ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 16 No. 3
July 2016
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

10.18697/ajfand.75.ILRI03

AFLATOXIN B1 OCCURRENCE IN MILLET, SORGHUM AND MAIZE FROM FOUR AGRO-ECOLOGICAL ZONES IN KENYA

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ABSTRACT

Aflatoxin-contaminated food is a public health concern. Contamination of staple foods in Kenya has in the past led to loss of human lives as well as condemnation of large quantities of food, contributing to food insecurity. This study investigated the occurrence of aflatoxins in maize, millet and sorghum from five counties in Kenya (Kwale, Isiolo, Tharaka-Nithi, Kisii and Bungoma) representing four agro-ecological zones (AEZs). Samples were collected from rural households in two phases between February and October 2014. Using competitive enzyme-linked immunosorbent assay (ELISA), 497 maize, 205 millet and 164 sorghum samples were screened for the presence of aflatoxin B1. Overall, 76% of maize, 64% of millet and 60% of sorghum samples were positive for aflatoxin B1. Of these, the proportion of samples with aflatoxin B1 levels above the Kenya Bureau of Standards limit of five parts per billion was 26% for maize, 10% for millet and 11% for sorghum. In samples collected during the second phase, there were significant differences in the mean levels of aflatoxin contamination between the agro-ecological zones ($p<0.05$); maize from Kisii and Bungoma, representing temperate AEZ, had the lowest mean contamination, whereas millet and sorghum from Tharaka-Nithi (humid) and Isiolo (semi-arid), respectively, had the highest mean contamination. Continued exposure to aflatoxins via food in Kenya poses a threat to human health.

Key words: mycotoxins, contamination, cereals, exposure, East Africa, aflatoxicosis, aflatoxins, seasons

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INTRODUCTION

Cereals, including maize, sorghum and millet, are important staple foods in Kenya. They are used as human food and animal feed and are a source of processed foods such as cooking oil and breakfast cereals. Maize is also potentially an export crop, although in recent years Kenya has been a net importer of maize with minimal exports, mainly to other East African Community countries [1, 2]. However, under conditions of high temperature and humidity, maize, sorghum and millet form ideal substrates for aflatoxin-producing fungi. Occurrence of aflatoxins has been reported in these commodities in a number of sites in the country [3, 4, 5].

Aflatoxins are fungal toxins produced by *Aspergillus flavus, A. parasiticus* and *A. nomius* moulds infesting crops and forage. Naturally occurring aflatoxins include aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2 [6]. Aflatoxins and other mycotoxins in crops and animal source foods are classified as one of the urgent agricultural related health problems globally [7]. According to the International Agency for Research on Cancer (IARC), aflatoxin B1 is a class 1 human carcinogen and its toxicity results from its ability to bind to deoxyribonucleic acid and proteins, forming adducts such as aflatoxin B1-lysine in albumin [6, 8]. Human exposure to aflatoxins has known health effects including acute aflatoxicosis and, following chronic exposure, liver cancer. Liver cancer is a serious health problem in Africa, with 8.19 cases reported per 100,000 inhabitants [8]. In sub-Saharan Africa, where food-borne aflatoxin exposure is high, stunting is also common and aflatoxins may contribute to this, as studies have shown an association between childhood stunting and aflatoxin exposure [9]. In livestock, exposure is associated with reduced feed intake, reduced feed efficiency and reduced weight gain [9]. Apart from the health impacts, aflatoxins can also impact negatively on the economy, for example, if contaminated food products above legal limits are tested and destroyed [10].

East Africa's tropical and sub-tropical climate, including conditions such as high temperature and relative humidity, favours the growth of aflatoxin-producing moulds and consequently aflatoxin production. In order to reduce exposure, the Government of Kenya has set limits for aflatoxins in food and feed. The legal limit of total aflatoxin contamination in cereals is 10 parts per billion (ppb), whereas that of aflatoxin B1 is 5 ppb. The limit in feed of total aflatoxin is 10 ppb [11]. Levels up to 100 times the legal limit were found in home-grown maize tested during one of the largest aflatoxicosis outbreaks in Kenya [12]. That led to campaigns regarding aflatoxin contamination in maize in Kenya. However, there is need to highlight chronic incidences of aflatoxin poisoning from other susceptible foods including peanut, millet and sorghum. This paper presents the results of a study that determined the occurrence of aflatoxin B1 in millet, sorghum and maize sourced from five counties representing four agro-ecological zones (AEZs) in Kenya. The study was part of a larger project investigating aflatoxin in the feed–dairy value chain in Kenya.

