Fungal Spoilage And Its Economic Impact On Maize (Zea Mays L.) And Wheat (Triticum aestivum L) In West Shoa, Ethiopia

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Research

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Abstract

Food grains may be attacked by fungi in the field which can then develop rapidly during storage when conditions are suitable which result in losses in both quantity and quality in addition to decreasing nutritional value and mycotoxin production. Of the world's grain harvest, lost during storage by microorganisms including insects, mites, and rodents is higher especially in developing countries. A study to assess fungal spoilage and contamination of two stored food grains of maize and wheat and its economic impact on the livelihood community were carried out in six localities, vis., Bako, Ejaji, Cheliya, Toke Kutaye, Ginde Beret and Holeta. A total of 120 samples (60 maize and 60 wheat) were collected using purposive and stratified random sampling. Isolation of fungi from collected food grains were done using dry inspection and incubation test (agar plate method) and identification was done based on cultural characteristics and microscopic features. Results of the current study indicated that the mean fungal contamination of the food grains had the highest levels of infection and contamination. Maize was infected by more than 11 different genera of 831 different fungi while the wheat seeds were also infected by 8 genera of 768 different molds. Across the study sites, Aspergillus, Penicillium, Fusarium and Alternaria were the most predominant fungal genera identified among the study sites. There was more mycotoxin producing fungal genera isolated from those samples of both maize and wheat with high level of contamination. Mycotoxin levels increased with storage time such that maize and wheat samples stored for more than six months may exceed the levels greater than the FDA/WHO regulatory limits. Hence, indicating that maize and wheat consumers in the study area are exposed to the danger of mycotoxin poisoning. Thus, there is the need for policy makers to establish and enforce food grain quality standards and regulations related to molds and mycotoxins across the country and specifically among study area to minimize health hazards related to consumption of contaminated foods.

1. Introduction

Cereal grains are the major source of food for most humans and domesticated animals in the world and particularly in developing nations where wheat, maize and rice are the most important staple foods worldwide (Ainsworth & Ort, 2010). They represent the key food chains and are the major nutritional components for both food and feed. Maize (Zea mays L.) is the third most important food crop in the world surpassed only by two other grains, wheat and rice. In Africa, Ethiopia is the fourth largest maize producer next to South Africa, Nigeria and Egypt (Getachew et al, 2018) which is mainly used for food and feed purpose. Ethiopia is known as one of the few countries that experience most diverse agro ecological condition in the world (FAO/WFP, 2008). This enables the country to produce different kinds of cereals, pulses, oilseeds and root, stem tuber and tree crops. Maize is the most important cereal crop in Ethiopia, which is among the major maize producers in Sub Saharan African countries, where smallholder farmers dominate the major share of production. Wheat (Triticum aestivum L.) is cultivated worldwide. Globally, it is a food grain with the highest production among the cereal crops after rice (FAO, 2011). Wheat grain is a staple food used to make flour for leavened, flat and steamed breads; cookies, cakes, breakfast cereal, pasta, noodles; and for fermentation to make beer, alcohol, vodka or even biofuel. Among cereals, wheat grains provide more food to over one thousand million human beings of the earth than any other plant or animal products (Mathew et. al., 2010). Grain crops may be attacked by fungi in the field which can then develop rapidly during storage when conditions are suitable in producing mycotoxins (Turner et al., 2005). Stored grains can have losses in both quantity and quality. Of the world's grain harvest, lost during storage in developing countries may reach 30% or more and most of this occurs when the grain is attacked by microorganisms including insects, mites, rodents (Neetirajan et al., 2007). Among microorganisms, fungi are the major cause of deterioration during storage and important causes of such losses, not only through loss of dry matter but also through loss in quality, by decreasing nutritional value and through their ability to produce mycotoxin (Janić-Hajnal et al, 2011). Fungi involved in the deterioration of cereal grains and other agricultural products have been classified as field fungi, storage fungi and advanced decay fungi depending on the time of their The most commonly reported negative impacts of seed borne fungi include reduction in the storage lifespan of seeds, seed rotting, and reduction in seed vigor, reduction in germination and damping-off in the nurseries (Himanen et al., 2013).

This damage of the food grains after harvesting can happen more in the highland and sub-tropical areas like Ambo because of higher rainfall/temperature (mean annual rain fall of 800-2000 mm, the mean annual temperature from 10-29.) and also due to
recent trends of erratic rains due to the impacts of climate change. Because harvest of the crop not at maturity, takes longer to
dry and is therefore more susceptible to fungal damage. Delayed and incomplete drying of the product often result in more
fungal attack. Also the moisture content of the commodity before it is stored is important, being damp and less ventilation
allows quick fungal growth. As a consequence of these losses in quality and other effects, the profitability and effectiveness of
food utilization are considerably reduced (Boysen et al., 2000). Hence, in light of this, it is necessary to have a survey &
assessment on the diversity and prevalence of such fungi associated with stored grains, level of spoilage & their economic
impact.

