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The Long-Term Influence of Agricultural Training on Aflatoxin Levels in Selected Maize Farms in Nandi County, Kenya

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ABSTRACT

Purpose: The purpose of the study was to evaluate the long-term influence of the training on aflatoxin levels.

Methodology: In this study a comparison in aflatoxin levels was made between farms where training had been done against those that were not. The samples from 30 farms where training had been done and 30 other farms where training was not done were analyzed.

Findings: The overall mean total aflatoxin levels were 0.5464 ±0.16124 and 1.1034 ±2.4849 ppb respectively. Despite the low mean total aflatoxin levels, the analysis of the aflatoxin levels between farms where training had been done and farms where training was not done, showed no significant difference with p >0.05. One of the maize samples out of the 60 farms analyzed had a total aflatoxin level of 14.23ppb. There were also numerous samples whose moisture content exceeded the regulatory limits. Additionally, population dynamic and change in attitude had influenced the six year-long term impact of the training. A further comparison between agricultural training and most of the detoxifying methods in use, gives preference to the training over routine decontamination methods as a preventive measure. Therefore, other than wait to detoxify the contaminated maize using the costly methods which hardly remove all the toxins, regular farm trainings are recommended, precisely every planting season to improve consistence in Good Agricultural Practice application and hence aflatoxin mitigation.

Unique Contribution to Theory and Policy: This study comprises of the theory of planned behavior, which takes into consideration the quick farm income obtained from tea farming that changed the attitude of some farmers.

Keywords: *Aflatoxin Levels, Agricultural Training, Population Dynamics, Maize, Decontamination Methods*

JEL Codes: *C44, E27, N57, O13, Q01, Q18*

INTRODUCTION

Maize is known to be the most widely produced and consumed staple food by majority of households in Kenya with about 90% of the households depending on it (Koskei *et al*., 2022a; Ouma & De Groote, 2018). It has also been estimated that an African adult consumes about 400g of maize-based foods per day, compared to less than 10g per day in developed countries. Moving forward, trends project that production and use of this commodity shall continue to grow (FAO, 2019). The fact that various fungal strains produce aflatoxins that camouflage in maize and maize products into animal and animals' products which serve as sources of food, pose a potential health risk. This increases the prevalence of aflatoxins in maize farming zones.

There has been a high prevalence of aflatoxin infections in maize supply chain especially during farming operations as reported by the Council for agricultural science and technology on interpretive summary of mycotoxins (CAST, 2019). Aflatoxins pose a major health concern to both human and animals (Kumar *et al*., 2017). This include the various aflatoxicoses outbreaks that were experienced in Kenya which led into fatal cases as evidenced in the numerous outbreaks in the year 2004/2006 (Omara *et al*., 2021a). The outcome of these cases were acute toxicity, immunosuppression, mutagenicity, teratogenicity and formation of carcinogenic compounds in man and livestock (Dhakal *et al*., 2024). Presence of aflatoxins in maize has become a setback on trade and general economy which limits value addition through manufacturing of contaminated foodstuffs and feed from maize as well as develop the irreversible health effects in man and livestock (Ezekiel *et al*., 2019). Training on Good agricultural practices has been known to prevent aflatoxin contamination and hence lower the levels of aflatoxins (Marete *et al*., 2019). A good example is in a case study done in the year 2017 in Nandi County, Kenya where total aflatoxin was evaluated before and after the training. A reduction in the mean aflatoxin levels from 1.918 to 1.301 ppb confirmed that the training in deed had an influence (Marete *et al*., 2019). However, various climatic, environmental and the socio-economic factors remain the limiting factors in predicting how frequent these trainings ought to be to continuously control the levels of aflatoxins. On the other hand, most of the physical and chemical decontamination methods only removes part and not all the toxins. They are also not ecofriendly (Colović *et al*., 2019). As a result, prevention of mycotoxins production is the only approach that is suitable to reduce aflatoxin contamination in food and animal feeds (Bulent *et al*., 2006)

