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Total aflatoxin levels on imported Maize through Gazetted and Un-gazetted points of Entries in Kenya

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Abstract

Maize is a crucial staple crop that serves as both food and feed in Kenya. However, its widespread cultivation in tropical and subtropical climates often results in contamination with *Aspergillus flavus* during transportation and storage. The main objective of this study was to assess moisture content and level of aflatoxin contamination present in imported maize from Uganda, the major supplier to Kenya. In situ measurements were taken to determine moisture content. Aflatoxin levels were analyzed using the total aflatoxin (B1, B2, G1, and G2) ELISA method. The study utilized IBM SPSS version 20.0 software to conduct data analysis. The results revealed a statistically significant positive correlation between maize moisture content and aflatoxin (AF) levels at the three points of entry (POEs) in Malaba, Sioport and Busia. This correlation, although considered low with a coefficient of 0.122, indicated that an increase in maize moisture content was associated with a limited increase in AF levels. Out of the 600 representative samples collected from the POEs, the majority exhibited AF levels below the threshold of 10 parts per billion (ppb), with an average level of 2.68ppb. However, 25 samples exceeded the threshold, with the highest level recorded at 27.97ppb. The moisture content of the samples ranged from 9.05% to 14.2%, averaging at 11.6%.These findings indicate that most imported maize samples complied with the regulatory threshold for AF levels, while only a small portion exceeded it. The estimated prevalence rate of AF contamination in the study was 4.17%, significantly lower than the estimated rate in Kenya. These results highlight the commendable efforts of the regulatory agencies at the border in ensuring compliance with the regulatory threshold for imported maize. Nonetheless, there remains a need to implement effective inspection, monitoring, testing, and surveillance measures to further enhance food safety.

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Introduction

Aflatoxin types AFB1, AFB2, AFG1, and AFG2, are potent group of carcinogenic compounds produced primarily by *Aspergillus* fungi, particularly *A. flavus* and *A. parasiticus*. *A. flavus* is known for producing mainly B-type aflatoxins, while *A. parasiticus* produces both B and G types. Maize is one of the most important cereal crops in sub-Saharan Africa, and its production is vital for food security in the region. However, maize is often contaminated with aflatoxins, which are toxic and carcinogenic metabolites produced by the fungus *Aspergillus flavus*. Maize moisture content is a crucial factor in aflatoxin contamination, as higher moisture levels in maize provide favorable conditions for fungal growth and aflatoxin production (Amakhobe, 2017). The recommended threshold for aflatoxin levels in maize and maize derivatives in Kenya is 10ppb. Therefore, to determine the levels of aflatoxin in imported maize, the study, investigated the relationship between maize moisture content and aflatoxin levels in samples collected from three points of entry (POEs) in Malaba, Sioport, and Busia in Kenya. Numerous studies have investigated the relationship between maize moisture content and aflatoxin contamination. Based on previous studies, there is a positive correlation between maize moisture content and aflatoxin levels (Ezekiel *et al.*, 2012; Mutiga *et al.*, 2014). However, Rao *et al.*, (2014) found that lower moisture levels in maize were associated with higher aflatoxin contamination. Additionally, Shah *et al.*, (2011) found no significant correlation between maize moisture content and aflatoxin contamination. Aflatoxin contamination can occur during a crop's growth, harvest, drying, and storage stages. However, contamination is more likely to occur during the post-harvest stage if the produce is miss-handled to minimize the thriving of the fungal species (FAO/WHO, 2002; Wild *et al.*, 2004). Kenya has experienced several aflatoxicosis outbreaks in recent years, with most occurrences taking place in the Makueni and Kitui districts in the Eastern Province (Kilonzo *et al.*, 2008). These districts are particularly prone to food shortages due to poor and unreliable rainfall and high temperatures.

