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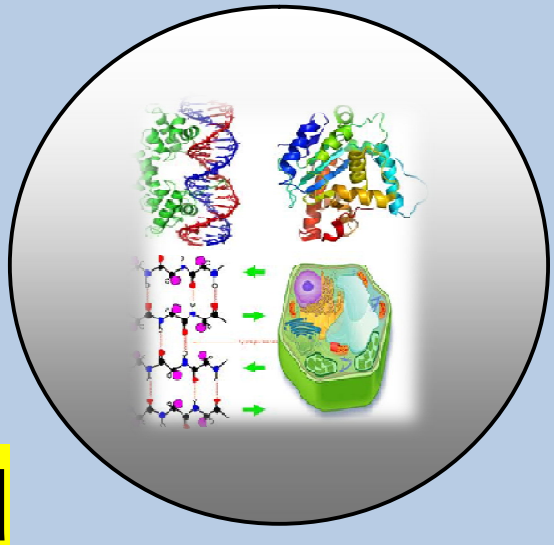

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RESEARCH PAPER

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Detection of Toxin Producing Fungi on the Corn Kernel during Storage at Jimma and its Surrounding District

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ABSTRACT

Corn ear before harvest and during storage were infected by different fungi. The current study was aimed to evaluate the species of corn spoiling fungi that produce toxin and determination of the factors that contribute for corn deterioration at storage. The results of this studys howed that, 288 fungi species were isolated, which were belonging to 7 different geneses. Species of Aspergilus (105 to 125%) and Penicillium (76to 90.5%), Fusarium (43 to 51.2%) including dominant fungi were identified as internal and surface mycoflora of the analyzed sample. Alternaria spp. (28.1 to 33.3%) and Rhizopus (19 to 22.6%) genus as internal and surface mycoflora were the next abundance among other species. Based up on the media type used, Aspergilus spp had the highest occurrence of 43 (35.5%), 21 (33.3%), 41(39.4%) while Penicillium spphad the frequency 34 (28.1%) 12 (19.0%), and 30(28.8%) on primary isolate media czapick dox agar (CDA), potato dextrose agar (PDA), and sabouraud dextrose agar (SDA) respectively in corn kernel. The frequency of Alternaria and Rhizopus reduced in corn kernel to11 (9.1%), 10 (15.9%) and 7 (6.7%) CDA, PDA and SDA while the rest spp varies in number based up on the media type. The risk of mold and contamination of the corn kernel depends on the complex interaction of several factors which include moisture content, temperature, fungal species composition and their interaction with different organisms.

Key words: Spoilage, Alternaria spp, Penicillium spp. and Fusarium.

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INTRODUCTION

Maize (*Zea mays* L.) is a cereal crop grown at hot and rainy area throughout the world **(**Abedi-Tizaki and, Sabbagh2011, Fandohan, *et al*., 2003). Maize plays an important role in the diet of millions of African people due to its high yields per hectare, its ease of cultivation and adaptability to different agro-ecological zones, versatile food uses and storage characteristics **(**Fandohan, *et al*., 2003).The corn has an important food sources for both human and animal nutrition (Abdullah, *et al*., 2000 and Saleemi *et al., 2012).*Corns are economically very important to get money, food supply systems and imperative as raw material for feed production.

Numerous reports in different literature indicate that corn at the time of harvest and storage often contains various fungi alive within the kernels (Hesseltine and Bothast, 1976). So far we have been almost exclusively concerned with the negative roles. Fungi influence our lives in many ways (Lee and Magan, 2000). Some genera of moulds are able to produce mycotoxins that contaminate raw materials, feed or food. Cereals, before being consumed as food, go through the processes of cultivation, harvesting, drying, preparation and marketing (including storage) all under natural conditions, and therefore, often involve microbiological contamination and infection(Abdullah, *et al*.,2000) . Similarly, the parasitic fungi cause serious diseases in crop plants and also pose hazards to animals and humans whenever they infect consumable crops (Ogaraku, 2010).In addition, most consumable crops are disposed to fungal infection. The most prominent types of fungi attacking commodities are species of *Aspergillus*, *Penicillium* spp known in toxin production and *Rhizopus*, etc (Ogaraku, 2010).