MATERIAL AND METHODS

Study Area

This study is a follow up on what was carried out by Marete and others in the year 2017 in Nandi County, Kenya. In this study, mean total aflatoxin levels was determined before and after the training for 82 samples. However, in the follow up study, a random selection of 30 farmers who

had been trained in the previous study and whose maize sample had also been collected for analysis in Marete et al study was recruited (Marete *et al*., 2019). Thirty other untrained farmers in the neighborhood, were considered as part of the study. Maize samples from these households were collected for aflatoxin analysis. The study regions included Kaptumo (n=20), Kipkaren (n=20) and Kilibwoni (n=20), see figure 1. The three regions had been further subdivided each into two subregions; Mwangaza (n=10) and Keteba (n=10), Sobetab Gaa (n=10) and Toret Gaa (n=10) and Kisob Katanin (n=10) and Toletany (n=10), respectively.

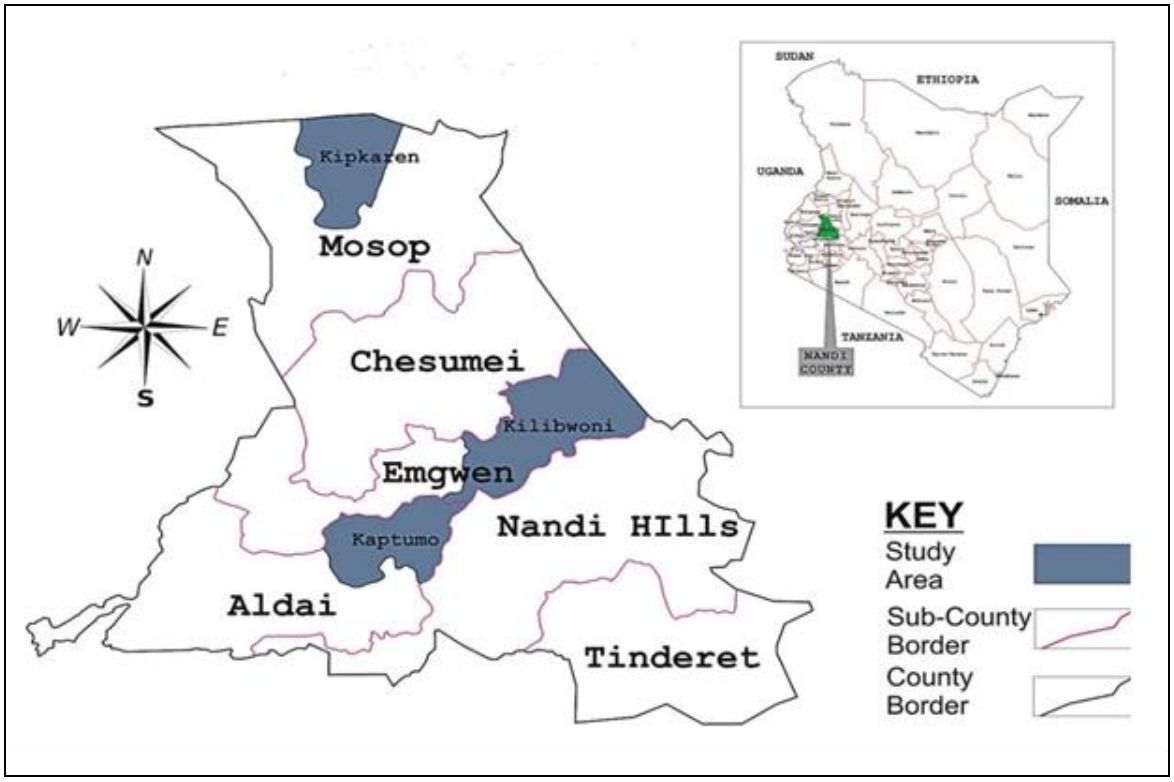

Figure 1*: Map of Kenya showing locations of farms selected for the study (Adopted from Marete et al., 2019)*

Ethical approval

This study sought an approval from the Animal Use, Biosafety and Ethics Committee and also from the National Commission for Science Technology and Innovation (NACOSTI) with a License No. NACOSTI/P/24/35010 to conduct research in Nandi County, Kenya

Reconnaissance

Before the main study a pre-visit was carried out to introduce the study to the locals and also identify the trained farmers from the previous cohort.

