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Occurrence of Fungi Associated with Stored Wheat Grains (*Triticum aestivum*) in Shashemene and Arsi Negelle Districts, Ethiopia

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ABSTRACT: Storage fungi damage grains in several ways: they reduce germinability, produce undesirable odour and kernel discoloration, reduce the nutritional value and also produce mycotoxins injurious to the health of the consumers. A study was conducted to assess the prevalence of fungi associated with stored wheat grains under the storage conditions of farmers in Shashemene and Arsi Nagelle districts from June to July, 2013. Stratified random sampling technique was used to collect wheat grains from the study sites of Arsi Nagelle and Shashemene districts. Two peasant associations from each district and 10 House Holders from each of the peasant associations, with a total of 40 House Holders were purposively drawn for sample collection. A Malt Extract and Potato Dextrose Agar were used for the isolation of fungi. Fungi were identified based on the microscopic examination of spore morphology and culture characteristics of the isolates. A total of 898 fungal isolates belonging to five genera and three unidentified taxa were obtained. The isolated mycoflora were dominated by the morphotaxa of *Aspergillus* (45.54%) and *Penicillium* (29.18%), respectively. It was concluded that stored wheat from the study areas could be contaminated by storage fungi and therefore, awareness creation and training should be given to the farmers on better and improved storage techniques.

KEYWORDS: Contamination, Fungi, Prevalence, Wheat - grain

1 INTRODUCTION

Wheat plants at all stages of growth are subject to numerous injuries and stresses, which interfere with their normal functioning and development. Each year about 20% of the wheat that otherwise would be available for food and feed is lost due to diseases [1]. Seed health plays an important role for successful cultivation and yield exploitation of a crop species. Among various factors that affect seed health, the most important are the seed borne fungi that lower seed germination and reduce seed vigor resulting in low yield [2]. Healthy seed plays an important role not only for successful cultivation but also for increasing yield of crop. Seed-borne pathogens of wheat are responsible to cause variation in plant morphology and also reducing yield up to 15-90 % if untreated seeds are grown in the field [3].

Wheat (*Triticum aestivum*) is cultivated worldwide. Globally, it is a food grain with the highest production among the cereal crops after rice [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 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 [5].

The post harvest loss of wheat grain has been found to be the highest during storage [6]. Stored grains can have losses in both quantity and quality. Losses occur when the grain is attacked by microorganisms and other organisms including insects,

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mites, rodents and birds [7]. It is therefore, very appropriate to protect wheat grains at all stages of handling, from the time of harvest, through storage, transportation and processing, up to the time they are ready to be consumed [8].

Post harvest spoilage by filamentous fungi (moulds) is one of the most important threats to processed and stored food products worldwide. Discolorations, deterioration in quality, reduction in commercial value and mycotoxin production have been linked to mould contaminated foods [9]. This situation is made worse in the tropics where the warm and humid climates provide these micro-organisms with favourable conditions for their spread and subsequent establishment in numerous substrates. Over sub-Saharan Africa, the phenomenon could be of great concern, especially in areas where food shortages have compelled people to consume low grade food items, even if moulds are visible as contaminants [10].

Post harvest handling of staple grains and the prevailing environmental factors are key determinants of the impacts fungi may have on the grain quality including germinability. It is important to remember that harvested grain and contaminating fungi are alive and respiring slowly even under dry, safe storage conditions [11]. Consequently, they produce metabolites that are toxic to animals and humans under favourable conditions; the ingestion of which poses significant health risks to humans and animals [12].

About 5% of the world's grain harvest is lost during storage but in developing countries such losses may reach 30% or more. Fungi are 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 [13]. In addition, estimated losses of grain, especially of staple food grains in store, from all causes varies widely. They may amount to 10% worldwide [14] but can reach 50% in tropical regions [15].

Three genera, *Fusarium*, *Penicillium* and *Aspergillus*, all potential mycotoxin producers, could be considered the most significant toxigenic fungi growing in processed and stored foods [10]. Due to their capability to develop in a wide range of environmental conditions, fungi in the genus *Aspergillus* are comparatively more widespread than others [16]. Fungal growth and mycotoxin production in cereals is influenced by various factors. Climatic conditions, especially temperature and humidity, play a very important role in this process [17]. Microscopic fungi and their metabolites, mycotoxins, are often found as contaminants in agricultural products before or after harvest as well as during transportation or storage.

