Isolation and Characterization of Post-harvest fungi from wheat (*Triticum aestivum*, L.) collected from Bale and West Arsi zones of Oromia, Ethiopia.

By:

Daniel Bogale Likelebet

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Office of Graduate Studies

Adama Science and Technology University

July,2020

Adama, Ethiopia

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Advisor: Dr. Teshome Geremew Biru .

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APPROVAL SHEET

We, the undersigned, member of the board of Examiners of the final open defense byDaniel Bogale Likelebet have read and evaluated his thesis entitled "Isolation and Characterization of post-harvest fungi from wheat collected from Bale and West Arsi zones of Oromia Regional State, Ethiopia" and Examined the candidate. This is therefore to certify that the thesis has be en accepted in par partial fulfillment of the requirements of degree of Masters of Science in A pplied Microbiology program for the award of Master Science in Applied Microbiology.

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Declaration

I hereby declare that this M.Sc. thesis entitled "**Isolation and Characterization of post-harvest fungi from wheat grain collected from Bale and West Arsi zones of Oromia Regional State, Ethiopia"** is my original work and has not been presented for a degree, diploma or certificate in any other university, and all sources of material used for this thesis have been duly acknowledged.

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Advisor's Approval sheet

To: Department of Applied Biology

Subject: Thesis Submission

This is to certify that the thesis entitled "**Isolation and Characterization of post-harvest fungi from wheat grain collected from Bale and West Arsi zones of Oromia Regional State, Ethiopia"** submitted in partial fulfillment of the requirement for Master's degree in Applied Biology (Applied Microbiology) program for the award of Master Science in Applied Biology (Applied Microbiology) has been carried out by **Daniel Bogale Likelebet, ID No pgr/18342/11** under my supervision. Therefore, I recommended that the student has fulfilled the requirements and hence hereby he can submit the Thesis to the Department.

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APPROVAL SHEET

We, the undersigned, member of the board of Examiners of the final open defense byDaniel B ogale Likelebet have read and evaluated his thesis entitled “Isolation and Characterization of post-harvest fungi from wheat collected from Bale and West Arsi zones of Oromia Regional State, Ethiopia” and Examined the candidate. This is therefore to certify that the thesis has bee n accepted in par partial fulfillment of the requirements of degree of Masters of science in App lied Microbiology program for the award of Master Science in Applied Microbiology.

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Dedication

I dedicate this project to GOD my creator, my strong pillar, my source of inspiration, wisdom, knowledge and understanding above all my everything. I also dedicate this work to my mother Etaferahu Eshetu She has separated me in her life and her love always with me throughout my work and my brothers Solomon Bogale, Ephrem Bogale and my sister Tizita Bogale and my best wisher Walelign Aklilu, Negash Asfaw and his wife Bekelech Uma.

ACKNOWLEDGEMENT

Above all I would like to express my heart felt gratitude to my advisor Dr. Teshome Geremew for his help, follow up, motivation, constructive feed back, comments and for sharing his proje ct. Next, I would like to thank Dr. Zerihun Belay who kindly assisted me how to isolate fungi from different sources. Then I would like to thank Adama Science and Technology University, instructors, Library attendants and all Biology Department lab assistants for their help in all my research work. The last but not least I would like to thank Temesgen Asefa for his important information and idea in my work. Finally, I am deeply grateful to Jonathan, Alicia and all my brothers and friends for their Salary support in all my MSc. class and Thesis Research.

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LIST OF ACRONYMS AND ABBREVIATIONS

FAO	Food and Agricultural Organization
GDP	Gross Domestic Product
GHI	Global Harvest Initiative
Masl	Meters above sea level
NGO	Non-governmental organization
RH	Relative humidity
WFP	World Food Program

ABSTRACT

Wheat is an important industrial and food grain, which is ranked second among the most impo rtant cereal crops in the world. In Ethiopia, wheat is one of the most important market oriente d commodity and a major source of income. Due to Lack of post-harvest technologies, an aver age 15-20% loss is recorded. So this research is aimed to isolate and identify Post-harvest fungi associated with Wheat grain collected from Bale and West Arsi zones of Oromia, Ethiopi a. A total of 144 Wheat grain samples were collected from January- April in 2017/2018 by cot a, random and incremental sampling method from four districts. Fungi isolation was done on Potato Dextrose Agar amended with 0.01% chloramphenicol. Fungal colonies were purified b y sub cultured on PDA. Morphological and microscopic characterization of the isolates was done based on fungal identification manual (Wang et al 2016). The data were recorded and analyzed by using SPSS version 20 statistical software. From 2160 wheat grains, 786 fungal isolates were recorded with 36.39%. Among the fungi isolated from current wheat samples, th e genus Fusarium was the most prevalent (86.39%) followed by Aspergillus (7.25%), Penicilli um (5.21%) and Muchor and Rhizopus (1.14%). Grains collected from open market shw highi nfection(41.33%) followed by residents' house (36.48%) and warehouse (30.51%). Bale Zone showed relatively higher mean percent of fungal prevalence (37.78%) followed by west Arsi Z one (35.36%). Among the districts, Hassasa Keble showed highest mean percent of fungal pre valence (48.69%) followed by Agarfa (37.77%), Dodola (37.22%) and ArsiNegelle (20.18%). Generally, the of the fungi of each genus was highly variable across the sampling areas. From the study, awareness creation, cumulative integrated management, monitoring, and precaution ary measures are recommended.

Keywords: *Agarfa, Fusarium,* Mycotoxin, Spoilage fungi, Post-harvest

1. Introduction

1.1. Background and Justification

Wheat is an important industrial and food grain, which is ranked second among the most impo rtant cereal crops in the world next to rice, and traded internationally (Falola et al., 2017). The area coverage, production, and productivity of wheat is increasing through time and fluctuatin g as a result of population growth, changing food preferences and a strong urbanization trend (Dubale Befiqadu, 2018). The economy of most African countries is depending on the agriculture. Ethiopia ranks 31st in wheat production in the world with 4.2 million quintals produced on 1.7 million hectares of land (Degye Goshu et al., 2019) In the country, it, contributes about 43% of GDP (Gross Domestic Product) which generates 90% of export value and supply 70% of the industrial raw materials for domestic industries (Abdu Mohammed et al., 2016). In Africa, wheat is a major component of many other foods including breads, porridge, cracker and biscuits (Kolawole et al., 2013).

Wheat and wheat products represent 14% of the total calorie intake in the country which makes wheat the second-most important food behind maize (19%) and ahead of teff (10), sorghum (11%) and enset (12%) (FAO and WFB ,2014 a). In Ethiopia, wheat ranks fourth after teff, maize and sorghum in area coverage and third after maize and teff in total production (CSA, 2014 a). In the country wheat is one of the most cereal crops in terms of the area of land allocated (1.6 million hectares), volume produced (3.9 million tons) and the number of farmers engaged in its production (4.7 million farmers) with a productivity of 2.4 tons per hectare (CS A, 2014 a). On other report of (CSA, 2018) indicated that the total production of wheat in 201 7/2018 was 267.8 million quintals and 277.7 million quintals in 2018/2019 (CSA, 2019). Based on the report, there is 3.67% change in production between the two production seasons. The highlands of the central, south-eastern and northwest parts of the country are the main wheat growing areas of Ethiopia; and regionally, wheat production comes from Oromia (57.4%), Amhara (27%), SNNP (8.7%) and Tigray (6.2%) of the national production (CSA, 2014 b).

Wheat has many purposes, better than other cereal crops produced in the country. In Ethiopia, wheat g arain is used in the preparation of a range of traditional food products such as the traditional staple pancake (“injera”), fermented bread (“*dabo*”), non fermented bread (“ambash

a/kitta"), boiled grain ("nifro"), roasted grain ("*kolo"*), snacks made from bread flour ("dabok olo"), cracked and boiled grain ("*kinche*"), porridge ("*genfo*"), local fermented beer ("*tella"*), distilled local spirit("*areki"),* and several other local food items (Dubale Abate, 2018). This shows that wheat is an important market-oriented commodity and a major source of income for many wheat growers in Ethiopia.

Despite of its all importance, there are many problems that hinder wheat production and marketing in the country. Wheat production is diminished by biotic and abiotic stresses all over the world. Pathological diseases are the most important limiting factor of wheat production by causing severe losses in yield and quality. Wheat can be infected by fungi and Insects as well as, other contaminant and bacteria. Among these, different fungi pathogens are the most prominent and pose a great challenge to wheat production (Aakash and Rajib, 2010).

1.2. Statement of the Problem

In Ethiopia, wheat is grown in different agro-ecological zones. The areas vary in-terms of weather conditions, wheat varieties grown and crop management practices. The crop contributed a great deal to the country as source of food and income but diseases and other biotic constraints continuously ravage it. Among the biotic factors, fungi are the principal organisms associated with crop seeds. A complex of seed-borne fungi including the genera of *Tilletia, Ustilago, Bipolaris, Fusarium, Alternaria, Drechslera, Stemphylum, Curvularia, Cladosporium, Rhizop us, Aspergillus and Penicillium* have been reported as the most frequent seed borne fungi of w heat throughout the world (Suproniene et al., 2011). Three genera, Fusarium, Penicillium and Aspergillus are considered as the most significant toxigenic fungi growing in processed and stored wheat foods where storage fungi has been the most poorly understood pathosystems in Stored crop (Essono et al., 2007). Yield loss assessment studies have been carried out in fewer areas and they are largely based on data from field surveys. Lack of post-harvest technologies causes on an average 15-20% losses due to pests and climatic factors (Tadele, 2016). As a result, there is a need to assess the incidence and frequency of storage fungi in wheat in the study area.

1.3. Objective of the Study

1.3.1. General Objective

The general objective of this study was to determine post harvest fungi associated with wheat collected from two zones of Oromia Regional State, Ethiopia

1.3.2. Specific objectives

- ✓ To isolate and characterize fungi associated with wheat using morphological and microscopic method.
- ✓ To evaluate prevalence of spoilage fungi associated with wheat.
- ✓ To Characterized Wheat Spoilage Fungi.

1.4. Significance of the Study

The finding of this study will benefit wheat farmers by raising awareness to minimize fungal invasion and contamination in wheat. It also benefits researches in the area of mycology and plant pathology by providing scientific data on the occurrence and prevalence of post-harvest fungal contamination in wheat in the study site and throughout the country. Moreover, it will direct policy makers to design appropriate preventive and control strategies.

2. Review Literature

2.1. Description of the Fungi

Fungi are more closely related to animals than to plants and are placed with the animals in the monophyletic group of opisthokonts. (Celio et al,2006). Analyses using molecular phyloge netics support a monophyletic origin of fungi. (Tedersoo et al ,2018). The taxonomy of fungi is in a state of constant flux, especially due to recent research based on DNA comparisons. These current phylogenetic analyses often overturn classifications based on older and sometimes less discriminative methods based on morphological features and biological species concepts obtained from experimental mattings. (Hibbett et al, 1997).

There is no unique generally accepted system at the higher taxonomic levels and there are frequent name changes at every level, from species upwards. Efforts among researchers are now underway to establish and encourage usage of a unified and more consistent nomenclature (Hi bbet et al, 2007*)*. Fungal species can also have multiple scientific names depending on their life cycle and mode (sexual or asexual) of reproduction. The 2007 classification of Kingdom Fungi is the result of a large-scale collaborative research effort involving dozens of mycologists and other scientists working on fungal taxonomy. It recognizes seven phyla, two of which the Ascomycota and the Basidiomycota are contained within a branch representing subkingdom

Dikarya, the most species rich and familiar group, all including all the mushrooms, most food-spoilage molds, most plant pathogenic fungi, and the beer, wine, and bread yeasts. The accom panying cladogram depicts the major fungal taxa and their relationship to opisthokont and unikont organisms, based on the work of Philippe Silar,The Mycota (Hibbett et al, 1997). A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research (Tedersoo et al. 2018. The lengths of the branches are not proportional to evolutionary distanc es.

