxins have been associated with various diseases; aflatoxicosis in livestock, domestic animals and humans worldwide. Aflatoxicosis is poisoning which resulted from ingestion of food or feed contaminated with aflatoxins. It is a potent liver carcinogen in rodents and human beings. Aflatoxin poisoning is reported from all over the world in almost all domestic and non-domestic animals such as cattle, horse, rabbit, and other primates (Chauhan *et al.*, 2016). There are four generally accepted dietary aflatoxins; B1, B2, G1 and G2 (AFB1 (Aflatoxin B1), AFB2, AFG1, AFG2 respectively). The metabolites, M1 and M2, are also found in milk. The toxicity rate is B1 > G1 > G2 > B2. Aflatoxin M1 has been identified in the milk of dairy cows consuming AFB1-contaminated feeds (Kehinde *et al.*, 2014).

Aspergillus flavus and *A. parasiticus* are associated with many diseases of human, most severe of which is invasive aspergillosis. It also causes diseases in insects as well as in crops (Magoha *et al.*, 2014). Agricultural commodities contaminated with toxigenic fungi like *A. flavus* can be injurious for animals and human health. Production of mycotoxins is species specific; therefore, proper identification and characterization of fungi is of prime importance to develop preventive strategies (UNICEF, 2012).

It has been reported that, because of their low molecular weight, aflatoxins upon ingestion can be rapidly adsorbed in the gastro-intestinal tract through a non-described passive mechanism, and then quickly appear as a metabolite in blood after just 15 minutes and in milk as soon as 12 hours post-feeding (Eva *et al.*, 2011). High exposure of aflatoxin over a relatively short time causes acute aflatoxicosis. Although acute aflatoxicosis occurs on a case by case basis, large outbreaks have been reported in Africa and chronic aflatoxicosis due to low dose aflatoxin exposure over a long period of time is reported more prevalent than acute aflatoxicosis. The most well established health effect of chronic exposure are hepatocellular carcinoma impaired child growth and immune suppression (Azziz-Baumgartner *et al.*, 2005).

The genus *Aspergillus* and *Penicillium* that occurs in wide range of products and grows in wide range of condition (Ruadrew *et al.*, 2013) produces ochratoxin A (OTA). This OTA is classified as possible carcinogen (Group 2B) to humans by the International Agency for

Research on Cancer (IARC 1993). They are immune-suppressive nature, teratogenic and have fertility inhibition, mutagenic and carcinogenic effects (Ruadrew *et al.*, 2013).

3.5 Factors influencing fungal infection of maize

The favoring conditions for mycotoxin production relate mainly to poor hygienic practices during transportation, improper storage, processing, high temperature and moisture content and heavy rains (Binyam, 2016). These conditions are typically observed in most African countries including Ethiopia (Bhat and Vasanthi, 2003). The demand for the storage of food substances has been increased due to the increasing population. Researchers have found a variety of factors which favors fungal infection and mycotoxin production. Those are grouped as physical, chemical, and biological factors. Physical factors include environmental conditions viz temperature, relative humidity, and insect infestation while chemical factors such as fertilizers as well as biological factors depend on the interactions between the colonizing toxigenic fungi and the substrate, in fact some plant species are more susceptible to colonization while environmental conditions may increase the vulnerability of others are more resistant (Marroquín *et al.*, 2014). In other ways thus factors can be either intrinsic (moisture content, water activity, substrate type, plant type and nutrient composition), extrinsic (climate, temperature, oxygen level; drying, blending, addition of preservatives, handling of grains), processing or implicit which includes insect interactions, fungal strain and microbiological ecosystem (Gabriel and Puleng, 2013; Assefa and Geremew, 2018). With the presence of fungal spore in the grain wit favorable conditions, this get proliferate easily.

Major factors affecting the risk of fungal infection are temperature, insect injury and infestation, drought stress, and water activity (determined by kernel moisture/developmental stage) and pre- and postharvest handling. These factors do not influence independently but most often there are complex interactions. Generally, these factors can be grouped in to biotic and abiotic. Abiotic factors constitute a great concern to the proliferation of fungi fungus and mycotoxin development. It includes environmental factors including seasonal variation, agricultural practices, and Postharvest operations. Biotic factors such as storage insects and fungal interaction leads to fungal contamination and subsequent mycotoxin production (Fandohan *et al.*, 2003).

3.6 Control of Fungal contamination

Good Agricultural Practice is a collective set of international codes of practices which are concerned with all aspects of primary food production, including environmental protection and sustainability, economics, food safety, food quality and health security (Magan *et al.*, 2003).

3.6.1 Pre-harvest control

Field preparation and cultivation practices play a central role in the management of fungal diseases and associated problems. The burial of residue plant material from a previous planting season by deep ploughing can reduce the primary inoculum that causes infections (Blandino *et al.*, 2010). This is important when crops are affected by the same group of mycoflora and crop rotation with legumes, brassicas and potato could significantly reduce fungal levels (Gilbert and Tekauz, 2011). Limiting plant stress to increase plant vigour by adhering to optimum plant dates, preventing drought stress and the optimal use of fertilizers have reduced fungal infection in a number of grain crops (Parsons and Munkvold, 2010).

Chemical elicitors are not environmentally safe and also failed to reduce mycotoxins, for instance, *Fusarium* contamination in maize (Small *et al.*, 2012). As chemical pesticides and fungicide regulations are restricted, to reduce human exposure and prevent environmental pollution, biological control has become more popular. In Ethiopia, under greenhouse conditions, complete disease suppression of *Fusarium* root and crown rot of sorghum was reported by using *Bacillus* spp. (Idris *et al.*, 2007).

Disease resistance is the reliable and environmentally safe management practice to reduce fungal diseases in grain crops. Maize hybrids genetically modified with crystal (Cry) genes from the *Bacillus thuringiensis*, known as Bt-maize, reduced the feeding of stem borers and resulted in lower infection by fungi such as *F. verticillioides* and *F. proliferatum* and subsequently reduced FUM contamination. Fumonisin detoxification has also been achieved by genetic modification of maize with a degradative enzyme originating from *Exophiala spinifera* and *Rhinochrysiella atrovirens* (Ilze *et al.*, 2016).

