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Impact of local pre-harvest management practices in maize on the occurrence of *Fusarium* species and associated mycotoxins in two agro-ecosystems in Tanzania

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ABSTRACT

Knowledge on the presence of mycotoxins in Africa is fragmentary, although it can be assumed that both concentrations and prevalence in food commodities is high. The present study focuses on the presence of *Fusarium* species and their associated mycotoxins in maize from two geographically distant agro ecological systems in Tanzania. In a two-year survey, both *Fusarium* species and concomitant mycotoxins were surveyed in the Northern highlands (Hanang district) and the Eastern lowlands (Kilosa district). Parallel with this, a questionnaire on agricultural practices in both agro-ecosystems was included in this study. This allowed us to put the presence of the toxigenic *Fusarium* species and their mycotoxins within a relevant agricultural framework.

Fusarium verticillioides, *Fusarium graminearum* and *Fusarium poae* were the predominant species in both locations although the population in the Eastern lowlands was slightly more complex comprising also *Fusarium culmorum*, *Fusarium avenaceum* and *Fusarium sporotrichioides*. The predominant presence of *F. verticillioides* resulted in a high prevalence of fumonisins in both regions. The importance of *F. graminearum* in the population was reflected by the presence of deoxynivalenol in the mycotoxin analysis. Although the agricultural practices differed significantly amongst both locations, only few significant correlations were detected between mycotoxin presence and crop rotation, storage conditions, and insect control measures.

1. Introduction

Maize is the most important cereal grown and consumed in Tanzania, providing 60% of dietary calories and more than 50% utilizable proteins to the population. The crop is cultivated in all 21 regions of mainland Tanzania, predominantly by smallholder farmers in the rural areas, on about two million hectares or 45% of the cultivated area. The consumption of maize is estimated to be 112 kg annually per capita equivalent to 308 g per day per capita (Katinila, Verkuijl, Mwangi, Anandajayasekeram, & Moshi, 1998; Mboya, Tongoona, Derera, Mudhara, & Langyintuo, 2011).

Unfortunately, maize production in Africa is known to be highly vulnerable to contamination with toxigenic fungi and their secondary metabolites, called mycotoxins. Mycotoxins attract worldwide attention because of their impact on human health, animal productivity and economic losses (Bhat, Rai, & Karim, 2010; Wagacha & Muthomi, 2008). Mycotoxin formation occurs during crop growth in the field and during storage. Field toxigenic fungi predominantly enclose *Fusarium* spp. (also *Aspergillus* could occur on mature, dry kernels) whereas storage fungi comprise mainly *Penicillium* and *Aspergillus* spp (Bhat et al., 2010; Logrieco, Bottalico,

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Mule, Moretti, & Perrone, 2003). The most important toxins from an agricultural and human health point of view comprise fumonisins (FB_1, FB_2), type-A trichothecenes (including T-2 toxin and HT-2 toxin), type-B trichothecenes (including deoxynivalenol (DON)), aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2), ochratoxins (OTA) and zearalenone (ZEA) (Pitt, Taniwaki, & Cole, 2013; Shephard, 2004; Wagacha & Muthomi, 2008). In the field, predisposing conditions leading to fungal growth are high temperature and humidity, poor soil fertility, drought and insect damage, monsoons and unseasonal rains during harvest. Poor harvesting, drying and storage practices, improper transportation, marketing and processing contributes to fungal growth of mainly storage fungi. The former conditions and practices prevail in Africa and on top of that, diets consist mainly of maize, which entails high daily exposure to mycotoxins (Bhat et al., 2010; Bhat & Vasanthi, 2003; Thompson & Henke, 2000; Wagacha & Muthomi, 2008). Vomiting, diarrhea or other gastro-intestinal problems and immunosuppression are general symptoms of mycotoxicosis in humans (Bhat et al., 2010). In addition, mycotoxins are known to be potentially carcinogenic, mutagenic, teratogenic and neurotoxic (Bryden, 2007; Frisvad, Smedsgaard, Larsen, & Samson, 2004; Gelderblom et al., 2001; Rheeder et al., 1992; Riley et al., 2001; Wagacha & Muthomi, 2008). Children that are chronically exposed to mycotoxins show signs of impaired growth (Gong et al., 2002; Gong et al., 2004; Kimanya, De Meulenaer, Roberfroid, Lachat, & Kolsteren, 2010). In addition, Marasas, et al. (2004) suggest that fumonisin consumption is a risk factor for development of neural tube defects in unborn children and related birth defects such as craniofacial abnormalities. Beside these direct health risks, mycotoxin contamination of the food chain has also an enormous economic impact. Losses from rejected shipments and lower prices for inferior quality can be devastating for developing countries export markets. Direct costs to farmers include reduced income as a result of crop losses, lower prices for inferior quality, increased livestock mortality and reductions in livestock productivity, fertility and immunity. The cost of reduced labor force due to illness and costs from hospitalization or other health care services are problems that are often overlooked (Bhat & Vasanthi, 2003; Bryden, 2007).