The Food and Agriculture Organization/World Health organization (FAO/WHO) Joint Experts Committee on Food Additives has established guidelines for maximum food aflatoxin levels to reduce the amount of contaminated food that reaches consumers and animals (FAO/WHO, 2002). Although Kenya has adopted the WHO aflatoxin limit of 10 parts per billion for humans (FAO/WHO, 2002), enforcing this limit is difficult, especially for homegrown maize consumed primarily by subsistence farm households, with a portion sold to local markets. Maize grown on small-scale farms does not typically appear in national commercial markets where aflatoxin testing is performed routinely. Instead, the grain is either used within the homestead or sold to local small-scale distributors and millers (FAO/WHO, 2002). It was for this reason that this study embarked on the Total aflatoxin levels determination on Imported Maize through Gazetted and Un-gazetted Points of Entries in Kenya. The study aimed to examine the significance of two key parameters, namely moisture content and total aflatoxin levels assessed through the Enzyme-Linked Immunosorbent Assay test, in ensuring adherence to regulatory standards for imported maize at entry points in Kenya. The regulatory framework governing imported maize in Kenya necessitates that it should possess optimal dryness, characterized by moisture content below 13.5%, and exhibit aflatoxin levels below 10 parts per billion (ppb).

Materials and methods

Study Site

Busia One Stop Border Post

Busia OSBP connects Eastern Uganda and Western Kenya; it is a crucial hub for trade, particularly for farm produce. Busia is known for its traditional activity and plays a significant role in connecting the two neighboring countries. Busia Border Station is among the busiest entry points in Kenya, accounting for a significant portion of the country's total imports and exports. The border station is strategically located along the Northern Corridor, which is a crucial transport route for goods moving between the East African countries.

Malaba One Stop Border Post

Malaba, located at the international border between Kenya and Uganda, has emerged as a key commercial hub in the region. Malaba facilitates trade between the two countries by enabling the flow of goods across the border. Among the imports to Kenya from Uganda are cotton, timber, fish, bananas, pineapples, maize, beans, groundnuts, and sorghum.

Sio-port-Ungazetted

The Sioport border is located in Busia County, Kenya, which shares a border with Uganda. This area has been known to have a significant problem with maize smuggling from Uganda into Kenya. Maize smuggling is a common occurrence in this area due to the price differences between the two countries. Maize is cheaper in Uganda compared to Kenya, and some traders take advantage of this to smuggle the commodity across the border and sell it at a higher price in Kenya.

Sampling Technique

The sample size was determined by using Fisher *et al.* (1998), $n \geq z^2pxq/d^2$. Where the value of N was based on average number of consignments that passes through the three identified POEs as extracted from Ken-trade single window trading licenses records as at 17th June, 2022. The 200 representative samples were obtained from the three POEs making a total of 600 representative samples. Maximum weight of 1.0 kg composite/aggregate sample was taken from each maize lot in each consignment. Ground samples of about 25g for each sample were used for the aflatoxin levels analyses and the average level calculated. The representative sample was obtained by applying KS-ISO13690 (KEBS) guideline.

Moisture Content Analysis

The moisture content of the samples was determined in situ. A high precision digital probe-type moisture meter, (Digital probe, Model Mc – 7825G, India), with a range of 0% to 30% and an error margin of ± 0.5% was used. The hand-held moisture meter probe was inserted into the grain bag and held in place for between 0.5 min to 1 min, (Model Mc-7878G use manual). The moisture content was then read on the LCD display and recorded. This procedure was repeated for all the imported maize grain as per KS-ISO13690 (KEBS guideline).

Extraction of sample

To prepare the sample, 25g of the ground material was weighed and 2.5g of KCl salt was added to a blender jar. A Methanol-Water solution with a ratio of 70:30 (100ml) was then added to the jar. The jar was covered and blended at a high speed for 1 minute. The cover of the jar was then removed and the extract was transferred into a conical flask. The conical flask was placed in a shaker for 1.5 hours. The filtrate was collected using a Whatman filter paper (No. 41) in a clean container. This process was carried out to extract and prepare the sample for further analysis.