In Ethiopia, most of wheat production takes place in relatively cooler areas of Arsi and Bale zones followed by Shashemene and Arsi Nagelle districts. In these areas, most of the harvested wheat grains are generally stored in sacks of different sizes and quality. Wheat grains are often contaminated with many kinds of fungal agents before or after harvesting and depending on the storage conditions and prevailing environmental factors. A lot of information is available regarding the mycoflora associated with harvested wheat grains based on reports from several countries including Germany [18]; Nigeria [19]; Poland [20] and Russia [21].

However, information concerning the magnitude of mycoflora associated with stored wheat grains in Shashemene and Arsi Negelle districts is scarce. Furthermore, assessment of the prevalence of the storage fungi associated with wheat grains under the storage conditions practiced by farmers would generate important information. Therefore, this study was conducted to assess the prevalence of fungi associated with stored wheat grains under the storage conditions of farmers in Shashemene and Arsi Negelle districts.

2 MATERIALS AND METHODS

2.1 STUDY AREA

The study was conducted at two geographic locations, namely, Shashemene and Arsi Negelle districts, both of which are located in the West Arsi Zone, Oromia National Regional State.

Shashemene district is bordered by Seraro in the west, Arsi Negele in the north and north east, Arsi zone in the east and Southern Peoples' Regional State in the South and the South East. The district lies within the geographic coordinates of 7° 05' - 7° 19' N and 38° 23'- 38° 41' E. It covers an area of 768.88 km^2 with the altitudinal range of 500 to 1700m above sea level and the farmers in the district practice mixed crop-livestock production system [22]. The major crops in the district are maize, wheat and barley from among the cereals, horse beans and haricot beans from Pulses and potatoes from vegetables. The district receives an annual rainfall of 700–950 mm, and has an annual temperature range of 12–27^{o}C. The total human population of the district is 285,176 [23].

Arsi Nagelle district is bordered on the South by Shashemene, on the southwest by Lake Shala which separates it from Seraro district, on the West, on the North by Adami Tullu and Jido Kombolcha districts with which it shares the shores of Lakes Abijatta and Langano, and on the East by the Arsi Zone. The district lies within longitudes and latitudes of 7° 19'N to 7°

40'N and 38° 30'E to 38° 53'E. Arsi Nagelle district lies within the altitudinal range of 500 to 2000m above sea level, and agricultural practices are mainly mixed crop-livestock production system [22]. Maize and wheat are the most important cereal crops grown in the district. It has erratic type of bimodal rainfall. The district receives an annual rainfall of 500-760 mm and an annual temperature of 10-25 C^0 [23].

2.2 SAMPLING STRATEGY AND SAMPLE COLLECTION

2.2.1 SAMPLING STRATEGY

Stratified random sampling technique was used to collect samples of wheat grains from the study sites of Arsi Negelle and Shashemene districts. Two peasant associations (PAs) from each district namely Edola Burka (EB) and Ebicha (E) of Shashemene district and Ali Wayo (AW) and Adaba Tita (AX) of Arsi Nagelle district were selected. The selection of the districts, the PAs and the households (HHs) was based on accessibility and the presence of surplus producing farmers. From each PA, 10 HHs and a total of 40 HHs who were known to produce surplus wheat grains and thus save grains after harvest for at least five to six months were purposively selected for sample collection. The selection of the PAs and HHs in both the districts was facilitated by key informants composed of experts in the respective district offices of Agricultural Development and development agents (DAs) of each PA. PAs that produce wheat as major cereal crop were identified by the key informants.

