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AFLATOXIN DETERMINATION IN SELECTED SPICES IN THE GREATER ACCRA REGION, GHANA

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ABSTRACT
This research was carried out to determine the level of aflatoxin present in common spices sold in Nima, Agbogbloshie and Tema Central markets in the Greater Accra Region of Ghana. Spices are used in everyday food preparation to enhance food properties and for medicinal and traditional purposes. The aflatoxin concentration was determined using the Neogen Reveal Q+ test kit and the mobile assay. Aflatoxin was present in all the cloves, cumin, negro pepper and calabash nutmeg spice samples used for the study. 100% of the spices sampled in this study recorded concentrations above the EU acceptable aflatoxin concentration limit which is below 5ppb. Calabash nutmeg spice recorded the highest mean aflatoxin concentration (78.4ppb) while clove spice recorded the lowest mean aflatoxin concentration (11.2ppb). Tema Central market recorded the highest aflatoxin mean concentration (55.0ppb) followed by the Agbogbloshie market (27.6ppb) and Nima market (21.3ppb). The study identifies a major threat to food safety and calls for attention to good handling and storage of spices to reduce health hazards associated with aflatoxins in the Greater Accra Region.

Keywords: Aflatoxin, *Aspergillus* species, Concentration, Mycotoxin, Spices.

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INTRODUCTION
Spices are mostly dried parts of a plant that have a special flavour used in food and drinks preparation, production of cosmetics, medicines, perfumes and in certain religious rituals (Orban, 2014). Spices provide nutritional benefits and as well enhance foods therefore consumers always prefer their usage in their foods. According to Yankey (2014), common spices consumed in Ghana include *Zingiberofficinale (*ginger*), Parkiabiglobosa* (dawadawa*)*, *Syzygium aromaticum* (clove), *Tetrapleura tetraptera* ("prekese"), *Cuminum cuminum* (cumin - locally called "nkitinkiti") and *Piper guineese* (Ashanti pepper - locally called "sorowisa").

Spices are cultivated all over the world however large-scale production is done in the tropics where climatic conditions such as high ranges of temperature, humidity and rainfall exist (Aziz *et al*., 1998). Fungal and mycotoxin contamination of food products such as spices often occurs in these kinds of environments and conditions. Research by Elshafie *et al*. (2002) revealed that *Aspergillus niger, Aspergillus flavus, Penicillium and Rhizopus*are among the common fungal species that contaminate spices.

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Aflatoxins are secondary metabolites of mycotoxins produced by toxigenic *Aspergillus* species which have been identified to cause cancer, mutation, death and organ diseases in humans and animals (Ogungbemile, Etaware and Odebode, 2020). Aflatoxins are heat resistant and are not eliminated by normal cooking procedures hence one of the lethal and dangerous food contaminants (Kishore, Pande and Manjula, 2002). Fungal and mycotoxin contamination of spices may pose serious health implications for humans considering how spices are added to almost every meal prepared especially for public consumption. Also, fungal and mycotoxin contamination of spices may lead to negative implications on trade (Afolabi *et al.,* 2019). To protect consumers from these harmful effects of Aflatoxin and boost trade, there should be periodic surveillance of foods consumed by humans for foodborne disease agents as a contribution towards understanding the menace of foodborne illnesses and diseases. Unfortunately, there is a lack of research that focuses on the safety of spices, especially in Ghana. This research, therefore, focuses on determining the level of aflatoxin present in common spices in markets across the Greater Accra Region of Ghana.

MATERIALS AND METHODS

Sample Collection

Spice samples were collected from the Greater Accra Region of Ghana. The Region is one of the 16 regions of Ghana and consists of two metropolitan assemblies namely, Accra and Tema Metropolitan Assemblies. Samples were collected from two markets in the Accra Metropolis namely Nima and Agbogbloshie markets. In the Tema Metropolis, samples were collected from the Tema Central market.

Before sample collection in these markets, interaction with the spices vendors was first carried out to reveal the most patronized spices by customers. Cloves, negro pepper, cumin and calabash nutmeg were identified and considered for this study. A random sampling method was used to identify various spices vendors in the markets. A sample size of 10 vendors from each market and a total of 4 spices were sampled with four replications of each spice sample, making a total of 48 spice samples. All the 48 spice samples were procured from the vendors, bagged into sterile bags, labelled and transported to the Spanish laboratory complex of the University for Development Studies for aflatoxin analyses.

