Low Mycotoxins Content at Harvest, High in Stored Maize: Harvesting and Storages Practices Implications in Two Agro Ecosystems of Tanzania

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Abstract

A survey was conducted to establish association of pre-storage and storage practices on occurrence of multiple mycotoxins in northern highland and eastern lowland maize based agro-ecosystems in Tanzania. Four hundred (400) households from 80 villages, 40 from each of the two agro-ecosystems were randomly selected for semi structured interviews to establish maize harvesting and storage practices. From each household, approximately 1kg of maize was collected at maize maturity before they were harvested and another 1kg maize collected from at least 6 months storage. The five household samples from each village were reconstituted to make one composite sample representing a village. Standard procedure was used for mycotoxins extraction. Compound quantification was done using Ultra-high performance liquid chromatography/time-of-flight mass spectrometry (UHPLC/TOFMS). More than 70% of farmers in the Eastern lowland used grain hardness as an indicator of grain maturity. Delayed harvesting of 4 to 12 weeks after maturity was observed across the two agro-ecosystems. More than 60% farmers shelled maize mechanically by beating on floor, in bags and elevated platforms. Most important storage insect pests were confused flour beetle (Tribolium confusum Jacquelin du Val) (100%), followed by 80% larger grain borer (Prostephanus truncutus L). Fourteen fungal species were detected and only 12 were present in both agroecosystems. Penicillium brevicompactum and F. culmorum were not detected in samples from northern highland while F. tricinctum and F. equiseti were not detected in the eastern lowland zone. With exception of F. graminearum, all other species were more abundant in eastern lowland than northern highland. In eastern lowlands, aflatoxin contamination in samples stored for six months was ten times higher than in samples collected at harvest. Significant ($p \leq 0.05$) positive and negative correlations between mycotoxins and storage practices were obtained. The study suggests that pre-storage and storage practices applied by subsistence farmers in the two agro-ecosystems need to be fine-tuned to reduce mycotoxins risk the two maize based agro-ecosystems.

Keywords: Pre-storage, storage, subsistence farming, mycotoxins, agro ecological zones

Introduction

Maize (*Zea mays* L.) is a major cereal used as a staple food and feed worldwide (Temu *et al.,* 2010). Under subsistence farming, maize has the simplest value chain comprising mainly the input supply and farmers who are consumers. Yet time from farm to folk is possibly the longest because after harvest, maize is usually stored for a year round until the next harvest cycle. Such storage of maize creates a man-made ecosystem in which the quality and nutritive value may change partly due to storage pests like insects and moulds (Magan, 2007). Apart from its inherent pre and postharvest vulnerability to mould infection, harvest and storage practices can exacerbate their effects (Miller, 2008). As a result fungal infection is of concern because it causes yield loss and contaminates maize with mycotoxins (Miller, 2008).

Mycotoxins are secondary metabolites produced by certain species of fungi. Depending on their adaptability to ecological conditions, the mycotoxin producing fungi are grouped as field

fungi (*Fusarium* and *Alternaria* species) (Gallo *et al.,* 2016) and storage fungi (*Aspergillus* and *Penicillium* species) (Lasram *et al.,* 2016). Field fungi can colonize the ripening grains on standing crop in the field prior to harvesting while storage fungi are more dominant in stored products although in a very low percentage they may be found in grains before harvesting. Field fungi are usually arrested after harvest because they require high relative humidity for growth, normally above 95% (aw>0.95) (Mannaa and Kim, 2017). Storage fungi are adapted to grow under low moisture condition (relative humidity range 70 and 90% (aw<0.95) (Lasram *et al.,* 2016). This classification is not strict as the field fungi can grow in stores and storage fungi grow in the field provided the environmental conditions permit. Presence of storage fungi before harvest constitutes an inoculum transferred in stores.

Therefore, to minimize the effects of mycotoxin contamination, prevention is the best option because complete removal of toxigenic fungi in food systems is difficult, nearly impossible. In this context, availability of strategies to reduce pre-harvest contamination of mycotoxins through good agricultural practices (GAP) and Good Storage Practices (GSP) as recommended by Codex Alimentarius and is probably the best option to reduce mycotoxins in food systems in Africa. These recommendations however, need to be tailored to the local conditions and practices. Unfortunately, in Tanzania, reports to customize the GSPs based on Codex recommendations for producing maize are lacking. Lack of GSPs is possibly the cause of the high levels of fumonisin contamination in Tanzanian maize. Therefore, the need to customize effective post-harvest strategies to reduce mycotoxins in Tanzania is already a necessity which is not adequately addressed.

Materials and Methods

Description of study area

The study was conducted in Northern Highland (NH) and Eastern Lowland (EL) agro ecological zones of Tanzania. The eastern lowland (6°S and 8°S, and 36°30'E and 38°E) is characterized of flat plateaus (0-900 m a.s.l) in the eastern part. The northern highland (4°S and 25°S and 84°E and 45°E) sits on (1000 and 1500 m a. s. l.) .

Sampling and inventory of pre-storage and storage practices

A two stage sampling was conducted in Kilosa (eastern lowland) and hanang' (Northern highland districts, Morogoro and Manyara regions respectively. The sampling involved 40 villages of each district and from each village, five households were randomly selected to make a total of 400 households. From each household, approximately 1kg of maize was collected at maize maturity before harvested and another 1kg maize collected from at least 6 months storage (Orsi *et al.,* 2000). Immediately the samples were sent to the laboratory, followed by air drying to maintain field status and frozen for 24 hours to kill insects before they were kept at 4°C until analysis. A questionnaire was used to establish used harvesting and storage practices of maize and common insect pests. Questionnaires were supplemented by direct observations at each household where types of storage structures, method of construction, and symptomatic occurrence of moist environment, mould growth, insect infestation, and rodents were recorded. Identification of storage insect pests was done by the researchers based on available literature during the survey and on respondents' description and ability to recognize the indicated pests from amongst other species in pictorial aids (Lever, 1976).

