Nig. J. Biotech. Vol. 35 No. 2 (2018): 130-138
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org
DOI: https://dx.doi.org/10.4314/njb.v35i2.16

Evaluation of the aflatoxin contamination and proximate composition of groundnut (*Arachis hypogea* L.) infected by *Aspergillus* spp.

***Ekhuemelo, C. and Abu, S. O.**

Department of Crop and Environmental Protection, Federal University of Agriculture, P.M.B. 2373 Makurdi, Benue State, Nigeria.

Abstract

Aflatoxins B_1 concentration and the proximate composition of groundnuts consumed in six Local Government Areas (LGAs) of Benue State in the Southern Guinea Savannah agro- ecological zone of Nigeria were evaluated. Seed health testing was conducted and associated fungi were identified. Aflatoxin B_1 (AfB_1) produced by *Aspergillus flavus* were detected and the concentrations quantified using the Enzyme Linked Immunosorbent Assay (ELISA) method. Proximate content of seeds was determined using standard methods of Official Analytical Chemists. *Aspergillus flavus* Link and *Aspergillus niger* van Tiegh were the associated fungi found in contaminated groundnut seeds with 15.67 % and 7.67 % occurrence respectively. A combination of both fungi had 4.67 % occurrence. The proximate content of groundnut seed samples ranged from 0.35 % to 40.21 % across the samples analyzed. The proximate analysis indicated that groundnut samples from Agatu LGA had significantly (P □ 0.05) higher protein and fat content of 29.11 % and 40.21 % respectively compared with samples from other LGAs samples. Groundnut samples from Makurdi LGA had significantly (P □ 0.05) higher ash content while samples from Ohimini recorded significantly (P □ 0.05) higher crude fibre. The highest moisture content (9.37 %) was from groundnut seeds from Otukpo LGA. The contamination of the groundnut samples by AfB_1 resulted in a significant (P □ 0.05) reduction in the nutritional quality of the groundnut seeds.

Keywords: Aflatoxin B_1, groundnut, seeds, quality, contamination.

*Corresponding author Email chiekhuemelo@gmail.com

Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed and cash crop for farmers of the arid and semi-arid tropics (Achola, et. al. 2017). Groundnut seeds are consumed boiled, roasted, as paste, or the oil extracted for cooking. The oil is used to make margarine and mayonnaise. Groundnut is an important ingredient in confectionary products (such as peanut butter, groundnut cake, cookies and snack nuts) and groundnut soup (Ibrahim et. al. 2013). Groundnut shells are used as fuel in local communities, as soil amendment and as livestock feed (Ibrahim et. al. 2013).

Groundnut is grown in countries located between 40° North and South of the equator (Ajeigbe et. al. 2014). Nigeria is the largest groundnut producer in West Africa contributing about 10 % of total global production (Ajeigbe et. al. 2014). Benue State which is known as the food basket of Nigeria grows groundnut as one of the cash crops. Although groundnut production in Benue State, Nigeria is a profitable business, its production has been hampered by the presence of plant pathogens. These pathogens utilize the nutrients in the groundnut for growth and produces mycotoxins at moisture content levels above 10 % (Aliyu and Kutama, 2007; Ani et. al. 2013; Ajeigbe et. al. 2014).

Aspergillus flavus contaminates groundnut seeds on the field through the soil, flowers, insect damage or during storage under favourable moisture content (Diener et. al. 1987).

Mycotoxins are fungal secondary metabolites that contaminate food and feedstuff especially cereals and legumes (Atanda et. al. 2013). Mycotoxins pose health threats when consumed by man and animals. Aflatoxin is the mycotoxin produced by *Aspergillus flavus* and *A. parasiticus* through the polyketide pathway (Smith and Moss, 1985). *Aspergillus flavus* is a major fungus infecting stored grains such as wheat, peanuts, soybeans, corn, groundnut and oil foods (CDC, 2004). *Aspergillus flavus* is known to be the main producer of aflatoxin in stored produce (Rodrigues et. al. 2007). Aflatoxin contamination of groundnut seeds contribute to the nutritional decline of groundnut quality resulting in reduced food security in sub-Saharan Africa (Emmott, 2013). Aflatoxin B1 (AfB_1) is a major aflatoxin found in food stuff reported to induce hepatocarcinogenicity and hepatitis B viral infection in humans (Sweeney and Dobson, 1998; Lee et. al. 2000; Lee et. al. 2004; Jonathan et. al. 2011). Aflatoxin is also reported to cause genotoxicity in reproductive and blood cells (Fapohunda et. al. 2008; Ezekiel et. al. 2011). Significant correlation was earlier established between aflatoxin intake and liver cancer in Swaziland (Richard et. al. 1993). The US Centers for Disease Control and Prevention estimated that 4.5 billion people are chronically exposed to aflatoxin through the consumption of staple foods (Emmott, 2013). Williams (2011) reported that about 40 % of commodities in African markets had aflatoxin levels above the acceptable international standard level of 10 – 20 ppb. About 450 million US dollar is reportedly lost yearly by African groundnut exporters as a result of aflatoxin contamination (Rijsberman, 2014).