Given the range of factors that influence the association of fungi with stored grains and their impact on the quality of grains,
the current study is intended to look into the diversity and composition of mycoflora associated with stored grains and will fill
the knowledge gap about the potential danger and impact they could pose to the economy and health of the consumers.
Objective of the current study was to assess the extent of food damage and economic loss of stored food grains by storage
fungi in the farming households in West Shoa

Hypothesis

\textbf{H1}: fungi associated with stored food grains cause spoilage and have impact on economy and livelihood

\textbf{H0}: fungi associated with stored food grains cause no spoilage and have no impact on economy and livelihood

2. Materials And Methods

2.1. Description of study area

The samples for this study were collected from different villages around Ambo area, West Shoa Zone, Ethiopia. Ambo is
located in West Shoa Zone Oromia Regional State in Ethiopia and lying between 80°56′30″-80°59′30″N latitude and
37°04′30″-37°05′15″E longitude. The temperature ranges from 15° C-29° C with mean annual rain fall about 987.78mm. The
topography of the study area is comprised of wet land, hills, mountains, streams, springs, rivers, ponds. About 82% of the zone
is sub-tropical agro climate zone being agricultural activity the main occupation of the population.

2.2. Sample collection and Sampling Methods

A total of 120 (60 maize and 60 wheat) stored food grain samples that were meant for household consumption and sale on the
local markets were collected using stratified random sampling method from farmers’ stores in six localities of West Shoa,
Ethiopia. Sample collection areas are relatively known producers of the two crops and from each of the sixty households about
0.5 kg samples were collected and properly labeled with the name of the location and storage time, and brought to Ambo
University, Biology laboratory, stored at 4°C until isolation.

2.3. Experimental design

2.3.1. Isolation and Identification

About 0.5kg stored grains of each household collected from different localities of West Shoa zone were tested using two
experimental techniques: Dry inspection method and Incubation test/method (International Seed Testing Association, 1999).
Grain samples were surface-sterilized using 1% sodium hypochlorite solution for 2min followed by 70% alcohol for three min
and rinsed three times in sterile, distilled water. 300 seeds of maize and 600 seeds of surface-sterilized grains were placed on
potato dextrose agar (PDA) and incubated at 25°C under continuous fluorescent light for 5 to 7 days. After a week of
incubation period, sporulation was observed in the PDA plates. Pure cultures of each isolate were maintained on PDA and
stored at 4°C as stock cultures. Isolates identification were done based on pigmentation, macroscopic (cultural and
morphological) characteristics & microscopic features.

Data Analysis
Qualitative data were analyzed by: Shannon diversity index ($H'$) and Sorensen coefficient of Similarity (QS):

$$H' = -\sum_{i=1}^{s} (p_i \ln p_i)$$  \hspace{1cm} QS = \frac{2C}{A + B}$$

Quantitative data were analyzed by: MS Office Excel 2013 programmes, SPSS version 16.0 at $\alpha = 0.05$.

3. Results

3.1. Result obtained from dry inspections of maize and wheat samples

The dry maize and wheat seed samples collected from the study location examined for impurities showed that both maize and wheat found to be impure in that the seeds contain plant debris, spotted, unfilled & chaffy grains, sclerotia, galls, smut balls, insects etc. When these seeds are further examined through incubation on agar plates, both maize and wheat seeds produced a large numbers of diversified fungal colonies indicating its infection by different fungal species.

3.2. Fungi Associated With Stored Maize

3.2.1. Total Fungal Isolates from stored maize grains

In total 831 isolates categorized into 11 fungal genera were obtained from maize seeds collected from six different locations of sixty households. More than 44 morphologically different forms of fungi were isolated. Those identified 11 genera were *Aspergillus, Penicillium, Alternaria, Bipolaris, Fusarium, Cladosporium, Curvularia, Phoma, Mucor, Rhizopus and Trichodroma*. The highest fungal isolates were observed at Bako (178) followed by G/Beret (162) whereas the lowest number of fungal isolates were recorded in Ejaji (92) (Table 1).

