

DETECTION AND IDENTIFICATION OF MAJOR STORAGE FUNGAL PATHOGENS OF MAIZE (*ZEA MAYS* L.) IN JIMMA, SOUTHWESTERN ETHIOPIA.

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ABSTRACT: *Maize (Zea mays L.) is the third most important food crop in the world produced on nearly 100 million hectares. Maize is attacked by more than sixty diseases and a number of species of insect pests and microorganisms in the field as well as in the storage. Fungi are among the principal causes of deterioration and yield loss on farmers' maize during the storage period. Among the storage fungal pathogens Aspergillus, Fusarium and Penicillium are the most predominant species attacked maize grain and resulting in production of harmful products of Mycotoxins. The study was conducted at the Jimma University College of Agriculture and Veterinary Medicine in plant pathology laboratory. Three maize varieties and two levels of disinfection were used and arranged in complete block design with five replications. The highest frequency of Aspergillus spp. (40.4%) at farmer preserved seed with surfacelly disinfected kernels on agar plate were recorded. The highest relative density of Fusarium spp. (51%) was only recorded on agar plate test on the farmer preserved seed which was not surfacelly disinfected. The lowest germination percentage (62%) were recorded on the farmer preserved seed which was not surfacelly disinfected. The Aspergillus spp are the most dominant fungi followed by Fusarium spp. were isolated in this study as well as in Ethiopia. These fungi are important in producing secondary metabolites which are carcinogenic to both humans and animals.*

KEY WORDS: *Aspergillus; Fusarium; Penicillium; Mycotoxins, Maize*

INTRODUCTION

Maize is the third most important food crop in the world surpassed only by two other grains, wheat and rice (Kyenpia *et al.*, 2009). The growing demand for food consumption in developing countries alone is predicted to increase by around 1.3% per annum until 2020 (Ortiz *et al.*, 2010). By 2050 demand for maize will double in the developing world, and maize is predicted to become the crop with the greatest production globally, and in the developing world by 2025 (Rosegrant *et al.*, 2008). In Ethiopia maize is the first most important cereal crop in terms of its production accounting for 27.77% (6.07 million) of 86.06 % (18.81 million tons) of the cereal production (CSA, 2012). However, its cultivation in the world is limited by diseases which cause grain loss of about 11% of the total production (Suleiman and Omafè, 2013). Fungi are among the principal causes of deterioration and loss of maize grain (Ominski *et al.*, 1994). Kossou and Aho (1993) reported that fungi could cause about 50-80 % of damage on farmers' maize during the storage period if conditions are favorable for their development.

During storage, several kinds of fungi can remain associated to corn seeds either causing their deterioration or simply remain viable to infect germinating seedling. The fungi genera typically found in stored grains are *Aspergillus*, *Penicillium*, *Fusarium* (Orsi *et al.*, 2000; Castellari *et al.*, 2010) and some xerophytic species, several of them with capabilities of producing toxins (Castellari *et al.*, 2010). The development of these fungi can be affected by moisture content of the product (Gtorni *et al.*, 2009). Temperature, storage time and degree of fungal contamination prior to storage, insect and mite activity facilitate fungi dissemination (Suleiman and Omafè, 2013).

There is a general increase in consumption of contaminated grain with mycotoxins which causes different health problems including death (Lerda *et al.*, 2005; Vess *et al.*, 2007). The rank of fungi is second after insects as the cause of deterioration and loss of maize (Uzma and Shahida, 2007). *Aspergillus flavus* becomes systemic and produces Aflatoxin and Vivesces in seedling of maize and damage stored corn. *Fusarium* invade more than 50% of maize grain before harvest and produce Mycotoxin (Uzma and Shahida, 2007), while *Aspergillus flavus* is a food contaminant and capable of producing Aflatoxin (Charity *et al.*, 2010).