Study design and sample size determination

During a pre-visit conducted before the main study, it was established that out of 82 trained and interviewed farmers in Marete et al study, only 45 farmers could be found in their original locations, with their maize farming pieces of land intact. See Table 1. The rest had either sold, leased out, transferred the pieces of land through inheritance or passed on due to covid 19. Additionally, out of these 45 trained farmers only 33 of these had maintained the maize farming system while the 12 trained farmers had already changed completely to tea farming with claims that it attracted more income than maize farming. Therefore, to avoid being bias, five trained farmers were sampled in each and every substation within the study area. As a result, the sample size (n) for each region included equal number of both trained farmers and untrained farmers. That is, for every farmer who had been trained, an untrained farmer was picked from an adjacent farm to make up a control cohort.

Table 1: The influence of population dynamics and change in attitude in the study area

No	Farm Location	Substation	Sample size (n) used by Marete *et al* in the year 2017	Sample size (n) obtained during reconnaissance study	Sample size (n) used in the year 2023
1	KAPTUMO	MWANGAZA	16	6	5
2		KETEBA	20	7	5
3	KIPKAREN	SOBETAB GAA	15	5	5
4		TORET GAA	10	5	5
5	KILIBWON	KATANIN	12	5	5
6		TOLETANY	9	5	5
	Farmers Interviewed and whose samples taken for analysis		**82**	**33**	**30**

This study therefore sought to evaluate the long-term influence of the training by comparing the aflatoxin levels of the 30 farms where training was conducted in the year 2017 against 30 other farms where training had not been done by the year 2023.

Sample collection

250grams of maize sample was collected from each farmer by the help of a scooping spoon that picked from the sides and both upper and lower parts of the sacks that had maize. Moisture content for each sample was immediately taken by use of moisture content meter. The samples were then transferred and sealed into sterile khaki bags. Each of the bags was labelled with a unique code for identification. The sealed bags were transported for aflatoxin analysis in the mycotoxin laboratory, Department of Public Health, Pharmacology and Toxicology, University of Nairobi. While awaiting analysis, they were stored away from light, heat and moisture (Marete *et al*., 2019).

Sample preparation

A grinder was used to grind the sample of maize into a fine powder after which 5 grams of each ground sample was weighed and placed in a falcon tube where 25ml of 70% methanol was added giving a ratio of 1:5 (w/v). This was mixed thoroughly by shaking using a voltex or shaker in a sealed container at room temperature for a minimum of 2 minutes. This particulate matter was allowed to settle for 5 minutes then filtered 5-10 ml off the extract through whatman No.1 filter paper. The filtrate collected was now ready for testing (Helica, 2023).

Total aflatoxin Determination

Aflatoxin levels in both the standard dilutions and prepared samples were carried out in the mycotoxin laboratory, using a competitive enzyme-linked immuno-sorbent assay (ELISA) kit of Product Number-KIT5007 (941AFL01M-96) in duplicates with the following test procedure as outlined by the manufacturer test instructions.