Mycotoxins are considered as unavoidable contaminants of food, therefore, the goal is to minimize contamination of maize and maize products by application of good agricultural practices (GAP) during production and-, harvest and good storage practices (GSP) during storage. These include growing resistant varieties, crop rotation, fertilization, insect management, irrigation, proper drying and removal of damaged kernels. A promising long-term strategy is breeding for resistance (Wagacha & Muthomi, 2008). But so far, high levels of genetic resistance have been difficult to achieve (Clements & White, 2004; Munkvold, 2003a). The knowledge that mycotoxins have serious effects on humans, animals and countries' economies has also led to the establishment of regulations on mycotoxins levels in food and feed. Worldwide, approximately 100 countries had developed specific limits by the end of 2003, representing approximately 87% of world inhabitants (FAO, 2004). Still, the majority of African countries have no specific mycotoxins regulations. Even for the few countries with established regulations, enforcement is limited due to reliance on subsistence farming and home produced food (FAO, 2004; Shephard, 2008).

In this context, this paper is presenting results of an inventory of local agricultural practices and their linkage with the presence of mycotoxigenic *Fusarium* species and their associated mycotoxins (FB1, FB2, DON, ZEA, T-2 toxin and HT-2 toxin), in two maize producing agro-ecological zones (AEZ) of Tanzania. The results of this study are useful for guiding the establishment of workable agricultural based strategies to prevent mycotoxins contamination of maize and minimize related human exposures in Tanzania.

2. Materials and methods

2.1. Research design

The study was conducted in two AEZ of Tanzania; Eastern lowlands (Morogoro region, Kilosa district) and Northern highlands (Manyara region, Hanang district). Both zones are main maize growing areas.

Kilosa is one of the districts in Morogoro region, which lies between 6°S and 8°S and 36°30′E and 38°E, consisting of mostly flat lowland. The area experiences an average of eight months of bimodal rainfall distribution whereby in good years short rain starts from October to January, followed by long rains in mid-February through May. Mean annual rainfall ranges around 600 mm in lowlands and average temperature is about 25 °C. In Kilosa, more than 80% of the population depends on agriculture and the district offers a variety of agro-ecological conditions for farming. Thus a variety of food crops is grown, including maize, rice, millet, cassava, beans, bananas and cowpeas. The surplus produces of these food crops are also used as cash crops. The crops are predominantly grown by small-scale farming (average farmland is less than one hectare). In addition, farming is characterized by limited use of inputs such as improved seeds, fertilizers and/or manure, and the majority of the farmers use hand hoes for cultivation (Benjaminsen, Maganga, & Abdallah, 2009; Morogoro Region Socio-Economic Profile, 1997).

Hanang is one of the districts in Manyara region, which is located within 3°S and 6°S and 33°E and 38°E. Its elevation is between 1000 m and 2000 m above sea level. Climate in highlands is more temperate with an average temperature of 20 °C. The zone usually experiences two rainfall seasons during the year, with short scanty rains during the months of October to December and long rains from February to May. The average rainfall in highland zone varies from 700 mm to 900 mm. In Hanang, growing maize in association with beans or pigeon peas is the most common cropping system. Pigeon peas are considered a commercial crop as less than 10% of production is consumed at home. The farming is usually semi-mechanized as a majority of the farmers use animal force for ploughing, planting and transportation of harvests (Investment and Socio-Economic Profile Manyara Region, 2013; Nkonya et al., 1998).

2.2. Field sampling and sample size

A two stage sampling was conducted during 2011/12 and 2012/13 cropping seasons (Zeller, Schwarze, & van Rheenen, 2002). The 2011/12 sampling involved 40 villages scattered around both districts to represent different agro-ecological conditions. Five maize growing households were randomly chosen from each village and approximately one kg of maize was collected from each household. Samples were collected from different points in the batch until approximately one kg was obtained. Per village, the five samples were composited to maintain one sample of one kg. The composite samples were sent to the laboratory, air dried to maintain field status, frozen for 24 h to kill insects and kept at 4 °C until required for analysis. The second sampling in 2012/13 was done analogously, only then ten villages were selected scattered over each district and four households per village participated.