2.2.2 SAMPLE COLLECTION

Collection of wheat grain samples was conducted from June to July, 2013. In both districts, it was observed that farmers stored their wheat grains in sacks. In each of the PAs, 5% of the storage sacks of each HHs were sampled [24]. The storage sacks for sample collection were randomly selected by the researcher followed by sampling by hand in order to obtain primary samples. Primary samples were obtained by taking handful of grains from the different depths (top, middle and bottom) of the sacks by inserting the hand into the sack with open palm and then closing once at the point of sampling and then withdrawing as closed. The number of primary samples obtained from a HH varied according to the number of storage sacks found in the store of the HH. Sample drawn from the different storage sacks in a HH were combined to form a composite sample from the HH. Then study samples were drawn from the composite sample. Grain samples were collected in air tight plastic sacks immediately upon drawing them in order to prevent alteration of moisture content of the seeds. The samples were transported to the laboratory of Wondo Genet College of forestry and Natural resource and stored at 25^{o}C until analyses were performed.

2.3 MEDIA PREPARATION

Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA) were used to isolate fungi. Before pouring plates streptomycin was added at a concentration of 50 mg /L of the medium to suppress the growth bacteria.

2.4 ISOLATION OF FUNGI

From each grain sample, twenty wheat kernels were randomly picked and surface sterilized in 34% H_2O_2. Before the actual surface sterilization, the kernels were briefly sprayed with 70% ethanol in order to reduce surface contaminants of the kernels. Then, the kernels were transferred to a Petri dish with 34% H_2O_2 for 3 min with constant stirring [25].Then they were rinsed three times in sterile water followed by blotting on sterile filter paper under aseptic condition. Five surface sterilized seeds were then randomly picked and placed on MEA and PDA. Plates were labeled and incubated upright at 25°C for 6- 10 days to allow growth of fungal colonies on the medium. Plates were inspected daily for the growth of fungi beginning a few days after incubation. Fungi that grew out of each grain were isolated and purified on to sterile plates of MEA and PDA. During isolation, data on the frequency of isolation and sampling site were recorded. Pure cultures of the isolates were maintained on MEA slants at 4 ^{0}C.

2.5 IDENTIFICATION OF FUNGI

Identification of the fungal isolates was based upon culture characteristics and spore morphology. The cultural characteristics included the color of the upper and reverse sides of the cultures, mycelial color formation such as green, dark, brown, grey, yellow or other colors, colony diameter, shape of colony margin, mycelial growth patterns such as fluffy aerial

hyphae, appresssed or submerged hyphae, formation of aerial hair-like tufts of hyphae. Identification was based on the International manual on the identification of fungi of agricultural and environmental significance [26] were used to provisionally identify the isolates to the genus level. Lacto phenol cotton blue staining solution was used for staining of non-pigmented fungal spore for microscopic examination purpose. Conidial morphological characteristics used for the microscopic identification include origin of conidia, shape and color of the spores, septation, presence or absence of specialized appendages on the spores.

Based on culture characteristics and spore morphology, the isolated fungi were categorized into morphotaxa identified to the genus level, while those which could be separated into distinct groups based on culture characteristics but could not be identified to any of the known genera were recognized as unidentified taxa. Morphotaxa within the genus were designated as morphotaxa 1, 2, 3, etc.

2.6 DATA ANALYSIS AND INTERPRETATION

Data obtained from the research work was presented using tables as percentages. The relative frequency (RF, expressed as a percentage) was calculated as the total number of isolate from a single taxa divided by the total number of isolates from taxa obtained from all seeds incubated [27]. It was used to determine the most frequently isolated taxa among the rest of the taxa.

$$RF = \frac{\text{Number of isolates of a taxon}}{\text{Total number of isolates of all taxon obtained from all incubated seeds}} \times 100$$

3 RESULTS AND DISCUSSION

3.1 GENERA OF FUNGI ISOLATED FROM STORED WHEAT GRAINS

A total of 31 morphotaxa belonging to five genera and three unidentified morphotaxa were identified from the isolates. In relation to their prevalence, the dominant genera were *Aspergillus (*45.54%), followed by *Penicillium* (29.18%), *Altarnaria* (12.14%), *Fusarium* (9.69%) and *Bipolaris* (1%), respectively (Table 1). The unidentified morphotaxa were comprised of 21 isolates and accounted for only 2.45% of the total collection of fungi isolated (Table 1).