Aflatoxin Detection

The detection and quantification of aflatoxin were performed following the Neogen Reveal Q+ protocol. The analysis was carried out using analytical grade diluents and reagents. The spice samples were ground into a powdery form. 10g of the powdered sample was weighed into a glass bottle and 30ml of 65% ethanol was added to the weighted sample. After, the glass bottle was covered and shaken for five minutes to homogenize. The sample extract was then filtered into a jar. 500ml of the diluent was pipetted into the red sample cup. After, 100ml of the sample extract was then added to the diluent in the red sample cup. The mixture obtained was mixed by pipetting up and down five times after which 100ml of the mixture obtained was further transferred into the clear sample cup.A test strip was placed in the solution in the clear sample cup and allowed to develop for about six minutes. The strip was analyzed using m-Reader and the results were recorded and stored on the m-Reader.

Data Analysis

Results after the aflatoxin analyses were analyzed using GenStat edition 12. Analysis of Variance (ANOVA) tests and Tukey'sstudentized range in the LSD test was

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used to determine which of the means were statistically significant at $p<0.05$.

RESULTS

Aflatoxin concentration of spices

Aflatoxin was successfully determined in all the spice samples collected in this study. In theNima market, cumin recorded the highest mean aflatoxin level (40.2ppb) followed by negro pepper (23ppb), calabash nutmeg (12.9ppb) and clove (9.0ppb). In the Agbogbloshie market, clove also recorded the lowest mean aflatoxin concentration (9.0ppb) however calabash nutmeg recorded the highest mean aflatoxin concentration (63.0ppb). Inthe Tema Central market also, calabash nutmeg recorded the highest mean aflatoxin concentration (160.0ppb) while clove recorded the lowest mean aflatoxin concentration (15.0ppb) just like the other markets. Furthermore, there was no significant difference between the aflatoxin levels of the spices in each market aside from the Tema central market where there was a significant difference between the levels of aflatoxin in calabash nutmeg and the other spices in the market (Table 1).

Mean aflatoxin concentration ofspices across the markets

The aflatoxin levels of the spices ranged between 4.89ppb and 240.08ppb. Calabash nutmeg recorded the highest aflatoxin mean concentration (78.41pp) whiles clove recorded the lowest aflatoxin mean concentration (11.2ppb). Furthermore, there was a significant difference between the aflatoxin concentration of each of the spices (Figure 1).

Mean aflatoxin levels of various markets.

The aflatoxin analysis revealed that the mean concentration of aflatoxin in spices collected from the Tema Central market was the highest (55.01ppb) followed by the Agbogbloshie and Nima markets at 27.60 and 21.3ppb respectively. There was however no significant difference between the levels across the three markets. (Figure 2).

DISCUSSION

The common spices identified after interaction with the vendors were cloves, negro pepper, cumin and calabash nutmeg. These spices are suddenly popular amongst Ghanaian vendors and are used for various uses such as in the preparation of traditional medicines for people suffering indigestion, toothaches and also for aphrodisiac preparation. Osei-Djarbeng*et al*. (2014) suggested in their research that the recent high consumption and demand for locally made spices for refreshing drinks such as “sobolo” during their production process is creating more interest in these spices and should be well exploited, especially for their safety.

The presence of aflatoxins in all of these spices is a threat to food safety and predisposes Ghanaian consumers to the harmful effects of aflatoxins such as damage to the liver, DNA mutation, cancer and death. The problem is further aggravated when the levels of aflatoxin realized in these spices are compared to the acceptable level of aflatoxins in spices set by food safety regulatory agencies. All spices, thus 100% of the spices sampled in this study recorded concentrations above the EU acceptable aflatoxin concentration limit which is below 5ppb. Also, the Codex Alimentarius Commission sets the recommended and acceptable aflatoxin concentration in spices below 10ppb (Codex Alimentarius, 1995). Comparing the aflatoxin concentration of the sampled spices in this study to the 10ppb set by the Codex Alimentarius, 83% of the spices sampled in this study exceed that mark.

Aside from the high exposure of aflatoxins to consumers who prefer spices in their meals and local drinks, this further means only 17% of the spices can be confidently exported and sold to customers in jurisdictions that enforce

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the codex limit. This directly affects trade since food crops that exceed the tolerable limits will not pass certification and approval and will eventually be called off the market if identified.

Aflatoxin contamination occurs both under pre-and post-harvest conditions. Information gathered during interaction with vendors revealed that most of the spices had been stored for months and some close to a year. According to Iqbal *et al*. (2011), it has been identified in spices that increasing storage increases aflatoxin levels, especially under improper storage conditions. This hence is one of the factors that influenced the presence of aflatoxin concentration in all the sampled spices. In addition, improper handling habits of spices by the vendors also contributed to the high concentration of aflatoxins in the samples as supported by the findings of Hammami *et al*. (2014) and Ahmed and Asghar (2021).