2.3. Isolation and identification of *Fusarium* species by real time PCR

From each sample, three randomly picked grains were surface-sterilized for 30 s in 1% NaOCl, washed for 30 s with 70% EtOH, washed with distilled sterile water, dried for five minutes, placed on PDA plates (potato dextrose agar, Oxoid Belgium, 39 g PDA/l) and subsequently purified as previously described in Landschoot et al. (2011). The obtained fungal species were categorized as *Fusarium*, *Penicillium*, *Aspergillus* or other based on macroscopic (color, reverse color and mycelium) and microscopic (conidiophores shape) characteristics. For *Fusarium* species determination, a mycelium plug taken from the fully grown PDA slants was transferred to liquid GPY-broth (10 g glucose, 1 g yeast and 1 g peptone, Oxoid Belgium) and incubated for seven days at 25 °C (Landschoot et al., 2011). After the incubation period, the mycelium was transferred to eppendorf tubes, centrifuged for a short spin and freeze-dried overnight at −70 °C. The freeze-dried material was crushed and DNA extraction was performed with the Invisorb® Spin Plant Mini Kit (STRATEC Molecular, Germany) according to the manufacturers' instructions. Species detection by means of real-time PCR was performed using the GoTaq® qPCR Master Mix (Promega, USA) for the species *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium avenaceum*, *Fusarium poae*, *Fusarium sporotrichioides*, *Fusarium verticillioides*, *Fusarium proliferatum*, *Fusarium equiseti* and *Fusarium tricinctum*. PCR was performed on a 7000 Sequence Detection System (Applied Bioscience), using the following cycling protocol: 2 min at 50 °C, 10 min at 95 °C, 42 cycles of 95 °C for 15 s and 62.5 °C for 1 min, 15 s at 95 °C, 20 s at 60 °C and 15 s at 95 °C (Nicolaisen et al., 2009). A no template control and a dilution series of five known template concentrations (1^{-4} μg−1 μg) were added to establish a standard curve for each species. Results were visualized with the 7000 System Sequence Detection Software (SDS), version 1.2.3., by Applied Biosystems.

2.4. Analysis of mycotoxins in maize samples by UHPLC/TOFMS

Mycotoxins were extracted from finely ground maize grains using a QuEChERS-based approach (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003; Frenich, Romero-Gonzalez, Gomez-Perez, & Vidal, 2011; Rasmussen, Storm, Rasmussen, Smedsgaard, & Nielsen, 2010; Rubert et al., 2013). By means of ultra-high performance liquid chromatography (UHPLC)/time-of-flight mass spectrometry (TOFMS), the presence of FB1, FB2, DON, ZEA, T-2 toxin and HT-2 toxin was analyzed. Kamala et al. (2015) evaluated the simultaneous contamination of multiple mycotoxins, validated this method of multi-mycotoxin analysis and determined limits of detection (LOD) and limits of quantification (LOQ). Ground and homogenized blank maize samples were spiked with a multi-standard working solution at different concentration levels to assess linearity and unspiked, blank maize samples were implemented to assess the matrix variability. The multi-standard stock solution and the spiked maize samples were prepared as described in (Ortiz, Van Camp, Mestdagh, Donoso, & De Meulenaer, 2013), using standards as solid pure extracts of FB1, FB2, DON, ZEA, T-2 toxin and HT-2 toxin, supplied by Sigma–Aldrich (St. Louis, MO, USA). Generating the extracted ion chromatograms was done using TargetAnalysisTM software (Bruker Daltonics, Germany). Identification of the ions was based on retention time deviation, mass accuracy and SigmaFitTM algorithm, which is a rate for the agreement of the theoretical and measured isotopic.

2.5. Inventory of local agricultural practices

Parallel to the isolation and characterization of the *Fusarium* population and associated mycotoxins, farmers were asked a series of questions to establish an inventory of local agricultural practices applied in maize production. The questionnaire mainly focused on preharvest practices (e.g. use of variety, land preparation, planting, cropping pattern, fertilizer type and application, presence and management of pests and harvesting) since *Fusarium* species are generally considered as ‘field fungi’ and require high moisture content for growth and mycotoxin production (Logrieco et al., 2003; Miller, 1995).

The data obtained through the questionnaires were processed into a database in the statistical software SPSS version 21.0. Descriptive statistics such as frequencies, percentages, means and standard deviation were computed to observe the distribution characteristics. Significant differences in crop management practices between the two studied agro ecological zones were evaluated by means of non-parametric tests for two independent variables (Mann–Whitney U test).

3. Results

3.1. Local maize production practices commonly used in Hanang district and Kilosa district

3.1.1. Management of crop residues and tillage practice

In this survey, all maize produced by the farmers was rain-fed. Crop residues are mostly ploughed down or used as animal feed (Fig. 1A). Since most households in Hanang are pastoralists, crop residues are used to feed the animals. In Kilosa residues are mainly ploughed down. Farmers in both AEZ commonly use conventional tillage to prepare their fields (Fig. 1B), although it is significantly more practiced in Kilosa district than in Hanang district (95% and 65% respectively). In last mentioned, minimum tillage is also practiced by 30% of the farmers.

3.1.2. Seeds for planting

Most households participating the survey use improved seeds for planting. However, farmers in Kilosa (87.5%) make significantly more use of improved maize seeds than farmers in Hanang (62.5%). On the other hand, there are significantly more households in Hanang (20% vs. 2.5%) that use a combination of both local and improved maize seeds for planting. Farmers in both AEZ obtain their seeds for planting primarily from a registered private dealer (72.5% in Hanang and 50% in Kilosa), but saving seeds for the next cropping season is also a common practice (30% of the farmers in both districts). The results of this questionnaire revealed that maize characteristic, such as good husk cover, cobs bending down at maturity and early maturity, aren't a determining factor in the choice of maize variety.

