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ABSTRACT

Aflatoxins are toxic and carcinogenic fungal metabolites. Aflatoxin B_1 is the most toxic compound and has been classified as a Group I carcinogen by the International Agency for Research on Cancer (IARC). This paper reports on a decade long analysis of a total of 1,168 groundnut samples brought to the Aflatoxin and Food Chemistry laboratory of the National Agricultural Research Institute (NARI) and analyzed for their aflatoxin content using a Thin Layer Chromatography (TLC). The results indicate that 58% of the entire samples during the period are within the acceptable limit of the Codex Alimentarius, which is <15 (ppb). A fluctuating rise and fall in the levels of aflatoxin with the highest mean of 112 ppb observed in 2011 and the lowest 8.55 ppb in 2018. Out of 103 samples in 2018, 81% were found to be within the permissible level (<15 ppb) of the Codex Alimentarius. The aflatoxin control intervention programs are geared toward improving the market value of groundnuts from The Gambia on the international market.

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The Trend of Aflatoxin Contamination Levels in Groundnuts from 2008-2018 in The Gambia

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ABSTRACT

Aflatoxins are toxic and carcinogenic fungal metabolites. Aflatoxin B_1 is the most toxic compound and has been classified as a Group I carcinogen by the International Agency for Research on Cancer (IARC). This paper reports on a decade long analysis of a total of 1,168 groundnut samples brought to the Aflatoxin and Food Chemistry laboratory of the National Agricultural Research Institute (NARI) and analyzed for their aflatoxin content using a Thin Layer Chromatography (TLC). The results indicate that 58% of the entire samples during the period are within the acceptable limit of the Codex Alimentarius, which is <15 (ppb). A fluctuating rise and fall in the levels of aflatoxin with the highest mean of 112 ppb observed in 2011 and the lowest 8.55 ppb in 2018. Out of 103 samples in 2018, 81% were found to be within the permissible level (<15 ppb) of the Codex Alimentarius. The aflatoxin control intervention programs are geared toward improving the market value of groundnuts from The Gambia on the international market.

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I. INTRODUCTION

The Gambia, a West African country, with a total land area of 11,300 square km, bordering the North Atlantic Ocean enclosed by Senegal and is by far the smallest country on mainland Africa. The country has a relatively flat landscape, open savannah woodland characterized by a tropical climate. The dry season starts from November to May, and an erratic rainy season from June to October, with an average annual rainfall varying from 500 to 1200 mm per annum. It has a maximum temperature range of 24-32°C and a minimum temperature of 18°C. These climatic factors are conducive for the growth of aflatoxin-producing fungal species, which often leads to aflatoxin contamination of agricultural produce and products.

Groundnut (*Arachis hypogaea L.*) is an vital oilseed crop belonging to the family Leguminosae and is an essential crop both in subsistence and commercial farming systems (Chivenge *et al.*, 2015). Rarely referred to as peanut in The Gambia, Groundnuts has been the main cash crop of the country since the 1960s.

Mycotoxins are fungal metabolites contaminating up to 25% of the human food supply (CAST, 2003). Aflatoxins (AFs) are a group of toxic secondary metabolites produced by several *Aspergillus flavus* and *Aspergillus Parasiticus* and to a lesser degree *Aspergillus nomius* (Jallow *et al.*, 2018). Aflatoxin contamination of food products is a common problem in tropical and subtropical regions of the world, especially in the developing countries, more so in Sub-Saharan Africa. Research has shown that poor agricultural practices, drought stress, insect damage, and poor storage coupled with environmental conditions of warm temperatures and humidity favor the growth of fungi (Thrasher, 2012).

