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Actors' post-harvest maize handling practices and allied mycoflora epidemiology in southwestern Ethiopia: Potential for mycotoxin-producing fungi management

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Summary

Maize plays a key role in household food security in Ethiopia, but its benefit has been limited with high post-harvest losses. This study was initiated to assess post-harvest practices and associated fungi pathogen epidemiology along the maize supply chain in southwestern Ethiopia. The study was conducted in five purposively selected districts and a three-stage sampling procedure was employed for selection of the target groups. In total, 342 participants from different actors were interviewed using a semi-structured questionnaire. Maize samples were collected every month from 63 randomly selected actors for mycological analysis during six months storage period. Post-harvest loss was estimated to be 31% and loss during storage was identified as a critical loss point. Comparing all biological agents, loss due to fungal pathogens in the store ranked on top. Moisture content at loading stage could not increase the shelf life of the commodity. Germination tests showed a significant ($P < 0.01$) decrease as storage duration increased, while mould incidence on cobs and kernels significantly ($P < 0.05$) increased. In total, seven fungal genera were isolated, characterized and identified, with *Fusarium, Penicillium* and *Aspergillus* being predominant. Most of the post-harvest practices are not effective in reducing post-harvest losses. Especially, farmers' traditional storage structures can be influenced by external climatic conditions and make the grains liable to develop mould during the rainy season. This research, therefore, highlights the need to design, develop or modify existing storage technologies that reduce post-harvest loss due to mycotoxin-producing fungal pathogens. Furthermore, post-harvest drying to obtain optimum moisture content is also crucial to reduce losses.

Keywords: Mould, post-harvest management, post-harvest loss, storage fungi, stored maize

Introduction

Food security is a major challenge in sub-Saharan African countries. Whilst increasing production through crop intensification has been suggested, the reduction of post-harvest losses (PHLs) has received little attention. Globally, PHLs has been estimated at one-third of the production but it could be even higher in sub-Saharan Africa (FAO, 2011). The magnitude of PHLs due to deterioration of quality seems to be at a level similar to quantity losses. For instance, the caloric loss is estimated at 24% of all food produced (Lipinski et al., 2013). This suggests that PHL reduction can play a key role to improve food, nutritional security and household income. It is also considered as the easiest and cheapest option for resource conservation (Yahia, 2008).

The food security and economic wellbeing of Ethiopia, in general, depends on agriculture. Maize is considered amongst the top commodities contributing to food security due to its wide adaptability, high production, productivity and relatively cheap calories compared to other cereals. As a result, it has been included in the national food security strategy via intensive agriculture systems (Abate et al., 2015). Maize production and productivity in Ethiopia has doubled in less than two decades and is the second in sub-Saharan Africa in yield/ha (Abate et al., 2015). However, this boost in production and productivity threatens to be negated by high PHLs, further affecting food security. For instance in Africa, maize PHLs were estimated at 14% to 36% (Tefera, 2012). Losses in weight of the same commodity in eastern and southern Africa was estimated at 17.5% (Rembold et al., 2011) and 41% to 80% for maize stored for six months using farmers traditional storage in Ethiopia (Sori and Ayana, 2012). In Ghana, up to 15% weevil attack was reported for five weeks stored maize (Baidoo et al., 2010).

Maize PHLs occur along the whole activity chain including harvesting, drying, shelling, transport to store, storage, transport to market and processing for consumption (Rembold et al., 2011). As a result, different research recommended commodity handling system analysis as the rational step in identifying suitable tactics for reducing PHLs along the activity chain (Kitinoja and Gorny, 1999; Kader, 2005). However, several authors have reported maize PHLs in Ethiopia in general and in southwestern Ethiopia in particular without considering those chain of activities and analysis handling systems (Ashagari, 2000; Dubale et al., 2012; Sori and Ayana, 2012; Befikadu, 2014). Consequently, issues leading to high PHLs were not fully identified and characterized along the activity chain in order to reduce losses. Identifying locally available post-harvest (PH) technologies and practices along the maize PH activity chain should be the first step in designing loss reduction strategies.

An efficient PHL reduction strategy for maize basically depends on the ecological conditions of storage which includes, physical, chemical and biological characteristics of the maize grain; the storage period and type; and functional characteristics of the facility (Golob et al., 2002; IFPRI, 2010; Dubale et al., 2012; Befikadu, 2014). Despite the realization of the importance of storage, the potential impact of destructive storage pests (especially fungi) that cause quality deterioration leading to quantity, nutritional and financial losses has not been well researched (Fourar-Belaifa et al., 2011). Furthermore, maize PHL by fungal pathogens is not only of economic importance, but is also a public health concern due to the possible production of mycotoxins (Golob, 2009). *Aspergillus, Fusarium* and *Penicillium* spp. are the top three mycotoxins producing fungi in food and feed in the tropics and sub-tropics along production chains. Generally, contaminated kernels by consuming those mycotoxins, particularly aflatoxin, resulted in illness, death, immunological suppression, liver cancers and nutritional interference (Rundbergeta et al., 2002; Jonathan et al., 2004; Stumpf et al., 2013; Kiarie et al., 2016).

Study findings reported that on-farm storage practices and structures in southwestern Ethiopia, such as *gombisa,* can make maize susceptible to different types of damage, including storage pests and mould development (IFPRI, 2010; Dubale et al., 2012; Befikadu, 2014).

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Furthermore, research findings reported maize PHL based on point data taken once or twice, and sample collection for loss assessment without considering the full storage duration until the product was depleted. At the same time, previous researches only focused at the farmer level without considering other actors along the maize supply chain that play a key role in maize transactions. Therefore, the current research was designed to assess maize post-harvest handling practices and the fungal pathogens dynamic associated with maize stored by producers, collectors, and wholesalers in selected districts of the Jimma zone in southwestern Ethiopia.

Materials and methods

Study site

The study was conducted in Jimma Zone, which is situated in southwest Ethiopia at 7°15´ and 8°56´ N latitude and 36°00´ and 38°38´ E longitude. The elevation of Jimma Zone ranges from 800 to 3360 m.a.s.l. The agro-ecological setting includes highlands (15%), midlands (67%) and lowlands (18%) (CSA, 2009; ZoFEDO, 2013). For the current study, five districts namely Dedo from the highlands; Kersa, Omonada and Mana from the midland; and Sokoru from the lowlands' agro-ecology were purposely selected based on their high maize production potential and varied agro-ecological conditions (Fig. 1).