Microbial crop pathogens including fungal pathogens have the ability to infect a few or a wide range of serial species causing varying magnitude of quantitative and qualitative losses in crops cultivated in different ecosystems. Global losses caused by crop diseases have been estimated to range from 9% to 14.2% of potential yield (Orke *et al.* 1994). The estimates of losses made later indicated that about 14.1% of produce may be lost due to crop diseases with a monetary value of $220 billion per annum, the developing countries suffering more losses compared with developed countries (Agrios, 2005). The loss assessments have been made for different types of diseases with different levels of accuracy. However, irrespective of the levels of accuracy, the estimates of losses emphasize the imperative need for measures to be taken urgently to avoid the losses to the extent possible. To achieve this aim, different mechanisms of crop disease management viz., exclusion, eradication and immunization are adopted to formulate short- and long-term disease management strategies (Narayanasamy 2002). The effectiveness of crop disease management systems depends heavily on the rapid, reliable and sensitive detection of microbial crop pathogens and accurate diagnosis of the diseases caused by the putative pathogen(s) detected in infected crops. The present study is primarily concerned with the detection and identification of toxin producing mold development in ears of corn during storage.

MATERIAL AND METHODS

Study Area and period

The study was conducted in Oromia Regional state, Jimma Zone, Jimma and its surrounding district including Seka, Dedo, Yebu and Serbo (Figure 1) from September 2012 to March 2013. Jimma Zone is located at about 350km from Addis Ababa, and it is bordered on the south by the Southern Nations, Nationalities and Peoples Region (SNNPR), in the northwest by Illubabor Zone, on the north by East Welega, and on the northeast by west Shewa Zone of the Oromia Regional State. It covers a total area of 18,412.54 square kilometers; and lies within 7 45'N Latitude and 35 ^{0} 30'E – 37 ^{0} 30'E Longitude. The altitude of the area ranges from 1300m to 2100m. The mean annual rainfall of the area is between 1800 mm to 2300 mm with maximum rainfall between months of June and September. The annual mean temperature of the area is between 15 20 ^{0} C and 22 ^{0} C. Based on figures from the CSA in 2005, the zone has an estimated total population of 2,773,730, of whom 1,382,460 are men and 1,391,270 are women; 340,666 or 12.3% of its population are urban dwellers (CSA, 2005).

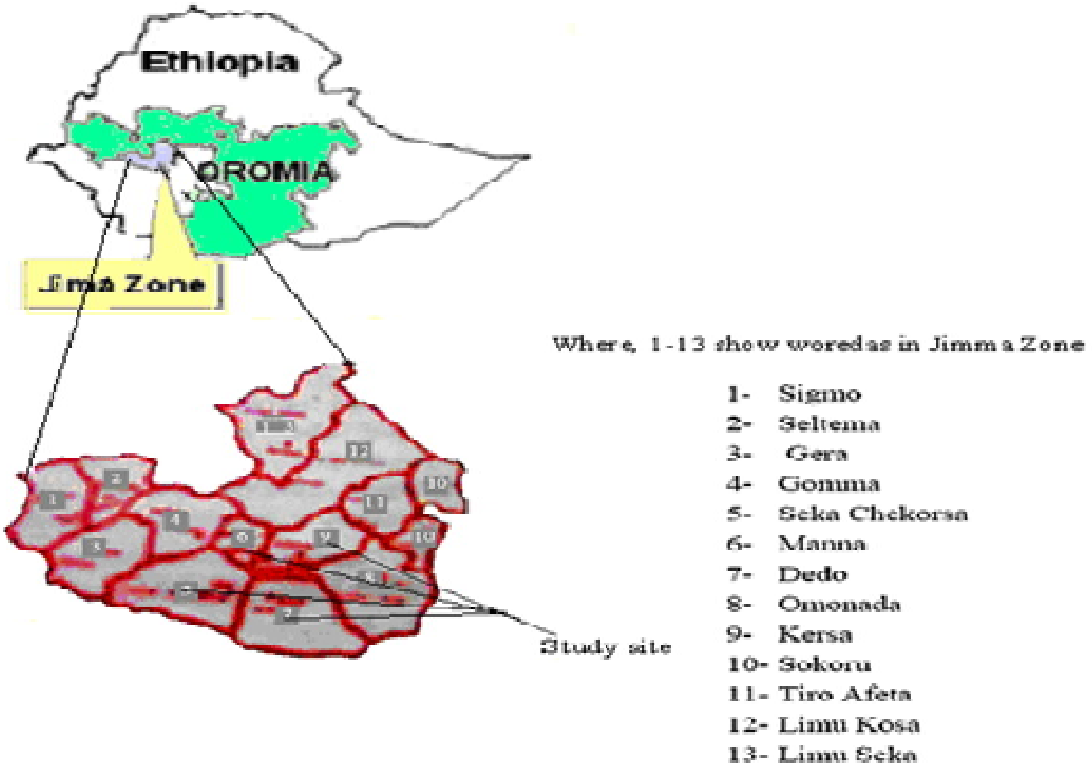

Figure 2. Study area map: The figure shows that Ethiopia and study area, Jimma town and its surrounding district where sample collection took place for this study (Reshad and Ketema, 2011).

