Detection of Fungal Pathogen and loss Assessment of Maize (*Zea mays L.)* seeds at Storage Conditions Around Jimma, South western Ethiopia

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Abstract

*Maize is the primary staple crop and it's grown nearly in all agro-ecological zones of Ethiopia. Despite the steady production of maize over the past several decades, post-harvest losses of maize remained significant. Post-harvest handling, poor infrastructure, weather variability, biotic factors such as insect pests, bacteria, viruses, and fungi, often aggravate such losses. There is an increasement in consumption of contaminated grain with mycotoxins which causes different health problems including death. Therefore, the present study was designed to quantify postharvest losses and detection of fungal diseases infecting maize seeds at storage conditions. Loss assessment were done by taking two hundred seed from each five sample of maize seeds collected randomly. The study was conducted at the Jimma University College of Agriculture and Veterinary Medicine in plant pathology laboratory. The experiment had five treatments and three replications with completely randomized design (CRD) arrangements. The overall Maize grain losses was **32.3**% whereas **20.7%, 2.6%** and **9.0%** where loss of Insect damage, broken and mould respectively. The highest postharvest losses were recorded in retailer seed from Limmu area **77.5**% losses. Among the storage fungal pathogens Fusarium, Aspergillus and Penicillium are the most predominant species attacking maize seed and resulting in reduction in seed germination. The highest fungal frequency were Fusarium spp. of the three major storage pathogens of maize seed collected. In the agar plate were recorded 56.66 %, 40.67% and 23.33%, Fusarium spp., Aspregilus spp. and Penicillium spp. respectively. In Blotter method the highest fungal frequency was Aspregilus spp. with 39.33% followed by Fusarium spp.and Penicilium spp. with 36.67% and 16.0% respectively. Therefore, giving attentions to those pathogens associated with postharvest fungal pathogen will help in the reduction of molds and mycotoxins development.*

Keywords: Aspregilus, Fusarium, Frequency, Maize, Post-harvest loss, Penicillium

Introduction

Maize (*Zea mays L*) is the third most important cereal crop worldwide after 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). It is a basic staple food grain for large parts of world including Africa, Latin America, and Asia (Yaouba *et al.,* 2012). Maize is the leading cereal crops in terms of production in Ethiopia, with 8.40 million tons produced in 2017/2018 on 2.13 million hectares of land. The national average yield of maize is estimated at 3.94 t/ha and approximately 88% of maize produced in Ethiopia is consumed as food, both as green and dry grain (CSA, 2018).

In tropical and subtropical countries, a large proportion of the grain (such as maize) is harvested and stored under hot and humid conditions, and most farmers lack proper knowledge, equipment and methods of drying grains (Weinberg *et al.,* 2008). Maize, like other stored products is hygroscopic in nature and tends to absorb or release moisture. Even if properly dried after harvest, exposure to moist and humid conditions during storage will cause the kernel to absorb water from the surroundings (Devereau *et al.,* 2002), leading to increased maize moisture contents, which results in enhanced deterioration. Temperature and moisture content of the cereal grains are the two key features affecting the resulting quality of the grain, biochemical reactions, dry matter losses, allowable storage times and overall storage management of the grain (Lawrence and Maier, 2010). One of the key constraints to improving food and nutritional security in Africa, however, is poor post-harvest management that leads to losses of 20–30 %, with an estimated monetary value of more than US$4 billion annually (FAO, 2010). Additionally, Tefera (2012) reported that maize grain postharvest losses in Africa are estimated to range 14 to 36% from harvest to consumer market. Traditionally maize grain is stored both in- and outdoors by Ethiopian farmers for consumption and to sell in the later months of the year depending on the quantity produced per household.

Post-harvest loss leads to an inadequate food intake and it could be manifested by seed loss, monetary loss, food loss and loss of reputation which in turn affect marketing. Post-harvest losses can be caused by mechanical damage and injury, physiological processes, poor handling, lack of processing, inadequate packaging, poor logistics and sub-optimal storage conditions (Chakraverty *et al.*, 2003). Some estimates would suggest the magnitude of post-harvest losses in Ethiopia to be tremendous; for example depending on the type of post-harvest handling method, the losses could range between 5 and 19% for maize, 6 and 26% for millet, 6 and 23% for wheat, and 5 and 20% for teff (Dereje, 2000). However, around 47 maize diseases were recorded in maize and 25 more diseases were recorded in the past few years which are the major limiting factors of its production in Ethiopia (Carlos, 1984). From those storage disease caused by *Fusarium* spp and *Aspergillus flavus* are the major maize disease in Ethiopia (Tegegne *et al*., 2009). Maize cultivation in the world is limited by diseases which cause grain loss of about 11% of the total production (Suleiman and Omafe, 2013). Therefore, the objectives of this study were to detect fungal diseases infecting maize seeds and to quantify post harvest losses of maize at storage conditions

