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Problems of seed-borne fungal diseases affecting sorghum grain (*Sorghum bicolor* L. Moench) in two districts of Oromia, Ethiopia

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Abstract

Mycological examination of 48 samples of sorghum grains obtained from farmers' households in two districts (Dawa Chefa and Kemise) of Northeastern Ethiopia resulted in 1087 fungal isolates separated into 55 morphotaxa (3 of which unidentified) belonging to 13 genera. The genera include *Aspergillus, Alternaria, Arthrinium, Bipolaris, Botrytis, Cercosporella, Cladosporium, Colletotrichum, Curvularia, Fusarium, Penicillium, Phoma* and *Trichoderma*. *Aspergillus*, dominated (46% of all the mycoflora) followed by *Penicillium* (15.7%) and Bipolaris having the lowest occurrence (0.7%). About 45% of the samples of the sorghum seeds were found infected by more than one fungus, more isolates were obtained in samples collected from mid altitude zones whereas highly diversified fungi samples were observed in low agro-ecological zones with the highest Shannon diversity index obtained from Kemise district (H'= 1.99864) whereas highly diversified (H'=2.20718) morphotaxa were encountered at Qello.

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Introduction

Food security has a primary focus not only in Ethiopia but all over Africa and Asia/Latin America whereas, small farm systems providing food for more than 70% of the global population (World Economic and Social Survey, 2011). Although agriculture sector in Ethiopia expected to have a growth rate of 8.1% in 2011/12 (African Economic Outlook, 2010). The fact that most of agricultural producers of Ethiopia are subsistence farmers with small land holdings (a mean of 1.2 hectares), with 55.13% holding less than one hectare, the smallholder farmers account for the majority of the rural population - more than 85% - who rely on agricultural production for livelihood. As the smallholder agricultural production is characterized by low output, poor access to land, and poor access to inputs, poor irrigation system, little access to know-how (risk management, technology and skill), low level of market orientation, poor infrastructure and institutional factors, the task of sustainable economic development is really challenging (MoFED, 2005; Moti, 2007). Through strengthening these traditional small food production systems with modern knowledge, technology and economic support, the countries like Ethiopia can attain sustainable food production (World Economic and Social Survey, 2011). But these subsistence farmers all over the world are facing problems originating from degradation of natural environment, pests and diseases and factors arising from external sources like climate change. The most commonly reported negative impacts of seed borne fungi include spoilage of food, reduction in the storage lifespan of seeds, seed rotting, and reduction in seed vigor, reduction in germination and damping-off in the nurseries (Lilja *et al.,* 1995). Also, seed-borne diseases have been found to affect the growth and productivity of crop plants. The seed-borne fungi cause reduction in seed viability and vigor, low percentage of germination and discoloration (Subbaiah, *et al*, 1982). Other impacts affecting livelihoods are food poisoning in man and animals due to the production of mycotoxins by certain species (aflatoxin, produced by the common storage fungus *Aspergillus flavus* causing liver collapse in certain domestic animals); lung diseases such as asthma and skin allergies especially aspergillosis (e.g. handling mouldy straw or prolonged exposure when emptying infested products/vessels/containers).

Sorghum (*Sorghum bicolor* L. Moench) is an important staple food, particularly in the semi-arid tropical regions of Africa and Asia, also an important feed grain and fodder crop in the Americas and Australia. It is one of the major cereal crops, which ranks fifth after rice, wheat, maize and barley in terms of importance and production constituting the main food grain for over 750 million people in the semiarid tropics of Africa, Asia and Latin America. Sorghum is known for withstanding harsh environmental conditions including high temperature, moisture deficit and water stagnation but is susceptible to chilling (Sleper *and Poehlm*., 2006). The crop is suited to less fertile degraded soil and hot, dry agro-ecological areas where it is difficult to grow other grain crops and is truly a multi-purpose crop with grain as the highly valued product (Seetharama *et al.*, 2005). Sorghum is the third widely cultivated crop, next to teff (*Eragrotis teff*) and maize (*Zea mays*) in Ethiopia, growing on more than one million ha of land (CSA 2008 / 9). A crop grown over a wide range of ecological habitats in the country, in the range of 400-3000 masl (Teshome *et al.*, 2007) and being drought resistant C4 plant cultivated during the dry season, it can withstand the rigors of rainfall and temperature variability due to climate change.

