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Impact of companion cropping on incidence and severity of maize ear rots and mycotoxins in Western Kenya

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Mycotoxins are harmful to health and mainly arise from ear rots, affecting maize in the field. This work analysed the effect of the cropping system on ear rot and final effect on mycotoxins from four sub-counties (districts) of western Kenya, Butere, Kisumu, Siaya and Vihiga, where plots comprising maize planted either as pure stand or in mixture with legumes, predominantly common bean treated as "Maize Monocrop" (MM), were used as control for those of climate-smart push-pull strategy treated as "Push-Pull" (PP). Symptomatic and asymptomatic maize ear samples were analysed for total aflatoxin (AF), total fumonisins (FB), deoxynivalenol (DON) and zearalenone (ZEA) using Enzyme-Linked Immunosorbent assay (ELISA). Cropping system had very high significant effect on ear rot incidence and severity. In general, low incidence was observed in PP (7.3 %) than MM (20.8 %). Similar trend was also observed on ear rot severity in PP and MM as follows: diplodia (1.15 and 1.85), gibberella (0.62 and 0.84), aspergillus (0.09 and 0.25), fusarium (0.19 and 0.68) and penicilium (0.03 and 0.05). A high proportion of ZEA (100%), AF (93.3%), DON (80.0%) and FB (65.9%) were observed in symptomatic samples than in ZEA (90.3%), DON (51.6%), FB (38.7%) and AF (3.2%) in asymptomatic samples. Low ear rot incidence and severity was more in PP than MM, and proportion of mycotoxins on asymptomatic ears; suggesting the potential of cropping system in managing ear rots and ultimately limiting mycotoxins. Thus the study highlighted the need to adopt cropping systems to deal with mycotoxins, and also recommends surveillance and awareness on emerging mycotoxins: ZEA and DON.

Key words: Push-pull, maize monocrop, aflatoxins, fumonisins, deoxynivalenol, zearalenone.

INTRODUCTION

Maize ear rots are fungal infections with worldwide distribution and presence in all agro-ecologies where maize is grown (Dragich and Nelson, 2014). Key fungal genera prominent for maize ear rot infections in sub-

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Saharan Africa include *Aspergillus*, *Fusarium*, *Sternocarpella* and *Penicilium* (Kapindu et al., 1999), causing estimated yield losses ranging between 10 and 30% (Kapindu et al., 1999; Ajanga and Hillocks, 2000; Bigirwa et al., 2007). Ravages caused by the ear rots are aggravated by mycotoxins, which are increasingly becoming the main sources of grain losses globally. Four agriculturally important mycotoxins with high occurrence are fumonisins, zearalenone, deoxynivalenol and aflatoxins (Gxasheka et al., 2015). These mycotoxins pose high risks to human and animal health (Zain, 2011), and have led to stringent regulation of their levels in food and feed in global grain trade (Otsuki et al., 2001).

A number of pre- and post-harvest measures have been advanced to manage ear rots and mycotoxin contamination in grain crops (Hell et al., 2008), with sorting as a post-harvest measure being key. Studies have directly linked mycotoxin reduction to removal of fungal ear or kernel rot (Balconi et al., 2014), making sorting a primary tool for management of mycotoxins and quality enhancement of the harvested crop (Wild et al., 2016). Moreover, uses of normal grain cleaners, largely in developed economies, have resulted in 50-60 % reduction of fumonisins and aflatoxins in some countries (Malone et al., 1998; Pacin and Resnik, 2012). Further improvement on grain sorting has also been made through development of high-capacity electronic optical sorters which target kernel discoloration and mycotoxin fluorescence (Pearson et al., 2010). Similar results have been obtained in Africa, where for instance, reports indicate 40% reduction of aflatoxin concentration through removal of moldy, damaged and broken grains in Benin (Fandohan et al., 2005). In addition, reports indicate 20% reduction of fumonisins in Tanzania, Kenya and South Africa as a result of sorting (Kimanya et al., 2009; van der Westhuizen et al., 2010; Mutiga et al., 2014). However, owing to the fact that 80 % of arable land (Wiggins, 2009) and 75% of total maize production (Nyoro et al., 1999) are from smallholders farmers, mycotoxin recycling and high exposure to consumers are preeminently promoted by alternative use of infected ears by fungal rot (Bigirwa et al., 2007; Mukanga et al., 2011). Since ear rot attacks begin in the field, pre-harvest measures can ensure achievement of good yields and grain quality in terms of mycotoxin reduction (Munkvold, 2003).