3.5.2 Post-harvest control

Mycotoxins detoxification can be achieved through physical or chemical processes. Chemical degradation of DON has been achieved by NH_3 , CaOH , Cl , HCl , O_3 and NaOH (Abramson *et al.*, 2015, Young *et al.*, 2005 and Wilson *et al.*, 2005).

However, the large-scale application of these methods limited by cost, safety issues and impact on grain quality. Biological detoxification; enzymatic degradation of mycotoxins or modification of their structure, offers an alternative method to reduce the mycotoxin content in food and feed products (Karlovsky, 2011).

4. MATERIALS AND METHODS

4.1 Description of sampling area

Maize samples were collected from four major maize producing woredas of East and West Shewa zone of Oromia, Ethiopia (Figure 1). The woredas were Bako Tibe and Dano from Western Shewa; Adami Tullu and Arsi Negelle from Eastern Shewa with different agroecological location. Bako Tibe is located in the West Shewa Zone of the Oromia Region, on the all-weather highway between Addis Ababa and Nekemte with an elevation of 1743 meters above sea level (masl). Bako is the administrative center of Bako Tibe woreda. Dano is bordered on the southwest by the Jimma Zone, on the north by Cheliya, and on the southeast by Nono; part of the boundary with the Jimma Zone is defined by the Gibe River. It has an elevation between 1701 and 1827 masl. Adami Tulu or Zway is a town and separate woreda in central Ethiopia. It is located on the road connecting Addis Ababa to Nairobi in the East Shewa Zone of the Oromia Region of Ethiopia with an elevation of 1643 masl. Arsi Negele is bordered on the south by Shashemene area, on the southwest by Lake Shala which separates it from Shala, on the west from the Southern Nations, Nationalities and Peoples Region, on the north by east shewa with which it shares the shores of Lakes Abijatta and Langano, and on the east by the Arsi Zone. It has an elevation which ranges from 1500 to 2300 masl (Woreda's Agricultural and Rural Development Office).

From each woreda two kebeles and a town were selected for sample collection. The kebeles were Seyu Gambella, Sayoo and Dano Shenan from Dano woreda; Odda Anshura, Anneno Shisho and Adamu Tulu town from Adami Tulu woreda; Bako town, Dambi Dimma and Dambi Gobbu from Bako Tibe woreda and Arsi Negelle town, Hadah Bioo and Rafuu Hargisa from Arsi Negelle woreda.

agroecological location, source of collection, storage duration after harvest, means of harvest, moisture content during collection, storage condition (type of storage) and use of fertilizer and compost were recorded for correlation analysis.

The grain moisture content was measured using an electronic moisture tester (HOH-Express-HE-50, Germany) immediately after collection. Maize samples were packed in polyethylene bag, labeled and stored at 4°C until needed for laboratory analysis.

4.3 Fungal isolation and morphological characterization

From each sample, 15 maize grains were randomly selected and surface sterilized by treating with 70 % ethanol for 2 minutes. Maize grains were rinsed three times with sterile distilled water. Potato Dextrose agar (PDA) (Hi-Media Laboratories Ltd. Mumbai, India) medium amended with 0.01% chloramphenicol was used for plating experiments. Five surface sterilized grains were aseptically placed on a Petri dish containing PDA in triplicate and incubated upright at 25°C for 5-7 days.

Fungal colonies were picked up with a sterile needle and purified by transferring to PDA medium. The pure isolates were then transferred and preserved to PDA slant on Potato dextrose agar slants at 4°C.

Fungal isolates were grouped to genus level based on fungal identification manual (Barnett and Hunter, 1998). Isolates were observed based on colony growth rates, texture, degree of sporulation, color of mycelia, shape of conidial heads, vesicles, the number of branching points between vesicle and phialides, phialides and conidia were observed for the primary screening of isolates to genus level according to Navi *et al.*, (1999). Microscopic characterization of the fungal isolates was also done by making the slides of different fungal isolates and identification was done by comparing the slide cultures with a manual (Sarah *et al.*, 2016). Iodine glycerol solution/dye (0.5%) and lacto phenol cotton blue dye were used to perform slide culture technique (Patrick *et al.*, 2010). The frequency of fungal infection and total microbial load were calculated according to AOAC (1995).

$$\text{Frequency (\%)} = \frac{\text{number of beans from which the genus isolated}}{\text{Total number of beans analyzed}} \times 100$$

$$\text{Percent of fungal infection (\%)} = \frac{\text{number of beans from which a fungus isolated}}{\text{Total number of beans analyzed}} \times 100$$

4.4 Data analysis

For statistical evaluation of the data, SPSS software version 20 was used to generate descriptive statistics. Pearson correlation was used to assess the relationship between fungal infection and moisture content of the samples. To determine the influence of the environmental predictive factors on the response factors, percentage of fungal incidence Kruskal-Wallis test ($\alpha = 0.05$) was performed.

5. RESULTS AND DISCUSSIONS

5.1 Fungal isolation from maize

Mycological analysis of the maize samples showed that the overall fungal incidence was 54.1% (Table 1). The result showed that the genus *Fusarium* was the predominant fungus (73.08%) and *Penicillium* (8.48%), *Muchor* and *Rhizopus* (7.5%), *Aspergillus* (5.38%) and others unidentified (5.54%) (Figure 2A). Dano woreda showed higher mean percent of fungal infection (68.15%) followed by Adami Tullu (60.37 %), Bako Tibe (50%) and Arsi Negelle (37.78%) (Figure 2B). West Shewa zone showed higher percent of fungal incidence (59.07%) than East Shewa zone (49.07%). The result of this study was lower than the finding of Amare (2010) who analysed fungal incidence on maize samples collected from Adama, Ambo and Dire Dawa and reported 94% *Aspergillus*, 76.5% *Fusarium* and 64% *Penicillium*. However, the result of fungal prevalence obtained in this study is higher than recent findings of Negasa *et al.*, (2019) in Bako, Western Shewa, 39.4% *Fusarium* and 26.7% *Aspergillus* from maize.