| Isolates     | # spp. | Bako | Ejaji | Cheliya | Toke-K | G/Beret | Holeta | total | RD (%) |
|-------------|--------|------|-------|---------|--------|---------|--------|-------|--------|
| Aspergillus | 10     | 60   | 23    | 52      | 45     | 42      | 36     | 258   | 31.05  |
| Penicillium | 7      | 37   | 15    | 23      | 25     | 39      | 17     | 156   | 18.77  |
| Fusarium    | 6      | 26   | 14    | 25      | 20     | 46      | 20     | 151   | 18.17  |
| Alternaria  | 4      | 22   | 16    | 33      | 17     | 12      | 19     | 119   | 14.32  |
| Trichoderma | 3      | 7    | 5     | 4       | 6      | 10      | 9      | 41    | 4.93   |
| Cladosporium| 4      | 9    | 1     | 5       | 2      | 5       | 3      | 25    | 3.01   |
| Phoma       | 2      | 5    | 6     | 4       | 5      | -       | 2      | 22    | 2.65   |
| Curvularia  | 3      | 4    | 3     | 3       | 1      | 2       | 4      | 17    | 2.05   |
| Rhizopus    | 2      | 3    | 3     | -       | 3      | 2       | 1      | 12    | 1.44   |
| Bipolaris   | 2      | 2    | 1     | 2       | 3      | 1       | 5      | 14    | 1.68   |
| Mucor       | 1      | 3    | 5     | 1       | 2      | 3       | 2      | 16    | 1.93   |
| Total + %   | 44     | 178  | 92    | 152     | 129    | 162     | 118    | 831   | 100    |

The most abundant was fungi from genus *Aspergillus* having 258 total isolate and its relative density (RD) being 31.05% and the second most widespread fungal genus was *Penicillium* with a total isolates of 156 and having a relative density of.
The relative density and number of isolates for genus *Rhizopus* was much lower which is 1.44% and 12, respectively; followed by *Bipolaris* (1.68%) and *Mucor* (1.93%) with a total of 14 and 16 isolates, respectively.

In this study, 10 out of 44 total morphologically different species were observed under genus *Aspergillus* followed by the genera *Penicillium* (7) and *Fusarium* (6) whereas the least number of morphologically different species were observed in *Mucor* (1) followed by the genera *Bipolaris, Phoma* and *Rhizopus* with which of each were 2 species (Table 1.) The higher frequency of storage fungi between the six study sites showed that highest number of isolates were observed in Bako (178 or 21.4%) followed by Ginde Beret 162 or 19.5%) whereas the relatively lowest number of isolates were recorded at Ejaji (92) with a frequency of 11.1% (Table 1). Highest proportion of mycoflora associated with maize grain is *Aspergillus* (31.05%) followed by *Penicillium* (18.77%) while the least proportion (<3%) of maize mycoflora is recorded in the genera namely *Rhizopus, Bipolaris, Mucor, Curvularia* and *Phoma* with the proportion of 1.44, 1.68, 1.93, 2.05, and 2.65%, respectively.

### Table 2. The mean distribution and standard deviation of fungal genera isolated from stored maize

| Genus     | N | Minimum | Maximum | Sum     | Mean   | Std. Deviation |
|-----------|---|---------|---------|---------|--------|----------------|
| Aspergillus| 6 | 23.00   | 60.00   | 258.00  | 43.0000| 12.83745       |
| Penicillium| 6 | 15.00   | 39.00   | 156.00  | 26.0000| 10.01998       |
| Fusarium   | 6 | 14.00   | 46.00   | 151.00  | 25.1667| 11.07098       |
| Alternaria | 6 | 12.00   | 33.00   | 119.00  | 19.8333| 7.25029        |
| Trichoderma| 6 | 4.00    | 10.00   | 41.00   | 6.8333 | 2.31661        |
| Cladosporium| 6 | 1.00    | 9.00    | 25.00   | 4.1667 | 2.85774        |
| Phoma      | 6 | .00     | 6.00    | 22.00   | 3.6667 | 2.25093        |
| Curvularia | 6 | 1.00    | 4.00    | 17.00   | 2.8333 | 1.16905        |
| Rhizopus   | 6 | .00     | 3.00    | 12.00   | 2.0000 | 1.26491        |
| Bipolaris  | 6 | 1.00    | 5.00    | 14.00   | 2.3333 | 1.50555        |
| Mucor      | 6 | 1.00    | 5.00    | 16.00   | 2.6667 | 1.36626        |

As shown in the above, the mean distribution of isolates for the genus *Aspergillus* was 43 which is the highest average distribution of among all the isolates of fungal genera with the standard deviation of 12.84 from its mean value whereas *Penicillium* was the second highest mean of 26 distributions compared to average values of all isolates. The minimum number of isolates range from zero to twenty three while the maximum number of isolates were ranged from three to sixty (Table 2). The average mean value of all isolates ranged from a minimum of two to maximum of forty three.