Aflatoxin testing is necessary to reveal levels of aflatoxin contamination entering local food systems through the markets and is necessary in the search for control measures since consumption of contaminated groundnut could be detrimental to human health. There is dearth of information on aflatoxin contamination levels in market-stored groundnut in the central agricultural zone of Benue State and Makurdi the Benue State Capital where large quantities of groundnut are cultivated and sold. This study investigated aflatoxin contamination in stored groundnut and the nutritional content of contaminated groundnut from six Local Government Areas of Benue State Nigeria known for large scale groundnut production.

Materials and Methods

Study area

The study was conducted in Apa, Agatu, Okpokwu, Ohimini, Otukpo LGAs located within the Central agricultural zone and Makurdi the Benue State capital. Benue State is located between latitude 8°-10° N and longitudes 6° - 8° E in the Southern Guinea Savannah Agro-ecological zone of Nigeria. The State shares boundary with other States of Nigeria and Cameroon: Nasarawa State to the North, Taraba State to the East, Cross - River State to the South, Kogi State to the West and Cameroon Republic to the South East of Nigeria (Figure 1).

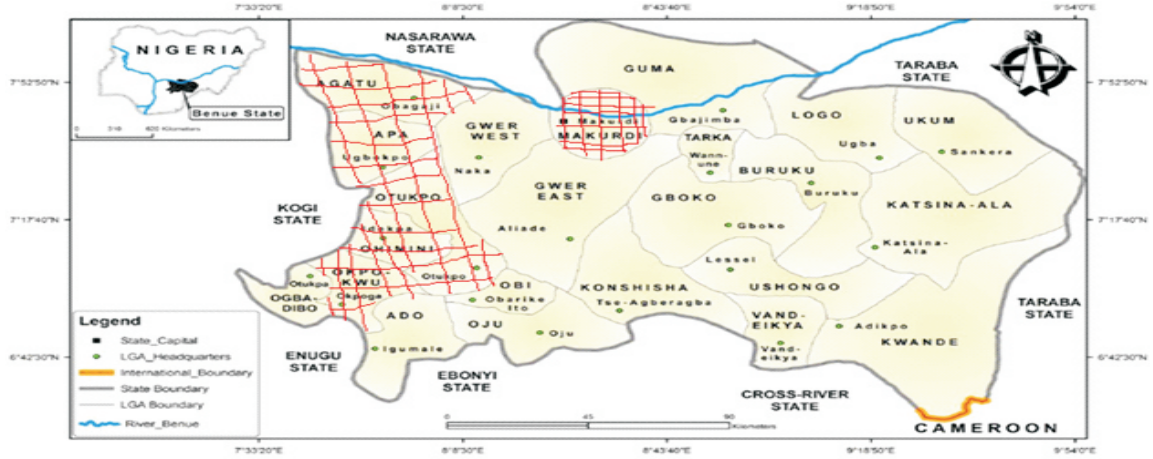

Figure 1: Map of Benue State showing study area

Sample collection and preparation for analysis

Two common groundnut accessions ('Cameroun' and 'Olamu') were collected from the six LGAs and screened for the presence of fungi and aflatoxin AfB_1. A total of fifty four groundnut samples of unshelled groundnut pods stored for between one and two years were collected from wholesalers and retailers in June 2017 from eighteen markets (three markets in each LGA) where groundnut is sold. The samples were bulked together and taken to the laboratory in sterile polyethene bags for aflatoxin and nutritional content analysis. The seeds were shelled by hand, placed in dry containers at ambient temperature before use in each of the experiments.