Mycological assays and multi-mycotoxins quantification

For the purpose of isolating fungi from maize grains, surface sterilization, inoculation of maize grains into potato dextrose agar medium and purification of culture was done according to Landschoot *et al.* (2011). Morphological criteria (Leslie *et al.,* 2006) was used to distinguish the different fungal genera contaminating the grain. The obtained fungal isolates were categorized as *Fusarium, Penicillium, Aspergillus* or others based on macroscopic (colour, reverse colour and mycelium) and microscopic (conidiophores shape) characteristics. Polymerase Chain Reaction (PCR) techniques according to Nicolaisen *et al.* (2009), Luo *et al.* (2009) and Cruz and Buttner (2008) were used to

identify species belonging to genus *Fusarium*, *Aspergillus* and *Penicillium* respectively. Quantification of mycotoxin was done according to Degraeve *et al.* (2016).

Sample preparation and subsequent mycotoxins extraction followed a Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) approach developed by Anastassiades *et al.,* (2003) and later modified for cereals (Rasmussen *et al.,* 2010; Rubert *et al.,* 2013). Mycotoxin standards, as solid pure extracts, of aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), ochratoxin A (OTA), deoxynivalenol (DON), fumonisin B1 (FB1), fumonisin B2 (FB2), T-2 toxin (T-2), HT-2 toxin (HT-2) and zearalenone (ZEN) were supplied by Sigma-Aldrich (St. Louis, MO, USA). To establish a standard curve, mycotoxin free grounded maize samples were spiked with the toxins at eight concentration levels: 5 ppb, 10 ppb, 25 ppb, 50 ppb, 75 ppb, 100 ppb, 150ppb and 200 ppb and later analyzed for multi-mycotoxins (Ortiz *et al.,* 2013). Quantification of multi-mycotoxins in maize samples was done according to Degraeve *et al.* (2016).

Data analysis

The questionnaire was coded posting the data in the SPSS 16 followed by calculating descriptive statistics; means and standard deviations. Non parametric tests were used to determined mean differences of the storage practices applied in the two agro ecosystem. Differences in occurrence of multi mycotoxins in the two agro ecosystems for the samples collected at harvest and those collected from 6 months storage were calculated.

Results

Determination of physiological maturity

It was established that farmers in both agro-ecosystems used drying leaves, stalk and bending cobs as plant indicator of maize physiological maturity. No differences (p=0.13) between the two ecosystems were observed except the use of grain hardness which more than 70% of farmers in the Eastern lowland used as an indicator of grain maturity (Fig. 1).

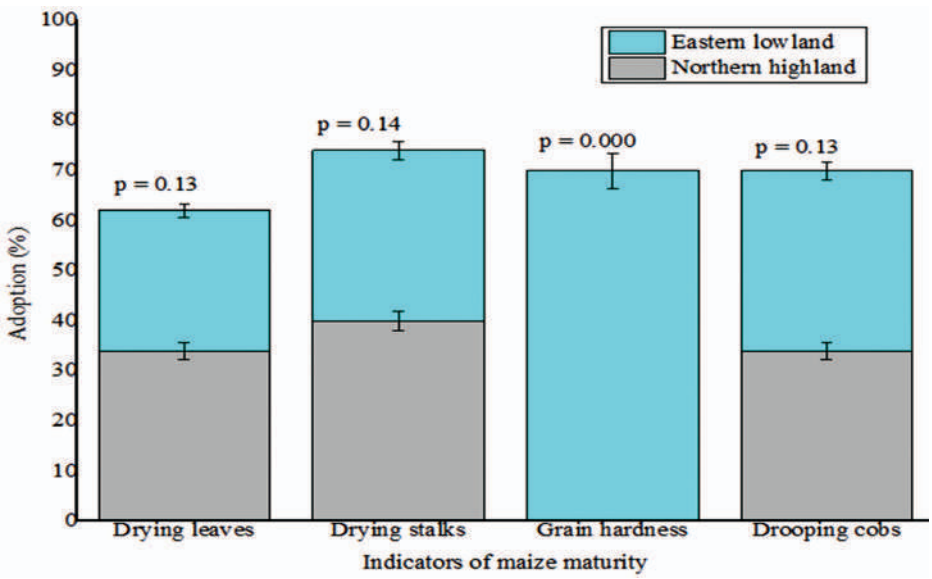

Figure 1: Indicators of maize maturity as used by smallholder farmers in Northern highland and Eastern lowland

Timing of harvest after physiological maturity and the source of labor

The Figure 2A indicates that between 35% and 50% of the farmers harvest maize 3 to 4 weeks after physiological maturity. Less than 10% harvested maize 12 weeks after

physiological maturity. During harvesting, females were the main source of labor in eastern lowland, while 60% farming households in northern highland outsourced harvesting labor (Fig. 2B).

maize. While 40% farmers in Northern highland sorted and used damaged maize as animal feeds, 50%, 10% and 5% farmers in eastern lowland use the damaged maize as food, preparing local brew and sale respectively.