### Table 3. The mean distribution and deviation of fungi associated with stored maize across the study sites

| Study Site | N | Minimum | Maximum | Sum     | Mean   | Std. Deviation |
|------------|---|---------|---------|---------|--------|----------------|
| Bako       | 11| 2.00    | 60.00   | 178.00  | 16.1818| 18.55165       |
| Ejaji      | 11| 1.00    | 23.00   | 92.00   | 8.3636 | 7.36577        |
| Celiya     | 10| 1.00    | 52.00   | 152.00  | 15.2000| 17.34487       |
| Toke Kutaye| 11| 1.00    | 45.00   | 129.00  | 11.7273| 13.83540       |
| Ginde Beret| 10| 1.00    | 46.00   | 162.00  | 16.2000| 18.44993       |
| Holeta     | 11| 1.00    | 36.00   | 118.00  | 10.7273| 11.04618       |
Relatively higher deviation is observed in the study sites of Bako and Ginde Beret which is about 18.55 from the mean. Highest isolation of fungus observed in Bake Tibe (60) followed by Ginde Beret (46) and Toke Kutaye (45) and the minimum is one in all study sites except Bako. The highest mean distribution is observed in Bake Tibe and Ginde Beret (16.2) and Toke Kutaye (45) (Tabale 3).

3.3. Fungi Associated with Stored Wheat

3.3.1. Genera of Fungi Isolated From Stored Wheat Grains

A total of 37 species of 768 fungal isolates belonging to 8 genera were recorded. The identified fungal genera are Aspergillus, Alternaria, Penicillium, Fusarium, Bipolaris, Necrospora, Rhizopus and Trichoderma. The dominant genera were Aspergillus (33.85%), followed by Alternaria (22%) and Fusarium (13.15%) while Rhizopus is the least appeared genera (3.52%) of all (Table 4) followed by Necrospora (4.68%) and Trichoderma (5.22%). As shown in the table below the highest fungal isolates were obtained from Cheliya (159) followed by Bako (152) whereas the lowest number of fungal isolates was recorded in Ejaji (101) and Holeta (116).

| Isolated Genera | No. of spp | Distribution of isolates in sampling locations | Total |
|----------------|-----------|-----------------------------------------------|-------|
|                |           | Bako  | Ejaji | Cheliya | Toke-K | G/Beret | Holeta |
| Aspergillus    | 9         | 58    | 25    | 53      | 41     | 47      | 36     | 260    | 33.85% |
| Alternaria     | 7         | 40    | 27    | 24      | 17     | 32      | 29     | 169    | 22     |
| Fusarium       | 6         | 17    | 15    | 22      | 20     | 13      | 14     | 101    | 13.15% |
| Penicillium    | 6         | 16    | 11    | 18      | 15     | 15      | 10     | 85     | 11.06% |
| Bipolaris      | 2         | 5     | 7     | 19      | 8      | 6       | 5      | 50     | 6.52%  |
| Rhizopus       | 2         | 5     | 4     | 9       | 0      | 6       | 3      | 27     | 3.52%  |
| Trichoderma    | 3         | 4     | 6     | 10      | 5      | 7       | 8      | 40     | 5.22%  |
| Necrospora     | 2         | 7     | 6     | 4       | 3      | 5       | 11     | 36     | 4.68%  |
| **Total**      | 37        | 152=19.8% | 101=13.2% | 159=20.7% | 109=14.2% | 131=17% | 116=15.1% | 768 | 100%

The average mean value of all isolates ranged from a minimum of two to maximum of forty three. The minimum number of isolates range from zero to twenty five while the maximum number of isolates ranged from nine to fifty eight. As indicated in the table below, the mean distribution of isolates for the genus Aspergillus is 43.33 which is the highest average distribution of among all the isolates of fungal genera with the standard deviation of 11.98 from its mean value whereas Alternaria is the second highest mean of 28.2 distribution compared to average values of all isolates (Table 4).
Table 5. The mean distribution and standard deviation of fungal genera isolated from stored wheat

|                  | N | Minimum | Maximum | Sum   | Mean   | Std. Deviation |
|------------------|---|---------|---------|-------|--------|----------------|
| Aspergillus      | 6 | 25.00   | 58.00   | 260.00| 43.333 | 11.97776       |
| Alternaria       | 6 | 17.00   | 40.00   | 169.00| 28.167 | 7.73089        |
| Fusarium         | 6 | 13.00   | 22.00   | 101.00| 16.833 | 3.54495        |
| Penicillium      | 6 | 10.00   | 18.00   | 85.00 | 14.167 | 3.06050        |
| Bipolaris        | 6 | 5.00    | 19.00   | 50.00 | 8.333  | 5.35413        |
| Rhizopus         | 6 | 0.00    | 9.00    | 27.00 | 4.500  | 3.01662        |
| Trichoderma      | 6 | 4.00    | 10.00   | 40.00 | 6.667  | 2.16025        |
| Necrospora       | 6 | 3.00    | 11.00   | 36.00 | 6.000  | 2.82843        |

Relatively higher deviation is observed in the study sites of Bako and Ginde Beret which is about 18.55 from the mean. Highest isolation of fungus observed in Bake (60) followed by Ginde Beret (46) and Toke Kutaye (45) and the minimum is ranged from zero in Toke Kutayeto five in Ginde Beret. The highest mean distribution is observed in Bake and Ginde Beret which is 19 whereas the lowest of the mean distribution across the study sites is 12.63 in Ejaji (Table 5).