The level of toxicity of aflatoxin varies with the type present, with the order of toxicity being AFB_1

> AFG_1 > AFB_2 > AFG_2. The B_1 and G_1 refer to the blue and green fluorescent colors respectively produced under ultraviolet light on Thin Layer Chromatography (TLC) plates, while the subscript numbers -1 and -2 indicate major and minor compounds respectively according to the migration distances on the TLC plates. AFB_1 is the most toxic and epidemiological studies has associated it with liver cancer and acute hepatitis, thus classified by the International Agency for Research on Cancer as a Class 1 human carcinogen (IARC 2002). The toxin is teratogenic, mutagenic, immune suppressive, and, therefore, very hazardous to both human and poultry health (Gridthai *et al.,* 2010). A study by Williams (2011) shows that people with a high aflatoxin biomarker status in The Gambia and Ghana were more likely to have active malaria. Aflatoxins show high chemical stability and may remain throughout the food chain even after fungi are removed by normal manufacturing and packaging processes (Lee *et al.,* 2015).

The Gambia suffers from prevalent aflatoxin contamination of raw groundnuts and subsequently reduces the export potential of the country. This is evident by the inability to attract international premium markets like the European Union and the United States, whose threshold for total aflatoxin B_1 is 2 ppb and 20 ppb, respectively. As a consequence, farmers in the country are gradually reducing their groundnut cultivation and shifting to rice, cereals, and other horticultural crops. However, most human exposure to the toxins comes from contaminated nuts, grains, and their derived products. Exposure to aflatoxins can also happen through milk and milk products, including breast milk, especially in areas where the poorest quality nuts or grains are used for animal feed. Also, aflatoxin M_1 (AFM_1) is a bioconversion product of aflatoxin B_1 (AFB_1) metabolism.

Studies have shown that exposure to aflatoxin can also affect the developing fetus through the placental barrier and further react with cellular macromolecules leading to the potential for biological effects *in utero* (Wild *et al.,*1991). Another means of exposure of aflatoxin in infancy can occur through the type of weaning food (grain-based foods) as well as early introduction of children to the family meals such as groundnut soup, maize and sorghum porridge which might be contaminated with aflatoxins (Wild *et al.*, 2000; Turner *et al.*, 2003; Turner *et al.*, 2005). Most exposure to aflatoxin through the consumption of contaminated foods is a combination of unawareness, poverty, and lack of enforcement of standards by authorities.

(Turner *et al.*, 2003) Detected aflatoxin albumin adducts 93% of sampled children (6-9years) in The Gambia and provide evidence that immunoglobin A (IgA) in saliva may be reduced because of high dietary levels of aflatoxin exposure. The study confirmed that children in rural areas of The Gambia are frequently exposed to a high level of aflatoxin. This paper reports the results of aflatoxin levels in groundnut samples targeted for exportation that were analyzed by the Aflatoxin and Food Chemistry laboratory at the National Agricultural Research Institute (NARI) in The Gambia from 2008 to 2018.

II. MATERIALS AND METHODS

A total of 1,168 groundnut samples were received at the Aflatoxin and Food Chemistry Laboratory (AFCL) of NARI and are sampled according to standard protocol for the Official Control of Mycotoxins in Food EU (2003). The 20 kg sack(s) received are sub-sampled with a riffle sample divider to half and kept for references for a stated period, and the other half further sub-sampled to 5 kg, which is ground using a blender and thoroughly mixed using a hand spatula. A 200 g of the ground sample weighed in a separate blender, and 400 ml distilled water added and blended for 5 min into a slurry. The blender is rinsed and dried, then 100 g of the slurry was weighed and 250 ml methanol, 100 ml hexane, and 2 g of NaCl was added and blended again for about 3 mins. It was filtered using a Whatman filter paper of 32 cm and 50 ml collected. Exactly 150 ml of distilled