Calabash nutmeg spice recorded the highest mean aflatoxin concentration among the four spices. This finding is supported by Ezekiel *et al*. (2013) and Ameho *et al*. (2017) who also observed high levels of aflatoxin in calabash nutmeg compared to other spices in their studies. The reason for the consistently high levels of aflatoxin in calabash nutmeg is not understood, however, it could be that it lacks inhibitory properties which may be present in other spices. Per Essawet *et al*. (2017), certain spices are known to have inhibitory factors such as spice oil and other secondary metabolites that prevent or reduce attack, colonization and subsequent production of mycotoxins by fungi. Also, some spices are reported to have inhibitory activity toward fungi by interfering with the production of aflatoxin (Kocic-Tanackov *et al*., 2012).

In all three markets, clove spices recorded the lowest aflatoxin concentration. Roamgnoli *et al.* (2007) posits that the poor growth rate of aflatoxin producing fungi in cloves can be attributed as the reason for the low level of aflatoxin often revealed in cloves. Thanaboripat *et al.* (1997) also reported that clove has the potential of inhibiting the growth of *Aspergillusflavus* and aflatoxin production.

There were variations realized in aflatoxin concentrations of similar spices across the various markets. For instance, cumin recorded an aflatoxin concentration of 40.2ppb in the Nima market, 23.0ppb in the Agbogbloshie market and 28.0ppb in the Tema Central market. Only clove recorded 9.0ppb both in the Nima and Agbogbloshie markets. This variation identified in similar spice from a different location is common and has been identified in other studies among other spices such as ginger, calabash nutmeg, cumin, cloves and curry (Martins *et al*., 2001; Erdogan, 2004; Ameho *et al*., 2017). Such variations can be a result of differences in weather conditions of the areas, storage conditions and food safety habits.

All the markets recorded high means of aflatoxin levels and there was no significant difference between these levels. Conditions such as bad sanitation, bad infrastructure and bad storage conditions in the markets facilitate aflatoxin contamination in every other food crop sold in the market including spices.

CONCLUSION

The study shows aflatoxin contamination in cloves, cumin, negro pepper and calabash nutmeg spices in the Nima, Agbogbloshie and Tema Central markets of the Greater Accra Region, Ghana. Furthermore, all the spices exceeded the acceptable aflatoxin concentration limit in spices set by the EU while only 17% were below the Codex Alimentarius standard. Also, improper handling, long term storage of spices, poor drying and inadequate proper storage facilities

influenced the aflatoxin contamination in these spices. The study identifies a major threat to food safety and suggests an aflatoxin awareness campaign among food crop vendors by authorities in charge of food safety in the country and the provision of education on the reduction and elimination of aflatoxin in food.

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APPENDICES

Table 1: Aflatoxin concentration of spices in various markets

Spices	Nima	Agbogboloshie	Tema Central
Clove	9 ± 0.750[a]	9 ± 1.437[a]	15 ± 4.817[a]
Calabash nutmeg	12.9 ± 2.632[a]	63 ± 50.64[a]	160 ± 52.10[b]
Cumin	40.2 ± 23.05[a]	23 ± 0.748[a]	28 ± 4.490[a]
Negro pepper	23 ± 2.167[a]	16 ± 4.147[a]	17 ± 4.898[a]
P-value	0.282	0.469	0.006
LSDs	35.92	78.3	81.3
CV%	109.5	184.1	95.9

Means with the same letters in the same column are not statistically significantly different from each other at a p-value < 0.05.