Different *Fusarium* species associated with maize grain were identified in Ethiopia by Tesfaye and Dawit (1998). Factors such as moisture content of the products (Gtorni *et al.*, 2009), temperature, storage time and degree of fungal contamination prior to storage, insect and mite activity facilitate fungi dissemination (Suleiman and Omafe, 2013). During storage, several kinds of fungi can remain associated to maize grains either causing deterioration or simply remain viable to infect germinating seedling (Castellari *et al.*, 2010). Tesfaye and Dawit (2000) found that three species of *Fusarium* (*F. moniliforme*, *F. subglutinans*, and *F. graminearum*) to be highly associated with maize samples around Shashemene and Alemaya. A previous survey by Dawit (1982) showed that *Fusarium* was the most common genera in maize grain samples.

Yesuf *et al.*, (2015) also reported that more than 50 % of *Fusarium* contamination in Sorghum, Maize, Common bean, Coffee, Mung Bean and Cowpea in South Omo and Segen Peoples Zone of Ethiopia. Misgana and Yesuf (2016) showed that *Fusarium* head blight was the most important wheat disease.

This study best fits with Chemedi *et al.*, (2018), a research conducted in southwestern Ethiopia and the genus *Fusarium*, *Penicillium* and *Aspergillus* were found highly dominant.

Also Yirga (2018) reported 70 % of *Fusarium* wilt disease in Sesame in Northern Ethiopia. Generally, there is high *Fusarium* contamination in different parts of the country. This is due to the boundary less distribution of the fungus and survival in different agro ecological locations. Additionally, since *Fusarium* is storage mold and all the samples were collected from 1-5 month storage, it showed the higher prevalence. Moreover, the other field fungi recorded were attempted due to poor drying before harvest.

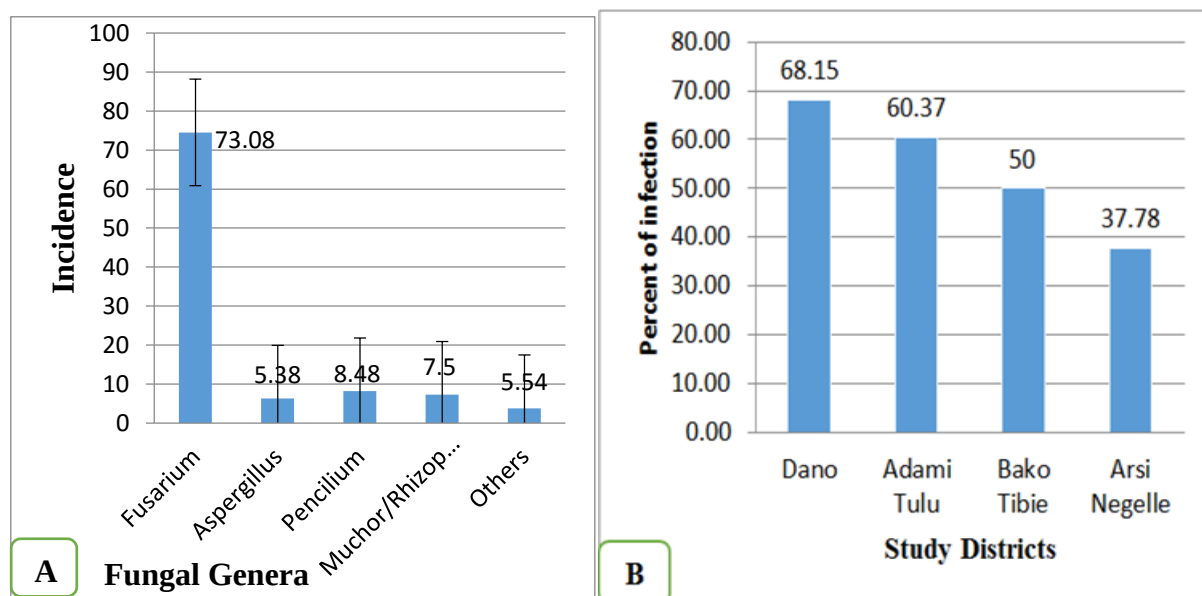


Figure 2: Incidence of fungal genera (A) and percent fungal of infection on maize in four woredas (B).

Table 1: Mean fungal percent of infection on maize sampels obtained from 12 kebeles

Kebele	Mean infection	N	Standard error
Adami Tullu Town	48.8	6	± 3.24
Anneno Shisho	60.0	6	± 4.66
Arsi Negelle Town	46.6	6	± 1.40
Bako Town	55.5	6	± 2.56
Dambi Dima	31.1	6	± 1.46
Dambi Gobu	63.3	6	± 2.75
Dano Shenen	63.3	6	± 3.24
Hadah Bioo	33.3	6	± 1.31
Odda Anshura	72.2	6	± 3.73
Rafuu Hargisa	33.3	6	± 1.72
Sayoo	73.3	6	± 3.61
Seyu Gambela	67.7	6	± 2.45
Total	54.1	72	± 3.15

Correlation analysis of moisture content, storage duration and fungal percent of infection is presented in Table 2. The analysis showed that moisture content and storage duration were negatively correlated with fungal prevalence ($r = -0.13$). Moisture content, storage duration in month and fungal percent of infection were found to be statistically insignificant ($p > 0.05$). Environmental conditions and climatic factors are the most important to the contamination of maize grains before and after harvest. The grain moisture content and temperature potentially affects the growth of mycotoxigenic fungi and spread of infection to the maize grain before and after harvest (Kana *et al.*, 2013). But according to this study the factors are negatively correlated with fungal contamination and even if there is weak correlation among them, there are considerable fungal incidence recorded. This was due to the smaller the sample size. Since the samples were collected after harvest; during storage when there is moisture to the grain, the fungi gets favorable opportunity for growth and specifically plays a role for *Fusarium* contamination.