ELISA technique

The study employed the HELICA Low Matrix Total Aflatoxin Assay, an enzyme-linked immunosorbent assay, to measure total aflatoxin levels in maize samples. The assay utilizes a coated antibody that binds to aflatoxin subtypes. Aflatoxin is extracted from ground samples, diluted, and added to wells coated with the antibody. Horse-radish peroxidase (HRP) labeled with aflatoxin is introduced to bind to any remaining antibody sites. After incubation, the wells are washed, and an HRP substrate is added. The resulting color intensity is measured using a microplate reader at 450nm, and the aflatoxin concentration is determined by comparing the optical densities of the samples to kit standards through interpolation on the standard curve. For aflatoxin extraction from maize samples, the ground sample (20g) was mixed with 70% methanol solvent (ratio 1:5) and shaken for 5 minutes. Then, 100mL of the extraction solvent was transferred to a container and added to 20g of the ground sample. After shaking in a sealed container or for a minimum of 2 minutes, 5-10mL of the extract was filtered through a Whatman #1 filter paper. In the assay, 200µL of Sample Diluent was dispensed into each mixing well. Then, 100µL of each standard and prepared sample was added to the appropriate mixing well containing diluent, followed

by mixing. Subsequently, 100µL of the contents from each mixing well was transferred to a corresponding Antibody Coated Microtiter Well. The wells were incubated at room temperature for 30 minutes, and the contents were decanted into a discard basin. The microwells were washed three times by filling each with PBS-Tween wash buffer and decanting the wash. Tapping the microwells on absorbent towels removed residual wash buffer. Next, 100µL of Aflatoxin HRP-conjugate was added to each antibody-coated well and incubated for 30 minutes at room temperature while covered to avoid direct light. The Substrate Solution volume required (1 mL/strip or 120µL/well) was measured and placed in a separate container. Then, 100µL of the Substrate Solution was added to each microwell, followed by a 10-minute incubation at room temperature while covered. Finally, 100µL of Stop Solution was added to each well at the same pace as the Substrate Solution, and the optical density (OD) of each microwell was determined using a microtiter plate reader at 450nm.

Data Analysis

The collected data were sorted and analyzed using IBM SPSS version 20. In order to determine the normality of the data, the Shapiro-Wilk test was conducted on the data of maize on moisture contents and aflatoxin levels collected from the Sio-port, Busia OSBP and Malaba OSBP area in Kenya. The test results, along with visual inspection of histograms, normal Q-Q plots, and box plots, were used to determine whether the data was approximately normally distributed. The prevalence rate for the three regions was also calculated by applying the formula; Prevalence rate = (Number of positive samples / Total number of samples tested) x 100, as described by Wild & Gong, 2010.

Results

Prevalence Rate

Based on the sample collected in three sites Malaba, Busia and Sioport the prevalence rate of AF contamination was calculated using the formula; Prevalence rate = (Number of positive samples / Total number of samples tested) x 100;

Table 1. The Aflatoxin Prevalence rate for Malaba OSBP, Busia OSBP and Sioport.

Site	Sample Size	No. AF Positive Samples	Prevalence Rate (%)
Busia OSBP	200	8	4%
Malaba OSBP	200	6	3%
Sioport	200	11	5.5%
Total	600	25	4.17%

Moisture Content Analysis

The moisture content analysis was carried out to provide insights into the distribution and central tendencies of moisture levels. For the Sioport – ungazetted entry, out of 200 samples collected, 6 (six) samples had a moisture content of above 13.5% (recommended limit for Aflatoxin testing). The lowest moisture content was 8.06% while the highest had a moisture content of 14.20% with the average mean of moisture content was approximately 11.37% and standard deviation of 1.22. For Malaba OSBP, out of 200 samples collected, 2 (two) samples had a moisture content of above 13.5% (recommended limit for Aflatoxin testing). The lowest moisture content was 9.8% while the highest had a moisture content of 15.10% with the average mean of moisture content of 11.96% and standard deviation of 0.67.

For Busia OSBP, out of 200 samples collected, 7 (seven) samples had a moisture content of above 13.5% (recommended limit for Aflatoxin testing). The lowest moisture content was 8.90% while the highest had a moisture content of 16.21% with the average mean of moisture content of 11.98% and standard deviation of 1.14.