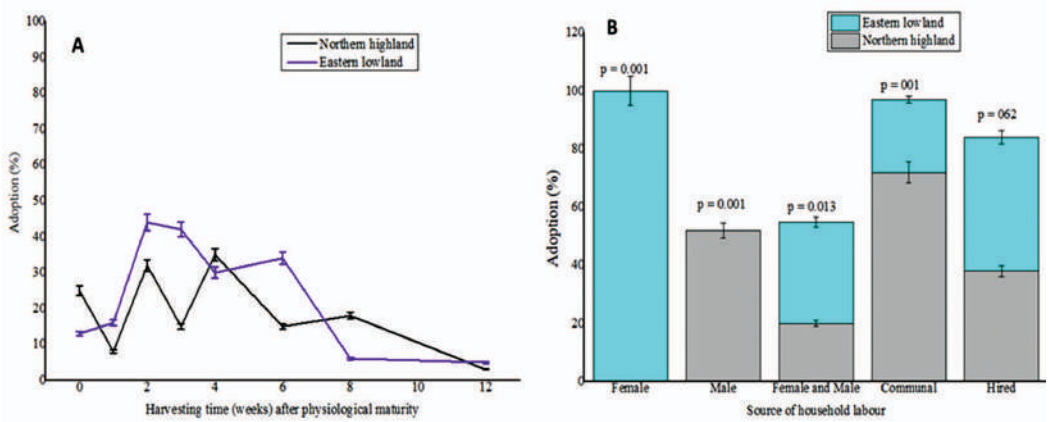

Figure 2; Timing of harvest after physiological maturity in northern highland and eastern lowland

Sorting purpose before storage

Figure 3 presents results on sorting purposes. In these practices, significant differences ($P \leq 0.05$) between the two ecosystems were detected on the number of households involved. The results in Figure 4A, show that farmers in the two ecosystems sorted maize before storage. They differed in the utilization of damaged

Drying and Shelling surfaces

Farmers in both ecosystems shelled maize mechanically by beating on elevated tunnels (Fig. 4A) and on floor (Fig. 4b). Handling of maize during beating varied significantly ($P \leq 0.05$) between agro ecological zones. The majority (80%) in Northern highland shelled maize by beating on a raised platform and

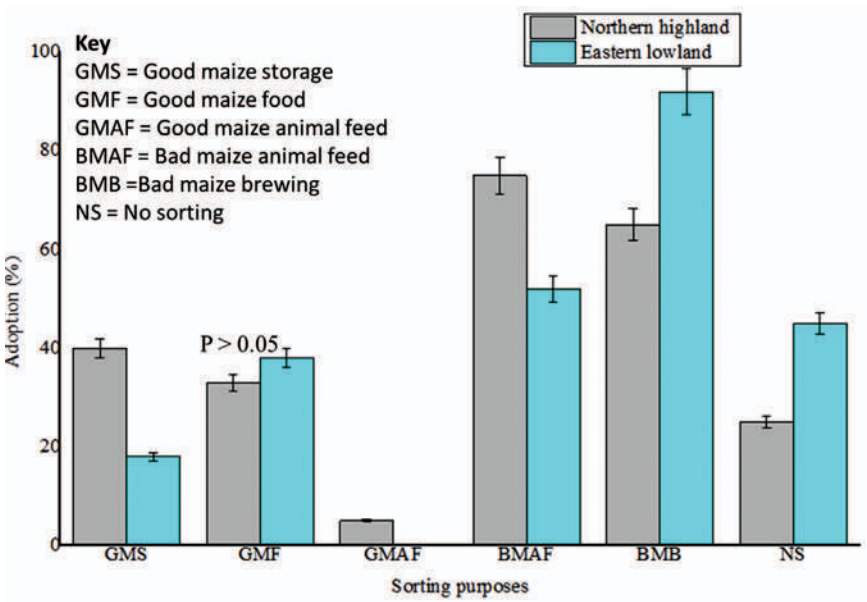

Figure 3: Maize sorting purposes

using motorized tractor operated engines. While 60% of the farmers in Eastern Lowland shelled maize by beating in poly bags, 65% of farmers in Northern highland shelled maize by beating on bare ground (Fig. 4B).

Common pests

In the two agro ecosystems the number of people who reported insect and bird pests as an important problem did not differ statistically ($p>0.05$). However half of the farmers in the

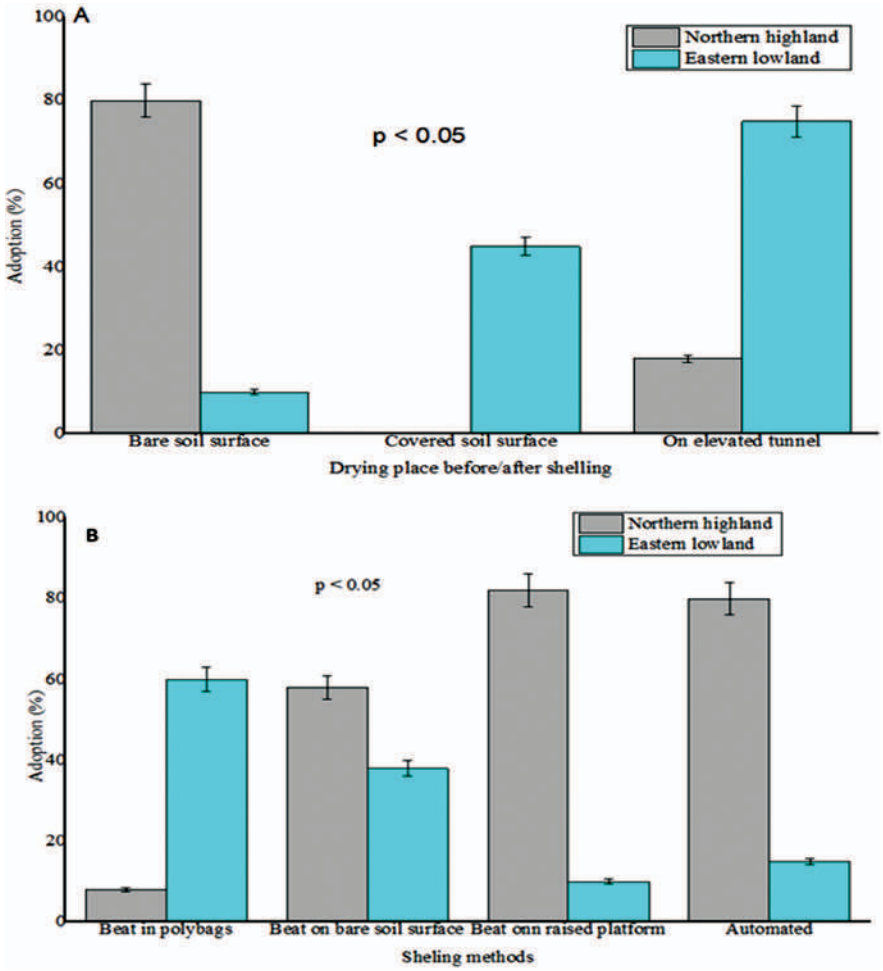

Figure 4; Sorting, drying and shelling practices in Northern highland and Eastern Lowland Storage Facilities