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METHODS

Study site selection

To choose the survey sites, a map of agro-ecological zones (AEZs) in Kenya was used [13] and the counties in each AEZ were listed. Random selection was used to choose one county each from the semi-arid, humid and sub-humid zones and two counties in the temperate zone, being an area with high maize growing and dairy keeping activity. The study targeted farmers growing cereals and keeping dairy cattle, and thus no sites were selected from the arid zone which is not favourable for crop and dairy farming. The counties selected were: Isiolo (semi-arid), Tharaka-Nithi (humid), Kwale (sub-humid), Bungoma (temperate), and Kisii (temperate). One sub-location from each county was then randomly selected (Figure 1).

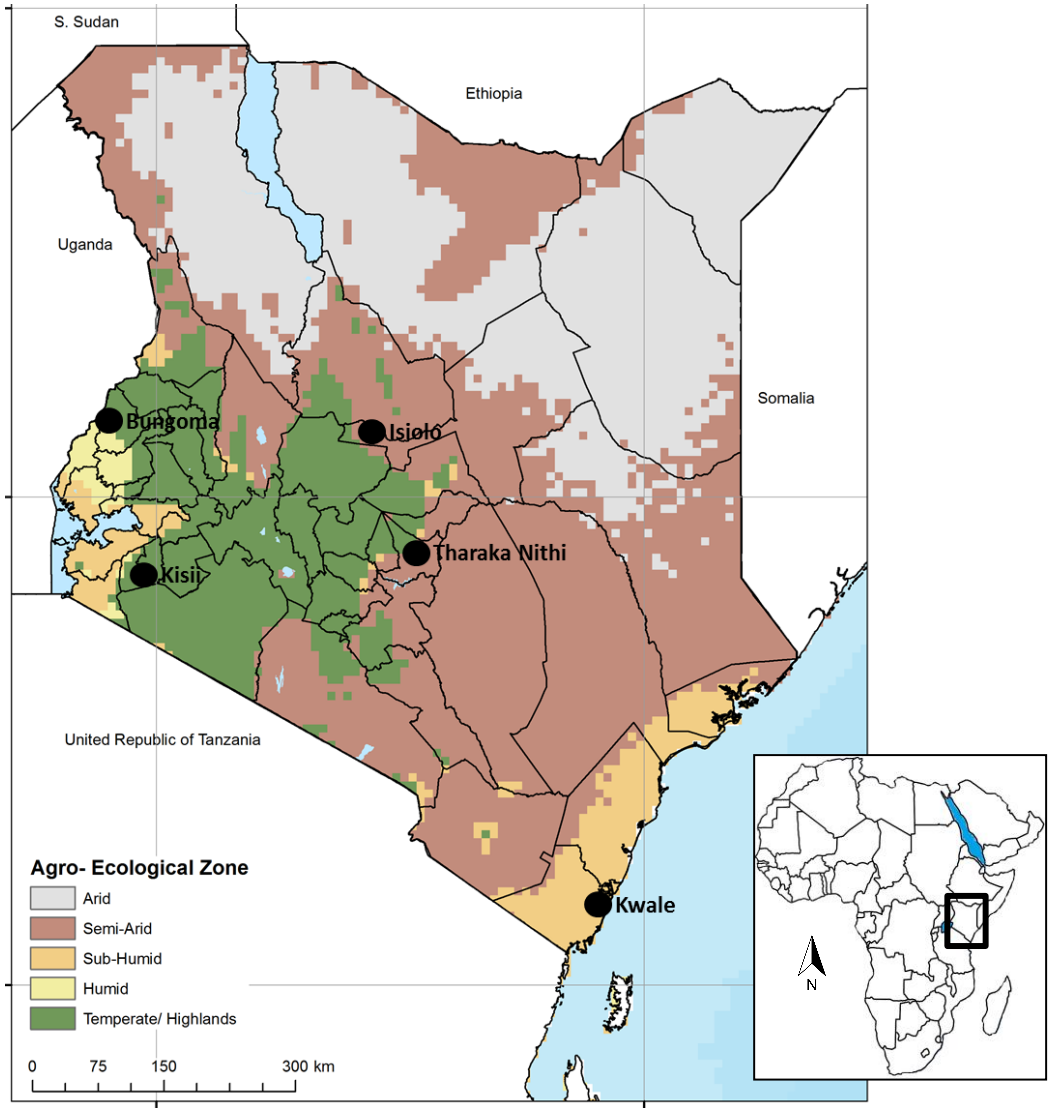

Figure 1: Spatial distribution of study sites according to agro-ecological zones in Kenya (IIASA/FAO 2012; Map produced by ILRI 2013)