Generally, factors including fungal taxon, humidity, rainfall, insect damage, drought, irrigation and maize germplasm influence incidence of maize ear rots in the field (Parsons, 2008). Gibberella and diplodia ear rots, caused by *Fusarium graminearum* and *Stenocarpella maydis*, respectively, are encouraged by high rainfall, susceptible crop genotypes, continuous maize cultivation and poor crop residue management (Marasas, 2001). The recurrence of fumonisin and aflatoxin contamination in tropical regions, make fusarium and aspergillus ear rots, caused mainly by *Fusarium verticillioides and Aspergillus flavus*, respectively, of high concern; although diplodia ear rot exceed them on incidence in several studies in maize growing regions (Bigirwa *et al.,* 2007; Mukanga *et al.,* 2010). Among the favorable factors for *Fusarium* and *Aspergillus* ear rots are water stress, insect damage and nutritional status of soil (Marasas et al., 2001). Empirically, strong correlation of ear rots with insect attack, and correlation of silk-cut symptom with incidence of immature thrips population has been reported (Parsons, 2008; Ajanga and Hillocks, 2000). Thus reduction in insect damage has resulted in reduced ear rots and mycotoxin attacks. These measures are aimed to control damage to crops by insect pests, thus contributing to the management of ear rots and mycotoxin attacks on harvested crop (Munkvold et al., 1997).

Push-push technology, a novel companion cropping system where maize is intercropped with insect repellent forage legumes in the genus *Desmodium* and with grasses such as *Brachiaria* planted around this intercrop effectively controls stemborers, the key pests of cereals in eastern Africa (Khan et al., 2014; Midega et al., 2015a,b). The technology, which is practiced by over 130,000 smallholder farmers in eastern Africa to date, has the potential to contribute to management of ear rots and mycotoxin contamination in maize in the region. This study analyze the influence of push-pull technology on incidence and severity of maize ear rots in maize and quantify the level, incidence and range of mycotoxins on fungal infected (symptomatic) and clean (asymptomatic) maize ears.

MATERIALS AND METHODS

Study site

The study was conducted in Butere (0° 09' to 0° 20' S, 34° 29' to 34° 33' E), Vihiga (0° to 0° 15' S, 34° 30' to 35° 0' E), Kisumu (0° 15' to 0° 25' S, 34° 55' to 34° 67' E) and Siaya (0° 26' to 0° 18' S, 33° 58' to 34° 33' E) sub-counties (districts) of western Kenya, where push-pull technology has been widely disseminated and has been practiced by smallholder farmers since the year 2000 (Figure 1) (Khan et al., 2011). The study sites are characterized by a bimodal rainfall pattern and forms part of the larger grain basket of Kenya. The region has the highest concentration of smallholder farmers who grow maize largely in mixed stands with legumes and in combination with livestock (Khan et al., 2011). Occurrence of ear rots is one of the key constraints affecting growing and utilization of maize in the region (Ajanga and Hillocks, 2000). Other serious constraints also include insect pests, principally cereal stemborers, striga weeds and poor soil fertility (Midega et al., 2015b). Studies have shown a strong and positive correlation of ear rots (r=0.87) with insect damage (Ajanga and Hillocks, 2000). The current study was conducted in farmers' fields during the short (September to December) rainy season of 2014 and the long rainy season (March to August) of 2015, with treatments comprising maize grown either in push-pull or in sole stands (monocrop). In both plots, maize was planted at inter and intra-row spacing of 75 and 30 cm, respectively. The push-pull treatment had maize intercropped with greenleaf desmodium (*Desmodium intortum* (Mill.) Urb.), with *Brachiaria* cv Mulato II grown as a border crop around this intercrop at a spacing of 50 cm within and 50 cm between rows. Farmers in the sample districts planted their local maize varieties, ‘Nyamula’ and ‘Jowi’