3.1.3. Improvement of soil fertility

There is a significant difference in fertilizer application between both AEZ. In Kilosa, only 22.5% of the households apply fertilizer, while 65% of the farmers in Hanang do (Fig. 1C). The majority of the households in former region apply fertilizer before planting (62.5%), by broadcasting or banding. It's clear that, if fertilizer is applied, farm yard manure is most commonly used. Other methods to improve soil fertility are crop rotation, mulching, shifting cultivation and the application of plant green manure. But only 45% of the farmers in this survey confirmed to use one of these techniques (Fig. 1D). However, households in Kilosa district take significant more measures to improve soil fertility. 62.5% of the farmers use at least one method, with the use of plant green manure as the most common one, followed by the application of mulching. In Hanang, only 17.5% of the households use an alternative measure to improve the soil fertility.

3.1.4. Cropping system

Mixed cropping is the most common cropping system in both AEZ. In Hanang district, all participating households practice this system. Maize is mixed cropped with legumes and sometimes sunflowers and pumpkins. In Kilosa district, 50% of the farmers indicated to practice a mixed cropping system of maize with

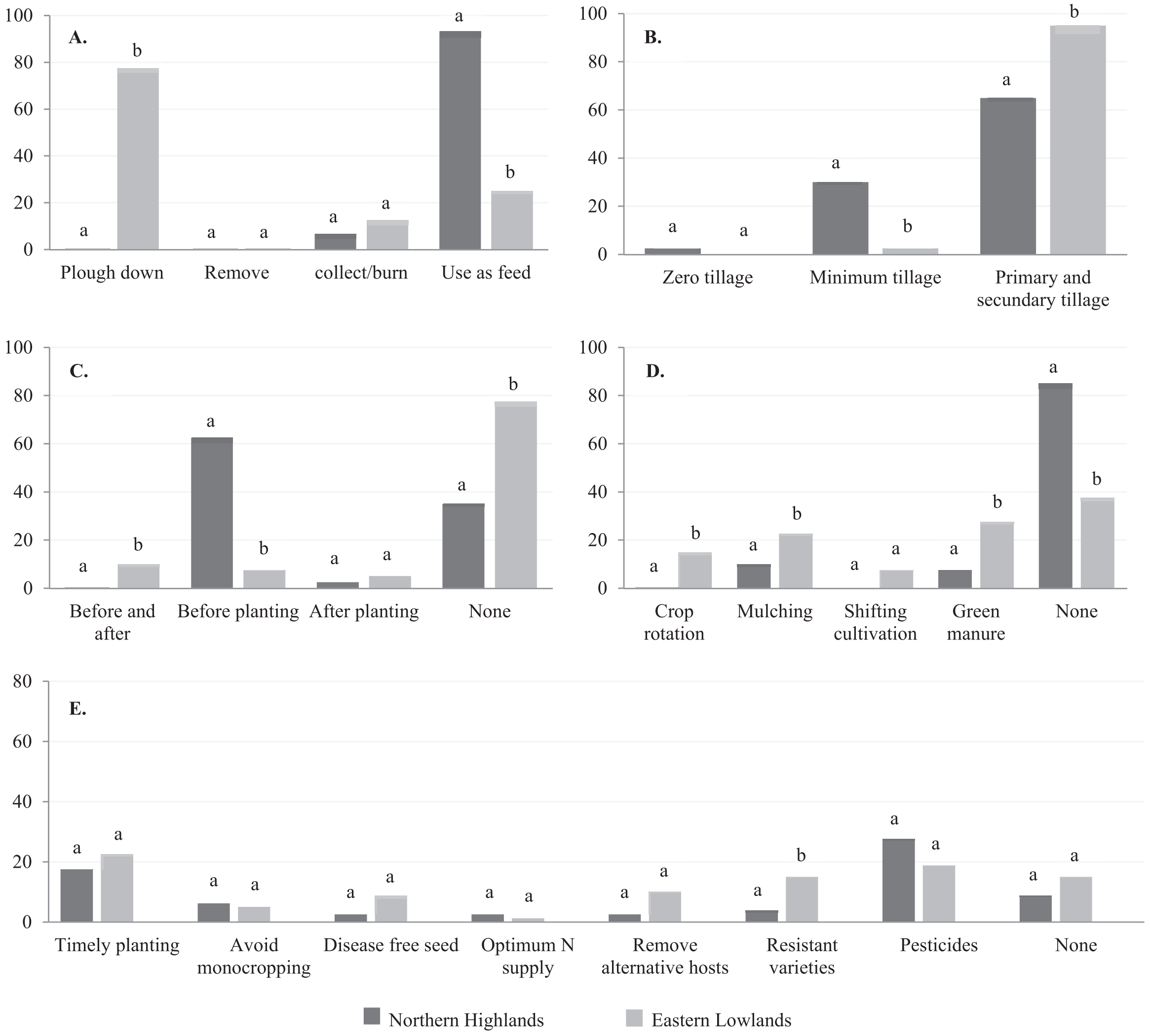

Fig. 1. Comparison of household agricultural practices between highland and lowland AEZ of Tanzania (in %). Different letters above bars represent paired differences after a Mann-Whitney-U test (p = 0.05). A: management of crop residues; B: tillage practices; C: fertilizer use; D: cultivation practices; E: pests and disease control measures.