Evaluation of Seed Infection

Seed health testing was done by blotter method. The groundnut seeds were sterilized in a 10 % Sodium hypochrorite solution (NaOCl) for one minute. One hundred seeds were used for each LGA giving a total of 600 seeds. Ten seeds were placed in a Petri dish containing moist double layer filter paper. After seven days, the mycelium growing out of the seeds were inoculated into fresh Petri dishes and incubated for seven days to obtain pure cultures. Fungal growth was identified by preparing slides and viewing spores under compound microscope (X40 magnifications). Isolated fungi were identified using a reference manual (Watanabe, 2010). Infection levels were recorded as the percentage of infected seeds in a sample. The experiment was laid out in a completely randomized design with ten replicates.

Detection of Aflatoxin B1

Aflatoxin (AfB_1) quantity present in the groundnut samples was detected using the Enzyme Linked Immunosorbent Assay (ELISA) method at the Aflatoxin laboratory of the Department of Crop and Environmental Protection of the Federal University of Agriculture, Makurdi, Nigeria.

One hundred grams of groundnut seeds from each LGA were blended separately. Twenty grams (20g) of each of the blended groundnut seeds were mixed with 100 ml of 70% methanol containing 5g Potassium chloride in Waring Commercial blender until homogeneous. The extract was transferred into a 250 ml conical flask and placed on an orbital (Model ORBI-Shaker) shaker for 30 minutes. The extract was filtered using Whatman No 1 filter paper and diluted in 1:10 phosphate buffer saline in Tween-20 (1 ml of extract and 9 ml of buffer). The setup was left to stand for 10 hours after which analysis of each sample was done.

Levels of Aflatoxin B1

The determination of the Aflatoxin B1 levels in groundnut samples was done by adding 1.5 µl of Aflatoxin B1- Bovine Serum Albumin (AfB_1-BSA) to 15ml of carbonate Buffer, mixed using a vortex and the mixture poured into wells and incubated for one hour at 37°C. One hundred and fifty microlitres (150µl) of BSA was added into all the wells and incubated for 30 minutes. A mixture of BSA (1ml) and AfB1 standard (2.5 µl) was vortexed and 100 µl poured into Standard wells. Six millilitre of BSA was mixed with 1 µl of Anti- Serum. Fifty microlitre (50µl) of the mixture was pipetted into both the Standard and sample wells. Ninety microlitre (90 µl) of BSA and 10 µl of the initial sample extract were added into all the sample wells. One hundred microlitre (100 µl) of the diluted Anti-Serum was added to some of the Standard wells and incubated for an hour.

Ten millilitre (10ml) of BSA was mixed with 2.5 µl of Anti- rabbit and 150 µl of the mixture poured into all the wells and incubated for 1 hour for 37°C. Finally, 150 µl of a mixture obtained by dissolving fifteen milligrams (15mg) of PNPP in 30ml of 10% diethanolamine at pH 9.8 was poured into all the wells, incubated for 20 minutes and AfB_1 levels quantified using a spectrophotometer.

Determination of proximate composition

Samples of the infected and uninfected groundnut seeds were analyzed for moisture, ash, crude fibre, crude protein, crude fat and oil using the methods of AOAC (1990).

Determination of Ash Content

A silica dish with lid was ignited in a muffle furnace at 600 °C for 15 minutes. It was then cooled in a desiccator and weighed. Two grams of finely grounded dried groundnut sample was placed into the pre - weighed dish. The weight of the dish and the sample was taken

The dish and its content was charred on a heater in a fume cupboard until smoking ceased. The dishes were then placed in a muffle furnace and heated at 550°C for 16 hours until a whitish grey ash was obtained. The dishes were again placed in desiccators to cool at room temperature and each dish containing the sample ash was weighed and the weight of ash was calculated using the formula:

%Ash = c-a/b x 100.....Equation 1

Where

a = weight of empty dish

b = weight of dish divided by sample before ashing

c = weight of dish divided by ash

Determination of Crude Fibre

Three grams of sample was weighed and oil extracted in a Soxhlet unit with petroleum spirit and decanted three times. The extracts were air dried and transferred to 1litre conical flask. Two drops of an anti - foam and twenty milliliters of dilute Sulphuric acid (0.128M) was mixed and boiled gently for 30 minutes then allowed to stand for one minute. The sample-acid mixture was poured through a Buchner funnel fitted with Whatman No 1 filter paper. Boiling water was then poured into the funnel and drained by suction. The insoluble matter was washed with boiling water until free from acid, then further washed with 200ml of 0.313M Sodium hydroxide solution and boiled for 30 minutes. The mixture was allowed to stand for one minute and filtered through the filter in the funnel. Thereafter it was washed successively with boiling water and 1% hydrochloric acid until the washings were neutral to litmus. The insoluble matter was washed twice with alcohol, thrice with acetone and then transferred to a crucible. It was then dried at 100 °C to a constant weight, cooled in a desiccator, weighed, ashed in a muffle furnace at 550° C for 1hour and then cooled and weighed again. The percentage crude fibre was then calculated using the formula:

%Crude fibre = a-b/c x 100.....Equation 2

Where

a = weight of insoluble matter

b = weight of ash

c = weight of sample

Determination of moisture content

Two grams of thoroughly mixed groundnut samples were transferred to a pre - weighed empty dish and lid and the dish reweighed with its content. The lid was removed and the dish placed in an oven at 100°C for 6 hours. The dish containing the dried sample was removed from the oven, the lid replaced, cooled in a desiccator for one hour and reweighed. The percentage moisture content was then calculated using the formula:

% Moisture content = a-b/c x 100.....Equation 2

Where

a = weight before drying

b = weight after drying

c = weight of sample taken

Determination of crude protein

Few glass beeds (anti-bump) were placed into the number of Kjeldahl flasks to be used after they have been washed, rinsed and dried and 8 g of Copper Sulphate added. Two grams (2g) of sample was weighed and transferred into the Kjeldahl flask, 30 ml of conc. Sulphuric acid was added and gently swirled. The flask was placed on a heating device in the fume chamber; gentle heating was applied until initial frothing ceased. The sample was heated strongly until the solution became light blue-green in colour. The sample was further heated for another 1 hour, cooled to about 40°C and 100 ml distilled water added, allowed to cool and transferred into a 250 ml volumetric flask. The markham semi-micro distillation unit was steamed for 20 minutes and 5 ml of sample digest pipetted into the unit, 7 ml of sodium hydroxide was then added. The unit was closed and the ammonia steam-distilled into 5 ml boric acid indicator mixture, about 20 ml of distillate was collected, titrated with 0.1 M hydrochloric acid or 0.05 M Sulphuric acid until the green colour changed to purple.

1ml of 0.1ml Cl or 0.05 M H_2SO_4 = 0.0014gN

Percentage Nitrogen (crude protein) was calculated using the formula:

% N = Titre × 0.0014 × 100/ 2 (sample weight).

Analysis of data

The data generated from these investigations were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS, 2009). The test of significance was carried out using Fishers Least Significant Differences (FLSD) at 5% level of probability.

Results

Data presented in Table 1 shows the fungi isolated from groundnut seeds from six LGAs of Benue State. Groundnut seeds were significantly (P∪ 0.05) infected by *Aspergillus flavus* in 15.67 % of the samples, 7.67 % of the samples were infected by *A. niger* alone while 4.67% of the samples were infected by a combination of *Aspergillus flavus* and *A. niger*.

Table 1: Incidence of *Aspergillus* spp isolated from groundnut samples from six LGAs of Benue State.

Fungi	Incidence (%)
A. flavus	15.67
A. niger	7.67
A. flavus/A. niger	4.67
LSD (0.05)	4.71

Table 2 shows the aflatoxin AfB_1 concentration detected in stored groundnut seeds from six LGAs of Benue State. The concentration of AfB_1 detected in groundnut seeds was significantly higher (P= 0.05) in Okpokwu LGA (11.02 µg Kg^{-1}) compared with a concentration of 5.92 µg Kg^{-1} detected in groundnut seeds from Otukpo LGA. There was no significant differences (P = 0.05) between the AfB_1 concentrations detected in groundnut seeds from Apa (8.29 µg Kg^{-1}), Agatu (8.62 µg Kg^{-1}), Ohimini (9.71 µg Kg^{-1}) and Makurdi (10.41 µg Kg^{-1}). The highest aflatoxin contamination was found in Okpokwu LGA while the lowest contamination was from Otukpo LGA.