Test Procedure

The reagents were brought to room temperature one hour prior to use. The PBS-Tween powder was reconstituted to wash buffer. Dilution wells were placed in micro well holders for each of the sample and standard that was to be tested. The concentrations of the standards: 4.0, 2.0, 1.0, 0.5, 0.2 and 0.0 ng/mL were also mixed in 70% methanol. An equivalent number of antibody-coated micro titer wells were placed in another microwell holder. Each reagent was mixed by swirling before use. 200µL of the Aflatoxin-Horse Reddish Peroxidase conjugate was dispensed into each dilution well. 100 µL of the standard and test samples were added to the appropriate mixing well that contained the conjugate using pippete tips. The mixing was done by priming a pipettor three times before the microtiter plate was incubated at room temperature for 20 minutes. An appropriate wavelength (450nm) was selected for the ELISA. 100 µL of the content from each

mixing well was transferred to a corresponding antibody-coated microwell using a new pipette tip and incubated at room temperature for 15 minutes. The contents of the microwells were discarded into a basin. The wells were then washed in buffer which was thereafter decanted into a discard bin. The wash was repeated three times. The microwells were tapped on a layer of absorbent towels to remove the residual buffer. The substrate (120 µL/ well) was measured in a separate container. 100 µL of the substrate was added to each microwell covered to avoid direct light and incubated at room temperature for 5 minutes. The stop solution (120 µL/ well) was measured and placed into a separate container. 100 µL of the solution was added in the same pace and sequence as for the substrate reagent. The optical density (OD) of each microwell was determined using a 450nm filter on a microtiter plate reader using ELISA Machine from Thermo Fisher Scientific Model Number: 355 of serial number: 3550904870. The optical density was recorded for each microwell and thereafter percentage-binding (% B) for each standard and sample was calculated as a percentage of the zero binding (%B/BO), setting the zero standards as 100% binding (%B/BO). (Helica, 2023)

Data Analysis and Processing

Hypothesis Testing for significance difference of the mean aflatoxin levels

The following steps were used as outlined in unpaired t-test**:**

i) **Defined the Hypotheses**

a)**.** (H_0) –There is no significant difference between the two means of the two samples, i.e H_0: $\mu_1 = \mu_2$

b). (H_1) –There is a significant difference between the two means of the two sample and that the difference is unlikely to be because of sampling errors. i.e H_1: $\mu_1 \neq \mu_2$

Assumptions

a)**.** The total aflatoxin means of both the farms are normally distributed

b). The sample observations are independent

ii) **Unpaired student statistics**

$t^* = (\hat{y}_1 - \hat{y}_2)/\{S^2p(1/n_1+1/n_2)\}^{1/2}$

Where S^2p = the pooled variance obtained using the following formula

$S^2p=\{(n_1-1)\ S^2_1+(n_2-1)\ S^2_2\}/\ [\ (n_1-1) + (n_2-1)\}$

Note: The degrees of freedom = $(n_1 + n_2 - 2)$ for t critical.

iii) Decision rule

Reject Ho: $\mu_1 - \mu_2 = 0$ if t*> t(α/2=0.025,58) with 95% confidence

t(α/2=0.025,58) = **±2.002**

Accept H_1: $\mu_1 - \mu_2 \neq 0$ if t*< t(α/2=0.025,58) with 95% confidence

But:

t(α/2=0.025,58) = **±2.002** (Daniel *et al*., 2009; Student, 1908)

RESULTS

Determination and comparison of aflatoxin levels between trained farmers and untrained farmers.

Unpaired student t test was computed and was confirmed with R program and online statistical program for social sciences (Daniel *et al*., 2009; Student, 1908)**.** See table 2.

Table 2: Aflatoxin and moisture content levels for all the thirty Farms in Nandi County Kenya.

