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Atoxigenic *Aspergillus flavus* (Aflasafe KE01) application reduces Fumonisin contamination in maize in lower Eastern Kenya

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Abstract

Management of fumonisin contamination in maize has been a challenge largely because there are no effective management measures for *Fusarium* ear rots. Maize varieties that are resistant to fumonisin contamination are also lacking. This study was conducted to determine the efficacy of atoxigenic *Aspergillus flavus* (Aflasafe KE01) on fumonisin contamination of maize. The study was carried out in four sub-counties in lower Eastern Kenya. Twenty-four maize fields were selected in each sub-county; where 12 fields were treated with Aflasafe KE01, while 12 fields comprised the untreated controls. Aflasafe KE01 was applied at a rate of 5 kg/ha and 10kg/ha by hand broadcasting in maize fields two to three weeks before tasselling of maize. Fumonisin level in the maize samples was determined using Accuscan Pro-reader enzyme-linked immunosorbent assay (ELISA). The results showed that application of Aflasafe KE01 reduced fumonisin in the maize from the Aflasafe KE01 treated fields by up to 68% compared to samples from untreated fields. About 62.5% of the maize fields treated with 5kg/ha of Aflasafe KE01 met the European Commission regulatory threshold of ≤2ppm for total fumonisin as compared to about 45% from the control fields. This indicates that Aflasafe KE01 is a potential biopesticide for the management of fumonisin in maize. Therefore, efficacy of Aflasafe KE01 to reduce fumonisin contamination of other key staples in Kenya should also be evaluated.

Key words: Aflasafe KE01, atoxigenic *Aspergillus* sp., fumonisins, maize.

Introduction

Maize is the staple crop in Kenya, contributing up to 21% of the total value of primary agricultural commodities (Kang'ethe *et al*., 2020). According to FAO statistics, maize crop occupies 48.5% of arable land (FAOSTAT, 2019). However, maize is prone to mycotoxins such as aflatoxins and fumonisins (Cinar and Onbasi, 2019). Fumonisins are toxic metabolites produced by *Fusarium verticillioides* and *Fusarium proliferatum* and is common in maize-based food and feed (Fandohan, 2003; Kamle *et al*., 2019). In Kisii county, a study by Alakonya *et al.* (2009) reported fumonisin B1 levels of 3,600–11,600ng/g in maize. Acute aflatoxicosis outbreaks in humans in Kenya have been reported from time to time and in different parts of the county (Korir and Bii, 2012; Muthomi *et al*. 2012; Okoth and Kola, 2012). Fumonisins are carcinogenic and have been linked to oesophageal cancer (Kimanya, 2015). Fumonisin epidemics occur commonly in dry years and are favored by warm, dry weather during grain-filling stage of maize growth. Droughts at the beginning of the growing season and wet weather during pollination and silking stages can favor fumonisin synthesis in harvested maize grain (Ariño *et al*., 2009). To reduce human exposure and deaths caused by fumonisin, there is an urgent need to manage/control its metabolism in maize. Fumonisins are managed by prevention of *F. verticillioides* infection.

To manage mycotoxigenic fungi and mycotoxin production, different approaches have been used including physical, chemical and cultural methods. These methods range from the use of synthetic fungicides (UlHaq *et al*., 2020), breeding for resistant maize cultivars (Lanubile *et al*., 2014) and cultural approach to avoid pre- and post-harvest attack of fungi (Munkvold, 2003; Njeru *et al*., 2019). However, agronomic practices for fumonisin content reduction are ineffective when conditions for fungal growth are optimal (Robertson-Hoyt *et al*., 2007). Biological control methods have become popular because of their ability to reduce the primary fungal soil inoculum and the infection rate in root systems of maize plants (Etcheverry *et al*., 2009).

Non-pathogenic *Fusarium* strains have been moderately applied as biocontrol

agents in suppressing the growth of toxigenic strains of *F. proliferatum* and *F. verticillioides* in maize (Luongo *et al.*, 2005). This method uses competitive exclusion which is made possible by the presence of toxigenic and atoxigenic strains of *A. flavus* populations. Application of non-toxigenic inoculants in the soil around the crops ensures competition with toxigenic strains for infestation sites on the growing plant. This is achieved by identifying and successfully introducing harmless atoxigenic strains that show competitive gain over toxigenic strains. Atoxigenic strains of *A. flavus* virtually eliminate the highly toxigenic strains thereby reducing aflatoxin contamination (Agbetiameh *et al.*, 2019; Senghor *et al.*, 2020). The objective of this study, therefore, was to assess the effectiveness of atoxigenic *Aspergillus flavus* (Aflasafe KE01) on the reduction of fumonisin contamination in maize in Lower Eastern Kenya.

