Effects of Fertilization on Aflatoxin Concentration in Fresh and Stored Groundnuts (*Arachis hypogaea* L.)

Evans A. Alenyorege, Felix K. Abagale, Albert Yawson, Nelson Opoku

.

Abstract—**The study aimed at determining aflatoxin concentration in groundnuts cultivated using Yaralegume and Humate Green OK fertilizers at four (4) communities in the Lambusie-Karni District of the Upper West Region of Ghana. In all, twenty four (24) fresh and stored groundnut samples were analysed using standard fluorometric method and procedures. All groundnut samples tested contained detectable concentrations of total aflatoxins with two fresh samples producing concentrations of 3.59 and 4.58 ppb which were within the Limit of Quantification (LOQ). Fresh and stored groundnuts contained aflatoxins in the range of 3.59 - 13.21 ppb and 10.43 - 93.43 ppb respectively indicating significantly ($p < 0.05$) high concentrations after storage. Aflatoxin concentration in fresh groundnuts where fertilizer was not applied increased from 34.19-62.05 % after storage whilst it reduced significantly from 24.41 - 19.23 % and 37.40 - 18.72 % in fields where Yaralegume fertilizer only and a combination of Yaralegume and Green OK fertilizers were used. Two stored samples however recorded aflatoxin levels of 93.43 ppb and 52.92 ppb which were noted to be above Ghana Standards Authority maximum allowable limit of 20 ppb. The potential of reducing the concentration of aflatoxins in fresh and stored groundnuts cultivated using the Yaralegume fertilizer and a combination of Yaralegume and Humate Green OK fertilizers was observed.**

Evans A. Alenyorege *Department of Agricultural Mechanization and Irrigation Technology, University for Development Studies, Tamale-Ghana.*

Felix K. Abagale *Department of Agricultural Mechanization and Irrigation Technology, University for Development Studies, Tamale-Ghana.*

Albert Yawson *Department of Agricultural Mechanization and Irrigation Technology, University for Development Studies, Tamale-Ghana.*

Nelson Opoku *Department of Biotechnology, University forDevelopment Studies, Tamale-Ghana.*

Index Terms —Fertilization, Aflatoxin, Groundnuts, Fluorometric

I. INTRODUCTION

In spite of the significance of groundnuts (*Arachis hypogaea* L.) as food, the incidence of aflatoxin contamination has the ability to limit its importance in the human diet since food safety is a fundamental measure for food security in sub-Saharan Africa where major food losses, health challenges and human fatalities have stemmed from contamination of major staples by fungal pathogens [1], [2]. One of the major problems in groundnut production and supply globally is aflatoxin contamination, which is of great concern in groundnuts and groundnut products consumption as this toxin can cause carcinogenic, immunosuppressive, hepathogenic and teratogenic effects in humans and animals [3]. Infection of groundnut by *Aspergillus flavus* occurs not only under post-harvest but also during cultivation and harvest conditions [4].

The Food and Agriculture Organization in 2002 estimated that 25 % of food crops worldwide were affected while the Center for Disease Control in 2004 reported that more than 4.5 billion people in the developing world are exposed to aflatoxins. The incidence of these toxins in Ghanaian groundnuts and other crops has been well documented for many years. Awuah and Ellis [5] found groundnut samples from 21 selected markets in 10 regions of Ghana with high levels of the toxin; infection was found in 31.7 % of the damaged kernels examined, and 12.8 % of the undamaged kernels.

Several approaches to reducing or managing aflatoxin contamination have been proposed [6], [7], [8]. They include; pre-harvest, at-harvest and post-harvest management practices; use of tolerant seed varieties and biocontrol measures. On-farm tests have been conducted in several countries in Asia and Africa to investigate not just technologies, such as the use of varieties that are tolerant or resistant to *Aspergillus flavus*, but also cultural practices, such as the use of soil amendments, and post-harvest handling on yield and aflatoxin contamination. Although increased levels of aflatoxin contamination in post-harvest groundnut samples have been reported [9], the advent of readily available fertilizers

ISSN: 2278 – 7798

has brought about the reduction or termination of many pathogenic diseases through improved plant resistance, disease escape, altered pathogenicity, or microbial interactions. Efficient fertility programs can enhance plant resistance to pathogens, reduce the impact of environmental stress, and increase quality of the food and feed that are produced. With the availability and utilization of some organic and inorganic fertilizers in groundnut production, an assessment of their effect on aflatoxin contamination of groundnuts as a measure of quality was important.