2.2. Major Post-harvest Fungal Diseases of Wheat

About 5% of the world's grain harvest is lost during storage but in developing countries such losses may reach 30%. According to the same report, fungi are important causes of such losses, not only through loss of dry matter but also through quality loss, by decreasing nutritional value and through their ability to produce mycotoxins (Magan and Aldred, 2007). Consequently, fungi

produce metabolites which are toxic and the ingestion of which poses significant health risks to humans and animals (Bhat and Vasanthi, 2003). In addition, estimated losses of grain, in store varies widely. It accounts for 10% worldwide but can reach 50% in tropical regions (Hall, 1970). In Ethiopia the frequently used post-harvest loss figure ranges from 20-30% (Bisrat and Laike, 2016). Fungi within seeds reduce seed germination and seedling emergence which lead to less vigorous seedling. Seed-borne fungi pathogen present externally or internally may cause seed abortion, seed rot and seed necrosis (Khanzada et al., 2002). Some plant pathogenic fungi kill seedling

2.2.1. Mycotoxins

Mycotoxin contamination is another big challenge, which makes the food unsuitable for human consumption or animal feed. A large amount (25% - 40%) of cereal grains are contami nated by the mycotoxins produced by storage in the fungi world. Molds and mycotoxins cause dry matter as well as quality loss, and are a hazard in the food value chain (Magan and Aldred, 2007). Mold formation in stored grain can produce different mycotoxins, which are toxic chemicals unsuitable for human consumption. In addition, mold generates other problems besi des mycotoxins, such as dry matter loss, odor, and a loss of nutritional value. Thus, the presen ce of active mold in stored grain can greatly limit grain usage due to quality loss and mycotoxi n formation. Mycotoxins, such as aflatoxins, ochratoxins, trichothecenes, zearalenone, and fu monisins, developed mainly from fungi, such as *Fusarium, Aspergillus, and Penicillium*, are common in grain in most countries (Quezada et al., 2006). Mold can develop on the crop in the field as field fungi, as well as during storage as storage fungi, thus creating two bases of mycotoxins owing to mold formation. Mold growth during grain storage is dependent on grain moisture content, temperature, gas composition, relative humidity (RH), and fungus contamination during field harvest and storage (Fleurat-Lessard, 2017). Reducing the grain moisture content to less than 13% and relative humidity to less than 60% during storage is crucial to limit mold activity (FAO, 2017). Thus, mold formation needs to be properly addressed during storage to minimize grain losses (Ashish et al., 2018).

Losses can be minimized by physically avoiding then try of insects and rodents, and maintaini ng the environmental conditions that avoid growth of microorganisms. The knowledge of

control points during harvesting and drying before storage can help in reducing losses during the storage of cereals. Taking the timely preventive actions for biotic and abiotic factors can be very effective in reducing the losses during storage (Ashish et al., 2018). Shortly after they emerge, whereas others cause serious disease epidemics after being transmitted from seeds to seedlings. It also affects the growth and productivity of wheat (Weber et al., 2001).

In Ethiopia, particularly in Oromia, most of wheat production takes place in relatively cooler areas of Arsi and Bale zones, Shashemene and ArsiNegelle districts. In these areas, most of the harvested wheat grains are generally stored in sacks of different sizes and quality. Wheat grains are often contaminated with many kinds of fungal agents before or after harvesting and depending on the storage conditions and prevailing environmental factors (Adisa, 1994). The major contributing problems for high postharvest losses relates with poor postharvest infrastru cture and marketing systems, poor research and improvement capability, and insufficiencies in guidelines and information sharing (Dubale, 2018).

2.3. Major Mycotoxins that produced by different species of fungi

2.3.1. Aflatoxin

The four major aflatoxins are called B_1, B_2, G_1, and G_2 based on their fluorescence under UV light (blue or green) and relative chromatographic mobility during thin-layer chromatography. Aflatoxin B_1 (Fig.2.1) is the most potent natural carcinogen known and is usually the major aflatoxin produced by toxigenic strains. It is also the best studied: in a large percentage of the papers published, the term aflatoxin can be construed to mean aflatoxin B_1. However, well over a dozen other aflatoxins (e.g., P_1. Q_1, B_{2a}, and G_{2a}) have been described, especially as mammalian biotransformation products of the major metabolites (Peterson, et al 2001). The classic book *Aflatoxin: Scientific Background, Control, and Implications*, published in 1969 (Goldblatt.,1969) is still a valuable resource for reviewing the history, chemistry, toxicology, and agricultural implications of aflatoxin.

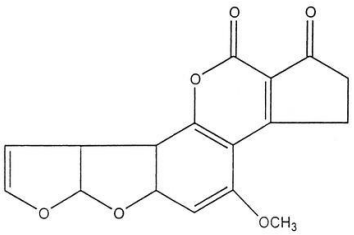

Figure 2.1 Aflatoxin

Aflatoxins are difuranocoumarin derivatives produced by a polyketide pathway by many strains of Aspergillus flavus and *Aspergillus parasiticus*; in particular, *Aspergillus flavus* is a common contaminant in agriculture. *Aspergillus bombycis*, *Aspergillus ochraceoroseus*, *Aspergillus nomius*, and *Aspergillus pseudotamari* are also aflatoxin producing species, but they are encou ntered less frequently (Goto, et al. ,1996).

Many substrates support growth and aflatoxin production by aflatoxigenic molds. Natural contamination of cereals, oilseeds, nuts, tobacco, and a long list of other commodities is a common occurrence. Like the genetic ability to make aflatoxin, contamination is highly variable. Sometimes crops become contaminated with aflatoxin in the field before harvest, where it is usually associated with drought stress. Even more problematic is the fate of crops stored under conditions that favor mold growth. In storage, usually the most important variables are the moisture content of the substrate and the relative humidity of the surroundings. Aflatoxin contamination has been linked to increased mortality in farm animals and thus significantly lowers the value of grains as an animal feed and as an export commodity. Milk products can also serve as an indirect source of aflatoxin. When cows consume aflatoxin-contaminated feeds, they metabolically bio transform aflatoxin B_1 into a hydroxylated form called aflatoxin M_1 (Van and Magee, 2001).

2.3.2. Citrinin

Citrinin (Figure 2.2.) was first isolated from *Penicillium citrinum* subsequently, it was identifie d in over a dozen species of *Penicillium* and several species of *Aspergillus* (e.g., *Aspergillus terreus* and *Aspergillus niveus*), including certain strains of *Penicillium camemberti* (used to produce cheese) and *Aspergillus oryzae* .

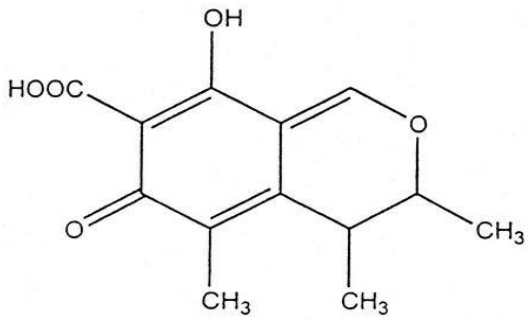

Figure 2.2 citrinum

Citrinin acts as a nephrotoxin in all animal species tested, but its acute toxicity varies in different species (Hendry and Cole, 2001).

2.3.3. Ergot Alkaloids

The ergot alkaloids are among the most fascinating of fungal metabolites. They are classified as indole alkaloids and are derived from a tetracyclic ergoline ring system. Lysergic acid, a structure common to all ergot alkaloids, was first isolated in 1934. The clavines have ergoline as a basic structure but lack peptide components; the lysergic acid alkaloids include ergotamin e and lysergic acid amide (ergine). The structure of ergotamine is shown in (Figure 2.3).

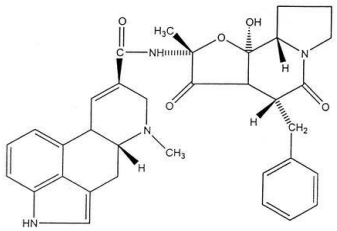

Figure 2.3 Alkaloids

These compounds are produced as a toxic cocktail of alkaloids in the sclerotia of species of *Cl aviceps*, which are common pathogens of various grass species. The ingestion of these scleroti a, or ergots, has been associated with diseases since antiquity. An Assyrian tablet dated to 600

B.C.E., referring to a “noxious pustule in the ear of grain,” is believed to be an early reference to ergot. The human disease acquired by eating cereals infected with ergot sclerotia, usually in the form of bread made from contaminated flour, is called ergotism or St. Anthony's fire. Two forms of ergotism are usually recognized, gangrenous and convulsive. The gangrenous form affects the blood supply to the extremities, while convulsive ergotism affects the central nervo us system (Bennett and Bentley, 1999).

2.3.4. Fumonisins

toxins are produced by over 50 species of *Fusarium* and have a history of infecting the grain of developing cereals such as wheat and maize.(*Cornely, 2008).*They include a range of mycotoxi ns, Figure 2.4 such as: the fumonisins, which affect the nervous systems of horses and may cause cancer in rodents; the trichothecenes, which are most strongly associated with chronic and fatal toxic effects in animals and humans; and zearalenone, which is not correlated to any fatal toxic effects in animals or humans. Some of the other major types of *Fusarium* toxins include: beauvercin and enniatins, butenolide, equisetin, and fusarins (Schaafsma and Hooker, 2007)

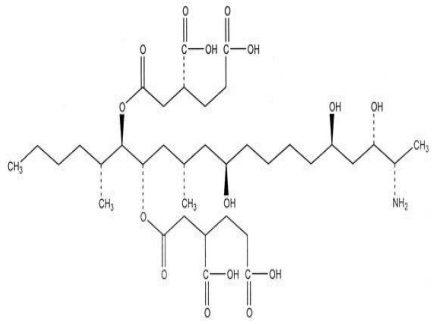

Figure 2.4: fumonisins.

2.3.5. Ochratoxin

is a mycotoxin that comes in three secondary metabolite forms, A, B, and C. All are produced by *Penicillium* and *Aspergillus* species. The three forms differ in that Ochratoxin B (OTB) is a no chlorinated form of Ochratoxin A (OTA) and that Ochratoxin C (OTC) is an ethyl ester form Ochratoxin A. (Mateo et al 2007). *Aspergillus ochraceous* is found as a contaminant of a wide range of commodities including beverages such as beer and wine. *Aspergillus carbonariu s* is the main species found on vine fruit, which releases its toxin during the juice making

process. OTA has been labeled as a carcinogen and a nephrotoxin, and has been linked (figure 2.5).

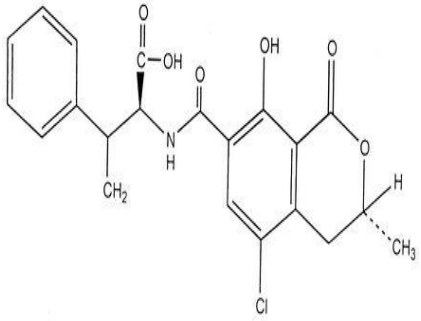

Figure2. 5: Ochratoxin

2.3.6. Patulin

Is a toxin produced by the *P.expansum*, *Aspergillus*, *Penicillium*, and *Paecilomyces* fungal spe cies. Although patulin has not been shown to be carcinogenic, it has been reported to damage the immune system in animals. In 2004, the European Community set limits to the concentrati ons of patulin(Figure 2.6) in food products. They currently stand at 50 μg/kg in all fruit juice concentrations, at 25 μg/kg in solid apple products used for direct consumption, and at 10 μg/kg for children's apple products, including apple juice. (Moss, 2008).

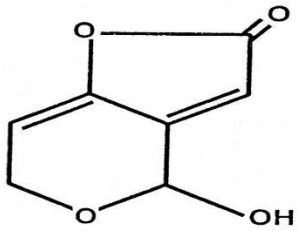

Figure2. 6: Patulin

2.3.7. Zearalenone.

Zearalenone (ZEN), also known as RAL and F 2 mycotoxin, is a potent estrogenic metabolite produced by some *Fusarium* and *Gibberella* species (Brezin et al,2013). Particularly, is produ ced by *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium cerealis*, *Fusarium equiseti*, *F usarium verticillioides*, and *Fusarium incarnatum*. Figure 2.7. Several *Fusarium* species produ ce toxic substances of considerable concern to livestock and poultry producers, namely deoxyn

ivalenol, T 2 toxin, HT 2 toxin, diacetoxyscirpenol (DAS) and zearalenone. Zearalenone is the primary toxin, causing infertility, abortion or other breeding problems, especially in swine. Ze aralenone is heat stable and is found worldwide in a number of cereal crops, such as maize, bar ley, oats, wheat, rice, and sorghum.(*Tanaka et al,1988)*

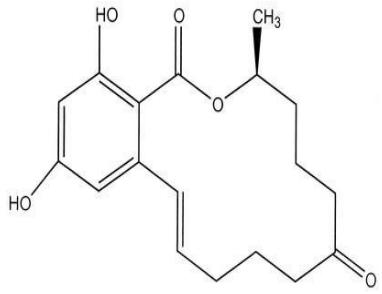

Figure 2.7: Zearalenone.