Materials and Methods

Description of Study Area

The study was conducted at Jimma University College of Agriculture and Veterinary Medicine in the plant pathology laboratory in 2017. The experimental location was in Jimma zone, Ethiopia which is found at about 345 km from Addis Ababa in South west and lies between 36° 10´ E longitude and 7° 40´ N latitude. The zone has an elevation ranging from 880 to 3360 meters above sea level (m.a.s.l.). The area experiences annual average rainfall of 1000 mm for 8 to 10 months. The temperature of Jimma zone varies from 8-28°C. The average annual temperature is 20°C.

Maize Sampling Method

A total of 5 samples (~1 kg of each samples; two samples from farmer seed and three samples from retailer seed) of maize seeds harvested in 2016 were collected by using random sampling method. Two farmer seeds from near Jimma town Buyo kofele kebele with local name of orome and Mazoria area (Jimma to Agaro road) with the common name of Kenya and from Jimma town three retailer owner bishishe area one kg of maize seed from each were bought. The retailer seed were come from different areas, the one from Limmu area, Nono Benja and Gibe area.

Experimental materials, treatments and design

This experiment was designed to assess postharvest loss of maize seeds and to isolate fungal pathogens associated on maize grain. The treatments of this experiment were five maize samples collected in three replications with completely randomized design (CRD) arrangements.

The Treatment were

1. RS (N) = Retailer seed from Nono Benja area
2. FS (O) = Farmer seed from Buno Kofele Kebele, with a common name Orome
3. RS (L) = Retailer seed from Limmu area
4. FS (K) = Farmer seed from Mazoria area, with a common mane Kenya
5. RS (G) = Retailer seed from Gibe area

Figure 1. Maize Farmer Seeds

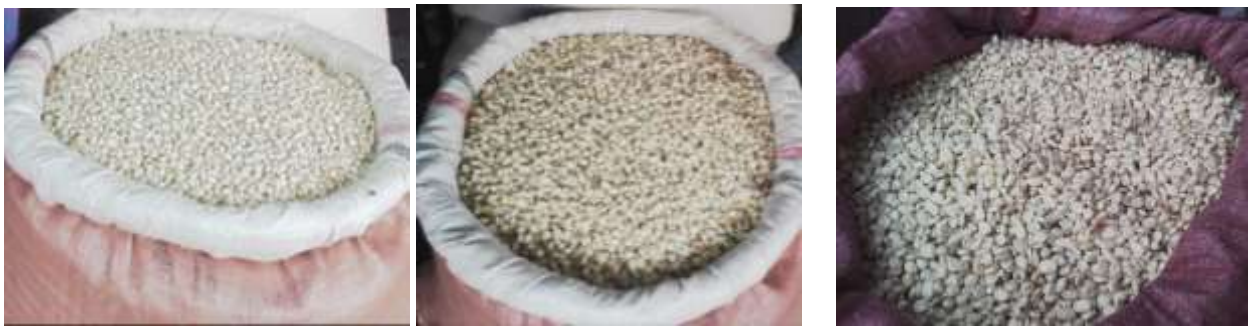


Figure 2. Maize Retailer Seed

Postharvest Loss Assessment

The postharvest losses assessment of the maize seeds were done by taking two hundred seed from each five sample collected and identified (divide) into insect damage, broken (physical damage), mold (color change) and normal seed and finally calculate the total loss.

Figure 3. Postharvest loss assessment of five sample maize grains

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, 1990).

Agar plate method

Thirty maize grains from each sample were surface-sterilized by immersion in 1% sodium hypochlorite solution in perti dish for 1 minute, and washed three times with sterilized distilled water, the grains were dried with sterilized filter paper in a laminar flow hood and placed on potato-dextrose agar medium for sample (10 grain / each plate). After incubation for 5 to 7 days at 25°C, the fungi which grown out from the grains were sub cultured to another medium PDA, then incubated for 5 to 7 days. Finally 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 producers of agar plate were followed. For this method, a total of thirty maize grains in three replication were used. Per subsample, 10 kernels were aseptically placed in each blotter plate which is moistened with sterile distilled water. Hence each blotter plats were incubated under ultraviolet light in alternating cycles of 12-h light/darkness for 7 days at 25°C. And then , the fungi which grown out from the grains were sub cultured to another medium PDA, then incubated for 5 to 7 days .Finally identify fungal colonies under microscope, germination percentage of each blotter were recorded .