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Sample size calculation

The number of households to be sampled was calculated based on an expected positive proportion of 72%, at the 95% confidence level with a 10% desired level of precision [14, 15]. A design effect of 0.05 and 0.2 was applied to account for clustering at sub-location and village level, respectively. This increased the sample size to 321 dairy farms. This was distributed equally across the five counties resulting in 64 farmers to sample per county. To achieve a balance between villages and the number of farmers per village, the aim was to sample eight villages per sub-location and within each village, eight farmers. Sampled farmers were those growing cereals and keeping livestock, and they were randomly selected from a sampling frame that included all the farmers who fit the criteria in selected villages. The sampling frame was constructed by the local administrators and extension officers.

Sampling

In order to account for seasonal variation in aflatoxin contamination, sampling was carried out in two phases. The first phase was done in February and March 2014 and the second in July and October 2014. The first phase sampling took place in the dry season and the second phase in the rainy season. Dry season sampling was expected to coincide with the cereal growing period where there would be household cereals stored for three months or more from the previous season. Rainy season sampling was expected to coincide with the cereal harvest period and, therefore, availability of freshly harvested cereals. Per household, about 500 g each of millet, sorghum and maize intended for human consumption were sampled in brown paper bags. A scoop sterilized in sodium hypochlorite was used to sample. The grain was mixed by stirring and a sample drawn from the top, middle and bottom of the bags. The samples were transported in boxes and stored at 4°C in a cold room. The analysis was carried out at the Department of Public Health, Pharmacology and Toxicology, University of Nairobi and the Biosciences eastern and central Africa–International Livestock Research Institute Hub mycotoxin laboratory, Nairobi.

Aflatoxin analysis

To extract and quantify aflatoxins, samples were ground using Romer Series II Mill (Romer Labs Inc., 1301 Stylemaster Drive Union, MO 63084). Aflatoxin testing was carried out using Helica Low Matrix Aflatoxin B1 competitive enzyme-linked immunosorbent assay (ELISA) kit (Helica Biosystems Inc., Santa Ana, CA - Catalogue Number 981BAFL01LM-96). Aflatoxin B1 was extracted from 5 g of ground samples using 80% acetonitrile following the manufacturer's instructions. Samples that were above the upper standard concentration in a given assay were re-diluted and the assay repeated so that it fell within the lower (1 ppb) and upper (20 ppb) standards.

Statistical analyses

For statistical analyses, R® (version 3.1.3) and Stata® 13 (StataCorp LP, Texas, USA) software were used. The data did not follow a normal distribution, so geometric means as well as arithmetic means were calculated. When calculating geometric means, all non-detectable samples were assigned the value of 1 ppb, which is the lower assay limit. To compare proportions of aflatoxin contamination amongst counties and AEZs, Chi Square Test and Fisher's Exact Test were used. Kruskal–Wallis rank sum test was used in

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analysis of variance of aflatoxin levels amongst counties and AEZs. Wilcoxon sign-rank test was used to compare the level of aflatoxin contamination of the samples collected during the dry and rainy seasons. Significance was reported at 95% confidence interval.

Ethical approval

Ethical approval for the study was acquired from the International Livestock Research Institute (ILRI) (approval number ILRI-IREC2013-09). All participants were informed about the purpose of the study and gave their informed consent to participate before sampling was carried out.

RESULTS

In total, 286 farmer households were visited. Of these, 37 were from Kwale, 56 from Isiolo, 65 from Tharaka-Nithi, 64 from Kisii and 64 from Bungoma. The target of 64 farmers per county was not attained due to a lower number of dairy farmers in the villages than anticipated. In Waa sub-location of Kwale County, only six villages fulfilled the criteria of livestock keeping and in some, less than eight farmers kept dairy cows. In Isiolo County, Lonkopito sub-location had only seven villages, totalling to 56 farmers. From these households, 205 millet, 164 sorghum and 497 maize samples in total were collected and analysed. The samples were generally home grown except in Isiolo (semi-arid) where all the households had market-sourced maize.