According to the current study, the most prevalent genus was *Aspergillus (*45.54%) and followed by *Penicillium* (29.18%). This result is in agreement with the study conducted by Simerda [29]. The high prevalence of these genuses might be due to their diversified, capacity to grow on all possible substrates and a wide range of temperature and humidity [5]. However, *Bipolaris* and the taxon that remained unidentified were the least prevalent. *Bipolaris* was solely isolated from grain samples collected from the PAs of Shashemene district. *Alternaria* and *Fusarium* were also distributed across all the PAs although their prevalence was more in Shashememe district than in Arsi Nagelle district (Table 1). The variation could be related to the differences in environmental factors. In this regard, Shashemene receives more rainfall and thus more relative humidity in the air and in the store, which preferable for occurrence of *Bipolaris*. Moreover, the PAs that were selected lie towards the highest part of the district and might be more humid and wetter than the lower parts of the Arsi Nagelle district.

3.2 FUNGAL ISOLATES FROM STORED WHEAT GRAINS AND THEIR DISTRIBUTION

The distribution of the storage fungal isolates between the two sampling districts showed that higher number of isolates (558 or 62.14%) were recovered from Shashemane district while lower number of isolates (340 or 37.86%) were obtained from Arsi Nagelle district (Table 1).

Among the PAs of Shashemene district, the highest number of fungal isolates was recovered from Edola Burka (321 or 35.7%) while the lowest number of isolates were obtained from Adaba Tita (167 or 18.04%). At both district and PAs levels, more number of isolates was recovered from Shashemene and its two PAs while lower number was recovered from Arsi Nagelle and its two PAs. Within district variation in terms of the number of fungal isolates was also higher between the two PAs from Shashemene (84) than between those of Arsi Nagelle (16) (Table 1).

The difference in the number of fungal isolates between the two districts could be related to the differences in environmental factors that influence the association of fungi with the grains both in the field and in the store. In this regard, Shashemene receives more rainfall and thus more relative humidity in the air and in the store. Moreover, the PAs that were

selected lie towards the highest part of the district and might be more humid and wetter than the lower parts of the district. On the other hand, Arsi Nagelle receives less amount of precipitation and therefore relatively drier and less humid than Shashemene, thus explaining the lower number of fungal isolates than from Shashemene. Likewise, the PAs of the district selected for sample collection lie relatively at lower elevation and thus drier than sample PAs from Shashemene thereby explaining lower number of isolates recovered; this is concur with the study of [28] as altitude increases diversity also decreases and vice versa.

Table 1. Fungal genera obtained from stored wheat grains collected from the study area

Genera	No of morphotaxa	Origin and distribution of isolates				Total No of isolates	Relative Frequency (%)
		Shashemene		Arsi Negelle			
		Edola Burka	Ebicha	Ali Wayo	Adaba Tita		
Aspergillus	12	122	98	108	81	409	45.54
Penicillium	10	91	85	33	53	262	29.18
Alternaria	5	52	27	25	5	109	12.14
Fusarium	3	37	16	12	22	87	9.69
Bipolaris	1	6	3	-	-	9	1
Unidentified groups	3	12	8	-	1	21	2.45
Total	34	321 (35.7%)	237 (26.4%)	178 (19.82%)	162 (18.04%)	898	
	Total	558 (62.14%)		340 (37.86%)			100

4 CONCLUSION AND RECOMMENDATIONS

4.1 CONCLUSION

In this study, a total of 31 morphotaxa of fungi belonging to five genera and three unidentified morphotaxa were identified. The prevalence of the morphotaxa of each genus was highly variable across the districts and PAs that were considered for sample collection. *Aspergillus* and *Penicillium* were the dominant genera of storage fungi, respectively where as *Bipolaris* were the least prevalent encountered on stored wheat grains collected from the study area. Most of the morphotaxa encountered during the current study occurred in more than one PAs while a few were confined to one or two of the sampled PAs. In this regard, Edola Burka of Shashemene district was the most diverse PA. According to the results of this study, it was concluded that stored wheat grain from the study area could be contaminated by storage fungi.

4.2 RECOMMENDATIONS

According to the results of the current study the authors recommended that awareness creation and training should be given to the farmers on better and improved storage techniques. The author also recommended further investigation in this area should look into how the diversity and prevalence of storage fungal communities on stored food grains changes in time and space. Further study should be also focus on the investigation of toxin produced by these storage fungi and in relation to the health status of human being in the study area.

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