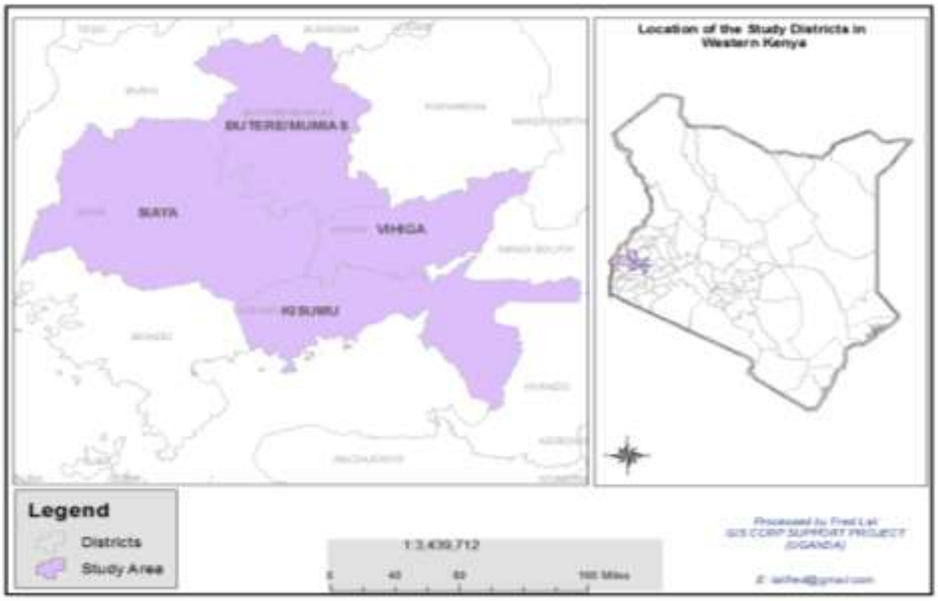

Figure 1. Map showing study sub-counties (districts) in western Kenya. org/10.1016/j.jscs.2010.06.006.

(Midega *et al.,* 2015b), with only a small proportion planting medium maturity hybrids WH505.

Sampling and determination of incidence and severity of maize ear rot

A total random sample of 224 maize plots was picked from a sampling frame comprising push-pull and maize monocrop plots of equal number (112) in the study sub-counties. Each plot was surveyed for ear rot incidence and severity through a randomized sampling process during each cropping season, short rain and long rain. Siaya sub-county was however not surveyed during the second cropping season as only a few farmers planted maize during the season. At the beginning of harvest, 100 maize cobs were randomly picked per plot from which ear rot incidence was determined by physical count as described by Mutitu et al. (2003). A score of severity on a scale of 0-5 where: 0= No infection, 1=1-10%, 2=11-25%, 3=26-50%, 4=51-75% and 5=76-100% infection was used to estimate severity (Jeffs, 2002). Compendium with well-illustrated photographs of maize ear rots was used and tested before identification and characterization. Samples of infected (symptomatic) and clean (asymptomatic) maize ear were also collected and transported in a coolbox to the laboratory at the international centre of insect physiology and ecology (*icipe*), Thomas Odhiambo Campus at Mbita Point in western Kenya. These samples were further dried in an open air to moisture level of 13% measured using moisture meter (Model KM-36G, AWR Smith Process Instrumentation cc, South Africa). Samples were then hand shelled and milled before storage in a refrigerator (-4°C), waiting further mycotoxin analysis.

Mycotoxin extraction and analysis

Mycotoxin extraction and assay was conducted using ELISA commercial kit (Helica Biosystem Inc., Fullerton, CA, USA) for total aflatoxin, Cat. No.941AFLO1M-96; total fumonisin, Cat. No.951FUM01C-96; zearalenone, Cat. No.951ZEA01N-96; and deoxynivalenol, Cat. No.941DON01M-96 as described by Gutleb et al. (2015). A total of 20 mg of sub-samples of maize flour was extracted in 100 ml of solvent. Total aflatoxin was extracted in 70% methanol, total fumonisin and zearalenone in 90% methanol, and deoxynivalenol in distilled water. The mixture was blended and filtered with Whatman filter paper number one. Filtrate was used to measure for total aflatoxin directly, but diluted with distilled water for total fumonisins (1:20) and deoxynivalenol (1:10), and 70% methanol for zearalenone (1:10). The ELISA assay was conducted according to manufacturer's instructions. Optical density (OD) of the reaction for AFB, FB, ZEA and DON was measured using a microplate reader (*EZ* Read 400, biochrom) at a wavelength of 450 nm. Standard curve for each mycotoxin tested was generated using OD of five standards with known concentration as provided in the kit. Test (unknown samples) value was then determined by interpolation from the standard curve. Sample was tested within the range of 1-20 ppb for total AF; 100-6,000 ppb, total FB; 15-500 ppb, ZEA; and 500-10,000 ppb, DON. Sample which had exceeded upper limit of quantification was subjected to additional extract dilution. The final result was converted from parts per billion (ppb) to equivalent microgram per kilogram (µg/Kg).