Table 2: Correlation analysis between moisture content, storage duration and fungal percent of infection.

		Moisture Content	Duration in month	Percent of infection
Moisture Content	Pearson Correlation	1	-.142	-.008
	Sig. (2-tailed)		.233	.947
	N	72	72	72
Duration in month	Pearson Correlation	-.142	1	-.202
	Sig. (2-tailed)	.233		.088
	N	72	72	72
Percent of infection	Pearson Correlation	-.008	-.202	1
	Sig. (2-tailed)	.947	.088	
	N	72	72	72

Maize storage type analysis result revealed that storage using of plastic bag showed higher fungal percent of infection (69.78%) followed by gotera (51.11%) and fertilizer bag (49.63%) (Figure 3). Plastic bags are widely used for storage in Ethiopia and before harvest, the product has to be dried well because, further drying is impossible during storage as there is no air ventilation. Moreover, since it has not climatically controlled structure, resulting in high moisture leakage during the rainy season and the common formation of mold on stored maize (Dubale, 2014). However, the use of fertilizer bag was predominantly recorded.

When plastic bags are closed well, air tight storage results, with all its advantages and disadvantages. Plastic bags do not offer much protection against rodents, and they can be pierced by sharp seeds during transport and penetrated by insects which can act as a carrier for contamination. This can be reduced by putting bag of tightly woven cotton inside the plastic bag. Plastic becomes weak or brittle after continued exposure to the sun and therefore no plastic package will last indefinitely. Fertilizer bags cannot be used unless they have been very thoroughly cleaned (Tilahun, 2007). The logic behind the higher incidence of fungal infection to the plastic bags was due to the less air ventilation and aeration.

Gotera storage system is also widely utilized in Ethiopia. In this study 51.1% of fungal infection of maize grains was recorded which shows higher prevalence and this was due to poor drying of the grains before harvest.

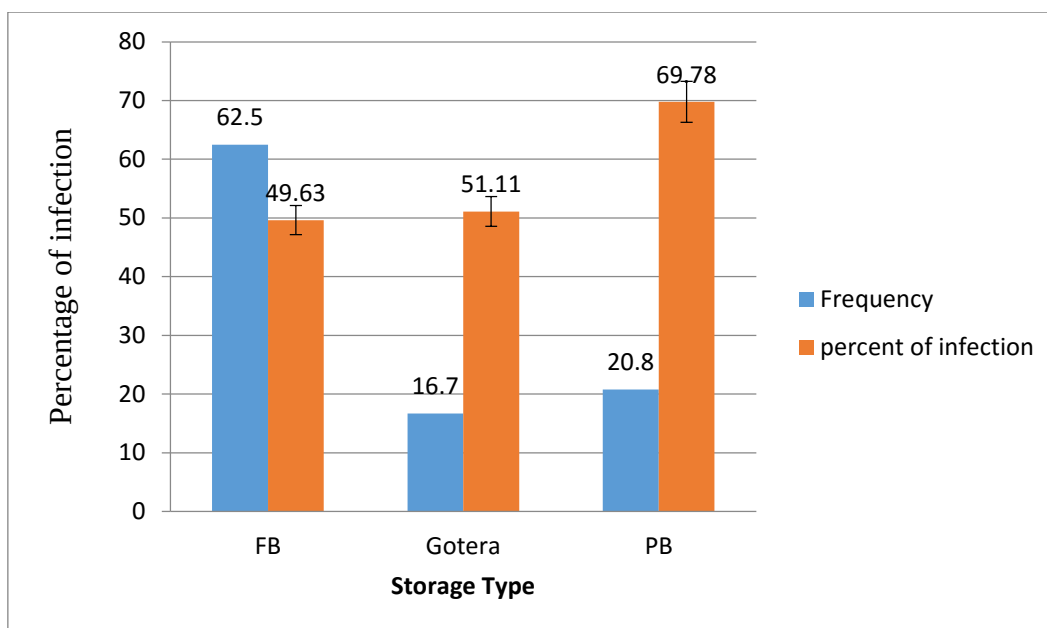


Figure 3: Maize storage type versus percent of infection (FB= Fertilizer bag, PB= Plastic bag).

The maize samples were obtained from four different sources (household, warehouse, open market and store). Grains collected from warehouse were more infected (58.88%) followed by store (55%), open market (54.28%) and household (52.78%) (Figure 4). This indicates that there were lesser aeration with higher level of infection in the warehouse and store. Moreover in the open market the grains have higher probability of contact with dust, soil and dung which also favors fungal growth.

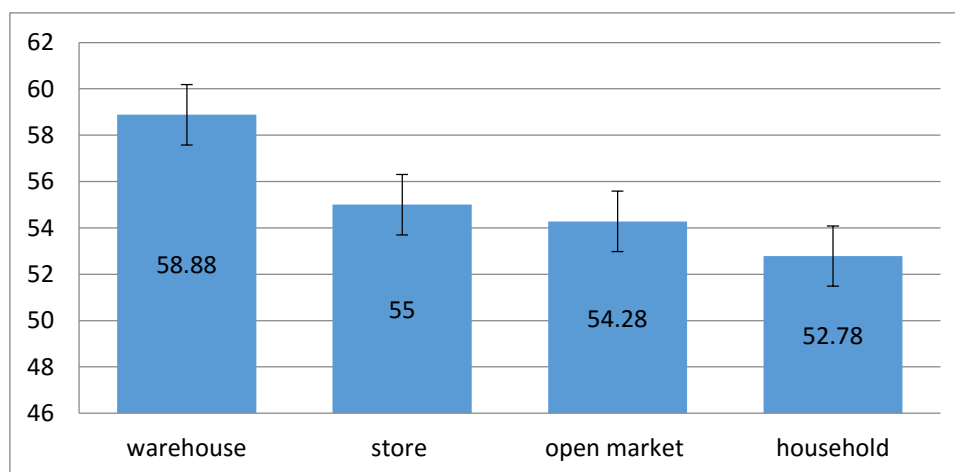


Figure 4: Sample source versus fungal percent of infection.