Mycological analysis

The Corns from the store of 42farmers were collected from and around Jimma town and transported to Jimma University, Natural Science College, and Department of biology, Microbiology and parasitology laboratory. Mycological analysis was done according to (Hesseltine and Bothast, 1974).A total of 84 ears of corn samples were collected. Immediately upon arrival of the ears at the laboratory, 10corn kernels were removed with sterile tweezers and placed on Potato dextrose agar (PDA), Czapek dox agar (CDA), sabouraud dextrose agar (SDA)in a Petri dish. The same procedures were repeated to remove kernels from the center, middle and butt region of the ear in all. 10 kernels and silks were examined from each of 84 ears. A total of 2520kernels weretested for mold infection.

Fungi identification

The growth on PDA, CDA, and SDA was examined critically after one week using prepared microscopic slides. Identification of fungi was made on the basis of their colony characters (diameter, color, fluorescence and texture of colonies) by growing on potato dextrose agarand microscopic characteristics on slide cultures with prepared spaceman mounted on Lacto Phenol cotton blue and identification of the fungal species with the aid of binocular compound microscope (40 xs) identification keys adopting using (Raper and Fennel, 1965; Brug*eet al*. 1977, Klich and Pitt, 1988; Singh *et al.*, 1991). Frequency, Dry matter loss and the moisture contents of the corn were evaluated (Mostafa, *et al*., 2011).

Respiration and Dry Matter Losses

Grain itself and the microbial contaminants respire slowly when stored dry. The dry matter loss was evaluated according to (Christensen and Meronuck, 1989).

Moisture Content

The absolute moisture content corn was measured (Lee and Magan, 2000) by collecting 14 different cornears from a single storage and the procedure is repeated similarly. Ears were split in half; kernels from the tip section of each of 10 ears were shelled and combined to form a sample. Similarly, samples of the butt fraction of ears were combined to form a sample. This resulted in 84 sample (10-11 corn ear; 8dates; tip versus butt) of whole kernel corn that were assayed for weight loss when dried in forced-draft ovens maintained at 105^{o}C for 24 hours (to assure full removal of heat volatile substances). In addition, vacuum oven drying at 105 C for 24 h and 135 C for 8 h was tested initially, but testing by these procedures was discontinued due to extensive condensation inside the vacuum chamber and sample charring problems, respectively

Storage temperature

The storage temperature and relative humidity was measured using thermometer during each sampling day.

Seed germination test

Seed germination test was conducted using standard procedures of ISTA 2006. The germinated seed were counted and percentage germination would be calculated.

% Germination = NO. Of germinated seed/ Total NO. of seed planted *100

The determination of differences between frequencies of fungi genera were done by Chi Square test, Also a data statistical analysis were performed by SAS (Statistical Analysis System) software.

RESULTS AND DISCUSSION

Filamentous fungi are ubiquitously present in our surroundings and they have both beneficial and harmful effects various places in nature. Fungi usually come from spores, which act like "seeds"(Fandohan *et al*.2003) of the fungus. Spores are microscopic, dust like particles that are almost everywhere in the environment (Limonard, 2007). The kernel spoiling fungi were isolated and identified as (Figure 2) based on their morphology and physiological activities. Because they are so widespread, it is impossible to prevent most fungi from inoculating the grain at some point in the production cycle, so the best strategy for preventing contamination is to avoid conditions that would allow these spores to germinate and grow.