Table 2: Aflatoxin (AfB_1) concentration in groundnut seeds from six Local Government Areas of Benue State.

Local Government Area	**AFB1 levels** (µg Kg^{-1})
Agatu	8.62
Apa	8.29
Makurdi	10.41
Ohimini	9.71
Okpokwu	11.02
Otukpo	5.92
LSD (0.05)	2.39

The proximate composition of groundnut seeds from the six LGAs is presented in Table 3. The protein content was significantly (P∪ 0.05) higher in groundnut seeds from Agatu (29.11%), Otukpo (29.10%), Makurdi (27.13%) and Okpokwu (26.69%) LGAs compared with Ohimini (26.03%) and Apa (25.38%) LGAs. Significantly (P∪ 0.05) higher fats and oil content were recorded from groundnut seeds from Agatu (40.21%), Apa (39.81%) and Okpokwu (39.76%) compared with groundnut seeds from Ohimini (38.62%) and Makurdi (38.12%) LGAs. Crude fibre was highest in groundnut seeds from Ohimini (1.19%) and lowest in groundnut seeds from Makurdi (0.35 %) LGA. The moisture content of groundnut seeds was lowest in seeds from Agatu LGA (8.10%) and highest in Otukpo LGA (9.37%). The ash content was significantly (P∪ 0.05) lower in groundnut seeds from Otukpo LGA (2.10 %) compared with other LGAs sampled.

Table 3: Proximate composition of stored groundnut seeds from Six Local Government Areas of Benue State, Nigeria.

Local Government Area	Proximate Composition (%)				
	Protein Content	Fats and Oil content	Ash content	Moisture Content	Crude Fibre Content
Agatu	29.11	40.21	2.42	8.10	0.57
Apa	25.38	39.81	2.42	8.65	0.79
Makurdi	27.13	38.12	2.58	8.37	0.35
Ohimini	26.03	38.62	2.52	9.03	1.19
Okpokwu	26.69	39.76	2.45	8.58	0.60
Otukpo	29.10	39.14	2.10	9.37	0.56
LSD (0.05)	0.52	0.68	0.17	0.14	0.10

Data of the proximate composition of groundnut seeds based on their infection status with *Aspergillus* spp is presented in Table 4. The non -infected groundnut seeds had significantly (P= 0.05) higher proximate values for crude protein, crude fibre, fats and oil compared with the infected groundnut seeds. Moisture content of 7.00% was recorded in the non-infected groundnut seeds and this was significantly (P∪ 0.05) lower compared with the infected groundnut seeds with a moisture content of 10.37%. There was no significant difference (P ∪ 0.05) between the ash content of the infected and non- infected groundnut seeds.

Table 4: Proximate composition of infected and non- infected groundnut samples

Status	Proximate composition (%)				
	Crude Protein	Fats and Oil	Ash	Moisture content	Crude fibre
Infected	25.45	35.98	2.41	10.37	0.52
Non-infected	29.03	42.56	2.41	7.00	0.82
LSD(0.05)	0.30	0.39	NS	0.08	0.06

Discussion

The study revealed the presence of *A. flavus* (15.67 %), *A. niger* (7.67 %) and the combination of *A. flavus* and *A. niger* (4.67 %) in the study area. The fungi isolated from groundnut seeds in this study have previously been reported as pathogens infecting various crops including groundnut (Aliyu and Kutama, 2007; Rodrigues et. al. 2007; Diedhiou et. al. 2014: Ekhuemelo and Yaaju, 2017). The isolation of *A. flavus* and *A. niger* in this study is in line with the report of Muhammad et al. (2004) in which the two *Aspergillus* spp had the highest rate of occurrence among the isolated fungi. Diener et al. (1987) observed the competitive relationship between *A. flavus* and *A. niger*. The prevalence of *A. flavus* in this study agrees with the report of Mensah and Owusu (2012) which also reported *A. flavus* as the most dominant fungal species occurring on

okra, tomato and pepper fruits from Accra, Ghana. Similarly, Ahene et al. (2011) and Ezekiel et al. (2013) identified *A. flavus* as the most frequently isolated fungal species in spice and spice products from Ghana and Nigeria. Although *Aspergillus flavus* and *A. parasiticus* are reported as the toxigenic strain of the genera *Aspergillus* that contaminates groundnut by the production of aflatoxin (Vabi et. al. 2016), Al-Abdalall (2009) reported aflatoxin B1 levels of 139.5 ppm – 302.0 ppm produced by strains of *A. niger* isolated from field bean in Saudi Arabia.