In Ethiopia various grain mold fungi including *Fusarium*, *Penicillim*, *Aspergillus*, and *Nigropora* spp. have been detected on maize samples collected from Hawassa, Areka, Billito, Shallo and Arsi-Negele. The populations of all the fungi were higher in samples collected from farmers' stores than in the samples collected from research and seed multiplication stores (Girma, 2009). *Aspergillus* and *Fusarium* were frequently isolated from damaged seeds and *Penicillim* spp were the second most frequent fungal species. Tesfaye and Dawit (1998) also identified four *Fusarium* species associated with maize grain in Ethiopia. The presence of Mycotoxins in grains and other staple foods and feedstuffs (Abera and Admasu, 1987; Dawit and Berhane, 1985) has serious implications for human and animal health and reduce seed quality by discoloration of the seeds. Despite the prevalence and seriousness of these storage fungi causing losses to the grain of maize crop in the stored in Ethiopia, adequate studies have not been made. Information on detection and identification of these fungi is limitedly available. Hence much remains to be done on the detection of storage maize fungal pathogens are required. This study was initiated with the specific objective: to detect and identify the major storage fungal pathogens of maize grain stored at different storage conditions.

MATERIALS AND METHODS

Description of the Study Area

The study was conducted at Jimma University College of Agriculture and Veterinary Medicine in the plant pathology laboratory in 2014. The experimental site is located at 350 kilometers from Addis Ababa, at 36°50'E longitude, 7°40' N latitude and at an altitude of 1,780 meters above sea level. It is situated in the mid-altitude tropical belt of south-western Ethiopia.

Experimental design, materials and treatments

This experiment was designed to isolate fungal pathogens associated on stored maize grain of different varieties with surface disinfecting and without disinfecting. The sample seeds of the varieties were collected one variety (Melco), from Jimma Agricultural Research Center, one Chemically treated, with moisture content of 12.5%, germination (97%) and purity of 98% variety (BH660) from Jimma Zone Jimma Farmers Cooperative Union and one variety from farmer preserved seed at near Jimma town. The treatments of this experiment were six in five replications with completely randomized design (CRD) arrangements.

The treatments were:

1. MW1= the variety Melco with surface disinfecting
2. MW2= the variety Melco without surface disinfecting
3. BH660W1= the variety BH660 with surface disinfecting
4. BH660W2= the variety BH660 without surface disinfecting
5. FPSW1= the farmer preserved seed with surface disinfecting
6. FPSW2= the farmer preserved seed without surface disinfecting

Maize sampling method

A total of 3 samples (~0.5 kg of Melco and BH660, and one cob of farmer preserved seed) of maize grain harvested in 2013 were collected at 3 locations in the Jimma zone around Jimma town, in the maize grain storages of (Jimma Agricultural Research Center, Jimma Zone Jimma Farmers Cooperative Union and Farmer preserved seed at near Jimma town). All samples were taken from dried maize grain storages.

Incubation tests

The seeds were incubated for a certain period in the agar plate and blotter test under specific environmental conditions in order to allow pathogens on the seed to grow. Different fungi were identified by features such as the form, length and arrangement of conidiophores, size, septation and chain formation of conidia (Warham *et al.*, 1990).

Agar plate method

For this method, three maize variety kernels with and without surface disinfecting were used and 10 grains of each treatment were aseptically placed on potato dextrose agar (PDA) plate according to (McGee, 1994) procedures. Firstly, from each sample 250 maize kernels; in 5 replications of 50 seeds were selected. To remove the chemical from the chemically treated seed of BH660, kernels were thoroughly washed in running water. For the surface disinfecting treatments, maize kernel sample was surface disinfected by 1% Sodium-hypochlorite for one minute and rinsed three times in sterile distilled water for 30 seconds each. From both surface disinfected and non-disinfected samples, 10 kernels per petri plate (9 cm diameter plates) containing PDA (potato dextrose agar) were aseptically placed. The plates were incubated at 26 °C for 7 days and the growth stage of colonies were determined periodically. Finally after 7 days of incubation, the total number of fungal colonies, frequency of isolation of fungi (%), relative density of isolated fungi (%) and incidence of fungi (%) were recorded and calculated.

Blotter method

In this method, similar washing and surface disinfecting procedures of agar plate were followed. For this method, a total of 250 maize kernels in 5 replication and each replication contained 50 kernels were used. Per subsample, 10 kernels were aseptically placed in each blotter plate which are moistened with sterile distilled water. Then each blotter plate was incubated under ultraviolet light in alternating cycles of 12-h light/darkness for 7 days at 20- 25 °C . Finally each petri dishes (blotter plate) were examined under stereo-binocular microscope for fungi isolation based on identification key and each fungal colonies were recorded. In addition to fungal isolation, germination percentage of each blotter were recorded after three and seven days of incubation.

Data collection

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

$$\text{In (\%)} = \frac{\text{Number of infected grain}}{\text{Total number of grain}} \times 100$$

Isolation Frequency (IF): For each fungus, the proportion of samples that yielded its isolates were determined and expressed as percent by using the following formula: (Marasas *et al.*, 1988).