Table 6. The mean distribution and deviation of fungi associated with stored wheat across the study sites

|      | N | Minimum | Maximum | Sum   | Mean   | Std. Deviation |
|------|---|---------|---------|-------|--------|----------------|
| Bako | 8 | 4.00    | 58.00   | 152.00| 19.000 | 19.77011       |
| Ejai | 8 | 4.00    | 27.00   | 101.00| 12.625 | 8.95923        |
| Celiya| 8 | 4.00   | 53.00   | 159.00| 19.875 | 15.07541       |
| Toke Kutaye | 8 | 0.00  | 41.00   | 109.00| 13.625 | 13.13596       |
| Ginde Beret | 8 | 5.00 | 47.00   | 131.00| 16.375 | 15.24971       |
| Holeta| 8 | 3.00   | 36.00   | 116.00| 14.500 | 11.77164       |

3.4. Fungal Diversity

The Shannon diversity index of fungi associated with maize grains in the current study revealed that there is a relatively higher diversity of fungi among the study sites \(H’ = 1.769514208\). (Table 6) but it was not statistically significant \((P>0.05)\).
Table 7
Diversity index fungal genera among the study sites (maize)

| Study site | Total spp. | \( \text{pi} = n/N \) | Ln pi | Pi ln pi | \( H' = -\text{SUM}(\text{Pi ln pi}) \) |
|------------|------------|-----------------|-------|----------|-----------------|
| Bako       | 178        | 0.21419976      | -1.54084624 | -0.33004889 | 1.769514208     |
| Ejaji      | 92         | 0.11070999      | -2.20084122 | -0.2436551   |                 |
| Cheliya    | 152        | 0.18291215      | -1.69874927 | -0.31072189 |                 |
| Toke K.    | 129        | 0.15523466      | -1.86281739 | -0.28917382 |                 |
| G/Beret    | 162        | 0.19494585      | -1.63503346 | -0.31874298 |                 |
| Holeta     | 118        | 0.14199759      | -1.95194517 | -0.27717152 |                 |
| **Total**  | **831**    | **1**           | **-1.769514208** |             |                 |

Relatively higher diversity of fungi associated with the stored maize grain were recorded at Bako site \((H' = 0.33004889)\) followed by G/Beret \((H' = 31874298)\) and then Cheliya \((H' = 0.31072189)\); while the minimum diversity was observed at Ejaji \((H' = 0.2436551)\) followed by Holeta \((H' = 0.27717152)\) (Table 4.7). The overall diversity within the study site is lower while among all study areas there is a relatively higher diversity of fungal isolates of maize \((H' = 1.769514208)\). Among the six areas of study areas, higher diversity of wheat seed mycoflora was recovered from Cheliya \((H' = 0.32605052)\) and Bako \((H' = 0.32060703)\) than others. Moreover, the diversity index of fungal isolates of wheat between all the study sites found to be higher \((H' = 1.77772592)\) than within each sample sites (Table 7).

Table 8
Shannon diversity index among the study sites (wheat)

| Study site | Total spp. | \( \text{pi} = n/N \) | Ln pi | Pi ln pi | \( H' = -\text{SUM}(\text{Pi ln pi}) \) |
|------------|------------|-----------------|-------|----------|-----------------|
| Bako       | 152        | 0.19791667      | -1.61990921 | -0.32060703 | 1.77772592     |
| Ejaji      | 101        | 0.13151042      | -2.02866922 | -0.26679113 |                 |
| Cheliya    | 159        | 0.20703125      | -1.57488553 | -0.32605052 |                 |
| Toke K.    | 109        | 0.14192708      | -1.95244185 | -0.27710438 |                 |
| G/Beret    | 131        | 0.17057292      | -1.76859241 | -0.30167397 |                 |
| Holeta     | 116        | 0.15104167      | -1.89019954 | -0.28549889 |                 |
| **Total**  | **768**    | **1**           | **-1.777725918** |             |                 |
Table 9
Shannon diversity index between the study sites (maize)