water was poured into a separating funnel, then the 50 ml filtrate and 25 ml of chloroform were respectively added, and the separating funnels topped then slightly shake. Uncapped, allowed to settle, filtered, eluate collected in a small beaker containing two anti-bumping granules for calm boiling, then heat to dryness in a water bath. The dried beaker allowed to cool and about 5-8 ml chloroform pipetted into it, a dropper was used to rinse its wall to cleanse off any aflatoxin then the chloroform was subsequently filled in a vial containing two anti-bumping granules. The vials are evaporated to dryness using an electric vial-rack heater. After dryness and the vials allowed to cool, a 0.25 ml of benzeneactonitrile (98:2 v/v) solution was pipetted into them and vortexed using an electrical shaker. Samples are spotted using an assiette-fix for (5-10--40) µl respectively on the TLC silica gel coated glass plates of 20 cm x 20 cm and thickness layer of 0.25 mm against the standard (1-3-5-7-10-15-20) µl and labeled using a pencil on top of the plate. The TLC plate is gently lowered into an already loaded tank with (diethyl: ether: Methonal: water) (94:4.5:1.5) v/v positioned on a flat surface and covered with the lid. After about 45 - 60 mins, when the plate in the tank has absorbed the solvent and must have reached the line marked at three quarter length of the plate, the plates are removed and allowed to dry up for about a minute then illuminated below the TLC machine or lamb and viewed under long-wave of UV 366nm in the dark room. The fluorescence intensities of aflatoxin B_1 spots in samples were compared with those of spots of standard in terms of color and Rf. Aflatoxin B_2, G_1, and G_2 spots were compared by the same procedure. The concentration of aflatoxin B_1 was calculated as part per billions of aflatoxin/ kilograms of sample as $(S \times Y \times V)/(W \times Z)$, where S is the amount (µl) of the aflatoxin B_1 standard required to match the unknown spot, Y is the concentration of the aflatoxin B_1 standard (µg/ml), V is the amount (µl) of the final dilution of sample extract, W is the amount (µl) of the spotted sample extract required to match the fluorescence intensity of S (the B_1 standard), and Z is the weight (g) of the sample. The same equation was used to determine the amounts of other aflatoxins, such as B_2, G_1, and G_2. The same equation was used to determine the amounts of other aflatoxins, such as B_2, G_1, and G_2.

III. PREPARATION OF THE STANDARDS

After the preparation of standard solutions of each aflatoxin, their concentrations were determined by using a Helios gamma UVG spectrophotometer.

The working standard solution was also prepared by diluting mixed standards (AFB_1, AF G_1 of 1.0 ppb and AFB_2, AFG_2 of 0.5 ppb) in vials and evaporated then filled with benzeneacetonitrile (98:2v/v). Preliminary tests under the UV fluorescence were conducted to estimate the intensity of the aflatoxin standards.

IV. STATISTICAL ANALYSIS

The absolute aflatoxin contamination values obtained from the TLC were recorded in Microsoft Excel sheets and transported into GraphPad Prism for analysis. The experimental results were analyzed using GraphPad Prism (V.5) for the effects of various factors on the results at 5% probability and 95% confidence interval.