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

Relative Density of Fungi: For each fungus, the relative density of isolated fungal species was calculated by using the following formula: (Marasas *et al.*, 1988).

$$\text{RD (\%)} = \frac{\text{Number of isolated fungi species}}{\text{Total number of fungi}} \times 100$$

Seed Germination (%): The percentage of kernels that germinated was determined separately for samples from surface disinfected and non-disinfected maize grains on blotter method. The relative reduction for germination also recorded by comparing the relative amount of germination capacity reduced for the varieties. The seed germination was calculated by using the following formula:

$$\text{G (\%)} = \frac{\text{Number of grain germinated}}{\text{Total number of grain}} \times 100$$

Where: G= Seed germination

Statistical analyses

The germination percentage after 3 and 7 days of incubation and total number of fungal colonies per plate were analyzed by using SAS software version 9.2 software (SAS, 2009) software. Analysis of variance (ANOVA) was performed using general linear model (GLM) procedure.

RESULTS

Total Colony Forming Units/Plate

In this experimental work, higher significant difference of total fungal colonies among the different treatment combinations of the maize varieties and surface disinfection on both agar plate and blotter method was recorded. On agar plate, the highest fungal colony (28) followed by (23) fungal colonies was recorded at FPSW2 and FPSW1 respectively. On the other hand, the lowest fungal colony (10) was recorded at BH660W2 which is statistically non significant with BH660W1 (Table 1). On blotter test the highest fungal colony (6) per plate on MW2 and the lowest fungal colony (1) per plate on MW1 was recorded (Table 1). In general, on both agar plate and blotter methods significant difference among varieties and surface disinfecting was recorded except on the variety BH660.

Table 1. The total fungal colony forming units/plate of three maize variety kernels with and without surface disinfecting at agar plate test and blotter test methods.

Treatments	Total Fungal Colonies Per Plate	
	Agar plate	Blotter Test
MW1	15.0 ^c	1.4 ^d
MW2	17.0 ^c	6.2 ^a
BH660W1	11.2 ^d	3.8 ^b
BH660W2	9.6 ^d	3.8 ^b
FPSW1	23.4 ^b	2.6 ^c
FPSW2	27.6 ^a	3.6 ^b
Mean	17.3	3.45
LSD (0.05)	2.64	0.75
CV (%)	11.70	16.65

Where: MW1 = Melco with surface disinfecting; MW2 = Melco without surface disinfecting; BH660W1 = BH660 variety with surface disinfecting; BH660W2 = BH660 variety without surface disinfecting; FPSW1 = Farmer preserved seed with surface disinfecting; FPSW2 = Farmer preserved seed without surface disinfecting; LSD = Least Significant Difference; CV = Coefficient of Variation; Values following by the same letter within the column or row are not significantly different at 0.05 probability level

Fungi Incidence Associated with Maize Grain

A total of 110 fungi isolates were recovered from three maize variety samples in six treatment combinations which is collected in three maize storage conditions, were harvested during 2013 cropping season. In this study, 100% incidences of fungal infection were recorded at all treatments under agar plate. Whereas under blotter test the highest incidences of 50% were recorded at FPSW2 (farmer preserved seed without surface disinfecting) followed by 48% and 46% at MW2 (Melco without surface disinfecting) and FPSW1 (farmer preserved seed with surface disinfecting) respectively (Figure 1.).