The differences between agro ecosystems on the practices involved in storage of maize were significant ($P\leq0.05$). In northern highlands close to 98%, 50%, 20% and 45% store maize in cribs, on floor, bags on floor and bags on raised platform respectively (Fig. 5). On the other hand, 5%, 40%, and 20% farmers in the eastern lowland store maize in Cribs (Fig. 5), and in bags placed on floor (Fig. 5) and raised platforms.

eastern lowland ranked moulds as an important problem followed by rodents. The problem of insect and birds pests was equally ($p>0.05$) experienced by farmers in both agro ecosystems (Fig. 6).

Occurrence of insect pests

Direct observation of this study revealed that eastern lowland had the greatest diversity of storage insect pests. These included; Confused flour beetle (*Tribolium confusum* Jacquelin

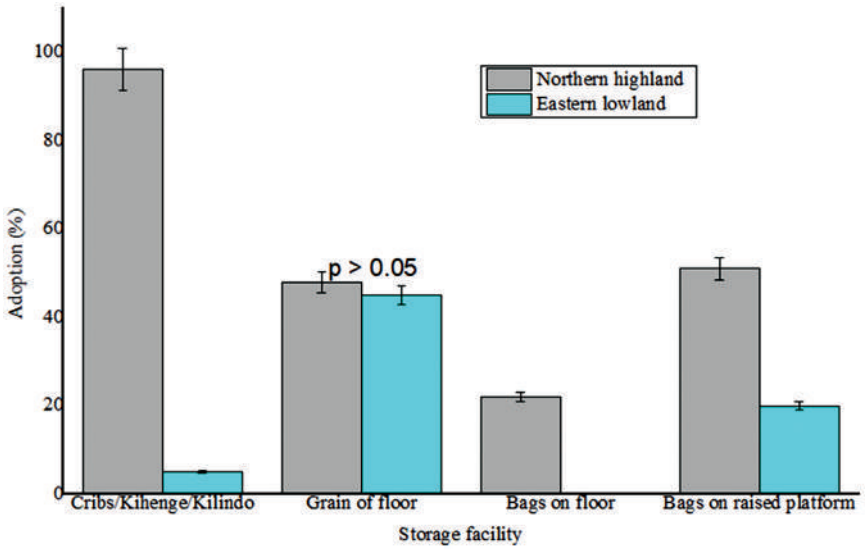

Figure 5; Maize storage in bags placed of floor and cribs

du Val) which occurred most frequently (100%), followed by 80% Larger grain borer (*Prostephanus truncutus* L), 78% Red flour beetle (*Tribolium castaneum* Herbst), 42% Laser grain weevil (*Sitophilus zeamais* L), 25% Areca nut weevil (*Araecerus fasciculatus* syn) and 14% Saw toothed grain beetle (*Oryzaephilus surinamensis* L.) (Fig. 7). The northern highland had the least diversity of identified storage insect pests, including 54% larger grain borer (*P. truncutus* L) followed by 22% Laser grain weevil (*S. zeamais* L).

Occurrence of fungal species

Of the 14 fungal species identified in this study, the occurrence of 9 fungal species varied significantly ($p<0.05$) between the two agro ecosystems. All the 14 species detected in this study only 12 were detected in each of the agro ecosystems. *Penicillium brevicompactum* and *F. culmorum* were not detected in samples from northern highland while *F. tricinctum* and *F. equiseti* were not detected in the eastern lowland zone. With exception of *F. graminearum*, all other species were more abundant in eastern lowland than northern highland (Fig. 8).

