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Isolation and enumeration of fungi and determination of contributing factors to fungal spoilage in maize (*Zea mays* L.) originated from east and west Shewa Zones of Oromia, Ethiopia

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ABSTRACT

Fungal spoilage in maize resulted in grain deterioration and mycotoxin contamination. In Ethiopia, maize production is prone to losses due to mold contamination. The purpose of this study was to isolate fungi and determine the contributing factors to fungal spoilage in Maize. 72 maize samples were collected based on incremental sampling method. Fungal isolation was done on Potato Dextrose Agar amended with 0.01% chloramphenicol. Fungal colonies were purified by sub-cultured on PDA. Morphological and microscopic characterization of the isolates was done based on fungal identification manual. From 1080 maize grains, 613 fungal isolates were recorded with 54.1% mean value where the majorities were belongs to the genus *Fusarium*. Grains collected from warehouse were more infected (58.88%) followed by store, open market and household. Overall, the prevalence of fungal infection in the study sites was high. Awareness creation, cumulative integrated management, monitoring, and precautionary measures are recommended.

Introduction

Cereals represent a substrate that facilitates mold growth and contamination. Maize (*Zea mays* L.) is the most commonly contaminated cereal followed by wheat, sorghum, oat, barley and rice (Jelka et al., 2017). Maize is an agricultural crop of worldwide importance grown both for the food industry and for other purposes (Jan et al., 2012). Fungal contamination becomes an issue of special concern during rainy seasons characterized by substantial temperature variations which favor mold contamination and mycotoxin production

(Pleadin et al., 2013). In substantial proportions of maize-producing areas worldwide, the crops are subjected to contamination from mycotoxins (Tomoko et al., 2007; Jelka et al., 2017).

In most parts of Africa, the mycotoxin hazard is very high because the limited food supply has forced people not to reject any material that can be used as food, even if the organoleptic quality of the food has been changed by molds. The prevailing malnutrition and poverty jeopardize people's susceptibility to very low levels of mycotoxins. This situation is further aggravated

by the warm humid tropical conditions and improper drying and storage practices (Dejene et al., 2004) which provide optimal conditions for mold growth and subsequent build-up of mycotoxins within a short spell of time (Samuel et al., 2011).

In Ethiopia, maize is being grown in different parts of the country (Yibrah and Dereje, 2015). Fungal spoilage is the most retarding factor for maize yield loss in terms of quality and quantity where the occurrence of maize fungal contamination in developing countries gets global attention (Maria et al., 2013). Fungal spoilage jeopardizes both human and animal health (Pleadin et al., 2013). Members of the fungal genera *Aspergillus*, *Fusarium*, and *Penicillium* cause frequent and problematic contamination of foods and feeds (Jolly et al., 2011, Temesgen and Teshome, 2018). These are found in many feeds and foodstuffs especially in plants during pre and post-harvest, transportation, processing and storage and are detected in cereal crops (Ezekiel et al., 2014).

More than 9 million smallholder households grow maize in Ethiopia at present (Tsedeke et al., 2015) where maize production is higher and is the second highest in Sub-Saharan Africa. According to World data atlas, maize production of Ethiopia increased from 2.34 million tons in 1998 to 8.12 million tons in 2017 growing at an average annual rate of 7.57 % (Hadush et al., 2017). In terms of internal distribution of maize production, Oromia (61%), Amhara (20%) and Southern Nations Nationalities and Peoples Region (12%) are the dominant areas of maize cultivation (CSA, 2013). Accordingly, 80% of the maize produced by smallholders is used for household consumption without being processed, 10% for sale and the remaining 10% for seed and other purposes (Rashid et al., 2013). The majority of farmers (more than 93.3%) use traditional maize storage containers that expose their stored grains to attack by storage pests, molds and or other factors. The average actual loss per household is about 12 % of the average total grain production (Dubale, 2018).

In Ethiopia the major contributing factors for high postharvest losses relates with poor postharvest infrastructure and marketing systems, poor research and improvement capability, and insufficiencies in guidelines and information

sharing (Dubale, 2018). However there are limited researches on the prevalence of fungi on maize and its prevention approaches in Ethiopia. Therefore, this study aims to magnify fungal prevalence and determine the contributing factors for fungal spoilage on maize.

Materials and methods

Description of sampling area

Maize samples were collected from four major maize producing districts of East and West Shewa zone of Oromia, Ethiopia (Fig. 1). Bako Tibe and Dano districts from Western Shewa; Adami Tullu and Arsi Negelle districts from Eastern Shewa zone were used in the study. From each district two kebeles and a town were selected for sample collection. The kebeles were Seyu Gambella, Sayoo and Dano Shenen from Dano district; Odda Anshura, Anneno Shisho and Adamu Tulu town from Adami Tulu district; Bako town, Dambi Dimma and Dambi Gobbu from Bako Tibe district and Arsi Negelle town, Hadah Bioo and Rafuu Hargisa from Arsi Negelle districts. All the study areas were selected based on maize production potential.

