ASSESSMENT OF POSTHARVEST FUNGAL DISEASES OF MAIZE (*Zea mays* L.) AND ASSOCIATED FACTORS IN SOME AREAS OF JIMMA ZONE, SOUTHWESTERN ETHIOPIA

MSc THESIS

BY

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ASSESSMENT OF POSTHARVEST FUNGAL DISEASES OF MAIZE (*Zea mays* L) AND ASSOCIATED FACTORS IN JIMMA ZONE, SOUTHWESTERN ETHIOPIA

A Thesis

Submitted to School of Graduate Studies Jimma University College of Agriculture and Veterinary Medicine in Partial Fulfillment of the Requirements for the Degree of Master of Science in Postharvest Management (Durable Crops)

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DEDICATION

This thesis manuscript is dedicated to my beloved parents and esteemed sister and brothers for all the sacrifices, wishes and praiseworthy to my success in all my endeavors.

STATEMENT OF AUTHOR

First, I declare that this Thesis is my original work and all sources or materials used for this Thesis have been duly acknowledged. This thesis is submitted in partial fulfillment of the requirements for MSc degree in Postharvest Management at Jimma University. I earnestly declare that this thesis is not submitted to any other institutions anywhere for the award of any academic degree, diploma or certificate.

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BIOGRAPHICAL SKETCH

The author, Hailegiorgis Nigussie Meshesha, was born on January 13, 1985 at Kelech Kele Kebele, Southwest Shoa zone of Oromia Regional State. He attended Elementary school in Kelech Guda and Secondary School Education at Sebeta Comprehensive School. He successfully passed the Ethiopian School Leaving Certificate Examination (ESLCE) in 2005, and joined Jimma University College of Agriculture and Veterinary Medicine in 2006. After three years of study, he graduated with BSc degree in Crop Science. After his graduation, he was employed as an Extension Expert in Setema Woreda Agriculture Bureau from September 2008 to August 2010. In September 2011, he joined the School of Graduate Studies of Jimma University College of Agriculture and Veterinary Medicine to pursue a graduate study leading to a Master of Science Degree in Postharvest Management (Durable Crops).

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ABBREVIATIONS

BH-660	Bako hybrid maize
CDA	Czapex dox agar
CSA	Central Statistical Agency
DDT	Di-chloro-Diphenyl- Trichloro ethane
FAO	Food and Agriculture Organization
GP	Germination percentage
m.a.s.l.	Meters above sea level
M.C	Moisture content
PAs	Peasant Associations
PDA	Potato dextrose agar
SPSS	Statistical Package for the Social Sciences
SSA	Sub-Saharan Africa

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ASSESSMENT OF POSTHARVEST FUNGAL DISEASES OF MAIZE (*Zea mays* L) AND ASSOCIATED FACTORS IN JIMMA ZONE, SOUTHWESTERN ETHIOPIA

ABSTRACT

Maize (Zea mays L*) is one of the major crops that are produced in different regions of Ethiopia. Despite the favorable environmental conditions for its production in Jimma Zone, maize is infected by several fungal diseases before and after harvest, due to poor pre- and post-harvest practices/handling. Thus, this study was conducted to assess the distribution of postharvest fungal diseases and associated factors, identify the major causal pathogens of maize in Jimma zone, southwestern Ethiopia during the period 2011 and 2012. This study was conducted at harvest and storage in three maize producing districts of Jimma zone, namely, Sekoru, Omonada and Dedo, respectively, representing low-, mid- and high- altitutde agroecological areas. Three Peasant Associations (PAs) per district and five farmers per PAs were randomly selected, 90 maize samples (45 from the field) and 45 from storage facilities were collected, and brought to Plant Pathology laboratory of Jimma University College of Agriculture and Veterinary Medicine. Fungal isolation and identification from maize kernels were made on Potato Dextrose Agar (PDA) and Czapex Dox Agar. The result showed that mean maize grain damage caused by mould and weevil were 37.8 and 20.9%, respectively, while the mean damage caused by mould, weevil and stalk borer amounted 28.9%, 19.3% and 23.3% respectively. The incidence of mould infected cobs at harvest in the field showed significant difference among PAs in Omonada but non significant for PAs in Sekoru and Dedo districts with respective mean incidence of 14.7%, 19.8% and 20.1% for Sekoru, Omonada and Dedo. Fungal contamination was evaluated from samples collected from field and storage. A total of 1462 fungal isolates were identified from 90 maize cob samples. The most frequent isolated fungi from Sekoru, Omonada and Dedo, respectively, were* Fusarium *(86.7, 100 and 100%),* Penicillium *(86.7, 100 and 73.3%),and* Drechslera *(66.7, 53.3 and 86.7%) species followed by* Cladosporium *(26.7, 46.7 and 40%) and* Aspergillus *(33.3, 13.3 and 26.6%) species; from maize samples collected in the field at harvest. Whereas* Aspergillus *(100, 100 and 93.3%),* Fusarium *(93.3, 93.3 and 86.6%),* Penicillium *(80, 73.3 and 100%) species, were most frequent in maize cob sampled from storage in Sekoru, Omonada and Dedo, respectively. This study indicated that, in the field, maize cobs damaged by birds, stalk borers and weevils frequently showed mould growth in kernels, more favored by continuous rainfall upon delayed harvesting resulted in higher crop damage. Further more, improper drying, poor storage structures and storage practices accompanied by rodents attack and weevil infestation aggravated fungal mould contamination in Jimma zone. Thus, it is important to create awareness and sensitize maize producing farmers about high mould contamination and fungal damage, along with possible health hazards of toxigenic fungi in humans and animals upon consumption of such contaminated food grains that needs concentrated efforts to ensure improved pre- and post-harvest handling of maize.*

1. INTRODUCTION

Maize (*Zea mays* L.) is one of the four major food crops of the world and in terms of production; it has the first position in production level in the world (Pingali, 2001; Anderson *et al*., 2004; Emily and Sherry, 2010). It is the most important cereal food crop in sub-Saharan Africa (SSA), particularly in eastern and southern Africa accounting for 53% of the total area covered by cereals (FAO, 2010) and 30-70% of the total caloric consumption (Langyintuo *et al*., 2010). In Ethiopia, maize is the staple food and one of the main sources of calories particularly in the major maize producing regions of the country (Girma *et al*., 2008). It ranks first in total production and yield per hectare, and next to tef in area coverage being grown across varied agro-ecological zones of the country (CSA, 2007).

The magnitude of postharvest loss in Ethiopia is tremendous ranging from 5% to 19% for maize (Dereje, 2000). Such figure is quite large and no wonder that great majority of people are food insecure. The immediate victims of food insecurity have traditionally been farmers who are the very producers of the crop. In the field as well as in the store, many pests and parasites attack maize. Insects are most often considered as the principal cause of grain losses (Ali *et al*., 2007). However, fungi are also the second important cause of deterioration and loss of maize (Scudamore and MacDonald, 2000). Maize is one of the cereals most susceptible to fungal contamination (Wilson *et al.*, 2006).

Fungi could cause about 50 to 80% of damage to maize on farms and during storage if conditions are favorable for their development and its nutritional characteristics also expose it to the constant attack of fungi and insect pests (Ali *et al*., 2007). The major genera commonly encountered on maize in tropical regions are *Fusarium*, *Aspergillus* and *Penicillium*. These are the major causes of concern because they consist of species capable of producing a wide spectrum of compounds (mycotoxins) known to be toxic to humans and animals (Orsi *et al*., 2000).

In a country like Ethiopia where production and productivity are low and postharvest loss is quite high, much effort should be made to generate a means that would boost production and minimize loss. The postharvest aspect includes proper handling practices, proper storage facilities and management practices, appropriate packaging techniques and transportation systems (Shimelis, 2001). According to Abebe and Bekele (2006), the results of a survey conducted in major grain producing areas of Ethiopia indicated that majority of farmers using traditional storage containers exposed their stored grains to attack by storage pests and/or other factors.

It is therefore necessary to study the fungal contamination of maize in the field and during storage thus making the grains unusable for consumption. The predisposing factors of infection include improper drying, farmers' production practices, early and delayed harvesting and poorly constructed storage structures. Maize predisposed to some of these factors has a high probability of fungal infection, which may, presumably enhance the development of mycotoxins. There is lack of accurate data on the frequency and relative importance of mycoflora associated with maize in southwest Ethiopia. Because of these reasons, it has not been possible to develop effective management strategies to prevent fungal infection and reduce postharvest losses. Therefore, it is imperative to assess the distribution and importance of postharvest fungal diseases along with detection of the major fungal groups associated with maize in Jimma zone, Southwest Ethiopia.

Objectives of the study

General objective

- To study the distribution and importance of postharvest fungal diseases, identify the major causal pathogens and associated factors on maize in Jimma zone

Specific objectives

- To determine the occurrence and distribution of postharvest fungal diseases on maize and associated factors/ practices influencing the disease infection, and
- To identify major causal pathogens at harvest and in the storage.

2. LITERATURE REVIEW

2.1 Maize production in Ethiopia

In Ethiopia, maize is one of the major cereal crops grown for its food and feed values. It is a dominant food crop in Ethiopia and majority of the population depends on it as sources of energy (Demissie *et al*., 2008). The production and productivity of maize has increased since the development of high yielding hybrid varieties by the Ethiopian Institute of Agricultural Research Center (Bako Agricultural Research Center). Of all food crops, maize has received special attention owing to its wide cultivation and its great significance among food crops. In 2007/08, maize production was 4.2 million tons, 40 percent higher than teff, 56 percent higher than sorghum, and 75 percent higher than wheat production. In addition to the highest total production per annum and the highest per-hectare yield, maize is also the single most important crop in terms of number of farmers engaged in cultivation. The vast majority of Ethiopian farmers are small-scale producers. Estimates shows about 94 percent of Ethiopian farmers rely on less than 5 hectares of land, of which 55 percent cultivate less than 2 hectares (Rashid and Negassa, 2010). Eight million smallholders were involved in maize production during 2008/09 production season, compared to 5.8 million for teff and 4.5 million for sorghum, the second and third most cultivated crops in Ethiopia (CSA, 2007). The rates of increase in maize production and its share in the total cereal output have been at 3.27% and 1.92%, respectively. Average yields have also increased from9.6 q/ha in 1961 to 22.29 q/ha in 2007, growing at an annual rate of 1.62% (Getachew *et al.*, 2010). The crop has been selected as one of the national commodity crops to satisfy the food self-sufficiency program of the country to feed the alarmingly increasing population (Demissie *et al*., 2008).

2.2. Fungi associated with maize kernels

Maize kernels are known to be attacked by various types of seed-borne pathogens of which, fungi account for 75% of reported cases of association. Fungi have also been found to cause maximum damage such as abortion, rot, necrosis, discoloration, mycotoxin contamination, and reduced germination and vigor (Shetty, 1988). Infected seeds act as media for survival of these fungi as well as their dispersal to disease free areas (Agarwal, 1981). Fungi that are associated with food grains are classified into field fungi and storage fungi mainly based on their ecological requirement for growth. According to the classification by Placinta *et al*. (1999), field fungi were considered plant pathogenic and storage fungi to be saprophytic. The first group requires grain moisture above 20% in cereals and often causes ear rot and toxin production before harvest. The most important are from the genera of *Alternaria*, *Cladosporium*, *Fusarium* and *Dreschslera* (Scudamore, 1993). *Fusarium* species are the most important field fungi of maize worldwide and are known to produce over 100 secondary metabolites that can adversely affect human and animal health (Visconti, 2001). *Fusarium verticillioides* has been found to be the most wide spread and most frequent in preharvest and stored maize in Nigeria (Essien, 2000) and Ethiopia (Tesfaye and Dawit, 2000). The major storage fungi that are associated with maize grains comprise about a dozen species of *Aspergillus* and several species of *Penicillium* (Northolt *et al*., 1995). The most important mycotoxins producing fungi after harvest also belong to these genera. *Penicilliam* are important in the temperate climates whereas *Aspergillus* predominate in the tropics (Northolt *et al*., 1995). Fungal infection can result during growing, harvesting, storage, transport and processing. The main fungal species associated with maize are *Aspergillus flavus*, *Fusarium verticillioides*, and *F. graminearum*. *Aspergillus flavus* can infect maize pre and postharvest. Although *Fusarium* species are predominantly considered as field fungi, it has been reported that *fumonisin* production can occur postharvest when storage conditions are inadequate (Marin *et al*., 2004).