2.4. Occurrence and Diversity of Major Fungi Species in Wheat

Fungi pathogens were isolated from wheat stems, heads, straw, grains and soil based on cultur al and morphological characteristics and during storage seed born fungi predominates. Seed bo rne fungi spp. reported in wheat including such as *Alternaria, Aspergillus, Cladosporium, Cla viceps, Cochliobolus,Curvularia, Dilophosphora, Drechslera, Didymella, Fusarium, Gaeuman nomyce, Gibberellazeae,Hymenellas, Lasiopiplodia, Leptosphaeria, Monographella, Mycosph aerella,* (Klyszejko et al., 2005; Kuwite et al., 2010).There is a significant difference in isolati on frequency of individual fungal genera among the agro ecological zones (Muthomi et al., 20 12). There is high diversity in each fungal genus, especially within the genera of Fusarium and Aspergillus in tropical and subtropical regions, where warm and humid weather as well the availability of stalks and alternate hosts provide optimum conditions for mold growth (Horn et al., 199).

2.5. Suitable Factor for Fungal Disease Dissemination

2.5.1. Harvesting

Harvesting is the first step in the grain supply chain and is a critical operation, which decides the overall crop quality. In the developing countries, harvesting is performed mainly manually using hand cutting tools such as sickle, knife, scythe, cutters. Harvesting timing and method di ctates the losses during the harvesting operations. A large amount of losses occurs before or during the harvesting operations,ifitisnotperformedatadequatecropmaturityandmoisturecontent. Tooearly harvesting of crop at high moisture content increases the drying cost,making it susce ptible to mold growth, insect infestation, and resulting in a high amount of broken grains and low milling yields (Khan, 2010).However, leaving the matured crop un-harvested results in high shattering losses, exposure to birds and rodents attack, and losses due to natural calamities (rain, hailstorms etc.) (Baloch, 2010). Most of the harvesting is performed manually in the developing countries, which is a highly labor intensive and slow process.

2.5.2. Threshing and Cleaning

The purpose of the threshing process is to detach the grain from the panicles. This is achieved through rubbing, stripping, or impact action, or using a combination of these actions. The operation can be performed manually (trampling, beating), using animal power, or mechanical threshers. Grain spillage, incomplete separation of the grain from chaff, grain breakage due of excessive striking, are some of the major reasons for losses during the threshing process (Khan, 2010). Delay in threshing after harvesting of crop results in significant quantity and quality loss, as the crop is exposed to atmosphere, and is susceptible to rodents, birds, and insect attack (Alaviet al., 2012). High moisture accumulations in the crop lying in the field ma y even lead to start mold growth in the field. The cleaning process is performed after the thresh ing to separate wholegrainsfrombrokengrains and other foreign materials, such as straw, stones, sand, chaff, and weed seed. Winnowing is the most common method used for cleaning in the developing countries. Inadequate cleaned grains can increase the insect infestation and mold growth during storage, add unwanted taste and color, and can damage the processing equipment. A large amount of grains are lost as spillage during this operation, and grain losses during winnowing can be as high as 4% of the total production (Sarkar et al., 2013).

2.5.3. Drying

Drying can be performed naturally (sun or shade drying) or using mechanical dryers. Natural drying or sun drying is the traditional and economical practice for drying the harvested crop, and is the most popular method in developing countries. Sometimes, whole crop without threshing is left in the field only for drying. Grains lying in the open for sun drying are eaten by birds and insects, and also get contaminated due to mixing of stones, dust, and other foreign materials. Unseasonal rains or cloudier weather may restrict the proper drying, and the crop is stored at high moisture, which leads to high losses due to mold growth (Abass et al., 2014). Some farmers use plastic sheets for spreading the grains, which reduces the contamination with dust and makes the collection of grains easy. Mechanical drying addresses some of the limitations of natural drying, and offers advantages, such as reduction in handling losses, better control over the hot air temperature, and space utilization (Alavi et al., 2012).

2.5.4. Storage

Storage plays a vital role in the food supply chain, and several studies reported that maximum losses happen during storage (Aulakh et al., 2013; Majumder et al., 2016). In most places, crops are grown seasonally and after harvesting, they are stored for short or long periods as food reserves, and as seeds for next season. Studies report that in developing countries about50%-60% of the grains are stored in the traditional structures at the household and farm level for self-consumption and seed. This facilitates fungal contamination, growth and mycotoxin production (Grover and Singh, 2013).

2.5.5. Transportation

Transportation is an important operation of the grain value chain, as commodities need to be moved from one step to another, such as field to processing facilities, field to storage facilities, and processing facilities to market. Lack of adequate transportation infrastructure results in damage of food products through bruising and losses due to spillage. Poor road infrastructure along with these improper and poorly maintained modes of transportation results in large spillage and high contamination in developing countries. Multiple movement of crop is another major reason for high transportation losses (Shah, 2013). Low quality Jute bags are used

commonly during transportation and even storage, which results in high spillage rates due to leakage from the sacks.

2.6. Storage Losses in Developing Countries

The maximum amount of losses occurs during the storage of crops. Storage losses can be classified in two categories: direct losses, due to physical loss of commodities; and indirect losses, due to loss in quality and nutrition. The loss in quality results in value loss of the product, and sometimes leads to total rejection. The rejection rate depends upon the individual's economic status and cultural background. For example, a subsistence farmer may consume the damaged food to some extent, whereas, affluent customers may reject even slightly damaged grain. Some loss happens in the form of spillage from leaky sacks, which can be identified when the store is emptied and the spilt grain remains on the floor (Boxall, 2002).

The storage losses are affected by several factors, which can be classified into two main catego ries: biotic factors (insect, pest, rodents, fungi) and abiotic factors (temperature, humidity, rain) (Abedin et al., 2012). Moisture content and temperature are the most crucial factors affecting the storage life. Most of the storage molds grow rapidly at temperatures of 20 40 ◦C and relativ e humidity of more than70%. Low moisture keeps the relative humidity levels below 70% and limits the mold growth. In the traditional storage structure, temperature fluctuations due to wea ther changes cause moisture accumulation either at the top or bottom of the grains'bulkdepend ingonthedirectionofairconvection.

Quality of grains before storage is another critical factor affecting the storage losses. Mechanical damage during harvesting and threshing can result in bruised areas on grains, which may serve as centers for infection and cause deterioration (Shah, 2013).

2.6.1. Insect Infestation

Among all the biotic factors, insect pests are considered most important and cause huge losses in the grains (30-40%) (Tapondjou et al., 2002). Nowadays, it is found in most parts of Africa and is considered the most threatening pest, as it causes extensive damage in a very short time (Tefera et al., 2011). Patel et al (1993) observed about 25% losses by Rhizoper the dominicain wheat stored for 3 months under laboratory conditions.

2.7. Interventions to Reduce Post harvest Storage Losses.

Although a huge challenge, storage losses can be mitigated by use of efficient storage technologies, upgrading infrastructure and storage practices. World Food Program (WFP) with the help of the government and non-governmental organizations (NGOs) performed an Action Research Trial to demonstrate the impact of improved postharvest management practices and using new storage technologies on the crop loss after harvesting (Costa, 2014). The results concluded that irrespective of crop or storage periods, use of improved practices and new technologies resulted in about a 98% reduction in food loss (Costa, 2014). It was also observed that losses in the traditional storage structures were much higher than those reported in the literature, because the storage period was longer than that commonly used by farmers in these countries. Other than saving losses, the availability of low cost and effective storage structures can motivate farmers to store their grains and obtain high prices instead of selling right after harvesting when there is an abundant supply of grains.

2.7.1. Chemical Fumigation

Synthetic insecticides are used in several countries and play an important role in controlling the pests and reducing losses during storage of grains. Methyl bromide and phosphine are the most commonly used chemicals in developing countries (Shaaya et al.,1997). Shelling the grains and storing them in polypropylene bags after proper application of Actellic Super can effectively avoid the pest infestation for a few months of storage (De Groote et al., 2013). This practice has been widely adopted by farmers in African countries. Irrespective of their effectiveness, the synthetic insecticides suffer from limitations such as high costs, development of genetic resistance in the treated pests, health hazards due to toxic residues, and environmental contamination. Residuals from synthetic fumigants could cause considerable loss of seed viability (Mutungi et al., 2014). Use of these chemical fumigation methods is even challenging in the traditional storage structures used in the developing countries, as most of them are open store infestation. Another challenge with these chemicals is knowledge and training to apply these pesticides at the correct time and at the correct dose. The delayed treatment, adulterated chemicals, and incorrect dosage can reduce the efficacy of the treatment and result in high storage losses (Villers et al., 2010).

2.7.2. Natural Insecticides

Several plant species and their extracts have been used commonly as a traditional practice to protect the grains from insects in several African and Asian countries. Plant based chemicals and products would be biodegradable, environment friendly, and relatively safe for human hea lth. For instance, the leaves and oil extract from leaves of Chenopodium ambrosioides Linn. (Chenopodiaceae) has been found to be very effective in controlling the damage of cereal grains by the insects during storage.

2.7.3. Hermetic Storage

Airtight storage is gaining popularity as a storage method in developing countries, due to its effectiveness and avoidance of chemicals and pesticides. The method creates an automatic modified atmosphere of high carbon dioxide concentration. As the structures are airtight, the biotic portion of the grains creates a self-inhibitory atmosphere over time by increasing carbon dioxide concentration due to its respiration metabolism. Some studies have reported that the aflatoxin production ability of Aspergillus flavus is also reduced at high concentrations of CO_2 (Tefera et al., 2011). Hermetic storage has been observed to be very effective in avoiding the losses (storage losses less than 1%) during long distance (international) shipments also. Ease of installation, elimination of pesticide use, favorable costs, and modest infrastructure require ments are some of the additional advantages that make the hermetic storage options attractive (GHI, 2014).

Technology interventions and improved storage structures can significantly reduce store losses. However, it is important to understand that training of smallholders is equally as necessary as the technology dissemination. Along with making these technologies available at a reduced price, the government agencies and organizations have to ensure the development of facilities to provide information and training about the use and maintenance of these technologies in the local language, for successful adaptation and effective use of these technologies (Kitinoja, 2013).

3. Materials and Methods

3.1. Map of study area

Bale is one of the zones in the Oromia Region of Ethiopia. It is bordered on the south by the G anale Dorya River which separates it from Guji, on the west by the West Arsi Zone, on the north by Arsi, on the northeast by the Shebelle River which separates it from West Hararghe and East Hararghe, and on the east by the Somali Region. The zone is the second largest zone in Oromia National Regional State after Borena zone with a total area of 63,555km^2. It shares about 17.5% of total area of Oromia. It has 18 districts, 2 urban administrative centers, 20 urban kebeles an d 351 rural kebeles. Latitude**:** 7°06' 60.00"N Longitude**:** 40° 00' 0.00" E.

West Arsi is one of Oromia Regional state territory which is surrounded by one National regional it shares bounder line with East Shewa zone to the north, SNNPRS to the west and south, Arsi to the northeast, Guji to the south east and Bale zone to the east. Most parts of the zone have elevations of ranging from 1500 to over 3300m Shashemene town is the administrative center of the zone it located at 250km from Finfine and the total area of Zone is 12556km2 West Arsi Zone is located approximately at a distance of 250km from Finfine. It is located in the Rift Valley Region The Astronomical location of West Arsi zone lies between 6012'29" to 7042'55" latitude and 38004'04" to 39046'08 longitude. West Arsi Zone has 12 districts, 4 Urban Administrative, 332 peasant's association.