Sample type, size and sampling technique

The experimental materials used in this study were maize grains collected from twelve different areas. Sample sites were purposefully selected based on maize production potential according to Central Statistical Agency of the country and Oromia regional and zonal agricultural office.

A total of 72 maize samples of 1kg were collected from household, warehouse, open market and store based on incremental sampling method. Each selected districts were represented by two rural kebeles and one town sampling. During sampling process; sample history including, source of collection, storage duration after harvest, means of harvest, moisture content during collection, storage condition (type of storage) and the use of fertilizer and/or compost were recorded. The grain moisture content was measured using an electronic moisture tester (HOH-Express-HE-50, Germany) immediately after collection. Maize samples were packed in polyethylene bag, labeled and stored at 4°C until further analysis.

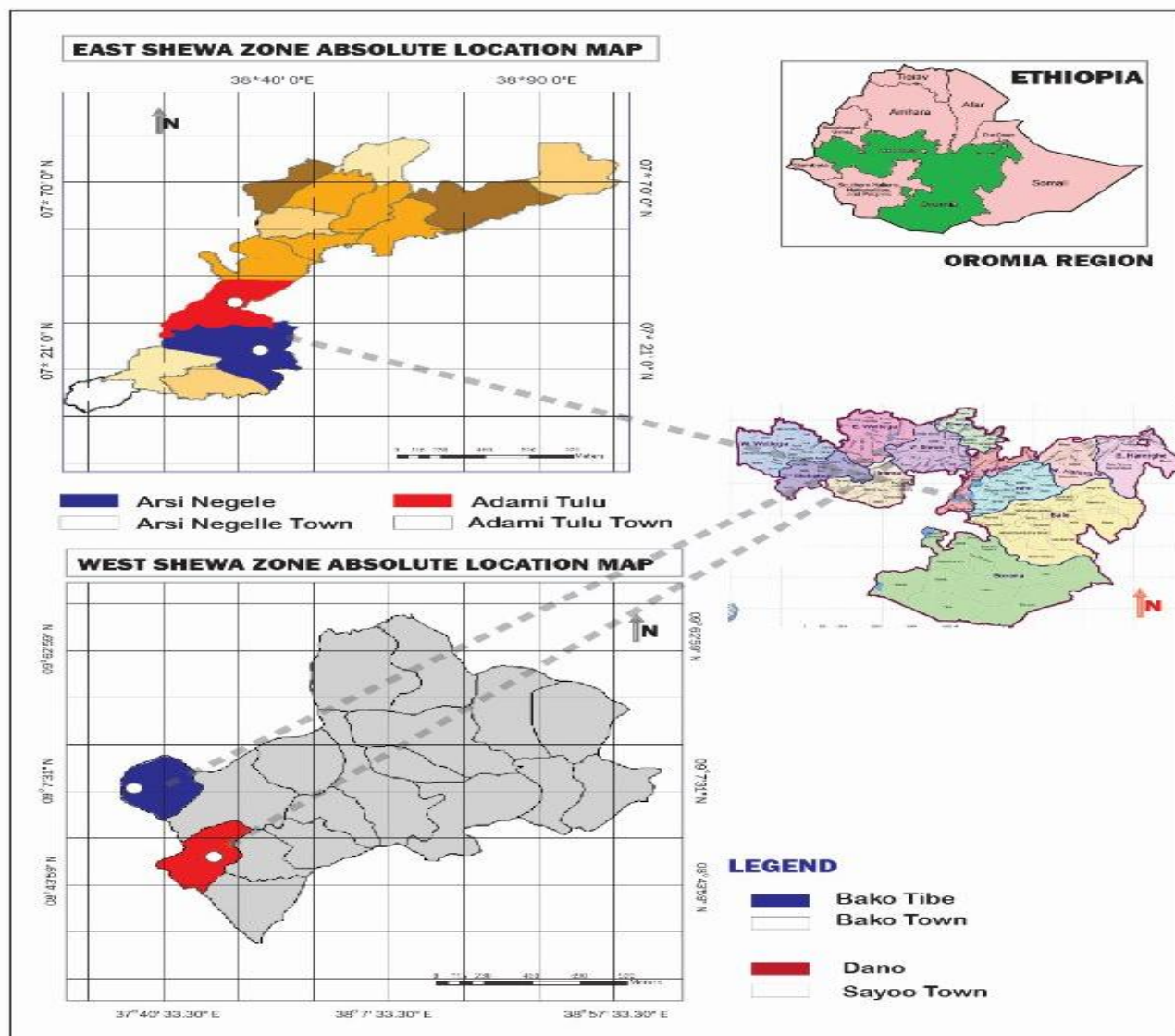


Fig. 1: Map of Ethiopia depicting the stud areas in Oromia regional state.