II. METHODOLOGY

A. Study Areas

The field experiments were conducted in four (4) communities; Samoa (N10.83208; W002.56059), Korro (N10.88371; W002.5688), Konguoli (N10.84229; W002.66427) and Hiinneteng (N10.85800; W002.69651) all in the Lambussie-Karni District of the Upper West Region of Ghana. The Lambussie-Karni District falls in the Guinea Savanna climatic zone and experiences two major seasons with a single maxima **(**short rainy season and a long dry spell). The rainy season starts from June to October each year and gives way to the dry season from November to May. The occurrence of drought or floods affects crop growth thereby culminating in reduced crop yields each year, as additional nutrients intake by the crops is impaired. The on-farm fertilization experiment was established in the rainy season of 2014. Planting was done in July 2014 and harvested in October 2014.

B. Experimental Description and Sampling

The experiment was laid out in a completely randomized design (CRD) with no fertilizer application as control treatment (T_1), full rate of Yaralegume (3.75 Kg/100 m^2) as second treatment (T_2) and a combination of Yaralegume at half rate (1.88 Kg/100 m^2) and Humate Green OK liquid fertilizer solution (3L/100 m^2) as third treatment (T_3). One groundnut variety (Shitaochi or Chinese) was planted to all experimental plots. Plot size per treatment was 10 m x 10 m (100 m^2). The plant spacing was 0.50 m inter-row and 0.10 m in-row. Groundnuts from each plot were harvested and sundried. From respective lots, 1.0 kg of dried groundnuts was picked as fresh samples (without storage) and the remainder stored in mini polypropylene bags for five (5) months (November, 2014 to March, 2015). After storage, 1.0 kg of groundnuts was picked as stored samples from respective lots. This was done in all four (4) communities. Subsequently, each of the fresh and stored samples per experimental unit per community was hand-shelled separately; working samples (100 g) were prepared accordingly and from which analytical samples (50 g) were taken for fluorometric aflatoxin analysis.

C. Quantification Analysis of Aflatoxins

The Romer FluoroQuant Afla Plus (COKFA3070) test kit was used for the analysis. It employed a solid-phase, single-step clean-up column followed by fluorometric analysis to determine aflatoxins in the groundnuts as illustrated by AOAC [10]. Acetonitrile (ACN) with de-ionized water (86:14) was used for extraction. This quantitative method was rapid, accurate and can be applied to individual samples.

D. Interpretation Criteria

Results represented the amount of total aflatoxins (B_1, B_2, G_1 and G_2) in parts per billion (ppb) present in the samples. Values less than the Limit of Quantification (LOQ) (2.4 ppb-5.0 ppb) are reported as <LOQ and those less than the Limit of Detection (LOD) (0.6 ppb-1.9 ppb) are reported as Non-Detect (ND).

E. Data Analysis

Data collected was subjected to one-way analysis of variance (ANOVA) using GenStat discovery edition 3 (VSN International Ltd). Statistically significant differences were reported at $p < 0.05$. If the overall F-test was significant ($p < 0.05$), then Fisher's Least Significant Difference (LSD) test was used to compute the smallest significant difference between two means and alphabetical notations used as superscripts to mark the differences at significant levels.

III. RESULTS AND DISCUSSION

A. Pre and Post-harvest Aflatoxin Concentration in Groundnuts

Aflatoxin concentration as in Table 1 were significantly low in fresh groundnuts (3.59 -13.21 ppb) and significantly high in stored groundnuts (10.43 – 93.43 ppb). All samples from all four (4) communities increased significantly in aflatoxin concentration after storage. The increase was over 75 % pronounced in samples from the control (T_1) plots with as high as 93.43 ppb and a low of 17.34 ppb depicting vulnerability to infection by *Aspergillus* species. This was also observed in the Yaralegume and Humate Green OK (T_3) treated plot, making samples from treatment plot 2 (T_2) reasonably safer and the Yaralegume fertilizer only more effective with storage. For fresh groundnuts, Yaralegume fertilizer only (T_2) treated plots produced samples that were less concentrated with aflatoxin in the range of 3.59 – 11.24 ppb. This treatment (T_2) in terms of protective effect is followed by the control (T_1) treatment (5.44 – 7.84 ppb) with the combined

ISSN: 2278 – 7798

effect of Yaralegume and Humate Green OK (T_3) treated plots producing samples with concentration in the range of 5.46 to 13.21 ppb.