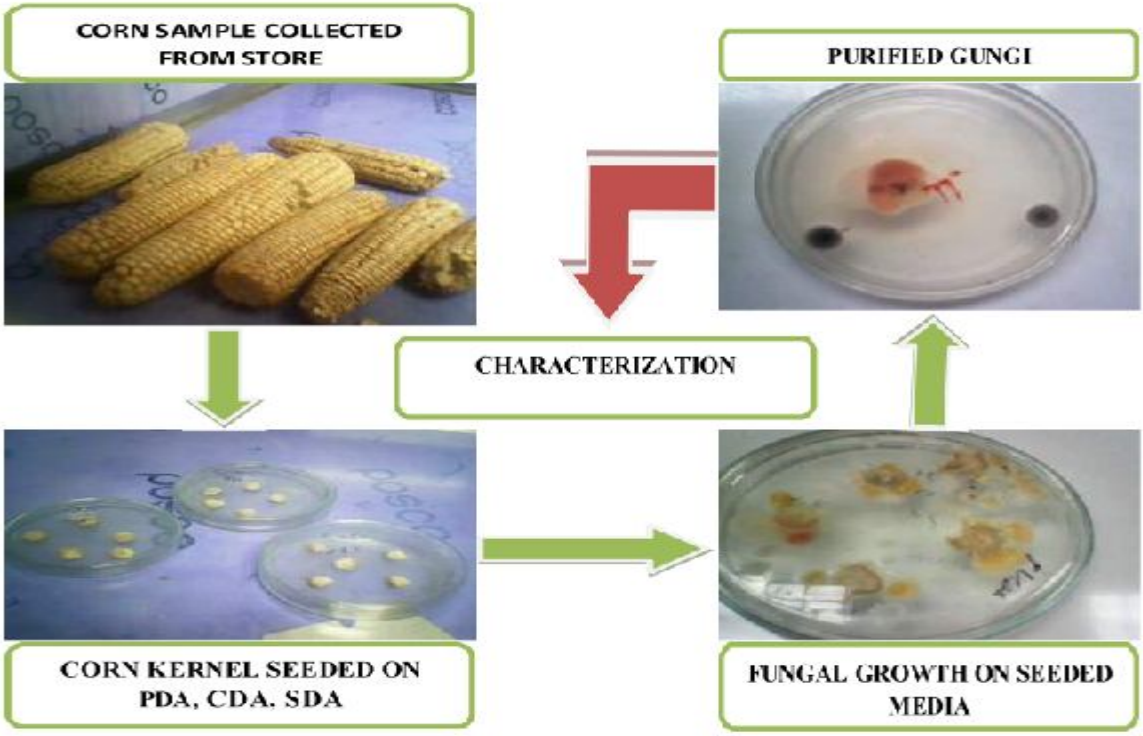

Figure 2. Diagrammatic illustration for fungal isolation and characterization of corn sampleCollected from the store.

Table 1. Fungal corn pathogen identification based upon morphological and physiological characteristics.

Corn sample	Colony Morphology	Microscopic morphology and other observed characters.	Presumptive Genus
1	Cottony, pink, purple, brown colonies	Conidiophores hyaline, simple, short or not well from hyphae, bearing spore masses at the apexes. Conidia	*Fusarium* spp
2	Greenish or blue green colonies	Conidia in long chains on repeatedly branched conidiophores resembling a brush like head. Conidiophores smooth, relatively Short. Penicillia mycelia arranged very irregular and asymmetrical with Branches of various lengths.	*Penicillium*sp
3	Dark greenish to black colony	Conidiophores pale brown, erect, , branched,2–3 times, at the apical parts, bearing catenulate conidia in each branch. Conidia ,blastosporous,often not well differentiated ,from branches, hyaline or pale brown, ovate, ellipsoidal, cylindrical, subglobose, irregular, in shape, apiculate at one end, often truncate at another end.	*Cladosorium spp*
4	Brown colony	Pycnidiaglobose, usually with cylindrical necks, Acovered with aerial Hyphae, peridium grayish brown. Conidiophores hyaline, simple, gradually tapering toward apexes.transverse septum and indistinct longitudinal patterns, often Mixed with hyaline, immature conidia.	*Diplodia spp.*
5	dark or brown colony	Pycnidiaglobose, brown, usually with cylindrical necks,A Conidiophores pale brown, simple or branched, bearing catenulate conidia at the apex and apical Fertile parts. Conidia catenulate, mostly up to 9 in a chain, often Branched. Conidia, porosporous, , acropetally developed,	*Alternaria spp*
6	Colonies with loose white to yellow mycelium rapidly becoming dark brown to black on the Developmentof conidia. Colonies light green Yellow. At maturity conidia are straw-like and yellow Green.	Black, brownish black, purple brown conidiophores and yellow to green Conidia with dark sclerotia. Microscopically Conidiophores arising from a foot-cell, catenate (Basipetal) conidia on phialides. (1or 2 series) on vesicle	*Aspergillus* sp.
7	White to dark grey colonies, fast growing and filling the petri dish with dense cottony mycelium, producing mass of sporangia	Nonseptate mycelium with root like rhizoids ; black columellate, sporangiophores, in clusters and dark sporangia containing dark to pale spores	*Rhizopus* sp.