Sorghum production in the world including Ethiopia is affected by different biotic and abiotic constraints among which seed-borne fungi of grain sorghum are considered as one of the most destructive biotic constraints in most sorghum growing regions of the globe. Diseases cuased by these fungi can affect all parts of sorghum most noteablly aboveground parts of the crop (Mathur and Manandhar, 2003) and also can determine the health condition of the seed and hence crop yield (Sultan *et al.,* 1991). A number of fungal species associated with sorghum, like Fusarium, *Aspergillus* and *Penicillium*, have been reported to produce mycotoxins that cause

mycotoxicoses of domestic animals and man (Moss, 1986). Sorghum seed mycoflora, in Burkina Faso had a total of thirty four fungal species belonging to 17 genera (*Alternaria* nees, *Bipolaris* shoem. *(syn. Drechslera* fide Ellis*), Botryodipldia* (sacc.) sacc., *Cercospora fres., Colletotrichum corda, Curvularia boedijn, Drechslera ito, Exserohilum leonard and suggs., Fusarium link, Gloeocercospora bain and Edgerton ex deighton, Macrophomina petrak, Myrothecium tode, Nigrospora zimm., Peronosclerospora (ito) shirai and K. hara, Phoma sacc., Sphacelotheca de bary, Tolyposporium woronin ex schroter)* (Neya *et al.,* 2008).

The aim of this study was to assess the diversity, prevalence and incidence of the seed-borne fungi associated with farmers' saved-grain samples of sorghum collected from Kemise and Dewachefa districts of Oromia Zone of Amhara Regional State of Ethiopia.

Materials and methods

Location of sampling

Sampling was conducted in Kemise and Dewa Chefa districts of Oromia Zone of Amhara Regional State from May to June, 2011 using stratified random sampling method. From each of the districts, two Peasant Associations (PAs) namely Qello and Tiyyo PAs from Kemise and Sheklla and Dindin PAs from Dewa Chefa were selected. The PAs were purposively selected in order to cover the major agro-ecological zones (Dindin and Tiyyo belonging to Mid highlands or *Weyna Dega* and Qello & Sheklla to lowland or *Kolla*) known for sorghum production (10°43'12" Latitude and 39°52'12" Longitude with an elevation of 1424 meters above sea level), a total of 48 households (HH) were included. Sampling of sorghum grain was done mainly from ground pit storages and from small baskets, bags or sacks of each household by taking about 0.5kg of grains from different depths (top, middle and bottom) of the storage system and mixed. The agar plate method recommended by ISTA (1996) was used for the isolation of fungi associated with the seeds of different locations.

Laboratory Method

From each sample, 50 sorghum seeds were randomly taken and briefly sprayed with 70% ethanol, surface sterilized with 10% sodium hypochloride (NaOCl) for five minutes, rinsed twice with sterile distilled water and dried on sterile filter paper in a laminar flow cabinet. The surface sterilized seeds were then inoculated onto Malt Extract Agar and Potato Dextrose Agar with five replicates of ten seeds per plate. Plates were turned upside down, labeled and incubated at room temperature (25^{o}C) for 5- 14 days to allow growth of fungal colonies on the medium. Identification was based on the International Course on the Identification of Fungi of Agriculture and Environmental Significance Manual (CAB, 2000).

Method of analysis

The morphotaxa were identified on the colony and mycelium characters, the relative frequency (RF, expressed as percentage of the total morphotaxa) was calculated as the total number of isolate from a single taxon divided by the total number of isolates from genera identified from all seeds incubated (Lv *et al.*, 2006).