Table 1: Aflatoxin Concentration in Fresh and Stored Groundnuts

Sample Identity		Aflatoxin Concentration (ppb)	
A	**B**	**Fresh Groundnuts**	**Stored Groundnuts**
HAT_1	HAT_1S	$7.84^d \pm 0.37$	$93.43^a \pm 0.42$
HAT_2	HAT_2S	$5.60^e \pm 0.41$	$11.00^{fg} \pm 0.10$
HAT_3	HAT_3S	$13.21^a \pm 0.69$	$14.10^e \pm 0.33$
KGT_1	KGT_1S	$7.43^d \pm 0.38$	$52.92^b \pm 0.41$
KGT_2	KGT_2S	$3.59^g \pm 0.57$	$14.71^{de} \pm 0.54$
KGT_3	KGT_3S	$9.18^c \pm 0.28$	$10.88^g \pm 0.20$
KOT_1	KOT_1S	$5.44^e \pm 0.03$	$17.34^c \pm 0.33$
KOT_2	KOT_2S	$4.20^{fg} \pm 0.42$	$10.43^g \pm 0.64$
KOT_3	KOT_3S	$5.46^e \pm 0.04$	$11.94^f \pm 0.98$
SAT_1	SAT_1S	$8.93^c \pm 0.28$	$10.43^g \pm 0.28$
SAT_2	SAT_2S	$11.24^b \pm 0.10$	$17.83^c \pm 0.25$
SAT_3	SAT_3S	$4.58^f \pm 0.11$	$15.62^d \pm 0.37$
LSD (0.05)		0.799	1.001
CV(%)		5.1	2.0

HA= Hiinneteng, KG= Konguoli, KO= Korro and SA= Samoa. A = Fresh groundnut samples and B = Stored Groundnuts samples. ^{a,b,c,d,e,f} *Means that do not share a letter are significantly different ($p < 0.05$).*

B. *Effect of Fertilization on Pre-harvest Aflatoxin Concentration in Groundnuts*

Application of fertilizers had an impact on the levels of aflatoxin as shown in figure 1 and 2. The Yaralegume only fertilizer (T_2) treatment reduced aflatoxin concentration by 28.57 % in Hiinneteng community, 51.68 % in Konguoli community and 22.79 % in Korro community. The reductions in Hiinneteng and Konguoli communities were respectively greater than 28 % and 42 % reduction achieved by Waliyar *et al.*[4] when they used cereal crop residues and Farmyard Manure (FYM) as treatments against pre-harvest aflatoxin infection. However, the combined effect of Yaralegume and Humate Green OK (T_3) could not reduce pre-harvest aflatoxin infection but rather increased it by 40.65 % in Hiinneteng community, 19.06 % in Konguoli community and 0.37 % in Korro community. Contrary to the above results, pre-harvest infection of groundnuts in the Samoa community reduced by 48.71 % in the Yaralegume and Humate Gren OK (T_3) treated plot and increased by 20.55 % in the Yaralegume Only (T_2) treated plot. In the absence of Humate Green OK, only Yaralegume fertilizer treatment (T_2) reduced pre-harvest aflatoxin infection in 3 out of the 4 communities in the range of 22.79 – 51.68 % whilst in combination (Yaralegume Humate Green OK), prehavest infection increased in the range of 0.37 – 40.65 %. This could be attributed to the fact that when fertilizers (organic and inorganic) are combined and introduced to the soil, a biological process called mineralization takes place where organic substances are converted to plant available inorganic forms [11].

C. *Effect of Fertilization and Storage on Post-harvest Aflatoxin Concentration in Groundnuts*

Aflatoxin concentration increased significantly in all 12 samples tested as presented in Table 1. This was however more pronounced in the samples from the control plots across three (3) out of the four (4) communities. Increased levels of aflatoxin contamination in post-harvest groundnuts samples have been reported [9], [12]. Post-harvest residual protective effect of fertilizers was observed in the samples from all the communities after analyses as shown in Figure 1 and 2. The combined treatment of Yaralegume and Humate Green OK (T_3) was 2.65 % more protective of groundnuts in storage than the Yaralegume only treatment (T_2). However, T_2 and T_3 were 69 % and 69.83 % more protective of groundnuts in storage against aflatoxin infection than the control (No fertilizer) treatment respectively.