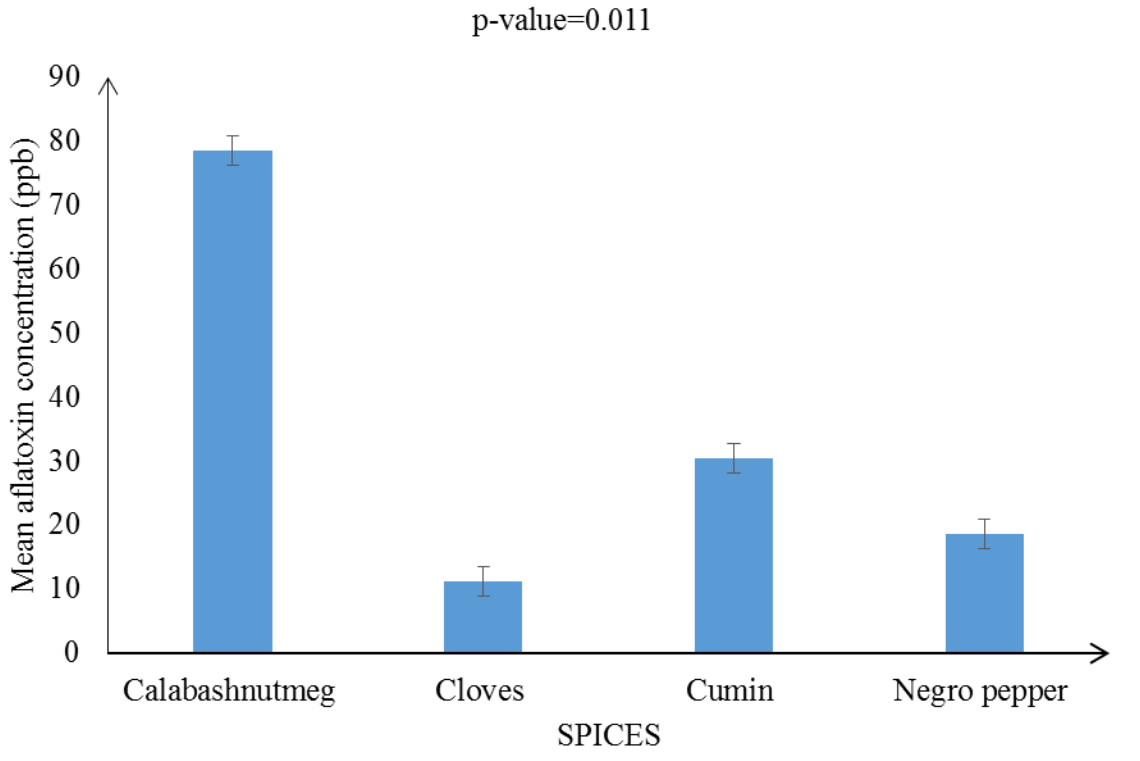

Figure 1: Mean aflatoxin concentration of spices across markets

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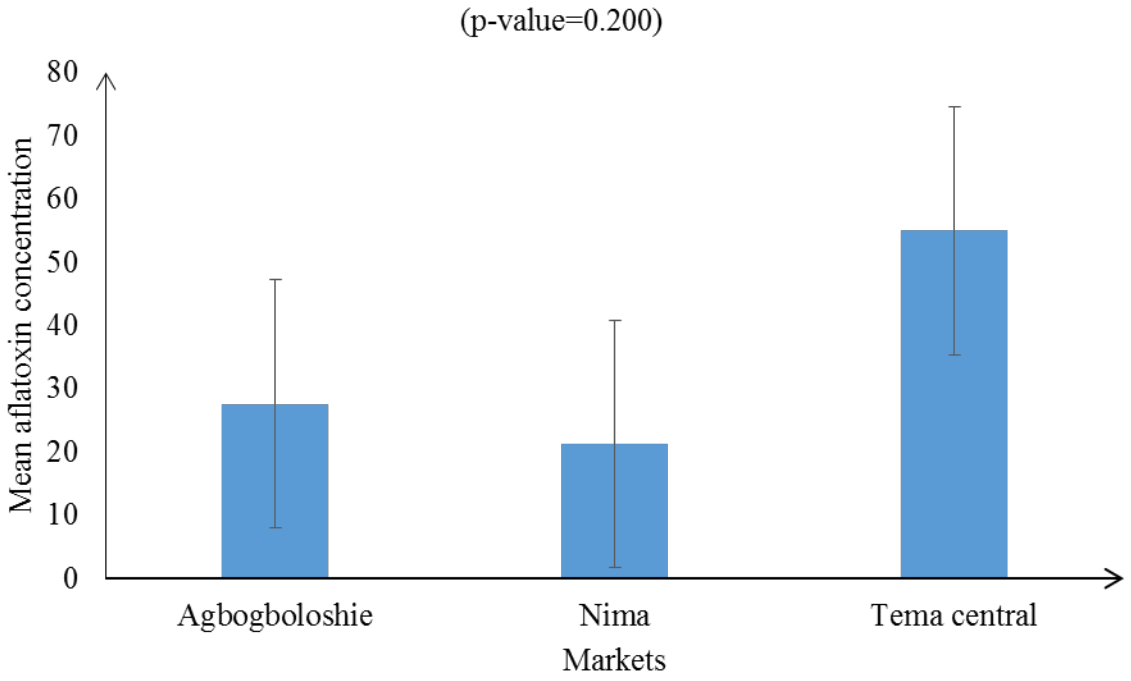

Figure 2: Mean aflatoxin concentration across the markets