

Research paper

Fungi Associated with Wheat (*Triticum* spp.) in South East Ethiopia under Storage Conditions

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Storage fungi are among the major factors causing post-harvest deterioration of crop produce worldwide. However, their identity, intensity and economic importance remains under studied in many parts of Sub-Saharan Africa including Ethiopia. Therefore, the current work was carried out with the objectives to determine the identity and incidence of major fungi associated with stored wheat in Southeast Ethiopia. For this purpose mycological analysis was carried out using 180 wheat grain samples collected from three major wheat growing zones of South East Ethiopia. Results of the mycological analysis revealed the contamination of wheat grains by fungal species in the study region. Among the different species identified, *Aspergillus flavus* and *Alternaria triticina* were the predominant species at different locations and storage time with different frequencies. The highest fungal incidence (98.62%) was recorded after six months storage of wheat grain.

Key words: Association, Fungal Incidence, Southeast Ethiopia, Storage Fungi, Wheat

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INTRODUCTION

Wheat (*Triticum* spp.) is among the most commonly cultivated cereal crops with over 755 million metric tons harvested each year [1]. It belongs to the genus *Triticum* of the grass family, Poaceae. The genus is originated in tropical South west Asia, where it occurs in wild as well as in cultivated forms [2]. Although the crop is widely cultivated at altitude ranging from 1500 to 3000m.a.s.l, in Ethiopia, the most suitable area falls between 1700 and 2800m.a.s.l [3]. The most important wheat growing areas of Ethiopia are the highlands of the central, southeastern, and northwestern regions of the country, which have a

bimodal rainfall. Most wheat is produced during the main rainy season, June to September, although some is produced during the light rain season, March to May. Virtually all wheat is produced under rain fed conditions. Wheat production practices vary from location to location. In most wheat areas wheat is planted only once per year at the onset of the main rainy season. In some areas, such as Sinana, wheat is also planted during the short rainy season in a different field than that used for main season wheat.

The two major wheat species grown in Ethiopia are durum wheat (*Triticum durum* L.) and bread wheat (*Triticum aestivum* L.). Durum wheat is indigenous to

Ethiopia and considered to be one of the centers of genetic diversity and bread wheat has been introduced to Ethiopia [4].

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 bio-fuel. Bread wheat accounts for approximately 20% of the totally consumed human food calories and provides the most stable food for 40% of the human population [5]. In Ethiopia rural households use wheat for preparation of local bread, boiled grain, roasted grain, porridge, or as a component of the traditional flat bread (injera). Wheat straw is used for roof covering and for animal feed although it is said to be inferior to straws of other major cereals [5].

Wheat is the fourth most important cereal crops in modern agriculture. Global harvests reached 755 million metric tons [1]. FAO's forecast for global wheat production in 2018 stands at 736.1 million tones 2.7 percent below the record output of 2017 [6]. Its production all over the world is 722 MMT [6]. Among the cereals, wheat is an important crop and widely cultivated in a wide range of altitude [7]. Ethiopia is the second largest producer of wheat only after South Africa in Sub-Saharan Africa. During the last 20 years, the area covered by wheat has increased from 0.77Mha in 1997 to 1.69Mha in 2018, and the crop ranks fourth among the cereal crops next to tef (*Eragrostis tef*), maize (*Zea mays*), and Sorghum (*Sorghum bicolor*) [3]. In spite of the production and yield increases, average grain yield of wheat is still low (2.74 t ha^{-1}), which is far less than potential yield of 8 to 10 t ha^{-1} [3].

Crop yields are dependent on interactions of socio-economical, biological, technological and ecological factors. The ideal daily temperature for wheat development varies from $20\text{-}25^{\circ}\text{C}$ for germination, $16\text{-}20^{\circ}\text{C}$ for good tillering and $20\text{-}23^{\circ}\text{C}$ for proper plant development [8]. The crop can be grown in most locations, where annual rainfall ranges from 250 to 1750 mm. About 75% of the wheat grown world-wide receives an average rainfall between 375 and 875 mm annually. However, too much precipitation can lead to yield loss from diseases, and poor root growth and development problems [8, 9].