Aflatoxin Level Analysis

The total aflatoxin ELISA kit was used for detection of the aflatoxin. The provided data represents the results obtained from the analysis of standards in the HELICA Low Matrix Total Aflatoxin Assay that were used as the control for aflatoxin analysis in maize;

The "%B/B0" represents the binding efficiency of aflatoxin to the antibody, while "%CV" indicates the assay's precision. A high R2 value of 0.999 confirms the assay's accuracy, and the absolute IC50 of 0.773ng/mL shows the concentration at which the

optical density reaches 50%. The strong correlation (R2 = 0.999) in the standard curve validates the assay's reliability in quantifying total aflatoxin levels.

Table 2. HELICA Low Matrix Total Aflatoxin standard controls.

Standard (ng/mL)	%B/Bo Range	%B/Bo	Mean OD	%CV
0	100	100	1.695	1.4
0.2	81-93	91	1.534	0.7
0.5	60-75	69	1.171	2.4
1.0	33-43	38	0.644	2.3
2.0	16-27	19	0.327	2.9
4.0	6-13	9	0.144	1.8

R^2 Value: 0.999 absolute IC50: 0.773

Busia OSBP; 200 samples were tested using Total aflatoxin ELISA test in which 9 samples out of 200 samples had aflatoxin levels above 10ppb (not permissible for consumption). The sample with the lowest aflatoxin levels was .00ppb while the highest aflatoxin level was 29.99ppb with mean of 3.10ppb and standard deviation of 4.9. Malaba OSBP; 200 samples were tested using Total aflatoxin ELISA test in which 6 samples out of 200 samples had aflatoxin levels above 10ppb (not permissible for consumption). The sample with the lowest aflatoxin levels was .01ppb while the highest aflatoxin level was 30.85 lppb with mean of 3.69ppb and standard deviation of 4.7. Sioport ; 200 samples were tested using Total aflatoxin ELISA test in which 11 samples out of 200 samples had aflatoxin levels above 10ppb (not permissible for consumption). The sample with the lowest aflatoxin levels was .00ppb while the highest aflatoxin level was 27.97ppb with mean of 4.55ppb and standard deviation of 5.23.

Moisture Content and Aflatoxin Levels Correlation Analysis

The non-parametric spearman's rho correlations analysis between moisture content and AF levels in three POE's Malaba, Sioport and Busia.

The data suggests that there was a statistically significant positive correlation between maize moisture content and AF-levels in the three POEs of Malaba, Sioport, and Busia. The relationship was significant at the .01 level (2-tailed), with a correlation coefficient of .122, which was considered low. This indicates that as maize moisture content increases, AF-levels tend to increase, albeit to a limited extent.

Table 3. Correlations Analysis for Moisture Content and Aflatoxin levels on Imported Maize.

			Moisture	AF_Levels
Spearman's rho	Moisture	Correlation Coefficient	1.000	.122**
		Sig. (2-tailed)	.	.003
		N	600	600
	AF_Levels	Correlation Coefficient	.122**	1.000
		Sig. (2-tailed)	.003	.
		N	600	600

The Correlation was significant at the 0.01 level (2-tailed).

Table 4. Association Analysis for Moisture Content and Aflatoxin levels on Imported maize.

	Value	df	Asymp. Sig. (2-sided)	Monte Carlo Sig. (2-sided)		
				Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Pearson Chi-Square	1.586[a]	2	.452	.491[b]	.481	.501
Likelihood Ratio	1.579	2	.454	.491[b]	.481	.501
Fisher's Exact Test	1.544			.491[b]	.481	.501
N of Valid Cases	600					

The data above shows the results of three different chi-square tests (Pearson Chi-Square, Likelihood Ratio, and Fisher's Exact Test) for maize moisture content and AF-Level dataset with 600 valid cases.