legumes and sunflower, 37.5% of the households practice mono cropping of maize and 12.5% indicates to use relay cropping of maize with legumes or sunflowers.

3.1.5. *Crop protection practices*

In both AEZ, weeding is done by 88.8% of the farmers, using a hand hoe. Only a minority (3.7%) doesn't remove the weeds from their fields. Methods indicated to reduce diseases and pests in farmers' fields are timely planting, avoid monocropping, use of disease free seed, application of optimal plant density and nitrogen supply, removing alternative hosts, use resistant varieties and pesticide application. Timely planting and the use of pesticides are the most commonly used measures in both AEZ. Still almost 25% of the farmers take no action (Fig. 1E). Farmers applying pesticides, mention Karate (lambda-cyhalothrin) and Actellic (pirimiphos-methyl + permethrin) as most common market pesticides. In Kilosa, significant more farmers mention the use of resistant varieties to combat pests and diseases than farmers in Hanang district. Also, in Hanang, most farmers apply only one measure, but in Kilosa most farmers combine at least two measures to reduce pests and diseases.

3.1.6. *Harvest*

In both AEZ, 8.8% of the farmers harvest their maize right after maturity. So the majority of the farmers let their maize dry in the stalks for a period from one to twelve weeks. Most farmers, in both Hanang and Kilosa district, harvest four weeks after maturity (40% and 35% respectively). All farmers confirm to harvest manually by picking cobs from the stalks. Farmers state that cobs in the field get damaged due to fungal diseases, insect pests, mice and rats, birds and mammalian.

3.2. *Identification of Fusarium species on maize grains*

In Hanang district, 90% and 72.5% of the households' maize is contaminated with *Fusarium* species, for growing season 2011/12 and 2012/13 respectively. In Kilosa district, respectively 70% and 67.5% of the households' maize is contaminated with *Fusarium*. Table 1 depicts the distribution of nine *Fusarium* species in maize samples from the two growing seasons per AEZ. For both sampling periods and in both AEZ, *F. verticillioides*, *F. graminearum* and *F. poae* were the most common species. Though, in Hanang district, contamination with *F. graminearum* and *F. poae* is respectively six times and 2.5 times higher than in Kilosa district for both growing

Table 1
Distribution of *Fusarium* species in maize (% of samples infected) from 2011/12 and 2012/13 growing season in two AEZ in Tanzania.

	2011/12		2012/13	
	Hanang (n = 20)	Kilosa (n = 20)	Hanang (n = 40)	Kilosa (n = 40)
F. graminearum	60	10	27.5	5
F. culmorum	–	5	–	–
F. avenaceum	–	5	–	–
F. poae	25	10	32.5	12.5
F. sporotrichioides	10	5	–	–
F. verticilioides	50	45	45	42.5
F. proliferatum	–	–	–	2.5
F. equiseti	–	–	–	–
F. tricinctum	–	–	–	2.5

Table 2
Monthly average rainfall in l (AR) and temperature in °C (AT) of two AEZ of Tanzania during a two-year survey. Resource: the National Bureau of Statistics, Tanzania, and the Tanzania Meteorological Agency.

	Northern highland zone						Eastern lowland zone					
	2011		2012		2013		2011		2012		2013	
	AR	At	AR	At	AR	At	AR	At	AR	At	AR	At
January	86.1	21.1	23.1	22.7	45	19.6	87.3	26.9	70.3	26.4	93	25.7
February	106.7	22	59.9	21.5	55	20.5	80.6	27.5	71.7	27.3	60	26.9
March	95.9	22.2	18.6	22.9	140	20.2	87.3	27.9	59.9	26.4	102	25.8
April	322.4	21.3	233	21.3	245	20.3	207	26.1	125	23.6	147	23.5
May	49.7	20.15	47.7	19.6	75	19.15	79.7	25.5	135	24.05	63	23.8
June	1.3	18.8	1.8	19	20	16.7	0	23.8	23.1	22.5	15	22.6
July	0	17.8	0.6	18.3	15	16.1	0	22.5	0	21.6	6	22.5
August	0.8	18.55	8.3	18.8	8	17.8	1	23	9.7	24.6	6	24.6
September	1.3	19.3	0.5	20.1	7.8	18.8	0	24.1	0	24.4	6	24.3
October	1.2	21.6	7.8	22.1	25	20.6	4.2	26.2	0	25.9	24	25.9
November	98.9	21.9	211	21.2	120	21.2	22.2	26.9	76.6	26.9	51	26.4
December	29.1	20.9	165	21.6	135	20.4	181	27.1	76	27.3	87	27.3
	793.4	**20.4**	**778**	**20.7**	**891**	**19.29**	**750**	**25.6**	**647**	**25.09**	**660**	**24.9**