Despite its importance as food and industrial crop, wheat production and productivity around the globe is affected by a number of factors including biotic and abiotic stresses as well as low adoption of new agricultural technologies [10]. Among the biotic stresses, diseases caused by fungi are the most important factors constraining wheat production. The grain production varies from year to year, and hence the grains should be stored strategically from years of overproduction for the use in year of under production. Also grain must be stored as point of production is not the point of

consumption and the time of production is not the time of consumption. Stored grains can have losses in both quantity and quality. Grain quality after harvest is influenced by a wide variety of biotic and abiotic factors and has been studied as a stored grain ecosystem. Losses occur when the grain is attacked by microorganisms and other organisms including insects, mites, rodents and birds. The grain losses in quantity and quality; can be in the form of depletion in seed viability, hardness, color, size and shape, grain weight and various biochemical parameters viz., protein, carbohydrate and vitamins under postharvest storages. The storage fungi damage the grains in several ways; they reduce the germination capacity, produce undesirable odor and kernel discoloration, decrease the food value and also produce toxins that are injurious to the health of consumers [2].

One of the basic strategies to produce certified and non-infected seeds is the identification of seed-borne pathogenic agents in wheat growing fields. If infested grain is used as seed, not only would the seed-borne diseases reduce crop yield but also the seed will be a source of inoculum [11]. They are responsible for both pre and post emergence death of grains, affect seedling vigor, and thus cause some reduction in germination and also variation in plant morphology [12-14]. Overall storage fungi have remained an important constraint to wheat production all over the world including in Ethiopia. As a result there is a need to assess the incidence and frequency of storage fungi in different areas and across agro-ecological zones. Thus, this study was designed with the following objective.

OBJECTIVE

To determine the identity and incidence of major fungi associated with stored wheat in Southeast Ethiopia

MATERIALS AND METHODS

Sample Collection

Wheat seed samples were collected from the major wheat growing zones (Bale, Arsi and West Arsi) of Southeastern Ethiopia from November 2017 to July 2018. Two districts per zone, three kebeles in each district and 10 households (HHs) from each kebele were selected for sample collection based on the wheat productions potential and ability to produce surplus wheat grains and save grains after harvest at least for six months. The selection of the woredas, kebeles and HHs in the three zones were facilitated by key informants composed of experts in the respective zone and woreda offices of Agricultural Development and development agents (DAs) of each kebele.

The primary samples were taken from three spots (top, middle and bottom) of each sack or store per HHs with the help of seed sampler and mixed to get composite sample. The study sample size of grains was adjusted to 1 Kg per HHs and taken to laboratory of plant quarantine and seed health research program at Holeta Agricultural Research Center. There were 30 wheat samples per woreda and totally about 180 samples were collected during harvesting and at three months interval for the period of six months. The geographic coordinate's of sample collection sites (latitude, longitude and altitude) were recorded using Geographic Positioning System (GPS) unit. In addition, planting and harvesting time, harvesting and threshing methods, storage condition or type, moisture content and date of sampling were documented. Weather parameters relative humidity and temperature of storage houses were recorded by using hygrometer.

Isolation and Identification of Fungal Species

Fifty seeds per sample were surface sterilized with 10% Chlorox solution to remove saprophytes for 3 min, followed by three times rinse in sterile distilled water for one minute each. Five surface sterilized seeds were then placed on each potato dextrose agar (PDA) media plates, and incubated for seven days at 25°C. Finally each petri-

dish were examined under stereo microscope for the observation of mycelia growth, fungal isolation and pure cultures of different out growing fungi were obtained by transferring fungal colonies to new PDA plates using sterile loop, and incubating the plates for seven days at 25°C. Pure cultures of each isolate was then stored at 4°C in vials containing 2.5 ml of sterile distilled water for further use.

Fungal isolates from the samples were morphologically identified to their genus based on [15] and to a species level using identification manuals [16-19].