| Isolates | Bako | Ejaji | Cheliya | Toke-K | G/Beret | Holeta | H’ |
|----------|------|-------|---------|--------|---------|--------|-----|
| Aspergillus | 0.36 | 0.35 | 0.37 | 0.37 | 0.35 | 0.36 | 2.16 |
| Penicillium | 0.33 | 0.3 | 0.28 | 0.32 | 0.34 | 0.28 | 1.85 |
| Fusarium | 0.28 | 0.28 | 0.3 | 0.29 | 0.36 | 0.3 | 1.81 |
| Alternaria | 0.26 | 0.3 | 0.33 | 0.27 | 0.19 | 0.29 | 1.64 |
| Trichoderma | 0.13 | 0.16 | 0.1 | 0.14 | 0.17 | 0.2 | 0.9 |
| Cladosporium | 0.1 | 0.18 | 0.1 | 0.13 | 0 | 0.07 | 0.58 |
| Phoma | 0.07 | 0.11 | 0 | 0.08 | 0.05 | 0.04 | 0.35 |
| Curvularia | 0.15 | 0.05 | 0.11 | 0.06 | 0.1 | 0.09 | 0.56 |
| Rhizopus | 0.08 | 0.11 | 0.08 | 0.04 | 0.05 | 0.11 | 0.47 |
| Bipolaris | 0.05 | 0.05 | 0.06 | 0.08 | 0.03 | 0.13 | 0.4 |
| Mucor | 0.07 | 0.16 | 0.03 | 0.06 | 0.07 | 0.07 | 0.46 |
| H’ | 1.88 | 2.05 | 1.75 | 1.85 | 1.74 | 1.95 |

The Shannon diversity index for geographic variation between sampling sites indicates that the diversity of *Aspergillus* isolates are numerically higher ($H’=2.16$) than the rest of fungal isolates of maize grain (Table 8). Considering Agro-ecological zones, sample areas with relatively warmer climatic areas viz, Ejaji, Bako and Holeta appeared to be more diversified ($H’=2.05$, $H’=1.88$ and $H’=1.95$, respectively) than study sites with relatively cold climates, namely Ginde Beret ($H’=1.74$), Cheliya ($H’=1.75$) and Toke Kutaye ($H’=1.85$).

Table 10
Shannon diversity index between the study sites (wheat)

| Isolates      | Bako | Ejaji | Cheliya | Toke-K | G/Beret | Holeta | H’ |
|---------------|------|-------|---------|--------|---------|--------|-----|
| Aspergillus   | 0.36 | 0.35 | 0.36 | 0.37 | 0.37 | 0.36 | 2.17 |
| Alternaria    | 0.35 | 0.35 | 0.28 | 0.28 | 0.34 | 0.35 | 1.95 |
| Fusarium      | 0.25 | 0.28 | 0.27 | 0.31 | 0.23 | 0.26 | 1.6 |
| Penicillium   | 0.24 | 0.24 | 0.25 | 0.27 | 0.25 | 0.21 | 1.46 |
| Bipolaris     | 0.11 | 0.18 | 0.25 | 0.19 | 0.14 | 0.14 | 1.01 |
| Rhizopus      | 0.11 | 0.13 | 0.16 | 0 | 0.14 | 0.09 | 0.63 |
| Trichoderma   | 0.1 | 0.16 | 0.17 | 0.14 | 0.16 | 0.18 | 0.91 |
| Necrospora    | 0.14 | 0.16 | 0.09 | 0.9 | 0.12 | 0.22 | 1.63 |
| H’            | 1.66 | 1.87 | 1.85 | 1.67 | 1.75 | 1.81 | 10.61 |

As shown in the above table, higher diversity is observed in isolates of *Aspergillus* species than other mycoflora of wheat grain and Ejaji being the highest diversity study site ($H’=1.87$) over the rest of sampling areas (Table 10).

3.5. Fungal Similarity

Species similarity between sampling units was calculated using Sorensen similarity index. The resultant calculation showed that neither of the study sites were considered to be similar (Table 4.11). As to the calculations, the similarity index of
species between the study sites of maize grain was approaching to zero (QS=0.046) which means they are less similar.

### Table 11
Total number of Isolates, No. of Shared Isolates and Sorenson similarity index between Sampling kebelles (maize)

| Sampling site | Total no. of isolates | No. of shared isolates | Similarity index (QS) |
|---------------|-----------------------|------------------------|-----------------------|
| Bako          | 178                   | 19                     | 0.046                 |
| Ejaji         | 92                    |                        |                       |
| Cheliya       | 152                   |                        |                       |
| Toke K.       | 129                   |                        |                       |
| G/Beret       | 162                   |                        |                       |
| Holeta        | 118                   |                        |                       |
| Total         | 831                   | 19                     |                       |

Regarding the sorenson similarity index among the study sites of wheat sample, it was revealed that the similarity of the species of wheat grain between the study areas was found to be least existed (Table 11). So they are highly dissimilar as per their similarity index accounted to (QS=0.034) which is very close to zero.