No	Region	Sub-region	Trained Farmers		Untrained Farmers	
			Total aflatoxin(ppb)	Moisture Content	Total aflatoxin(ppb)	Moisture Content
1	Kaptumo	Mwangaza	0.473	13.2	0.868	12.2
2			0.354	12.1	0.688	11.2
3			0.408	12.4	0.816	11.9
4			0.487	11.6	0.627	12.1
5			0.536	12.0	0.327	11.9
6		Keteba	0.514	12.6	0.589	12.4
7			0.487	12.2	0.587	12.3
8			0.492	12.0	0.498	12.3
9			0.527	12.0	0.629	12.2

10			0.427	12.3	0.561	12.0
11	Kipkaren	Sobetab Gaa	0.408	11.1	0.568	11.6
12			0.468	10.9	0.852	11.5
13			0.440	11.5	0.678	11.7
14			0.483	10.8	0.812	11.1
15			0.674	11.3	0.619	11.7
16		Toret Gaa	0.316	10.8	0.627	12.2
17			0.550	11.9	0.581	11.5
18			0.484	11.6	0.578	11.3
19			0.434	11.0	0.496	11.5
20			0.555	10.8	0.635	11.4
21	Kilibwoni	Kisob Katanin	1.21	12.2	0.564	12.9
22			0.623	12.5	14.23	12.8
23			0.663	12.2	0.529	12.8
24			0.533	11.9	0.560	12.8
25			0.597	11.9	1.3	13.6
26		Toleltany	0.579	13.4	0.587	11.8
27			0.653	13.4	0.630	14.5
28			0.602	12.5	0.567	12.1
29			0.778	13.0	0.801	12.4
30			0.637	16.3	0.699	13.6

Total	**16.39**	**363.4**	**33.1**	**365.3**
Mean	**0.5464**	**12.11**	**1.1034**	**12.18**
Standard Deviation	**0.16124**		**2.4849**	
t-Calculated	**1.733**			
t-Critical(df)=58 at 95% confidence	**±2.002**			

The relationship between the mean moisture content obtained and the mean aflatoxin levels in both farms where training was done and those that were not.

A part from these, some of the farms where maize had been stored, the moisture content was above 13%. A mean moisture content of 12.11% in farms where training was done resulted in a mean total aflatoxin level of 0.5464 ppb. Whereas a mean moisture content of 12.18% in farms where training was not done resulted in a mean total aflatoxin level of 1.1034 ppb. See figure 2.

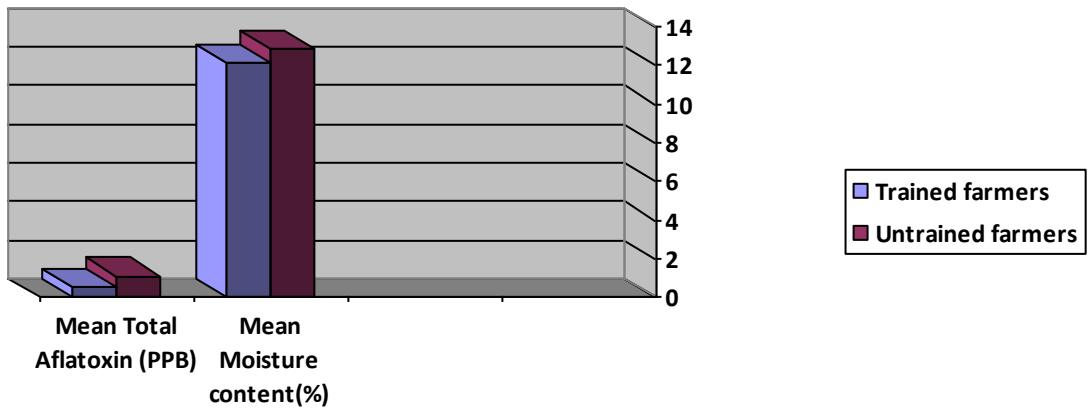

Figure 2: Relationship between the mean moisture content obtained and the mean aflatoxin levels for both farms

A hypothesis was set to evaluate the significant difference of the two total aflatoxin means to determine the long-term influence of the training as shown.