V. RESULTS

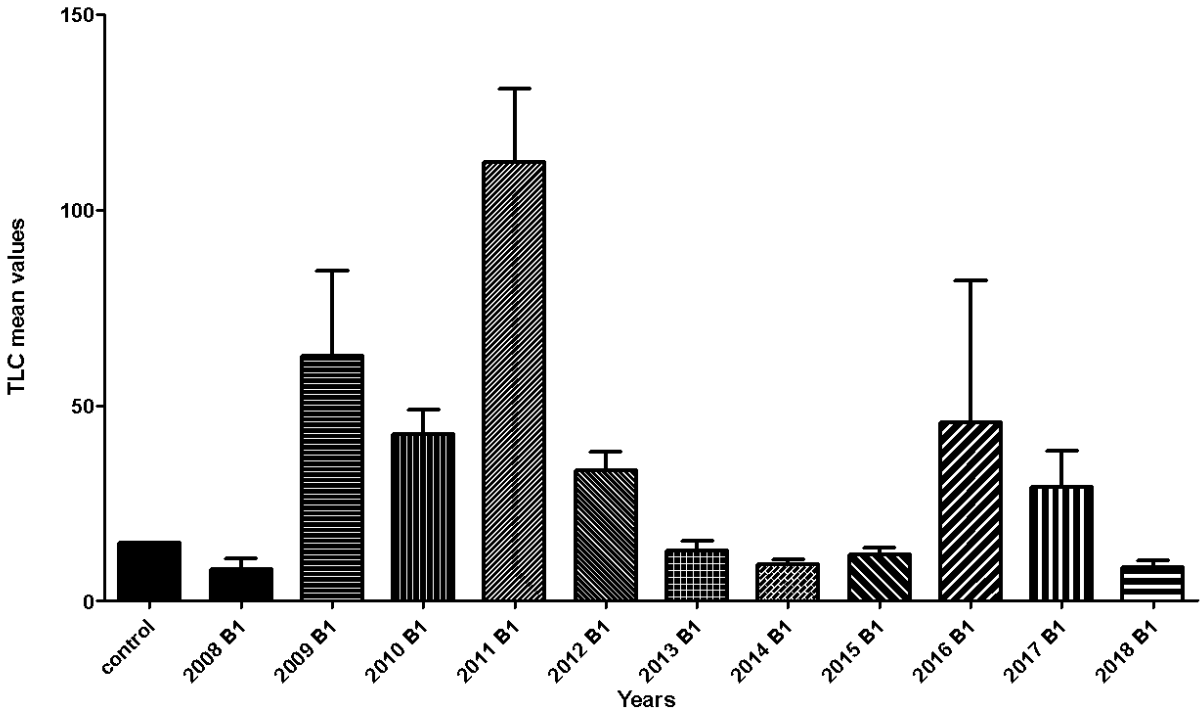

Figure 1: A graph is displaying aflatoxin B_1 contamination levels from 2008 to 2018.

Table 1: Variation (number, range, mean, and standard deviation values) of Aflatoxin B_1 contamination levels as detected by the TLC.

Years	Number of samples per year	Samples within permissible limits(15ppb)	Range of AFB_1 (ppb)	AFB_1 mean (ppb)	Standard Deviation
2008	29	22	1- 46.64	8.14	15
2009	94	55	1-1887	62.70	208
2010	276	131	0 - 950.57	42.71	103
2011	201	50	0 - 2851.71	112.05	266
2012	78	35	0 - 308.93	33.50	40
2013	89	70	0 - 142.59	12.92	24
2014	180	150	0 - 95.06	9.31	18
2015	97	73	0 - 66.54	11.80	18
2016	13	09	0 - 475.23	45.70	130
2017	08	04	0 - 109.32	29.11	26
2018	103	83	0 - 95.06	8.55	18

At a glance at the graph, it can be deduced that, there was a rise in aflatoxin values from 2009 to 2011 and then a sudden reduction in values until 2018. This can also be seen from the mean and standard deviation values of the various years as compared to the codex control mean of 15 ppb. All the years are significantly different from the control except for 2016 & 2017 due to its less experimental values. The greatest deviation was observed in 2016 with the biggest coefficient of variation (285.17%) from the control, followed by 2010 (240.93%) and 2011 (237.57%). A 95% confidence interval was used in all analyses. The respective years varied significantly from each other with P< 0.05. Dunns Multiple Comparison test was used to compare each year against each other. There were significant differences between some years and the control (2008, 2011, 2013, 2014, 2015, 2018), with 2011 having the highest significant difference (p< 0.0001).

VI. DISCUSSION

As per samples brought to the Aflatoxin and Food Chemistry Laboratory from 2008 to 2018, the aflatoxin content on groundnut kernels varied over the years of the study (Table 1). The highest mean level of aflatoxin content was in 2011 with 112ppb, while the lowest was in 2008 & 2018 with 8 ppb. In 2010, 276 samples were analyzed with a mean of 42.7 ppb. However, the mean contamination levels of aflatoxin in 2014, 2013, 2015, 2018 were lower than the maximum tolerable limits (<15 ppb). Those with permissible level (<15 ppb) of aflatoxin content were safe for human consumption, as mentioned in the document of the Codex Alimentarius Commission. However, the rest of the years were in the non-permissible level set for $AFB_1$with toxin content higher than the 15 ppb (Table 1). The results of AFB_1 occurrences were very prevalence over the years; out of the 1168 samples, 58% of the samples were within the acceptable limit of the Codex Alimentarius, which is <15 (ppb).