Seed Germination Percentage

The percentage of kernels that germinated was determined separately for samples from surface 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:

$$\textbf{\textit{Germination Percentage}}\ (\%) = \frac{Number\ of\ seedsgerminated}{Total\ number\ of\ seeds\ plated}\ x\ 100$$

Results and Discusion

The postharvest losses of maize grains were calculated and the overall Maize grain total losses is 32.3% whereas, 20.7% ,2.6% ,and 9.0% was loss of Insect damage, broken seed and mould respectively (table1). The highest postharvest losses were recorded in retailer seed from Limmu area 77.5% loss and almost 68.0% were from insect damage.

Table 1. postharvest losses of maize grain

No.	Treatment	Insect damage		Broken seed		Mould		Normal seed		Total Loss
1	RS (N)	36	18%	11	5.5%	21	10.5%	132	66%	34%
2	FS (O)	11	5.5%	0	0	17	8.5%	172	86%	14%
3	RS (L)	136	68%	6	3%	13	6.5%	45	22.5%	77.5%
4	FS (K)	17	8.5%	7	3.5%	32	16%	144	72%	28%
5	RS (G)	7	3.5%	2	1%	7	3.5%	184	92%	8%
	Total Mean		20.7		2.6		9.0		67.7	32.3

In this experiment, germination of the five sample maize grain which was treated with surface disinfection at 7day incubation on blotter test. The highest seed germination was recorded on FS (K), RS (G), RS (N) and FS (O), 93.33%, 90.0%, 86.67%, and 83.33% respectively, whereas the lowest germination 50 %was recorded on RS (L) (Table 2). The total fungal colony and seed viability were showed negative relationships. As total number of fungal colony were increased seed viability were also decreased.

Table 2. Germination percentage of maize grain

No.	Treatment	No. seed germinated /replication						Germination %
		R1		R2		R3		
1	RS (N)	8	80%	8	80%	10	100%	86.67
2	FS (O)	9	90%	8	80%	8	80%	83.33
3	RS (L)	5	50%	4	40%	6	60%	50
4	FS (K)	9	90%	10	100%	9	90%	93.33
5	RS (G)	9	90%	9	90%	9	90%	90.0

Incidence and Frequency of fungal species

Incidence of fungal infection on each sample of Agar plate Method was calculated by using the following formula:

$$\textbf{\textit{Incidence Prerentage}}(\%) = \frac{Number\ of\ infected\ seeds}{Total\ number\ of\ seeds}\ x\ 100$$

A total of 195 fungal Isolate were recorded from the five samples collected and 100% incidence were recorded from the entire sample collected in agar plate method (Table 3). In a single seed per petridish there were many fungal isolates recorded. Whereas in blotter method a total of 153 fungal Isolate were recorded, except FS (O) farmer maize seed sample that records 80% incidence, and the other entire records 100% incidence (Table.4).

Table 3. Number of fungal colony in Agar plate methods

No	Treatment	Number of fungal colony												Total
		R1				R2				R3				
		Fusa.spp	*Asp* spp	*Peni.* spp.	Oth spp.	*Fusa* spp	*Asp* spp	*Peni* spp.	Oth spp.	*Fusa* spp	*Asp* spp	*Peni* spp.	Oth spp.	
1	RS (N)	6	5	2	1	6	3	1	1	7	5	2	0	39
2	FS (O)	4	5	0	1	4	3	3	1	5	4	2	1	33
3	RS (L)	7	5	4	0	3	6	3	2	6	4	3	2	45
4	FS (K)	5	3	2	0	8	2	3	1	8	4	2	2	40
5	RS (G)	4	5	3	0	5	3	3	2	7	4	2	0	38

Fusa.spp = *Fusarium* spp. Asp.spp. = *Aspergillus* spp. Peni. Spp. = *penicillium* spp. Oth spp. = Other species.

Table 4. Number of fungal colony on Blotter Method

No	Treatment	Number of fungal colony												Total
		R1				R2				R3				
		Fusa.spp	*Asp* spp	*Peni.* spp.	Oth spp.	*Fusa* spp	*Asp* spp	*Peni* spp.	Oth spp.	*Fusa* spp	*Asp* spp	*Peni* spp.	Oth spp.	
1	RS (N)	3	5	2	1	4	5	1	1	2	5	2	0	31
2	FS (O)	2	2	0	0	3	4	1	1	3	5	2	1	24

3	RS (L)	4	5	2	1	4	5	2	1	4	4	3	2	37
4	FS (K)	4	3	1	1	5	2	2	2	5	2	1	2	30
5	RS (G)	5	2	1	2	4	5	2	0	3	5	2	0	31

Fusa.spp = *Fusarium* spp. Asp.spp. = *Aspergillus* spp. Peni. Spp. = *penicillium* spp.
Oth spp. = Other species