Fungal isolation and morphological characterization

From each sample, 15 maize grains were randomly selected and surface sterilized by treating with 70 % ethanol for 2 minutes. Maize grains were rinsed three times with sterile distilled water. Potato Dextrose agar (PDA) (Hi-Media Laboratories Ltd. Mumbai, India) medium amended with 0.01% chloramphenicol was used for plating experiments. Five surface sterilized grains were aseptically placed on a Petri dish containing PDA in triplicate and incubated upright at 25°C for 5-7 days. Fungal colonies were picked up with a sterile needle and purified by transferring to PDA medium. The pure

isolates were then transferred and preserved on PDA slant at 4°C.

Fungal isolates were grouped to genus level based on fungal identification manual (Barnett and Hunter, 1998; Sarah et al., 2016). Isolates were observed based on colony growth rates, texture, degree of sporulation, color of mycelia, shape of conidial heads, vesicles, the number of branching points between vesicle and phialides. Iodine glycerol solution (0.5%) and lacto phenol cotton blue dye were used to perform slide culture technique (Patrick et al., 2010). The frequency of fungal infection and total microbial load were calculated according to AOAC (1995).

Data analysis

For statistical evaluation of the data, SPSS software version 20 was used to generate descriptive statistics. Pearson correlation was used to assess the relationship between fungal infection and moisture content of the samples. To determine the influence of the environmental predictive factors on the response factors Kruskal-Wallis test ($\alpha = 0.05$) was performed.

Results and discussion

Fungal isolation from maize

Mycological analysis of the maize samples showed that the overall fungal incidence was 54.1% (Table 1). The result showed the genus *Fusarium* was the predominant (73.08%) followed by *Penicillium* (8.48%), *Muchor* and *Rhizopus* (7.5%), *Aspergillus* (5.38%) and others unidentified (5.54%) (Fig. 2A). Dano district showed higher mean percent of fungal infection (68.15%) followed by Adami Tullu (60.37%), Bako Tibe (50%) and Arsi Negelle (37.78%) (Fig. 2B). West Shewa zone showed higher percent of fungal incidence (59.07%) and also East Shewa zone showed considerable fungal prevalence (49.07%). The result of this study was lower than the finding of Amare (2010) who analysed fungal incidence on maize samples collected from Adama, Ambo and Dire Dawa and reported 94% *Aspergillus*, 76.5% *Fusarium* and 64% *Penicillium* fungal prevalence. However, the result of fungal prevalence obtained in this study is higher than recent findings of Negasa et al., (2019) in Bako, Western Shewa, 39.4% *Fusarium* and 26.7% *Aspergillus* from maize.

Fusarium species associated with maize grain were identified in Ethiopia (Tesfaye and Dawit, 1998). Factors such as 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 maize grains either causing deterioration or simply remain viable to infect germinating seedling (Castellari et al., 2010). Tesfaye and Dawit (2000) founded three species of *Fusarium* (*F. moniliforme*, *F. subglutinans*, and *F. graminearum*) to be highly associated with

maize samples around Shashemene and Alemaya. A study by Yesuf et al., (2015) also reported more than 50% of *Fusarium* contamination in Sorghum, maize, common bean, coffee, mung bean and cowpea in South Omo and Segen Peoples Zone of Ethiopia.

This study best fits with Chemedet et al. (2018), a research conducted in southwestern Ethiopia and the genus *Fusarium*, *Penicillium* and *Aspergillus* were found highly dominant. There is higher *Fusarium* contamination in different parts of the country. This is due to the boundary less distribution of the fungus and survival in different agro ecological locations. Additionally, since *Fusarium* can cause systemic infection to maize it can lead to kernel infection and showed higher prevalence. Moreover, the other storage fungi recorded were attempted due to poor drying before harvest.

Correlation of environmental factors with fungal prevalence

Correlation analysis of moisture content, storage duration and fungal percent of infection showed that moisture content and storage duration were negatively correlated with fungal prevalence and found to be statistically insignificant ($p > 0.05$). Environmental conditions and climatic factors are the most important to the contamination of maize grains before and after harvest. The grain moisture content and temperature potentially affects the growth of mycotoxigenic fungi and spread of infection to the maize grain before and after harvest (Kana et al., 2013). But according to this study the factors are negatively correlated with fungal spoilage and even if there is weak correlation among them, there is considerable fungal incidence recorded. Since the samples were collected after harvest; during storage when there is moisture to the grain, the fungi gets favorable opportunity for growth and specifically plays a role for *Fusarium* contamination.