The Shannon diversity index measuring both diversity and evenness can be used to look at the level of species (morphotaxa) diversity and evenness of distribution (Kent and Coker, 1992). Two components of diversity are combined in the Shannon diversity index: the number of species and equitability or evenness of allotment of individuals among the species (Krebs, 1985). The Shannon diversity index (*H'*) was used to characterize diversity of morphotaxa in a community (in a site) which accounts for both abundance and evenness of the morphotaxa present were calculated (Kent and Coker, 1992). However, care should be taken in interpreting because, usually Shannon diversity index place most weight on the rare species in the sample (Krebs, 1985) and is also moderately sensitive to sample sizes. The Sorensen similarity coefficient was applied to qualitative data and widely used because of; it gave more weight to the species that were common to the samples rather than to those that only occur in either sample (Kent and

Coker, 1992).

Results

Seed-borne fungal isolates recovered from grains of sorghum

A total of 1087 seed-borne fungal isolates (Table 1) were recorded from four different PAs, the highest (355) were recovered from Tiyyo PA (Kemise) while the lowest number (194) was recorded at Dindin (Dawa Chefa).

Table 1. Total fungal isolates recorded from stored grains of sorghum collected from four different Pas.

District	PA	No. of fungal isolates	%
Dawa Chefa	Shekla (low land)	293	26.9
	Dindin (mid altitude)	194	18.0
Kemise	Qello (low land)	245	22.5
	Tiyyo (mid altitude)	355	32.6
	Total	1087	100

Table 2. Abundance, relative frequency and incidence of the fungal genera isolated from sorghum grain samples collected from the study area.

Genus	No. of fungal isolates	Relative frequency % of the genus	Incidence of the genus %
Alternaria	63	5.8	2.6
Arthrinium	31	2.9	1.3
Aspergillus.	500	46	20.8
Bipolaris .	8	0.7	0.3
Botrytis .	14	1.3	0.6
Cercosporella .	24	2.2	1.0
Cladosporium .	55	5.1	2.3
Colletotrichum	10	0.9	0.4
Curvularia .	16	1.5	0.7
Fusarium	62	5.7	2.5
Penicillium [c]	171	15.7	7.1
Phoma	56	5.2	2.3
Trichoderma	37	3.4	1.5
Unidentified taxa	40	3.6	1.7
Total	1087	100	45.2

The identified morphotaxa, on the basis of spore morphology and culture characteristics, belonged to 13 genera eg *Alternaria, Arthrinium, Aspergillus, Fusarium*, *Bipolaris*, *Botrytis*, *Cercosporella*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Phoma, Trichoderma* and *Penicillium* (Table 2). *Aspergillus* was the most prevalent and abundant genus with the largest number of isolates (500), also had the highest relative frequency (46%) and about 20% of incidence on sorghum grains (Table 2). The second most prevalent and abundant genus was *Penicillium* represented by 171 isolates with a relative frequency of 15.1% and incidence (7.1%). Other genera with RF of 5% and above were *Alternaria*, *Fusarium*, *Phoma* and *Cladosporium,* while the unidentified taxa were represented by 40 isolates and accounted for 3.6% of the total number of isolates.

While *Aspergillus* was the most prevalent genus in the grain samples from all the four PAs followed by *Penicillium* (Table 3), other (four) genera viz, *Aspergillus, Cladosporium, Penicillium* and *Phoma* were obtained in all the sampled PAs, while *Arthrinium, Cercosporella* and *Bipolaris* were unique

to the samples collected from Qello. Also, *Curvularia, Colletotrichum* and *Fusarium* were absent from the two mid altitude (Weyna Dega) zones, namely Dindin and Tiyyo, while *Trichoderma* was the only genus which was unique to the seed samples collected from these sites. Likewise, seed samples collected from low (Kolla) altitude (Sheklla and Qello) were free from Trichoderma, also interesting, the three genera, *Colletotrichum*, *Curvularia* and *Fusarium* were found to be unique to the samples obtained from Sheklla and Qello (Table 3).