seasons. When comparing contamination with *F. verticillioides*, Kilosa exhibits a slightly lower contamination grade in both growing seasons. *F. sporotrichioides* occurred less common and was only isolated from maize of growing season 2011/12. Only few *F. culmorum* and *F. avenaceum* species were isolated from maize collected in Kilosa district during growing season 2011/12.

3.3. *Fusarium mycotoxins in maize grains*

Table 3 depicts the prevalence of the different *Fusarium* mycotoxins in the two regions covered in the survey. It is clear that fumonisins are the most common *Fusarium* mycotoxin contaminating the maize samples, followed by DON. ZEA, HT-2 toxin and T-2 toxin are also present but to a lesser extent (data of T-2 and HT-2 are not shown because of very low prevalence).

Descriptive statistics on the mycotoxin concentrations in our maize samples (Table 4) reveal that median concentrations for fumonisins in both growing seasons and both AEZ are below the regulatory limit of 1000 μg/kg. In 2011/12, ten percent of the Kilosa samples contain concentrations above the limit set for fumonisins, whereas in 2012/13, 27.5% of the Hanang samples and 20% of the Kilosa samples contain concentrations above the regulatory limit with the highest concentrations respectively 95804 μg/kg and 10633 μg/kg. DON median concentration for growing season 2011/12 is below the EU regulatory limit of 750 μg/kg in both AEZ, and even maximum concentrations don't exceed this limit. But for growing season 2012/13 median and maximum concentrations for DON are remarkably higher and exceeding regulatory limits (note: in Kilosa only one sample contained DON with a concentration of 23586 μg/kg). ZEA was found in two samples from Kilosa district from growing season 2011/12 at very low concentrations. For growing season 2012/13, ZEA is detected in two samples from Hanang' district, with one sample exceeding regulatory limits, and in one sample from Kilosa district also exceeding regulatory limits (depending on country, limits for ZEA in maize vary from 50 to 1000 μg/kg).

3.4. *Linking mycotoxigenic fungi, mycotoxins concentrations and agricultural practices in two AEZ of Tanzania*

To investigate in detail the impact of agricultural practices and location on the *Fusarium* population and mycotoxins, a detailed linkage analysis was carried out. Most of the agricultural practices had no remarkable influence on the *Fusarium* population nor the mycotoxins presence (see Supplementary Table S1). But factors with significant impact on the *Fusarium* population and mycotoxins

Table 3
Prevalence of *Fusarium* mycotoxins in maize (% of samples containing different mycotoxins) of two AEZ of Tanzania over two growing seasons.

	2011/12		2012/13	
	Hanang (n = 20)	Kilosa (n = 20)	Hanang (n = 40)	Kilosa (n = 40)
Fumonisin B	50	95	90	77.5
Deoxynivalenol	40	20	35	2.5
Zearalenone	–	10	5	2.5

Table 4
Concentrations of Fumonisin B, DON and ZEA in maize from two AEZ in Tanzania over two growing seasons. All data are expressed in μg/kg.

		2011/12		2012/13	
		Hanang (n = 20)	Kilosa (n = 20)	Hanang (n = 40)	Kilosa (n = 40)
Fumonisin B (μg/kg)	Max	197	6947	95804	10633
	Min	11	35	19	13
	Mean	67	866	6423	856
	Median	36	376	371	43
	SD	70	1651	18661	2155
DON (μg/kg)	Max	770	586	25651	23583[c]
	Min	33	143	156	–
	Mean	298	303	8970	23583[c]
	Median	271	241	4880	23583[c]
	SD	266	195	9486	–
ZEA (μg/kg)	Max	–	9[a]	2032	3663[c]
	Min	–	9[a]	33	–
	Mean	–	9[a]	1032[b]	3663[c]
	Median	–	9[a]	1032[b]	3663[c]
	SD	–	0.3	1413	–

[a] Two samples contain approximately 9 μg/kg zearalenone, explaining the same value for max, min, mean and median.
[b] Only two samples contain zearalenone, explaining the same value for mean and median.
[c] Only one sample contains deoxynivalenol/zearalenone, explaining the same value for max, mean and median.

are represented in Table 5. The table depicts the important role of the AEZ on both population and mycotoxins. In addition, also some agricultural practices influence mycotoxin presence. Especially successful insect control measures help to reduce levels of *Fusarium* toxins, while storage practices and implementation of well-considered crop rotation might reduce *Fusarium* toxins presence as well.