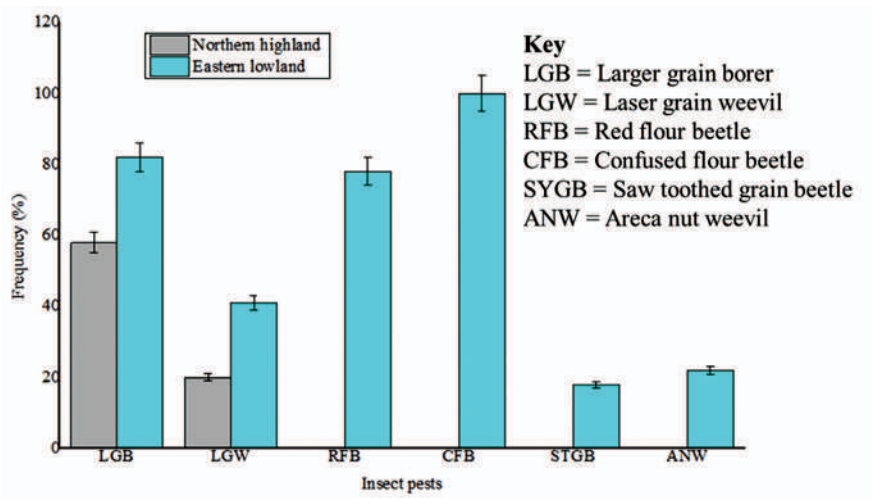

Figure 6: Common pests in northern highland and eastern lowland agro ecosystems

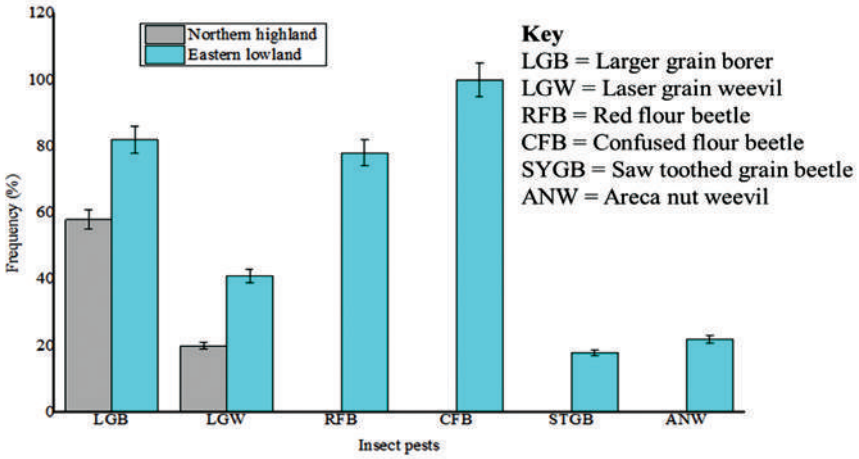

Figure 7: Common insect pests of maize in northern highland and eastern lowland agro ecosystems

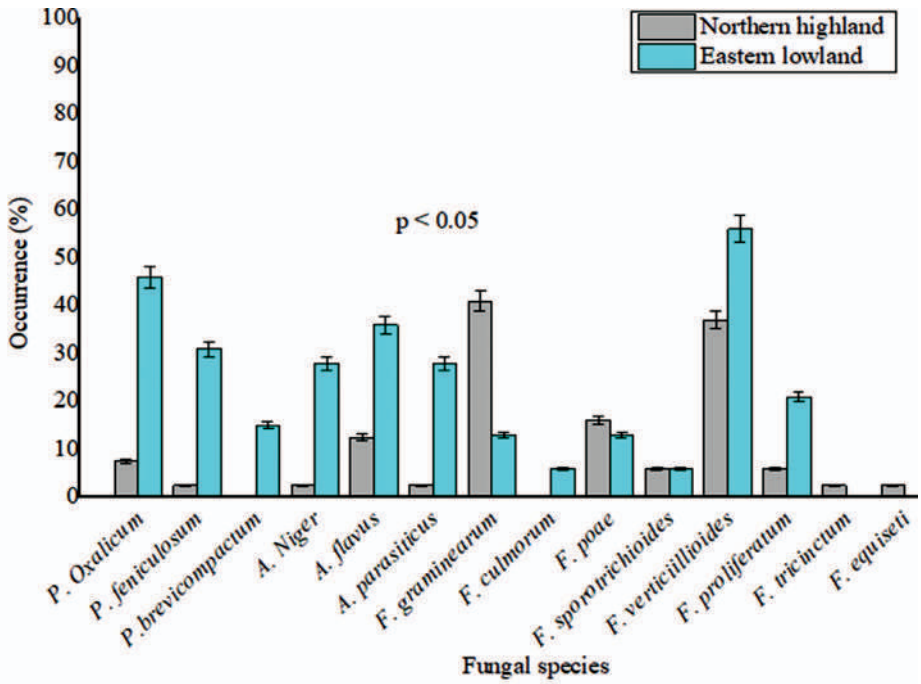

Figure 8: Occurrence of fungal species in the eastern lowland and northern highland agro ecosystems of Tanzania

Occurrence of multi-mycotoxins at harvest and storage

Six types of mycotoxins were detected in samples collected at harvest in both northern highland and eastern lowland (Table 1). Three more mycotoxins (HT-2, T-2, and OTA) were detected in maize of eastern lowland after 6 months of storage. At harvest, occurrence of FB1 in northern highland was 5 times higher than its occurrence in samples collected from 6 months of storage in the same location (Fig. 9). Similarly, DON was higher in both agro ecosystems than its occurrences in samples from 6 months storage. At harvest the occurrence of FB1 in Eastern lowland was lower by 45% than its occurrence in samples collected from 6 months of storage. Occurrence of FB2 in northern highland from samples collected at harvest was the same (42%) as its occurrence in samples from 6 months storage. However FB1

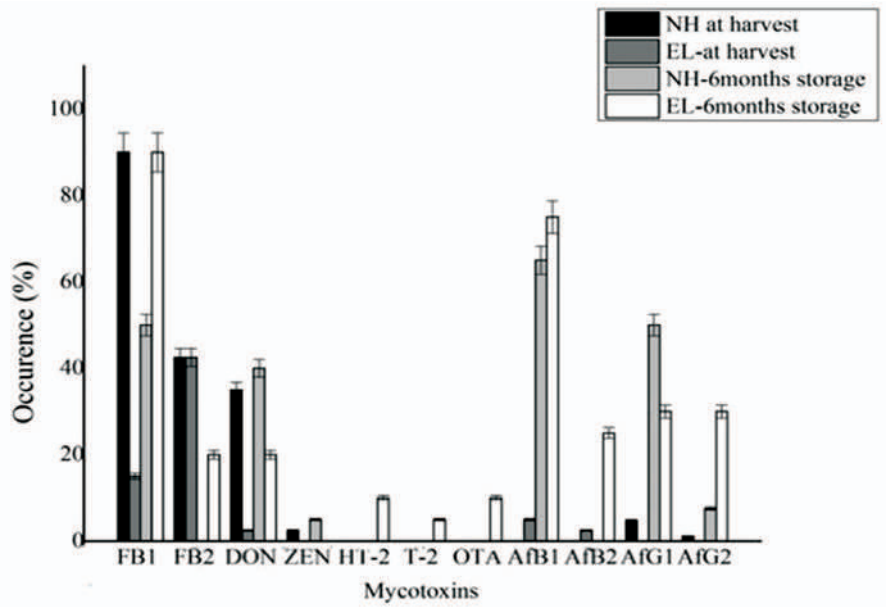

Figure 9: Differences of occurrence of multi mycotoxins between samples at harvest and six months storage in Northern lowland and Eastern lowland; AFB1 = aflatoxin B1, AFB2 = aflatoxin B2, AFG1 = aflatoxin G1, AFG2 = aflatoxin G2, OTA = ochratoxin A, DON = deoxynivalenol, FB1 = fumonisin B1, FB2 = fumonisin B2, T-2 = T-2 toxin, HT-2 = HT-2 toxin and ZEN = zearalenone