Data analysis

Effects of cropping system and season on ear rot incidence were analyzed using generalized linear model, while ear rots severity were analyzed by analysis of variance using R software version 3.3.1 (R Core Team, 2013). Mean, frequency and percentage of samples contaminated with mycotoxins were presented by simple descriptive statistic using SPSS version 22 (IBM Corp, 2013).

RESULTS

Effect of cropping system on incidence and severity of maize ear rots

The study revealed that cropping system had a significant effect ($p \leq 0.001$) on the incidence of ear rots as shown in Table 1. Generally, there was high total ear rot incidence (20.8%) observed with sole maize than with push-pull system (7.3%). Similarly, there were significantly higher ($p \leq 0.001$) incidences of each of the ear rot types with sole maize than with push-pull. The sequence of incidence of ear rot types in sole maize and push-pull from highest to lowest was as follows: *Diplodia* < *Gibberella* < *Aspergillus* < *Fusarium* < *Penicillium*. The respective incidences of ear rots in sole maize and push-pull were 7.31 and 3.33%, *Diplodia*; 4.48 and 1.30%, *Gibberella*; 2.09 and 0.65%, *Aspergillus*; 0.51 and 0.21%, *Fusarium*; and 0.40 and 0.11%, *Penicillium*. The results in Table 2 showed that the severities were also significantly different ($p \leq 0.001$) between the two cropping systems; however, they seemed to be influenced by incidence of the ear rots. For instance, *Diplodia* and *Gibberella* ear rots had the highest severities of 1.85 and 1.15 in sole maize, and 0.84 and 0.62 in push-pull, when compared to other ear rots. However, for *Aspergillus* and *Fusarium* ear rot this seemed not to be the case, as *Aspergillus* had higher incidence than *Fusarium*, yet it had the lower severity rating (0.25 and 0.09) than *Fusarium* (0.68 and 0.19) with sole maize and with push-pull, respectively. Conversely, insignificant and least severities of 0.05 and 0.03 were reported in *Penicillium* ear rot with sole maize and push-pull, respectively.

Influence of cropping season on incidence and severity of maize ear rots

Short rainy season reported the highest (16.0%) incidence of total ear rot compared to the long rainy season (10.1%) (Table 1). However, incidence of *Penicillium*, *Diplodia* and aspergillus ear rots ear rots was significantly higher ($p \leq 0.01$) in the long rainy season than in the short rainy season. Conversely, incidence of *Diplodia* ear rots was significantly higher ($p \leq 0.001$) in the short rainy season than in the long rainy season. In terms on incidence levels, the highest ear rot incidence was recorded in *Diplodia* in both short (8.02 %) and long (3.12 %); while the lowest incidence was recorded in *Penicillium* in the short (0.05 %) and long (0.7 %) rainy seasons. Severities of ear rots were mostly insignificant with season, except for *Penicillium* ($p \leq 0.043$). There was no significant observable interaction between cropping system and season on incidence and severity of all types of ear rots (Tables 1 and 2).

Ear rots and mycotoxin incidence levels

Incidence of mycotoxins was higher in symptomatic than asymptomatic ears samples (Table 3). Based on mycotoxin type, the order of decreasing incidence on symptomatic samples was zearalenone (ZEA) (100%), total aflatoxin (AFB) (93.3%), deoxynivalenol (DON)(80.0%) and fumonisins (FB) (65.9%). However, asymptomatic ear samples had unexpected high incidence of ZEA (90.3%) and DON (51.6%). The mycotoxin ranges were also wider in symptomatic (ZEA, 18.7-688 µg/Kg, AFB, 0.35-28.9 µg/Kg; DON, 0-18,260 µg/Kg; and FB, 0-8,280 µg/Kg) than in asymptomatic (AFB, 0-11.7 µg/Kg; DON, 0-4,360 µg/Kg; FB, 0-6,460 µg/Kg; and ZEA, 0-405.8 µg/Kg) samples. The respective average levels of four quantified mycotoxins, ZEA, AFB, DON and FB, were 274.3, 6.1, 3,672 and 4,193.9 µg/Kg, respectively on symptomatic samples, showing high levels of mycotoxins compared to 48.4, 0.39, 633.9 and 1,616.8 µg/Kg on asymptomatic ears samples. The incidences of samples with mycotoxins beyond acceptable levels were also high; higher on symptomatic ears with 46.7, 28.9, 50.0 and 56.8% reported for ZEA, AFB, DON and FB, respectively. On asymptomatic samples, incidences were as low as 3.2% for AF and ZEA, and 19.4% for DON and FB.