i) Defined the Hypotheses;

a). H_0: $\mu_1 = \mu_2$

b). H_1: $\mu_1 \neq \mu_2$

Assumptions made were that the aflatoxin levels of individual farms are normally distributed and that the sample observations are independent. The overall mean total aflatoxin levels in maize farms where training was done and those that were not were 0.5464±0.16124 and 1.1034± 2.4849 ppb respectively. The t-statistic was computed using unpaired student t test as outlined in Basic Concepts and Methodology for the Health Sciences and confirmed with R program and online statistical program for social sciences. (Daniel *et al*., 2009; Student, 1908)

ii) Unpaired student statistics

$t^* = (\hat{y}_1 - \hat{y}_2)/\{S^2p(1/n_1+1/n_2)\}^{1/2}$

Where S^2_p = the pooled variance obtained using the following formula

$S^2_p=\{(n_1-1)\ S^2_1+(n_2-1)\ S^2_2\}/\ [\ (n_1-1) + (n_2-1)\}$

$S^2_p= \{(30-1)\ 0.16124^2+(30-1)\ 2.4849^2\}/\ [\ (30-1) + (30-1)\}$

$S^2_p= (0.7539517904+179.06711229)/\ 58$

$S^2_p= 3.1003631738$

$t^*= (1.1034- 0.5464)/\ \{3.1003631738\ (1/30+1/30)\}^{1/2}$

$t^*= 0.557/\ \{3.1003631738\ (1/30)\}^{1/2}$

$t^*= 0.557/\ (0.10334543912666)^{1/2}$

$t^*= 0.557/\ 0.3215$

$t^*= 1.733$ppb

Where the degrees of freedom = $(n_1 + n_2 - 2)$ for t critical.

The critical-value for a t-statistic of 1.733 and 58 degrees of freedom at 95 % confidence level is approximately ±2.002. Since the calculated t-value (1.733) ppb is within the critical t-value range (-2.002 to +2.002), the null hypothesis was retained.

DISCUSSION

Aflatoxins are resistant to heat and can withstand normal cooking temperatures (Yau *et al*, 2018). Even the advanced methods used to make brews hardly remove all the toxins in the liquor (Nkwe *et al*., 2005; Shephard *et al*., 2005). A good example is a case study in Kenya where mycotoxins were isolated in beer (Mbugua and Gathumbi, 2004). Despite the low levels in the means 0.5464 ±0.16124 and 1.1034 ±2.4849 ppb and the slight difference of aflatoxin means between the farms where training was done and those that were not, there is no enough evidence to support the difference in significance at p >0.05 using independent Student t-test (Daniel *et al*., 2009). In the previous study, an analysis of 82 samples was done for the mean total aflatoxin. A mean total

aflatoxin levels of 1.301 ± 1.5011ppb was obtained after the training (Marete *et al.*, 2019). However, based on the sample size obtained after the reconnaissance study, it was not possible to make deductions based on comparison between the mean of 0.5464 ±0.16124 for 30 samples and the mean obtained of 82 samples obtained in the previous study. This confirms that the sample size was not representative. This also calls for the need to make frequent follow-ups to minimize deviations in sample size.

Despite the mean total aflatoxin levels obtained being much far below the regulatory limits, one of the maize samples analyzed had a total aflatoxin level of 14.23ppb. This exceeds the regulatory limit of 10ppb as set by the Kenya Bureau of Standards (KEBS, 2018). It is also important to note that in this study only sixty farms had been sampled in the entire county which had a projected population of 951,460 obtained in National Census report as of July 1, 2023 (KNBS, 2021). The positive sample and the numerous samples whose moisture content exceed the regulatory limits are indicators that serve as a warning because the farm with a positive sample does not feed only a single family, but many other households within the communities around and also the fact that various aflatoxin strains are able to camouflage and cause a wider coverage of detrimental effects to the surrounding communities and livestock (Williams *et al.*, 2004). During the analysis, a relationship between the mean moisture content and mean total aflatoxin levels was also noted. This indicated that the higher the mean moisture content, the higher is the mean total aflatoxin. This confirms the influence moisture content has towards toxins production and the necessary action needed on the training of farming practices to reduce aflatoxin levels.