Materials and methods

Description of the study area

The study was conducted in Nzambani sub-county (Kitui County), Kathiani sub-county (Machakos County) and Wote and Kaiti sub-counties (Makueni County) in lower Eastern parts of Kenya (latitude between 4°N to 4°S, longitude 34° to 41°E). These regions receive an average rainfall of between 150 mm to 650 mm p.a. On average, Machakos and Kitui counties receive 500 to 700mm p.a and 500 to 1050 mm p.a. respectively. The soils in these areas are sandy to loamy sand texture with low organic matter contents, low water retaining capacity and low plant nutrients thus making is susceptible to erosion (Gachimbi *et al.*, 2005). Makueni county has several agro-ecological zones (AEZs) with altitudes ranging from 790-1770masl and receives about 600-1050mm of average annual rainfall (Jaetzold *et al.*, 2010).

Experimental design and application of atoxigenic *Aspergillus flavus* (Aflasafe KE01)

The farms were selected randomly within each sub-county. The experiments were conducted using maize planted by the farmers who consented to take part in the study. Each of the four sub-counties had 24 maize fields where 12 fields were treated with Aflasafe KE01 while the other 12 were control fields. Within

each area, control fields were a maximum of 100m from treated fields. Aflasafe KE01 was obtained from the International Institute of Tropical Agriculture (IITA). Six of the individual farmers' farms were treated with Aflasafe KE01 at an application rate of 5 kg/ha while the other six were treated with 10 kg/ha. Aflasafe KE01 was broadcast by hand in selected fields 2-3 weeks prior to tasselling of maize. The experiment was carried out in one maize cropping season across the four sub-counties. Data collected from the experiment included the population of *Fusarium* species in the soil and grain samples and the fumonisin levels in the maize grains.

Collection of maize cob samples

Maize cobs were sampled from the maize fields to quantify the amount of fumonism present in grains. Maize cobs were sampled at harvest ensuring a minimum of eight cobs from each farm. Maize cobs were picked from the farm using a zigzag approach. The maize cobs were sun dried avoiding contact with the ground. Thereafter the dry maize was manually shelled by hand and dried in an oven at 45^{o}C for two days and then crushed to fine powder using a Bunn coffee mill grinder (Bunn omatic Corporation, Spring Field Illnois, USA). The ground maize sample was thoroughly mixed for fumonisin analysis. The samples were stored in the refrigerator at 4 ^{o}C.

Analysis of fumonisins in maize kernels

AccuScan Neogen Reveal Q+ was used for fumonisin analysis. A sample of 10g of ground maize was mixed with 50ml of 65% ethanol then shaken vigorously in a centrifuge for three minutes and allowed to settle and then sieved using a Whatman No. 1 filter paper. Red and clear sample cups were placed into a labeled sample rack. Two hundred microlitres of the sample diluents was placed in the red dilution cup and mixed with 100µL sample extract in a sample cup and mixed by a pipette up and down five times. One hundred microlitres of the diluent sample extract was then transferred into a new well labeled clear sample cup. The new reveal Q+ for fumonisin test strip was placed into the sample cup with the test strip coming into contact with the liquid and the timer set for 6 minutes after which it was removed from the sample cup and read within 1 minute by fully

inserting in the AccuScan pro-reader which automatically analyzed the cartridge. The test strips were read in the Reveal AccuScan or Reveal AccuScan III Reader within one minute of completion of the 5-minute incubation. Reaveal Q+ was designed for quantitative analysis of fumonisin with a limit of detection that ranges from 0.3 to 6ppm. Samples with quantities above 6ppm were determined through serial dilution and the result acquired multiplied by the number of dilutions. After each dilution, the above procedure on taking reading from the Reveal AccuScan III Reader was followed.

Data analysis

The data on fumonisin level was subjected to analysis by GenStat 15th edition to determine significant difference in fumonisin levels in Aflasafe KE01 treated and untreated maize fields. Differences between treated and untreated fields were separated using Fishers protected LSD ($p \leq 0.05$). Fumonisin categorization levels provided by Food and Drug Administration (FDA) of ≤ 2 = low, 2-4 = medium and >4 = high fumonisin level (above acceptable level in maize) was used to analyze fumonisins.

Results

Efficacy of field application of atoxigenic *A. flavus* (Aflasafe KE01) in reducing fumonisin contamination of maize grains at harvest

Maize sampled at harvest from Aflasafe KE01 treated fields were contaminated with varying levels of fumonisin. About 63.5% of the maize fields treated with 5kg/ha and 10kg/ha of Aflasafe KE01 met the threshold set by European Commission (≤2ppm) for fumonisin (Table 1). A total of 66.6% of the maize samples met the standard set by the US FDA (≤4ppm) while 33.3% was above the set standard of (≤4ppm) in the maize samples from fields treated with 10kg/ha of Aflasafe KE01 (Table 1). Generally, there was a reduction in fumonisin levels due to the application of Aflasafe KE01. There was significant difference in fumonisin levels in maize sampled from Aflasafe KE01 treated and untreated maize fields from Kaiti and Nzambani using the 10 kg/ha application rate with a 60 % and 65.5% decrease in the fumonisin level respectively (Table 2).