Table 3. Relative frequency of the identified fungal genera isolated from saved sorghum grains samples collected from the study area.

Genus	RF(%) of the genera in each of the sampled PAs				Overall RF (%)
	Sheklla	Dindin	Qello	Tiyyo	
Alternaria	1.1	0	3	1.7	5.8
Arthrinium	0	0	2.9	0	2.9
Aspergillus	14.5	10.5	5.8	15.2	46
Bipolaris	0	0	0.7	0	0.7
Botrytis	1.3	0	0	0	1.3
Cercosporella	0	0	2.2	0	2.2
Cladosporium	0.9	1.7	0.6	1.9	5.1
Colletotrichum	0.6	0	0.4	0	1
Curvularia	0.8	0	0.6	0	1.4
Fusarium	3.6	0	2.1	0	5.7
Penicillium	1.7	3.3	2.4	8.3	15.7
Phoma	1.1	1.8	0.8	1.4	5.1
Trichoderma	0	0.1	0	3.3	3.4
Unidentified	1.3	0.6	1	0.8	3.7
Total	26.9	18	22.5	32.6	100

Alternaria was obtained in grain samples collected from all the PAs except Dindin, the maximum incidence of the genus was recorded in seed sample of Qello (3%) followed by Tiyyo (1.7%), while *Fusarium* was found only in samples collected from Sheklla and Qello with higher RF recorded from samples from Sheklla (3.6%), *Botrytis* was observed in samples from Sheklla only, not found in samples from Dindin, Qello and Tiyyo. *Cladosporium* was observed in seed sample of Tiyyo (1.9%) and Dindin (1.7%), while the low record was encountered in Qello and Sheklla samples, (0.6%) and (0.9%) respectively. The genera *Arthrinium, Bipolaris and Cercosporella* were observed in one sample area (Qello) only were not detected in the seed samples of Sheklla, Dindin and Tiyyo while *Phoma was* observed in all the four sites.

Table 4. Fungal genera encompassing morphotaxa, total isolates and relative frequency(RF) of seed-borne genera recovered from the study sites.

Fungal Genera	No. of Morphotaxa	Sampling Sites				Total No. of Isolates	RF (%)
		Sheklla	Dindin	Qello	Tiyyo		
Aspergillus	15	158	114	63	165	500	27.3
Penicillium	8	19	36	26	90	171	14.6
Fusarium	5	39	0	23	0	62	9.1
Cladosporium	5	10	17	7	21	55	9.1
Alternaria	3	12	0	32	19	63	5.5
Arthrinium	1	0	0	31	0	31	1.8
Bipolaris	2	0	0	8	0	8	3.6
Botrytis	1	14	0	0	0	14	1.8
Cercosporella	3	0	0	24	0	24	5.5
Colletotrichum	2	6	0	4	0	10	3.6
Curvularia	2	9	0	7	0	16	3.6
Phoma	3	12	20	9	15	56	5.5
Trichoderma	2	0	1	0	36	37	3.6
Unidentified taxa	3	14	6	11	9	40	5.5
Total	55	293	194	245	355	1087	100

Distribution and relative frequency of morphotaxa isolated from stored sorghum grains

The relative frequencies of morphotaxa for each genus revealed that the morphotaxa of *Aspergillus* were the dominating fungi encountered, isolates of *Aspergillus* were separated into 15 distinct morphotaxa which covers the highest number, most rich among the identified genera (Table 4), while six of these morphotaxa were isolated from grain samples collected from all the sites, two were unique to each of the districts (i.e., one to Dawa Chefa district and one to Kemise district).

Table 5. Distribution and relative frequency of morphotaxa of the genus *Aspergillus*.