Determination of Grain Contamination Frequency

The percentage of grains infected (incidence) (Table 2) and also the percentage of samples (isolation frequency) (Table 1) for each fungal species was recorded and calculated [20].

Data Analysis

Data on frequencies and incidence of seed infection by fungal species for samples collected from different locations of the three zones was subjected to simple descriptive analysis and data were analyzed by using the SAS computer package, version 9.3 [21].

Data Collected

Incidence of fungi (In): Incidence of fungal infection on each sample was calculated by using the following formula:

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

Isolation Frequency (IF): For each fungus, proportion of samples that yielded its isolates was determined by using the following formula [20].

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

RESULTS AND DISCUSSIONS

Fungi Associated with Stored Wheat in Southeastern Ethiopia

The current survey work revealed the contamination of wheat by multitude of storage fungi in Southeast Ethiopia. The following fungi species associated with wheat grains: *Fusarium oxysporium*, *Fusarium moniliformae*, *Fusarium graminearum*, *Botrytis* sp., *Bipolaris victoriae*, *Bipolaris sorokiniana*, *Aspergillus flavus*, *Aspergillus niger*, *Phoma* sp., *Tilletia indica*, *Eppicocum nigrum*, *Cladosporium macrosporium*, *Alternaria triticina*, *Rhizopus* sp., *Penicillium chrysosegenum* and *Nigrum* sp. were isolated and identified.

One of the dominant species isolated from the collected samples was *Aspergillus flavus*. The major distinction of this fungus was that its colonies are characterized by yellow to dark, yellowish-green pigments, consisting of a dense felt of

conidiophores or mature vesicles bearing Phialides over their entire surface separating from the other species of *Aspergillus* [17 and 19]. This observation was also confirmed by [22], who reported *A. flavus* colonies as being initially yellow, turning to yellow-green or olive green with age and appearing dark green with smooth shape and having rapid growth (Figure 1).

Another dominant species of fungi isolated was *Alternaria triticina* which is characterized with dark grey colony but may also be white, olive green, brown, or almost black and conidiophores are dark to olive brown and smooth, arise singly or un-branched. The distinctive character was light brown, tapered to a beak, conidia with transverse and longitudinal septa [23]. The current study also confirmed the production of light brown, tapered to a beak, conidia with transverse and longitudinal septa and having quick growth (Figure 1).

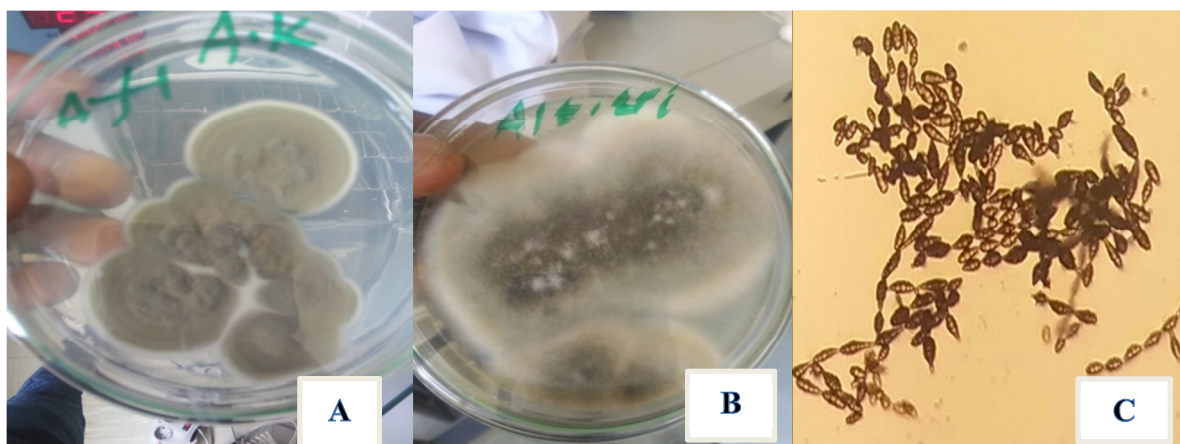


Figure 1. Two Dominant Species of Fungi Isolated from Wheat Samples from Southeast Ethiopia. *A. flavus* (A); *A. triticina* (B) and Conidia of *A. triticina* (C)