DISCUSSION

Maize ear rots reduce grain yields and quality, with some of the causative pathogenic fungi producing mycotoxins that pose health risk to humans and livestock (Mukanga et al., 2010). Such ear rots are thus an important component of the myriad factors responsible for the high rates of food insecurity and health complications among smallholder farm families in sub-Saharan Africa. There is evidence that attack of maize by the ear rots and mycotoxins begin before the crop is harvested (Mukanga et al., 2011), and the attack is aggravated by grain handling and storage conditions (Mutiga et al., 2015). Indeed, incidence of ear rots in the study region, pre-harvest, often exceeds 20% (Ajanga and Hillocks, 2000), as confirmed by the current study. Notably, results of the current study, which to the best of our knowledge is the first study that directly relates ear rots and mycotoxins with cropping system under field conditions, demonstrated that maize grown under the push-pull cropping system suffered significantly less ear rots than sole maize, reducing the incidence level to 7.3%.

Attack of maize by stemborer pests has been shown to predispose the grains to ear rots and mycotoxin attack. Studies by Ajanga and Hillocks (2000) reported positive and high correlation between stemborers and incidence of ear rots in maize. Additionally, an interplay of other factors such as increase of organic matter (Alakonya et al., 2008), cover cropping (Tédihou et al., (2012), and intercropping (Vincelli, 1997; Flett and Ncube, 2015) have been reported to reduce ear rot incidence in maize. The push-pull cropping system effectively controls stemborers in maize (Khan et al., 2014; Midega et al., 2015a, b), improves soil organic matter content (Midega et al., 2005) and provides other soil improvement benefits. Therefore

Table 1. Effects of cropping system, season and their interaction on percentage incidence of ear rot disease.

Factor	Level	Gibberella x̄±SE	Fusarium x̄±SE	Penicillium x̄±SE	Aspergillus x̄±SE	Diplodia x̄±SE	Total incidence x̄±SE
System	Push-Pull (PPT)	1.30±0.1	0.12±0.1	0.11±0.04	0.65±0.1	3.22±0.2	7.30±0.3
	Maize Monocrop (MM)	4.48±0.2	0.51±0.2	0.40±0.1	2.09±0.2	7.31±0.3	20.8±0.4
Season	Long rains (LR)	2.02±0.1	2.67±0.1	0.70±0.1	1.38±0.1	3.12±0.2	10.1±0.3
	Short rains (SR)	3.00±0.2	0.22±0.2	0.05±0.04	0.96±0.1	8.02±0.3	16.0±0.4
System x Season							
	PP- LR	3.21±0.2	0.20±0.1	0.22±0.1	1.58±0.15	4.7±0.2	13.9±0.4
	PP- SR	2.19±0.2	0.26±0.1	0.21±0.03	1.05±0.14	4.93±0.3	12.1±0.3
	MM –LR	2.19±0.2	0.26±0.2	0.21±0.1	1.05±0.2	4.93±0.3	12.1±0.5
	MM-SR	2.19±0.3	0.26±0.3	0.21±0.1	1.05±0.2	3.93±0.5	12.1±0.6
Source of variation							
System		***	***	***	***	***	***
Seasons		Ns	***	***	**	***	***
System x Season		Ns	Ns	Ns	Ns	Ns	Ns

LR = Long rain; SR = Short rain; MM=Maize Monocrop; PP=Push-Pull; ns=not significant; x=Interaction,
x̄±SE, Standard error of the mean.Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05.

Table 2. Mean severity of ear rots disease by cropping system, season and their interaction.