Maize storage type analysis result revealed that storage using plastic bag showed higher fungal percent of infection (69.78%) followed by gotera (51.11%) and fertilizer bag (49.63%) (Fig. 3). Plastic bags are occasionally used for storage in Ethiopia. The higher percentage of fungal infection observed on maize stored in plastic bag may be due to lack of ventilation and

accumulation of water vapor in the plastic bag which leads to germination of mold on stored maize (Dubale, 2014). Therefore, the reason behind the higher incidence of fungal infection in the plastic bags was due to the lesser air

ventilation. Gotera storage system is also widely utilized in Ethiopia. In this study 51.1% of fungal infection of maize grains was recorded which shows higher prevalence and this was due to poor drying of the grains before harvest.

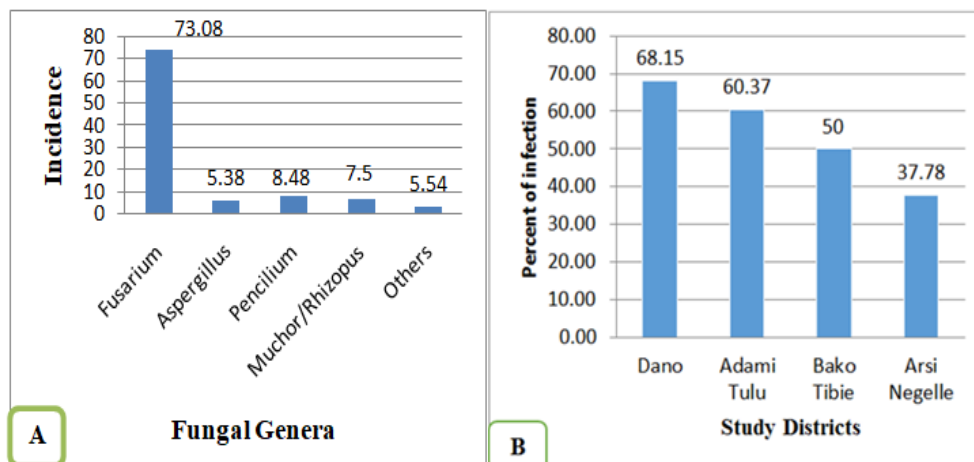


Fig. 2: Incidence of fungal genera (A) and percent of fungal infection on maize in four districts (B).

Table 1. Mean fungal percent of infection on maize sampels obtained from 12 kebeles

S.No.	Kebele	Mean infection	N	Standard error
1	Adami Tullu Town	48.8	6	±3.24
2	Anneno Shisho	60.0	6	±4.66
3	Arsi Negelle Town	46.6	6	±1.40
4	Bako Town	55.5	6	±2.56
5	Dambi Dima	31.1	6	±1.46
6	Dambi Gobu	63.3	6	±2.75
7	Dano Shenen	63.3	6	±3.24
8	Hadah Bioo	33.3	6	±1.31
9	Odda Anshura	72.2	6	±3.73
10	Rafuu Hargisa	33.3	6	±1.72
11	Sayoo	73.3	6	±3.61
12	Seyu Gambela	67.7	6	±2.45
	Total	54.1	72	±3.15

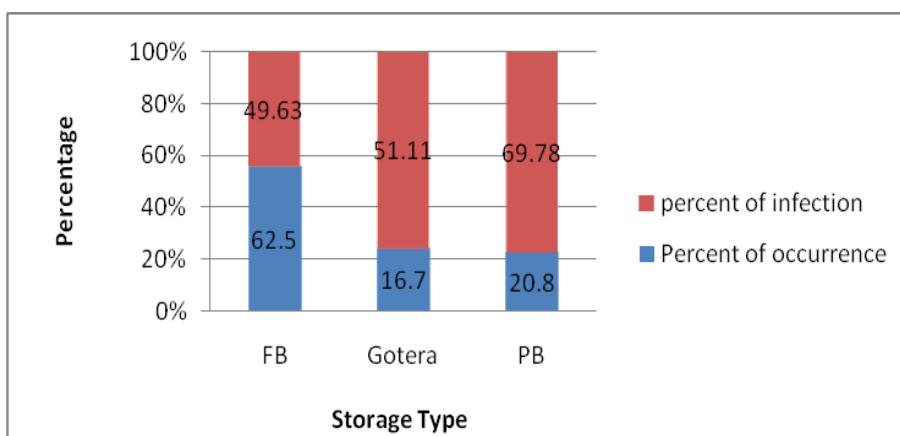


Fig. 3: Maize storage type versus percent of infection (FB= Fertilizer bag, PB= Plastic bag). The percent of occurrence indicates the percentage of the storage types used by the society in the study area.

The maize samples were obtained from four different sources (household, warehouse, open market and store). The grains collected from warehouse were more infected (58.88%) followed by store (55%), open market (54.28%) and household (52.78%) (Fig. 4).

This indicates that there were lesser aeration with higher level of fungal infection in the warehouse and store. Moreover in the open market the grains have higher probability of contact with dust, soil and dung which also favors fungal growth.