Combating aflatoxin in The Gambia is highly aimed at generally to have a food commodity acceptable to the international markets. As a result, awareness to reduce aflatoxin is ongoing, such as to adopt good agronomical large-scale farming system and mechanize postharvest treatments. The central targets of the outcomes are exportation, international trade, and meeting aflatoxin regulations, especially of the importing country. Rather than the direct consumers in the local communities where the immediate human health effects are most evident. As stated by (Turner *et al.*, 2003), detected aflatoxin albumin adducts 93% of sampled children (6-9years) in Gambia showed that immunoglobin A (IgA) in saliva might be reduced because of high dietary levels of aflatoxin exposure.

A significant drop in the number of samples is observed in 2016 and 2017 with only 13 and 08, respectively. The fall of sample numbers is mostly because of the country's political status and policies that allowed foreign buyers of groundnut who are not concern with aflatoxin contamination levels to buy directly from farmers through their agents, whose markets are mostly in Asia. This competition limits the access of local groundnut export companies to the local peanut market. Out of 103 samples in 2018, 81% were found to be within the permissible level (<15 ppb). The trend in the decrease in AFB_1 contamination level from 2016 to 2018 is promising, and if the ongoing strategies to combat aflatoxin contamination are maintained, it will not only attract premium prices, but also hope to improve the nutritional benefits which will protect consumers from potential public health threats posed by aflatoxin. Thus, a continuation of aflatoxin control intervention programs like sensitization of good agronomic practices, provision of better transport and storage facilities, public awareness campaigns which targets with low socio-economic status, and basic educational level to improve the peanut production in the country. Due to the thermal stability of aflatoxins, physical treatment by heat

results in only small changes in their levels (Tripathi and Mishra 2010). Chemical treatments using solvents can extract these compounds causing a minimal effect on nutritional quality; however, this technology is still impractical and expensive; therefore not an option for ordinary Gambian farmers, besides inducing odors and flavors. Ammonization is also used as an effective and practical application for the decontamination of agricultural products containing aflatoxins (Allameh *et al.,* 2005). A study by Bhatnagar and the team has demonstrated that the addition of neem leaf extract above 10% (v/v) effectively inhibited the production of aflatoxin (Bhatnagar *et al.*, 1988). Drought can lead to cracking of the pods of peanut and ingress by *A. flavus* and *A. parasiticus* resulting in significant aflatoxin accumulation. Delayed harvest, late irrigation rain, and dew during warm periods are associated with increased aflatoxin levels. Aflatoxin increases were greater on crops receiving over 50 mm of rain during boll opening (Cotty and Jaime-Garcia 2007). Groundnut exposed to high temperature during pod maturation and rainfalls on windows is additional susceptibility factors to fungus growth. Some farm implements may also play a part in aflatoxin contamination in the country. Along the production stages, modern machines like groundnut lifters are often used which enables immediately packing of the undried uprooted groundnuts, unlike the previous practices when the farmer(s) will uproot per plant and instantly dry before packing.

The used of TLC technique for aflatoxin analysis is obsolete and losing recognition by the importing countries, therefore, the government of The Gambia and relevant stakeholder and authorities should provide modern equipment like the High-Performance Liquid Chromatography (HPLC) and Ultra Performance Liquid Chromatography (UPLC) which provides precision for the routine aflatoxin analysis.

VII. CONCLUSION

The ongoing strategies being implemented by Gambian authorities with relevant stakeholders in collaboration with the Partnership for Aflatoxin Control in Africa (PACA) in the country have been encouraging. However, there is a huge missing link in the groundnut value chain production that needs to be checked and regulated to protect consumers' livelihood from potential public health threats posed by aflatoxin and pave the way for better trade opportunities. Much is needed in building both human and resource capacity in the aflatoxin mitigation process. Farmers whose groundnut produces are low with aflatoxin contamination should be recognized and handsomely rewarded. A proper surveillance and monitoring system throughout the groundnut value chain should be introduced and enforced. More especially, a system that will provide a well-structured procedure of groundnut production to minimize aflatoxin contamination meant for both consumption and exportation.

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