ISSN: 2278 – 7798

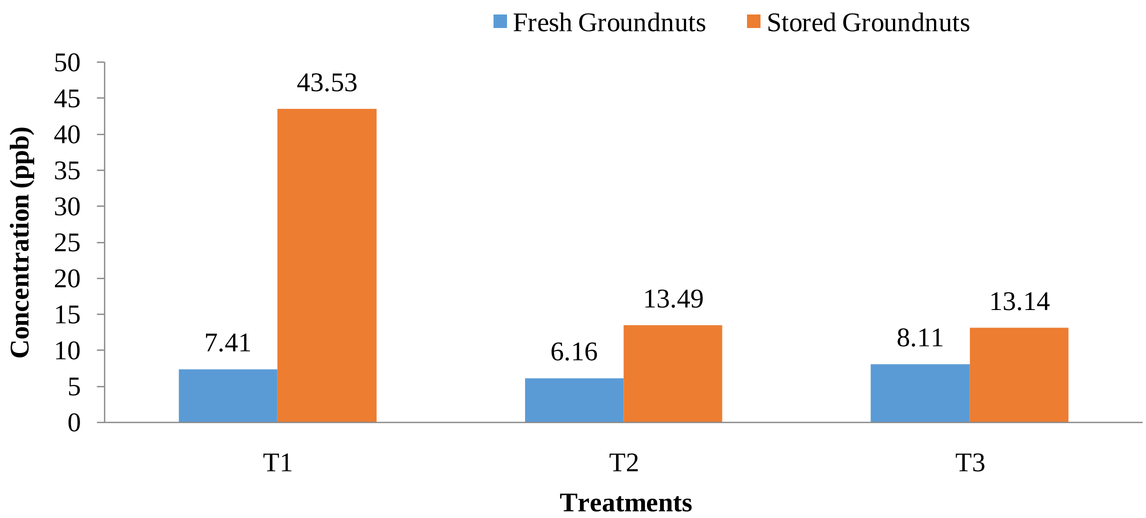

Figure 1: Treatment Effect on Aflatoxin Concentration in Fresh and Stored Groundnuts