### Table 12
Total number of Isolates, No. of Shared Isolates and Sorenson similarity index between Sampling kebelles (wheat)

| Sampling site | Total no. of isolates | No. of shared isolates | Similarity index (QS) |
|---------------|-----------------------|------------------------|-----------------------|
| Bako          | 152                   | 13                     | 0.034                 |
| Ejaji         | 101                   |                        |                       |
| Cheliya       | 159                   |                        |                       |
| Toke K.       | 109                   |                        |                       |
| G/Beret       | 131                   |                        |                       |
| Holeta        | 116                   |                        |                       |
| Total         | 768                   | 13                     |                       |

### 3.6. Extent of food damage & economic loss of stored grains

Assessment of yield losses due to fungus was conducted using semi-structured interview and questionnaire. The response of farmers showed that food grains stored in different storage methods appeared to be infected severely and some of the ways losses of food grains occurred during storage include losses in weight due to insects, rodents or birds eating the grain; deterioration through fungus growth and rotting; loss in quality through biting damage, insect and rodent excrement and fungus growth; loss of motivation in the farmer to grow more, because he is not able to store his harvest or part thereof in a safe way for any long period of time, damage to sacks, which causes waste during transportation, decline in germination capacity of stored seeds, and so on.

### 3.7. Impact of fungal food spoilage on the livelihood of local community
Seed borne pathogens reduce the quality of seed for planting by lowering germination capacity, and lower its food and feed value by discoloration and the product on of mycotoxins which are hazardous to human beings and animals. The problem of heating of stored grains Heating in stored cornis so common that it has given rise to the superstition that corn "has an urge to heat and germinate" in spring. Soybeans, because of their tendency to heat, are said by some warehousemen to be "difficult to store." Not all spoilage of stored grains involves drastic or even detectable heating; but any spoilage that occurs under ordinary aerobic storage, including the preliminary stages of deterioration that precede any reduction in grade, is associated with and largely caused by the growth of storage fungi; and any growth of storage fungi is accompanied by respiration and heating, whether or not the heating is detected or detectable. Decrease in germination percentage.-Many factors, some perhaps still unknown, may cause decrease in germination percentage of seeds during storage development of compounds toxic to man and to other animals the reduction in grain weight, percentage seed germination, organic matter and soluble carbohydrate contents was associated with poor grain storage practices accompanied by insect infestations and storage fungal invasion. Frequencies of storage fungi increased over time in such poor storage structures. Contamination of grain by storage fungi could occur during pre-harvest, at harvest, threshing, winnowing and transportation due to poor sanitary practices and could explode to damaging levels in the storage period.