2.3. Factors influencing fungal infection of maize kernels

Factors that influence the incidence of fungal infection in maize invertebrate vectors, damaged grain, inoculums load, substrate composition, fungal infection levels, prevalence of toxigenic strains and microbiological interactions. Insects vector fungi and cause damage that allows the fungi to gain access, increasing the chances of fungal contamination, especially when loose-husked maize hybrids are used (Dowd, 2003). Many researchers have found that poor harvesting practices, improper, storage and drying can lead to fungal growth and toxin proliferation (Reddy *et al.,* 2001; Bankole and Adebanjo, 2003; Ravikiran *et al.,* 2005).

High incidence of insect attack was reported to be positively correlated with fungal contamination of maize in Benin (Setamou *et al*., 1998; Fandohan *et al*., 2008). Storage pests, in particular *Sitophilus zeamais* Motschulsky, play an important role in the contamination of foods with fungi, especially those that produce toxins (Hell *et al*., 2008; Lamboni and Hell, 2009). Undoubtedly, old grain within storage or spilled grain near a storage site is common sources of insect re-infestation.

2.3.1 Abiotic factors

2.3.1.1 Moisture content

If grain moisture content is too high, even the best aeration equipment and monitoring management will not keep the grain from spoiling. All microorganisms, including molds, require moisture to survive and multiply. If the moisture content of a product going in to the store is too low, microorganisms will be unable to grow provided that the moisture in the store is also kept low.

Going into storage at the proper moisture content does not guarantee grain will remain at that initial moisture content. Grain may be rewet because of storage roof or sidewall leaks. Moisture can also enter through downspouts from a bucket elevator or through hatches that have been left open. Moisture condensation can also cause localized increases in grain moisture content. Condensation, particularly on storage roofs and sidewalls, is common when warm grain (10^{o}C or above) is cooled during cold weather (-1^{o}C or less), or when hot grain from a dryer is cooled inside a storage (Probst *et al.*, 2007; David and David, 1998). Condensation can be minimized by providing adequate exhaust vents in the cooling storage. Due to excessive humidity, multiplication of fungi particularly *Aspergillus species*, which produce dangerous toxins (Aflatoxins), will make grain unfit for human consumption

(Fandohan *et al*., 2008). In general, it is essential that all grains are below their safe moisture content before they enter the store. 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 time storages are required. The sitting and ventilation of the store are important. Condensation of moisture can cause storage problems.

2.3.1.2 Grain initial condition

After harvesting, farmers should clean all the materials used in the process of harvesting and store them properly, away from sources of contamination and insect breeding places. The same materials may be needed during the proceeding operations like, to transport cobs from the crib for threshing or to transport grains to the store. If the materials are not cleaned properly, they can easily contaminate clean grains or become source of pest infestation since at times pest infestation starts from the field.

Currently, most of the maize grown in Ethiopia is harvested by hand. Considering the need for a farmer to keep the cobs clean, to dry the cob immediately and avoid infestation of the harvested cobs, a farmer makes the following preparations: Make sure the drying place or equipment is clean and disinfected, ready to receive the cobs, remove old grain and dirt from anything that will come in contact with the good or new grain; This includes harvesting tools, carts, wheel barrows, bags and baskets, where possible, fumigate them or at least treat them with boiling water to kill insects or their eggs. This is done in order to avoid infection of new grain by insects and their eggs and organize enough labour to reap and carry the cobs to the drying place. The safe moisture content for any particular grain may vary slightly depending on the variety. Higher temperatures require lower moisture content maxima (Hayma, 2003). Often the products are dried in the field as much as possible. During pre-storage drying period, and sometimes even before harvesting, the grains may easily become infested with insects. When the grains have reached the safe moisture content, they can be stored permanently.

2.3.2 Biotic factors

Damages of grains or loss of grains vary generally and are a function of pest and insects, climate, system of harvesting, system of processing, storage, and handling. The agents causing deterioration of stored grains also include micro-organisms (fungi and bacteria), rodents and birds (Mathew, 2010). Most of the fungal pathogens of maize crop are seed-borne. During prolonged storage of grains decrease in field fungi and increase in storage fungi has been reported (Sinha, 1979). *Alternaria, Helminthosporium, Fusarium* and *cladosporium* are major fields fungi, *Aspergillus* and *Penicillium* are storage fungi. *Aspergillus flavus* and *Penicillium* species were found to be the most predominant fungi on maize kernels (Hafiz, 1986; Sauer and Tuite, 1987).

2.4 Losses of maize kernels

According to FAO (1998), food production cannot satisfy the increasing food demand unless attention is focused on reducing postharvest losses. Postharvest operations for cereal grains follow a chain of activities starting in farmers' fields and leading eventually to cereals being supplied to consumers in a form they prefer. When determining the losses that may occur in this chain it is conventional to include harvesting, drying in the field and/or on platforms, threshing and winnowing, transport to store. The losses occur mainly because of improper storage (Ishrat and Shahnaz, 2009). Fungi affect the quality of grain through increase in fatty acid, reduction in germination, mustiness and finally spoilage of grain. Survey of literature shows that a number of fungi viz., *Aspergillus* species, *Fusarium* species, *Helminthosporium* species, and *Penicillium* species, have been reported from maize seed (Anne *et al*., 2000; Mohammed *et al*., 2001; Desjardin *et al*., 2006; Tulin and Askun, 2006). Grain losses vary from one geographic location to another and from storage to storage, depending on original grain conditions, season, and associated organisms. Adequate management of insects and molds that attack and destroy harvested grain has always received less attention than pest management efforts on crops in the field. There is no justification for such behavior, as losses of grain in storage are often equal to cereal grain losses in the field.

2. 5. Management of fungal contamination of maize

2.5.1 Crop management strategies

Controlling or reducing infection and regulating the factors that increase the risk of contamination of maize in the field will go a long way in controlling fungal contamination. Management practices that reduce the incidence of fungal infection in the field include timely planting, optimal plant densities, proper plant nutrition, avoiding drought stress, controlling other plant pathogens, weeds, insect pests, and proper harvesting (Bruns, 2003). In Africa, crops are cultivated under rain fed condition, with low levels of fertilizer and practically no pesticide application. These management practices promote infection with *Aspergillus spp* in fertility stressed plants. Any action taken to interrupt the probability of silk and kernel infection will reduce fungal contamination (Diener *et al*., 1987). Preharvest measures that are efficient in reducing fungal infection rate are the same as those that will enhance yields. Crop rotation and management of crop residues also are important in controlling fungal infection in the field. Tillage practices, crop rotation, fertilizer application, weed control, late season rainfall, irrigation, wind and pest vectors all can affect the source and level of fungal inoculum, maintaining the disease cycle in maize (Hell *et al*., 2008; Diener *et al*., 1987).

2.5.2 Timely harvesting

Extended field drying of maize could result in serious grain losses during storage (Borgemeister *et al*., 1998; Kaaya *et al*., 2006), and as such harvesting immediately after physiological maturity is recommended to combat fungal infection problems. Kaaya *et al*. (2006) observed aflatoxin levels increased by about four times by the third week and more than seven times when maize harvest was delayed for four weeks. However, after early harvesting products have to be dried to safe levels to stop fungal growth. Leaving the harvested crop in the field prior to storage promotes fungal infection and insect infestation, this is a common practice in Africa often due to labour constraints, and the need to let the crop dry completely prior to harvest (Udoh *et al*., 2000).

2.5.3 Rapid drying

Moisture and temperature influence the growth of fungi in stored commodities. Fungal infection can increase 10 fold in a three day period, when field harvested maize is stored with high moisture content (Hell *et al*., 2008). The general recommendation is that harvested commodities should be dried as quickly as possible to safe moisture levels of 10 – 13 % for cereals (Probst *et al.*, 2007). Achieving this through simple sun-drying under the high humidity conditions of many parts of Africa is difficult. Even, when drying is done in the dry season, it is not completed before loading grains into stores as observed by Mestre *et al*. (2004) and products can be easily contaminated with fungi. There are several technologies to increase the efficacy of grain drying and reduce the risk of toxin contamination even under low-input conditions; these are the use of drying platforms, drying outside the field, drying on mats (Hell *et al*., 2008). Farmers should be able to determine the actual moisture content of their products.

2.5.4 Postharvest management practices

Traditional storage methods in Africa can be divided into two types, namely temporary storage that is mainly used to dry the crop and permanent storage that takes place in the field or on the farm. The latter includes containers made from plant materials (woods, bamboo, thatch) or mud placed on raised platforms and covered with thatch. The stores are constructed to prevent insect and rodent infestation and to prevent moisture from getting into the grains. It is difficult to promote new storage technologies, such as the use of metal or cement bins, to small-scale farmers due to their high cost. Many farmers nowadays store their grains in bags, especially polypropylene which are not airtight, but there is evidence that this method facilitates fungal contamination and aflatoxin development (Hell *et al*., 2000a; Udoh *et al*., 2000).

2.5.5 Disinfestations methods

Smoking is an efficient method of reducing moisture content and protecting maize against infestation by fungi. The efficacy of smoking in protecting against insect infestation was found to be high. About four to twelve percent of farmers in the various ecological zones in Nigeria used smoke to preserve their grains, and this practice was found to be correlated with lower fungal contamination in farmers' stores (Udoh *et al*., 2000). Farmers use local plant products for controlling insect infestation, past studies have looked at the use of these substances for the control of fungi mostly proving their efficacy in-vitro (Hsieh *et al*., 2001), but these products have not proven there efficiency in farmers stores. There is need to review the efficiency of the multiple products used by farmers and tested by researcher to get a complete picture about their potential in reducing toxin contamination. Use of pesticides to control fungal contaminations and their efficacy, have been reviewed by D'Mello *et al*. (1998), but their use by farmers in Africa is not always well practiced and deaths due to pesticide use have been reported. Extension workers should educate farmers on the importance of using recommended chemicals for specific crops at appropriate concentrations and within a safe delay before consumption.

2.5.6 Physical separation and hygiene

Fungi are unevenly distributed in a seed lot and may be concentrated in a very small percentage of the product (Whitaker, 2003). Sorting out of physically damaged and infected grains (known from colorations, odd shapes and size) from the intact commodity can result in 40-80% reduction in fungal infection and aflatoxins development levels (Park, 2002; Fandohan *et al*., 2005; Afolabi *et al*., 2006). The advantage of this method is that it reduces toxin concentrations to safe levels without the production of toxin degradation products or any reduction in the nutritional value of the food. This could be done manually or by using electronic sorters. Clearing the remains of previous harvests and destroying infested crop residues are basic sanitary measures that are also effective against storage deterioration. Cleaning of stores before loading in the new harvests was found to be correlated with reduced fungal contamination and aflatoxins levels (Hell *et al*., 2000b). Separating heavily damaged ears i.e. those having greater than 10% ear damage also reduces aflatoxin levels in maize (Setamou *et al*., 1998). Wild hosts, which constitute a major source of infestation for storage pests, should be removed from the vicinity of stores (Hell *et al*., 2000b).

2.5.7 Harvesting

Timing of harvest can have major consequences for the ultimate levels of fungal contamination. In general, earlier harvest results in lower infection of fungi (Jones *et al.*, 1981). While grain dries slowly in the field, moisture content remains high enough to allow continued development and toxin production by fungi that infect kernels preharvest. Physical damage to grain during harvest and transportation contributes to the potential for fungus to develop. Field shelling in a mechanical combiner subjects the kernels to direct physical contact with the moving parts of the harvesting equipment. This damage and the vulnerability of the grain to toxigenic storage fungi can be reduced by adjusting the combiner’s cylinder speed and clearance (Herum, 1987).

2.6 Postharvest management

2.6.1 Drying

After harvest, reducing grain moisture by artificial drying is a valuable tool for arresting fungal development. The objective of grain drying is to reduce moisture content to the extent that molds, both oxygenic and non-toxigenic, are not able to grow or remain physiologically active. Artificial drying can involve natural gas burners (50° to 82°C) or ambient or low-temperature drying. This process is most successful between 4° and 15°C and low relative humidity between 55% and 75% (Wilcke and Morey, 1995). Grain with significant ear rot or head scab symptoms from the field should be dried at high temperature as quickly as possible to minimize the risk of fungal development. The lower the moisture contents in storage, the lower the risk of fungal development.