For each fungus, the proportion of samples that yielded its isolates were determined and expressed as percent by using the following formula:

$$\boldsymbol{IF}\ (\%) \quad = \frac{Number\ of\ samples\ of\ occurence\ of\ fungal\ species}{Total\ Number\ of\ samples} x100$$

where: **IF** = Isolation Frequency percentage

In the laboratory work the highest frequency were *Fusarium* spp. (70.0%) on farmer preserved seed kernels from Mazoria area followed by (63.33%) on retailer seed come from Nono Benja area. The highest frequency of *Aspregilus* spp. on retailer seed come from Limmu area (50%) and followed by (43.33%) on retailer seed come from Nono Benja area which is on agar plate test. *Penicilium* Spp. (33.33%) was recorded at retailer seed from Limmu area (Table 5). Moreover, *Fusarium* spp. were the highest fungal frequency of the three major storage pathogens of maize seed which recorded 56.66 % followed by *Aspergillus* spp. 40.67% and the lowest frequent pathogen was *Penicillium* spp. with 23.33%.

Table 5. Isolation frequency of fungal species on agar plate methods

No	Treatment	Number of fungal colony							
		Fusarium spp.		*Aspergillus* spp		*Penicilium* spp.		Other spp.	
		Fungal colony	% IF	Fungal colony	% IF	Fungal colony	% IF	Fungal colony	% IF
1	RS (N)	19	63.33	13	43.33	5	16.67	2	6.67
2	FS (O)	13	43.33	12	40	5	16.67	3	10
3	RS (L)	16	53.33	15	50	10	33.33	4	13.33
4	FS (K)	21	70	9	30	7	23.33	3	10
5	RS (G)	16	53.33	12	40	8	26.67	2	6.67
	Mean		56.66		40.67		23.33		9.33

In Blotter test method almost all treatments were recorded *Aspregilus* spp was the highest frequency, except farmer seed from Mazoria area (Trt 4), then followed by *Fusarium* spp. and *Penicilium* spp. respectively (Table 6). In this laboratory work over all mean of the highest fungal frequency was *Aspregilus* spp. with 39.33% followed by *Fusarium* spp.and *Penicilium* spp. with 36.67% and 16.0% respectively.

Table 6. Isolation frequency of fungal species on Blotter methods

No	Treatment	Number of fungal colony							
		Fusarium spp.		*Aspergillus* spp		*Penicilium* spp.		Other spp.	
		Fungal colony	% IF	Fungal colony	% IF	Fungal colony	% IF	Fungal colony	% IF
1	RS (N)	9	30.0	15	50.0	5	16.67	2	6.67
2	FS (O)	8	26.67	11	36.37	3	10.0	2	6.67
3	RS (L)	12	40.0	14	46.67	7	23.33	4	13.33
4	FS (K)	14	46.67	7	23.33	4	13.33	5	16.67
5	RS (G)	12	40.0	12	40.0	5	16.67	2	6.67
	Mean		36.67		39.33		16.0		10.0