Overall, the proportion of positive samples with aflatoxin B1 levels above the Kenya Bureau of Standards limit of 5 ppb was 26% for maize, 10% for millet and 11% for sorghum (Figure 2). Based on Wilcoxon sign-rank test, rainy season sorghum from humid ($p<0.01$) and semi-arid ($p<0.05$) zones had significantly higher aflatoxin contamination levels compared to samples collected during the dry season. Dry season maize from the temperate zone had significantly higher aflatoxin contamination levels compared to rainy season maize ($p<0.05$).

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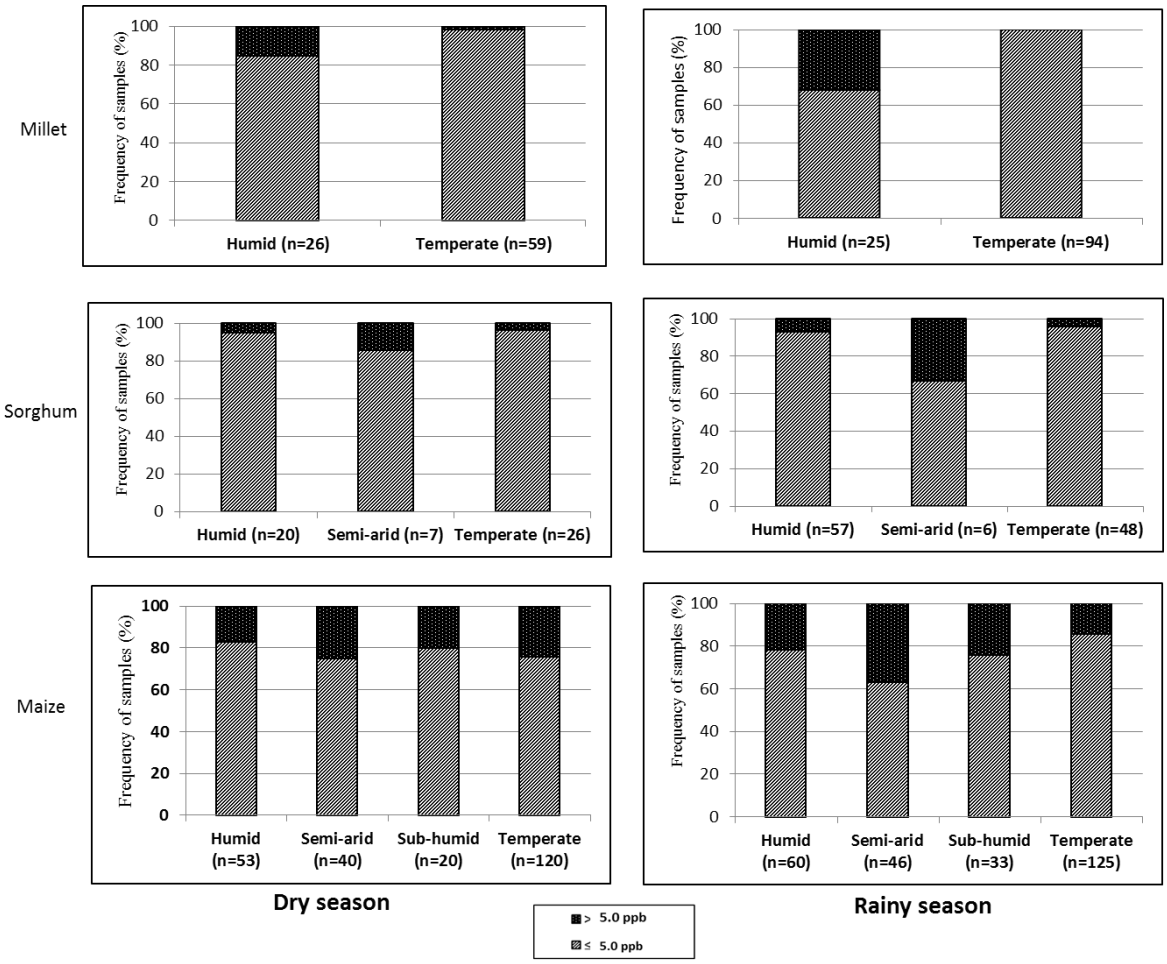

Figure 2: Percentage of samples in the two categories of levels of aflatoxins plotted against agro-ecological zones

Contamination levels of samples collected during the dry season

Mean levels of aflatoxin were not significantly different between AEZs in the samples collected during the dry season. Most samples of millet and sorghum were sourced from temperate and humid zones; the latter zone had the highest proportion of millet samples contaminated above legal limits ($p<0.05$).