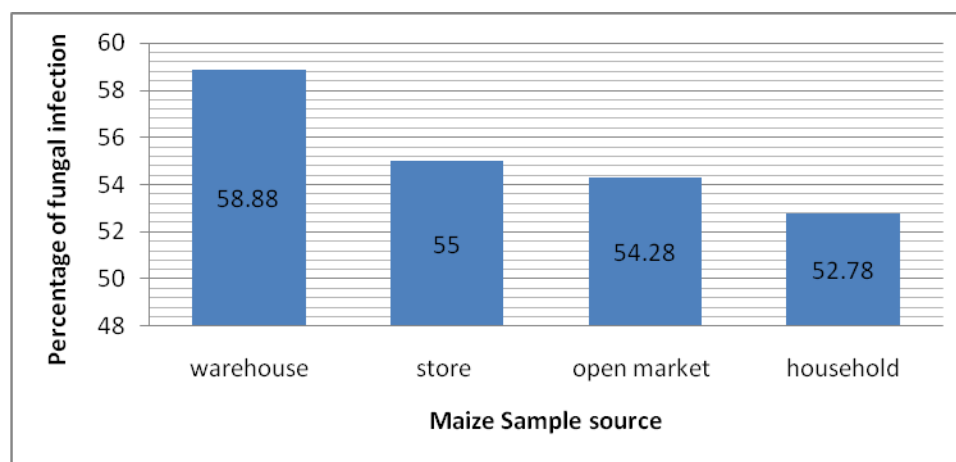


Fig. 4: Sample source versus fungal percent of infection.

Maize samples moisture content obtained in this study ranges from 11 to 17%. Fungal infection ranges from 13.3 to 100%. The mean moisture content was 14.16% and mean percent of fungal infection (54.1%). The minimum moisture content with 80% fungal infection was obtained from west shewa zone, Dano district of Dano shenen Kebele on manually harvested and gotera stored maize. In other way, the higher moisture content value was recorded from east shewa zone of Arsi Negele district in the town from manually harvested maize stored on fertilizer bag for two month. Moreover, Adami tulu town and Arsi Negele town represented the minimum and maximum mean moisture content value. Based on the ANOVA analysis, the relationship between moisture content and fungal percent of infection is statistically insignificant ($p > 0.04$).

The main factors which greatly affect grain storability include grain moisture content, storage temperature, insects infestation and molds invasion which are highly interrelated. If grain moisture content is too high monitoring other conditions will not prevent the grain from fungal spoilage. The grain should be placed in the storage at less than 14% moisture wet basis and preferably

13% for greater safety (Dubale, 2014). Pockets of high moisture grain or inclusion of green leaf material with the grain can affect quality of all the grain in storage because of moisture movement (Dubale, 2014).

According to National Agricultural Commodities Marketing Association Standards (Queensland, Australia), the maximum moisture limits for trading and storage of maize grains is 14% (Dubale, 2014). Aeration will slow the rate of deterioration of high moisture grain, but if the moisture is more than two or three percent above the limits, it should be dried before long term storage (Laura, 2019). Early harvesting of grain at higher moisture produces higher quality and higher yield of grain, but those advantages are lost unless aeration and drying are used to minimize losses in storage (Dubale, 2014).

In this study, the mean moisture content (14.16%) was found above the standard limit of National Agricultural Commodities Marketing Association Standards and associated with higher fungal contamination. However, the association of mean moisture content with mean percentage of fungal infections is statistically insignificant ($p = 0.09$). As

the moisture content increases above the standard levels, storage fungi invasion increases (Laura, 2019). The reason for the moisture content above the safe limit was due to initial exposure of sampled grains to different ambient temperature and relative humidity. The statistical insignificance may be due to the variation of fungal contamination in samples.

From the current finding, there was no significant difference between the use of fertilizer and compost during maize production in relation to fungal infection, although 54.1 % of fungal infections were recorded from maize produced using fertilizer use and 55.65 % from maize produced using compost. Nutrients are the determinant factors for growth and productivity, particularly under adverse conditions (El Kinany et al., 2018). According to Tsedeke et al. (2015), Ethiopian farmers have historically used organic fertilizers (such as farmyard manure, compost, crop residue, and household refuse) for agricultural production and today commercial fertilizer use is the dominant input that goes with modern varieties where all of Ethiopia's mineral

fertilizer is imported. Organic fertilizers have higher favorable condition for fungal growth because compost addition enhanced hyphal growth and sporulation (Wei et al., 2018).

The storage duration versus fungal percent of infection analysis revealed that grains stored for one month showed 63.7% (Fig. 5). Thus, storage duration and percent of fungal infection have weak negative correlation with statistical insignificance ($p=0.23$). This implies as the storage time increases the incidence of fungal contamination decreases. At normal condition, as the storage period increases, the incidence and frequency of all fungal species will also increase which may be due to increase of relative humidity in the storage that favors the rewetting of the stored maize grains (Negasa et al., 2019). In contrary, this study found that as the storage time increases the mean percent fungal contamination decreases. This was due to the higher moisture at harvest season and longer storage of the grain in the fertilizer bag provides aeration thereby reduce fungal contamination. More over this variation may be due to the grains varied fungal exposure.