Dodola is a town in southeastern Ethiopia. Located in the West Arsi Zone of the Oromia Region, this town has a latitude and longitude of 06°59′N 39°11′E, with an elevation ranging from 2362 to 2493 meters above sea level. It is the administrative center of Dodola woreda.

Agarfa city of country Ethiopia lies on the geographical coordinates of 7° 17' 0" N, 39° 49' 0" E. Latitude and Longitude. The town is situated 980 m above sea level. It is named after the Hasanamba temple. The urban population in 2011 was 133,436.Area: 26.52 km²Elevation**:** 980 mWeather**:** 26°C, Wind SW at 13 km/h, 73% Humidity

Arsi Negele is one of the woredas in the Oromia Region of Ethiopia. It is named after its administrative center, ArsiNegele. Part of the Mirab Arsi Zone located in the Great Rift Valley ,Arsi Negele is bordered on the south by Shashamene Zuria, on the southwest by Lake Shala w hich separates it from Shala, on the west from the Southern Nations, Nationalities and Peoples

Region, on the north by Misraq Shewa with which it shares the shores of Lakes Abijatta and L angano, and on the east by the Arsi Zone . Top two wheat-producing zones (Bale and West Arsi) form Oromia regional state were selected based on production potential according to CSA data of the country. From the two zones, four districts were selected each including two kebeles an d one districst main town. Twelve samples were taken from each sample sites (Table 3.1 and fi gure 3.1)

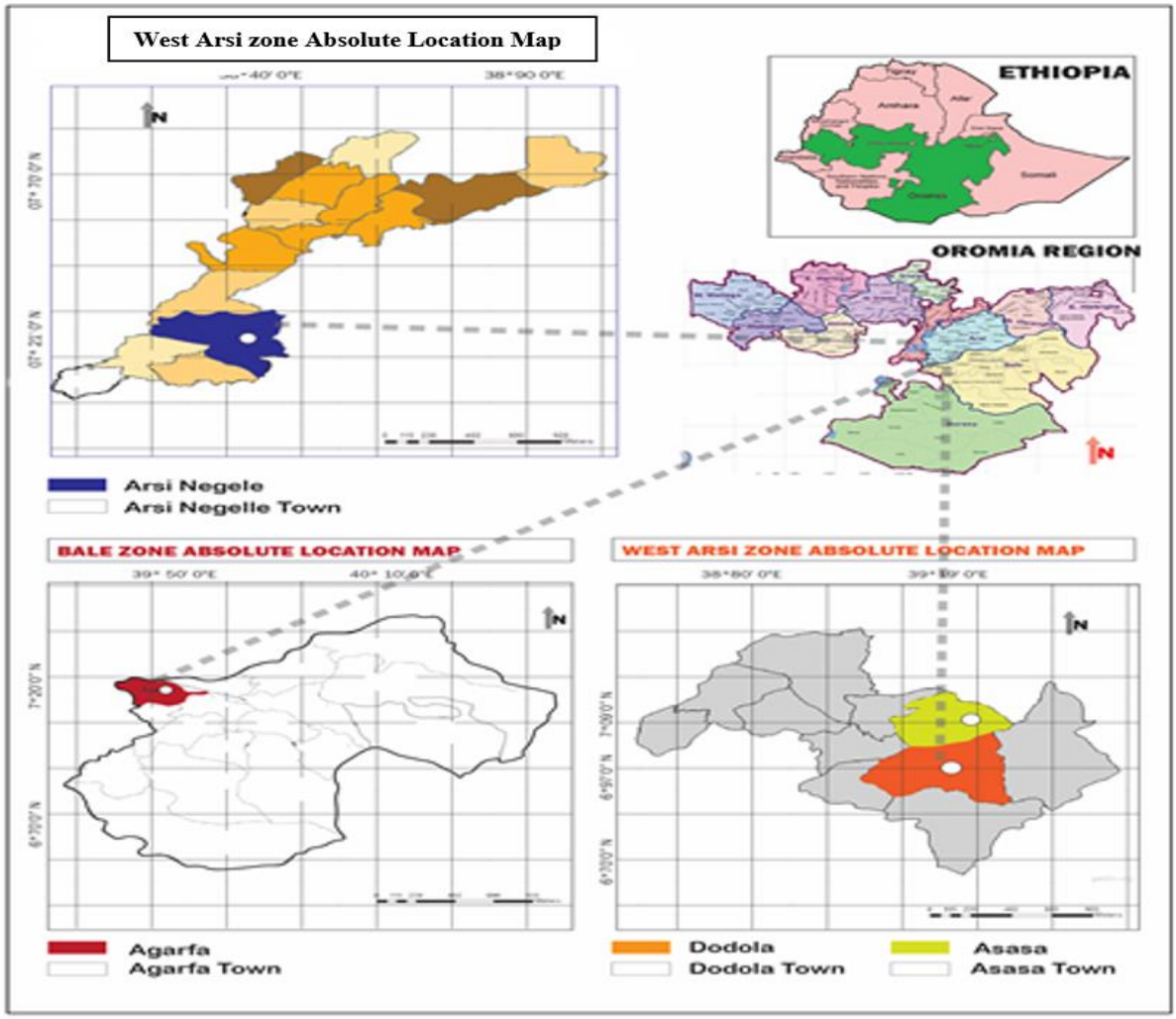

Figure 3.1 Map of the study Area.

Table 3.1: Sample zones, woredas and kebeles

S. no	Zones	Sampling Districts	Kebele	Number of samples	Remark
	Bale zone	Agarfa	Ilani	12	
			Ali	12	36
			Agarfa town	12	
	West Arsi zone	Dodola	Deneba	12	
			Berisa	12	36
			Dodola town	12	
		Hasassa	BuchoWokenso	12	
			Debara Walati	12	36
			Hasassa Town	12	
		ArsiNegele	Ali Weyo	12	
			Mako Odda	12	36
			ArsiNegelle Town	12	
	Total number of samples			144	

A total of 144 (36 samples from one district)

Samples were collected based on incremental sampling method, the sample collectors went to the central part of the sample site and took the first household to the east direction as sample one and jump to the third household for sample two and while finishing the east direction, return

to the center and went to west direction in similar manner and continues to south and north until the sample number was attained. Woreda level Agriculture office experts and Agricultural extension workers were involved in the sampling process. In the sampling process, sample history including location, average annual rainfall, temperature, relative humidity, altitude, climatic condition of the area, duration of crop storage after harvest, moisture level during collection, storage condition (type of storage) were recorded. Samples were packed in polyethylene bags with appropriate labeling and stored at 4^{o}C until needed for further analysis. The moisture content of the samples was measured using an electronic moisture tester (HOH-Express-HE-50, Germany) and recorded immediately after collection.

Table 3.2: Mean moisture content and fungal infection score of the study areas

Collection Type	**Frequency of collection**	**Percent collection**	**Valid Percent**
Open Market	10	6.9	6.9
Residents House	108	75.0	75.0
Ware House	26	18.1	18.1
Total	144	100.0	100.0

3.2. Fungal isolation and morphological characterization

From each sample, 15 wheat grains were randomly selected and surface sterilized by treating 70 % ethanol for 2 minutes (Navi et al., (1999) and Wang et al., (2016). The grains were rinsed three times with sterile distilled water. Potato Dextrose agar medium amended with 0.01% chloramphenicol was used for plating experiments with controlled bacterial outgrowth. Five surface sterilized grains were aseptically placed on a Petri dish containing Potato Dextrose agar and incubated upright at 25°C for 5-7 days (Figure 3.2). Every fungal isolate were grouped to genus level based on macro and micro morphology by using fungal identification manual (Barnett and Hunter, 1998). Every microbial colony that appeared on the wheat grains

were counted as Aspergillus, *Penicillium, Mucho, Rhizopus and Fusarium.* Fungal colonies were picked up with a sterile needle and purified by transferring to Potato dextrose agar medium. Pure isolates were cultured on Potato dextrose agar slants in the refrigerator at 4°C for further study (Figure 4.2, C). Purified isolates were maintained on PDA slant and spores maintained in 2 mL 0.2 % agar + 0.05 % Tween 80 for further analysis.

Percent of fungal infection (%) = number of grains from which the fungus isolated/total number of grains analyzed] X 100

Isolates were morphologically and microscopically analyzed based on colony growth rates, texture, degree of sporulation, color of mycelia, shape of conidial heads, vesicles, the number of branching points between the vesicle and phialides, phialides and conidia was observed for the primary screening of isolates to genus level according to (Barnett and Hunter, (1998), Navi et al., (1999) and Wang et al., (2016)

3.3. Slide Culture Preparation

Sterile 1.5% water agar (7 to 8 ml) was poured into sterile petri dishes and allowed to solidify. A sterile 22-mm2 cover glass was prepared. The desired nutrient agar medium (10 ml) was poured into a second 60-mm Petri dish, allowed to solidify, and cut with a sterile stainless steel spatula into blocks approximately 5 to 8 mm2. One block was aseptically removed and placed on the cover glass. Inoculation of the agar block on one or more sides with fungal hyphae or conidia was followed by placement of a second sterile cover glass on top of it. After the Petri dish lid was replaced, the completed modified slide culture (Figure, 3.2, D) was incubated at the 25^{o}c until adequate growth and conidio genesis were occurred. The top cover glass was lifted off with forceps and wetted on the specimen side with a drop of ethanol (70 to 90%).One drop of fungus mounting medium (e.g., lacto phenol cotton blue) was applied to the specimen, and the cover glass was lowered gently onto the slide, specimen side down and observed conidial growth and structure under microscope.

3.4. Data analysis

Statistical evaluation of the data was done by SPSS software version 20. To assess correlations, Spearman rank correlation was performed. To test the influence of the environmental predictive factors on the response factors, percentage of fungal incidence (a non-parametric Kruskal-Wallis test ($\alpha = 0.05$)) was done.

4. Result and Discussion

4.1. Prevalence of Fungi Isolated from Wheat

From 2160 wheat grains, 786 fungal isolates were recorded with 36.39% mean value. Among the fungi isolated from current wheat samples, the genus *Fusarium* was the most prevalent (86.39%) followed by *Aspergillu*s (7.25%), Penicillium (5.21%) and *Muchor* and *Rhizopus* (1. 14%), (Figure 5.1). Bale Zone showed relatively higher mean percent of fungal prevalence (37.78%) and also West Arsi Zone showed considerable mean fungal prevalence (35.36%). West Arsi Zone of Hassasa District showed higher mean percent of fungal prevalence (48.69%) followed by Agarfa (37.77%), Dodola (37.22%) and ArsiNegelle District (21.85%) (Figure 5.2). Moreover, Hassasa Town and Mako Odda Keble were showed the higher (56.66%) and lower (16.11%) mean fungal infection respectively.

The result of this study agrees with the findings of Fenta Negasa et al., (2019) who reported that *Fusarium* spp.Was the most prevalentspoilage fungi followed by *Aspergillus spp*. It also best fits with Chemeda et al. (2018), in which the genera *Fusarium, Penicillium* and *Aspergillus* we re dominantly recorded. Moreover, Temesgen and Teshome (2019) have reported higher *Fusa rium* sp. contamination and prevalence in maize from Oromia Regional State. There is higher *Fusarium* contamination in different parts of the country. This is due to the boundary le ss distribution of the fungus and survival in different agro ecologies.

The result of this study is deviated from the findings of the authors reported that *Aspergillus* was the most prevalent genus constituting 98.3% from Bale and 81.7% from West Arsi after three months of storage. In contrary to the current finding, Asela et al., (2020) reported 6.67% *Fusarium* from West Arsi and 0% from Bale. But both research works reported the prevalence of storage fungi; *Aspergillus* and Fusarium. Dinku andAbdella (2014) reported the dominant g enera, *Aspergillus* (45.54%), followed by *Penicillium* (29.18%), *Altarnaria* (12.14%), *Fusariu m* (9.69%) and Bipolaris (1%) were isolated from wheat in Shashemene and ArsiNegelle.Cere al products provide important nutritional substance for human health (Rakicka and Sturdik, 2013). The quality and safety are of major concern due to increasing occurrence of chronic diseases associated with consumption of contaminated cereals throughout the world (Al-Defiery et al., 2015). Wheat represents a strategic raw material of human nutrition. In developing

countries with poor post-harvest activities and huge losses, post-harvest fungal contamination of cereals is frequently reported. For instance, Kolawole et al., (2013) have reported the abundance of *Aspergillus spp, Fusarium spp, Penicillium spp and Rhizopus spp* in wheat grain s collected from Lagos, Nigeria. Other researches also reported 65% prevalence of wheat disea se (Fusarium head blight) caused by Fusarium graminearum or gibberellazeae in Ethiopia (Mis gana Mitiku and Yesuf Eshete (2016).