2.6.2 Proper storage management

Mold development can arise in storage because of moisture variability with the grain mass or moisture migration that results from rapid grain cooling in the storage. Open or closed structures are frequently used, especially in developing countries, and their tendency to promote fungal infection problems depends on the extent to which grain moisture and temperature can be maintained at low levels. Inadequate storage facilities are major causes of mycotoxin problem in grain produced in developing countries (Hell *et al*., 2000a; Adejumo, and Raji, 2007). Storage facilities should be thoroughly cleaned before the new crop is stored, because grain residue will often harbor large populations of storage molds. Storage temperature is the most critical factor in managing growth of fungi problems in dried grain (Wilcke and Morey, 1995). Ideally, grains should be cooled after drying and maintained at 1° to 4 °C for the duration of storage. At this temperature fungal metabolism is minimal.

Temperature control is achieved by aerating the grain when outside air temperature is within the desired range and humidity is low. Aeration is essential for maintaining grain quality in storage, by controlling temperature and evaporating moisture that has migrated and condensed in the store. Because of unpredictability associated with stored grain, no matter how carefully it is dried and aerated, frequent observations are necessary to head off developing problems with mould growth. Observations should include inspection for overall temperature, crusts or mold on the grain, moisture in the bin, moldy odor, and warm spots. If any problems are detected, steps should be taken immediately to reduce the temperature, aerate the bin, break up hot spots or removed spoiled grain (Munkold, 2003).

Insect activity in stored grain promotes the development of fungi, so controlling insects will help reduce the risk of molds and mycotoxins (Klich, 2007). Insect control in stored grain requires an integrated approach, including sanitation, good control of grain moisture and temperature, frequent monitoring, and chemical treatments. Sanitation includes cleaning the grain and the empty bin to remove fines, broken kernels, and other debris that provide breeding sites and food for storage insects. The area around the storage also should be kept clean and free of vegetation (Holscher, 2000).

3. MATERIALS AND METHODS

3.1. Description of the study areas

The study was conducted in Jimma University Agriculture and Veterinary medicine, (JUCAVM) College located at 356 km Southwest of Addis Ababa at about 7^0 33’N latitude and 36^0 57’ E longitude and altitude of 1710 meter above sea level (m.a.s.l). The mean maximum and minimum temperature are 26.8^0C and 11.4^0C, respectively and the mean maximum and minimum relative humidity is 91.4% and 39.92% respectively (BPEDORS, 2000). At the time of investigation the average temperature and relative humidity of the laboratory was 24 $\pm$ 0.5 and 59 $\pm$ 0.5 respectively. In the present study, three districts namely Dedo, Omonada and Sekoru (Fig.1.) were purposively selected to represent areas of high, intermediate and low altitude maize producing agro-ecologies of Jimma zone.

Figure 1: Map showing the study sites-Sekoru, Omonada and Dedo ‘*woredas’* in Jimma zone of Oromia Regional State in Southwestern Ethiopia (2011/12)

3.2 Disease and damage assessment

Survey and data collection on the preharvest and postharvest practices of maize producing farmers were conducted with actual observation during disease assessment and using a questionnaire in the three selected districts of Jimma zone. The assessments were conducted at two stages, the first one was at harvesting time while the crop was in the field and the second was four months after storage for the same farmers. In each district, 15 farmers were randomly selected in three major maize growing *kebeles* (Peasant Associations). At each stage, maize samples were collected from each field or store, taken to the laboratory at Jimma University College of Agriculture and Veterinary Medicine to examine fungal infections and damages, followed by isolation and identification of the associated fungal genera.

3.2.1 Assessment of maize infection and damage at harvest in the field

The survey at harvesting stage was conducted in 2011/12 crop season when almost all the maize crop completed physiological maturity and ready for harvest. Maize cobs with sign of infection were diagnosed on 45 (15 per plot) randomly selected maize stalk by walking diagonally in transect across each sample field. The number of healthy and infected cob (incidence) was recorded per plot. Besides, damage by insects (weevil and stalk borer), birds, rats and others were assessed and recorded. Detailed information on the crop variety, harvesting time and method, drying; and storage time and structure etc were gathered. At the time, nine maize cobs with sign of infection were randomly sampled from the farmers' fields.

3.2.2 Assessment of fungal infection in the storage

The second assessment was conducted during the storage time, four month after the storage of maize cobs in the traditional storage structure known as '*Gombisa'* or '*Gotera'* of the same sample farmers used for the first survey. In this case, about 25 to 30 maize cobs sampled from different directions and layers of pile in '*Gombisa*' were considered for kernel infection and damage assessment. Maize kernels on each cob were diagnosed and number of damage and apparently healthy cobs per sample '*Gotera*' was recorded. The storage conditions and handling practices of each sample farmer were examined and noted.

During this assessment stage, three infected cobs were randomly sampled per *Gombisa* and placed in paper bags, transported and stored in the laboratory at JUCAVM. A total of 405 cobs (9 cobs x 5 farmers x 3 PAs x 3 districts) were collected from the storage.

In addition, questionnaires were used to collect farmer's knowledge and experiences on postharvest handling of maize, *vis-a-vis* detailed information on variety, harvesting time and method, drying materials; and storage structures/ facilities. In the three selected '*woredas*', 165 respondents (farmers) were randomly selected in nine peasant association (PA) using simple random sampling method.

3.3 Laboratory assessment of maize kernels for damage

Three of nine maize cobs were randomly picked, shelled and bulked together, and then 150 kernels were sampled and used for damage analysis such as mould infection and insect infestations. The number of infected (mouldy) and infested kernels, and healthy ones were counted and recorded. Insect attack was evaluated for damage by weevils and stalk borer for field samples while only weevil for storage samples) was examined and counted with naked-eye or assisted by hand lens. At the same time, moisture content of the sample grains was measured using calibrated moisture tester (Villeneuve la Garenne codex – 92396-France) immediately after shelling the kernels from the three cobs.

3.4. Isolation and identification of fungal moulds from maize kernels

The fungal moulds were isolated and identified to the genus level from maize kernels sampled from each field during the disease assessments at harvest and storage periods, following recommended media and standard isolation procedures.

3.4.1 Isolation on culture media

The maize kernels were plated on two types of media: Potato dextrose agar (PDA) and Czapex dox agar (CDA) (Singh *et al.*, 2003). The media were prepared by dissolving 39 gm of commercially formulated dehydrated into one liter of distilled water. The mixtures were boiled while stirring with a magnetic stirrer for 10 minutes to completely dissolve the powdered agar and then autoclaved at 121°C for 15 minutes to sterilize the media. The liquid media were maintained under aseptic condition and allowed to cool to about 50°C, then streptomycin sulphate powder (0.1gm/1) were added to suppress bacterial growth and the media were poured into sterilized petri dishes. The agar media were then allowed to cool and solidifying before being used for plating the maize kernels.

Maize kernels from each sample lot were surface sterilized with 5% sodium-hypochlorite solution for one min and rinsed twice with sterile distilled water and dried in a laminar flow cabinet, then aseptically plated on the solidified media (3 plates for each medium). The plates were incubated under alternating periods of 12 h darkness and 12 h of daylight at 25 ^{o}C+2 for seven days (Singh *et al.*, 2003).

3.4.2. Purification and identification of fungi

The colonies emerged from each plated kernels were purified and sub-cultured on the recommended media, namely Czapex dox agar (CDA) for *Aspergillus* and *Penicillium*, and PDA for *Fusarium* and others (Nelson *et al.*, 1983; Pitt and Hocking, 2006). The plates were incubated at 25^{o}C under similar conditions for 10 days. The mould group growing out of the plated kernels was provisionally identified to the genus level first based on colony characteristics (mainly color on both sides of the plates and shape) and morphological appearance of conidiophores and conidial appearance under the microscope (400x) in reference to Marasas *et al*., (2001) and Pitt *et al*. (2004).

The isolation frequency (Fq), relative density (Rd) (Marasas et *al*., 2001) and the incidence of genera/ species isolated were calculated as follows:

$$\text{Frequency (\%)} = \frac{\text{Number of samples in which genus/species occurred}}{\text{Total number of samples}} \times 100$$

$$\text{Relative density (\%)} = \frac{\text{Number of isolates of genus/species}}{\text{Total number of fungi/ genus isolates}} \times 100$$

$$\text{Incidence (\%)} = \frac{\text{Number of kernels in which genus/ species occurred}}{\text{Total number of kernels}} \times 100$$

3.5. Kernels germination test

For this particular test 50 maize kernels per sample were kept in Petri-dishes (9 cm) lined with filter paper (Whatman No.1) moistened with distilled water and incubated on clean laboratory bench at room temperature (25°C) for seven days. The germinated seeds were visually examined for appearance of radicle and/or plumule and the percent germination was computed (Ogendo *et al*., 2004) as follows:

$$\text{Germination (\%)} = \frac{\text{Number of germination kernel}}{\text{Number of kernels plated}} \times 100$$

3.6. Insect damage assessment

Insect damage was assessed by count method. One hundred seeds were randomly taken from each maize sample and the number of insect damaged and un-damaged (weevils and stalk borer for field samples while only weevil for storage samples) was examined and counted assisted by hand lens. The percentage of insect damaged seed was then calculated (Wambugu *et al*., 2009).

$$\text{Insect Damaged kernels (\%)} = \frac{\text{Number of damaged kernels}}{\text{Total number of kernels}} \times 100$$

3.7 Statistical analysis

The Statistical Package for the Social Sciences (SPSS) Version 16 was used to calculate the means and standard errors. Data on fungal invasion, insect damage kernels and stalk borer damaged kernels were analyzed using descriptive statistics. Frequency and percentage values of variables were also computed to observe their distribution. Percentage data were square root transformed prior to statistical to stabilize the response variance to the normal distribution.

4. RESULTS

4.1. Fungal infection symptoms on maize kernels at harvest in the field and storage

Almost all of the respondents pointed out that there was a common maize cobs disease that locally so call it *'fakata'*. Some of the farmers collect maize cobs invaded by mould that they call it *'fakata'* separately and sell it for the purpose of locally processed alcohol, though it can bring health problem to humans.

During this assessment in the farmers' field, it has been recorded the symptom of '*fakata*' (Plate.1 a) almost in all farms and based on the opinion of the respondents; it cause huge loss to maize as compared to other diseases (Plate.1 b). From assessed districts, farms' fields in Sekoru, which is located in the lowland agro-ecology, were observed to be well managed as compared to those in Omonada and Dedo. In general, maize cobs which were initially damaged by birds in the field frequently showed mould invasion than those without bird damage. Moreover, the invasion of mould starts always from maize kernels damaged by weevil, stalk borer and birds as observed in the fields (plate 2a and b).

Plate 1: Maize cobs with symptoms and signs of moulds '*fakata*' (a); maize cobs infected by mould '*fakata*' sorted out before store (b); cobs infested by weevil in the field (c); maize cobs damaged by bird and followed by mould growth (d) and stalk borer collected at survey in the field.

Plate 2: Fungal mould deterioration of maize kernels (after shelling) from cob samples sampled in the field and in local storage structure (*Gombisa*) in Jimma zone, 2011-2012. (a) Damaged maize kernels by stalk borer, (b, c) showing signs of mould infection and (d) healthy maize kernels.

Some of the assessed fields were weed infested and the maize cobs sampled from thereof were more heavily colonized by mould and maize stalk borer. Moreover, maize cobs from those poorly managed farms were small and the kernels on the cobs were scattered (plate 3 A, B, C and D). Maize is prone to fungal contamination, particularly when produce comes into contact with soil during harvesting, threshing, and drying (Plate 3 E, F and G). Contamination can also occur when cobs are in storage due to pest infestation and poor conditions that lead to accelerated growth rates of fungi.