4. Discussion

Our results indicate that maize in both AEZ during growing season 2011/12 and 2012/13 is contaminated by predominantly *F. verticillioides*, *F. graminearum* and *F. poae*. Though, Hanang district has a higher contamination grade with 81% of the samples contaminated, compared to 69% in Kilosa district. Infection with *F. verticillioides* is slightly higher in Hanang, whereas infection with *F. graminearum* and *F. poae* is respectively six times and 2.5 times higher. This phenomenon could be linked with differences in climate between Northern highlands and Eastern lowlands. For both AEZ, monthly average rainfall and temperature, during growing season 2011/12 and 2012/13, are presented in Table 2. Northern highland AEZ experienced an average monthly temperature of 20.1 °C, which is more favorable for the growth of *F. graminearum* and *F. poae* than the average monthly temperature of 25.2 °C in the Eastern lowland AEZ. Different reports mention the impact of temperature and rainfall on species distribution of the *Fusarium* complex (Aguin et al., 2014; Doohan, Brennan, & Cooke, 2003; Mateo, Mateo, & Jimenez, 2002; Munkvold, 2003b; Parry, Jenkinson, & Mcleod, 1995). The high incidence of *F. verticillioides* in both regions could be explained by the drought experienced during grain filling and maturity phase (Miller, 2001). On the other hand, *Fusarium* contamination in Kilosa district is more complex since also low incidence of *F. culmorum*, *F. avenaceum*, *F. proliferatum* and *F. tricinctum* is measured. *F. sporotrichioides* is isolated from maize of both districts in growing season 2011/12. Multi-mycotoxin analysis of the maize samples showed some interesting results. The dominance of *F. verticillioides* is confirmed by the mycotoxin analysis, pointing out fumonisins as the most common *Fusarium* mycotoxin in both regions. Though, the incidence of maize samples contaminated with this mycotoxin is greater than the incidence of maize samples infected with its concomitant fungi. This could be due to the differences in sampling method for both analyses. One g of ground and homogenized maize sample was used for the mycotoxin analysis, while for the species determination only three maize grains were needed. As result, it is possible that not all species present in the sample are isolated and identified. As cited in the introduction, the majority of African countries have no specific mycotoxins regulations, but the following regulations are most commonly applied (FAO, 2004): 1000 μg/kg is the maximum limit for fumonisins, limits for DON vary from 300 to 2000 μg/kg and 750 μg/kg is the limit for DON used by countries in the EU, limits for ZEA in maize vary from 50 to 1000 μg/kg. Median fumonisin concentration for both regions in both growing seasons stays under the regulatory limit of 1000 μg/kg. Though, significant more maize samples in growing season 2012/13 contain concentrations exceeding this limit. Wide ranges of fumonisin concentrations in maize have been reported before (Atukwase, Kaaya, & Muyanja, 2009; Kedera, Plattner, & Desjardins, 1999; Kimanya et al., 2010). DON has a greater incidence in Hanang district, according to the greater incidence of *F. graminearum* in that region. Median and maximum DON concentrations for growing season 2011/12 in both AEZ don't exceed the EU regulatory limit, but DON concentrations for growing season 2012/13 are remarkably higher and exceeding regulatory limits. ZEA, also produced by *F. graminearum*, was only found in few samples. The optimal temperature for fumonisin and DON production is 20 °C and for ZEA it is below 10 °C. This explains

Table 5
Linkage analysis of agricultural practices, location, *Fusarium* population and mycotoxins. P-values are indicated in the table.

	Fusarium	FB1	FB2	Total FB	DON	ZEA
Previous crop	NA	0.080[a]	0.475	0.074[a]	0.035[b]	0.035[b]
Solution for storage problems	0.817	0.038[b]	0.065[a]	0.033[b]	0.156	0.985
Success of treatment to protect maize from insect damage in storage	0.864	0.040[b]	0.012[b]	0.037[b]	0.484	0.773
AEZ	0.022[b]	0.036[b]	0.007[b]	0.0259[b]	0.003[b]	0.578
Village	0.020[b]	0.097[a]	0.043[b]	0.079[a]	0.025[b]	0.560

[a] Significant at level 0.1.
[b] Significant at level 0.05.

the low incidence of ZEA in this study (Domijan, Peraica, Jurjevic, Ivic, & Cvjetkovic, 2005). The high incidence of maize contaminated with sometimes high concentrations of different mycotoxins, leaves the habitants of both districts highly exposed to mycotoxin contamination and the associated health implications due to their daily consumption of maize.