Sample history record analysis founded three maize harvest/dehulling methods in the study area; manual, mill and by using oxen. From these, manual harvest methods have highest percent of fungal infection (60%), followed by an oxen (50%) and mill (32.59%). Manual harvest increases the probability of maize grains to be in contact with soil thereby favors fungal infection. Small quantities of spores of storage fungi may present on grain going into storage or may be present on spilled grain present in harvest, handling and storage equipment or structures (Aurimas, 2017).

Maize samples moisture content obtained in this study ranges from 11 to 17% and fungal infection ranges from 13.3% to 100% (See Appendix 6). The mean moisture content was 14.16% and mean percent of fungal infection 54.1% (Table 3). The minimum moisture content with 80% fungal infection was obtained from west shewa zone, Dano district of Dano shenen Kebele on manually harvested and gotera stored maize. In other way, the higher moisture content value was recorded from east shewa zone of Arsi Negele district in the town from manually harvested maize stored on fertilizer bag for two month. Moreover, Adami tulu town and Arsi Negele town represented the minimum and maximum mean moisture content value. Based on the ANOVA analysis, the relationship between moisture content and fungal percent of infection is statistically insignificant ($p=0.94$).

Table 3: moisture content and percent of infection record of the maize samples.

S.No	Kebele	Mean moisture content (%)	Percentage of infection
	Adami Tulu Town	13.16	48.8
	Anneno Shisho	14.13	60.0
	Arsi Negele Town	15.18	46.6
	Bako Town	13.38	55.5
	Dambi Dima	14.34	31.1
	Dambi Gobu	14.58	63.3
	Dano Shenen	13.37	63.3
	Hadah Bioo	13.74	33.3
	Odda Anshura	14.16	72.2
	Rafuu Hargisa	14.46	33.3
	Sayoo	14.18	73.3
	Sayoo Gambela	14.70	67.7
	Mean	14.16	54.1

The main factors which greatly affect grain storability include grain moisture content, storage temperature, insects infestation and molds invasion which are highly interrelated. If grain moisture content is too high, even with good aeration and monitoring management will not prevent the grain from spoilage. High moisture grain should not be stored long-term. The grain should be placed in the storage at less than 14% moisture wet basis and preferably 13% for greater safety (Dubale, 2014). Accepted moisture limits for trading and storage of grains are generally below the limits at which molds develop. Pockets of high moisture grain or inclusion of green leaf material with the grain can affect quality of all the grain in storage because of moisture movement (Dubale, 2014).

According to National Agricultural Commodities Marketing Association standards (Queensland, Australia), the maximum moisture limits for trading and storage of maize grains is 14% (Dubale, 2014). Aeration will slow the rate of deterioration of high moisture grain, but if the moisture is more than two or three percent above the limits, it should be dried before long term storage. Early harvesting of grain at higher moisture produces higher quality and higher yield of grain, but those advantages are lost unless aeration and drying are used to minimize losses in storage (Dubale, 2014).

Moisture content below the safe limit in starchy cereal seeds such as wheat, barley, rice, corn and sorghum prevents invasion by storage fungi regardless of how long the grains are stored (Laura, 2019). In this study the mean moisture content (14.16%) is above the standard limit and associated with higher fungal contamination. However, the association of mean moisture content with mean percentage of fungal infections is statistically insignificant ($p = 0.09$). There also is a variation in moisture content through a grain mass. Storage fungi will grow with suitable moisture and will not be according to the average moisture content of the grain mass. These moisture content limits for safe storage imply that nowhere in the bulk of grain is the moisture content higher than that specified (Laura, 2019).

All micro-organisms, including molds, require moisture to survive and multiply. If the moisture content in a product going to be stored is too low, microorganisms will be unable to grow provided that the moisture in the store container is also kept low (Tilahun, 2007). Moisture should therefore be prevented from entering the storage structures. The safe moisture content is to some extent related to the storage time. Moisture levels above safe moisture content can be tolerated if only short times are required. The sitting and ventilation of the store

are important. Condensation of moisture can cause storage problems. If the walls of a store are cooled below their dew point by low night temperature, condensation can occur and increase the moisture in the layers of the stored grain near the edge of the store. It is important to remember that the stored grains are alive and respiring giving off moisture as well as heat (David and David, 1998). And the reason for the moisture content above the safe limit was due to initial exposure of sampled grains to different ambient temperature and relative humidity. Most of the samples showed that higher value of moisture content above the safe limit and this was due to inappropriate drying before harvest and absence of ventilation. At this situation, storage fungi get higher chance of proliferation.

From the current finding, there was no significant difference between the use of fertilizer and compost in relation to fungal infection, although 54.1 % of fungal infections were recorded from fertilizer use and 55.65 % from compost (Table 4). Nutrients are the determinant factors for growth and productivity, particularly under adverse conditions (El Kinany *et al.*, 2018). According to Tsedeke Abate *et al.*, (2015), Ethiopian farmers have historically used organic fertilizers (such as farmyard manure, compost, crop residue, and household refuse) for agricultural production and today commercial fertilizer use is the dominant input that goes with modern varieties where all of Ethiopia's mineral fertilizer is imported. Despite, organic fertilizers have higher favorable condition for fungal growth because compost addition enhanced hyphal growth and sporulation (Wei *et al.*, 2018).

The storage duration versus fungal percent of infection analysis revealed that grains stored for one month showed 63.7% infection and grains stored for five month showed 23.3% fungal infection (Figure 5). Thus, storage duration and percent of fungal infection have weak negative correlation with statistical insignificance ($p=0.23$). This implies as the storage time increases the incidence of fungal contamination decreases. At normal condition, as the storage period increases, the incidence and frequency of all fungal species will also increase which may be due to increase of relative humidity in the storage that favors the rewetting of the stored maize grains (Negasa *et al.*, 2019). In contrary, this study found that as the storage time increases the mean percent fungal contamination decreases. This was due to the higher moisture at harvest season and longer storage of the grain in the fertilizer bag provides aeration thereby reduce

fungus contamination. Additionally, the samples of five month storage were with low frequencies and represented by only two.