Morphotaxa of *Aspergillus*	PA				Total	RF %
	Kolla Zone		Weyna Dega Zone			
	Sheklla	Qello	Dindin	Tiyyo		
morph.1	13	0	5	0	18	3.6
morph.2	32	9	18	16	75	15
morph.3	6	2	0	19	27	5.4
morph.4	8	4	15	3	30	6.0
morpha.5	0	2	0	12	14	2.8
morph.6	38	0	10	8	56	11.2
morph.7	1	0	0	10	11	2.2
morph.8	17	7	18	16	58	11.6
morph.9	1	4	7	25	37	7.4
morph.10	36	6	12	5	59	11.8
morph.11	2	0	4	8	14	2.8
morph.12	1	0	0	6	7	1.4
morph.13	1	0	3	1	5	1.0
morph.14	2	9	15	3	29	5.8
morph.15	0	20	7	33	60	12.0
Total	158	63	114	165	500	100
Total(Zone)	221		279			

Table 6. Distribution and relative frequency of morphotaxa of *Penicillium*.

Penicillium morphotaxa	PAs				Total	RF %
	Sheklla	Dindin	Qello	Tiyyo		
morph.*1*	-	-	-	8	8	4.68
morph.*2*	3	-	4	3	10	5.85
morph.*3*	7	14	4	34	59	34.50
morph.*4*	4	-	6	13	23	13.45
morph.*5*	-	-	-	11	11	6.43
morph.*6*	3	16	9	8	36	21.05
morph.*7*	-	-	-	6	6	3.51
morph.*8*	2	6	3	7	18	10.53
Total	19	36	26	90	171	100

The rest seven morphotaxa were obtained from two or three PAs from both the districts (Table 4). *Penicillium* was second and *Fusarium* and *Cladosporium* were third in terms of morphotaxa diversity having eight, five different morphotaxa, respectively. *Penicillium* was second to *Aspergillus* in terms of incidence of occurrence with about 7.1 % (Table 4), however, the incidence of *Penicillium* was

much lower than that of *Aspergillus*. *Penicillium* had relative frequency of 14.6% (8 out of 55), followed by *Cladosporium* and *Fusarium* 5 (9.1%) while three of the genera, viz, *Alternaria*, *Cercosporella* and *Phoma* had RF of 5.5% each with 3 total morphotaxa. The lowest percentages of frequency with a single morphotaxon (1.8%) were observed in *Arthrinium* and *Botrytis*. The rest four genera namely *Trichoderma*, *Colletotrichum*, *Curvularia* and *Bipolaris,* had RF of 3.6% each. Three morphotaxa with a relative frequency of 5.5% were left unidentified.

Table 7. Distribution and relative frequency of morphotaxa for *Cladosporium*.

Cladosporium morphotaxa	PA				Total	RF %
	Shekla	Dindin	Qello	Tiyyo		
morphotaxon.1	4	-	-	4	8	14.55
morph.2	3	-	4	3	10	18.18
morph.3	-	12	-	5	17	30.91
morph.4	3	-	-	6	9	16.36
morph.5	-	5	3	3	11	20.00
Total	10	17	7	21	55	

Distribution, relative frequency and abundance of morphotaxa from sorghum grains among the PAs

Aspergillus was the largest genus both in terms of the number of morphotaxa recognized and total number of isolates (500) obtained, of them 221 were recorded from lowland or Kolla agro-ecology in both Dawa Chefa and Kemise districts which comprised of 15 different morphs. Among them, *Aspergillus* morph.2 was the most abundant represented by 75 isolates with RF of 15%. This morphotaxon was isolated from all the PAs in the study with little marked variation in the number of isolates (Table 5). *Aspergillus* morph.15, *Aspergillus* morph.10, *Aspergillus* morph.8 and *Aspergillus* morph.6 were represented by 60 to 56 types (12 to 11.2 %) of the isolates. *Aspergillus* morph.13 and *Aspergillus* morph.12 were very rare with the RF of 1% and 1.4%, respectively (Table 5). *Aspergillus* morph. *1*, morph *5*, morph *7* and morph *12* were recorded only in two of the sample sites, of which *Aspergillus* morph*1* was unique to Dawa Chefa district while *Aspergillus* morph*5* was restricted to Kemise district.