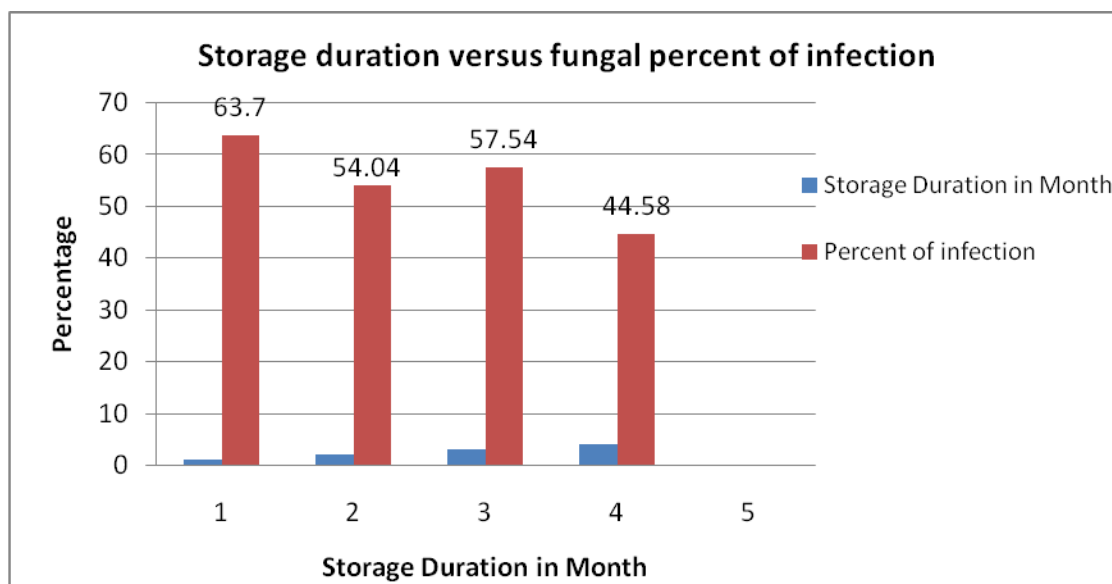


Fig. 5: Mean comparison of percentage of storage duration in month with fungal percent of infection (STD for storage duration=1.08 and percent of infection = 26.79), ($p=0.29$).

Fungal characterization

From the total of 1080 maize grains analyzed about 613 (56.8%) were found contaminated with different

fungi. Every fungal colony that appeared on the grains were counted as the genus *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Fusarium* and others based on morphological and microscopic

observation. From the 613 isolated fungi 73.1% were *Fusarium* followed by 8.5% *Penicillium*, 7.5% *Mucor/Rhizopus*, 5.4% *Aspergillus* and 5.5 % others unidentified fungi (Table 2). Odda Anshura Kebele from Adami Tullu district and Arsi Negelle Town

from Arsi Negelle District showed the higher and lower mean percentage of *Fusarium* infection respectively. In contrary, Odda Anshura and Bako town showed no fungal infection for *Aspergillus* and *Penicillium* respectively.

Table 2. Fungal genera isolated from maize samples collected from different kebeles.

Kebele	Fungal incidence (%)				
	<i>Fusarium</i>	<i>Penicillium</i>	<i>Aspergillus</i>	<i>Mucor/Rhizopus</i>	Others
Seyu Gambela	80.79	5.55	4.88	2.38	6.38
Sayoo	62.2	3.66	2.56	2.08	29.48
Dano Shenen	69.84	3.33	9.72	2.38	0
Odda Anshura	94.16	12.96	0	4.81	0
Anneno Shisho	65.04	2.39	6.66	27	0
Adami Tulu town	65.21	27.73	8.05	7.79	2.08
Bako Town	78.67	0	3.03	14.69	3.59
Dambi Dima	78.19	14.3	5.41	2.08	0
Dambi Gobu	84.18	5.12	4.06	4.06	2.56
Arsi Negelle Town	58.82	16.27	7.22	15.59	2.08
Hadah Bioo	74	5.55	20.43	0	0
Rafuu Hargisa	87.2	4.16	4.16	4.38	0
Standard Error	±1.28	±0.93	±0.6	±0.93	±0.97

Morphological and microscopic characteristics of the isolates showed that the genus *Fusarium* was dominantly isolated from samples collected from the twelve study sites. Morphological characteristics of pure culture isolates were used as a primary screening for fungal identification

(Fig. 6 and 7). As most of the isolates were *Fusarium*; presence of whitish mycelium and numerous non-branched microconidia were recorded. The microscopic image of the isolates was captured from slide culture preparations (Fig. 8).