There is higher *Fusarium* contamination in the study that indicate will be associated with exposure of the grains for *Fusarium* during harvest, transportation, storage and others. Moreover, the prevalence of storage fungi is reported by many scholars in many cereals in the country which is indicative of the countries post-harvest management shortcomings.

4.2. Correlation of Independent Variables with Fungal Incidence

In this study moisture content of wheat grains, storage duration after harvest, source of sample, and storage type were tested for their correlation with fungal incidence in stored wheat. Correlation analysis of moisture content with fungal percent of infection showed that moisture content and fungal prevalence were negatively correlated and found to be statistically insignificant (p=.087) (Table 4.1). Environmental conditions and climatic factors are the most important determinant factors to the contamination of grains before and after harvest. Grain moisture content and temperature determines the growth of fungi in grains before and after harvest Kana et al., 2013). In this study, even if there is weak correlation, there is considerable fungal incidence recorded. Since the samples were collected after harvest; post-harvest fungi get favorable opportunity for growth and specifically plays a significant role for *Aspergillus* and *Penicillium* contamination. A research by Asela et al (2019) reported fungal incidence after six months of wheat storage in South east Ethiopia was significantly associated ($p<0.05$) with two of the independent variables, temperature and relative humidity of the storage. However, there is no significant association ($p<0.05$) with grain moisture content of ($\leq$13.5% and >13.5%) and storage type of wheat seed. Besides the insignificant correlation, there is incidence of multitude of storage fungi recorded. Absence of statistical association between fungi presence and moisture doesn't necessarily mean no fungal prevalence. It seems all wheat samples were well dried during harvest and no rewetting had happened any time after harvest. Moreover, the genus

Fusarium will possibly dwell in plants before storage and such insignificance will be due to this reason

Table 4.1: Correlation analysis result of moisture content and fungal prevalence

		Moisture Content	Percent of Infection
Moisture Content	Pearson Correlation	1	-.074
	Sig. (2-tailed)		.377
	N	144	144
Percent of Infection	Pearson Correlation	-.074	1
	Sig. (2-tailed)	.377	
	N	144	144

Individually, wheat samples moisture content measured in this study ranges from 8.9 (Agarfa, Ali Keble) to 17.86 (Arsi Negele, Ali Weyo Keble) and the prevalence of fungal infection ranges from 6.67% to 100%. The mean moisture content was 12.57% and mean percent of fungal infection (36.39%). The relationship between moisture content and fungal percent of infection was statistically insignificant ($p>0.05$). The moisture content below which micro-organisms cannot grow is referred to as the safe moisture content and in wheat the accepted safe limit of moisture content is set 13.5% (Dubale Abate, 2018; Tilahun, 2007). In this study the mean average safe moisture content was found to be 12.75% which was normally in accepted range, where the fungi will have reduced proliferation.

Table 4.2. Mean moisture content and fungal infection score of the study areas

Zone District	Kebele	Mean Moisture Content	Mean Percent of fungal Infection
Bale	Agarfa Town	11.97	37.22
Agarfa			
	Ilani	12.2	42.77
	Alli	11.88	33.33
West arsi	Dodola Town	12.58	28.89
Dodola			
	Deneba	12.4	41.11
	Barrisa	13	41.66
Hasassa	Hasassa Town	12.06	56.66
	Debara Walati	12.9	54.44
	Bucho Wokenson	12.5	34.99
Arsi negelle	Arsi Negele Town	13.29	21.66
	Ali Woyo	13.23	22.77
	Mako Odda	12.84	16.11

In this research, wheat samples stored for one, two and three months are used and wheat grains stored for three month showed higher mean percent of infection (37.11%). With an increase in storage duration, the incidence and frequency of all fungal species will also increase due to an increase in relative humidity in the storage that favors the rewetting of the stored grains (Negasa et al., 2019). This may be associated with the exhaustion of nutrients and accumulation of toxic metabolites produced by the fungi themselves (Niaz et al., 2011). And over a storage period of three months, the grains' mycoflora will be enriched by growth of the phyto pathogenic fungi, due to storage conditions (Dudoiu et al., 2016).

Storage type of grains also plays a significant role for fungal contamination and unfortunately in this research, all the wheat samples were obtained from sack storage. Thus, the storage type effect for fungal contamination of wheat grains is evaluated in all the samples (36.39%). In Ethiopia, particularly in Oromia, most of wheat production takes place in relatively cooler areas of Arsi and Bale zones followed by Shashemene and ArsiNegelle districts. In these areas, most of the harvested wheat grains are generally stored in sacks of different sizes and quality (DubaleBefikadu , 2018).

The wheat samples were obtained from three different sources (Table 4.2). The grains collected from open market were more infected (41.33%) followed by residents' house (36.48%), and ware house (30.51%). This indicates that the more exposed to soil, the more chance of fungal contamination. Moreover, in the open market the grains have higher probability of contact with dust, soil and dung which also favors fungal growth. Also, residents house and warehouse will have poor management and since contact with rodents and insects.

Table 4.3: Sample collection source (Collection type)

Collection Type	**Frequency of collection**	**Percent of collection**	**Valid Percent**
Open Market	10	6.9	6.9
Residents House	108	75.0	75.0
Ware House	26	18.1	18.1
Total	144	100.0	100.0

4.3. Fungal Characterization

From the total of 2160 analyzed wheat grains a total of 786 were found to be contaminated with multitude of storage fungi which account for 36.39%. Morphological and microscopic ch aracterization result showed the following fungal genera; *Fusarium, Aspergillus, Penicillium,* and *Muchor/Rhizopus* (Wang et al., (2016). (Figure4.3) and (Table 4.3).

4.3.1. Aspergillus species

4.3.1.1. Microscopic Appearance

Hyphae are septate (2.5 - 8.0 micrometers in diameter); an unbranched conidiophore arises from a specialized foot cell. The conidiophore is enlarged at the tip, forming a swollen vesicle. Vesicles are completely or partially covered with flask-shaped phialides (formerly referred to as sterigmata) which may develop directly on the vesicle (uniseriate form) or be supported by a cell known as a metula (biseriate form). The phialides produce chains of mostly round, sometimes rough, conidia (2 - 5 micrometers in diameter)

4.3.1.2. Macroscopic Appearance

Table: 4.4: Show macroscopic feature of *Aspergillus*

Surface	Initially white and then any shade of yellow, green, brown, or black, depending on species. Texture is velvety or cottony
Reverse	White, goldish, or brown.
Growth Rate	Rapid, mature within 3 days; some species are slower growing.

4.3.1.3. Distinguishing Features

Aspergillus spp. is easily recognized by its conidiophores terminating in an apical vesicle and, at the opposite end, in a basal foot cell inserted into the supporting hyphae. Phialides are attached directly to the vesicle (uniserate) or an intervening cell called a metula (biseriate); these structures may cover the entire surface (columnar head); conidia in chains. The identification of species or species groups depends primarily on colony color and the form of conidial heads. The appearance of the species commonly isolated in the clinical laboratory is normally typical on Sabouraud Dextrose Agar or Potato Dextrose Agar. In reference works, descriptions are often of colonies grown on PDA (Samson, et al 2011).

4.3.2. The colony morphology of *Penicillium* species

4.3.2.1. Macroscopic Features

The colony morphology of *Penicillium* species exhibit rapid growth and fully mature in 5 days. The surface appearance is powdery, the colony color varies with species but usually green, blue-green or grey green often with white edge, and the reverse is pale cream 0n Wheat Media.Conidiophores can be simple or branched, conidia range from 0.14 -2.62micrometer, w hich extends as a chain the entire structure, and conidia arrange in chain.

4.3.2.2. Microscopic Features

Penicillum species septate hyphae (1.5 to 5 µm in diameter), simple or branched conidiophores , metulae, phialides, and conidia are observed. Metulae are secondary branches that form on conidiophores. The metulae carry the flask-shaped phialides. The organization of the phialides at the tips of the conidiophores is very typical. They form brush-like clusters which are also referred to as penicilli. The conidia (2.5-5µm in diameter) are round, unicellular, and visualized as unbranching chains at the tips of the phialides.

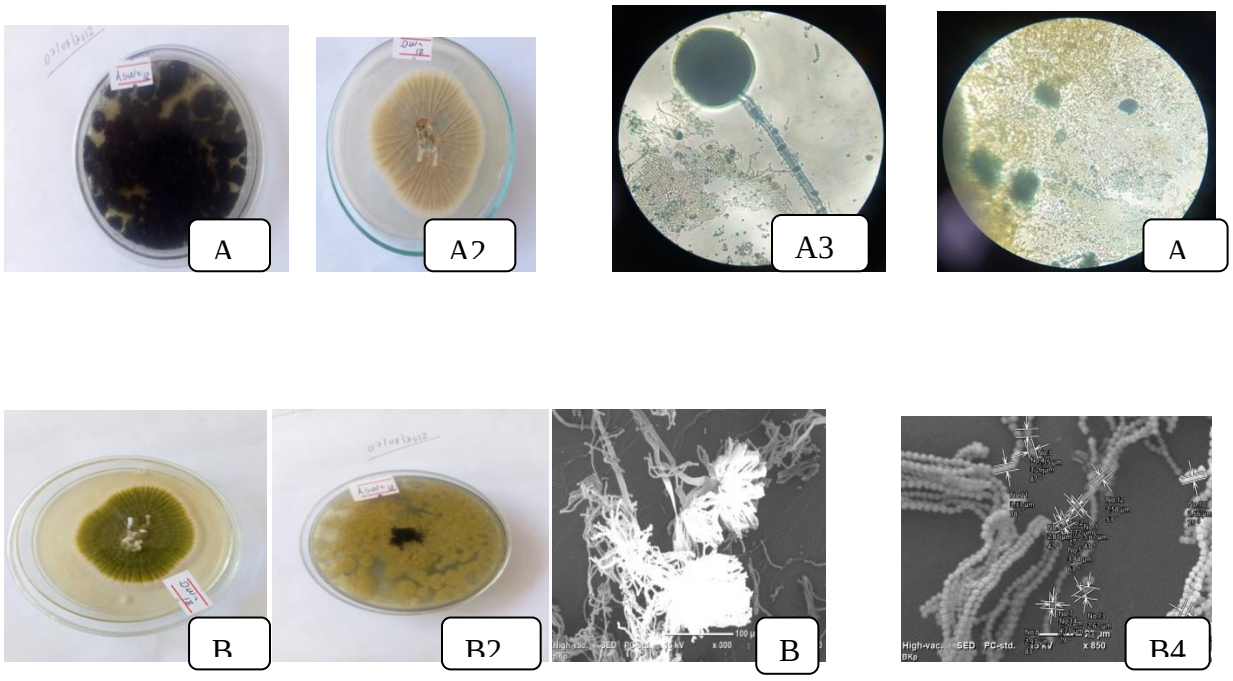

Figure 4.1: (A) *Aspergillus* colony features on PDA (A1-Top, A2-Reverse, A3-conidia and A4-spore; (B) Penicillium colony features on PDA (B1-Top, B2-Reverse, B3-conidia and B4

Scanning Electron microscope Characteristics of *Penicillium* spore.

4.3.3. Fusarium species

4.3.3.1. Macroscopic Features

fusarium spp. grows rapidly on PDA at 25°C and produce woolly to cottony, flat, spreading colonies. The only slow-growing species is *Fusarium dimerum*. From the front, the color of the colony may be white, cream, tan, salmon, cinnamon, yellow, red, violet, pink, or purple. From the reverse, it may be colorless, tan, red, dark purple, or brown.