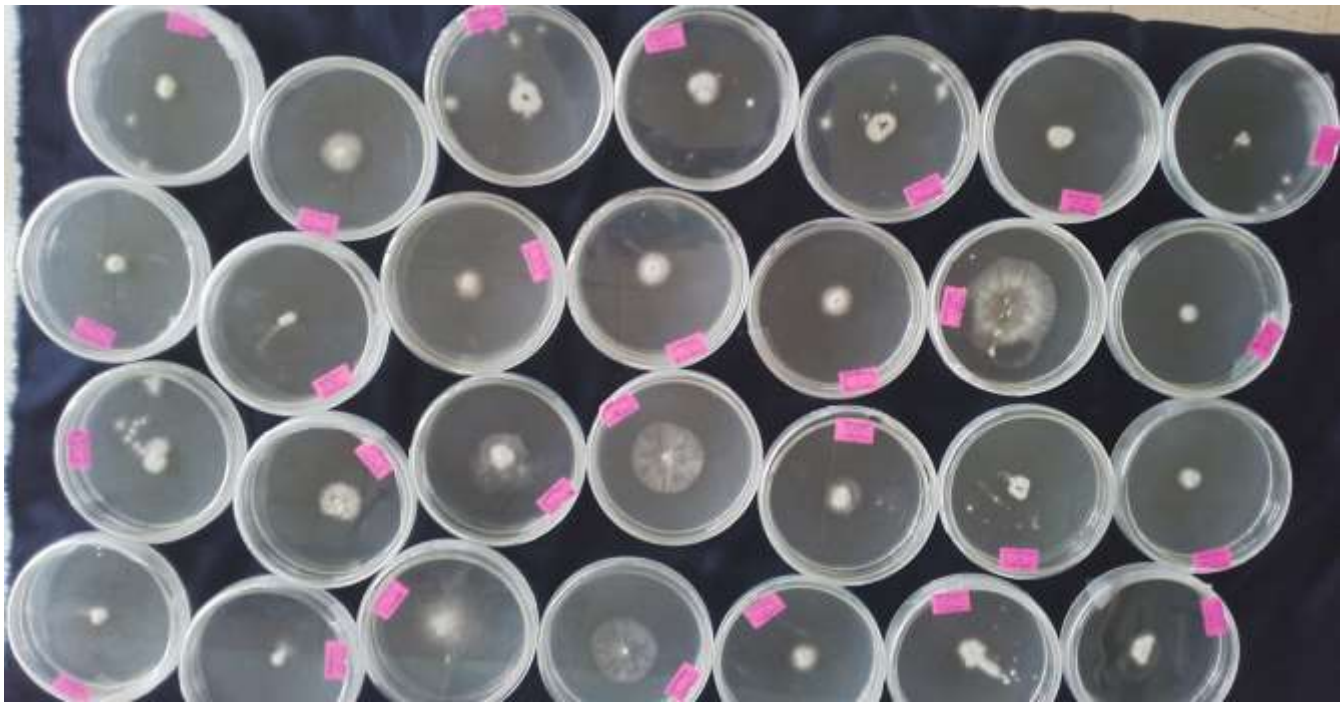


Figure 4. Recultured Isolates of two days after plated

Figure 5. Recultured Isolates of five days after plated

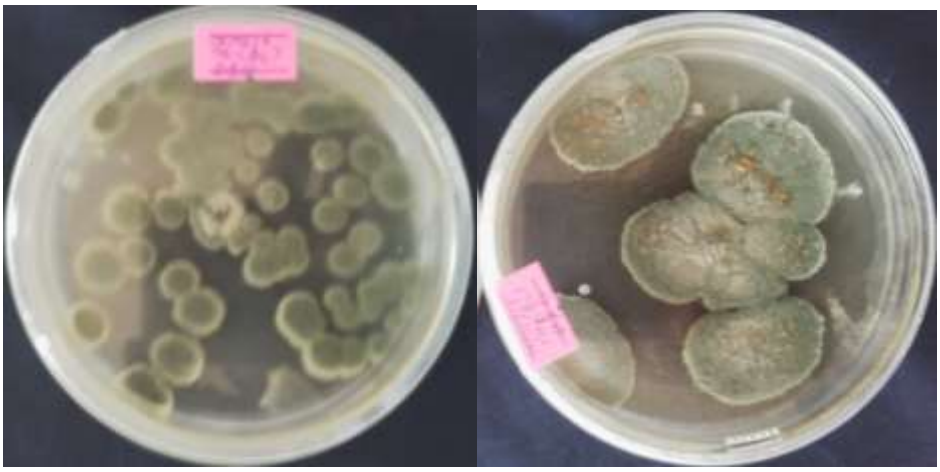


Figure 6. *Penicillium* spp.

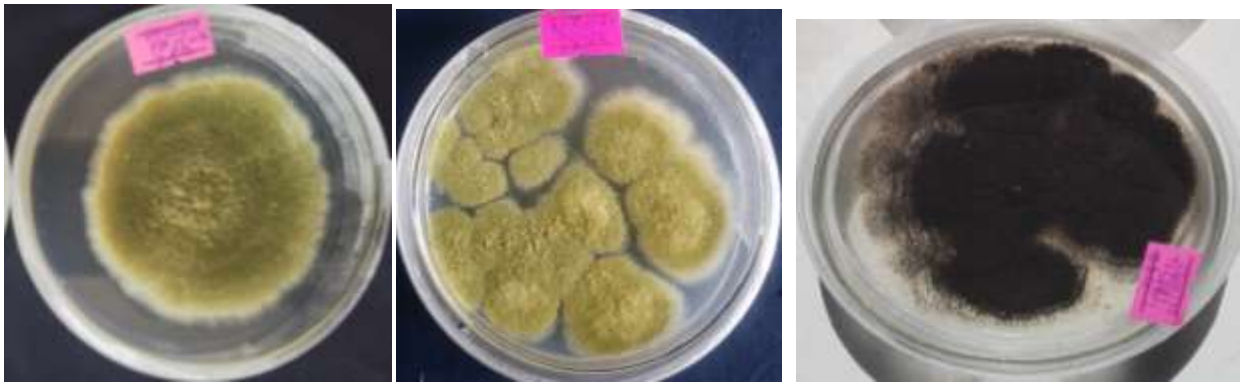


Figure 7. *Aspergillus* spp.

Figure 8. *Fusarium* spp.