The AfB_1 concentration detected in groundnut in this study was below the acceptable limit of 20 µg Kg^{-1} for AfB_1 set by the Standards Organization of Nigeria (SON) in all LGAs studied. This result is similar to the findings of Ousman (2015) in which High Performance Liquid Chromatography (HPLC) analyses of AfB1 from groundnut samples from six markets in Central region of Ghana were below the consumption acceptable limit of 20 ppb of Ghana Bureau of Standards.

The low levels of aflatoxin contamination in this study may be attributed to the low moisture content of the groundnut seeds which was not favourable for fungi growth. Kurmar et al. (2002) attributed the predominance of A. flavus on groundnut seeds to improper drying of groundnut on the field. Similarly, Aliyu and Kutama (2007) reported the presence of fungi in groundnut samples collected from market silos from Kano State, Nigeria and attributed their infection to high moisture content. Conversely, Atanda et al. (2013) reported *A. flavus* as a storage fungus that grew on grains with lower moisture content. The low concentration of aflatoxin may also be due to the presence of atoxigenic strains of *Aspergillus* spp. Ahene et al. (2011) also encountered atoxigenic A. flavus strains on spices and spice products in Ghana. However, Samson and Reenen-Hoekstra (1988) observed that the quantity of mycotoxins produced by toxigenic fungi was dependent on environmental factors such as temperature, water activity and oxygen. Oyebanji and Efiuwevwere (1999) also reported significant positive correlation between moisture content and aflatoxin contamination in maize seeds. Atanda et al. (2013) had earlier reported that the ambient conditions in tropical Africa favoured the optimum moisture content for the growth and toxin production of toxigenic fungi accounting for high prevalence of mycotoxins in Africa. Similarly, Villers (2014) reported that post - harvest storage of produce for months in hot humid climate resulted in exponential growth of aflatoxin.

The reduction in the nutritional content of the infected groundnut seeds in the present study agrees with the report of Amusa et al. (2003) and Amusa et al. (2006) in which African Star apple fruits and guava fruits infected by fungi had significant deterioration of nutrient resulting in a significantly lower percentage nutrient composition in infected fruits. Emmott (2013) noted the decline of groundnut quality resulting from aflatoxin incidence. Adelaja (1997) attributed this reduction in nutrient content to the utilization of the nutrients by the fungi for growth and survival.

Conclusion

This study has shown that groundnut seeds from the six Local Government Areas of Benue State were contaminated with *Aspergillus flavus* and *A. niger* singularly and in combination. Groundnut seeds were contaminated with AfB_1 within the acceptable limits of 20µg/kg for grain commodities and foodstuff in Nigeria. This implies that the consumption of groundnut from the study area do not pose a health threat to the population in the study area. However the consumption of groundnut from the study area could result in nutritional deficiencies due to their reduced nutrient content. Interventions are needed to further eradicate contamination thereby preventing nutrient degradation of groundnut in the study area.

References

Achola, E., Tukamuhabwa, P., Adriko, J., Edema, R., Mwale, S.E., Gibson, P., Naveen,P., Okul, V., Michael, D. and Okello, D.K. (2017). Composition and variation of fatty acids among groundnut cultivars in Uganda Afr. Crp. Sci. J. 25(3): 291 – 299.

Adelaja, B.A. (1997). Observations on the pests and diseases of *Chrysophyllum albidum* in Nigeria. Proceedings of a National Workshop on the Potentials of the Star Apple, 27th January,

Nigeria, pp: 117-121.

Ahene, R. E., Odamtten, G. T. and Owusu, E. (2011). Fungal and bacterial contaminants of six spices and spice products in Ghana. Afr. J. of Env. Sci. and Tech. 5(9): 633-640.

Ajeigbe, H. A., Waliyar F., Echekwu C.A., Ayuba K., Motagi B.N., Eniayeju, D. and Inuwa, A. (2014). A Farmer's Guide to Groundnut Production in Nigeria.Patancheru, Telangana, India: International Crops Research Institute for the Semi-Arid Tropics. 36pp.

