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MORPHOLOGICAL DIVERSITY OF FUNGI ASSOCIATED WITH STORED GRAINS OF MAIZE (*Zea mays L*) IN SHASHEMENE AND ARSI NAGELLE DISTRICTS, ETHIOPIA

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ABSTRACT: Maize (Zea mays L.) is a cereal crop grown throughout the world. Invasion of maize grains by fungi leads to losses in quantity and quality. The objective of the current study was to assess the diversity of fungi associated with stored maize grains under storage conditions of farmers in Shashemene and Arsi Nagelle districts. Stratified random sampling technique was used to collect maize grains from the study sites of Arsi Nagelle and Shashemene districts. Two peasant associations (PAs) from each district and 10 HHs from each of the PAs, with a total of 40 HHs were purposively drawn for sample collection. A 2% MEA and PDA plates were used for the isolation of fungi associated with the collected maize grains. Identification of the isolates to the genus level was performed on the basis of culture characteristics and spore morphology. A total of 767 fungal isolates belonging to ten genera and four unidentified taxa were obtained. Out of which 430 (56.06%) were from Shashemene and 337(43.94%) were from Arsi Nagelle. In general comparison, higher isolation rate (IR) was found at Shashemene than Arsi Nagelle. While, in case of Arsi Nagelle districts higher number of fungal isolates was at Ali Wayo 172 (22.43%) than Adaba Tita 165 (21.52%). Aspergillus spp. was the dominant that were represented by 284(37.027%) isolates from all sites. Penicillium was the second most frequently encountered genus where 196(25.55%) isolates was recovered from all sites. It was concluded that stored maize grain 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: Fungal communities, Grain quality, Morphotaxa, Post-harvest, Storage losses.

1 INTRODUCTION

Cereal grains are the major source of food for most humans and domesticated animals in the world [21], [22]. Maize (*Zea mays L*.) is a cereal crop grown throughout the world; and plays an important role in the diet of millions of Africans due to high yields per hectare, its ease of cultivation, adaptability to different agro-ecological zones, versatile food uses and storage characteristics [3]. It is also an important crop in much of the developing countries and grows over a wider geographical range and in a variety of environments than any other cereal crop and is the third most important crop on a global basis [7].

In Ethiopia, Maize is cultivated throughout the country in diverse ecological conditions covering wide geographic areas ranging from moisture stress areas to high rainfall areas and lowlands to the highlands [16]. In terms of area coverage, maize constitutes about 17% of the cultivated area which is over one million hectares [8], and 24% of the grain production in the country [15].

Grain storage conditions affect its quality. From observation of Shashemene and Arsi Nagelle districts farmers have various harvesting, processing and storing conditions. These farmers have developed a variety of storage practices. The most common storage methods are plastic bags, baskets and sacks. According to [6], storage is associated with a range of hazards. Mould spoilage, pest infestations and grain germination are the main problems. Food grains are subjected to contamination

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and subsequent colonization by microorganisms during different phases of development, harvesting, preparation, transport and storage [25].

Fungi are the major cause of deterioration during storage and cause total deterioration of grain mass. 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 invasion and colonization of grains and whether they occur before or after the harvest [14]. Storage fungi are those that subsequently grow after harvest in store, transport or processing [24].

Although there is a general knowledge that food grains can be affected by moulds in the field and store, the extent of contamination and diversity of fungi that do contaminate grains may vary depending on environmental factors prevailing in the field and store. The species of fungi that contaminate the grains of different crop species may not be the same. Even though maize is one of the major food grains in Ethiopia, there were no sufficient studies made on its deterioration in store and the diversity of fungi that are associated with it in the study area. In the current study, an attempt was made to assess the diversity of such fungi associated with stored grains of maize in Shashemene and Arsi Nagelle districts of West Arsi Zone.

2 MATERIALS AND METHODS

2.1.1 DESCRIPTION OF THE STUDY AREA

The investigation was conducted in two geographic locations, Shashemene and Arsi Nagelle districts known to produce maize grain which are located in the West Arsi Zone, Oromia National Regional State.