Participant selection

A three-stage sampling procedure was used to select participants. Jimma Zone was purposively selected from the southwest part of the country due to its high maize producing potential. Five districts ranking among top maize producers and representing variable agro-ecology from the aforementioned zone were selected purposely based on secondary data from Jimma Zonal Agricultural Office. After discussion with Agricultural Office experts and extension agents from each district, three *kebeles* (the lowest administrative region, Peasant Associations, PAs) were selected based on agro-ecological settings and the potential for maize production. Considering the total population of each PA, sample size (the number of participants) selected from the households was determined using sampling formula with a 95% confidence level (Yamane, 1967 as cited by Ajay and Micah, 2014) as indicated below.

$$n = \frac{N}{1 + N}(e)\,2$$

Where *n* is the sample size; *e* is the level of precision at 5% and *N* is the total number of maize producing household in selected PA.

After determination of sample size, a list of all household was collected from each PA and respondents were selected randomly using Minitab randomization software. Similarly, a number of collectors from each district and wholesalers from Jimma town were randomly selected and used for data collection. For triangulation and validation of the information collected, key informants from farmers, developmental agents, experts at districts and Zonal level were also interviewed. In order to acquire the desired depth of information, a total of 342 participants were involved in the study.

Data collection techniques

The study was conducted from January 2014 to June 2015. Both quantitative and qualitative data were collected from secondary and primary sources in the selected districts and communities. Secondary data were collected from the archives of various organizations. Semi-structured questionnaires covering socio-economic, demographic data, maize post-harvest handling practices and associated issues were collected during field surveys via village meetings, key informants, and individual interviews. House to house interviews were carried out with the help of developmental agents from each PA. Semi-structured questionnaires were prepared accordingly for each participant (farmers, key informants, traders and experts) to generate reliable data. Pre-test interviews were conducted before actual data collection at each study site and amendments were made before the final interview.

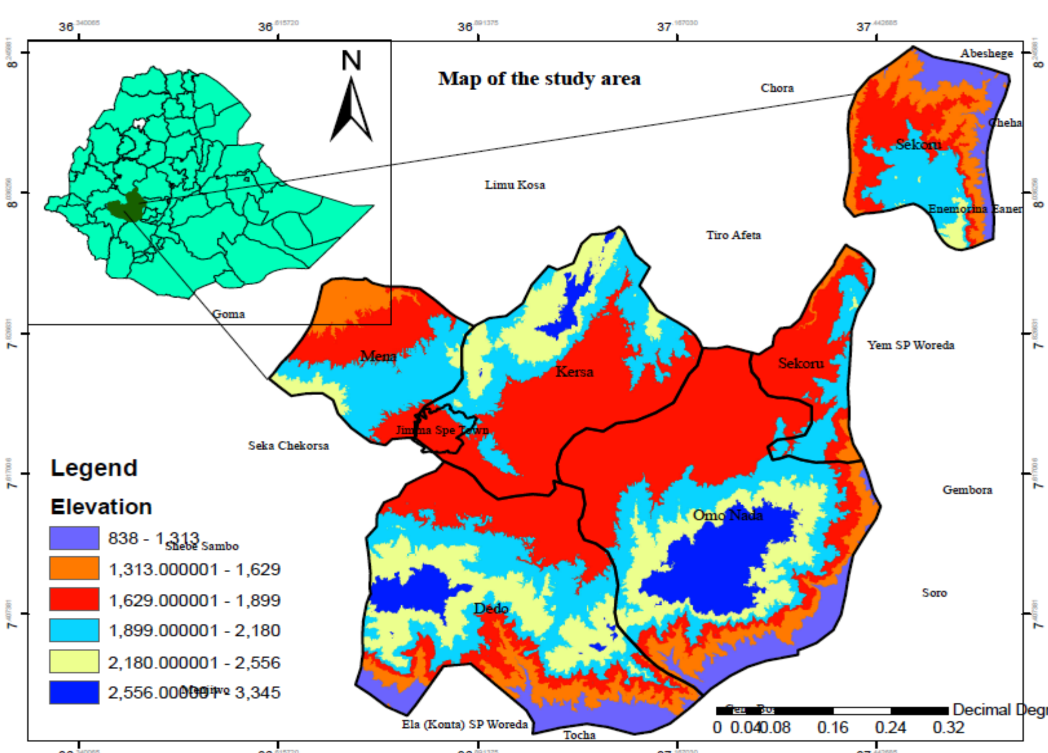

Fig. 1: Study area map with different agro-ecology of the study districts of Jimma Zone, Southwestern Ethiopia.

From each PA, three farmers were randomly selected from those growing the dominant BH-660 maize variety and storing their maize in a local storage structure, called *gombisa,* of uniform structure. In total, 45 farmers were included for the mycological study of the maize samples collection. Similarly, three local collectors from each district (15 in total) and three wholesalers from Jimma town were also included in the fungal pathogens assessment of maize kernels sample. Disease assessment and sample collection started at harvest and loading stage (farmers) then continued with monthly intervals up to six months, at which time most of the participants' stored product depleted from all actors store.

Experimental designs

A 3 × 6 factorial design was used for the determination of the germination test, mould incidence on cobs and maize kernels stored in the farmers' traditional storage structures. Three agro-ecological levels (highland, midland, and lowland) and six-months storage duration with monthly interval data collection were used at the farmer level. Three farmers from highland and lowland agro-ecological setting were used as replicates. However, the average of three districts used as replication for midland agro-ecology as more dominantly maize is produced in this agro-ecology setting. All factors including storage structures, maize variety, and management practices were kept uniform to minimize experimental error. Similarly, for the collectors 3 × 6 factorial design was used that included the three agro-ecological levels of the respective districts and six-month storage duration with a monthly interval for the determination of germination test and mould incidence of maize kernels. For all collectors, the same maize variety, open-weave sacks and management practices used were uniforms. In a similar manner, three collectors from each district were used as replicates. For the wholesalers, three actors were included in the study with six level of storage duration. A completely randomized design was used for determination of germination test, mould incidence on kernels, cobs and maize as samples were collected from Jimma town alone for wholesalers condition.

Germination test and moisture content

The germination test was undertaken by randomly selecting 150 maize kernels from each sample lot. The test was done in triplicate: 50 kernels per replication. Maize kernels were sown in 9 cm Petri-dishes lined with filter paper (Whatman No. 1), moistened with distilled water and then placed on a clean laboratory bench at room temperature (25 °C) for 7 days. The germinated seeds were visually examined for the appearance of radical and/or plumule and the germination percentage was computed following developed method (OGENDO et al., 2004).

$$\text{Germination (\%)} = \frac{a}{b} \times 100$$

Where a stands for a number of germinated kernels, and b stands for the total number of plated kernels.