Across all AEZs, about 20% of all maize samples had aflatoxin contamination levels above Kenyan legal limits. The proportion of maize contaminated above legal limits did not significantly differ amongst AEZs (Table 1). Millet from the humid zone had a significantly higher proportion testing above the legal limits ($p<0.05$).

Contamination levels of samples collected during the rainy season

Mean levels of aflatoxin were significantly different between AEZs in the samples collected during the rainy season. Tharaka-Nithi, representing the humid zone, was the only county with millet samples testing above the legal limit of 5 ppb and had five times higher mean of aflatoxin in millet compared to counties in the temperate zone (Bungoma and Kisii; $p<0.05$). In sorghum, samples from Isiolo (semi-arid) had the highest mean

level of aflatoxin contamination. Kwale (sub-humid) had the highest contamination of maize (Table 2).

Comparing the proportions of samples with aflatoxin contamination above the 5 ppb limit, maize from the semi-arid region had the highest proportion, humid and sub-humid zones moderate proportion while temperate zone the least. Sorghum from the semi-arid zone had a significantly higher proportion testing above the legal limits ($p<0.05$).

DISCUSSION

This study investigated the occurrence of aflatoxins in millet, sorghum and maize samples from five counties representing four AEZs in Kenya in the rainy and dry seasons. Although the sampling method aimed to collect a representative sample, the very heterogeneous distribution of aflatoxin in grains, challenges in sampling and variability of results can affect the reliability of results. Besides the AEZs, there are other factors not assayed in this study that affect colonization and production of aflatoxins by *Aspergillus* in grains, such as stress of the host plant during planting, different *Aspergillus* strains present in the soils and agricultural practices. The majority of samples in this study were within acceptable limits, although occasionally very high levels were detected; one sample had over 1600 ppb, which is 320 times the legal limit. In both rainy and dry seasons, millet and maize from the humid and sub-humid zones had consistently higher mean levels of aflatoxin than millet and maize from the temperate zone. This finding could be explained by the fact that high humidity favours colonization and production of aflatoxins in grains [16].

Aflatoxin levels in millet were comparable to levels found in Nandi (temperate zone) in 2011-12, when the arithmetic mean was 7.9 ppb and the range 0.1–6.4 ppb [17]. That study reported a much wider range (0.2–210.1 ppb) of sorghum contamination than this study; however, those samples had been obtained from markets, so the studies are not directly comparable [17]. The present study can be compared to one done in Ethiopia that found an arithmetic mean of 1.12 ppb aflatoxin B1 in millet. However, that study reported a much higher mean level (29.5 ppb) of aflatoxin B1 in sorghum [18]. Generally, there are few surveys done in East Africa reporting on aflatoxin contamination in sorghum and millet. The fact that the present study reports proportions with aflatoxins above legal limits shows the need to conduct further surveys and to also consider the role of sorghum and millet in contributing to aflatoxin exposure in East Africa.

In this study, aflatoxin contamination of maize at household level is of concern owing to high levels found. This is consistent with high levels that Okoth and Kola [3] found after testing market maize from Nairobi; only 17% of the maize was fit for human consumption. Maize collected from the sub-humid AEZ in the rainy season had the highest mean level of aflatoxin followed by maize from the semi-arid zone in the dry season. Moreover, rainy season maize from the semi-arid region had the highest proportion testing above legal limits. Isiolo (semi-arid) and Kwale (sub-humid) produce little maize, if any; however, imported grains could contribute to exposure to aflatoxin. Contamination levels could also increase due to poor transport and storage of the grains. Maize collected during the rainy season from Isiolo had almost double the mean aflatoxin

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in maize from Tharaka-Nithi, which is one of the main sources of maize for Isiolo. It is, therefore, important for the government to ensure that traders and millers test cereals before selling them to regions that do not produce, as well as train retailers and farmers on how to store cereals, even if they are not growing them, as contamination could increase during the transport and storage phases [19].

Maize from temperate regions had a relatively low mean contamination as compared to that from the semi-arid and sub-humid zones. This could be attributed to unfavourable climatic conditions for the fungal growth and, to a lesser extent, presence of low-aflatoxin-producing L strains of *Aspergillus* found in their soils [20]. A survey of maize collected from local mills in Bungoma and Kisii found a low proportion of samples contaminated above legal limits, with only 2% and 8%, respectively, exceeding the limit [21], results that were much lower than in the present study. A study within Makueni County, a semi-arid area that has had a previous outbreak of aflatoxicosis, found contamination of slightly more than a quarter (35.5%) of the locally grown maize exceeding legal limits [4]. This is comparable to what the present study found in the semi-arid zone of Isiolo, a similar AEZ.