2.1.2 CLIMATE AND AGRO-ECOLOGY OF THE ZONE

Topographically, the altitude of West-Arsi ranges from 500 meters to 3200m above sea level. The highland is temperate and cold climate (are locally called *Dega* or *Baddaa* ranges from 2300- 3200masl), midland (warm or locally called *Woinadega* or *Badadaree* –ranges from 1500 to 2300masl), and lowland are hot and arid (also called *Kola* or *Gamojjii*, ranges from 500 to 1500masl) each comprising 45.5%, 39.6% and 14.9% of the land area respectively. Average annual temperature in the zone varies between 15 and 20 °C [1].

2.1.3 SHASHEMENE DISTRICT

Shashemene district has an area of 768.88 km2 and bordered by Siraro in the West, Arsi Nagelle in the North and North East, Arsi zone in the East and Southern Peoples‟ Regional State in the South and the South East [9]. Most parts of the district have elevations that range from 1500-2300, except the Eastern section with altitudes over 2300m. Shashemene district lies within the altitude range of 500 to 1700m above sea level. Soil types in Shashemene district are clay loam and sandy loam, 32.5% vegetation covers and erratic type of bimodal rainfall [10].

2.1.4 ARSI NAGELLE DISTRICT

Arsi Nagelle district is bordered by Shashemene on the South, by Lake Shalla which separates it from Siraro district on the South West, on the West by the Southern Nations, Nationalities and Peoples Region, 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 altitude of this district ranges from 1500 to 2300 meters above sea level. Latitude 7° 20' N and Longitude 38° 09'E [23]. The soil type is mainly vertisol and alfisol with pH around 7.5[12].

2.2 SAMPLING STRATEGY AND SAMPLE COLLECTION

2.2.1 SAMPLING STRATEGY

Stratified random sampling techniques were used to collect maize grain from the study sites, Shashemene and Arsi Nagelle districts. From Arsi Nagelle two peasant associations (PAs) namely Ali Wayo and Adaba Tita, while from Shashemene districts Kore Rogicha and Chafa Guta were selected based on key and production capacity. From four PAs ten households (10HHs) were systematically selected in the analysis for the identification of fungi associated with the maize grain. A total of 40 HHs were purposively drawn for sample collection. Selection of the PAs and HHs in 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 where main cereal crop was maize were identified by the key informants. In each PA, HHs known to have surplus production that can be stored for at least four to six months after harvest were identified by the Das.

2.2.2 COLLECTION OF MAIZE GRAIN SAMPLE

Collection of grain samples was conducted during the months of June- July, 2011. 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 [13]. The storage sacks for sample collection were randomly selected by the researcher followed by sampling by hand using alcohol sterilized plastic bags 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 sacks with open palm and then closing once at the point of sampling. The number of primary samples obtained from a HH varied according to on 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 working samples were drawn from the composite sample. Grain samples were collected in air tight plastic bags immediately upon drawing them in order to prevent alteration of moisture content of the seeds. The samples were transported to the laboratory of WGCFNR and stored at 25^{o}C until analyses are performed.

2.2.3 ISOLATION OF FUNGI ASSOCIATED WITH STORED MAIZE GRAINS

Each Sample of maize about 50g was surface-disinfected. Two disinfectants were used: 80% (v/v) ethanol in water for 1 min and H2O2 34% (w/v) for 1 min. After decanting, the grain samples were washed three times in sterile distilled water and blotted on sterile filter paper under aseptic conditions. From each sample (50g) 50 grains were randomly picked and then inoculated in Petri plates (90 mm diameter, 10 grains/plate) containing Malt Extract Agar (MEA) and PDA using sterile forceps. One hundred kernels were used in each treatment and replicated four times. After sealing with parafilm and careful labeling, Petri plates were incubated at 25 ℃ 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 on to sterile plates of MEA and PDA. After incubation fungal colonies were counted. Fungi were sub-cultured on PDA and later identified.

During isolation, data on the frequency of isolation and sampling site was recorded. Pure cultures were maintained on MEA slants stored at 4 ℃. Pure cultures were grouped on the basis of their morphological features which may include culture morphology as well as conidial characteristics.

2.2.4 IDENTIFICATION OF THE FUNGAL ISOLATES

Identification was based on the [5] 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. Conidial morphological characteristics used for the microscopic identification include shape and color of the spores, septation, presence or absence of specialized appendages on the spores. Identification of filamentous moulds like *Aspergillus* and *Penicillium* spp. was based on microscopic examination of the sporulating parts of a colony using slide culture since it is too difficult identifying them by mounting only.