Plate 3: Some poorly managed maize farms showing weed infestation and poor harvesting practices in the fields. Sample (B) was sampled from farm (A) in the field and Sample (D) was sampled from farm (C), and drying practices pre-storage (E, F and G) in the field

4.2. Incidence and severity of fungal infection at harvest in the field

4.2.1 Mould incidence on maize cobs in the field

A total of forty-five maize stalk with cobs were assessed for each forty-five farmer's farm. Based on means of incidence, the highest mean of incidence was 29.60% which was recorded from farms located in Chalte Peasant Association (highland agro-ecology) and the lowest mean of incidence was (0.0%) which was recorded from farms located at Unkure Peasant Association (lowland agro-ecology) (Appendix table 5). When peasant association within each districts were compared in terms of mould incidence on maize cobs in the field, there was significant difference ($p<0.05$) among PAs from Omonada districts (intermediate) while there was no significant difference for PAs from Sekoru (Lowland) and Dedo (Highland). Accordingly, the highest incidence was observed in Nadasokote peasants' association (22.70%) (Table 1) where most maize fields have high planting density and rotated with crops share common disease. The management practice in this area was different from farm to farm, as it has been observed during field assessment. There were farms dominated by weed and not weeded while some farmers weeded only once and this can make favorable environment for both insect pest and disease to damage the maize. Most of maize farms from Nadasokote area were used to grow maize with irrigation year after year. During maize cob harvesting, in all the studied districts, farmers leave severely infected maize cobs right in the farm, which could be serving as source of inoculums for next season. In addition, there was unseasonal rainfall during the assessment and as a result harvesting time of the crop was delayed in all the districts.

Table 1: Mean incidence of mould (%) on maize at harvest from different agro-ecologies of Jimma zone (2011-2012)

District and its agro-ecology	Peasant Associations	Mean± SD	P value
Sekoru (lowland)	Unkure	12.8±9.9	0.327^{ns}
	Walmera	17.1±5.6	
	Bore	16.3±8.8	
Omonada (intermediate)	Nadasokote	$22.7±5.7^{a}$	0.011^{*}
	Bisogombo	$19.9±5.7^{a}$	
	Nadachala	$16.6±3.9^{b}$	
Dedo (highland)	Kata	19.1±5.0	0.412^{ns}
	Chalte	21.5±5.2	
	Belo	19.7±5.1	

**= significant, ns= non-significant, means within a column followed by the same letter are not significantly different at p<0.05*

Of the three studied *woredas* (districts), the highest mean incidence of mould was registered in Dedo (20.10%) and Omonada (19.70%), from highland and intermediate agro-ecology, respectively while the lowest mean incidence of mould recorded in Sekoru (14.90%), representing the lowland agro-ecology (Table 2).

Table 2: Mean mould incidence (%) on maize in Sekoru, Omonada and Dedo districts of Jimma zone (2011-2012)

Agro-ecology	Districts	Mean±	SD	p value
Lowland	Sekoru	14.9	$±5.8^{b}$	0.001^{**}
Intermediate	Omonada	19.7	$±3.5^{a}$	
Highland	Dedo	20.1	$±3.7^{a}$	
	Overall means	18.24	±5.1	

***= highly significant, means within a column followed by the same letter are not significantly different at p > 0.05*

4.2.2 Severity of mould infection and damage on maize kernels at harvest in the fields

The mean percentage of kernel maize sample from the field infected with or damaged by mould, weevil and stalk borer was 28.90%, 19.30% and 23.30% (Table 3), respectively. For freshly harvested maize samples in the field, mould infected kernels was the maximum which ranged from 16.0 to 50.4% and followed by kernels damaged by weevil and stalk borer that ranged from 8.8 to 53.2% and 18.0 to 35.7%, respectively (Table 3). For freshly harvested maize samples, maize kernels infected with mould and damaged by weevil were recorded in Omonada district (36.9% and 29.7%) districts, respectively and the mean percentage stalk borer damaged kernels recorded in Sekoru (25.8%) district was higher as compared to Omonada (21.5%) and Dedo (22.5%) districts (Table 3). Accordingly, the maximum loss due to mould invasion was recorded in Omonada district (36.9%), followed by Sekoru district (30.3%) and the least was recorded in Dedo district (19.9%) (Table 3), respectively.

Table 3: Percent maize kernels infected with mould and damaged by weevil and stalk borer in the field

Districts	Peasant Association	Mould infected kernels (%)	Weevil damaged kernels (%)	Stalk borer damaged kernel (%)
Sekoru	Unkure	37.4±13.3	25.5±16.2	35.7±13.6
	Walmera	29.2±8.7	13.6±5.8	18.0±3.7
	Bore	23.8±12.6	17.2±7.8	23.8±5.1
	Means	30.3± 11.5	18.8±9.9	25.8±7.5
Omonada	Nadasokote	50.4±9.5	53.2±6.3	20.6±11.1
	Bisogombo	39.1±5.1	27.1±9.4	20.5±2.2
	Nadachala	21.3±4.8	8.8±2.4	23.3±1.9
	Means	36.9±6.5	29.7±6	21.5±5.1
Dedo	Keta	16.0±3.6	8.9±2.3	22.8±2.6
	Chalte	26.9±4.5	10.3±5.1	20.8±4.1
	Belo	16.7±3.4	9.1±3.9	23.8±4.9
	Means	19.9±3.8	9.4±3.8	22.5±3.9
	Overall mean±SE	28.9±2.9	19.3±3.1	23.3±2.1

The highest damaged caused by birds on maize kernels was recorded in Bore (6.71%) peasant association of Sekoru districts. While the lowest was recorded in Nadachala (0.75%) peasant association of Omonada district (Fig.2).

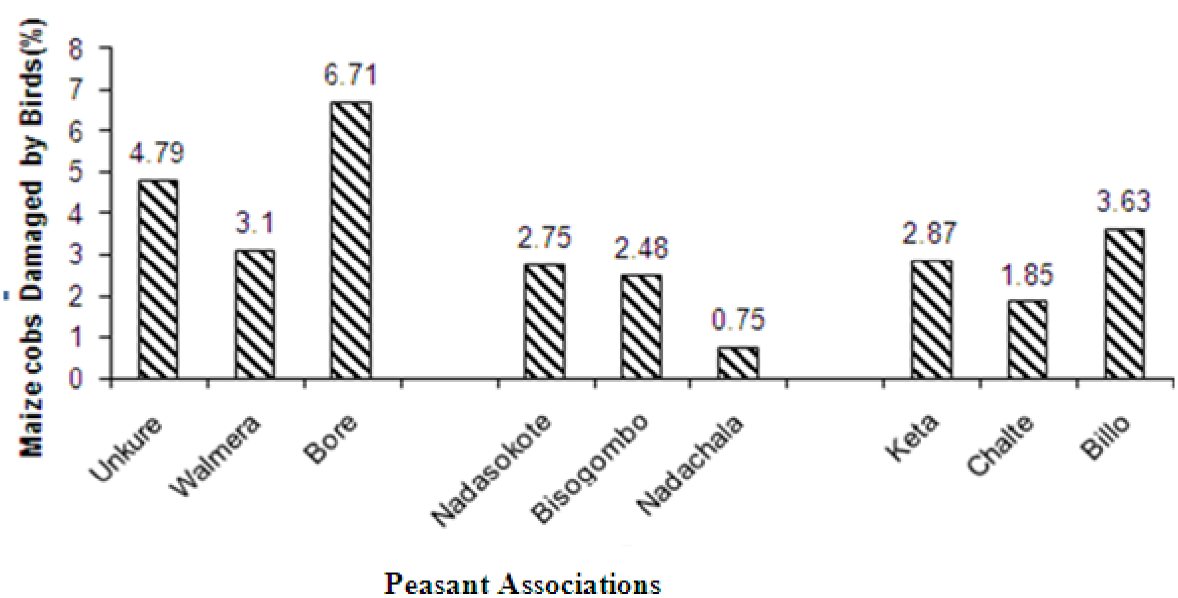

Figure 2: Percent maize cobs damaged by birds in the field

4.3. Severity of fungal infection of maize in traditional storage

4.3.1 Severity of mould infection on maize kernels (grain damage) in the storages

Overall mean percentage kernel infected with mould and damaged by insect pests (weevil) from samples of maize cobs collected from the storage was 37.80% and 20.90% (Table 4), respectively. The proportion of mould infected kernels was the maximum, which ranged from 29.80 to 47.00 %, followed by kernels damaged by weevil that ranged from 10.60 to 28.90, respectively.

Based on means of percentage of mould infected kernels, in each districts almost the severity was the same, at Dedo (38.6%) and Omonada (38.5%) and in Sekoru (36.5%) in sample collected from storage, where as weevil damaged maize kernel was higher in maize sample collected from Omonada (24.7%), Dedo (19.3%) and Sekoru (18.9%) districts.

Table 4: Percent maize kernels infected with mould and damaged by weevil during storage in sample Peasant Association in three districts of Jimma in 2011/12.

Districts	Peasant Association	Mould infected kernels (%)	Weevil damaged kernels (%)
Sekoru	Unkure	37.6±4.8	23.4±4.7
	Walmera	38.9±5.5	18.6±3.5
	Bore	32.9±3.5	14.6±3.4
	Means	36.5±4.6	18.9±3.9
Omonada	Nadasokote	29.8±4.2	19.7±7.0
	Bisogombo	47.0±7.1	25.5±8.9
	Nadachala	38.7±6.8	28.9±10.4
	Means	38.5±6.1	24.7±8.8
Dedo	Keta	39.7±7.3	10.6±3.4
	Chalte	43.4±7.1	26.8±8.1
	Belo	32.7±7.5	20.4±7.3
	Means	38.6±7.3	19.3±6.3
	Over all mean±SE	37.8±2.0	20.9±2.2

4.4 Moisture content of maize kernels from cobs sampled on the stalk in the field and storage

The mean moisture content of maize kernels from storage structures during the survey was 11.57%, ranged from 10.23 to 13.56% while the mean moisture content of fresh maize sample by the time of sampling was 15.74%, and ranged between 14.50 to 18.80% (Table 5).

Table 5: Moisture content of maize grains (%) sampled at harvest in the field and storage in three districts of Jimma Zone 2011/2012

		Maize kernels in the field at harvest	Maize kernel in storage
Districts	Peasant Association	Means±SE	Means±SE
	Unkure	14.76±0.39	10.89±0.22
Sekoru	Walmera	15.18±0.26	10.76±0.31
	Bore	14.50±0.14	10.92±0.18
	Nadasokote	15.60±0.48	13.56±0.39
Omonada	Bisogombo	15.54±0.21	11.76±0.42
	Nadachala	15.11±0.26	11.58±0.47
	Keta	16.16 ±0.53	11.84±0.34
Dedo	Chalte	16.04±0.22	10.23±0.25
	Belo	18.80±0.65	11.25±0.35
	Means	15.74±0.14	11.57±0.11

4.5 Germination test of Kernels

The germination percentage of maize kernels sampled from storage ranged from 32.4 to 64.6% and 54.2 to 89.2% for sample collected at harvest (in the fields) (Fig. 3 and 4). It indicates that the poorest viability of the maize kernels.