The moisture content of the sample maize was determined using a digitally calibrated moisture tester (Wile[55] TR serial number 554601 by Farm comp Agro-electronics, France) on the spot.

Mycological analysis

Six cobs from each farmer's store were brought for laboratory analysis. Similarly, two kilograms of maize kernels from every trader's store were sampled by deep probing at three layers of each sack, then mixed together and brought for mycological analysis under laboratory conditions.

Mould incidence on-cobs-maize

Sixty cobs were picked from a different level of each farm storage structure (top, centre, and bottom) and fed through PVC pipe fitted at the middle and bottom of the *gombisa* which allowed removal of sample cobs from the store. Twenty cobs sampled from each layer were used as replication (ATUKWASE et al., 2012). A visual inspection and scrutiny for kernel infections on each cob was made to record mould in order to calculate the incidence following (MEER et al., 2013).

$$\text{Mould incidence (MI) on-cobs-maize} = \frac{a}{b} \times 100$$

Where a stands for a number of infected cobs while b stands for a total number of cobs assessed.

Mould incidence on kernels

A blotter test was used to determine mould incidence on maize kernels and the test was carried out following developed procedures (FANDOHAN et al., 2003; HAJIHASANI et al., 2012).

$$\text{Mould incidence on kernel (\%)} = \frac{a}{b} \times 100$$

Where a stands for infected kernels, while b total number of kernels plated.

Isolation and identification of fungal genera

Fungal pathogens on the maize kernels were grown, isolated and identified to the genus level on a monthly basis until the six month of storage period following standard techniques and procedures (NARAYANASAMY, 2006; PITT and HOCKING, 2009; ATUKWASE et al., 2012). The isolated fungal genera frequency of occurrence and relative density were calculated (MOSTAFA and KAZEM, 2011; MEER et al., 2013).

$$\text{Fungal frequency (FF)} = \frac{nf}{nt} \times 100$$

Where nf number of particular fungal genera and nt a total number of samples/kernels.

$$\text{Relative density (RD)} = \frac{ng}{tg} \times 100$$

Where ng stands for a number of the specific isolated genus, while tg for a total number of the fungal genus.

Data analysis

Statistical Package for Social Sciences (SPSS) version 20.0 software was used for descriptive analysis of data (socioeconomics, maize PHM practices, the frequency of occurrence and relative density of fungal genera recorded). Whereas germination tests, mould incidence on kernels and on-cobs-maize were analyzed using SAS software version 9.0 after checking ANOVA assumptions. Analysis of Variance was carried out using general linear model (GLM). Wherever significant difference was observed, mean separation was carried out using Tukey's Honestly Significant Difference (HSD) test at the 5% probably level.

Results

Socio-economic characteristics

Most of the respondent farmers and traders were between 30 and 59 years old. But, about 78% of the experts participating were less than 30 years old. The majority of the respondent farmers and traders had primary education. More than 50% of the farmers had up to two decades' experience in maize production but only a few traders more than that. However, none of the participant experts had more than five years' experience in maize post-harvest management. Most of the

socio-economic characteristics did not show significant differences ($P > 0.05$) between surveyed agro-ecological settings (Tab. 1).
Survey results showed a majority of the participants (74%) had a family size of 5-10 and 58% of the farmers had less than five workforces in the household. On average, most of the participant farmers (68.4%) produced maize on less than 1 ha of land. Similarly, most of the farmers (51.3%) allotted up to 50% of their land for maize, while 34.7% of the farmers allocated up 75% out of their total land, the rest allotted one-quarter of their land for maize production. In the study areas, participant farmers mainly produce maize for household consumption (67.9%) while 32.1% of the producers used it both for consumption and for selling (as an income source). Most of the collectors collected maize from nearby PAs in their district. About 50% of collectors sold their maize to individual consumers. Wholesalers also sold to both local traders and consumers (33.3%) and Addis Ababa traders (50%). Low quality maize especially discoloration and irregularity of maize supply was the major trading problem mentioned by both collectors and wholesalers.

Maize post-harvest practices

In the current study, 10 post-harvest handling practices have been identified at producer level but harvesting, transportation, drying, storage and shelling are amongst the key activities carried out by producers (Fig. 2). Some of handling practices to maintain PH quality also carried out by traders too.

Harvesting

Maize harvesting in the study area started in September and lasted until December. Farmers used visual observation, crop calendar method, shelling and observing kernel dryness; and checking seed

Tab. 1: Socio-economic characteristics of the participants

Actor	Socio-economic characteristics	Agro-ecology				Statistical test	
		lowland	midland	highland	mean	χ^2-value	P-value
Farmer	Sex (%)					0.278	0.870^{ns}
	• Male	95.5	94.6	92.7	94.3		
	• Female	4.5	5.4	7.3	5.7		
	Educational level					3.921	0.687^{ns}
	• None	4.5	20.8	17.1	14.1		
	• Basic	18.2	15.4	14.6	16.1		
	• 1 to 6	59.1	46.2	53.7	53.0		
	• 7 and above	18.2	17.7	14.6	16.8		
	Age (years)					12.896	0.012*
	• Less than 30	9.1	5.4	24.4	13.0		
	• 30 to 59	81.8	86.9	70.7	79.8		
	• More than 60	9.1	7.7	7.3	8.0		
	Experience (years)					7.834	0.098^{ns}
	• Less than 10	22.7	7.7	17.1	15.83		
	• 10 to 20	31.8	24.6	29.3	28.57		
	• More than 20	45.5	67.7	53.7	55.63		
Traders	Sex					2.974	0.245^{ns}
	• Male	100.0	100.0	83.3			
	• Female	0.0	0.0	16.7			
	Educational level					8.922	0.063^{ns}
	• 1- 6	33.3	60.0	0.0	31.1		
	• 7- 10	50.0	30.0	33.3	37.8		
	• 11- 12	16.7	10.0	66.7	31.1		
	Age (years)					7.145	0.128^{ns}
	• less than 30	0.0	50.0	16.7	22.2		
	• 30 to 59	66.7	50.0	66.7	61.1		
	• 60 and above	33.3	0	16.7	16.7		
	Experience (years)					7.559	0.109^{ns}
	• Less than 10	16.7	70.0	83.3	56.7		
	• 10 to 20	66.7	30.0	16.7	37.8		
	• More than 20	16.7	0.0	0.0	5.6		
Experts	Sex					0.297	0.862^{ns}
	• Male	90.0	87.5	80.0	85.8		
	• Female	10.0	12.5	20.0	14.2		
	Age (years)					2.385	0.304^{ns}
	• less than 30	70.0	62.5	100.0	77.50		
	• 30 to 59	30.0	37.5	0.0	22.50		
	Experience (years)					1.179	0.555^{ns}
	• Less than 3	20.0	12.5	0.0	10.8		
	• 3 and 4	80.0	87.5	100.0	89.2		

Statistically significant at, $*P < 0.05$; ns = not significant.

hardness with the proportion of 61.1%, 28.8%, 8.8%, and 2.1%, respectively to determine the maturity of the crop for harvesting. However, none of the respondent farmers used the moisture testing method to harvest maize for safe storage. The moisture content at harvest and loading stage ranged between 16% to 28% which is far more than the optimum moisture content recommended for long term storage (Fig. 3).