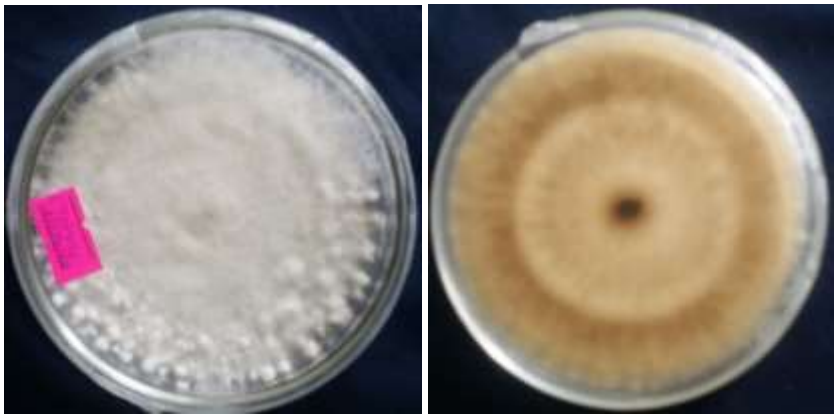


Figure 9. *Alternaria* spp.

Those fungal species indentified

Table 7. Morphological characteristics of fungal isolates from Maize Seed

Fungal Species	Texture	Vesicle shape	Colony color	Conidia shape
Apergillus niger	Smooth	subglobose	Brown to black	globose
Apergillus fumigatus	Smooth	clavate	Green	Short columnar
Apergillus flavus	Rough	radiate	Yellow to green	radiate
Fusarium oxysporum	Rough	Oval to cylindrical	White to purple black	intercalated
Penicillium spp.	Rough	Oval and circular	Grey to green	intercalated
Alternaria alternate	Rough	irregular	White	irregular