Fungal Contamination Frequency, Incidence and Their Distribution

Among the fungi isolated from current wheat samples, *Aspergillus flavus* was the dominant followed by *Alternaria triticina* (Table 1). In 180 wheat samples examined upon harvest, the highest frequency of *Aspergillus flavus* was recorded from samples collected from Bale (98.3%) followed by Arsi (96.7%) and West Arsi (81.7%). In addition, the highest frequency of *Aspergillus flavus* (95.0%), (91.7%) and (78.3%) was recorded from Bale followed by Arsi and West Arsi, respectively, after three months of storage. However, after six months of storage the highest frequency of *Aspergillus flavus* was recorded from Arsi and Bale equally (100.0% from each) followed by West Arsi (95.0%).

The percent of *Alternaria triticina* recorded was also the highest at Bale (71.67%) followed by Arsi (63.33%) and West Arsi (26.67%) upon harvest. But after three months of storage, the highest frequency was recorded from West Arsi (85%) followed by Arsi and Bale (80% from each). Similarly, after six months of storage, the highest frequency was recorded from west Arsi (88.33%) followed by Arsi and Bale with, 66.67% and 55%, respectively. The percent record of *Aspergillus niger* was highest from West Arsi (46.67%) followed by Arsi (50%) and Bale (35%) upon harvest. Also the highest frequency

was recorded from West Arsi (95%) followed by Arsi (76.67%) Bale (58.33%) and also the highest frequency from West Arsi (93.33%) followed by Arsi (68.33%) and Bale (31.67%) was recorded after three months and six months of storage period, respectively.

The other fungi species were the least prevalent species and with the record percentage of frequency was minimal. The percent record of *Fusarium oxysporium* from Arsi (6.67%) followed by West Arsi and Arsi (1.67% from each) upon harvest. After three months of storage, the record from West Arsi (6.67%) followed by Arsi (5%) and Bale (0%). But after six months of storage the record from West Arsi (8.33) followed by Bale (6.67%) and Arsi (0%). The percent record of *Fusarium moniliformae* from West Arsi (5%) followed by Arsi (1.67%) and Bale (0%) was recorded upon harvest. But, the highest frequency was recorded from Arsi (36.67%) followed by Bale (18.33%) and West Arsi (3.33%) and also from Arsi (66.67%) followed by Bale (63.33%) and west Arsi (36.67%) after three months of storage and six months of storage, respectively.

Fusarium graminearum was recorded from West Arsi and Arsi (6.67% from each) followed by Bale (5%) upon harvest. But, after three months of storage, the percent of record was from Arsi (16.67%) followed by West Arsi and Bale (5% from each). After six months of storage, the frequency from West Arsi and Arsi (10%) followed by Bale (6.67%) was record of *Phoma* sp. was from West

Table 1. continues

	W.Arsi	1.67	5.00	6.67	0.00	61.67	3.33	81.67	46.67	73.33	20.00	6.67	8.33	26.67	0.00	1.67	31.67
0	Arsi	6.67	1.67	6.67	1.67	0.00	6.67	96.67	50.00	23.33	15.00	0.00	1.67	63.33	0.00	15.00	28.33
	Bale	1.67	0.00	5.00	0.00	0.00	0.00	98.33	35.00	1.67	1.67	0.00	0.00	71.67	1.67	11.67	3.33
	W.Arsi	6.67	3.33	5.00	0.00	8.33	33.33	78.33	95.00	1.67	5.00	0.00	0.00	85.00	0.00	5.00	0.00
3	Arsi	5.00	36.67	16.67	0.00	0.00	5.00	91.67	76.67	5.00	0.00	0.00	0.00	80.00	1.67	11.67	1.67
	Bale	0.00	18.33	5.00	0.00	0.00	0.00	95.00	58.33	0.00	0.00	0.00	0.00	80.00	0.00	13.33	0.00
	W.Arsi	8.33	36.67	10.00	0.00	5.00	10.00	95.00	93.33	0.00	11.67	1.67	0.00	88.33	1.67	0.00	6.67
6	Arsi	0.00	66.67	10.00	0.00	0.00	1.67	98.33	68.33	0.00	1.67	1.67	0.00	66.67	5.00	5.00	8.33
	Bale	6.67	63.33	6.67	0.00	0.00	0.00	96.67	31.67	0.00	0.00	0.00	0.00	55.00	6.67	6.67	6.67