was not detected at harvest in eastern lowland although the detection was 20% after 6 months in the region. The storage samples had respectively 10%, 20% and 22% more AfB1, AfB2 and AfG2 than detection in samples collected at harvest in eastern lowland.

Control of storage pests

More than one third of the farmers in both ecosystems did not apply any measure to control storage pests. Significant differences ($p \leq 0.001$) between agro ecosystems were noted on practices applied to control storage pests. Close to 90% farmers in Eastern lowland applied ash to control storage pests while less than 10% farmers in Northern highland used this option. Fumigation (50% Northern Highland, 38% eastern lowland), removing remnants of previous crop in the store (22% Northern highland and 48% Eastern lowland), application of local herbs (35% in each location) and synthetic pesticides (40% Northern highland and 38% Eastern lowland) were the common practices (Fig. 10).

Linking storage practices to mycotoxin contamination

Linkage analysis established that there was a significantly positive correlations R=0.82, p=0.034 and R 0.47, p=0.043 among the number of households storing maize in synthetic polyethylene plastic bags and contamination of FB1 and FB2 respectively. Number of households which used the same storage method had significant negative correlation with DON R = -0.57, p=0.036. The correlation coefficients in Table 2 indicate that the number of households storing maize in cribs or (in Kiswahili *Vihenge*), had a significant negative correlation with FB2 (p=0.003), DON (p=0.002), AfB1 (p=0. 001), and AfG1 (p=0.003). The results point out that number of households which removed remains of previous crops in the store before introducing a new crop had a significant negative correlation with FB1 (p=0.012), FB2 (p=0.006), AfB2 (p=0.001) and AfG1 (p=035). Furthermore the number of households which were fumigating prior to storage had a significant negative correlation with FB2 (p=0.003), AfB1 (p=0.002), AfB2 (p=0.003), and AfG1 (P=0.004). The study has also established significant negatively

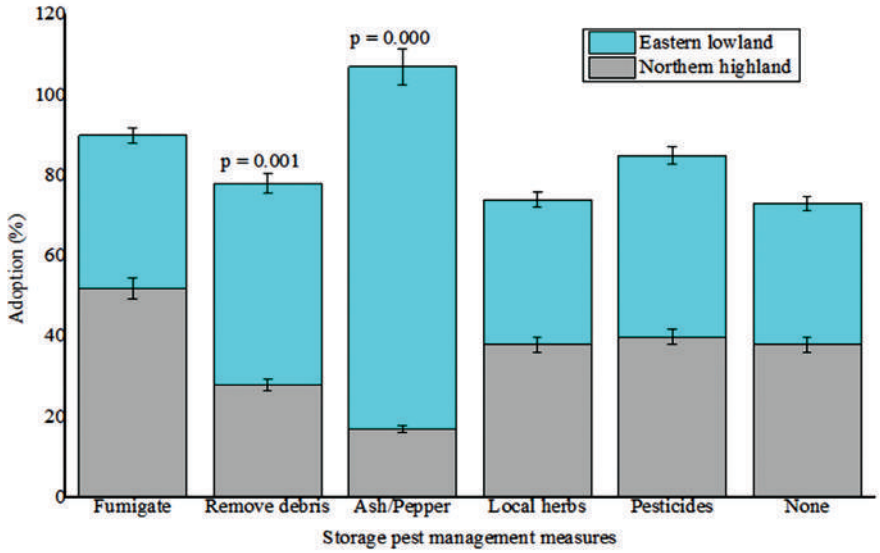

Figure 10; Storage practices, storage pests, insect pests and pest control in Northern highland and Eastern lowland

correlation between the number of households which sorted out maize before storage and FB1 (P=0.044), DON (p=0.005), OTA (p=0.049), AfB1 (p=003) and AfG2 (p=0.003). Significant negative correlations between number of farmers who controlled insect pests by applying ash or herbs like pepper and OTA (p=0.047), AfB1 (p=0. 045), AfB2 (p=0.038), AfG1 (p=0.036, and AfG2 (p=0.035) were noted. Contamination of AfB2, AfG1 and AfG2 were significantly negatively correlated ($p \leq 0.01$) with number of households which used synthetic pesticides to control storage insect pests. The link between number of farmers who used different storage structures, hygienic practices, approaches to control storage insect and contamination of ZEN, T2 and HT2 was insignificant or none existent (Table 2).

Discussion

The current study established that majority of farmers delayed harvesting up to 12 weeks increasing the risk to pre harvest mycotoxin contamination of maize. The study linked the delay with farmers' practice of determining maize physiological maturity and time of harvesting using unreliable indicators such as leaf and stem senescence. According to Thomas (2013), for a healthy plant, drying leaves, stalk and drooping (bending) cobs is a sign of senescence as well as maturity but these indicators are not always reliable because they can occur as a result of abiotic and biotic stress factors (Degraeve *et al.* 2016; Madege *et al.* 2018). The grain hardness which was a maturity indicator used by the majority farmers in eastern lowland indicated that grains had dried enough for harvesting. However the rate at which the kernel dries is highly influenced by the variety used, agronomic practices as well as the prevailing weather condition (Parthasaranthi and Jeyakumar 2013). Therefore, reliance on this indicator can lead to early or delayed harvesting. Harvesting too early may result in immature seeds that have poor vigor and vulnerable to insect and fungal attacks that increase chances of mycotoxins contamination (Gu *et al.* 2017; Martinez-Feria *et al.* 2019).