Table 1. Proportions (%) of fumonisin contamination levels falling under different categories for maize sampled at harvest from treated and untreated fields with atoxigenic *A. flavus* (Aflasafe KE 01).

			Fumonisin level (ppb)			
Rate of Aflasafe	**Sub-county**	**Treatment**	**≤ 2**	**2-4**	**>4**	**Range**
5kg/ha	Wote	Treated	83.3	0.0	16.7	0-6
		Untreated	66.7	16.7	16.7	0-11.5
	Kaiti	Treated	50.0	16.7	33.3	02-6.2
		Untreated	16.7	50.0	33.3	0-8.2
	Kathiani	Treated	16.7	50.0	33.3	0-6.5
		Untreated	16.7	33.3	50.0	0-8
	Nzambani	Treated	100.0	0.0	0.0	0-0.6
		Untreated	83.3	16.7	0.0	0-3.3
10kg/ha	Wote	Treated	83.3	0.0	16.7	0-4.1
		Untreated	100.0	0.0	0.0	0-0.6
	Kaiti	Treated	50.0	0.0	50.0	0-5.4
		Untreated	16.7	0.0	83.3	0-9.3
	Kathiani	Treated	50.0	16.7	33.3	0-7.5
		Untreated	83.3	0.0	16.7	0-7.5
	Nzambani	Treated	66.7	0.0	33.3	0-7
		Untreated	83.3	16.7	0.0	0-2.9

The fumonisin level categories are ≤ 2ppb is low, 2-4ppb is medium and >4ppb is high.

Table 2. Fumonisin level (ppb) in maize kernels sampled at harvest from atoxigenic *A. flavus* (Aflasafe KE01) treated and untreated fields in Kaiti, Nzambani Kathiani and Wote sub-counties in alternate seasons

	Treatment	**Sub county**			
		Kaiti	**Kathiani**	**Nzambani**	**Wote**
5 Kg/ha	Treated	2.7a	3.7a	0.25a	2.5a
	Untreated	3.7a	4.2a	0.8b	3.1a
% Reduction[a]		**27.0**	**11.9**	**68.7**	**19.3**
10 Kg/ha	Treated	2.4a	1.8a	1.0a	0.5a
	Untreated	6.0b	3.5a	2.9b	0.9a
% Reduction[a]		**60**	**48.5**	**65.5**	**44.4**

[a] Indicates reduction in levels of fumonisin in maize grains sampled from aflasafe KE01 treated and untreated fields. Means followed by the same letters within a column are not significantly different ($p \leq 0.05$) for each sub county.

Discussion

The higher fumonisin levels noted in untreated fields is as a result of dominant occurrence of *F. verticillioides* and *F. proliferatum* in maize samples from these fields. Most isolates of *F.verticillioides* and *F. proliferatum* are major fumonisin producers (Chulze *et al.*, 2015; Tsehaye *et al.*, 2017). The infection of maize grains with fumonisin in this study could be attributed to the high incidence of *F. verticillioides* and *F. proliferatum* (Misihairabgwi *et al.*, 2019). Additionally, weather in the lower Eastern parts of Kenya provides optimal conditions for the production of fumonisins in maize.

The results from this study showed that maize samples from treated fields had lower fumonisin levels compared to maize from untreated fields. This implies that the fumonisin level in maize grains was reduced due to the application of Aflasafe KE01. Lauren *et al.* (2004) reported a reduction in the population of *F. verticillioides* and *F. proliferatum*, and significant inhibition of fumonisin Bl (FBI) production by the presence of *F. gramineanum*. In a study by Kaur *et al.* (2010) strains of non-phytopathogenic *Fusarium* were combined with other biocontrol agents to obtain an effective reduction in fumonisin levels in crops. Other studies have reported a decrease in fumonisin levels in maize as a result of the interaction of *A. flavus* and *Fusarium* species (Dwivedi and Enespa, 2013; Camiletti *et al.*, 2018; Giorni *et al.*, 2019; Reis *et al.*, 2020). Single application of Aflasafe has been shown to be effective for up to 3 years and in several crops subsequently planted on the same plot (IITA, 2009). This implies that, Aflasafe KE01 should not necessarily be applied every season for effective control of the *Fusarium* species and fumonisim production in maize.

Conclusion

The level of Fumonisin in from Aflasafe KE01 treated fields were reduced by up to 68% compared to samples from untreated fields implying that Aflasafe KE01 is effective in reducing fumonisin contamination in maize and is therefore a promising biocontrol product in managing fumonisin contamination.

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