2.3 DATA ANALYSIS AND PRESENTATIONS

Microsoft Excel software programs were used in the calculations of treatment means and summary tables presented wherever required. Data obtained from the research work was presented using tables as percentages. The isolation rate (IR, is a quotient calculated by dividing the number of isolates obtained from maize grain by the total number of maize grain inoculated. This allows for the measurement of fungal species richness in a grains sample [18].

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 maize grains inoculated [18].

$$RF=\frac{\text{Number of isolates of a taxon}}{\text{Total number of isolates of all taxa obtained from inoculated maize grains}} X\ 100$$

3 RESULTS

3.1 FUNGI ISOLATED FROM MAIZE GRAIN SAMPLES

A total of 767 fungal isolates were recovered from 40 maize samples collected from all sampling sites. Higher number of fungal isolates 430 (56.06%) was isolated from maize grain samples collected from Shashemene district than from those collected from Arsi Nagelle district, 337 (43.94%) (Table.1). However, the data is not statistically significant at $\alpha = 0.05$(Table.1). Among the PAs, the highest numbers of fungal isolates was recovered from samples collected from Kore Rogicha PA, 272 (35.5%) while the lowest number of isolates, 158 (20.6%) was obtained from grain samples collected from Chafa Guta PA. The difference between PAs within each district with regard to the number of isolates was much larger (114 isolates) between Kore Rogicha and Chafa Guta PAs of Shashemene district while there was a very narrow difference (7 isolates) between the Ali Wayo and Adaba Tita PAs of Arsi Nagelle district (Table 1).

3.2 FUNGAL GENERA ISOLATED FROM STORED GRAINS OF MAIZE AND THEIR DISTRIBUTION AMONG THE SAMPLING SITES

The 767 fungal isolates were separated into 58 different groups that were recognized as morphotaxa. The morphotaxa were identified to 10 known genera while four morphotaxa could not be identified and thus provisionally kept as unidentified taxa. The identified genera include *Aspergillus*, *Penicillium*, *Phoma*, *Alternaria*, *Nigrospora*, *Trichoderma*, *Fusarium*, *Cephalosporium*, *Cladosporium* and *Absidia*. Among the genera, *Aspergillus* and *Penicillium* were the first and second most dominant genera represented by 284 isolates and having RF of 29 37.03% and 196 isolates and RF of 25.55%, respectively followed by the third dominant group Trichoderma represented by 131 isolates and RF 17.08%. *Aspergillus* was predominantly isolated from Kore Rogicha PA in Shashemene district with 158 of the 284 isolates of *Aspergillus* obtained in the study isolated from grain samples collected from the PA. This figure represents 55.63% of the *Aspergillus* isolates. The remaining 126 isolates of *Aspergillus* were obtained from samples collected from the remaining three PAs (Table 1). *Alternaria*, *Fusarium* and *Nigrospora* were represented by 66, 29 and 23 isolates, respectively. Furthermore, *Cephalosporium* and *Nigrospora* were not isolated from the two PAs of Shashemene district (Table 1).

Among the identified genera, Aspergillus and *Penicillium* were the most diverse (species rich) genera each represented by 19 and 17 morphotaxa, respectively. *Alternaria* and *Cephalosporium* were the least diverse genera encountered in the current study each represented by single morphotaxa (Table 1). *Phoma* was comprised of five morphotaxa but only eight isolates coming from three of the PAs from which samples were collected suggesting that were rare. *Cephalosporium* and *Nigrospora* were not only rare but were also isolated only from one and two PAs, respectively. The unidentified morphotaxa were composed of four distinct groups represented by only 10 isolates coming from all the PAs from which grain samples were collected (Table 1).

Table 1: Fungal genera encompassing the morphotaxa isolated from maize grain samples from Shashemene and Arsi Nagelle districts

Identified genera	Number of morphotaxa	Number of isolates	Origin of isolates			RF (%)	
			Shashemene		Arsi Nagelle		
Aspergillus	**19**	**284**	KR	CG	AW	AT	**37.3**
			158	28	56	42	
Penicillium	17	196	44	58	43	51	25.5
Alternaria	1	66	12	26	19	9	8.60
Phoma	5	8	2	3	3	-	1.04
Trichoderma	2	131	41	30	27	33	17.08
Fusarium	3	29	7	7	7	8	3.78
Nigrospora	2	23	-	-	12	11	3.00
Cephalosporium	1	5	-	-	-	5	0.65
Cladosporium	2	11	2	4	2	3	1.43
Absidia	2	4	1	-	2	1	0.52
Unidentified taxa	4	10	5	2	1	3	1.30
Total	58	767	272	158	172		