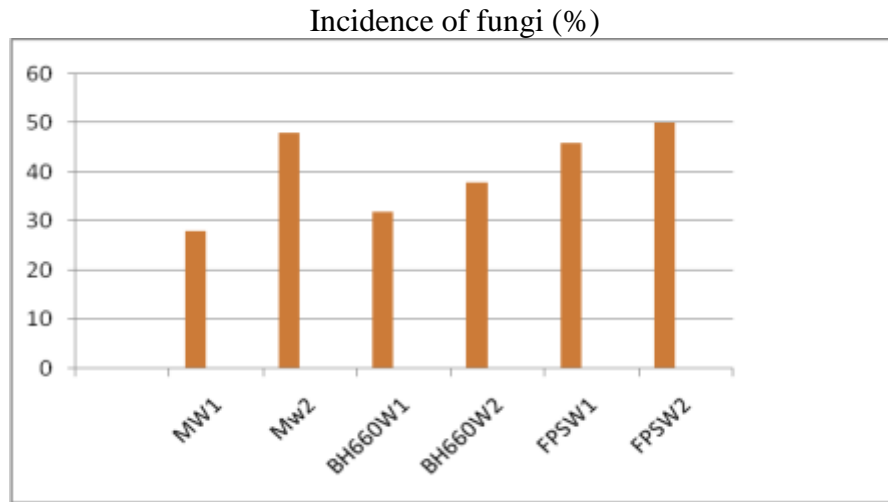


Figure 1. Incidence of fungi on combinations of three maize varieties with and without surface disinfection of stored kernels on blotter test.

Frequency of Isolation of Fungi

In this experiment the highest frequency of *Aspregilus spp.* (40.4%) at farmer preserved seed with surfaclyly disinfected kernels followed by (32%) on Melco verity which is non-surface disinfected kernels was recorded on agar plate test (Figure 2.). The highest frequency of *Fusarium spp.* (28%) and *Penicilium Spp.* (18%) were recorded both at FPSW2 at agar plate test (Figure 3). In general the highest fungal frequency of the three major storage pathogens of maize was recorded at agar plate method which was placed with farmer preserved seed without surface disinfecting. In blotter test method in all treatments the highest frequency was *Aspregilus spp.* followed by *Fusarium spp.* and *Penicilium spp.* (Figure 3.). In this experiment at blotter higher difference of fungal frequency especially on *Aspregilus spp.* was recorded among kernels which were surfaclyly disinfected and that of non-disinfected once (Figure 3.). In both agar plate and blotter test methods, a clear difference of fungal frequency of the fungi genera among varieties and their combination with surface disinfection and without disinfection was recorded.

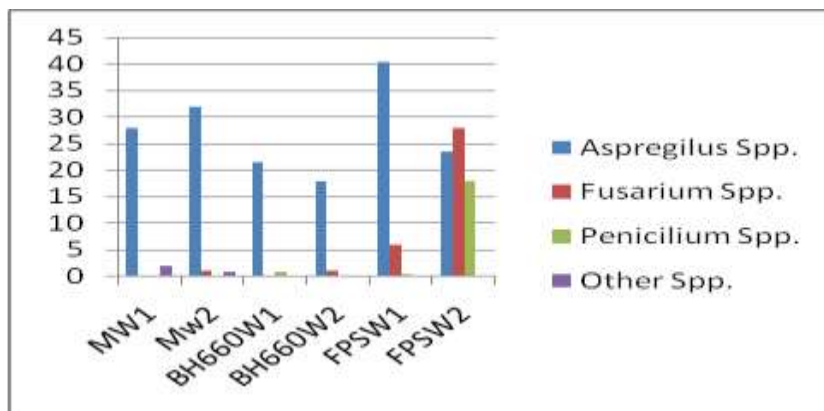


Figure 2. The frequency of fungal pathogens associated with stored maize kernels of three varieties and combined with and without surface disinfecting at Agar plate test.

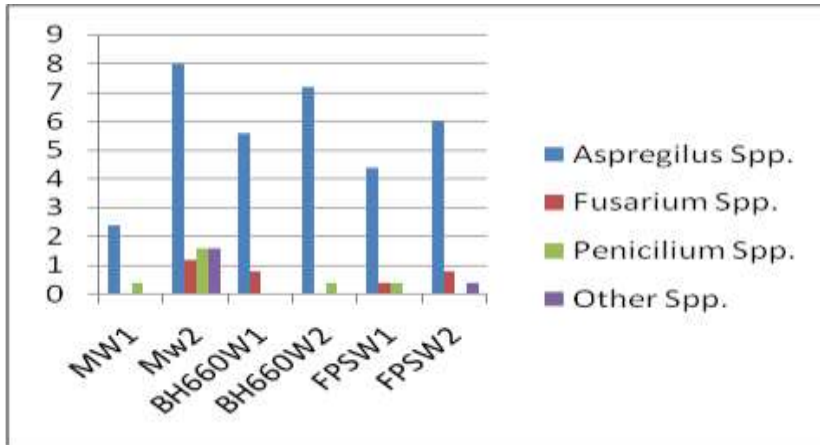


Figure 3. The frequency of fungal pathogens associated with stored maize kernels of three varieties and combined with and without surface disinfecting at Blotter test methods.