In both agro ecosystems, after removing the crop from the field, they undergo sorting and drying before shelling. Farmers sorted out damaged or mouldy maize for various purposes including food, animal feed and brewing. Consumption of damaged or mouldy maize has been reported in Tanzania (Kimanya *et al.,* 2008) and Kenya (Lewis *et al.,* 2005) where it was associated with aflatoxicosis. Utilization of damaged or mouldy maize as animal feed is reported in Kenya (Bhat *et al.,* 1997) and is associated with food borne

Table 1: Detected multi mycotoxins in maize samples collected at harvest and in storage after six months

Location	Range (µg/kg)	FB1	FB2	DON	HT-2	T-2	OTA	ZEN	AFB1	AFB2	AFG1	AFG2
EL-H	Highest	6590.18	4042.33	23582.51	nd	nd	nd	3663.32	nd	nd	2558.44	5.26
	Lowest	12.75	723.88	23582.51	nd	nd	nd	3663.32	nd	nd	20.28	5.26
EL-6mS	Highest	6946.97	270.47	585.81	nd	6.82	11.67	9.45	973.94	96.74	9.42	2.05
	Lowest	34.66	106.66	143.24	nd	5.56	11.67	9.02	7.38	0.69	0.58	0.59
NH-H	Highest	53540.52	42263.39	25651.42	nd	nd	nd	2031.55	nd	nd	5.21	1.79
	Lowest	544	18.55	156.49	nd	nd	nd	33.28	nd	nd	5.21	1.54
NH-6mS	Highest	197.04	197.04	770.19	16.91	nd	nd	nd	11.75	nd	1.12	1.18
	Lowest	11.41	11.41	32.85	16.91	nd	nd	nd	0.85	nd	0.56	0.61

Key; EL-H= Eastern lowland sampling at harvest, EL-6mS = Eastern lowland sampling from 6months stored grain. NH-H = Northern Highland sampling at harvest, NH-6mS = Northern Highland sampling from 6months stored grain, nd = not detected. ***AFB1 = aflatoxin B1, AFB2 = aflatoxin B2, AFG1 = aflatoxin G1, AFG2 = aflatoxin G2, OTA = ochratoxin A, DON = deoxynivalenol, FB1 = fumonisin B1, FB2 = fumonisin B2, T-2 = T-2 toxin, HT-2 = HT-2 toxin and ZEN = zearalenone***

Table 2: Linkage analysis of mycotoxins to storage practices based on pearson correlation coefficients

PRACTICE	FB1	FB2	DON	HT-2	T-2	ZEN	OTA	AfB1	AfB2	AfG1	AfG2
Polyethylene plastic bags	0.82*	0.47*	-0.57*	-0.30	0.09	0.09	0.59	0.40	0.39	0.33	0.37
Cribs	-0.29	-0.68**	0.38*	0.01	-0.09	-0.09	-0.19	-0.42**	-0.30	-0.55**	-0.19
Fumigating before storage	-0.32	-0.54**	0.12	0.04	-0.02	-0.02	-0.27	-0.43**	-0.44**	-0.38**	-0.30
Removal of previous crop	-0.52**	-0.58**	-0.25	-0.04	0.05	0.05	0.05	0.35	-0.86**	-0.37*	0.20
Sorting before storage	-0.41*	0.21	-0.46**	-0.08	0.01	0.01	-0.41*	-0.46**	0.05	0.14	-0.48**
Control pest by Ash /pepper	0.43	0.09	-0.29	-0.03	0.03	0.03	-0.39*	-0.48*	-0.57*	-0.51*	-0.59*
Use commercial pesticides	-0.36	-0.09	-0.31	0.07	-0.03	-0.03	-0.03	-0.04	-0.97**	-0.75**	-0.38*

*Key; **, * = Correlation is significant at 0.01 and 0.05 respectively.*

outbreak of mycotoxicosis. Saydenham *et al.* (1990) reported that consumption of mouldy maize either as direct food or via local brews is indicative of the magnitude of the problem of food insecurity in the rural places in many African countries. Previous studies have confirmed that sorting based on colour, visually by hand and based on weight by machine were more effective in reducing aflatoxins in peanuts than fluorescent sorting (Pelletier and Reizner, 1992). These results are further supported by findings regarding the use of near infrared transmittance kernel sorting technology which established negative correlation with fusarium kernel damage as well as fumonisins and deoxynivalenol (Kautzman *et al.,* 2015).

To allow maize dry after harvest, farmers in Northern highland heaped maize on a bare ground while farmers in eastern lowland dried maize on elevated tunnels. Drying maize before storage to reduce moisture content down to level not favorable for mould growth is common among smallholder farmers in East Africa (Lewis *et al.,* 2005; Kaaya *et al.,* 2006; Mwihia *et al.,* 2008). Previous reports show that, timely harvesting, sorting harvested maize-ears in the field, drying maize with and without husk on elevated bamboo tree platforms reduced postharvest fungal infection and risks of contamination of fumonisins (Ngoko *et al.,* 2003). These findings are also in agreement with the report that small holder maize in many tropical countries are subject to long pre harvest drying as well as long postharvest drying before they can be safely stored (Bodholt, 1985; Kaaya *et al.,* 2005). The majority of farmers shelled maize mechanically through beating maize in polybags, elevated tunnels and on covered and uncovered ground surfaces. Previous literature has established that mechanical shelling is known to cause kernel damage although the degree with which the grains are damaged would vary depending on the beating impact caused (Fandohan *et al.,* 2006). Hand shelling, although time consuming, can cause less damage than shelling using automobile power engines and beating (Chulze 2010). Vulnerability to damage in any shelling method may vary between maize varieties and degree of dryness (Nkakini *et al.,* 2007). Reports show that higher rate of grain damage due to mechanical shelling increases chances of mould growth particularly *Fusarium* and *Aspergillus* species (Diedhiou *et al.,* 2011; Fandohan *et al.,* 2006)