Table 8. Distribution and relative frequency of morphotaxa for *Fusarium*.

Fusarium morphotaxa	PA				Total	RF %
	Shekla	Dindin	Qello	Tiyyo		
morph.1	6	-	3	-	9	14.52
morph.2	-	-	8	-	8	12.91
morph.3	-	-	4	-	4	6.45
morph.4	-	-	3	-	3	4.84
morph.5	33	-	5	-	38	61.29
Total	39	0	23	0	62	

Penicillium was the second largest genus of the isolated fungi of the study, represented by 8 different morphotaxa, among which morph.3 was the most frequent morphotaxon with a RF of 34.50% (59) followed by morph. 6 having RF of 21.05% (36) (Table 6). Both of these and morph. 8, were distributed across all the four Sites. Furthermore, these two morphotaxa were most frequently isolated from both districts of PAs with mid highland (Weyna Dega) climatic zones, i.e., Dindin and Tiyyo; and 126 isolates out of the 171 total isolate were also obtained from these two PAs (Table 6). The remaining morphotaxa

were rare and restricted in distribution, for instance, three of the morphotaxa (*Penicillium* morph.1, morph.5, and morph.7) were restricted to Tiyyo PA of Kemise district where morph.7 was the least abundant (3.51%). Moreover, *Penicillium* morph.2 and morph.4 were recorded from all PAs; except Dindin PA (Table 6).

Among the ten (five to each) morphotaxa of the two genera; *Cladosporium and Fusarium,* none of them were distributed across all the four PAs. Out of the five morphotaxa of *Cladosporium*, only two morphotaxa viz *Cladosporium* morph.*2* and morph.*5* were distributed across the three sampling sites of PAs; while the rest of the three morphotaxa *(* morph.1*,* morph.3 *and* morph.4) were restricted to the two out of four PAs (Table 7). Regarding the abundance, distribution and relative frequency for the *Fusarium* morphotaxa, they were rare and restricted to one or two of the four sampling sites with lowland (Kolla) in both Kemise and Dawa Chefa districts. Among the five morphotaxa recognized in *Fusarium*, three morphotaxa (*Fusarium* morph.2, *Fusarium* morph.3 *and Fusarium* morph.4) were the rarest and restricted only to a single sampling area of Qello PA. The rest of the two morphotaxa were distributed across the two PAs of study sites. The highest RF was recorded for *Fusarium* morph.5 whereas the lowest RF was recorded for *Fusarium* morph.4 (Table 8). Apart from these four, other genera were represented either by a single taxon or represented by two or three morphotaxa. *Arthrinium* and *Botrytis* were both represented by a single morphotaxon each. Both of them were isolated from sample sites with Kolla in that *Arthrinium* having 31 isolates all encountered in Qello whereas *Botrytis* with 14 isolates was from Shekla (Table 9).

Table 9. Distribution, abundance and relative frequency of morphotaxa of the relatively less frequent and less abundant genera.