CONCLUSION

Most samples were safe, according to the Kenyan legal limit of 5 ppb, but a substantial proportion of samples exceeded this limit (26%, 10% and 11% of maize, millet and sorghum, respectively). This is of concern given the grave impact of chronic exposure to aflatoxins on health. Furthermore, there are uncertainties on the actual safety levels, evident from different regulations of mycotoxins in different regions; for example, when maize consumption is very high, standards developed for countries with lower maize consumption may not be appropriate [22]. It is important to focus on the risks for individual consumers from susceptible foods and further study the factors contributing to exposure and health impacts. Risk and economic assessments of aflatoxins on human and animal health will provide this information that is lacking in East Africa.

ACKNOWLEDGEMENTS

This study was a part of the FoodAfrica Programme which is mainly financed by the Ministry for Foreign Affairs of Finland contract no. 29891501 and the CGIAR Research Program on Agriculture for Nutrition and Health. The authors thank the participating villages and sampled households for their cooperation. The authors are grateful to Helica for providing kits at significantly reduced cost than their higher commercial price.

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Table 1: Levels of aflatoxin contamination in samples collected during the dry season

Region	Range (ppb)	Mean (ppb) Arithmetic mean	Mean (ppb) Geometric mean	Percent detected	Percent exceeding 5 ppb
Millet					
Kwale (sub-humid; n=1)	N/A	1.4	1.4	100	0
Tharaka-Nithi (humid;	<1.0 –	66.2	1.3	69.2	15.4
n=26)	1658.2	0.5	0.5	77.1	0
Kisii (temperate; n=35)	<1.0 – 3.0	0.9	0.4	75	4.1
Bungoma (temperate;	<1.0 – 13.8				
n=24)					
Sorghum					
Isiolo (semi-arid; n=7)	<1.0 – 11.9	2.0	1.0	57.1	14.3
Tharaka-Nithi (humid;	<1.0 – 23.1	1.5	0.4	85	5
n=20)	N/A	0.7	0.7	100	0
Kisii (temperate; n=1)	<1.0 – 12.3	0.9	0.4	84	4
Bungoma (temperate;					
n=25)					
Maize					
Kwale (sub-humid;	<1.0 – 19.2	3.5	2.1	95	20
n=20)	<1.0 –	67.3	2.7	50	25
Isiolo (semi-arid; n=40)	1137.4	23.9	1.3	75.4	17
Tharaka-Nithi (humid;	<1.0 – 774.7	8.9	1.6	77.8	25.4
n=53)	<1.0 – 371.5	3.5	1.3	71.9	22.8
Kisii (temperate; n=63)	<1.0 – 39.3				
Bungoma (temperate;					
n=57)					

N/A: not applicable

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Table 2: Levels of aflatoxin contamination in samples collected during rainy season

Region	Range (ppb)	Mean (ppb) arithmetic mean	Mean (ppb) geometric mean	Percent detected	Percent exceeding 5 ppb
Millet					
Tharaka-Nithi (humid; n=25)	<1.0 – 152.3	10.9	2.3	64	32
Kisii (temperate; n=52)	<1.0 – 3.0	0.1	0.8	21.1	0
Bungoma (temperate; n=42)	<1.0 – 2.9	0.6	0.5	97.6	0
Sorghum					
Isiolo (semi-arid; n=6)	<1.0 – 12.8	3.8	1.5	100	33.3
Tharaka-Nithi (humid; n=57)	<1.0 – 17.9	1.2	1.0	33.3	7
Kisii (temperate; n=19)	<1.0 – 16.4	0.9	1.1	10.5	5
Bungoma (temperate; n=29)	<1.0 – 91.7	3.5	0.4	96.5	3.4
Maize					
Kwale (sub-humid; n=33)	<1.0 – 394.1	28.9	2.8	97	24.2
Isiolo (semi-arid; n=46)	<1.0 – 120.7	9.6	2.2	97.8	37
Tharaka-Nithi (humid; n=60)	<1.0 – 536.8	23.6	1.4	88.3	21.7
Kisii (temperate; n=62)	<1.0 – 102.6	4.0	1.3	46.8	17.7
Bungoma (temperate; n=63)	<1.0 – 217.6	7.9	0.8	81	11.1

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