On the other hand, detoxifying strategies are critically known to remove toxins in food and feedstuffs whenever they are detected. Physical and chemical methods have been used as mycotoxin decontamination techniques for a long time. Some of the physical methods used include cleaning, use of high temperature, sorting, use of high pressure, sterilization, milling and cooking (Grenier *et al.*, 2014). Chemical methods include ammoniation and ozonation *(*Isikber *&* Athanassiou, 2015*;* Neal *et al*., 1998). However, some of these methods are not eco-friendly, the costs of operation are high and they produce less reliable results which may reduce the nutritional value or quality of foodstuff (Colović *et al*., 2019). This poses a potential health risk to both humans and animals (Koletsi *et al*., 2021). For instance, extraction as a method, removes vital nutrients and diminishes desirable qualities in the food material. It is not practiced in rural setups due to the associated costs and hazards in handling the solvents (Shapira and Paster, 2004). Some of these methods hardly remove all the toxins; for instance, heating reduces aflatoxin content to about 82–90% during cooking of porridge (Mutungi *et al*., 2008a). Nixtamalization is known to decontaminate 68–90% of aflatoxins (Pérez-Flores *et al*., 2011). In dry milling aflatoxin gets separated in the bran or fines (Mutungi *et al*., 2008b) with about 88% of the aflatoxin being removed from the main product (Scott *et al*., 1984). While in wet milling,

40% of aflatoxin is primarily separated in the fiber and about 40% in the germ (Njapau *et al*., 1998). Irradiation eliminates pathogens and mycotoxins in food. At higher doses, aflatoxin is reduced by 59–88 % (Ghanem *et al*., 2008). The ozone detoxifies the initial toxin concentration in flours, peanut, cereal grains and soybean with high efficiency of up to 66–95% (Loi *et al*., 2020; Torlak *et al*., 2016). As such, prevention of mycotoxin through training on good agricultural practices is the only approach that is suitable in controlling aflatoxin contamination in food and animal feeds (Bulent *et al*., 2006; Kwoba *et al*, 2023). These therefore call for frequent control measures on farm practices focused on aflatoxins prevention rather than advocating for decontamination methods that are not 100% effective.

CONCLUSION

Population dynamics and change in attitude, between the year 2017 and 2023 were the limiting factors. Despite the low levels of mean total aflatoxins, one of the maize samples analyzed had a total aflatoxin level of 14.23ppb in only 60 farms. The numerous samples whose moisture content exceeds the regulatory limits are also indicators that serve as a warning in maize supply chain within the entire County. A relationship between the mean total aflatoxin and the mean moisture content between farms where training was done and those where training was not done was also noted. A mean moisture content of 12.11% in farms where training was done resulted in a mean total aflatoxin level of 0.5464 ppb. This explains the influence moisture content has on aflatoxin levels. Whereas a mean moisture content of 12.18% in farms where training was not done resulted in a mean total aflatoxin level of 1.1034 ppb.

The Unpaired student t-test set to evaluate the hypothesis showed no significant difference in the mean total aflatoxin levels between the farms where training had been done and those that were not. This confirmed that the training did not have a long-term influence.

RECOMMENDATION

In view of all these, there is need for regular follow up on these agro ecological zones to monitor the levels of aflatoxins as recommended by kang'ethe *et al* (2017) and Marete *et al* (2019) and Kwoba *et al* (2025) with special reference to Nandi County so as to not only mitigate aflatoxins contamination in Kenya but also other agro-ecological zones in Africa. It is therefore recommended that these trainings be conducted on annual basis; during planting, harvesting and post-harvesting periods. Determination of aflatoxin levels in the selected farms to be done before and after the training every year or a comparison of aflatoxin levels between farms where training has been done to those that have not be used to evaluate the short-term influence of the training.

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