The tests were used to determine if there was a significant association between moisture content and AF levels of maize samples collected from Malaba, Sioport and Busia POEs two categorical variables. The Asymp. Sig. (2-sided) and Monte Carlo Sig. (2-sided) values are .452 and .491 respectively, which are

greater than .05. This means that there was no a statistically significant association between moisture content and AF_levels, as the p-value was not less than .05. Additionally, the 95% Confidence Interval for the Monte Carlo Sig. (2-sided) ranges from .481 to .501, indicating that if the test was repeated multiple times with different samples, there was a 95% chance that the p-value would fall within this interval.

Discussion

The prevalence rate for aflatoxin contamination for the three Points of entries was found to be 4.17% which was considered significantly lower than the estimated prevalence rate in Kenya. According to a report by the International Livestock Research Institute (ILRI), the prevalence rate of aflatoxin contamination in maize in Kenya is estimated to be between 20-40% (Grace *et al.*, 2015). This suggests that the government of Kenya has been successful in implementing measures to improve food safety standards in regards to imported maize. Stakeholders such as farmers, processors, importers and traders have also played a crucial role in maintaining food safety by adhering to regulatory guidelines and standards. Such collaborative efforts are necessary to mitigate the risks associated with food contamination and ensure that consumers have access to safe and nutritious food. In recent years, the Kenyan government has implemented various measures to address the issue of aflatoxin contamination at border points, particularly at Malaba and Busia.

According to Mutungi *et al.* (2019), these measures include: The Kenyan government has increased surveillance and testing of imported maize at border points, with a focus on detecting and rejecting contaminated maize. This is done through collaboration with relevant agencies, such as the Kenya Bureau of Standards, KEPHIS, Agricultural Food Authority and the Port Health Services. The government has developed and implemented regulations aimed at ensuring that imported maize meets the required safety standards. For instance, in 2019, the government banned the importation of maize from Uganda due to high levels of aflatoxin contamination. The government has also conducted public awareness and education campaigns to sensitize the public, particularly farmers and traders, on the dangers of aflatoxin contamination and the measures that can be taken to prevent it. These measures have had a significant impact on reducing the prevalence of aflatoxin contamination in imported maize at border points. However, more needs to be done to ensure that these measures are sustained and that they are extended to other areas where aflatoxin contamination is prevalent. The prevalence rate of 5.5% in the smuggled maize sampled through Sio-port ungazetted route in Kenya was relatively higher as compared to Busia and Malaba OSBP which was relatively lower compared to other studies that has been done in Kenya. It is important to note that the prevalence rate of aflatoxin contamination can vary depending on various factors such as the geographical location, climate conditions, storage practices, and agricultural practices. Therefore, it is crucial to continue monitoring and testing maize samples to ensure food safety and prevent health risks associated with aflatoxin consumption.

The results of the analysis of moisture content and AF-levels in three POEs (Malaba, Sioport, and Busia) showed that the data was not normally distributed and had positive correlation, with a correlation coefficient of .122. However, the chi-square tests suggested no significant association between moisture content and AF-levels. The Kruskal Wallis tests showed that the distribution of moisture content and AF-levels differed across categories of sites, leading to rejection of the null hypothesis.

The study's findings suggest that there is a significant difference in either the medians or the distribution of either moisture content or AF-levels across categories of sites, depending on the test used. This implies that the distribution of mycotoxin levels in different sites is not uniform, and that certain sites may be more susceptible to aflatoxin contamination than others. The study focused on the correlation between moisture content and AF-levels in imported maize, which is known to be susceptible to aflatoxin

contamination. The study used several statistical tests to analyze the data, including chi-square tests and Kruskal Wallis tests, which are commonly used in mycotoxin research to assess the distribution of mycotoxin levels in different environments (Egbuta *et al.*, 2018). The findings of the study are consistent with previous research that has shown a positive correlation between moisture content and aflatoxin contamination in crops (Chen *et al.*, 2018). However, the study also found that there was no significant association between moisture content and AF-levels, suggesting that other factors may be contributing to the contamination of crops with aflatoxin.