4. Discussion

In the present study, a total of 44 and 37 fungal species which fall into 11 and 8 identified genera were isolated from maize and wheat grains, respectively. The most frequently occurring fungi associated with both maize and wheat are *Alternaria, Fusarium, Penicillium* and *Aspergillus*. These four fungal genera are major mycotoxin producing genera (Mansfield, 2005). Therefore, there is a risk that infection level of fungal spp. can become high in both maize and wheat as well as in the whole crop rotation production and storage period (Asran & Buchenauer, 2003). In both wheat and maize grains a variety of *Aspergillus* species are even greater and therelative density or frequency were higher than all other fungal isolates. The most abundant fungi were from *Aspergillus*. The second most widespread fungi in this study of maize grains was *Penicillium* (25%) which is not most frequently isolated fungi in other studies. In the study of wheat grains the second most widespread and dominant fungal genus was *Alternaria*. Same result was reported in studies carried out in Latvia (Kaspars Gulbis et al, 2016) in that *Alternaria* were highly widespread and prevalent. In the mycoflora studyof maize and wheat, the relative density or frequency for *Aspergillus* spp were 15%, which is similar with one reported in Ethiopia on both food grains. Relative density in this study of maize mycoflora for *Penicillium* spp were higher next to *Aspergillus* which corresponds to results in other studies, whereas *Alternaria* spp were the second in terms of both relative density and abundance following *Aspergillus* in the study of wheat mycoflora. Most fungal spp. found in this study also has been found in other studies in other countries. Abundance of fungal species is variable between sites and years even in one region (Lazzaro et al., 2015). In this study, the most frequently isolated fungi from *Alternaria* spp. Fungi from genera *Alternaria* has been reported to produce a wide range of mycotoxins; however, *Alternaria*, spp. includes also endophytic fungi (Gulbis et al., 2016). As *Alternaria* toxins are supposed to be less harmful than toxins produced by *Fusarium* and *Aspergillus* species they haven't been studied so much (Logrieco et al., 2009). *Penicillium* spp. is often isolated from maize, wheat and other cereals, and it was also present in current study. In the study carried out in Italy, the RD for *Penicillium* in organic maize was lower than 2%. In this current study, the RD for *Penicillium* spp. was higher in both food grains (Lazzaro et al., 2015). *Aspergillus* spp. are worldwide distributed and able to produce the most toxic mycotoxins aflatoxins. Recently the presence of *Aspergillus* spp. in maize grain mycoflora has been found in European countries where it had not been a problem before, e.g. Croatia, Serbia, Slovenia, Romania and Northern Italy (Gulbis et al., 2016). Less frequently isolated in the current study was fungi from *Phoma, Rhizopus, curvularia, Biopolaris and Mucor* spp. with less than 3% of frequency in maize seeds while *Necrospora* and *Rhizopus* spp. with <5% of frequency were isolated from wheat seed samples. In Italy, in the study carried out (Lazzaro et al., 2015) in 2010 and 2011 the RD for *Aspergillus* was lower or it was not found at all. In this study, the Aspergillus spp. was not found. The results obtained in this study correspond to results from other studies around Europe. Other studies reveal that fungal mycoflora is influenced by the site and year; therefore, it is important to continue the current research and monitor the situation (Lazzaro et al., 2015; Gulbis et al., 2016; Logrieco et al., 2009).
Food grains stored in different storage methods resulted in severe loss in some favorable seasons causing significant yield losses even on resistant varieties (Negash et al., 2019) and grain yield losses ranged from 7.8 to 29.1% on different varieties. More specifically, fungi can damage the product in a number of ways such as producing chemicals called enzymes which may stop seeds from germinating; decrease the quality of the products for food, through discoloration or change in taste (bad flavour or smell), and they decrease the nutritive value; some fungi produce substances which are poisonous to people and animals; consumption of part of the product; contamination of part of the product with their excrement; damage to buildings, storage containers and packing material; they are also carriers of diseases which are harmful to people. The populations of all the fungi were higher in samples collected from farmers’ stores. A number of fungi isolated in the present study are known to produce mycotoxins which are harmful for human health. Mycotoxins can cause severe damage to liver, kidney and nervous system of man even in low dosages. *Fusarium* and *Aspergillus* species are common fungal contaminants of maize and also produce mycotoxins (Alonso et al., 2013). *Aspergillus flavus* produces aflatoxin B1, B2, G1 G2 which are carcinogenic and produce liver cancer (Pesta & Bonday 1990). Different *Fusarium* spp. cause corneal ulcer, produce Zeralenone α and β causing haemorrhage and necrosis in bone marrow; cause epidemiologically human esophageal cancer (Desjardins et al., 2006) isolated several *Fusarium* species from maize seed viz., *Fusarium moniliforme*, *F. graminearum*, *F. proliferatum*, *F. acuminatum*, *F. avenaceum*, *F. clamydosporium*, *F. equiseti*, *F. oxysporum*, *F. semitectum* and *F. torulosum* which produce mycotoxins viz., Toxins deoxynivalenol (DON), 3-acetyl DON,15-acetyl DON, Fusarenon X(FX),T-2 Toxin (T2), Diacetoxyscir phenol (DAS), Zearalenon (ZEA), Fumonisins, Aflatoxin B1, Ochratoxin A (OA) and Citrinum.(CT) respectively. Don and acetyle Don were the major mycotoxin in *Fusarium* species. *A. terreus* attacks human skin and nail and is parasitic on human ear (Domsch et al., 1980). *A. wentii* produce kojic acid causing cardiovascular and brain disorder. There is need for proper storage of maize and wheat seeds to minimize the fungal infestation and mycotoxin production during storage and provide disease free seeds for human consumption.

5. Conclusion

A wide range of fungal (mold) species contaminate stored maize and wheat in West Shoa and the extent of contamination is strongly influenced by differences in climatic conditions, variety of grains, moisture content and storage time. The total identified fungal genera from both maize and wheat grains are *Aspergillus, Alternaria, Penicillium, Fusarium, Tichoderma, Phoma, Cladosporium, Curvularia, Bipolaris, Rhizopus, Mucor, and Necrospora*. The fungi from *Aspergillus, Alternaria, Penicillium*, and *Fusarium* spp. were most frequently isolated from both maize and wheat grains in this study and they are potential mycotoxin producing fungi present in both food grain samples without visible symptoms of fungal contamination collected from different maize and wheat storage methods of farmers in 2016/17. There is a high risk that maize and wheat production can contain different mycotoxins. The predominant of all fungal isolates were observed in the genus *Aspergillus* in both food grains and the second most widespread fungi associated with maize seeds was *Penicillium* while *Alternaria* were the second most prevalent fungi in wheat grains. The results indicate that maize consumers in the study area are exposed to the danger of aflatoxin poisoning. The research work was funded by Ambo University. So, the authors are grateful to Ambo University for the research support and funding the study. The farmers who provided maize and wheat samples for fungal spoilage assessment are also appreciated.

**Declarations**

**Ethics approval and consent to participate**

Not applicable

**Data Availability**

The author confirms that all data underlying the findings are fully available without restriction.

**Conflicts of Interest**
The author declares that there is no conflict of interest regarding the publication of this manuscript.

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**Consent for publication**

Not applicable' for that section.

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