0=Upon harvest, 3=Three months of storage, 6=Six months of Storage, W.Arsi=West Arsi, *Foxy*=*Fusarium oxysporium*, *Fmon*=*Fusarium moniliformae*, *Fgra*=*Fusarium graminearum*, *Botry*=*Botrytis* sp., *Bvic*=*Bipolaris victorae*, *Bsor*=*Bipolaris sorokiniana*, *Afl*=*Aspergillus flavus*, *Anig*=*Aspergillus niger*, *Phoma* sp., *Tind*=*Tillitia indica*, *Enig*=*Eppicocum nigrum*, *Cmac*=*Cladosporium macrosporium*., *Atri*=*Alternaria triticina*, *Rhizo*=*Rhizopus* sp., *Pchr*=*Penicillium chryosegenum*, *Nigrum* sp.

The fungal contamination of samples indicated that highest incidence was observed at West Arsi (96.98%) followed by Bale (91.76%) and Arsi (86.43%), (Table 3). At district level, the highest incidence was recorded in Arsi Negelle (97.89%) followed by Shashamene (96.07%), Sinana (94.20%), Tiyo (90.18%), Gobba (89.31%) and Hetosa (82.69%) of fungal incidence respectively (Table 2).

The mean incidence of fungi varied with altitude range and the highest was in lower altitude (<2000 m.a.s.l) followed by higher altitude (>2500 m.a.s.l) compared to mid altitude (2000 m.a.s.l – 2500 m.a.s.l) with incidences of 96.93%, 93.03% and 88.30%, respectively (Table 2). The present finding, concur with the study of [24] which reported as that as altitude increases diversity of storage

fungi also decreases and vice versa.

[29] Also reported as the wheat production areas in Ethiopia with the lower altitude limit is roughly 2000 m.a.s.l, the present agro-climatological constraint on wheat area is not lack of rainfall but warm temperatures. Increasing temperatures by 1°C decreased wheat yields by 130 kg/ha, whereas an increase of 2°C reduced wheat yields by 270 kg/ha. In terms of wheat cropping and ignoring year-to year variation, the Ethiopian highlands (to which the wheat crop is so well-adapted) represent a relatively humid environment. Growing wheat under warmer conditions might require cultivars with greater heat tolerance as well as resistance to pathogens that prevail under warmer conditions [29]. The result indicating incidence of storage fungi varied with the relative altitude, humidity, temperature and months of the

storage during the study period (Table 3).

In addition fungal contamination also varied with storage period with the highest incidence of (98.62%) followed by (89.78%) and (86.77%) was observed after six months, upon harvest and three months of storage, respectively (Table 2) and incidence decreased from upon harvest to the first three months of storage and then increased after the second three months of storage. Fungal contamination of stored wheat varied with storage duration and an increasing trend of percentage incidence of different fungi of wheat seeds was also recorded as the storage period prolonged from three months of storage period to six months of storage.