Factor	Level	Gibberella x̄±SE	Fusarium x̄±SE	Penicillium x̄±SE	Aspergillus x̄±SE	Diplodia x̄±SE
System	Push-pull	0.62±0.09	0.19±0.05	0.03±0.01	0.09±0.03	0.84±0.1
	Maize momocrop	1.15±0.09	0.68±0.04	0.05±0.01	0.25±0.03	1.85±0.1
Season	Long rains	0.82±0.10	0.45±0.04	0.06±0.01	0.19±0.03	0.89±0.1
	Short rains	0.95±0.09	0.41±0.04	0.01±0.01	0.14±0.03	1.79±0.1
System x Season						
	PP – LR	0.58±0.1	0.23±0.06	0.06±0.02	0.11±0.04	0.45±0.1
	PP – SR	0.66±0.1	0.16±0.06	0.004±0.02	0.06±0.04	1.22±0.1
	MM-LR	1.06±0.1	0.68±0.06	0.07±0.02	0.27±0.04	1.32±0.1
	MM-SR	1.23±0.1	0.67±0.06	0.02±0.02	0.22±0.04	2.37±0.1
Source of variation						
System		***	***	Ns	***	***
Seasons		Ns	Ns	**	Ns	Ns
System x Season		Ns	Ns	Ns	Ns	Ns

LR = Long rain; SR = Short rain; MM=Maize Monocrop; PP=Push-Pull; x=Interaction; Ns=not significant;
x̄±SE, Standard error of the mean.Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05.

Table 3. Mycotoxin incidence and levels on symptomatic and asymptomatic ear samples.

Mycotoxin	Symptomatic ears				Asymptomatic ears			
	AFB	DON	FB	ZEA	AFB	DON	FB	ZEA
Total sample (N)	45	30	44	45	31	31	31	31
Positive N (%)	29 (93.3)	24 (80.0)	29 (65.9)	45 (100)	1 (3.2)	16 (51.6)	12 (38.7)	28 (90.3)
Range (µg/Kg)	0.35-28.9	0-18,260	0-8,280	18.7-688	0-11.7	0-4,360	0-6,460	0-405.8
Average (µg/Kg)	6.1	3,672	4,193.9	274.3	0.39	633.9	1,616.8	48.4

N, Number of ears sample; (%), Percent; AFB, Total Aflatoxin; DON, Deoxynivalenol; FB, Fumonisins (Total fumonisins B1+B2+B3); and ZEA, (Zearalenone); ML, Maximum Limit of concentration for mycotoxins.

the significant reduction in incidence of ear rots observed in the push-pull plots might have resulted from the multiple ecological benefits provided by the technology.

Planting seasons are important on disease forecasting and appropriate for decision by farmers (De Wolf et al., 2003). Maize is grown in seasons which have varied amount of rainfall and temperature, the two major factors for ear rot incidence and severity. In Uganda, Diploda was the most abundant ear rot found in areas receiving high rainfall (Bigirwa et al., 2006), thus Bigirwa et al., (2007) reported more ear rot during first season. There was similar observation in study, but during the second season and not first season which received high rainfall. This may be due to wet conditions at silking stage favourable for *Diplodia* and *Gibberella* infection and progression (Miller, 2001; Woloshuk and Wise, 2010); which was met when late rainfall cessation extended beyond silking stage in short rain (Mugo et al., 2016). Similarly, push-pull cropping system could as well promote cooler conditions due to high evapotranspiration from intercrop; thereby predisposing ears to potential infection with *Diplodia* or *Gibberella* ear rot as observed on insignificant by slightly high *Gibberella* and total ear rot incidence by interaction of push-pull and long rain season.

Fusarium mycotoxins are abundant in cereals and their products (Yazar and Omurtag, 2008), and are diverse in nature. They can cause food poisoning upon ingestion. Deoxynivalenol poisoning is characterized by diarrhea, vomiting, nausea, headache, dizziness and fever (Zain, 2011), while zearalenone is known to cause reproductive problems mostly in pigs, sheep and human beings (de Rodriguez et al., 1985; Smith et al., 1986; Kuiper-Goodman et al., 1987).

These two mycotoxins have received little attention due to their causal agents' devastation on wheat (Zain, 2011) than in maize, and less acute outbreaks compared to aflatoxin (Darwish et al., 2014) and fumonisin (Fadhohan et al., 2003) in the Sub-Saharan region. However, a likelihood of high population exposure might be seen from high average levels of zearalenone (3,663 µg/Kg) and deoxynivalenol (23,586 µg/Kg) tested from household maize samples in Tanzania (Degraeve *et al.,* 2016). Similarly, these two mycotoxins were found in high amount from our samples, with difference of less amount of mycotoxin on asymptomatic ear compared to symptomatic.

Conclusion

Conclusively, impact of cropping system on ear rot was evident and the system should be integrated with other management system to control ear rots and mycotoxins. The high incidence and amount of zearalenone and deoxynivalenol in these studies and elsewhere in sub-Saharan suggests that there is need for their surveillance by screening their presence and sensitizing of farmers on their management

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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