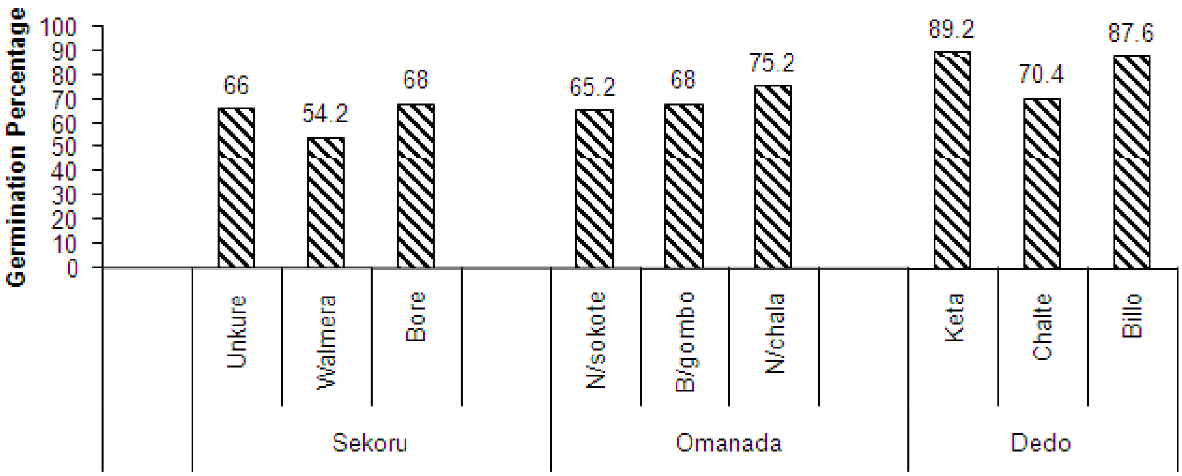

Figure 3: Germination percentage of maize kernels sampled from the field (at harvest) in Peasant Association, *woredas* and Agro-ecology of Jimma zone in 2011/12

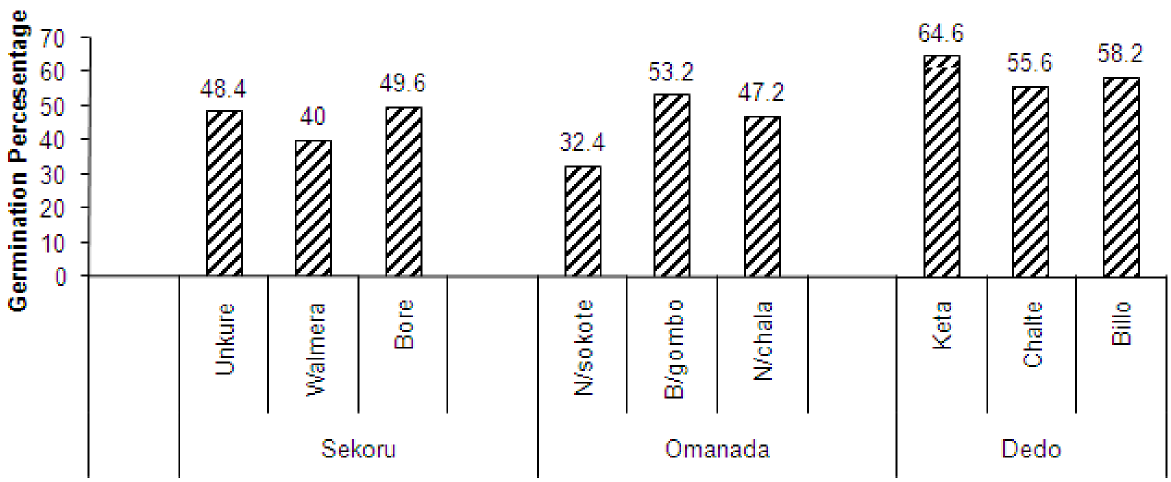

Figure 4: Germination percentage of maize kernels sampled from storage in Peasant Association, *woredas* and Agro-ecology of Jimma zone in 2011/12

4.6 Storage structures and postharvest practices affecting fungal infection of maize

The storage structures observed in the study areas were mostly roofed cribs constructed from woven twigs and bamboos standing on raised bed and some are plasted with cow dung or mud and the roofs made from sticks and then covered with grasses that appeared as small huts called '*Gombisa*' or *Gotera*' from forests (Plate:4). The storage structures were mostly arranged around the houses and a farmer can have one or more '*Gombisa'* depending on the amount of maize harvested. There was also a storage which was located within animal fence that increase mould development and facilitate the contamination of maize kernels. The storage practices were usually unshelled maize and maize together with other crops like sorghum; and teff. It was observed that maize stalk and/or teff straw were put beneath before piling the harvested maize cobs in the '*Gombisa*' that may facilitate mould development in the stored maize by serving as source of inoculum.

Many of the farmers in the study area do not sort out the infected maize cobs rather they store it with uninfected cobs. Some of the farmers sort the maize cob that appeared discolored or moldy at the household, but the discarded maize could still enter the food chain as animal feed. Other farmers reported mixing moldy maize with fresh maize to decrease the level of mold consumed. Others reported consuming moldy maize they produced as they knew it was safe, but they claimed they would never consume moldy maize from stores as that was unsafe.

Plate 4: Maize storage practices of farmers in different districts of Jimma zone

4.7. Survey Results

4.7.1. Maize harvesting practices

All the assessed farmers use variety of BH660 that released from the Bako Research Center and distributed by Agriculture Office of the districts. Farmers usually leave their maize in the field upon maturity so as it may dry, but the harvest season often overlap with the second rains and the result is often increased mould infection and even rotting of the cobs. Maize harvesting in the study area started in October and ended at the end of December and/or the beginning of January. All of the respondent farmers in the study area carry out harvesting by removing the maize cob from the standing stalk. During the assessment of the present study, harvesting maize was delayed due to the unseasonal rainfall and the main concern given to harvesting of tef than maize in order to prevent losses of tef due to shattering by rainfall. Hence, maize was exposed to rain during the harvesting season of 2011/12. Those farmers that produce maize in large quantity allow further drying in the sun before storage. All farmers responded that, they predict harvesting time of maize by checking the dryness leaf of the crops.

4. 7.2. Pre-storage practices

In the surveyed area, 82% of the farmers directly stored after harvest without sorting out the infected cobs from the healthy ones. This is merely due to fear of theft and problem of wild animals and only in some cases shortage of human resource to do job. Only some farmers (18%) pre-store their maize before they transfer to the permanent storage structure. They pre-store to make ready the storage, some of them pre-store maize cobs in the farm, and some of them pre-store at home on bare ground.

Duration of maize storage depends on the production capacity of farmers and it varies among the agro-ecological zones. In low and mid altitude areas maize was stored for about five to seven months because they cultivate maize on large size of land and use improved seeds year after year that can give better yield per hectare, but in case of high altitude areas the storage period is very short due to the limited cultivation of maize in those areas and low area coverage.

Most of the storage structures were located nearby homestead in the field. Almost all farmers use storage, which they construct from wood materials though some of the farmers store maize cobs under the roof for seed purpose. All farmers in the study areas keep their maize in storage with the husk. Farmers, especially those in highland areas, also select cobs for good husk cover before storing them for seed. It was learned from the assessment that, at times, maize cobs were stored with other commodities that could share common diseases. In this respect, 37% of the storages the study area maize was stored were with other commodities like sorghum, tef and stalk of maize.

4. 7.3. Storage problem

More than 85% of the farmers criticized about storage problems. Farmers noticed primarily insects, mould and rats at the beginning of storage. As the storage period of the maize cobs increase the number of farmers that complained about insects damaging their stored maize increased. In low altitude areas, the farmers complained about weevil infestation that start in the field and continues up to storage. Farmers also reported fungal disease as the second important storage problems that change the color of kernels. The problem of fungal contamination was higher specifically in stores wherein maize cobs are stored without sorting healthy ones from infected

Many farmers in the surveyed area did not remove maize that appeared discolored or moldy at the household level, and the discarded maize could still enter the food chain as animal feed. Other farmers reported mixing moldy maize with fresh maize to decrease the level of mold consumed. Others reported consuming moldy maize they produced, as they knew it was safe, but they claimed they would never consume moldy maize from stores as that was unsafe (13%). The farmers' response to storage problems varied. Corrective measures adopted by most farmers (86%) in the study area for the control of storage pests are mainly application of chemical pesticides like Actellic. Few farmers (14%) also apply chemical pesticides such as DDT and Malathion as a corrective measure following the examination for the presence of insect pests. Farmers also used traditional storage protectants that are prepared from plant materials like *green leaf* of *Chenopodium* and *Endod* (*Phytolacca dodecandra*); powder of *Abayi* (*Maesa lanceolata*) and *Shinfa* (*Lepidium atvuim*). Unknown amount of ground seed or leaf of the traditional pesticide is mixed in a litter of water and sprinkled once at the beginning of the storage, on cobs ready to be stored in *Gotera*. Though farmers pre-treatment their maize cobs before storage with the intent to control insect infestation, since weevil infestation begins right in the farm and the weevil veil itself in the maize grain, traditional pesticides may not be effective in controlling storage insect infestation.

4. 8 Mycological analyses

4.8.1. Isolation and identification of fungi

A total of 1462 fungal isolates grouped in five genera were recovered from maize cob samples collected from three selected maize producing districts that represent the three agro-ecologies of Jimma zone. The mycological investigation on maize samples revealed that *Fusarium* and *Penicillium* were the most frequent genera in maize samples at harvest while *Aspergillus*, *Fusarium*, and *Penicillium* were the most dominant genera in maize samples collected from the storage facilities (Figure 5 and 6), respectively. Other genera of fungi recovered were *Drechslera* and *Cladosporium*.

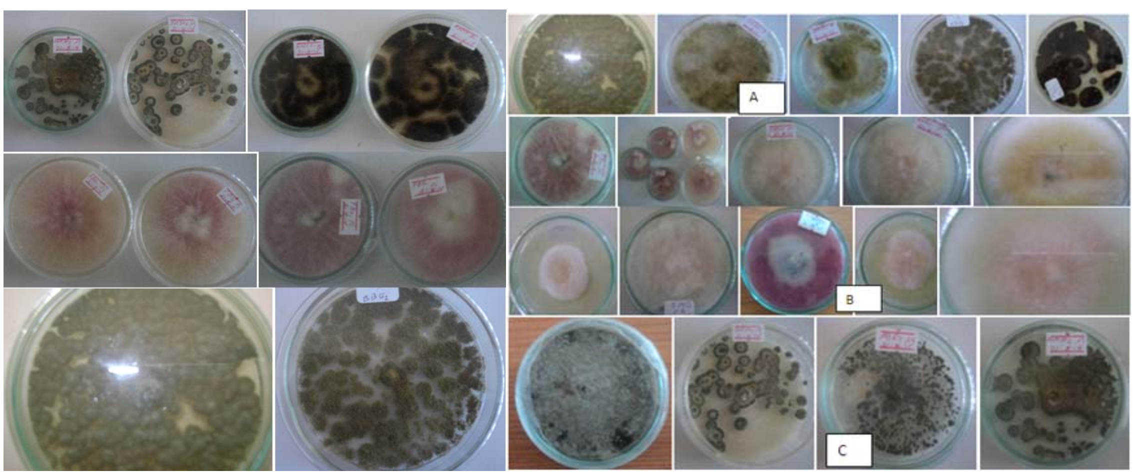

Plate 5: Pure culture of different fungal species from maize kernels: (A) *Aspergillus* species, (B) *Fusarium* species and (C) *Penicillium* species

Fusarium and *Penicillium* were the most predominant fungal genera isolated from the samples collected at harvest in the field. Whereas *Aspergillus* was the least recovered as compared to samples collected from field at harvest. *Aspergillus* was isolated from samples of each district, but the fungus was not recovered from some peasant associations (like Nadasokote and Nadachala of Omonada districts and Billo of Dedo). But in maize samples collected from Sekoru district which represent the lowland agro- ecology of the zone, *Aspergillus* was recovered from each peasant association with highest recovery being in samples from Unkure (Fig. 5).

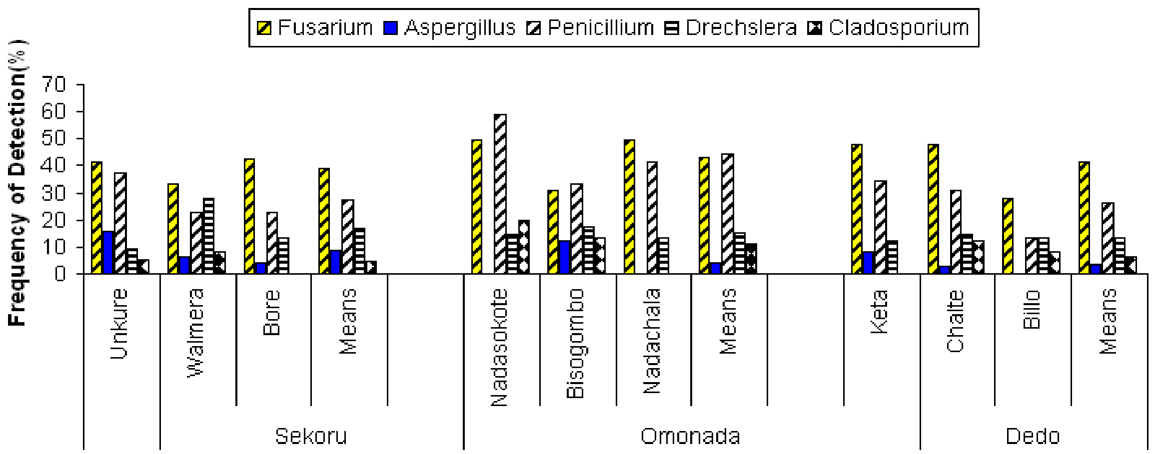

Figure 5: Distribution of different fungi recovered from maize kernels collected at harvest in the field in three districts of Jimma Zone in 2011/2012

Aspergillus was the predominant fungal genera isolated and followed by *Fusarium* for samples collected from the storage. Whereas, *Cladosporium* was the least recovered as compared to samples collected from the storage (Fig. 6).