This result agrees with findings reported by Habib [30]; who reported that the incidence of different groups of storage fungi boasted from the beginning of storage to the end of 180 days

of storage. The decreased percentage incidence of different fungi was recorded from upon harvest to the first three months of storage (after 90 days), which was resemblance with the result of Habib. Infection of fungi increased with the increase in storage time and the percentage of fungal infection start decreasing after longer storage period (after 120 days). This may be due to the exhaustion of nutrients or the accumulation of toxic metabolites produced by the fungi themselves [31]. The current study confirms similar result of previous work of Dudoiu [32] that reported after 60 days of storage, the percent of the field fungi, respectively species of *Alternaria* and *Cladosporium* decreased, and the development of stored grain specific fungi such as *Fusarium* spp., *Aspergillus* spp. and *Sclerotinia sclerotiorum*. Gradually, over a storage period of 90 days, the grains' microflora enriched by the incidence and growth of the phytopathogen fungi, due to storage conditions like high atmospheric humidity.

Table 2. Incidence of Fungi on Wheat Grains across Locations, Altitudes and Storage Months

Variable	Variable Class	N	Incidence (%)				
			Maximum	Minimum	Mean	Std Dev	Std Error
Zone	W.Arsi	180	100	60	96.98	7.23	0.54
	Arsi	180	100	12	86.43	18.80	1.40
	Bale	180	100	40	91.76	13.61	1.01
District	A.Negelle	90	100	60	97.89	6.50	0.69
	Shashemene	90	100	68	96.07	7.81	0.82
	Hetosa	90	100	12	82.69	19.55	2.06
	Tiyo	90	100	12	90.18	17.33	1.83
	Sinana	90	100	40	94.20	11.28	1.19
	Gobbaa	90	100	48	89.31	15.27	1.61
	Altitude	<2000	150	100	60	96.93	7.34
2000-2500		273	100	12	88.30	17.62	1.07
>2500		117	100	48	93.03	11.67	1.08
Storage month	0	180	100	12	89.78	15.89	1.18
	3	180	100	12	86.77	17.33	1.29
	6	180	100	74	98.62	4.19	0.31

N, number of samples in the three rounds; 0, period of time before storage; 3, three months after storage; 6, six months after storage; W.Arsi, West Arsi; A.Negelle, Arsi Negelle

CONCLUSION AND RECOMMENDATION

Bread wheat (*Triticum aestivum* L.) is one of the most important cereal crops in Ethiopia. It is widely grown in most regions of Ethiopia. However, its production is affected by abiotic and biotic factors. Among the biotic stresses, diseases caused by seed borne fungal agents or storage fungi, are among the main factors reducing yield and quality of wheat. Despite its importance, storage fungi has been one of the most poorly understood pathosystems in Ethiopia. As a result, the present study was conducted to determine the identity, incidence and frequency of storage fungi in major wheat growing zones to have a complete picture on the

importance of the storage fungi in the country. The major objective of the study was to contribute towards improved wheat production in Ethiopia through effective and sustainable management of storage fungi in the country.

Wheat samples analyzed in the current experiment were found to be contaminated by *A.flavus*, *A.niger*, *A.triticina*, *F.oxysporium*, *F.monoliformae*, *F.graminaerum*, *Botrytis* sp., *B.victoriae*, *B.sorokiniana*, *Phoma* sp., *T.indica*, *E.nigrum*, *C.macrosporum*, *Rhizopus* sp., *P.chrysosegenum* and *Nigrum* sp.. Among the species *A.flavus* and *A.triticina* were found to be the pre-dominant species followed by *A.niger*, *Phoma* sp. and *Nigrum* sp. The highest fungal contamination of samples was observed at West Arsi (96.98%) followed by

Bale (91.76%) and Arsi (86.43%). Across the surveyed districts, incidence of storage fungi ranged between 12% and 100%, with an average of 91.72%. The highest mean incidence was recorded in Arsi Negelle (97.89%) followed by Shashamene (96.06%), Sinana (94.20%), Tiyo (90.18%), Gobba (89.31%) and Hetosa (82.69%) districts, respectively. Fungal contamination also varied with storage period with the highest incidence (98.62%) followed by (89.78%) and (86.77%) was observed after six months of storage, upon harvest and three months of storage, respectively.

Last but not least, looking at the alarming rate of wheat production in the country to feed the ever increasing population, this study suggests that research on the biology, ecology and management of major storage fungi associated with wheat should be given due attention in the country.

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