Morphotaxa	Study sites				Isolates		RF %
	Dewa Chefa		Kemise		no of isolates	TOTAL	
	Shekla	Dindin	Qello	Tiyyo			
Alternaria morph.1	7	-	21	-	28	63	44.44
Alternaria morph.*2*	-	-	3	16	19		30.16
Alternaria morph.*3*	5	-	8	3	16		25.40
Arthrinium.	-	-	31	-	31	31	100.00
Bipolaris morph.*1*	-	-	5	-	5	8	62.50
Bipolaris morph.*2*	-	-	3	-	3		37.50
Botrytis.	14	-	-	-	14	14	100.00
Cercosporella morph.*1*	-	-	12	-	12	24	50.00
Cercosporella morph.*2*	-	-	5	-	5		20.83
Cercosporella morph.*3*	-	-	7	-	7		29.17
Colletotrichum morph.*1*	6	-	-	-	6	10	60.00
Colletotrichum morph.*2*	-	-	4	-	4		40.00
Curvularia morph.*1*	5	-	4	-	9	16	56.25
Curvularia morph.*2*	4	-	3	-	7		43.75
Phoma morph.*1*	4	16	7	6	33	56	58.93
Phoma morph.*2*	3	-	-	2	5		8.93
Phoma morph.*3*	5	4	2	7	18		32.14
Trichoderma morph.*1*	-	-	-	23	23	37	62.16
Trichoderma morph.*2*	-	1	-	13	14		37.84
unidentified morph.1	-	3	-	4	7	40	17.50
unidentified morph.*2*	6	3	7	5	21		52.50
unidentified morph.*3*	8	-	4	-	12		30.00

Alternaria, Cercosporella and Phoma were represented by three morphotaxa each, among the morphotaxa of *Alternaria*, the first morpholotaxon (morph. 1), isolated only from the two lowland (Kolla) sampling sites, has relatively the highest RF of 44.44% with a total number of 63 isolates. But rests of the morphotaxa of Alternaria were isolated from all sample sites, except Dindin. On the other hand, all the three morphotaxa of *Cercosporella* were isolated only from Qello PA of low land (Kolla) agro-ecology in Kemise district, out of which morph.1 had relatively high (24) number of isolates. The genus *Phoma* with three morphotaxa was recorded from all the PAs having 56 total isolates, except *Phoma* morph.2, which was isolated from two sample sites, the two morphotaxa (morph.1and morph.3) were recorded in all study sites, morph1 being relatively more abundant of the three (Table 9). Likewise, *Bipolaris*, *Colletotrichum*, *Curvularia,* and *Trichoderma* each was represented by two morphotaxa. Among them, both *Bipolaris* and *Curvularia* were isolated only from sample areas with low land (Kolla) agro-ecology in one or/and both district(s); i.e., *Bipolaris* was isolated from Qello in Kemise district whereas *Curvularia* was from Sheklla and Qello. *Trichoderma*, on the other hand, was encountered from mid highland (Weyna Dega) areas in both Dawa Chefa and Kemise districts. The genera *Bipolaris*, *Colletotrichum, Curvularia,* and *Trichoderma*, the morphotaxa of the first rank (morph.1) were relatively more abundant compared to the rest of the morphotaxa.

Table 10. The Sorensen similarity index among the Pas.

Sampled PAs	QS			
	Dindin	Qello	Tiyyo	Kemise
Sheklla	0.06571	0.10502	0.07716	
Dindin		0.06378	0.07286	
Qello			0.07667	
Dawa Chefa				0.02208

Diversity index of the fungi isolated

Among the isolates identified, the highest diversity of morphotaxa (15) was observed for *Aspergillus* with fast growing colonies of deep to light black, white, yellow, yellow-brown, brown to black, yellowish-green or shades of green colors and a dense felt of erect conidiophores terminated in vesicle. Some colonies showed granular, flat, often with grooves, bright to dark yellow-green and others with a compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads. On the other hand, *Penicillium* with 8 morphotaxa were identified by the typical morphology appeared in culture, fast growing, suede-like to downy, white with yellowish-green conidial heads which become grayish-pink to brown with age; some colonies are rough, glabrous, tan-colored and yeast-like and produced a diffusible brownish-red to wine red-pigment. The Shannon diversity index of seed-borne fungi revealed that higher diversity of seed-borne fungi was recorded at low land (Kolla) sites, while the mid highland site.