The molds were adapted to grow under low and intermediate moisture conditions. Normally invade kernels after harvest (Mostafa and Kazim, 2011).The fungus generate heat and moisture as they grow (Fandohan *et al*.2003).Grain temperature and moisture content determine the allowable storage time (AST) or how long grain can be kept before it spoils (Fandohan *et al*.2003). Notice that as grain moisture content increases for a given temperature, the allowable storage time for drying and storing decreases. Also, as temperature increases, allowable storage time decreases.

Table 2. Frequency of occurrence pathogenic fungi in the corn kernel isolated on different media.

Fungal Records	**Number of isolate % mold corn kernel and media type**		
PDA	**CDA**	**SDA**	
Aspergilus spp.	43(35.5%)	21 (33.3%)	41(39.4%)
Fusarium spp.	16(13.2%)	10 (15.9%)	17(16.3%)
*Penicilliums*pp.	34(28.1%)	12 (19.0%)	30(28.8%)
Alternaria spp.	11 (9.1%)	10 (15.9%)	7(6.7%)
*Cladosoriums*pp.	5(4.1%)	2 (3.2%)	4(3.8%)
*Rhizopus*spp.	9(7.4%)	7 (11.1%)	3(2.9%)
Diplodia spp.	3(2.5%)	1 (1.6%)	2(1.9%)
Subtotal=	**121**	**=63**	**=104**
Total	**=288**		

PDA=Potato dextrose agar, CDA=Czapek dox agar, SDA= sabouraud dextrose agar

The species of filamentous fungi occurring in corn and their frequency at storage was examined by monitoring 42 maize storage over a whole season (Table 1). Viable propagules of filamentous fungi were present in all corn samples at all times during the study. The most frequent species were *Aspergillus* spp and *Penicillium* spp, and *Fusarium* spp. *Alternaria* spp*, Rhizopus* spp, *Diplodia* spp*.* and *Cladosorium spp* occurred less frequently (Table 2 &Table 3).
Fungi (molds) are the major cause of spoilage in corn"(Fandohan *et al*.2003). Losses caused by fungi in cereal grains are related to a decrease in germination, discoloration of the seed, heating and mustiness, biochemical changes, possible production of toxin, loss of weight, loss of nutritive value, Poor milling quality, deterioration in flavor (simpanya, 2001). All these changes may occur without the mold becoming visible to the naked eye. Simpanya *et al*., (2001) supports the idea that infestation of food commodities by insect and fungal is common problem due to some agricultural practices that lead to fungal contamination. The Jimma zone hot temperature and the moisture contents of the corn storage texture favor the corn spoilage and toxin production.

Almeida *et al*., (2000) indicate that fungi are worldwide microorganism, although tropical climates favor the growth of toxigenic species on agricultural products, with consequent risk of mycotoxin contamination.

While the grain is still in the field molds invade kernels. The field molds cause the discoloration of cereal grains often observed in plants exposed to very moist weather during storage. Hesseltine and Bothast, 1977 reports indicate that corn at the time of harvest and storage often contains various fungi alive within the kernels.

Table 3.Isolation frequency and relative density of fungal genera isolated from corn samples (n=84) in Jimma town south western Ethiopia, in 20013.

Isolate	Number of Isolate	Isolate frequency (%), n=84	% Relative density Based upon total isolate(n=288)	Type
Aspergilus	105	125	36.5	
Fusarium	43	51.2	14.9	
Penicillium	76	90.5	26.4	
Alternaria spp	28	33.3	9.7	
Cladosorium	9	10.7	3.1	
Rhizopus spp	19	22.6	6.6	
Diplodia	6	7.1	2.1	
Mucor spp.	3	1.4	1.1	

In addition to affecting grain appearance, the mold may cause a decrease in the germination of the grain seeds. Storage mold damage is completed by the time the grain water content is decreased. Once the grain is dried, these molds die or become inactive. Despite the fact that over 2,520 individual kernels of corn were examined during this study. In this recent study, for detection of corn spoiling fungi at storage from Jimma and around woreda Southwestern Ethiopia; a large number of the surface-sterilized kernels showed the presence of different fungal species (Fig.2). The (Table 1, 2 &3) give the overall picture of the finding of the study, which is isolated fungal species.