Relative Density of Fungi

In this experiment the highest relative density of *Aspregilus spp.* was recorded on both agar plate and blotter test in all treatment combinations except at FPSW2 on agar plate method (Figure 4. and 5.). On the other hand, the highest relative density of *Fusarium spp.* was recorded only at agar plate on the variety farmer preserved seed which is surfacally non-disinfected. At agar plate test the highest density (99%) of *Aspregilus spp.* on MW1 followed by (96%) on BH660W1 was recorded. The highest *Fusarium spp.* (51%) was only recorded at agar plate test on FPSW2. In general the highest fungal relative density of the three most common storage fungi (*Aspregilus*, *Fusarium* and *Penicilium*) were recorded at gar plate on FPSW2 (Figure 5).

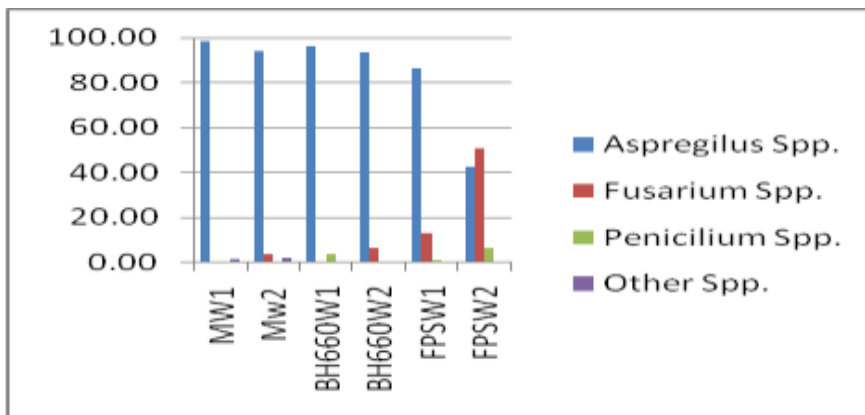


Figure 4. The relative density of fungal pathogens associated with stored maize kernels of three varieties and their combination with and without surface disinfecting at Agar plate test.

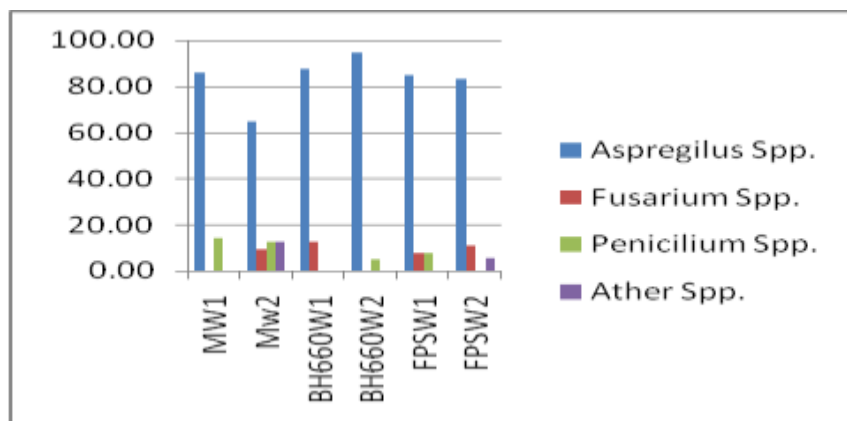


Figure 5. The relative density of fungal pathogens associated with stored maize kernels of three varieties and their combination with and without surface disinfecting at Blotter test method.

Germination Percentage (%)

In this experiment, germination of the three varieties shows significant differences among each other's, but there is no significance difference on the same variety which was treated with and without surface disinfection (Table, 2) at both 3 and 7 day after incubation on blotter test. The highest seed germination was recorded on MW1, MW2, BH660W2 and FPSW1, whereas the lowest germination (6% and 62%) was recorded on FPSW2 at 3 & 7 days after incubation respectively (Table, 2). This result shows that how much the storage fungal pathogens greatly affect the germination ability of maize grains. And it is in agreements with the idea of Quezada *et al.* (2006), Seed with high rates of fungal infection or insect damage have very low germination rates, which can be as low as 28% of original potential.

Table 2. Germination percentage of three maize varieties and their combinations with and without surface disinfecting at 3 and 7 days after incubation on blotter test.