Key: - - Absent, KR- Kore Rogicha, CG-Chafa Guta, AW- Ali Wayo, AT-Adaba Tita, RF - Relative frequency

4 DISCUSSION

FUNGI ASSOCIATED WITH STORED MAIZE GRAIN FROM THE TWO SITES

From two geographic locations varying number of fungal isolates was recovered. The altitude-mediated ecological diversity might expose the grain to a range of fungi [19], [20]. The observed difference in terms of the number of isolates between the two districts might be explained by the geographic and climatic differences between the districts. The current observation concurs with [17] stating that development of microscopic fungi on grain depends upon ambient humidity and temperature. Shashemene district may be more humid and cooler than the Arsi Nagelle district, a factor which might lead to higher level of infection of the grains both in the field and in the store.

The previous study by [26] strengthens our finding that fungi have in the course of evolution diversified to exploit a wide variety of habitats and different species hence require different conditions for optimal growth. Microbial metabolism is significantly influenced by the environmental conditions and toxin-producing fungi may invade food at pre-harvesting period, harvest-time, during post-harvest handling and in storage. For instance [27] showed that temperature and humidity/wetness are the main climatic factors influencing the development of *Fusarium* on cereals. Moreover, conditions favourable for *Fusarium* development are also favourable for mycotoxin production on cereal grains and high moisture favours the production of mycotoxins [11]. [19] Also concluded the dependency of fungal development on climate, plant and storage conditions. They said that climate represents the key agro-ecosystem driving force of fungal colonization, mycotoxin production and non-infectious factors (e.g. bioavailability of (micro) nutrients, insect damage, and other pests attack), that are in turn driven by climatic conditions also influence fungal colonization and mycotoxin production.

From the Shannon diversity index the diversity of fungi shows that higher diversity of fungi was obtained from Shashemen than Arsi Nagelle. This is because that higher diversity of fungal isolates and abundance decrease as altitude increases [4]. In terms of identified genera *Aspergillus* the most abundant and dominant genus. 284 (37.027%) isolates were obtained from all sites. Out of this 158 (20.60%) isolates were at Kore Rogicha while 28 (3.65 %) at Chafa Guta. *Penicillium* was the other most frequently encountered genus. 196 (25.55) isolates was obtained from all sites. From this 44 (5.73), 58 (7.56), 51 (6.64) and 43 (5.60) were Kore Rogicha, Chafa Guta, Adaba Tita and Ali Wayo respectively. The previous study by [2] strength our finding that he obtained fifteen species of fungi identified from the maize samples isolated from Ambo, Adama and Dire Dawa. According to his results, *Aspergilli* were the most frequent fungi, occurring in 94% of the samples. *Fusarium* spp. occurred in 76.5% of the samples while *Penicillium* spp. was found in 64% of the samples.

This clearly indicates that the two geographic locations are different not only in terms of species richness and abundance, but also due to the proportion of taxa they harbor. Generally, the highest number of morphotaxa was obtained from Shashemene than Arsi Nagelle. This observation may be due to several factors that may have been involved, most notably the geographic and climatic variations between the two sites. similar pattern of association of fungi with maize grain in that he isolated total fungal density ranged from 2.8×10^2 to 9.4×10^3 cf/ g in samples from Dire Dawa, 2.4×10^2 to 3.5×10^4 cfu/g in those from Adama, and 2.2×10^4 to 1.3×10^6 for samples from Ambo. He concluded that there were substantial variations in cfu/g among the samples regardless of their geographic origin. Furthermore, genetic variations of the maize samples and the floristic composition of the sites may have contributed to the observed differences [2]. However, such speculations need to be verified with further studies.

5 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

In our study Shashemene district and its selected peasant association harbored more fungal isolates and morphotaxa than Arsi Nagelle district and its peasant association. The highest distribution of the fungal isolates was recorded from Shashemene followed by Arsi Nagelle. Among the recovered fungi, *Aspergillus* and *Penicillium* were the first and second most diverse and abundant genera both in terms of number of isolates and recognized morphotaxa. Samples collected from Kore Rogicha PA of Shashemene district harbored the highest number of fungal isolates and morphotaxa. The implication of higher number and diversity of storage fungi with grain samples could be health risks to the public.