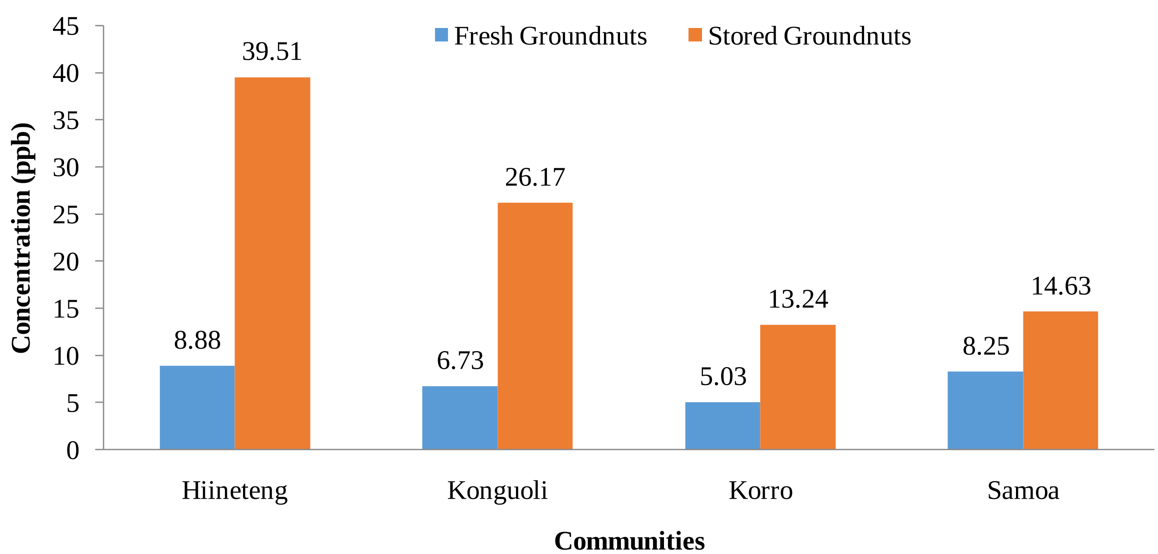

Figure 2: Aflatoxin Concentration of Fresh and Stored Groundnuts in Communities

IV. CONCLUSION

Detectable levels of aflatoxin were obsered in both fresh and stored groundnut samples in all communities. There existed significant difference in pre-harvest and post-harvest aflatoxin concentration as affected by fertilizer treatments. There was a significant increase in concentration with storage. The Yaralegume only treatment as a soil nutrient proved efficient in reducing pre-harvest aflatoxin contamination and providing effective residual protection to groundnuts in storage. The combined effect of Yaralegume and Humate Green OK as fertilizers performed poorly compared to the control of no fertilizer application in reducing pre-harvest aflatoxin concentrations with the former producing groundnut samples with higher aflatoxin concentration. However, Yaralegume and Humate Green OK treatment had a slightly effective residual

ISSN: 2278 – 7798

post-harvest capacity in suppressing aflatoxin increase in groundnuts than the Yaralegume only treatment with the control (no fertilizer) treatment being the least protective. The two fertilizers used proved generally to be effective in reducing pre-harvest and post-harvest aflatoxin concentration levels.

V. REFERENCES

[1] Gong, Y.Y., Cardwell, K., Hounsa, A., Egal, S., Turner, P.C., Hall, A.J., Wild, C.P. (2002). Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. *British Medical Journal* 325, 20-21.

[2] Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A. M., Ambrose Misore, A., DeCock, K. and Rubin, C. (2005). Aflatoxin Contamination of Commercial Maize Products during an Outbreak of Acute Aflatoxicosis in Eastern and Central Kenya. *J. Environ Health Perspect*. 113(12): 1763–1768.

[3] D'mello, J.P.F. (2003). Mycotoxins in cereal grains, nuts and other plant products. In: D'Mello J.P.F. (ed.) *Foods safety: Contaminants and toxins*. Wallingford: Cab International Publishing. pp. 65-88.

[4] Waliyar, F., Reddy S.V., Lava Kumar P., Reddy, B.V.S., Rai, K.N., Ashok, S. and Ravinder, R.C. (2007). Surveillance for natural contamination of mycotoxins in sorghum and pearl millet grains in Andhra Pradesh and Maharashtra states, India. Paper presented (Abstract page 294) in 2nd Asian congress of mycology and plant pathology, 19-22 December 2007, Osmania University, Hyderabad, Andhra Pradesh, India.

[5] Awuah, R. T. and Ellis, W. O. (2001). Effects of some packaging methods and protection with *Ocimum* and *Syzygium* powders on kernel infection by fungi. *Mycopathologia.* 154:29-36.

[6] Cardwell, K.F., and Cotty, P.J. (2000). Interactions among U.S. and African *Aspergillus* spp. strains: Influence on aflatoxin production. *Phytopathology* 90, S11.

[7] Bandyopadhyay, R., Kiewnick, S., Atehnkeng, J., Donner, M., Cotty, P.L. and Hell, K. (2005). Biological control of aflatoxin contamination in maize in Africa. *Proceedings of the Tropentag Conference on International Research for Development,* (11-13 *October 2005, Hohenheim, Germany).*

[8] Abbas, H.K., Zablotowicz, R.M., Bruns, H.A. and Abel, C.A. (2006). Biocontrol of aflatoxin in corn by inoculation with non-aflatoxigenic *Aspergillus flavus* isolates. *Biocontrol Science and Technology*. 16:437-449.

[9] Kladpan, S., Mahakachanakul, W., Yongmanitchai, V., Boonyaratanakornkit, M., and Chinbuti, A. (2004). Situation of aflatoxin contamination in groundnut products in Thailand in 2004. In: Proceedings of the 43rd Kasetsart University Annual Conference, Thailand, 14 February, 2005, pp. 557-564.

[10] AOAC (Association of Official Analytical Chemists). (2007). Official Methods of Analysis. 19th Edition, Association of Official Analytical Chemists, Arlington.

[11] Graham, R.D. (1983). Effect of nutrient stress on susceptibility of plants to disease with particular reference to the trace elements. *Adv. Bot. Res*. 10:221-276.

[12] Kaaya, A.N., Eigel, W and Harris, C. (2006) 'Groundnut Aflatoxin Levels on Farms and in Markets of Uganda in *Groundnut Science*, vol. 33.

ISSN: 2278 – 7798