Drying

The majority of the farmers (75.1%) practiced on-farm drying with the cobs still attached to the stalk (Fig. 2), creating favorable conditions for fungal infection. The drying process was usually done by heaping up or spreading out the cobs on bare ground for a couple of days which inadvertently creates favorable conditions for a mycotoxin-producing fungal contamination.

Transportation

About 55.0% of the respondent used animals as transportation means, while the remaining 45.0% used human labor (Fig. 2). However, spillage loss of the harvested product was high during transportation

Storage

The most common maize storage structure used by farmers across all agro-ecological settings was *gombisa* to store maize-on-cobs dominantly without sheaths. *Gombisa* is the type of circular granary and is made by interweaving locally available materials; mostly bamboo split by local artisans. The roof is covered with natural grass or thatch. In rare cases, the corrugated sheet is used. The most critical problem observed in *gombisa* was not climatically controlled structure, resulting in high moisture leakage during the rainy season and the common formation of mould on stored maize. Newly

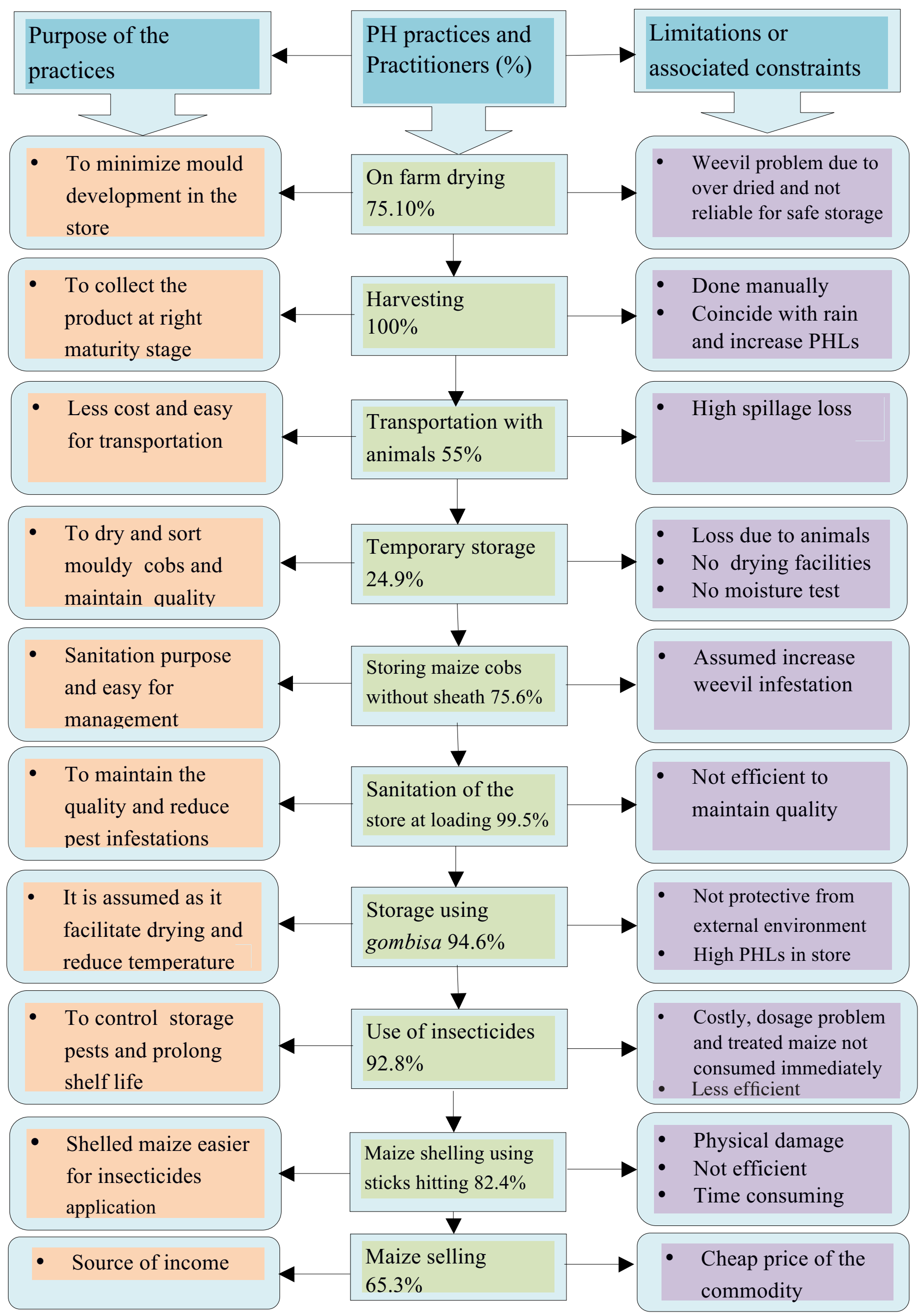

Fig. 2: Flow chart for maize post-harvest activity chain, its function, and associated constraints

constructed *gombisa* can be used for about 10 years and farmers use the same structure every year which serves as an inoculation source to enhance damage by insect pests and stored fungal pathogens.

About 94.8% of farmers stored their new maize separate from the old maize (if available) while, 5.2% of the farmers mixed it with the old maize. Participant farmers stored maize separately from other cereals to prevent from insect damage (73.6%), to avoid difficulty during storage management (11.9%) and to control mould problems (8.3%). The remaining farmers mixed maize with other cereals such as sorghum or *teff*. Maize was stored in different forms by farmers. De-husked cob was most common (75.6%), both as cob and shelled kernels (21.2%) and sheath kernels alone (2.6%). Maize cobs were stored on average for six months in *gombisa* then shelled and stored inside the house with sacks for a few months as it depleted mostly at six months.