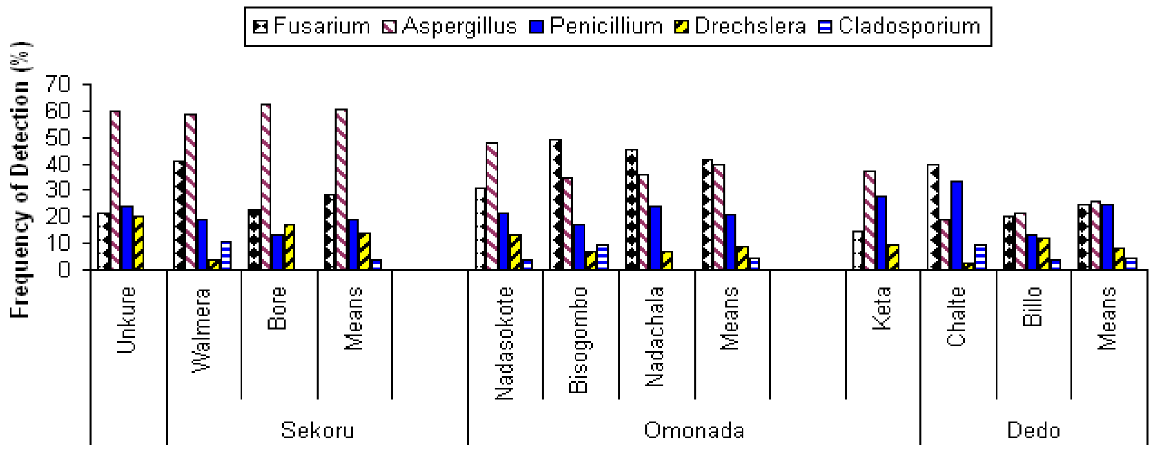

Figure 6: Distribution of different Fungi isolated from maize kernels samples collected from storage of three districts of Jimma Zone in 2011/2012

The most frequently detected fungal genera in Sekoru samples were *Fusarium* (86.7%) and *Penicillium* (86.7%). *Fusarium* predominated the total mycobiota in freshly harvested maize samples. The highest frequency of occurrence and fungal load were recorded in samples collected from Omonada and Dedo and *Fusarium* was detected in 100% of freshly harvested maize samples collected from those districts. On the other hand, *Penicillium* was detected in 100% of freshly harvested maize samples collected from Omonada district. *Aspergillus* was recovered from freshly harvested maize samples with low frequency in each district, Sekoru (33.3%), Dedo (26.6%) and Omonada (13.3%). *Drechslera* and *Cladosporium* were also encountered in maize samples collected from fields of different districts of Jimma zone. Moreover, there was also a variation among districts in terms of the occurrence of these fungi (Table 6 and 7).

The highest frequency of occurrence and fungal load were recorded in samples collected from Sekoru and Omonada. *Aspergillus* was the most frequently observed fungi species for samples collected from storage facilities in Sekoru (100%) and Omonada (100%). Of the two, *Aspergillus* species pre-dominated the total mycobiota of samples collected from storage facilities and followed by *Fusarium* and *Penicillium* species.

From total of the isolates of fungi, samples collected from Omonada accounted for about 38.50% whereas Sekoru and Dedo had 31.7% and 29.8% respectively in maize samples collected from the field at harvest. However , in case of samples from the storage Sekoru accounted for 36.30% which is the highest and 33.60% and 30.10% were isolated from samples of Omonada and Dedo respectively (Table 6 and 7).

Table 6: Occurrence of fungi in maize grain samples collected from fields of three woredas of Jimma zone, 2011/12 crop season

Fungi isolated	Sekoru			Omonada			Dedo			Overall means		
	Fq (%)	Rd (%)	In (%)	Fq (%)	Rd (%)	In (%)	Fq (%)	Rd (%)	In (%)	Fq (%)	Rd (%)	In (%)
Fusarium spp.	86.7	40.4	39.1	100	36.6	43.1	100	45.4	41.3	95.6	40.8	41.2
Aspergillus spp.	33.3	9.2	8.9	13.3	3.4	4	26.6	3.9	3.6	24.4	5.6	5.5
Penicillium spp.	86.7	28.4	27.7	100	37.7	44.4	73.3	28.8	26.3	86.7	31.6	32.8
Drechslera spp.	66.7	17.4	16.9	53.3	12.8	15.1	86.7	14.6	13.3	68.9	14.9	15.1
Cladosporium spp.	26.7	4.6	4.4	46.7	9.4	11.1	40	7.3	6.7	38.8	7.1	7.4
Total isolates	**218**			**265**			**205**			**688**		
Total samples	**15**			**15**			**15**			**45**		

Fq = frequency; Rd = relative density; In = incidence

Table 7: Occurrence of fungi in maize grain samples collected from storages in three woredas of Jimma zone, 2011/12 crop season

Fungi isolated	Sekoru			Omonada			Dedo			Overall means		
	Fq (%)	Rd (%)	In (%)	Fq (%)	Rd (%)	In (%)	Fq (%)	Rd (%)	In (%)	Fq (%)	Rd (%)	In (%)
Fusarium spp.	93.3	22.8	28.4	93.3	36.2	41.8	86.6	37.3	24.9	91.1	32.1	31.7
Aspergillus spp.	100	48.4	60.5	100	34.2	39.6	93.3	25.5	25.8	97.8	36.0	42.1
Penicillium spp.	80	14.9	18.7	73.3	18.1	20.9	100	15.5	24.9	84.4	16.2	21.5
Drechslera spp.	60	11.1	13.8	46.7	7.7	8.9	53.3	18.9	8.1	53.3	12.6	10.3
Cladosporium spp.	20	2.8	9.3	33.3	3.8	6.7	26.7	3	9.3	26.7	3.2	8.4
Total isolates	**281**			**260**			**233**			**774**		
Total samples	**15**			**15**			**15**			**45**		

Fq=frequency; Rd=relative density; In= incidence

The frequency of *Aspergilus* species in sample collected at harvest is least as compared to other fungal species where as the dominant in sample collected from storage facilities. The occurrence of fungal diseases was different in different agro-ecology of the zone. Based on means of frequency, the most dominant fungal genera were *Aspergilus*, *Fusarium* and *Penicillium* with the frequency of 97.8, 91.1 and 84.4, respectively for samples collected from storage facilities and, *Fusarium* and *Penicillium* with the frequency of 95.6 and 86.7, respectively for maize samples at harvest in the field. Moreover, fungal genera such as *Drechslera* and *Cladosporium* were also isolated (Fig. 7 and 8). The species of *Fusarium* and *Penicilium* were the predominant mycoflora with relative density of 40.80% and 31.60%, respectively for samples collected from the fields at harvest while, *Aspergilus* and *Fusarium* were the predominant mycoflora with 36.0% and 32.10% relative density, respectively for samples collected from the storage facilities. The species of *Drechslera (*14.9%) and *cladosporium* (7.1%) were isolated from freshly harvested maize samples and the same genera showed (12.6%) and (3.2%) occurrence from maize samples isolated from maize sample collected from storage in low relative density (Table 6 and 7).

Based on incidence, *Fusarium* species was the predominant with the highest incidence in being Omonada (43.1%) and the lowest in Sekoru (39.1%) for maize samples taken from the field. Next to *Penicillium, Fusarium* species was the most prevalent recorded, with incidence of 43.1% in Omonada and lowest incidence of 26.3% in Dedo (Table 6).

Based on overall means of fungal genera isolated, *Fusarium* species was the predominant genera recovered in freshly harvested maize samples with frequency of (95.6%) followed by *Penicillium* species (86.7%). *Drechslera* species took the third place with frequency of 68.9%. The least frequently recovered fungal genera from freshly harvested maize samples were *Aspergillus* and from this species, *Aspergillus flavus* might be the dominant one in freshly harvested maize samples. The species of *Drechslera* (68.9 and 53.3 %) and *Cladosporium* (38.8 and 26.7%) were the other most important fungal genera recorded in samples collected from freshly harvested maize and storage facilities respectively with high relative density (Table 6 and 7).

Within districts, the incidence of *Fusarium* species was highest in Dedo and Sekoru and *Penicillium* was highest in Omonada while *Aspergillus* species was highest in Sekoru and lowest in Dedo. The incidence of *Drechslera* species highest in Sekoru and it was the same in Omonada and Dedo whereas *Cladosporium* species was caused higher incidence in Omonada and followed by Dedo and the least in Sekoru. Overall among all the fungal genera isolated, *Fusarium* was the most dominant followed by *Penicillium* and *Drecheslera* species and the least was *Aspergillus* species in maize samples harvested from the field (Table 6).

On the other hand, for samples collected from the storage facilities, the incidence of *Aspergillus* species was highest in Sekoru and least in Dedo. While *Fusarium* species was highest in Omonada and least in Dedo. The incidence from *Penicillium* species, however, was highest in Dedo and followed by Omonada. The least fungal genera isolated was *Cladosporium*. With respect to isolates from the samples collected from the storage facilities, *Aspergillus* was the predominant species isolated followed by *Fusarium*, *Penicillium* and *Drecheslera* while the least was *Cladosporium* species (Table 7).

Fusarium species was recorded as the highest incidence across all the '*woredas'* followed by *Penicillium* species whereas the lowest incidence was recorded by species of *Aspergillus*. Other species of fungi were also recorded; *Drecheslera* and *Cladosporium* for samples collected at harvest in the field (Table 6). In case of samples collected from the storage, the highest incidence was recorded by *Aspergillus* species as compared to other fungal species across the selected districts of each agro-ecology. Specifically in Sekoru district the incidence was highest followed by Fusarium species. In Dedo district the level of incidence from the different isolated fungi was almost equal (Table 7).

5. DISCUSSION

In this study, fungal diseases of maize occur in the maize growing areas of Jimma southwestern Ethiopia with increasing order of importance varying in the extent of invasion among and within maize farms and distribution. These variations were mainly due to non-seasonal rain at the time of harvest, storing of infected and health cobs together, and poor handling of storage, infestation of insect pests and rodents predisposed grains to fungal infection. In general, poor harvesting and postharvest practices by farmers were the major factor for mould infection, deteriorations and loss of the crop in the region.

Farmers in the districts leave their maize in the field beyond physiological maturity to allow it to dry in order to facilitate direct storage into the store without sun drying after harvest. Most of the maize in the farms was over-dried as harvesting time was delayed and there was unseasonal rain for more than two weeks at the time of survey and sample collection. Almost in all districts the harvesting time of maize was delayed, which increased the probability of maize cobs to be infected by field fungi and damaged by insect pests. If there is rain while the cobs are in the field, kernels may be subjected to infection by fungi (Ochor *et al*., 1987). Similarly Alakonya *et al*. (2008) have reported that there was an increase in rotting whenever harvesting was delayed. As maize is left in the farm, favorable conditions for ear rot proliferation make the fungi to spread and cover the kernels with its mycelia. It has been reported that late planting of maize with harvesting in wet conditions favors disease caused by *Fusarium* species (Hell *et al.,* 2003; Abarca *et al*., 2001; Kaaya and Kyamuhangire, 2006) and the prevalence of these fungi are considerably increased in the seasons with wet weather (Al-Heeti, 1987). Under high humidity, initially dry seed develops water content conducive to mould growth on the kernels (Peter and Ramon, 2007). Delayed harvesting may exacerbate the problem of mould growth on the cobs, harvesting at physiological maturity when moisture content is high increases the risk of mould contamination during postharvest handling (Kedera *et al.*, 1994 and Nagler *et al.*, 1988).