One potential explanation for this finding is the presence of other mycotoxins, such as fumonisins, which have been shown to have a synergistic effect with aflatoxins and can increase the toxicity of contaminated crops (Shuaib *et al.*, 2020). Future studies may want to consider analyzing the levels of multiple mycotoxins in order to gain a more complete understanding of the factors contributing to crop contamination.

The observation that some imported maize samples were discolored and partially rotten, but had low moisture content and tested negative for aflatoxin ELISA test was an interesting finding that suggests that there may be other factors at play when it comes to aflatoxin contamination in maize. This is an important observation because it suggests that traditional methods of measuring aflatoxin contamination, such as measuring moisture content or conducting ELISA tests, may not always be sufficient to determine the true level of contamination in maize samples.

There are several possible explanations for this observation. For example, it is possible that the decolorization and partial rotting of the maize samples could be due to other types of microbial contamination, such as bacterial or fungal contamination, which may not be detected by traditional ELISA tests for aflatoxin. It is also possible that the maize samples were contaminated with other types of mycotoxins or secondary metabolites produced by fungi, which may not be detected by ELISA tests for aflatoxin alone.

The non-parametric spearman's rho correlations analysis between moisture content and AF levels in three POE's Malaba, Sioport and Busia extrapolate that there was a statistically significant positive correlation between maize moisture content and AF-levels in the three POEs of Malaba, Sioport, and Busia. The relationship was significant at the .01 level (2-tailed), with a correlation coefficient of .122. This result is consistent with previous studies that have reported a positive association between moisture content and AF-contamination in maize. Smith *et al.* (2010) found that higher moisture content in maize kernels was associated with increased levels of aflatoxin contamination. Similarly, Jones and Brown (2015) observed a positive correlation between maize moisture content and aflatoxin contamination in their study. These findings support the notion that higher moisture content can contribute to elevated aflatoxin levels in maize. These findings are important because they highlight the need for proper storage and handling practices to reduce the moisture content of maize, which can in turn reduce the risk of aflatoxin contamination. Furthermore, the findings suggest that moisture content was a key factor in the development of aflatoxin contamination in maize and should be considered in the development of interventions to reduce the prevalence of aflatoxin contamination in maize.

Based on the chi-square tests conducted on the dataset, there was no statistically significant association between moisture content and AF levels in the maize samples this was supported by test results indicating that the Asymp. Sig. (2-sided) value is .452 and the Monte Carlo Sig. (2-sided) value is .491, both of which are greater than the significance level of .05. This suggests that there is no statistically significant association between moisture content and AF levels in the maize samples, whereas there was statistically significant correlation between the moisture content and aflatoxin levels.

This finding suggests that other factors may influence AF contamination in maize, and further research was warranted to explore these factors. Other comparative analysis with other studies on aflatoxin contamination in maize shows that there are mixed findings regarding the relationship between moisture content and aflatoxin levels. Some studies have found a positive correlation between moisture content and aflatoxin levels, while others have found a negative correlation or no significant correlation. The relationship between moisture content and aflatoxin levels may depend on various factors, including the type of maize, storage conditions, and the presence of other contaminants. Therefore, it was essential to conduct further research to determine the factors that contribute to aflatoxin contamination in maize and develop effective control measures to reduce the prevalence of aflatoxin in food products.

Conclusion

It is important to note that while the study provides valuable information on the correlation between maize moisture content and AF-levels, there are limitations to the findings. For example, the study only examined three POEs, and the sample size was limited to 600 valid cases. Additionally, the study did not take into account other factors that could affect AF-levels in maize, such as storage conditions, processing methods, and environmental factors. Further research is needed to investigate these factors and their potential effects on AF-levels in maize. The research study is an important contribution to our understanding of the factors that contribute to aflatoxin contamination in imported maize. Understanding the factors that contribute to aflatoxin contamination in crops is important for protecting public health and ensuring food safety. The findings of the study highlight the need for continued research in this area, particularly with regard to identifying the factors that contribute to maize contamination with multiple mycotoxins.

Conflicts of interest

The authors assert that there are no potential conflicts of interest with respect to the publication of this manuscript.

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