Both collectors and wholesalers stored shelled maize with sacks inside a house-like structure made up of wood from different trees and roofed by a corrugated iron sheet. In most cases, the inside and outside wall were sealed by mud (81.5%) or cement (18.8%) and the floor was either cemented (56.3%), mud (25%) or mud covered with plastic (18.8%). Only 18.8% of the participant maize collectors store had windows and most of the stores had no ventilation system. However, half of the wholesalers' stores possessed windows and a ventilation system. Still the moisture content was not optimal (Fig. 3). In general, the storage structure of both collectors and wholesalers were not protected from insects, fungal pathogens, and other pests which resulted in high PHLs. In addition, sanitation was the main problem observed in stores belonging to traders.

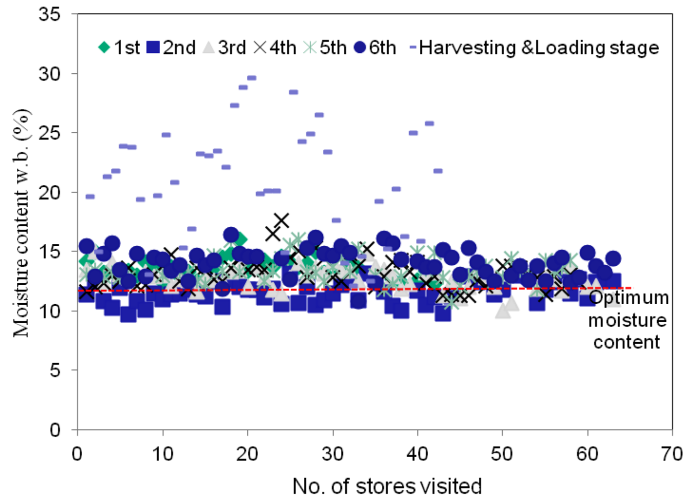

Fig. 3: The moisture content of maize measured with monthly interval from loading stage to six month of storage periods.

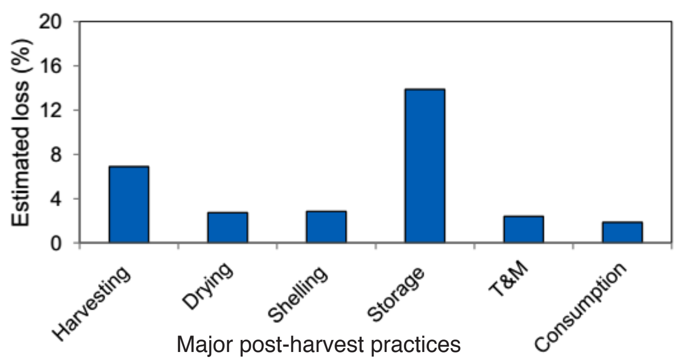

Fig. 4: Actors' maize PHL estimation for major activities. T & M = Transportation and marketing activities.

Shelling

Farmers in the study area shelled maize kernels from cobs manually. Farmers were hitting cob by stick inside the sack, finger palm shelling and hitting cob inside the house which physically damaged and made the kernels prone to fungal damage.

Post-harvest loss estimation and causes

Actors estimated 31% of maize PHL along the activity chain (harvesting, post-harvest drying, shelling, storage, transportation and selling) out of this, 14% happened during storage (Fig. 4). The proportion of PHLs due to biological agents is the most significant factor which starts as the crop reaches physiological maturity. Respondent estimated that the highest proportion of PHL (18%) was due to mould development followed by insect pest, rodents, wild animals and domestic animals in decreasing order.

Germination test

A significant ($P = 0.01$) difference was observed due to the interaction of storage duration and variation in agro-ecology which affected germination percent of maize stored in farmers' storage systems (Tab. 2). Similarly, highly significant ($P < 0.0001$) effect on the germination percentage of maize kernels stored under collector condition was observed without interaction between storage duration and agro-ecological variations (Fig. 5). Maize kernels stored under wholesaler stores were significantly ($P = 0.0012$) affected by storage duration (Tab. 2). The maximum and minimum kernels germination was 93.1 and 65.2% at lowland and highland agro-ecology, respectively.

Mycological analysis

Mould incidence

Both storage duration and variation in agro-ecology exhibited highly significant ($P < 0.0001$) effects on the MI of on-cobs-maize stored

Tab. 2: Mean separation for germination (%) test of stored maize kernels under farm and wholesaler conditions.

Actors	Agro ecology	Storage duration (months)						
		1	2	3	4	5	6	P-value
Farmer	Lowland	93.1 ± 1.7^{a}	$82.2 \pm 1.7^{b-d}$	83.3 ± 1.7^{bc}	$78.5 \pm 1.7^{c-f}$	$77.1 \pm 1.7^{c-f}$	71.2 ± 1.7^{fg}	0.01
	Midland	86.9 ± 1.7^{b}	86.2 ± 1.7^{b}	83.5 ± 1.7^{bc}	80.7 ± 1.7^{cd}	79.2 ± 1.7^{cd}	72.6 ± 1.7^{fg}	
	Highland	83.6 ± 1.7^{bc}	84.1 ± 1.7^{bc}	$78.1 \pm 1.7^{c-f}$	$74.6 \pm 1.7^{d-f}$	$72.9 \pm 1.7^{e-g}$	65.2 ± 1.7^{g}	
Wholesaler	-	91.9 ± 3.9^{a}	83.2 ± 3.9^{ab}	81.3 ± 3.9^{ab}	$76.7 \pm 3.9^{a-c}$	64.7 ± 3.9^{bc}	61.9 ± 3.9^{c}	0.0012

Values are mean ± SE of triplicate samples. Means followed by the same letter among columns and/or rows are not significantly different from each other at P < 0.05 for the farmer and along row for the wholesalers.

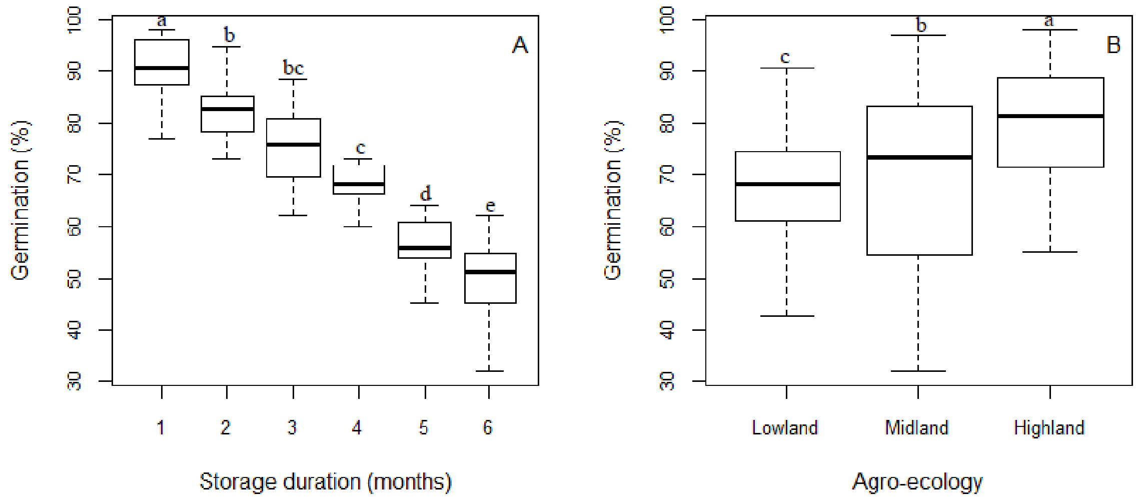

Fig. 5: Box plots for germination test of maize stored in collector stores A) varying storage duration and B) different agro-ecology. P < 0.0001 for both storage duration and agro-ecology**.** Box plots with the same letter(s) for each figure do not significantly different from each other at P < 0.05. Error bars are range values.