The Shannon diversity index for geographic variation between sampling sites indicated that the diversity of seed-borne fungi was higher in Kemise site than from Dewa Chefa site but not significant. Morphotaxa similarity between the PAs calculated using Sorensen similarity index, a relatively higher index was observed between Sheklla and Qello, with lowland (Kolla) , in both Districts than between Dindin and Tiyyo sites (Table 10). As far as the districts are concerned, the index value for similarity revealed the existence of less similarity between morphotaxa of both Kemise and Dawa Chefa districts.

Discussion

The present study revealed that 45% of the sorghum grains were infected by different species of fungi, this was common in both the mid-high land and low land areas in the two districts of Ethiopia. Also, in Libya,

sorghum grains exhibited the highest contamination (45%) among the cereals (Karim, 2006). About 39% of the sorghum seeds infected by fungi in Bangladesh (Karim (2005), while Islam *et al.* (2009) also reported 36% of the sorghum seeds collected from eight different locations in Bangladesh. The prevalence of fungi recorded in sorghum grains varied depending on the location of sample collection (geographic location) and agro-ecological zones (Karim, 2005). Seven species of fungi detected in sorghum seeds obtained from different locations of Punjab, India were A. *flavus*, *A. niger*, *A. tenuis*, *C. lunata*, *F. moniliforme*, *Helmintho-sporium* (*Bipolaris*) *sativum and Penicillium* spp. *F. moniliforme* (Randhawa *et al.*,1998). Neya *et al.*(2008) identified 34 fungal species belonging to 7 genera in Burkina Faso. Seed-borne mycoflora of sorghum from different parts of the world include *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium sp., Fusarium moniliforme, F.oxysporum, F. pallidoroseum, Drechslera tetramera, Nigrospora sp., Phoma sp., and Rhizopus sp.* (Ahmed *et al.,* 1992). Sultan and Magan, 2010) obtained 9 genera belonging to 26 species in sorghum seeds from Pakistan.

The results of the present study indicated that the morphological diversity of seed-borne fungi was rich in sorghum grains. The differences in the number of isolations in the two agro-ecological zones in sorghum grains may be due to climatic and geographic, from mid highland (Weyna Dega) areas (549 or 51%) , but within this environment there was variation eg 33% isolates was obtained from Tiyyo, while 18% was isolated from Dindin. Though there existed a slight altitudinal difference even within the two Weyna Dega PAs where Dindin has relatively higher altitude than Tiyyo, this results need further investigation. There existed a strong obvious connection between diversity of isolates and agroecological zones of the study sites where lowlands (Kolla) supported more diversified fungi than the midhighland (Weyna Dega) ones. This might be due to differences in altitude or probably because of host heterogeneity, where the isolates could have different host ranges, and biological adaptation of the fungi. Regarding the diversity of isolates, site Qello was most diversified which may be explained by differences in geographic location, altitude, habitat heterogeneity and diversity of community. These findings of the current study strengthen the idea stated that geographic location affects distribution of fungal diversity (Gore and Bucak, 2007).

Conclusion

A total of 55 morphotaxa belonging to 13 genera and a few unidentified taxa were recognized in the current study. The abundance and prevalence of the fungi varied considerably depending on the location and agro ecological condition of sites. The identified genera were *Aspergillus, Alternaria, Arthrinium, Bipolaris, Botrytis, Cercosporella, Cladosporium, Colletotrichum, Curvularia, Fusarium, Penicillium, Phoma and Trichoderma*. The incidence of fungal association with the grains of sorghum was about 45% of the incubated grains. Among the fungi isolated from the grain samples of sorghum, *Aspergillus was the* most abundant and the most diverse genus followed by the *Penicillium*. The other genera were relatively less abundant and less diverse. Locations in mid highland agro-ecological zones resulted in more number of seed-borne fungi than those with Kolla climatic zones, but morphotaxa (species) diversity was more in lower sites. Further work should be focused on molecular techniques for complete taxonomic identification, the production and effect of their mycotoxins.

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