Mechanical damage to grain and the amount of foreign material also affects allowable storage time. Clean grain and whole seeds are more resistant to mold (Fandohan *et al*.2003). For long-term storage grain should be dried as soon as possible after it comes from the field. A delay in drying decreases the allowable storage time. In study, means of incidences (Figure 1) *Aspergillus* spp. were the highest (125%), followed by *penicillium* spp. (90.5%), *fusarium* spp. (51.2%), *Alternaria* spp. (33.3%), *Rhizopus* spp. (22.6%) and, *Cladosorium(*10.7%) *Diplodia* (7.1) and *Mucor* spp. (1.4%). According to the reports (Mostefa and kazim, 2011)the means of incidences Fusarium spp. were the highest (35.2%), followed by Aspergillus spp. (2.9%), Penicillium spp. (1.1%), Rhizopus spp. (2.3%), Mucor spp. (1.4%), and Alternaria spp was indicated.

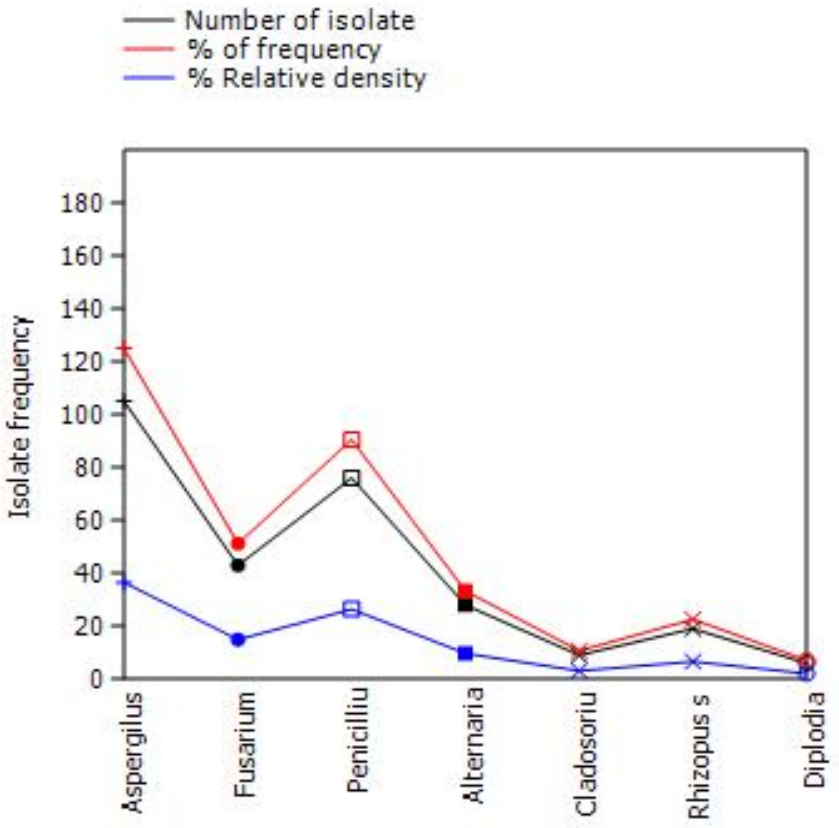

Figure 2. Frequency of seven fungal species during storage time, isolated from the corn kernel at Jimma and it's around woreda south western Ethiopia.

The mean fungal counts of the corn kernel samples were 40.9 (Table 3) and had over 48.62% of the mean frequency. The counts of fungal groups within corn kernel sample vary Significantly (CV=90.6%- 90.70%) (Table 3).

The Fungal spp isolated from 84 corn samples together with their rf frequency of Occurrences are shown in (Tables 1, 2, 3 and 4) and (Figure 2). The isolated organisms are *Mucor* spp., *Fusarium* spp., *Aspergillus* spp., *Rhizopus* spp. *Alternaria spp*, *Cladosorium spp, and Diplodiaand Penicillium* spp.

However, if the water availability is increased to 15-19% moisture content, activity (corn) spoilage fungi, particularly Fusarium spp., Aspergillus and Penicillium species grow, resulting in a significant increase in respiratory activity. This could result in an increase in temperature (Table 5) and sometimes spontaneous heating from the colonisation by a succession of fungi resulting in colonisation by thermophilic fungi and actinomycetes (Fleurat-Lessard, 2002; Magan*et al*., 2004).