A sclerotium, which is the organized mass of hyphae that remains dormant during unfavorable conditions, may be observed macroscopically and is usually dark blue in color. On the other hand, sporodochium, the cushion-like mat of hyphae bearing conidiophores over its surface, is usually absent in culture. When present, it may be observed in cream to tan or orange color, except for *Fusarium solani*, which gives rise to blue-green or blue sporodochia .

4.3.3.2. Microscopic Features

Hyaline septate hyphae, conidiophores, phialides, macroconidia, and microconidia are observe d microscopically. In addition to these basic elements, chlamydospores are also produced by *F usarium chlamydosporum*, *Fusarium napiforme*, *Fusarium oxysporum*, *Fusarium semitectum*, *Fusarium solani*, and *Fusarium sporotrichoides (* Singh, et al 2016).

Phialides are cylindrical, with a small collarette, solitary or produced as a component of a complex branching system. Monophialides and polyphialides (in heads or in chains) may be observed. Macroconidia (3-8 x 11-70 µm) are produced from phialides on unbranched or branched conidiophores. They are 2- or more celled, thick-walled, smooth, and cylindrical or sickle- (canoe-)shaped. Macroconidia have a distinct basal foot cell and pointed distal ends. They tend to accumulate in balls or rafts. Microconidia (2-4 x4-8 µm), on the other hand, are formed on long or short simple conidiophores. They are 1-celled (occasionally 2- or 3-celled), smooth, hyaline, ovoid to cylindrical, and arranged in balls (occasionally occurring in chains). Chlamydospores, when present, are sparse, in pairs, clumps or chains. They are thick-walled, hyaline, intercalary or terminal. (Zhang et al, 2006). Macroscopic and microscopic features, such as, color of the colony, length and shape of the macroconidia, the number, shape and arrangement of microconidia, and presence or absence of chlamydospores are key features for the differentiation of *Fusarium* species (Singh, et al 2016)

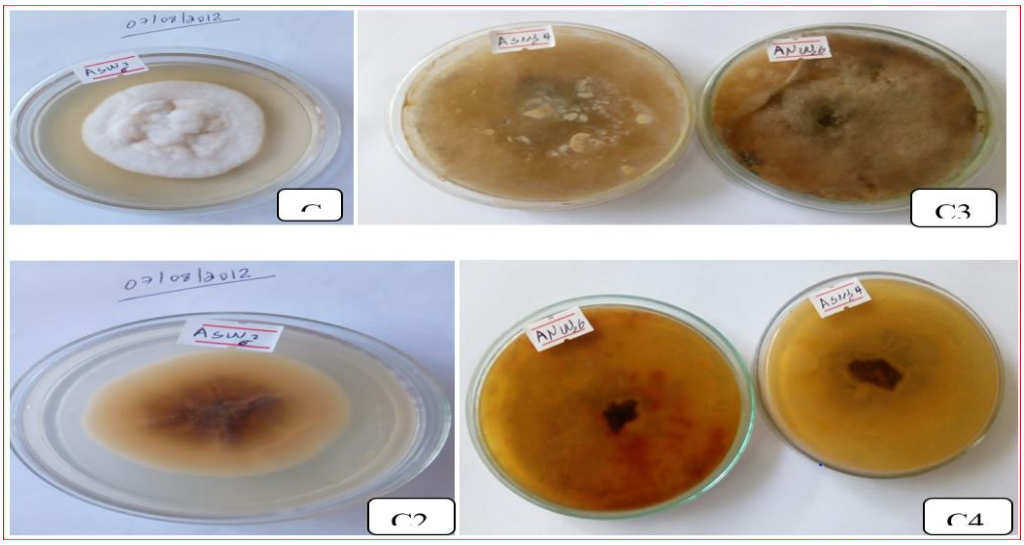

Figure 4.2: Fusarium colony features on PDA (C1 and C3-Top, C2 and C4-Reverse.

4.3.4. *Rhizopus* species

4.3.4.1. Macroscopic Features

Colonies of *Rhizopus* grow very rapidly, fill the Petri dish, and mature in 4 days. The texture is typically cotton-candy like. From the front, the color of the colony is white initially and turns grey to yellowish brown in time. The reverse is white to pale. Pathogenic species of *Rhizopus* can grow well at 37°C (Figure4.5, D).

4.3.4.2. Microscopic Features

No septate or sparsely septate broad hyphae (6-15 µm in diameter), sporangiophores, rhizoids (root-like hyphae), sporangia, and sporangiospores are visualized. Sporangiophores are brown in color and usually unbranched. They can be solitary or form clusters. Rhizoids are located at the point where the stolon's and sporangiophores meet. Sporangia (40-350 µm in diameter) are located at the tip of the sporangiophores. They are round with flattened bases. Apophysis is absent or rarely apparent and columellae are hemispherical. Sporangiospores (4-11 µm in diameter) are unicellular, round to ovoid in shape, hyaline to brown in color, and smooth or striated in texture

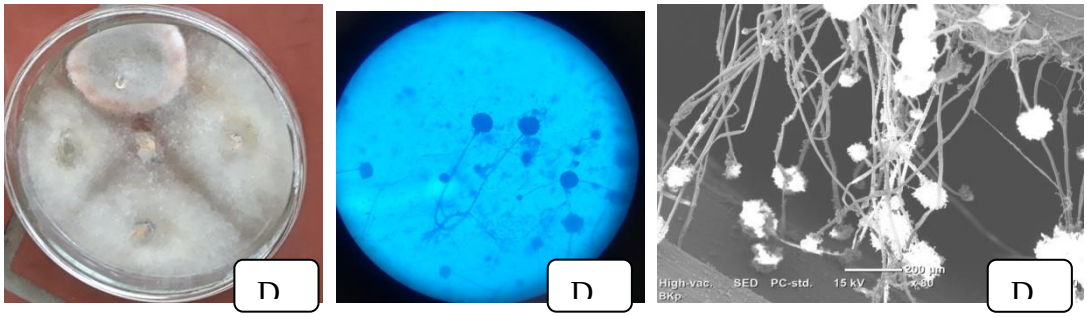

Figure 4.3: Rhizopus colony features on PDA, D1-Top, and D2 and C3 conidia

One of the dominant geniuses isolated from the collected samples was *Fusarium*. The major distinction of this fungus was that its colonies are characterized by presence of whitish mycelium (Figure 4.4 C) (Temesgen and Teshome, 2019). Morphological characteristics of pure culture isolates were used as a primary screening for fungal identification and by using microscopic slide culture technique, isolates confirmatory tests test revealed the distinctive characteristics of the genera

Table 4.5: Fungal prevalence in the study areas

Sample Area	**Fungal Genera Prevalence (%)**			
	Possible identified Fungi species			
	Fusarium sp	*Aspergillus sp*	*Penicillium sp*	*Muchor/Rhizopus sp*
Agarfa Town	22.22	0	12.78	2.22
Ilani	42.22	0.56	0	0
Alli	31.67	1.11	0.56	0
Dodola Town	28.89	0	0	0
Deneba	40.00	0.56	0.56	0
Barrisa	40.56	1.11	0	0
Hasassa Town	52.22	4.44	0	0
DebaraWalati	43.89	9.44	0	1.11
BuchoWokenson	26.11	5.0	3.89	0
ArsiNegelle Town	15.56	4.44	0	1.67
Ali Weyo	14.44	4.44	3.89	0
Mako Odda	19.44	0.56	1.11	0

5. Conclusion and Recommendations

5.1. Conclusion

This study examined the prevalence of fungi associated with wheat grain obtained from top two wheat producing zones in the Oromia regional state. Various environmental factors were tested for association with wheat fungal contamination and found to be statistically insignificant. Ho wever, there was considerable prevalence of four fungal genera in the study areas. Accordingl y, the mean moisture content was found to be safe for whet storage and reduce mycotoxin production. Wheat samples stored for one, two and three months are used and wheat grains stored for three month showed higher mean percent of infection (37.11%). Overall, the study revealed that the genus *Fusarium* was the dominant genera in the wheat samples followed by Aspergillus, *Penicillium and Muchor/Rhizopus*. Fungal mean incidence of 36.39% was recorded in this study and the prevalence of the fungi was highly variable across the sampling areas.

5.2. Recommendation

- Awareness creation and training should be given to the farmers on better and improved storage techniques to standardize the farmers, grains marketers and consumers to improve wheat storage facilities.
- Recent information on magnitude of postharvest loss and exemplary management practices in the country is still needed specifically covering value chain points.
- It is important to further explore peculiar factors that cause postharvest losses at each production levels that will require special attention.
- Use of holistic and bio-based antifungal strategies has to be focused.

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Appendices

S.No	Zone	District	Kebele	S	SS	MH	S/M	SM	MC	GA	IG	PI	AC	PC	FC	M/RC	% A	% P	% F	% M/R
1	Bale	Agarfa	Agarfa Town	Wheat	1	1	3	1	11.6	15	8	53.33	0	0	7	1	0.00	0.00	87.50	12.50
2	Bale	Agarfa	Agarfa Town	Wheat	1	1	3	1	14.46	15	7	46.67	0	7	0	0	0.00	100.00	0.00	0.00
3	Bale	Agarfa	Agarfa Town	Wheat	1	1	3	1	12.6	15	8	53.33	0	5	0	3	0.00	62.50	0.00	37.50
4	Bale	Agarfa	Agarfa Town	Wheat	1	1	3	1	12.9	15	10	66.67	0	0	10	0	0.00	0.00	100.00	0.00
5	Bale	Agarfa	Agarfa Town	Wheat	1	1	3	1	11.6	15	8	53.33	0	0	8	0	0.00	0.00	100.00	0.00
6	Bale	Agarfa	Agarfa Town	Wheat	1	1	3	1	10.73	15	7	46.67	0	7	0	0	0.00	100.00	0.00	0.00
7	Bale	Agarfa	Agarfa Town	Wheat	1	1	3	1	12.7	15	6	40.00	0	0	6	0	0.00	0.00	100.00	0.00
8	Bale	Agarfa	Agarfa Town	Wheat	1	1	3	1	10.06	15	4	26.67	0	4	0	0	0.00	100.00	0.00	0.00
9	Bale	Agarfa	Agarfa Town	Wheat	1	1	3	1	11.2	15	6	40.00	0	0	6	0	0.00	0.00	100.00	0.00
10	Bale	Agarfa	Agarfa Town	Wheat	1	1	3	1	13.06	15	2	13.33	0	0	2	0	0.00	0.00	100.00	0.00
11	Bale	Agarfa	Agarfa Town	Wheat	1	1	3	1	12.57	15	1	6.67	0	0	1	0	0.00	0.00	100.00	0.00
12	Bale	Agarfa	Agarfa Town	Wheat	1	1	3	1	10.16	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
13	Bale	Agarfa	Ilani	Wheat	1	1	3	1	12.7	15	9	60.00	0	0	9	0	0.00	0.00	100.00	0.00
14	Bale	Agarfa	Ilani	Wheat	1	1	3	1	13.03	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
15	Bale	Agarfa	Ilani	Wheat	1	1	3	1	12.4	15	10	66.67	0	0	10	0	0.00	0.00	100.00	0.00
16	Bale	Agarfa	Ilani	Wheat	1	1	3	1	15.06	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
17	Bale	Agarfa	Ilani	Wheat	1	1	3	1	12.07	15	15	100.0	1	0	15	0	6.67	0.00	100.00	0.00
18	Bale	Agarfa	Ilani	Wheat	1	1	3	1	12.16	15	3	20.00	0	0	3	0	0.00	0.00	100.00	0.00
19	Bale	Agarfa	Ilani	Wheat	1	1	3	1	11.5	15	5	33.33	0	0	5	0	0.00	0.00	100.00	0.00
20	Bale	Agarfa	Ilani	Wheat	1	1	3	1	12.16	15	1	6.67	0	0	1	0	0.00	0.00	100.00	0.00
21	Bale	Agarfa	Ilani	Wheat	1	1	3	1	11.5	15	15	100.0	0	0	15	0	0.00	0.00	100.00	0.00
22	Bale	Agarfa	Ilani	Wheat	1	1	3	1	10.26	15	3	20.00	0	0	3	0	0.00	0.00	100.00	0.00
23	Bale	Agarfa	Ilani	Wheat	1	1	3	1	11.2	15	15	100.0	0	0	15	0	0.00	0.00	100.00	0.00
24	Bale	Agarfa	Ilani	Wheat	1	1	3	1	14.56	15	1	6.67	0	0	1	0	0.00	0.00	100.00	0.00