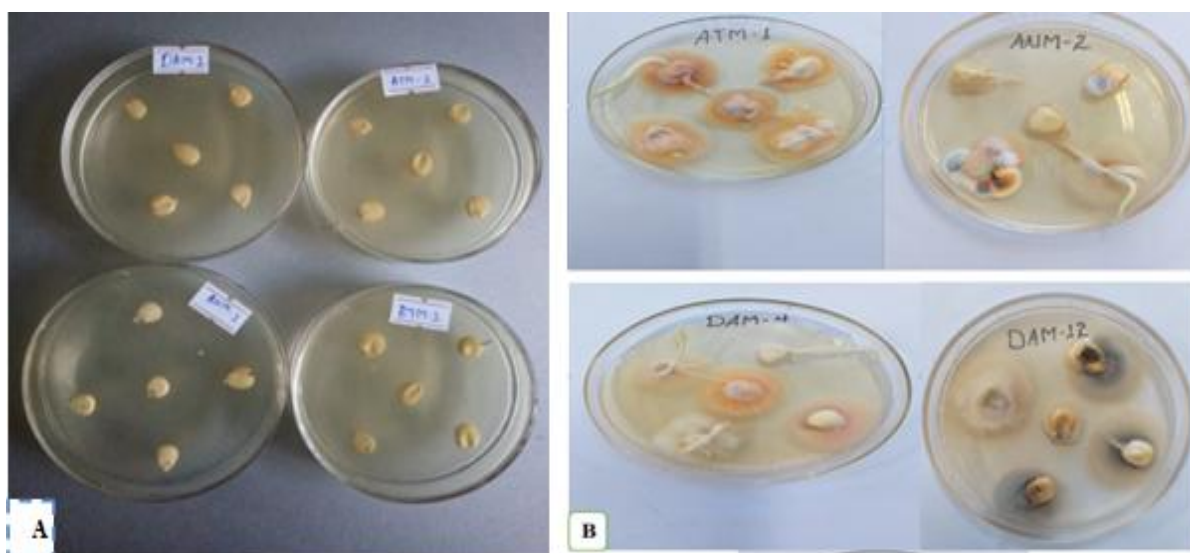


Fig. 6: Isolation of fungi from maize samples. A =inoculated maize sampled before incubation, B = fungal growth on maize samples after incubation for 3-5 days) (Photo Credit; Temesgen Assefa).

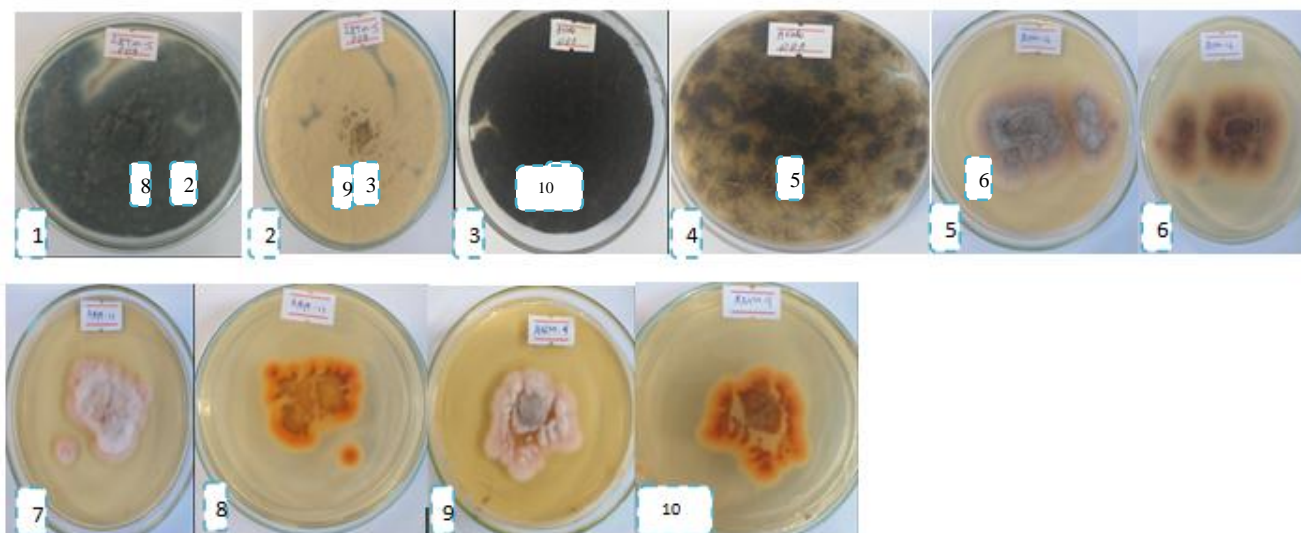


Fig. 7: Five day's old cultural characteristics of fungal pure cultures (1; *Aspergillus* Obverse side, 2 *Aspergillus* reverse side; 3, *Penicillium* Obverse and 4, *Penicillium* reverse side; 5, 7 and 9 *Fusarium* Obverse side and 6, 8, and 10 *Fusarium* reverse side) (Photo Credit; Temesgen Assefa).