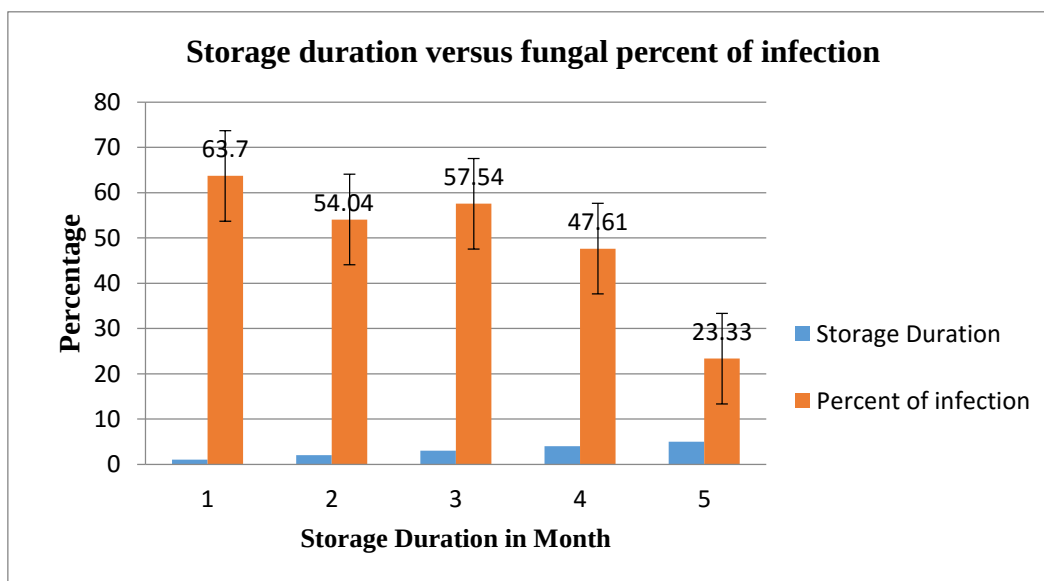


Figure 5: Mean comparison of percentage of storage duration in month with fungal percent of infection (STD for storage duration=1.08 and percent of infection = 26.79), ($p=0.29$).

5.2 Fungal characterization

From the total of 1080 maize grains analyzed about 613 were found contaminated with different fungi. Every fungal colony that appeared on the grains were counted as the genus *Aspergillus*, *Penicillium*, *Muchor*, *Rhizopus*, *Fusarium* and others based on morphological and microscopic observation. From the 613 isolated fungi 448 were *Fusarium* followed by 52 *Penicillium*, 46 *Muchor/Rhizopus*, 33 *Aspergillus* and 34 others unidentified fungi (Table 5). Odda Anshura Kebele from Adami Tullu woreda and Arsi Negelle Town from Arsi Negelle woreda showed the higher and lower mean percentage of *Fusarium* infection respectively. In contrary, Odda Anshura and Bako town showed no fungal infection for *Aspergillus* and *Penicillium* respectively.

Table 4: Fungal genera isolated from maize samples collected from different kebeles.

Kebele	Fungal incidence (%)				
	<i>Fusarium</i>	<i>Penicillium</i>	<i>Aspergillus</i>	<i>Muchor/Rhizopus</i>	Others
Seyu Gambela	80.79	5.55	4.88	2.38	6.38
Sayoo	62.2	3.66	2.56	2.08	29.48
Dano Shenen	69.84	3.33	9.72	2.38	0
Odda Anshura	94.16	12.96	0	4.81	0
Anneno Shisho	65.04	2.39	6.66	27	0
Adami Tulu town	65.21	27.73	8.05	7.79	2.08
Bako Town	78.67	0	3.03	14.69	3.59
Dambi Dima	78.19	14.3	5.41	2.08	0
Dambi Gobu	84.18	5.12	4.06	4.06	2.56
Arsi Negelle Town	58.82	16.27	7.22	15.59	2.08
Hadah Bioo	74	5.55	20.43	0	0
Rafuu Hargisa	87.2	4.16	4.16	4.38	0
STD	10.89	7.92	5.15	7.93	8.31
Standard Error	±1.28	±0.93	±0.6	±0.93	±0.97

Morphological and microscopic characteristics of the isolates showed that the genus *Fusarium* was dominantly isolated from samples collected from the twelve study sites. Morphological characteristics of pure culture isolates were used as a primary screening for fungal identification (Figure 6 and 7). As most of the isolates were *Fusarium*; presence of whitish mycelium and numerous non-branched microconidias were recorded. The microscopic image of the isolates was captured from slide culture preparations (Figure 8).

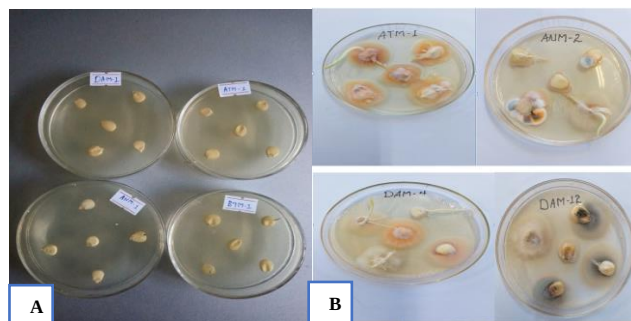


Figure 6: Isolation of fungi from maize samples (A, inoculated maize sampled before incubation, B, fungal growth on inoculated maize samples after incubation for 3-5 days).