After shelling, the farmers adopted different storages methods such as polybags and traditional structures like vihenge, vilindo and cribs. These traditional storage techniques are common in many parts of Sub Saharan Africa especially on small holder farms (Udoh *et al.,* 2000; Armah and Asante, 2006; Nduku *et al.,* 2013;). Many traditional storage structures are perforated hence depending on environmental conditions, the storage practices could have been creating specific microbial ecosystems because they may avail different environmental conditions for growth as well as production of mycotoxins. Studies in Nigeria reported high aflatoxins in maize stores in polyethylene synthetic bags (Udoh *et al.,* 2000). This is probably because grains kept in synthetic polythylene bags provide suitable temperature for insects and mould growth as well as ad libitum food supply which allows insects to be reproduced haphazardly (Pantenius, 1988). To reduce risks of insect damage associated with storage of maize in bags, efficacy of multiple layer hermetic bags have been tested (Villers 2014; Maina *et al.* 2016; Mallikarjunan *et al.* 2016;). Research has established that storage of maize in ventilated facilities like bamboo granary have lower fumonisins content (Hell *et al.,* 2000; Fandohan *et al.,* 2005). This could be an explanation for the negative correlation of mycotoxins with numbers of cribs observed in the current study. Prior to storage few farmers fumigated as part of hygiene and it was ascribed to low mycotoxins contamination. These results are in agreement with scientific findings that use Ammonia fumigants inhibited growth of A. flavus in maize stores with subsequent significant reduction in AfB1 (Duncan *et al.,* 1994).

Before harvesting and after harvesting, the crop sufferes from various pest attacks including rodents, insects and moulds. The farmers knowledge on rodents as major field pests is in agreement with observation on rice farming systems in the same location that rice farmers had substantial knowledge on the presence and

losses caused by rodents in their crop yield and general public health (Mulungu *et al.,* 2014). Similarly maize yield loss due to rodents, insect pests and mould has been reported previously (Mdangi *et al.* 2013; Liu *et al.* 2016; Danso *et al.* 2017). Attacks of these pests in stored maize have been associated with increased contamination of mycotoxins of maize (Degraeve *et al.* 2016; Madege *et al.* 2018; Madege *et al.* 2019). High infection of insects in stores of eastern lowland can be explained by the suitable growth factors created particularly relative humidity (60-80%) and temperature (25-30°C) which is typical characteristic of eastern lowland (Bakker-Arkema *et al.,* 1999). Increases of mycotoxins is related to insect attack because insects create wounds on maize kernels which become infection sites for fungi (Wu, 2006). Therefore, Storage practices that create optimal conditions for growth of mycotoxins producing fungi, predispose maize grains to biosynthesis of these toxins by *F. cerealis, F. poae, A. ochraceus, P. verrucosum, and P. nordicum* (Agriopoulou *et al.,* 2020).

The current study also established that the maize samples collected at harvest in the northern highland samples had the highest levels of Fumonisins and Deoxynivalenol which are commonly produced by *F. Verticillioides* and *F. graminearum*. During the same period the samples from the eastern lowland which were collected after six months storage had more than ten times higher contamination of aflatoxins than in maize samples collected at harvest. Aflatoxins are secondary metabolites of some *Aspergillus* species particularly of the section flavi; *A. flavus* and *A. parasiticus*. The observed differences in mycotoxins contamination between the two agro ecosystems can be associated with the ecological differences between the two zones that support different fungal growth and mycotoxins production potential. According to available literature, host susceptibility to fungal disease is directly influenced by ecological characteristics especially temperature and osmotic stress. These climatic variables determine infectivity, colonization, reproduction, survival, competitive ability, mycotoxicity and pathogenicity of both field and storage fungi (Drakulic and Ray 2017; Mannaa and Kim 2017). Temperature range of 15 to 30 and water activity ranging from 0.9 to 0.995 is optimum condition for production of Fumonisins in many cereals (Milani, 2013). The increase in aflatoxin levels from harvest, suggest that if conditions are permissive, aflatoxins can keep on increasing with storage duration in which case the public exposure is also acerbated. The increasing trend of aflatoxins along the maize value chain after harvest is reported in many countries in Africa (Kang'ethe, 2011; Asiki *et al.,* 2014; Nyangi, 2014; Akowuah *et al.,* 2015)

Strategies to reduce insect pest pressure using plant extracts, ash and pesticides were common on both northern highland and eastern lowland agro ecosystems. However, use of ash and herbs like *Tephrosia vogelii* L (or *utupa* in its Swahili acronym) leaves and fruits of Neem plant (*Azadracta indica* L) have been reported in some parts of Tanzania (Mihale *et al.,* 2009). However the efficacy of these herbs and many others are yet to be validated as reliable control strategies (Reddy and Muralidharan 2009). Application of ash in conjunction with cow dung and tobacco powder are also tested local alternatives of pest control (Mihale *et al.,* 2009).

Conclusions and recommendations

It can therefore be conclusive that pre-storage and storage practices applied by subsistence farmers in the two ecosystems are linked to increasing mycotoxins contamination along maize value chain. The practices that are linked to to reduced risk of mycotoxins should be improved to reduce mycotoxin contamination in maize.

Acknowledgement

The funding provided by VLiR-UOS to complete this study is highly appreciated.

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