Treatments	Germination (%) of stored maize kernels on Blotter Test at	
	After 3 Days of Incubation	7 Days of Incubation
MW1	48 ^a	84 ^{ab}
MW2	42 ^a	88 ^a
BH660W1	18 ^b	76 ^b
BH660W2	18 ^b	84 ^{ab}
FPSW1	12 ^{bc}	80 ^{ab}
FPSW2	6 ^c	62 ^c
Mean	24	79
LSD (0.05)	8.9	10.39
CV (%)	28.46	10.07

Where: MW1 = Melco with surface disinfecting; MW2 = Melco without surface disinfecting; BH660W1 = BH660 variety with surface disinfecting; BH660W2 = BH660 variety without surface disinfecting; FPSW1 = Farmer preserved seed with surface disinfecting; FPSW2 = Farmer preserved seed without surface disinfecting; LSD = Least Significant Difference; CV =

Coefficient of Variation; Values following by the same letter within the column or row are not significantly different at 0.05 probability level.

DISCUSSIONS

The results of this study were in agreement with (Girma *et al.* 2009), which is in Ethiopia various grain mold fungi including *Fusarium*, *Penicillium*, *Aspergillus*, and *Nigropora* spp. have been detected on maize samples collected from Hawassa, Areka, Billito, Shallo and Arsi-Negele. The populations of all the fungi were higher in samples collected from farmers' stores than in the samples collected from research and seed multiplication stores. *Aspergillus* and *Fusarium* were frequently isolated from damaged seeds and *Penicillium* spp. were the second most frequent fungal species. Tesfaye and Dawit (1998) also identified four *Fusarium* species associated with maize grain in Ethiopia. The major genera commonly encountered on maize grain in tropical regions are *Fusarium*, *Aspergillus* and *Penicillium* (Samson 1991; Orsi *et al.*, 2000). The development of these fungi can be affected by moisture content of the product (Gtorni *et al.*, 2009), temperature, storage time and degree of fungal contamination prior to storage, insect and mite activity facilitate fungi dissemination (Suleiman, and Omafe, 2013). During storage, several kinds of fungi can remain associated to corn seeds either causing their deterioration or simply remain viable to infect germinating seedling. The fungi genera typically found in stored grains are *Aspergillus*, *Penicillium*, *Fusarium* and some xerophytic species, several of them with capabilities of producing toxins (Castellari *et al.*, 2010). *Aspergillus*, *Fusarium*, *Penicillium* and *Cladosporium* are the predominant fungal genera associated with grain in storage (CAST, 2003). *A. flavus* can infect maize pre- and post-harvest and an increase in aflatoxin content can occur if the phases of drying and storage are poorly managed (Chulze, 2010). Several phytopathogenic species of *Fusarium* are found to be associated with maize including *F. verticillioides*, *F. proliferatum*, *F. graminearum* and *F. anthophilum* (Munkvold and Desjardins, 1997; Scott, 1993). Fungi from five genera (*Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria* and *Calviceps*) are responsible for the production of the great majority of the mycotoxins that are of agricultural relevance (Jones & Toal, 2003). The losses caused by seed fungi may occur during seed development, storage or germination. Damage may result from loss of seed viability or from seedling infection following germination (Griffin, 2010 as cited in Suleiman and Omafe, 2013). *Fusarium*, *Aspergillus* and *Penicillium* were the most common genera isolated from maize samples collected around Shashemene and Alemaya (Tsfaye, 1997; Tesfaye and Dawit, 1999, 2000). The researchers found three toxic species of *Fusarium* (*F. moniliforme*, *F. subglutinans*, and *F. graminearum*) to be highly associated with maize samples. A previous survey by Dawit (1982) also indicated that *Fusarium* was the most common genus in maize grain samples.

CONCLUSIONS

The farmer preserved seed and certified maize seed is highly infested with a number of fungal seed borne pathogens and this can affect germination capacity of the seed. Seed selection is

useful in improving seed germination and reducing the seed borne spectrum of most pathogens of maize. The *Aspergillus* spp. are the most dominant storage fungi followed by *Fusarium* spp. were isolated in this study as well as in Ethiopia. These fungi are important in producing secondary metabolites which are carcinogenic to both humans and animals. Since these storage fungi are very important in causing postharvest yield losses and production of Mycotoxins, accurate detections and identifications are crucial in order to develop an appropriate management strategies. In Ethiopia there is a great gap of experiments on stored maize pathogens. So, giving attentions to these pathogens may play a vital role in our country's development and transformation strategy.

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