under farm conditions (Fig. 6). Significant interaction between agro-ecology and differences in storage duration were observed in MI of maize kernels sampled from both farmer and collector stores (Tab. 3 and Fig. 7), respectively. For wholesaler storage systems, the MI on kernels differs significantly (P = 0.0017) with storage duration (Fig. 8).

Fungal genera

A total of seven fungi genera were isolated, characterized and identified in maize kernels from each actor's store, except for wholesalers; where six fungi genera were recovered. Genus *Fusarium* was the most common fungi based on the frequency of occurrence and relative density followed by *Penicillium* and *Aspergillus* throughout the study period and location (Tab. 4). In the current study, *Fusarium* had the highest frequency of occurrence and relative density during the first two months of storage then slightly decreased as storage duration increased. Comparison of the first month's data with the last month's exhibited a negative increment for *Fusarium* but a positive one for both *Penicillium* and *Aspergillus* (Tab. 5-7).

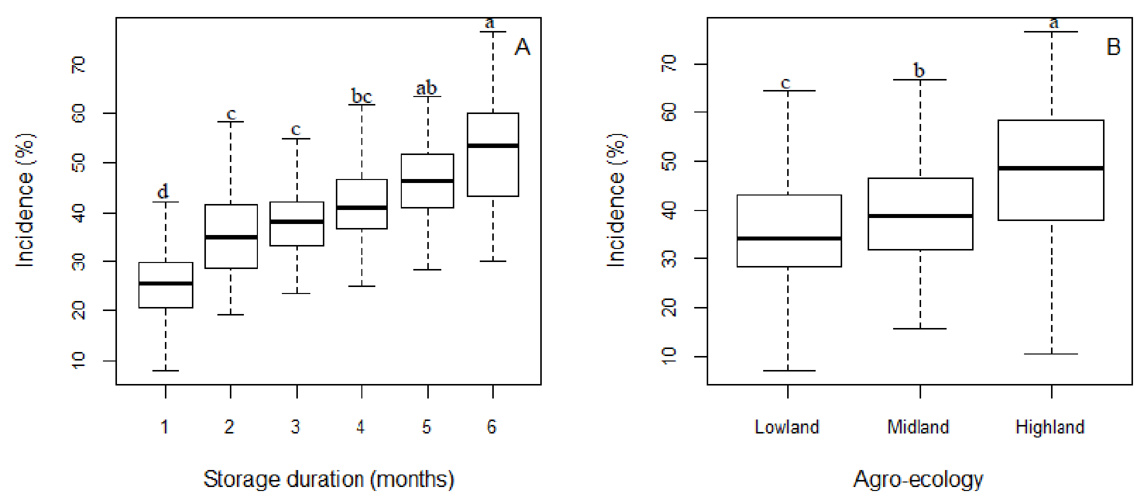

Fig. 6: Box plots for mould incidence of on-cobs-maize stored under farm conditions A) storage duration B) agro-ecology. P < 0.0001 for both storage duration and change in agro-ecology. Box plots with the same letter(s) for each figure do not significantly different from each other at P < 0.05. Error bars are range values.

Tab. 3: Mean separation for mould incidence (%) of maize kernels stored under farm condition

Agro-ecology	Storage duration (months)						P-value
	1	2	3	4	5	6	
Lowland	10.5±2.7^{k}	24.7±2.7^{hi}	30.9±2.7^{g-i}	37.8±2.7^{e-h}	45.2±2.7^{d-f}	51.8±2.7^{b-d}	0.001
Midland	17.1±1.6^{jk}	27.6±1.6^{hi}	34.2±1.6^{f-h}	41.7±1.6^{d-g}	49.6±1.6^{cd}	61.6±1.6^{b}	
Highland	21.8±2.7^{i-k}	28.4±2.7^{g-i}	41.9±2.7^{d-g}	49.2±2.7^{c-e}	59.9±2.7^{bc}	78.9±2.7^{a}	

Values are mean ± SE of triplicate samples. Means with the same letter(s) are no significantly different from each other along the columns and/or rows at P < 0.05.

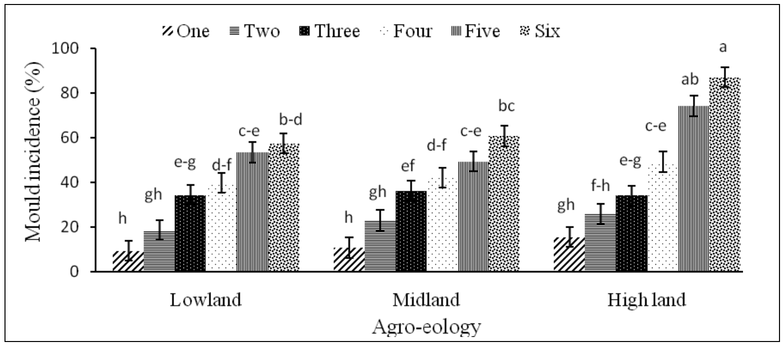

Fig. 7: Mould incidence of stored maize kernels in collector store-house for six months.
P-value = 0.005; Values are mean ± SE of triplicate samples. Means with the same letter(s) do not differ significantly from one another at $P < 0.05$, both for storage duration and agro-ecology.