5.2 RECOMMENDATION

Good storage is vital to minimize post-harvest losses. Therefore, integrate Management strategies like, cultural practices, physical, chemical and biological methods should be recommended. Awareness creation and training on post harvest handling of food grains to the farmers need to be organized in order to reduce potential health risks associated with the mycotoxin production by the fungi.

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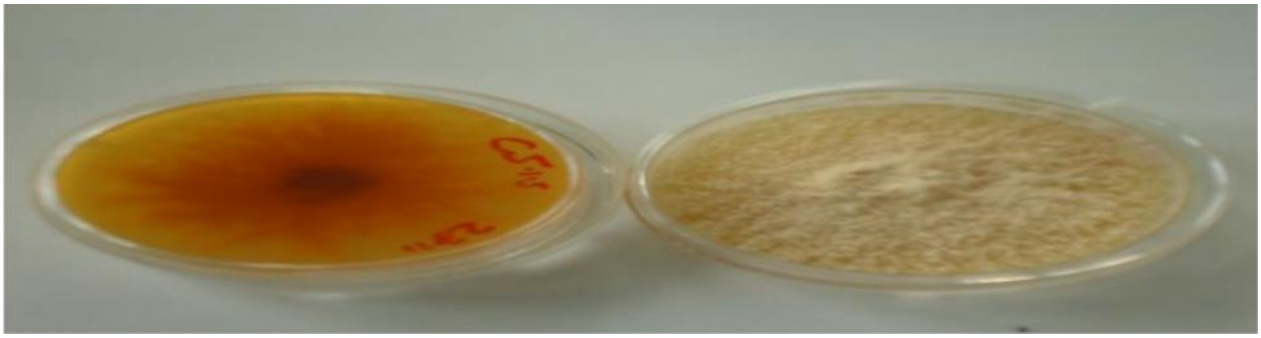
SOME OF REPRESENTATIVE PICTURES OF THE IDENTIFIED GENERA

Lower side view of Aspergillus sp.

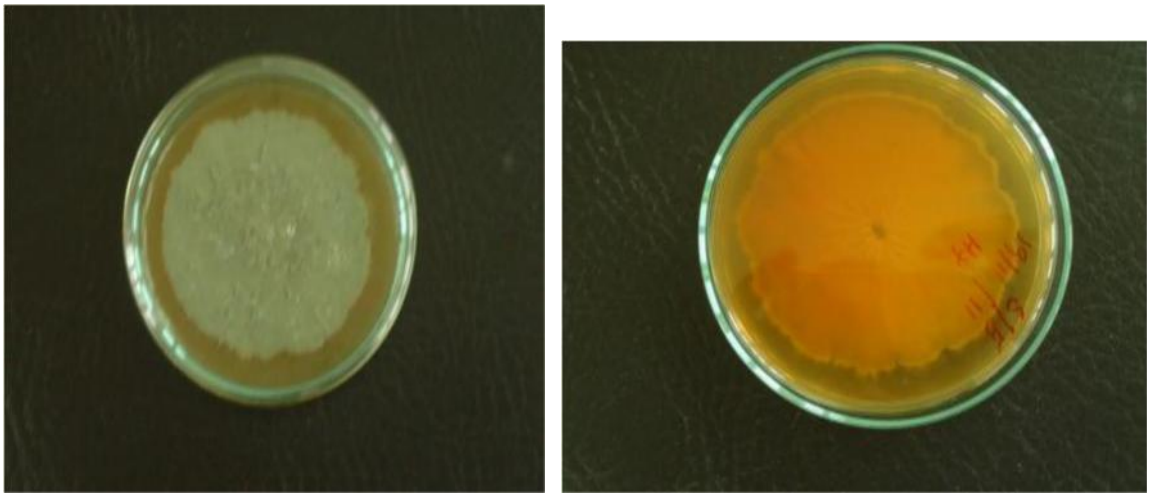
Upper side View of Aspergillus sp

Upper side view of Penicillium spp.

Lower side view of Penicillium sp.



Upper and lower side view of Fusarium spp.



Upper side view of Trichoderma sp. ***Lower side view of Trichoderma sp.***

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