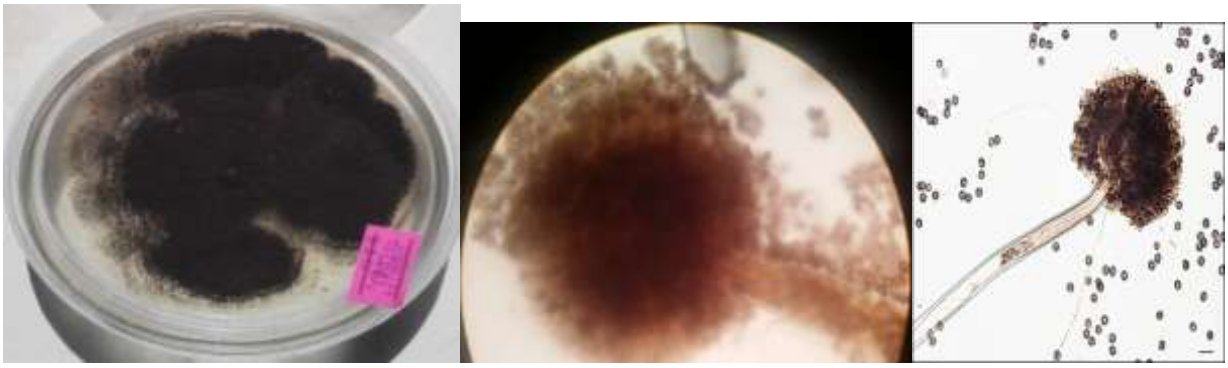


Figure 10 *Aspergilus niger*

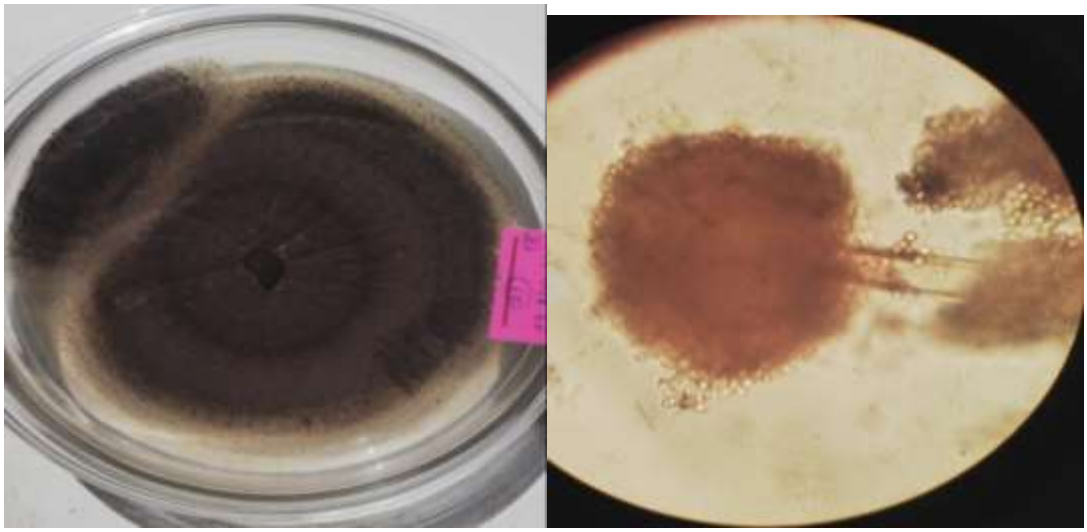
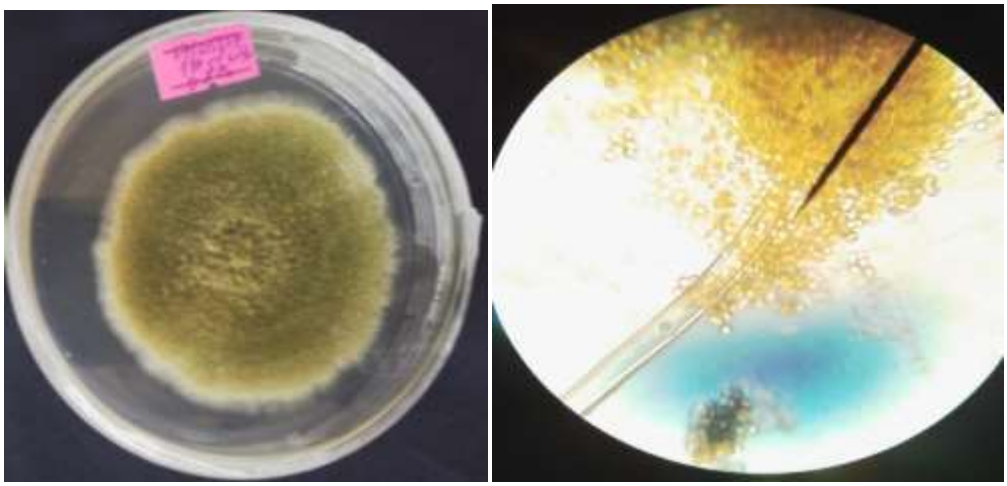