The results of this study revealed that mould incidence on maize cobs in the field, there was significant difference ($p<0.05$) among Peasant Associations (PAs) from Omonada districts (intermediate) while non-significant difference among PAs from Sekoru and Dedo. Maize cobs collected during this study were of poor quality often displaying severe insect damage and a range of discolorations. Cobs from districts of Sekoru, Omonada and Dedo, with 30.3%, 36.9%, and 19.9% of mould infected, respectively, and 18.8%, 29.7% and 9.4% of weevil damage and 25%, 21.5% and 22.5% of stalk borer damage, respectively for maize sample in the field, were severely deteriorated and often had profound discoloration with at times prominent signs of fungal infection, this indicates that insect pest, birds and other wild animals play great role for easy invasion of maize kernels by mould. Frequent observation of weevil was recorded in maize cobs during storage that the infestation was started in the farm.

In the case of maize sample collected from storage for the same districts, Sekoru, Omonada and Dedo 36.5%, 38.5% and 38.6% of mould infected, 18.9%, 24.7% and 19.3% of weevil damaged respectively. Similarly Kerstin *et al*. (2010) reported 10 to 12% loss of maize stored in traditional storage containers similar to *Gombisa* due to insect pests. Per household, average actual loss was reported about 12 % of the average total grain produce (Abebe and Bekele, 2006).

During harvesting, some farmers directly put the shelled cobs in the sack and transport to the home. Nevertheless, in the case of Omonada and Dedo '*woredas'*, the farmers remove the shell and put together with infected cobs there in the field for one or more days. Respondents from Sekoru said that, they used certified seed year after a year but some farmers from the assessed '*woredas'* used previous seed, which stored together with infected seed because of the high price of the seed. In addition, in all surveyed areas people sell infected maize kernels with cobs that could be the means for distribution and spread of fungal diseases, weevils; and the infected maize cobs were thrown in the field, which serve as sources of inoculums for infection. However, in the case of poor farmers they mixed it with other grain mill it and consume until that they store infected cobs with healthy one. Since the product was low, they store healthy cobs together with infected cobs in the storage that can serve as source of inoculums that can be disseminated by insect pest and other. During the second phase survey farmers store maize cobs with different crops with their straw (tef, sorghum and maize stalk) that can be share common disease and it can be source of inoculums.

Maize was typically stored either inside the houses or in various structures outside the homestead or in the field. During the survey it was observed that structures used for maize storage by small-scale farmers similar considerably across locations and nearly all of them were in a poor state of maintenance and hygiene except those producer that produce maize in large amount. The most common storage structures were open-air cribs made of tree poles and covered with a thatch roof allowing access to insects and rodents. Hence, these structures were prone to grain spoilage caused by insect and microbial contamination. It has been reported previously that storage structures differ in their ability to protect grains from fungal and insect infestation. Hell *et al*. (2000b) found that some types of farmers' storage structures also provided conditions that were more conducive to fungal infection in West Africa.

Percentage of maize kernels with mould infection was highest for each district than weevil and stalk borer damage. This might have resulted from accelerated insect-mediated spoilage of maize grains in storage. According to Cardwell *et al*. (2000) and Schulthess *et al*. (2002), insects play a big role in the vectoring of fungal spores and also provide entry holes to fungal organisms through their tunneling activity, both prior to and after harvest. Setamou *et al.* (1998) found that the percentage of maize grains infected with fungi increased correspondingly with increased insect damage in preharvest maize in Benin. Zuber *et al.* (1986) and Lamboni and Hell (2009) reported that insects that feed on maize ears in the field predispose kernels to *A. flavus* infection through the physical damage caused by their feeding.

High insect and fungal infestation was also prominent in maize samples (freshly harvested and sample from storage facilities) from Omonada district. The invasion of mould starts mostly from maize kernels damaged by weevil, larvae (stack borer) and birds as observed in the fields (plate 2a and b). Factors that influence the incidence of fungal infection include the presence of insect vectors, bird vectors, damaged grain and fungal infection levels (Horn, 2003). Insects and birds vector fungi and cause damage that allows fungal access to kernels and other crop tissues thereby increasing the chances of fungal contamination (Setamou *et al., 1998*). In the field, birds may damage a standing maize kernels in the ear making it suitable for fungal contamination usually *Aspergillus flavus* (Dick, 2008).

Insect damage may be the only way the fungus can enter under some conditions, or the fungus may be able to invade on its own under other conditions. The most prevalent insects affecting the crop in the field and storage included the weevil *Sitophilus zeamais* and the larger grain borer *Prostephanus truncatus* which have been also identified as major causes of maize yield losses in storage (Waktole and Amsalu, 2012; Schulthess *et al*.,2002 and Hell *et al*., 2000b).

The development of fungi can be affected by moisture content of the product, storage time, degree of fungal contamination rate prior to storage and insect and mite activity that might facilitate fungi dissemination (Giorni *et al*., 2009; Hell *et al*., 2000a) and Delayed harvest increased mold incidence and insect damage (Kaaya *et al.,* 2005). The study of the conditions that lead to the development of fungi during storage and in the field indicated that the grain moisture content is one of the most important factors (Hell *et al*., 2000b; Giorni *et al*., 2009). In maize, for instance, it was determined that a storage moisture content of 13% is sufficiently low to prevent fungus development.Thus, development of fungi can be affected by moisture content of the product, storage time, degree of fungal contamination rate prior to storage and insect and mite activity that might facilitate fungi dissemination and delayed harvest increased mold incidence and insect damage.

The lowest germination percentage (32.40%) was recorded in Nadasokote of the Omonada district for sample from storage, which correlated with the mould invasion percentage (Table 6 and 7) and the highest germination (64.60%) was recorded in Keta kebele of the Dedo district. Maize kernels invaded by fungi show poorly germinated. Invasion of maize seed by storage fungi will result in reduction in seed germination and discoloration of the germs (Lopez and Christensen, 1967) and severe invasion leads to moulding and grain weight loss (Prasad, 1992; Brown *et al*., 1995). Similarly, Mashilla (2004) reported that invasion of sorghum and maize seed by storage fungi resulted in reduced seed germination and discoloration of the germs. *Aspergillus* species has been found to cause maximum damage such as abortion, seed rot, physiological alteration and reduced germination and vigor (Shetty, 1988). Germination of seeds was completely decreased with the increase in storage time due to fungal infection (Ishrat *et al*., 2011). Considering a high incidence of fungal contamination of maize, it seems that the traditional methods of handling maize cobs during harvesting in the field, drying activity and transporting it to the storage leads to mechanical damages of kernels. In this condition, broken and infected kernels are more vulnerable to fungal attack than whole grains. Maize stored for long-time periods are more vulnerable than freshly harvested maize. Insect pests may also contribute to deteriorating the grains rapidly and increasing maize mycoflora during long-term storage (Hussein and Brasel, 2001).

Fungal contamination in maize differs between the different agro-ecological zones. These may be due to the prevailing climatic conditions (Probst *et al.*, 2007; Cotty and Jaime-Garcia, 2007), the cultivars grown in each zone, the cultural practices and ⁄ or the storage methods (Setamou *et al.*, 1998). Land management strategies and, particularly, crop rotation systems and factors such as genotype may influence crop infestation by *Aspergillus* species and the aflatoxin content of maize. Furthermore, crop management practices vary across the agro-ecology and these may contribute to risk of contamination (Cardwell and Henry, 2005; Hell *et al*., 2000a)

Consistently in this study, several mouldy fungi namely *Aspergillus*, *Penicillium* and *Fusarium* were isolated from high number of maize samples collected from freshly harvested maize samples and from storage facilities. Besides species belonging to *Drechslera* and *Cladosporium* were associated with maize in all the three-agro ecology in low frequency. *Fusarium* species were isolated from high number of freshly harvested maize samples (in forty maize samples). The highest frequency of *Penicillium* species in the freshly harvested maize samples could be due to shift in the grain fungi to storage fungi during transportation of samples. Alternatively, this could be due to their occurrence in the field as reported by Philiph and John (1970). Whereas, *Aspergillus* species were recovered from high number of samples collected from storage facilities (forty-three maize samples). Even though *Aspergillus species* is storage fungal disease, it has previously been reported in Nigeria albeit in preharvest maize (Bankole and Mabekoje, 2003). It is likely that preharvest infections greatly influence the mycoflora in storage (Hell *et al.,* 2003).

The distribution of *Aspergillus species* were the most dominant across agro-ecological zones in all the three agro-ecological zones in maize sample collected from the storage facilities. *Aspergillus flavus* also isolated from maize samples collected in the field at the harvest. Machinski et al. (2000) previously reported the presence of A. flavus in freshly harvested maize. Similarly higher frequencies of *A. flavus* in stored maize have been reported previously in Benin (Egal *et al*., 2005; Hell *et al.,* 2003). Penicillium and Fusarium species were more prevalent across the different surveyed agro-ecological zones for samples collected at harvest but for sample from storage Aspergillus species was predominant. The *Apergillus flavus* contamination in maize has been associated with drought combined with high temperature as well as insect injury (Betran and Isakeit, 2003). Insect activity is important in determining the likelihood of preharvest contamination (Cole *et al*., 1995). Poor harvesting practices, improper storage and less than optimal conditions during transport and marketing can also contribute to fungal growth and proliferation of mycotoxins (Bhat and Vasanthi, 2003; Wagacha, J.M. and Muthomi, J.W., 2008). The high frequencies of *Aspergillus species* can be explained by the occurrence of correspondingly in the plant debris and insects (Horn and Dorner, 1999; Jaime-Garcia and Cotty, 2004; Nesci and Etcheverry, 2002), which acts as the reservoir of inoculums for infection of kernels in the field.

6. SUMMARY AND CONCLUSION

Maize is one among the most important crops as a source of food and cash in maize growing areas of Ethiopia. However, maize incurs considerable losses both in the field and storage due to insect pests mainly weevil, fungal pathogens and other factors. In the study area, there is a little information mould infection and damage along with associated factors during various stages of postharvest; lack of data on the distribution, frequency and relative density of mycoflora on maize kernels. Because of these reasons, it has not been possible to develop effective management strategies to prevent fungal disease of maize kernels both in the field and in storage. Therefore, the present study was conducted to examine the distribution of post harvest fungal diseases, practices, and conditions that affect mould infection on maize kernels. Accordingly, to assess postharvest fungal diseases of maize, maize cobs were sampled from three districts of Jimma zone which are Sekoru (lowland), Omonada (intermediate) and Dedo (highland) maize producing agro-ecologies. Three PAs per districts were selected and five samples per PAs were collected. Preharvest and postharvest samples of maize cobs were collected in two series of surveys from randomly selected districts of Jimma during the 2011/12 crop season and a total of 90 samples of maize were collected; while the laboratory study were conducted at Jimma University College of Agriculture and Veterinary Medicine. In this study, the disease distribution, frequency, relative density and incidence was assessed and maize samples were collected and brought to the laboratory for further study from each districts. To isolate fungi, maize kernels were plated on Potato Dextrose Agar (PDA) and Czapex Dox Agar.

In all assessed farms, maize cobs first damaged by birds show mould invasion and weevil infestation than maize cob with no bird damage. The infestations of moulds were varying in some extent across the '*woredas*'.

During the survey, it was observed that structures used for maize storage by farmers similar across locations, but nearly all of stores were in a poor state of maintenance and hygiene; hence, conditions were conducive for insect infestation and fungal contamination of the stored kernels with cobs. Again, it was observed that weevil infestation was high in maize cob storage that the infestation was started in the farm. In some storage, there were other crops stored together with unshelled maize like sorghum; teff and maize stalk for fire wood purpose, which can be, facilitate mould development in the stored maize by serving as source of inoculants. Most of the farmers store healthy and infected maize cobs (by mould and insect) were stored together.

Infected or damaged kernels by mould and insect pest were varied among districts for both sample collected at harvest and storage facilities. The maximum loss due to mould invasion was recorded in Omonada (36.90%) and followed by Sekoru (30.30%) and the least was recorded in Dedo (19.90%) for sample from the farms and Dedo (38.60%), Omonada (38.50%) and Sekoru (36.50%). The overall mean for mould invaded kernels and weevil attacked kernel were 37.8%, and 20.90%, respectively for maize sample from storage and 28.90%, 19.30% and 23.30%, respectively for maize cob sample collected from farms at harvest.