Table4. Frequency, relative density and incidence of fungi species in maize from Jimma and around districts.

Parameters	Number of isolate	Lower conf.	Upper conf.	% of frequency	Lower conf.	Upper conf.	% Relative density	Lower conf.	Upper conf.
N	7	7	7	7	7	7	7	7	7
Min	6			7.1			2.1		
Max	105			125			36.5		
Mean	40.85714	13.42857	64	48.62857	16.3	76.37143	14.18571	5	22.22857
Std. error	13.99563	9.123119	19.31691	16.66767	10.81616	22.96965	4.865448	3.157908	6.697933
Variance	1371.143	582.619	2612	1944.679	818.9252	3693.232	165.7081	69.80667	314.0362
Stand. dev	37.02895	27.58568	62.64362	44.09852	32.78554	74.19254	12.87277	9.571184	21.5766
Median	28	-20	47	33.3	-23.9	55.9	9.7	-7	16.3
25 prcntil	9	-25	12	10.7	-29.8	14.3	3.1	-8.7	4.1
75 prcntil	76	47	133	90.5	56	158.4	26.4	16.3	46.2
Skewness	1.011354	-0.3296799	2.761674	1.010661	-0.338471	2.783746	1.015	-0.334257	2.641242
Kurtosis	-0.1340515	-6.073134	2.358513	-0.1369183	-6.128255	2.355291	-0.1257461	-6.117064	2.37867
Geom. mean	26.7721	-1.110385	40.46322	31.83175	-1.929263	48.47921	9.292658	-0.2249009	14.0724
Coeff. var	90.63029	53.06996	134.5355	90.68437	53.04701	134.6003	90.74457	53.07843	135.0073

Table.5. physical characteristic of corn sample used for isolation of fungal pathogen.

Sample examined / week	120 kernel Wet mass	120 kernel dry matter	% of moisture content	T^o of storage Material
Sw1=11	34.8	32.2	8.1	27
Sw2=10	32.3	28.7	12.6	29
Sw3=11	35.6	31.1	14.5	28
Sw4=11	34.4	29.6	16.2	30
Sw5=11	36.2	33.1	9.4	29
Sw6=10	35.1	32.1	9.35	29
Sw7=10	37.3	33.4	11.7	29
Sw8=10	35.3	31.4	12.4	27
Total=84 corn ear				

Notice

Sw1=sample one Sw2=sample of week two

Sw3=sample of week three, Sw4=sample of week four

Sw5=sample of week five, Sw6=sample of week six

Sw7=sample of week seven, Sw8=sample of week eight

Mold damage of the corn increases while, the moisture of grain at storage were>14%.Germination testing is considered as the most important quality; test in evaluating the planting value of a seed lot. The ability of seeds to produce normal seedling and plants later on was measured in terms of germination test. From forty (40) sowed of the corn kernel, the testing results indicate 3.9% germination in infected when compared to 13.2% in uninfected corn kernel. Laboratory methods then have been regarded where in the external factors are controlled to give the most uniform, rapid and complete germination. Test done at the laboratory conditions indicated that low germination when compared to the uninfected corn kernel.

CONCLUSION

Fungi isolates were identified by their cultural and morphological characteristics. These isolates include *Mucor* spp*, Fusarium* spp*, Rhizopus* spp*, Aspergillus* spp and *Penicillium* spp. In all analyzed samples, the most prevalent genera were *Aspergillus*, *Penicilium*and *Fusarium*, its dominance could have been that the maize was incorrectly managed and dried. Insufficient drying and precarious condition of storage could promote fungal growth as thefungal genera need water for growth. Corn improperly harvested and dried would remain of low quality no matter how well it is stored. In corn grains loss in quality and quantity before and during storage is largely affected by fungi. The development of fungi is influenced by the**:** Moisture content of the stored grain ,Temperature, Condition of the grain going into storage, Length of time the grain is stored, Amount of insect and mite activity in the grain. Fungi (molds) were the major cause of spoilage in corn grain. Losses caused by fungi in corn grains were related to ,a decrease in germination, discoloration of the seed, heating and mustiness, biochemical changes, expectation for possible production of toxins, loss in dry matter, Loss of weight, Loss of nutritive value, Poor milling quality, Deterioration in flavor.

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