Figure 11. *Aspergillus fumigatus*



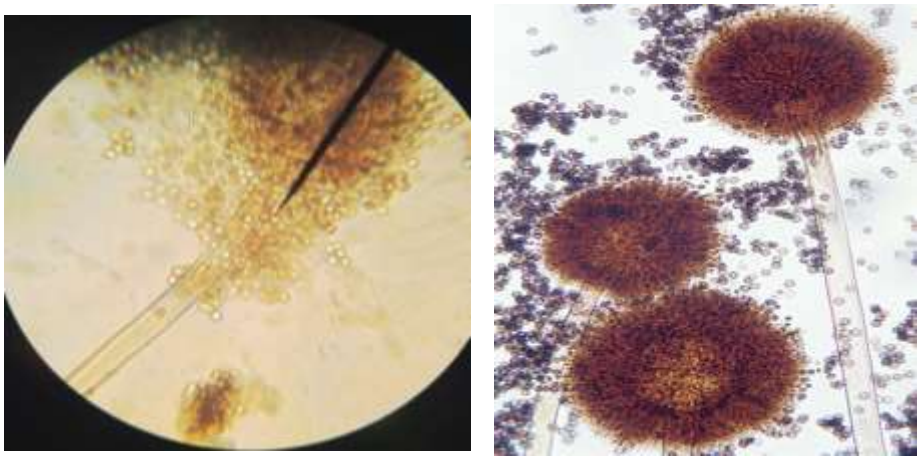


Figure 12. *Aspergillus flavus*

Figure 13. *Fusarium oxysporium*

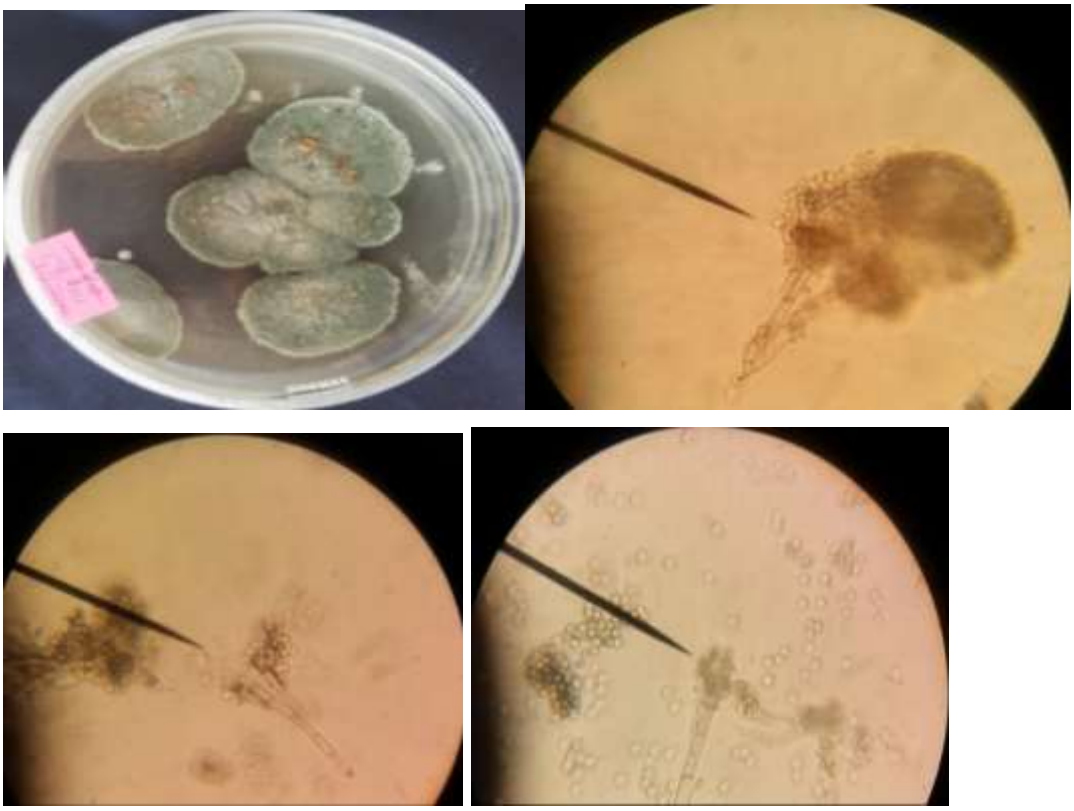


Figure 14. *Penicillium* spp

Figure 15. *Alternaria alternate*

According to the the study three major genera of fungi associated with stored maize seed were identified, those are:- *Fusarium, Penicillim, and Aspergillus*. The result agrees with Tsedaley and Adugna (2016), Ayalew (2010) and Camila *et al.* (2015) reports, which are the major genera commonly encountered on maize grain in tropical regions are *Fusarium, Aspergillus* and *Penicillium*. The result showed that, the genera *Fusarium* was the most frequently found and the genera *Aspergillus* was the second most diverse. This result agreed with Camila *et al.* (2015) reports the *Fusarium* genera was most frequently found but Tsedaley and Adugna (2016) reports the genera *Aspergillus* was most frequently found followed by *Fusarium*. According to the study *Fusarium* spp. were the highest fungal frequency of the three major storage pathogens of maize seed recorded. In the agar plate were recorded 56.66 %, 40.67% and 23.33%, *Fusarium* spp., *Aspregilus* spp. and *Penicillium* spp. respectively. In Blotter method the highest fungal frequency was *Aspregilus* spp. with 39.33% followed by *Fusarium* spp.and *Penicilium* spp. with 36.67% and 16.0% respectively. According to Ayalew (2010) reports fifteen species of fungi were identified from the maize samples. *Aspergillus* was the most frequent fungi, occurring in 94% of

the samples. *Fusarium* spp. occurred in 76.5% of the samples while *Penicillium* spp. was found in 64% of the samples. According to Dubale *et al*. (2014) reports the most common fungi such as *A. flavus*, *A. niger, D. halodes* and *F. oxysporum* recorded in almost all the four districts (Omo Nada , Kersa, Tiro Afeta and Sekoru) throughout 6 months of storage period. 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 (Dubale *et al.*, 2014). 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. Therefore, further studies are needed for identification of the major storage fungal pathogens that greatly affect viability of maize seeds and their management options.

Conclusion and Summary

The result of this study showed that all maize seeds collected from different storage conditions were highly infested with a number of fungal seed borne pathogens. Those fungal isolated can affect germination capacity of the seed and fungal diseases isolated may be highly hazardous as certain species of fungus produce mycotoxins, which are poisonous substances produced by moulds during their growth and development. Mycotoxins are highly stable compounds that cannot be destroyed through food processing, and the only way to avoid them is to prevent the fungal growth and proper storage conditions. Pre and postharvest handling practice reduce the infestation of fungal pathogens associated with maize grain. Seed selection is useful in improving seed germination and reducing the seed borne of most fungal pathogens of maize. *Aspergillus* species were the most dominant (highest frequency) storage fungi followed by *Fusarium* spp, *penicillium* spp. and *Alternaria* spp. were identified in the study. Those fungi are the most important accurate detections and identifications along with viability tests before supplying seeds to farmers are crucial in order to develop an appropriate management strategy. There is a great gap of experiments on stored maize seed pathogens. Therefore, giving attentions to those pathogens associated with postharvest fungal diseases may play a vital role in the reduction of molds and mycotoxins development.

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