A total of 1462 fungal isolates were recovered from 90 maize cob samples collected from three maize producing districts that represent the three agro-ecology in the zone. Five genera of fungi were identified from total of 90 maize cob samples. Forty-five of the maize cob samples were obtained from farmers' storage facilities and Forty-five samples of freshly harvested maize cob were collected from farmers' fields at harvest.

The mycological investigation of maize samples revealed *Fusarium* species, and *Penicillium* species to be the most prevalent genera in freshly harvested cob samples and *Aspergillus* species, *Fusarium* species, and *Penicillium* species, to be the most prevalent genera in maize samples collected from the storage facilities. Across agro-ecological zones, *Fusarium* species were the most predominant fungi identified, followed by species belonging to the genera of *Penicillium* and *Drechslera*, while species of *Aspergillus* and

Cladosporium were the least predominant one for sample collected in the field. But *Aspergillus* species were the most predominant fungi identified, followed by species belonging to the genera of *Fusarium* and *Penicillium,* while *Drechslera* and *Cladosporium* were the least predominant for sample collected from the storage.

Based on means of frequency, the most dominant fungal genera were *Aspergilus*, *Fusarium* and *Penicillium* with the frequency of 97.8%, 91.1% and 84.4% respectively for sample collected from storage facilities and *Fusarium* and *Penicillium* with the frequency of 95.6% and 86.7% respectively for freshly harvested maize sample from the farmer's farm. The genera of *Fusarium* and *penicilium* were the predominant mycoflora with 40.8% and 31.6% relative density respectively for sample collected from the farm and, *Aspergilus* species and *Fusarium* species were the predominant mycoflora with 36.0% and 32.1% relative density respectively for sample collected from the storage.

Based on these results maize kernels were mostly infected by toxin producer fungi such as *Fusarium species*, *Aspergillus species* and *Penicillium species. Aspergillus* species predominated the total mycobiota of sample collected from storage facilities and followed by *Fusarium* and *Penicillium* species. *Fusarium species* predominated the total mycobiota of freshly harvested maize samples. Across districts, the incidence of *Fusarium* species was highest in Dedo and Sekoru and *Penicillium* was highest in Omonada. *Aspergillus* species was highest in Sekoru and lowest in Dedo.

Pre-harvest measures that are efficient in reducing fungal infection are the same as those that will enhance yields. Crop rotation and management of crop residues also are important in controlling fungal infection in the field. However, biological control in conjunction with other management practices such as timely harvest, appropriate grain drying, avoidance of rewetting in storage and sorting holds the promise of offering a long-term solution to the problem of mould growth in maize and the consequent will effects on health. Furthermore, crop management practices vary across the agro-ecological zones and these may contribute to risk of fungal contamination.

Several storage factors that may help to reduce fungal infection in the stored maize in Jimma were identified in the present study; control of storage insects through the sorting out of damaged cobs, the use of appropriate storage insecticides and awareness of the farmers of the risk that insects and fungal contamination present to their maize. Insect pests play a big role in the vectoring of fungal spores and also provide entry holes to fungal organisms through their tunneling activity, both prior to and after harvest. Crop management practices vary across the agro-ecology and these may contribute to risk of contamination by fungal disease. These are the most important factors responsible for spreading and infestation of fungal diseases.

In general, the extent of fungal contamination of stored maize and maize in the field recorded in present study reveals that a concentrated effort is needed to ensure that improved pre- and post-harvest handling of maize in Jimma zone. Farmers are therefore advised to harvest their maize at physiological maturity to avoid further rotting and possible lethal contamination of kernels by fungi. In addition, insect activity in stored grain promotes the development of fungi, so controlling insects will help reduce the risk of moulds. Sorting out the infected cobs before store it with healthy cobs. Since, the present study was done only in some selected districts of the zone; it would be advisable to repeat the experiment in all districts to come up with comprehensive recommendations. It appears to be worthy of conducting on the mycotoxin content of maize since some of the fungal genera identified during this study was the one responsible for mycotoxin contamination in maize.

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8. APPENDICES

Appendix 1: ANOVA for mould incidence in the farm in Lowland agro-ecology

	S.V.	SS	Df	MS	F-value	P-value
Mould infection	Between Groups	159.227	2	79.614	1.147	0.327
	Within Groups	2915.014	42	69.405		
	Total	3074.241	44			

Appendix 2: ANOVA for mould incidence in the farm in Intermediate agro-ecology

	S.V.	SS	Df	MS	F-value	P-value
Mould infection	Between Groups	275.606	2	137.803	5.038	0.011
	Within Groups	1148.922	42	27.355		
	Total	1424.529	44			

Appendix 3: ANOVA Table for means of incidence of mould infected maize in the farmer's farms across the Peasant Association

S.V.	SS	Df	MS	F-value	P-value
Between Groups	390.027	8	48.753	2.455	0.031
Within Groups	714.824	36	19.856		
Total	1104.851	44			

Appendix 4: ANOVA for mould incidence on maize at the farm in Highland agro-ecology

	S.V.	SS	Df	MS	F-value	P-value
Mould infection	Between Groups	47.248	2	23.624	0.906	0.412
	Within Groups	1094.775	42	26.066		
	Total	1142.023	44			

Appendix 5: Means of Mould incidence within farmer's farm

Name of Farmers	Mean ± SD	p value
A/Diga	20.5 ±10. 2	0.000
A/Oli	11.5 ±1.9	
A/Biya	18.7 ±7.0	
A/Temam	0.00 ±.0	
Abdo	13.3 ±11.7	
Mubarik	21.3 ±2.7	
A/Jihad	16.3 ±10.7	
Sh/Kadir	16.3 ±4.6	
A/Dura	17.9 ±1.8	
Zinab	13.8 ±5.5	
A/Rayya	19.5 ±11.1	
Sh/Adam	14.4 ±6.7	
Sh/kamal	24.7 ±7.9	
Nasir	6.6 ±6.6	
A/Faji	16.4 ±3.1	
Biya	19.0 ±4.4	
Habib	20.3 ±7.7	
A/Zinab	25.9 ±5.4	
A/Temam	24.9 ±5.3	
Sh/Jamal	23.4 ±6.5	
A/Tamaam	22.2 ±4.7	
Biyyaa	14.2 ±5.4	
Hajii	21.3 ±5.4	
Awal	21.4 ±9.2	
Taju	20.5 ±1.1	
Mamadhawi	17.8 ±3.3	
Tajuu	16.9 ±5.0	
Naimo	15.4 ±5.7	
A/Zinaab	16.7 ±1.1	
Rida	16.5 ±5.9	
A/Garo	17.1±6.8	
A/Maca	15.1±2.0	
A/Jihad	19.4±1.0	
A/Nagaa	25.4±5.0	
A/Reshad	18.6±2.9	
A/Temaam	21.6±1.9	
A/Jebal	18.3±3.4	
Haile	29.6±4.9	
Nazif	17.2±2.6	
Zafis	20.8±0.8	
A/Jihad	22.6±6.7	
A/Fita	14.9±5.0	
Abdela	18.4±3.9	
Mohammed	19.7±5.4	
Zinab	23.1±0.6	
Mean	18.4±6.8	

Appendix 6. Questionnaire

Questionnaire on harvesting practices, storage handling and associated problems of maize in Jimma zone

Note to the Interviewer

This questionnaire is prepared to get feedback on Farmers' post harvest practices of maize in selected districts of Jimma. I would appreciate for all the cooperation made. Please introduce yourself and the objectives of the study to the interviewee very politely. Complete the questionnaire by circling the letter of the choice and filling in the open ended questions very patiently. One question may have more than one answer. Please don't forget to thank the interviewee after completing the interview.

Date: ______________________

Name of district: ______________________

Agro-ecological location __________________

Name of interviewee: _____________________

Name of interviewer: _____________________

I. Harvesting practices and harvesting problem

1. Which maize variety do you grow and store? ________________

2. How do you harvest maize?

a/ manual harvesting of the cob from standing stalk

b/ Other method (specify) _______________________

3. How do you judge that the maize is ready for harvesting?

a/ Visual observation

b/ Shelling and checking for seed hardness

c/ Other means (specify) ______________________

4. Time of harvesting maize after attaining physiological maturity?

a/one week

b/two week

c/three week

d/four week and more

5. What method do you use for transporting the harvested maize to drying site?

a/ Carrying on human shoulders or back of animals

b/ Wheel barrows

c/ Animal drawn carts

d/ Other means (specify)

6. How do you shell/ thresh the harvested maize?

a/ Beating the cobs with sticks inside sacks

b/ Finger-palm shelling

c/ Using mechanical shellers

d/ Other method (specify)

7. What tool/equipment do you use for transporting the shelled maize grain or cobs to storage containers?

a/ Carrying on human shoulders or back of animals

b/ Wheel barrows

c/ Animal drawn carts

d/ Other means (specify)

8. Do you dry maize after harvest by sun spreading it on drying floor?

a/ Yes b/ No.

9. If your response to question No. 8 is yes is the drying surface bare ground or prepared otherwise?

a/ Yes b/ No.

10. If your response to question No. 8 is no, what is the finishing material used for drying surface?____

11. Do you use the same drying floor year after year or you change the site?

____

12. If you do not use drying surface, how and where do you dry the grain?

____

13. How long would it take, at an average, to dry the grain to your satisfaction before taking it in to storage containers? _______________ days**.**

14. How do you decide that the grain is dry enough to be stored?

____

15. What type of maize storage container do you use?

16. Do you clean your storage containers and the surrounding before storing newly harvested grain?

a/ Yes b/ No.

17. Do you fumigate your storage container before taking new grains in?

a/ Yes b/ No.

18. If your response to question No. 16 is yes, what do you use for fumigating the grain?

a/ Smoking firewood

b/ Smoking pepper

c/ Smoking plant leaves (specify leaf type) ___________

d/ Others (specify) ______________________

19. Do you aerate your stored grain?

a/ Yes b/ No.

20. If your response to question No. 18 is yes, how and how often do you do it?

____

21. How do you inspect the stored maize to check for any sign of deterioration so that you could take measures on time?

____

22. How frequent do you inspect the stored grain?

____

23. What corrective measures do you take in response to your storage inspection if you find sign of disease (mould development)?

a/ Use pesticides

b/ Use of plant materials

c/ Use other traditional protectant (Specify) ____________________________

24. Describe briefly how you apply your treatment to the grain.

____

25. If you find other signs of deterioration different from fungal or disease attacks what measures do you take?

____

26. How much of your maize grain do you think you lose because of problems associated with post harvest practices start from field up to storage? And which factor of loss account
more? ____

27. What do you suggest the government or non-governmental agents should do in order to minimize post harvest loss and grain quality deterioration problems in your area? ____

28. Are there any additional points you would like to raise regarding maize post harvest activities like when to harvest, when to store in storage, etc (experiences to share to others, questions to ask, suggestions to make, etc)?

____

II. Storage practices

1. When is the harvested maize stored? Directly after harvest_____ Pre-storage_____

2. Why do you pre-store? ______

3. Where do you pre-store? Field_____ In the house_____

4. For how many months do you store? _____

5. What storage method do you use? List_____

6. Where is your storage structure located? Field ___In the house___ Courtyard_____

7. What construction material did you use? Wood___ Clay_____ Metal_____

8. For how many seasons have you used the store? _____

9. Do you store maize in the store every season? No, why? _____ Yes_____

10. Do you store other products in the store, together with maize? No_____ Yes, list?_____

III. Storage problems

1. Do you have storage problems? Yes____ No____

2. Which storage problem is the most important? Insects__ Rodents __Birds __Mould_ Others__

3. When did you observe this problem?

- At the beginning of storage
- After a few months
- At the end of storage

4. What did you do to solve this problem? List_____

5. Does the grain germinate in storage? Yes____ No____

6. Do you clean the storehouse before storage? Yes____ No____

7. Do you remove old grains? Yes____ No____

8. What else did you do to clean the store before storage? List_____

9. If you treated the storehouse before storage, what methods did you use? Ash_____ Sand_____ Insecticides (specify) _____Smoke_____ Manure____ (specify)

10. How did you store your maize? As grain _____In the husk _____ Dehusked ____Other_____

11. Did you use pesticides during storage? If yes, give name_____

12. Did you take any other precautions? List_____