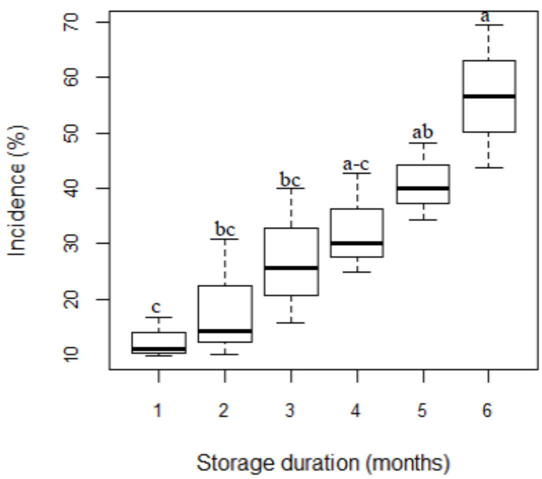

Fig. 8: Box plots for mould incidence on maize kernels collected from wholesaler warehouse. P-value = 0.0017. Box plots with the same letter(s) are do not significantly different from each other at $P < 0.05$. Error bars are range values.

Discussion

The present study identified 10 post-harvest handling activities in the maize PH supply chain. Generally, the moisture content at harvest and loading stage was not optimal to increase the shelf life of the stored product; it actually favored the development of mould in the store. High kernel moisture levels of above 12% increase the chances of fungal growth in the store (DUBALE et al., 2012). It was also suggested that for either mechanical or manual harvesting, the maize grain must be dried to safe moisture levels (FAO, 2011). Furthermore, most farmers in the study area did not use post-harvest drying but wait until the crop dries on the field. Unfortunately, this mostly coincides with rainfall which will also facilitate mould development when the maize is stored. Delayed harvesting causes maize ear rot and *Fusarium* spp. which are the principal pathogenic fungi responsible for causing rotting of maize ears (PITT and KOCKING, 2009). The report also indicated, *Aspergillus* spp. often encountered on maize kernels that were allowed to dry in the field before harvesting (OWOLADE et al., 2005). Producers commonly used domestic animals, both for transportation of harvested maize to stores and to market; loss due to spillage was the most common

Tab. 4: Frequency of occurrence and relative density of fungal genera associated to maize under different actors store

Actors	Fungi genera (%)													
	Penicillium		*Aspergillus*		*Fusarium*		*Phoma*		*Geotrichum*		*Cloudosporium*		*Drechslera*	
	Fr	Rd	Fr	Rd	Fr	Rd	Fr	Rd	Fr	Rd	Fr	Rd	Fr	Rd
Farmer	19.10	17.48	7.80	7.45	48.05	67.33	0.58	1.05	1.57	1.00	3.02	2.68	0.60	0.65
Collector	28.02	23.60	5.95	8.33	61.13	59.18	0.00	0.00	2.67	2.50	1.70	1.38	0.52	0.58
Wholesaler	35.70	20.22	31.02	16.68	36.38	44.95	11.12	1.85	0.00	2.37	6.67	2.53	0.00	0.00

Rd = relative density, Fr = frequency of occurrence

Tab. 5: Frequency of occurrence and relative density of major fungal genera associated to stored maize under farmers condition

Fungal genera	Parameters	Storage duration (months)						Mean	SD	Increment (%)*
		1	2	3	4	5	6			
Penicillium	Frequency	2.20	3.40	12.40	34.10	26.20	36.30	19.10	15.15	93.94
	Relative density	5.80	3.40	26.50	24.40	18.70	26.10	17.48	10.39	77.78
Aspergillus	Frequency	1.10	1.50	2.70	11.20	12.70	17.60	7.80	6.96	93.75
	Relative density	2.70	4.70	3.80	7.80	9.30	16.40	7.45	5.04	83.54
Fusarium	Frequency	67.40	29.10	27.00	66.60	72.60	36.60	48.05	23.00	-84.15
	Relative density	83.60	86.10	64.40	53.90	66.40	49.60	67.33	14.97	-68.55

Where SD = Standard deviation of mean, * = % increment calculated by subtracting last month data from first-month data, then divided the value by last month data and multiply by 100 for each fungal genera frequency of occurrence and relative density.

Tab. 6: Frequency of occurrence and relative density of major fungal genera associated to stored maize under collector condition

Fungal genera	Parameters	Storage duration (months)						Mean	SD	Increment (%)*
		1	2	3	4	5	6			
Penicillium	Frequency	6.90	12.50	23.80	41.80	24.90	58.20	28.02	17.39	88.14
	Relative density	8.50	9.70	20.80	39.00	21.20	42.40	23.60	13.07	79.95
Aspergillus	Frequency	1.80	0.40	3.30	4.00	12.00	14.20	5.95	5.22	87.32
	Relative density	0.40	0.40	10.60	5.50	17.30	15.80	8.33	6.77	97.47
Fusarium	Frequency	81.40	70.30	49.80	46.70	70.20	48.40	61.13	13.39	-68.18
	Relative density	91.10	83.20	47.50	39.60	52.10	41.60	59.18	20.31	-118.99

Tab. 7: Frequency of occurrence and relative density of major fungal genera associated to stored maize under wholesaler condition

Fungal genera	Parameters	Storage duration (months)						Mean	SD	Increment (%)*
		1	2	3	4	5	6			
Penicillium	Frequency	28.80	32.00	2.10	68.90	48.90	35.60	36.05	22.21	19.10
	Relative density	9.20	9.00	1.88	44.40	34.40	24.30	20.53	16.65	62.14
Aspergillus	Frequency	1.60	6.70	44.40	66.70	17.80	48.90	31.02	26.09	96.73
	Relative density	5.60	11.30	8.90	27.80	16.60	29.90	16.68	10.11	81.27
Fusarium	Frequency	60.90	60.90	8.10	12.11	57.80	30.60	38.40	24.73	-99.02
	Relative density	78.00	72.50	24.40	9.08	49.00	45.80	46.46	26.70	-70.31

Where SD = Standard deviation, * = % increment calculated by subtracting last month data from first-month data, then divided the value by last month data and multiply by 100 for each fungal genera frequency of occurrence and relative density.

problem observed when using domestic animals. Pack animals especially donkey and mules are used for transportation of goods in most parts of Africa and Ethiopia (FERNANDO and STARKEY, 2004; TOLERA and ABEBE, 2007).