25	Bale	Agarfa	Ali	Wheat	1	1	3	1	13.67	15	8	53.33	0	1	7	0	0.00	12.50	87.50	0.00
26	Bale	Agarfa	Ali	Wheat	1	1	3	1	11.84	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
27	Bale	Agarfa	Ali	Wheat	2	1	3	1	13.1	15	8	53.33	0	0	8	0	0.00	0.00	100.00	0.00
28	Bale	Agarfa	Ali	Wheat	2	1	3	1	8.9	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
29	Bale	Agarfa	Ali	Wheat	2	1	3	1	12.53	15	9	60.00	1	0	8	0	11.11	0.00	88.89	0.00
30	Bale	Agarfa	Ali	Wheat	2	1	3	1	9.19	15	1	6.67	1	0	0	0	100.0	0.00	0.00	0.00
31	Bale	Agarfa	Ali	Wheat	1	2	3	1	13.2	15	10	66.67	0	0	10	0	0.00	0.00	100.00	0.00
32	Bale	Agarfa	Ali	Wheat	1	2	3	1	13.2	15	1	6.67	0	0	1	0	0.00	0.00	100.00	0.00
33	Bale	Agarfa	Ali	Wheat	2	2	3	1	11.83	15	15	100.0	0	0	15	0	0.00	0.00	100.00	0.00
34	Bale	Agarfa	Ali	Wheat	2	2	3	1	11.89	15	2	13.33	0	0	2	0	0.00	0.00	100.00	0.00
35	Bale	Agarfa	Ali	Wheat	2	2	3	1	11.8	15	2	13.33	0	0	2	0	0.00	0.00	100.00	0.00
36	Bale	Agarfa	Ali	Wheat	2	2	3	1	11.46	15	4	26.67	0	0	4	0	0.00	0.00	100.00	0.00
37	Bale	Dodola	Dodola T.	Wheat	1	1	3	1	13.4	15	10	66.67	0	0	10	0	0.00	0.00	100.00	0.00
38	Bale	Dodola	Dodola T.	Wheat	1	1	3	1	12.16	15	1	6.67		0	1	0	0.00	0.00	100.00	0.00
39	Bale	Dodola	Dodola T.	Wheat	1	1	3	1	10.27	15	10	66.67	0	0	10	0	0.00	0.00	100.00	0.00
40	Bale	Dodola	Dodola T.	Wheat	1	1	3	1	12.13	15	7	46.67	0	0	7	0	0.00	0.00	100.00	0.00
41	Bale	Dodola	Dodola T.	Wheat	1	1	3	1	12.83	15	4	26.67	0	0	4	0	0.00	0.00	100.00	0.00
42	Bale	Dodola	Dodola T.	Wheat	1	1	3	1	12.7	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
43	Bale	Dodola	Dodola T.	Wheat	2	1	3	1	12.07	15	5	33.33	0	0	5	0	0.00	0.00	100.00	0.00
44	Bale	Dodola	Dodola T.	Wheat	2	1	3	1	13.8	15	4	26.67	0	0	4	0	0.00	0.00	100.00	0.00
45	Bale	Dodola	Dodola T.	Wheat	2	1	3	1	12.73	15	4	26.67	0	0	4	0	0.00	0.00	100.00	0.00
46	Bale	Dodola	Dodola T.	Wheat	2	1	3	1	12.3	15	2	13.33	0	0	2	0	0.00	0.00	100.00	0.00
47	Bale	Dodola	Dodola T.	Wheat	2	1	3	1	13.8	15	5	33.33	0	0	5	0	0.00	0.00	100.00	0.00
48	Bale	Dodola	Dodola T.	Wheat	2	1	3	1	12.76	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
49	Bale	Dodola	Deneba	Wheat	1	1	3	1	13.03	15	6	40.00	0	0	6	0	0.00	0.00	100.00	0.00
50	Bale	Dodola	Deneba	Wheat	1	1	3	1	10.86	15	5	33.33	0	0	5	0	0.00	0.00	100.00	0.00
51	Bale	Dodola	Deneba	Wheat	1	1	3	1	13.4	15	12	80.00	1	0	11	0	8.33	0.00	91.67	0.00
52	Bale	Dodola	Deneba	Wheat	1	1	3	1	11.33	15	8	53.33	0	0	8	0	0.00	0.00	100.00	0.00

52	Bale	Dodola	Deneba	Wheat	1	1	3	1	12.37	15	10	66.67	0	0	10	0	0.00	0.00	100.00	0.00
54	Bale	Dodola	Deneba	Wheat	1	1	3	1	11.76	15	1	6.67	0	0	1	0	0.00	0.00	100.00	0.00
55	Bale	Dodola	Deneba	Wheat	1	1	3	1	13.23	15	10	66.67	0	0	10	0	0.00	0.00	100.00	0.00
56	Bale	Dodola	Deneba	Wheat	1	1	3	1	13.16	15	2	13.33	0	0	2	0	0.00	0.00	100.00	0.00
57	Bale	Dodola	Deneba	Wheat	1	1	3	1	13.17	15	6	40.00	0	1	5	0	0.00	16.67	83.33	0.00
58	Bale	Dodola	Deneba	Wheat	1	1	3	1	12.13	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
59	Bale	Dodola	Deneba	Wheat	1	1	3	1	13.27	15	10	66.67	0	0	10	0	0.00	0.00	100.00	0.00
60	Bale	Dodola	Deneba	Wheat	1	1	3	1	11.13	15	4	26.67	0	0	4	0	0.00	0.00	100.00	0.00
61	Bale	Dodola	Barrisa	Wheat	1	1	3	1	12.47	15	10	66.67	0	0	10	0	0.00	0.00	100.00	0.00
62	Bale	Dodola	Barrisa	Wheat	1	1	3	1	14.23	15	10	66.67	0	0	10	0	0.00	0.00	100.00	0.00
63	Bale	Dodola	Barrisa	Wheat	1	1	3	1	13.03	15	4	26.67	0	0	4	0	0.00	0.00	100.00	0.00
64	Bale	Dodola	Barrisa	Wheat	1	1	3	1	12.83	15	1	6.67	0	0	1	0	0.00	0.00	100.00	0.00
65	Bale	Dodola	Barrisa	Wheat	1	1	3	1	12.77	15	15	100.0	0	0	15	0	0.00	0.00	100.00	0.00
66	Bale	Dodola	Barrisa	Wheat	1	1	3	1	11.3	15	6	40.00	0	0	6	0	0.00	0.00	100.00	0.00
67	Bale	Dodola	Barrisa	Wheat	1	1	3	1	13.2	15	4	26.67	0	0	4	0	0.00	0.00	100.00	0.00
68	Bale	Dodola	Barrisa	Wheat	1	1	3	1	13.73	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
69	Bale	Dodola	Barrisa	Wheat	1	1	3	1	15.27	15	5	33.33	1	0	4	0	20.00	0.00	80.00	0.00
70	Bale	Dodola	Barrisa	Wheat	1	1	3	1	12.06	15	3	20.00	0	0	3	0	0.00	0.00	100.00	0.00
71	Bale	Dodola	Barrisa	Wheat	1	1	3	1	12.67	15	12	80.00	1	0	11	0	8.33	0.00	91.67	0.00
72	Bale	Dodola	Barrisa	Wheat	1	1	3	1	12.46	15	4	26.67	0	0	4	0	0.00	0.00	100.00	0.00
73	W/Arsi	Hasassa	Debara W.	Wheat	1	1	3	1	13.67	15	8	53.33	1	0	7	0	12.50	0.00	87.50	0.00
74	WArsi	Hasassa	Debara W.	Wheat	1	1	3	1	12.33	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
75	W/Arsi	Hasassa	Debara W.	Wheat	1	1	3	1	12.43	15	10	66.67	3	0	6	0	30.00	0.00	60.00	0.00
76	W/Arsi	Hasassa	Debara W.	Wheat	1	1	3	1	11.33	15	4	26.67	2	0	3	0	50.00	0.00	75.00	0.00

77	W/Arsi	Hasassa	Debara W.	Wheat	1	1	3	1	15.5	15	15	100.0	0	0	15	0	0.00	0.00	100.00	0.00
78	W/Arsi	Hasassa	Debara W.	Wheat	1	1	3	1	12.1	15	9	60.00	3	0	6	0	33.33	0.00	66.67	0.00
79	W/Arsi	Hasassa	Debara W.	Wheat	1	1	3	1	12.2	15	15	100.0	1	0	14	0	6.67	0.00	93.33	0.00
80	W/Arsi	Hasassa	Debara W.	Wheat	1	1	3	1	13.23	15	1	6.67	1	0	0	0	100.0	0.00	0.00	0.00
81	W/Arsi	Hasassa	Debara W.	Wheat	1	1	3	1	12.8	15	10	66.67	5	0	5	0	50.00	0.00	50.00	0.00
82	W/Arsi	Hasassa	Debara W.	Wheat	1	1	3	1	12.8	15	8	53.33	0	0	6	2	0.00	0.00	75.00	25.00
83	W/Arsi	Hasassa	Debara W.	Wheat	1	1	3	1	12.73	15	11	73.33	1	0	10	0	9.09	0.00	90.91	0.00
84	W/Arsi	Hasassa	Debara W.	Wheat	1	1	3	1	13.66	15	7	46.67	0	0	7	0	0.00	0.00	100.00	0.00
85	W/Arsi	Hasassa	Bucho W.	Wheat	1	1	3	1	12.47	15	4	26.67	1	0	3	0	25.00	0.00	75.00	0.00
86	W/Arsi	Hasassa	Bucho W.	Wheat	1	1	3	1	11.83	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
87	W/Arsi	Hasassa	Bucho Wo.	Wheat	1	1	3	1	12.2	15	12	80.00	2	0	10	0	16.67	0.00	83.33	0.00
88	W/Arsi	Hasassa	Bucho W.	Wheat	1	1	3	1	13	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
89	W/Arsi	Hasassa	Bucho W.	Wheat	1	1	3	1	12.56	15	15	100.0	3	3	13	0	20.00	20.00	86.67	0.00
90	W/Arsi	Hasassa	Bucho W.	Wheat	1	1	3	1	12.7	15	5	33.33	1	0	0	0	20.00	0.00	0.00	0.00
91	W/Arsi	Hasassa	Bucho W.	Wheat	1	1	3	1	12.23	15	10	66.67	0	1	9	0	0.00	10.00	90.00	0.00
92	W/Arsi	Hasassa	Bucho W.	Wheat	1	1	3	1	13.03	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
93	W/Arsi	Hasassa	Bucho W.	Wheat	1	1	3	1	13.1	15	7	46.67	0	1	6	0	0.00	14.29	85.71	0.00
94	W/Arsi	Hasassa	Bucho W.	Wheat	1	1	3	1	14.5	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
95	W/Arsi	Hasassa	Bucho W.	Wheat	1	1	3	1	12.86	15	10	66.67	2	2	6	0	20.00	20.00	60.00	0.00
96	W/Arsi	Hasassa	Bucho W.	Wheat	1	1	3	1	9.5	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
97	W/Arsi	Hasassa	Hasassa T	Wheat	2	1	3	1	13.86	15	11	73.33	1	0	10	0	9.09	0.00	90.91	0.00
98	W/Arsi	Hasassa	Hasassa T	Wheat	2	1	3	1	10.56	15	6	40.00	0	0	6	0	0.00	0.00	100.00	0.00
99	W/Arsi	Hasassa	Hasassa T	Wheat	2	1	3	1	14.19	15	10	66.67	4	0	6	0	40.00	0.00	60.00	0.00
100	W/Arsi	Hasassa	Hasassa T	Wheat	2	1	3	1	11.83	15	6	40.00	0	0	6	0	0.00	0.00	100.00	0.00