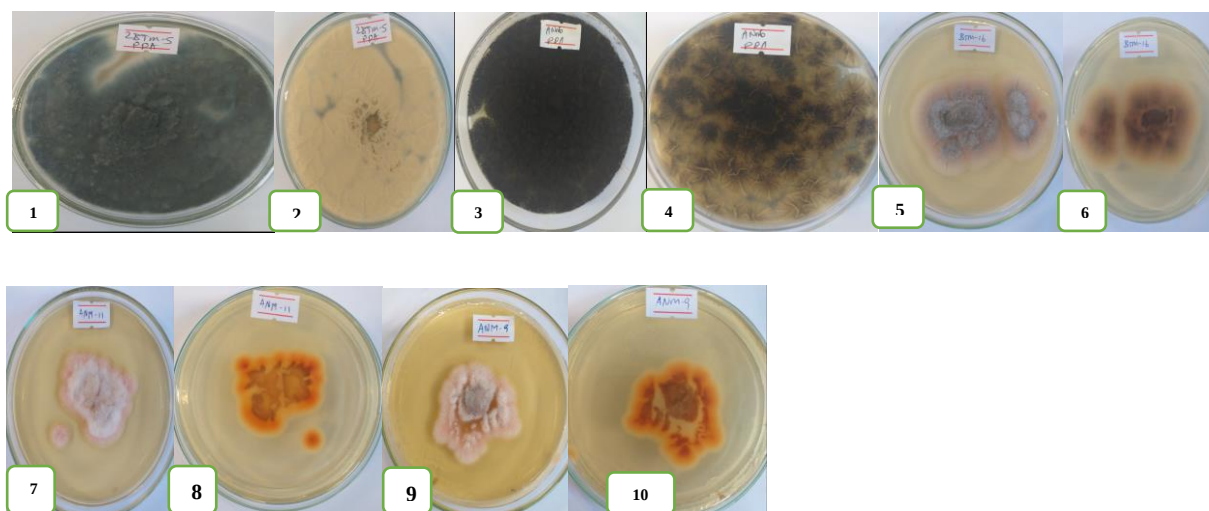


Figure 7: Five day's old cultural characteristics of fungal pure cultures (1; *Aspergillus* Obverse side, 2 *Aspergillus* reverse side; 3, *Penicillium* Obverse and 4, *Penicillium* reverse side; 5, 7 and 9 *Fusarium* Obverse side and 6,8, and 10 *Fusarium* reverse side).

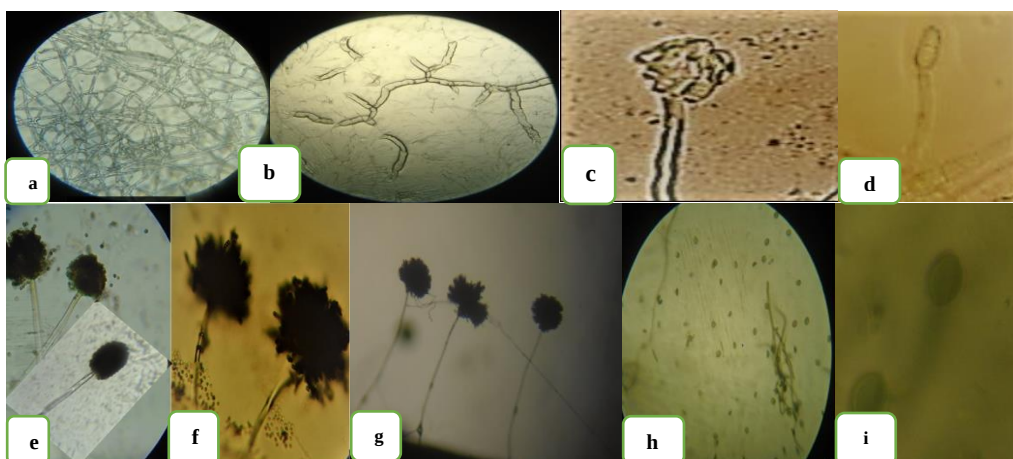


Figure 8: Microscopic examination of isolates (a, Two day *Fusarium* mycelia with 10 X magnification, b with 40 X magnification; c and d, Two day *Fusarium* macro conidia with 40 X magnification; e, *Aspergillus* spore; f, *Muchor* spore; g, *Rhizopus* spore; h and i, *Fusarium* Micro conidia). Note: Slide culture was performed on five day's old pure cultures and further slide cultured for two days.

6. CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

There was wider mycoflora contamination of maize in the study area. Despite the statistical insignificance, since the grain moisture content lies above the safe limit, the rate of fungal contamination was higher. Except some kebele's response for some fungal genera, most of the study kebeles have higher incidence of fungal contamination and Dano woreda showed higher mean percent of maize contamination among others.

The associated factors for mold growth in maize showed statistical insignificance due to various reasons and even if insignificant it showed considerable fungal spoilage in maize grains. The moisture content was above safe standard and before harvest appropriate drying of the grain has of special relevance to minimize mold growth and mycotoxin production. Besides fungal contamination, moisture content value of maize grains beyond 14% is not recommended for export purpose and so as to impose economic crisis.

Fusarium was predominantly recorded and since the samples were collected after harvest; during storage when there is moisture to the grain, the fungus gets favorable opportunity for growth and specifically plays a role for *Fusarium* contamination.

Generally, since molds in maize grains are reported in different parts of the country and has great impact on human and animal life, it is therefore, important to take preharvest and postharvest preventive measures for contamination. There is also need to standardize the farmers, grains marketers and consumers to improve their storage facilities.

6.2. RECOMMENDATIONS

From the study, the following recommendations were forwarded;

- ❖ Awareness creation of farmers, experts and agricultural extension workers about postharvest handling and the importance of diseases and their management is required.
- ❖ Use of holistic, cumulative integrated management, monitoring, and precautionary measures of the stored grain throughout the storage period.
- ❖ Maize breed type and mycoflora association studies have to be concerned which enables to identify resistant and or tolerant maize varieties and screening of effective bio-agents.
- ❖ Further study is needed on molecular identification of the isolates and of the mycotoxin analysis.

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APPENDICES

Appendix 1: Sample collection photos



Note: The first four photos indicate different storage conditions of maize and the last two pictures represent sample collected with polyethylene bag (Photo by; Temesgen Assefa, 2019).