G*ombisa* is the most dominant traditional storage structure used to store maize in cob form. Studies conducted in various areas of Ethiopia showed that most farmers across the country used *gotera, gombisa* and sacks to store maize (TADESSE and BASEDOW, 2004; DUBALE et al., 2012). Traditional granaries (cribs), usually made up of locally available materials such as timber, bamboo, etc. are used in humid countries both for drying and for the storage of maize (NUKENINE, 2010). Newly constructed *gombisa* can be used for up to ten years for the same purpose in the study area. HELL et al. (2000) reported most storage structures for maize in Benin were used for up to 5 years. This accelerated the risk of contamination with the increasing age of the storage structure. Maize storage with sheath was only common in lowland maize producing districts and farmers in the study area assumed it reduced weevil damage. A study conducted at Bako research centre, in western Ethiopia, showed good sheath cover is considered as protecting the ear from insect and fungi damage (DEMISSIE et al., 2008). In general, the traditional storage structure, *gombisa* provides less protection from pests and not effective in protecting the stored products from external climatic conditions (like rainfall and high temperatures) which facilitate the development of fungi in the store (NARAYANASAMY, 2006). On an average, after six months of storage, shelling is another main activity that is carried out to store the remaining kernels in the sack. However, shelling is totally carried out manually using stick hitting. This action causes physical damage or breakage, splitting or cracking of kernels which make them prone to fungal damage by other pests resulting in high PHL (TADESSE and BASEDOW, 2004; IFPRI, 2010). It has been reported that physical damage during shelling favors *Fusarium* infections and using mechanical shelling can reduce fumonisin levels by 57% - 65% in maize (FANDOHAN et al., 2003).

Current survey results revealed that maize PHL estimated at 31%. In Ethiopia, the major maize production challenge for farmers is high PHL, ranging from 15 to 30% (IFPRI, 2010) and up to 19% (ASHAGARI, 2000). In sub-Saharan Africa, about 37% of losses occur during storage and handling (LIPINSKI et al., 2013). It was also stated that poor PH management results in large amounts of maize loss after harvest (KAAYA et al., 2006). Declining of germination percentage along the storage duration was observed for all actors' storage systems; mainly due to the increment of mould which resulted in kernels quality loss. Therefore, the current study shows that high fungi damage resulted in reduction of maize kernel germination. This result is in accordance with BEFIKADU (2014) who reported that germination reduction of maize grain stored for six months in traditional farmers' storage was 98% to 68.5% under intermediate and 97.5% to 70.17% in lowland agro-ecology. Decrease in the germination percentage of stored maize to 28% after 180 days of storage at 35 ºC with 14% moisture content has been reported (TABATABAEI and NAGHIBALGHORA, 2013). SOMDA et al. (2008) and GOVENDER et al. (2008) also reported that fungal infection caused a reduction in germination rates of maize kernels.

For all storage systems studied, the trend in mould development rose along with the storage duration and agro-ecology. Both mould incidence on cobs and kernels were more significant in the highland region of the area studied and were also related to an increase of storage duration which coincided with the region's next rainy season. This may be because of higher relative humidity that favors fungal growth in less protected traditional storage structures. Insufficient drying and humid conditions favor the development of fungi in tropics (SULEIMAN et al., 2013). GROOT (2004) also stated that humidity is crucial for the development of fungi; even at low temperatures, some mould development may occur if the relative humidity of the air is high. Similarly, GARUBA et al. (2011) reported that occurrence and frequency of mould were higher on maize stored with a higher moisture content of 19.0%. Furthermore, damage caused by weevils can allow fungi to enter more easily and at the same time serve as an agent transferring fungi spores from infected to healthy grain (KANKOLONGO et al., 2009; SULEIMAN et al., 2013). Moreover, even if maize can be harvested during the dry season, the rainy season that follows will facilitate mould development in traditional storage structures which are not climatically controlled and allow entry of moisture from outside.

The present findings show that there were seven fungal genera associated with stored maize but *Fusarium, Penicillium* and *Aspergillus* are the most dominant ones. Tesfaye and Abate (2000) also reported that *Fusarium, Penicillium* and *Aspergillus* spp. were the most significant toxigenic fungal pathogens in Ethiopian maize. *Fusarium* was the most common of the above three. In a study conducted in the Jimma zone, *Fusarium, Penicillium* and *Aspergillus* spp. were identified from farmers' storage systems; in both *gombisa* and in sacks (Befikadu, 2014). However, this study confirmed that these fungi genera did not only dominate at producer level but also at collector and wholesaler level in the maize supply chain, including different agro-ecologies, storage periods and types. Kaaya et al. (2006) reported that predominantly *Fusarium, Penicillium,* and *Aspergillus* were identified from traders samples collected from different Ugandan agro-ecologies. However, a report from Cameron showed that the infection levels of stored maize were: *Aspergillus* (up to 96%), *Penicillium* (up to 63%) and lastly by *Fusarium* (up to 32%) (Tagne et al., 2003). Similarly, several studies have reported that these three fungi genera were the most significant in stored maize (Mostafa and Kazem, 2011; Bosah and Omorusi, 2014). However, most studies did not cover the whole commodity supply chain but focused on the producer level.

The findings showed that as the storage duration increased, the occurrence of most toxinogenic fungi, *Aspergillus* spp. and *Penicillium* spp. increased along the maize supply chain. *Aspergillus* species (particularly *Aspergillus flavous* and *A. parasticus*) are the major aflatoxin producing fungi species in food and feed in the tropics and sub-tropics along production chains (Kiarie et al., 2016), but are particularly significant in the storage phase (Jonathan et al., 2004). Depending on dose and duration of the exposure, aflatoxin can cause acute illness and death, immunological suppression, liver cancers and nutritional interference (Jonathan et al., 2004). Several species of *Penicillium* are able to produce mycotoxins in the storage phase (Rundbergeta et al., 2002). Types of *Fusarium* are also among the main fungal diseases that contribute to a loss in quality and the contamination of maize kernels with mycotoxins (Stumpf et al., 2013).

Conclusion

The present study clearly demonstrated that significant amount of losses occurred during post-harvest activities along the maize supply chains. Traditional storage structures in the study area did not protect the stored maize from external environmental conditions and pest problems. Thus, losses during storage were particularly significant and identified as a critical intervention point. The study also showed that, among the seven fungi genera identified, *Fusarium, Penicillium* and *Aspergillus* spp. were the predominant fungi occurring in all the maize sampled along the supply chain. At the same time, these were the top three fungi able to produce mycotoxins and cause health hazards both to humans and animals that feed on it. However, the trend showed that *Fusarium* spp. were slightly decreased over time but *Aspergillus* and *Penicillium* spp. increased rapidly with storage duration throughout the maize storage duration. In general, mould incidence both on-cobs-maize and kernels increased with storage duration for all storage situations studied.

In order to reduce PHLs it is important to implement good post-harvest practices such as selecting the optimal harvesting time, drying techniques and maize shelling method. In addition, traditional storage structures should be improved and disseminated to producers. Also, current research findings highlight the need to investigate quality loss that occurs along the supply chain. Furthermore, as the most dominant fungal pathogens isolated are able to produce secondary metabolites, further investigation is required to understand the multiple mycotoxin profiles along the maize supply chain using different types of storage structures.

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