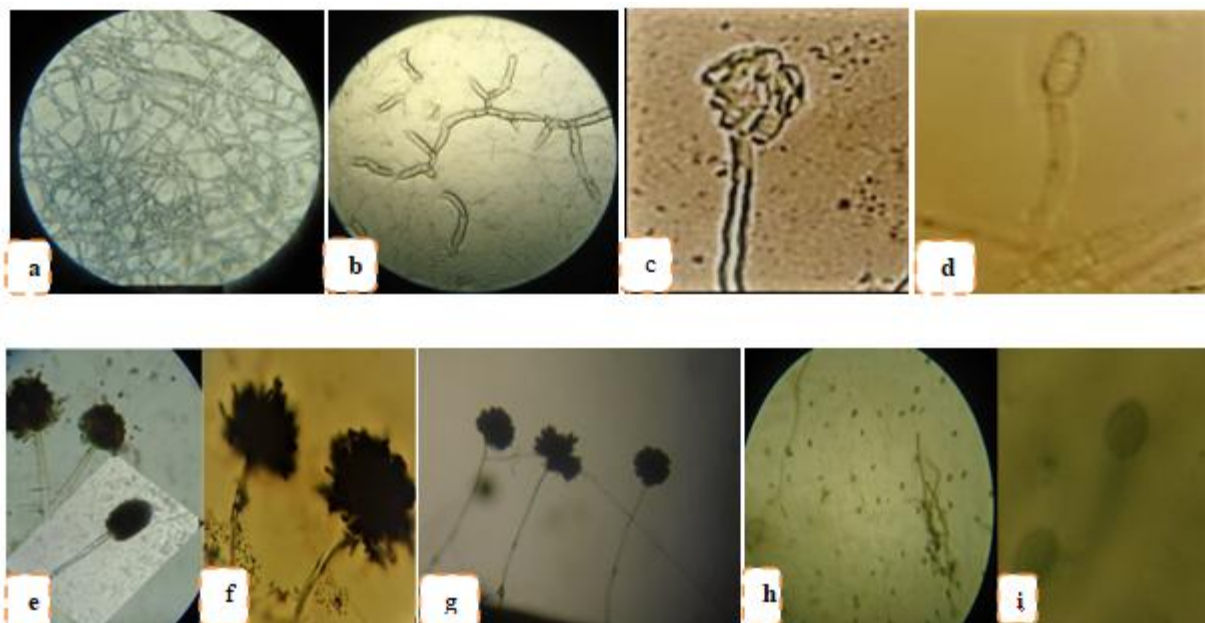


Fig. 8: Microscopic examination of isolates in slide culture, a- Two day *Fusarium* mycelia with 10 X magnification, b with 40 X magnification; c and d, Two day *Fusarium* macro conidia with 40 X magnification; e, *Aspergillus* spore; f, *Mucor* spore; g, *Rhizopus* spore; h and i, *Fusarium* Micro conidia). Note: Slide culture was performed on five day's old pure cultures and further slide cultured for two days (Photo Credit: Temesgen Assefa).

Conclusion and recommendations

Conclusion

There was wider mycoflora contamination of maize

in the study area. The rate of fungal contamination was higher. Most of the study kebeles have higher incidence of fungal contamination and Dano district showed higher percent of maize contamination among others.

The mean moisture content obtained in this study was above the National Agricultural Commodities Marketing Association Standards (Queensland, Australia) and this indicated inappropriate drying of the grains was before harvest which has special relevance to maximize mold growth and mycotoxin production.

The genus *Fusarium* was predominantly recorded in the study sites followed by *Penicillium*, *Mucor/Rhizopus* and *Aspergillus*. Even if statistically found to be insignificant various factors were tested for correlation with fungal spoilage and each of which showed to have an effect for fungal spoilage in maize. Generally, since molds in maize grains are reported in different parts of the country and worldwide, maize is one of the most reservoirs of fungi.

Recommendations

From the study it was recommended that awareness creation of farmers, experts and agricultural extension workers about postharvest handling and the importance of diseases and their management is required. Use of holistic, cumulative integrated management, monitoring, and precautionary measures of the stored grain throughout the storage period and Maize breed type and mycoflora association studies have to be concerned which enables to identify resistant and or tolerant maize varieties and screening of effective bio-agents. There is also need to standardize the farmers, grains marketers and consumers to improve their storage facilities. Moreover, further study is required on the molecular identification of the isolates and of the mycotoxin analysis.

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Conflict of interest statement

Authors declare that they have no conflict of interest.

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