Appendix 2: Fungal culturing and incubation



Photo by; Temesgen Assefa, 2019.

Appendix 3: Media preparation



Photo by; Temesgen Assefa, 2019.

Appendix 4: Microscopic examination and slide culture technique



Photo by; Temesgen Assefa, 2019.

Appendix 5: Pure culture preparation

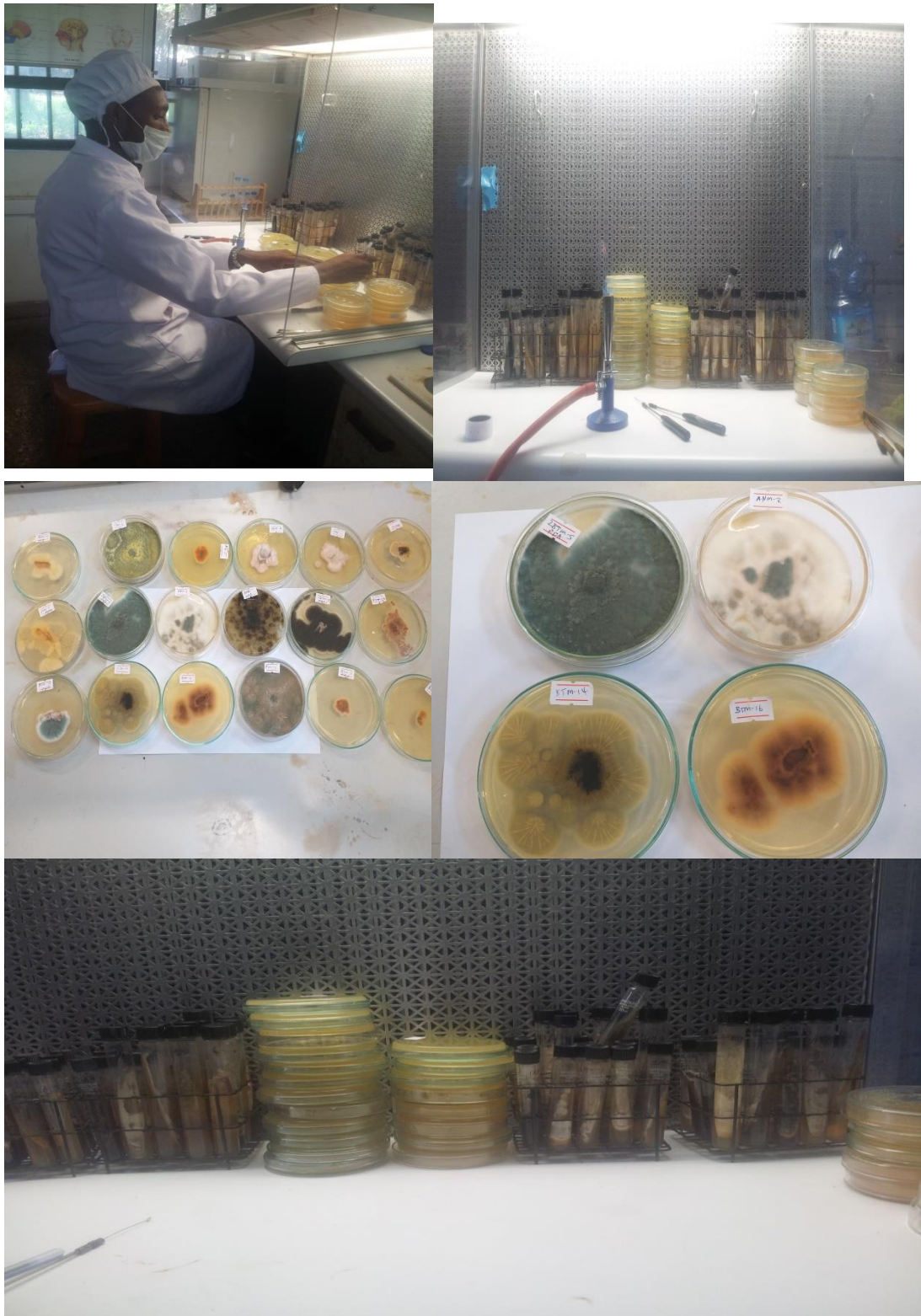


Photo by; Temesgen Assefa, 2019

Appendix 6: Mean moisture content and percent of infection

Moisture Content	Mean Percent of infection	N
11.0	80.0	1
11.7	26.6	1
12.0	46.6	1
12.4	53.3	1
12.6	73.3	1
12.7	86.6	1
12.7	46.6	1
13.0	26.6	1
13.1	40.0	1
13.1	53.3	1
13.3	100.0	1
13.3	80.0	1
13.3	40.0	1
13.4	100.0	1
13.4	40.0	2
13.6	25.0	4
13.6	56.6	2
13.6	33.3	1
13.7	33.3	2
13.7	80.0	1
13.7	33.3	1
13.8	86.6	1
13.8	33.3	2
14.0	78.3	4
14.0	33.3	1
14.1	40.0	1
14.2	20.0	1
14.2	63.3	2
14.2	46.6	1
14.3	33.3	1
14.3	66.6	2
14.4	100.0	2
14.5	50.0	2
14.6	46.6	3
14.6	53.3	1
14.6	51.1	3
14.7	82.2	3
14.7	40.0	1
14.7	26.6	1
14.8	53.3	1
14.8	26.6	2
15.0	62.2	3
15.6	100.0	1
15.7	26.6	1
15.8	46.6	1
16.0	60.0	1
16.3	53.3	1
16.3	53.3	1
17.0	40.0	1
Total	54.07	72

Appendix 7: Chemical composition of Potato Dextrose Agar

PDA (HIMEDIA (LOT: 0000331163; REF: M096-500G)) was used for fungal culturing with the following standard formulas with final PH 5.6 ± 0.2 (at room temperature).

Ingridients	Gms/liter
Potato infusion	200.00
Dextrose	20.00
Agar	15.00