101	W/Arsi	Hasassa	Hasassa T	Wheat	2	1	3	1	12.72	15	14	93.33	0	0	14	0	0.00	0.00	100.00	0.00
102	W/Arsi	Hasassa	Hasassa T	Wheat	2	1	3	1	10.96	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
103	W/Arsi	Hasassa	Hasassa T	Wheat	3	1	3	1	12.52	15	10	66.67	3	0	8	0	30.00	0.00	80.00	0.00
104	W/Arsi	Hasassa	Hasassa T	Wheat	3	1	3	1	11.36	15	5	33.33	0	0	4	0	0.00	0.00	80.00	0.00
105	W/Arsi	Hasassa	Hasassa T	Wheat	3	1	3	1	12.37	15	15	100.0	0	0	15	0	0.00	0.00	100.00	0.00
106	W/Arsi	Hasassa	Hasassa T	Wheat	3	1	3	1	10.23	15	4	26.67	0	0	4	0	0.00	0.00	100.00	0.00
107	W/Arsi	Hasassa	Hasassa T	Wheat	3	1	3	1	13.32	15	15	100.0	0	0	15	0	0.00	0.00	100.00	0.00
108	W/Arsi	Hasassa	Hasassa T	Wheat	3	1	3	1	10.8	15	6	40.00	0	0	6	0	0.00	0.00	100.00	0.00
109	W/Arsi	Arsi Negele	Arsi Negele T	Wheat	3	1	2	1	12.77	15	7	46.67	3	0	4	0	42.86	0.00	57.14	0.00
110	W/Arsi	Arsi Negele	Arsi Negele T	Wheat	3		2	1	15.1	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
111	W/Arsi	Arsi Negele	Arsi Negele T	Wheat	3	1	1	1	11.73	15	7	46.67	0	0	7	0	0.00	0.00	100.00	0.00
112	W/Arsi	Arsi Negele	Arsi Negele T	Wheat	3	1	1	1	13.3	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
113	W/Arsi	Arsi Negele	Arsi Negele T	Wheat	2	1	3	1	12.03	15	3	20.00	0	0	2	1	0.00	0.00	66.67	33.33
114	W/Arsi	Arsi Negele	Arsi Negele T	Wheat	2	1	3	1	15.83	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
115	W/Arsi	Arsi Negele	Arsi Negele T	Wheat	2	1	3	1	11.83	15	2	13.33	1	0	0	1	50.00	0.00	0.00	50.00
116	W/Arsi	Arsi Negele	Arsi Negele T	Wheat	2	1	3	1	12.76	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
117	W/Arsi	Arsi Negele	Arsi Negele T	Wheat	1	1	1	1	11.77	15	9	60.00	3	0	5	1	33.33	0.00	55.56	11.11
118	W/Arsi	Arsi Negele	Arsi Negele T	Wheat	1	1	1	1	14.7	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
119	W/Arsi	Arsi Negele	Arsi Negele T	Wheat	1	1	1	1	12.37	15	10	66.67	0	0	10	0	0.00	0.00	100.00	0.00
120	W/Arsi	Arsi Negele	Arsi Negele T	Wheat	1	1	1	1	15.3	15	1	6.67	1	0	0	0	100.0	0.00	0.00	0.00
121	W/Arsi	Arsi Negele	Ali Weyo	Wheat	1	1	3	1	12.47	15	9	60.00	0	0	9	0	0.00	0.00	100.00	0.00
122	W/Arsi	Arsi Negele	Ali Weyo	Wheat	1	1	3	1	14.26	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
123	W/Arsi	Arsi Negele	Ali Weyo	Wheat	1	1	3	1	11.87	15	5	33.33	1	3	1	0	20.00	60.00	20.00	0.00

124	W/Arsi	Arsi Negele	Ali Weyo	Wheat	1	1	3	1	14.46	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
125	W/Arsi	Arsi Negele	Ali Weyo	Wheat	1	1	3	1	10.43	15	5	33.33	0	1	5	0	0.00	20.00	100.00	0.00
126	W/Arsi	Arsi Negele	Ali Weyo	Wheat	1	1	3	1	15.4	15	3	20.00	0	0	2	0	0.00	0.00	66.67	0.00
127	W/Arsi	Arsi Negele	Ali Weyo	Wheat	1	1	3	1	11.1	15	5	33.33	0	1	4	0	0.00	20.00	80.00	0.00
128	W/Arsi	Arsi Negele	Ali Weyo	Wheat	1	1	3	1	15.63	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
129	W/Arsi	Arsi Negele	Ali Weyo	Wheat	1	1	3	1	11.53	15	5	33.33	1	1	3	0	20.00	20.00	60.00	0.00
130	W/Arsi	Arsi Negele	Ali Weyo	Wheat	1	1	3	1	17.86	15	2	13.33	2	0	¨0	0	100.0	0.00	0.00	0.00
131	W/Arsi	Arsi Negele	Ali Weyo	Wheat	1	1	3	1	11.47	15	6	40.00	4	1	1	0	66.67	16.67	16.67	0.00
132	W/Arsi	Arsi Negele	Ali Weyo	Wheat	1	1	3	1	12.4	15	1	6.67	0	0	1	0	0.00	0.00	100.00	0.00
133	W/Arsi	Arsi Negele	Mako Odda	Wheat	1	1	3	1	12.7	15	3	20.00	0	0	3	0	0.00	0.00	100.00	0.00
134	W/Arsi	Arsi Negele	Mako Odda	Wheat	1	1	3	1	14.56	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
135	W/Arsi	Arsi Negele	Mako Odda	Wheat	1	1	3	1	14.43	15	8	53.33	0	0	8	0	0.00	0.00	100.00	0.00
136	W/Arsi	Arsi Negele	Mako Odda	Wheat	1	1	3	1	14.13	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
137	W/Arsi	Arsi Negele	Mako Odda	Wheat	1	1	3	1	10.13	15	4	26.67	0	0	4	0	0.00	0.00	100.00	0.00
138	W/Arsi	Arsi Negele	Mako Odda	Wheat	1	1	3	1	13.76	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
139	W/Arsi	Arsi Negele	Mako Odda	Wheat	1	1	2	1	9.7	15	6	40.00	0	0	6	0	0.00	0.00	100.00	0.00
140	W/Arsi	Arsi Negele	Mako Odda	Wheat	1	1	2	1	13.36	15	0	0.00	0	0	0	0	0.00	0.00	0.00	0.00
141	W/Arsi	Arsi Negele	Mako Odda	Wheat	1	1	3	1	12.89	15	7	46.67	1	0	6	0	14.29	0.00	85.71	0.00
142	W/Arsi	Arsi Negele	Mako Odda	Wheat	1	1	3	1	13.53	15	2	13.33	0	0	2	0	0.00	0.00	100.00	0.00
143	W/Arsi	Arsi Negele	Mako Odda	Wheat	1	1	3	1	12.23	15	5	33.33	0	2	3	0	0.00	40.00	60.00	0.00
144	W/Arsi	Arsi Negele	Mako Odda	Wheat	1	1	3	1	12.76	15	3	20.00	0	0	3	0	0.00	0.00	100.00	0.00

Legend of the Above Table

Abbrevation	Expanded	Abbrevation	Expanded
ST	Sample Type	FC	Fus. Count
SS	Sample Source	M/RC	Muc/Rhiz Count
MH	Means of Harvest	% A	% Aspe
S.D/M	Store. Duraion./month	% P	% Pen
SM	Storage Material	% F	% Fus
MC	Moisture Content	% M/R	% Muc/Rus
GA	Grains Analyze	Bucho W.	Bucho Wokenson
PI	Percent of Infection	Debara W.	Debara Waltai
AC	Asper. Count	W/Arsi	West Arsi
PC	Penici. Count	T	Town

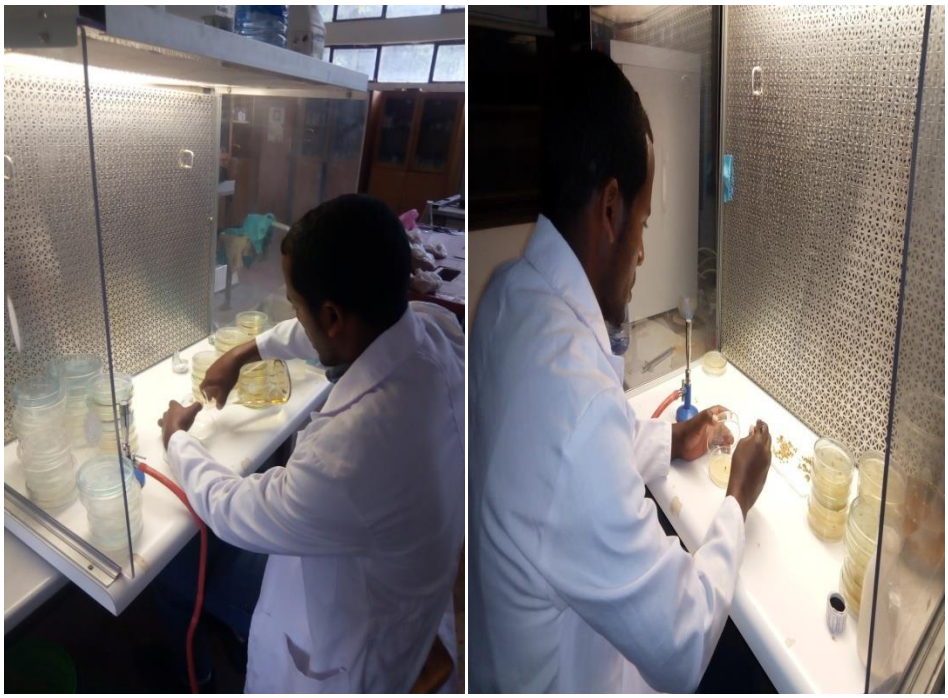

FIGURE 1. Media preparation and Sample inoculation.

FIGURE 2. Growth of fungi from wheat on Potato Dextrose Agar

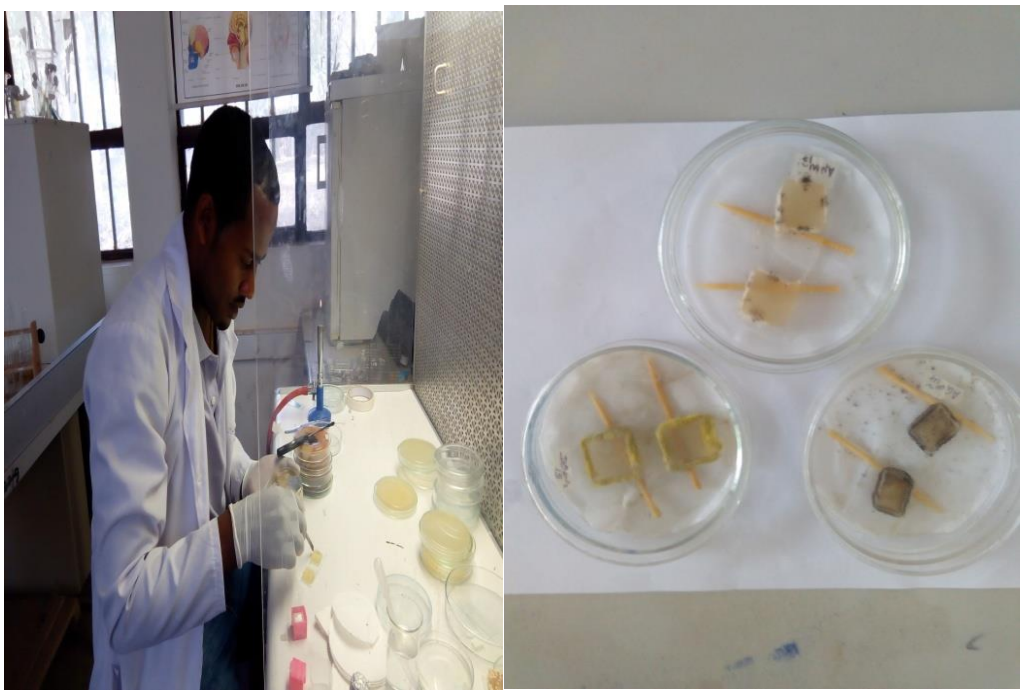

FIGURE 3. Show Slide culture preparation.

FIGURE 4. Show Pure culture identification.