Agricultural practices leading to fungal infection and associated mycotoxin contamination are present in both AEZ. Tillage systems and handling crop residues from previous growing cycle could have an influence on the fungal population dynamics in the soil as well as for the soil fertility. Minimum and conventional tillage are differentially practiced between the study sites. Most households in Hanang district are pastoralists and use crop residues to feed their animals, leaving the soil bare and susceptible to erosion (Owenya, Mariki, Kienzle, Friedrich, & Kassam, 2011), but reducing the primary inoculum for next cropping season. In addition, minimum and conventional tillage is commonly practiced. Most farmers in Kilosa district plough the crop residues from the former cropping season back into the soil as part of nutrient cycling, preventing survival of mycotoxigenic fungi on surface residues (Dill-Macky & Jones, 2000; Munkvold, 2003a). Skoglund and Brown (1988) and Cotton and Munkvold (1998) reported that survival of *Fusarium* species is greater in superficial residues compared to buried residues. Results show that most farmers use seeds which are certified and commercially sold, however, farm saved seeds are also commonly utilized in both AEZ. The questionnaire also revealed low fertilizer use among Kilosa farmers, which could be associated with their traditional means of cultivation. In Hanang, farmers are semi pastoralists and use farm yard manure to improve soil fertility, since most farmers have only low purchasing power for inorganic fertilizers. In Kilosa, only few households own a cattle herd to collect manure from. On the other hand, farmers take significant more alternative measures to improve soil fertility, for example, the application of green manure and mulching. These results have also been reported previously by Kaliba et al. (1998). Stressed plants are more prone to fungal infection, thus other stress factors such as drought and insect damage need to be avoided. But all maize in both AEZ is produced under rain fed conditions. Farmers do not have enough resources or the knowledge to irrigate their fields. Insects play an important role in the infection pathway of fungi as they act as wounding agents or as vectors spreading the fungi (Borgemeister et al., 1998; Cao et al., 2014; De Curtis et al., 2011; Dowd, 2003; Fandohan, Gnonlonfin, Hell, Marasas, & Wingfield, 2006; Kaaya, Warren, Kyamanywa, & Kyamuhangire, 2005). Though, one out of four households do not take any measures to reduce pests and diseases during maize production. Practices implemented by the other farmers are timely planting, to assure harvest before rainy season starts, and application of pesticides. In addition, certain maize characteristics, for example good husk cover, cobs bending down at maturity and early maturity, could be an important factor in protecting maize against insect damage or fungal infestation. Results of this research revealed that these characteristics aren't a determining factor in the choice of variety. Delayed harvesting of 4–12 weeks after physiological maturity is reported as a common approach to allow the crop to dry sufficiently in the field before harvesting. This could have negative implication in terms of increasing the odds of pest infestation. In addition, moisture content of the grains will not drop under 15% when drying on the stalk. Delayed harvesting of maize and its implication on mycotoxins is reported for several countries, e.g. Uganda (Kaaya et al., 2005) and Kenya (Mutegi, Ngugi, Hendriks, & Jones, 2009), and in review papers (Cao et al., 2014; Cotty & Jaime-Garcia, 2007; Munkvold, 2003a; Wagacha & Muthomi, 2008). Although former practices could be seen as favorable for fungal infection and mycotoxin production, most of the agricultural practices did not influence significantly the *Fusarium* population nor the mycotoxins presence in this study. Though, linkage analysis revealed that well-considered crop rotation, appropriate storage conditions and insect control measures might influence *Fusarium* toxins contamination. Well-chosen crops, resistant to *Fusarium* infection, could decrease the risk of infection, and subsequent contamination with mycotoxins, in maize by reducing primary inoculum in the field (Lipps & Deep, 1991). Crops indicated in this study as preceding or accompanying maize are legumes, sunflower, tomatoes and pumpkin. A well aerated and clean storage structure, free of insect pests and sources of fungal populations, is another important management aspect in preventing contamination of maize and save healthy seeds for next cropping season (Chulze, 2010; Fandohan et al., 2006; Kaaya et al., 2005; Pitt et al., 2013). The linkage analysis also revealed significant correlations between AEZ, *Fusarium* population and mycotoxin contamination. Differences in climatic conditions, soil characteristics or geographic conditions between both regions could outdo the effects of agronomic practices on *Fusarium* population and mycotoxin presence. In addition, a significant correlation between village, *Fusarium* population and mycotoxin presence was demonstrated.

5. Conclusion

The current study highlights the vulnerability of the habitant in Tanzania's Kilosa district and Hanang district to mycotoxins exposure, through the daily consumption of maize and maize based products, since childhood onwards. Maize samples from both locations are predominantly contaminated with *F. verticillioides*, *F. graminearum* and *F. poae*, which was reflected by the high prevalence and sometimes high concentrations of fumonisins and DON in the mycotoxin analysis. Although agricultural practices during maize cultivation differ significantly in both districts, only few correlations were found between maize management practices and *Fusarium* population nor mycotoxin presence. Differences in climatic conditions, soil characteristics or geographic conditions could possibly outdo the effect of agronomic practices on *Fusarium* population and mycotoxins presence. A similar, but more extensive study, integrating more participating households, could provide further insight necessary to address this problem on the level of the subsistent farmer.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.foodcont.2015.05.028.

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