Aliyu, B. S. and Kutama, A. S. (2007). Isolation and Identification of fungal flora associated with groundnut in different storage facilities. Sci. Wld J. 2(2): 34-36.

Amusa, N. A, Ashaye, O. E. and Oladapo, M. O. (2003). Biodeterioration of proximate composition of African star apple in storage and effect on its food value. Afr. J. of Biotech. 2(3):56-59.

Amusa, N. A., Ashaye, O. A., Amadi, J. and Oladapo, O. (2006). Guava Fruit Anthracnose and the Effects on its Nutritional and Market Values in Ibadan, Nigeria. J. of Appl. Sci. 6: 539-542.

Ani, D. P., Umeh, J. C. and Weye, E. A. (2013). Profitability and Economic Efficiency of Groundnut Production in Benue State, Nigeria. Afr. J. of Food, Agr. Nutr. and Develop. 13(4):1-15.

AOAC (Association of Official Analytical Chemistry) (1990). Official Methods of Analysis.15th edition Association of Official Analytical Chemists, Washington DC, USA.

Atanda, O., Makun H. A., Ogara, I. M., Edema M., Idahor K. O., Eshiett M. E and Oluwabamiwo, B. F. (2013). Fungal and mycotoxin contamination of Nigerian Foods and feeds In: Mycotoxins and Food Safety in Developing Countries.

CDC (Centers for Disease Control and Prevention). (2004). Outbreak of aflatoxin poisoning – Eastern and Central Provinces, Kenya. MMWR, Morb. Mort. Weekly Rep. January-July 53(34): 790-793.

Diedhiou, P. M., Zakari, A. H., Mbaye, N., Faye, R. and Samb, P. I. (2014). Control methods for post-harvest diseases of banana (*Musa sinensis*) produced in Senegal. Int. J. Sci., Env. and Tech. 3(5):1648 – 1656.

Diener, U. L., Cole, R. J., Sanders T. H., Payne, G. A., Lee, L. S. and Klich, M. A. (1987). Epidemiology of aflatoxin formation by *Aspergillus flavus*. Ann. Rev.of Phytopath. 25(1): 249-270.

Ekhuemelo, C. and Yaaju, M. D. (2017). Identification and management of fungi associated with crown rot of banana in Makurdi, Benue State, Nigeria. Nig. J. of Agr., Food and Env. 13(2): 50-55.

Emmott, A. (2013). Aflatoxins: Finding solutions for improved food safety. CGIAR Research program on Agricultural for Nutrition and Health. 2pp.

Ezekiel, C. N., Alabi, O. A., Anokwuru, C. P. and Oginni, O. (2011). Studies on dietary aflatoxin-induced genotoxicity using two in-vivo bioassays. Arc. of Appl. Sci. Res. 3(2): 97–106.

Ezekiel, C. N., Fapohunda, S. O., Olorunfemi, M. F., Oyebanji, A. O. and Obi, I. (2013). Mycobiota and aflatoxin B1 contamination of *Piper guineense* (Ashanti pepper), *P. nigrum* L. (black pepper) and *Monodora myristica* (calabash nutmeg) from Lagos, Nigeria. Int. Food Res. J. 20(1): 111-116.

Fapohunda, S. O., Ezekiel, C. N., Alabi, O. A., Omole, A. and Chioma, S. O. (2008). Aflatoxin mediated sperm and blood cell abnormalities in mice fed with contaminated corn. Myco. 36(4): 255–259.

Ibrahim, U., Ayinde, B. T., Dauda, H. and Mukhtar, A. A. (2013). Socio Economics factors affecting groundnut production in Sabon Gari LGA of Kaduna State, Nigeria. Int. J. of Food and Agr. Eco. 1(1): 41-48.

Jonathan, G., Ajayi, I. and Omitade, Y. (2011). Nutritional compositions, fungi and aflatoxins detection in stored 'gbodo' (fermented *Dioscorea rotundata*) and 'elubo ogede' (fermented *Musa parasidiaca*) from South western Nigeria. Afr. J. of Food Sci. 5(2): 105 – 110.

Kumar, K. V. K., Desai, S., Rao, V. P., Nur, H. A., Srilakshmi, P. and Thakur, R. P. (2002) Evaluation of an integrated management package to reduce pre-harvest seed infection by *Aspergillus flavus* in groundnut. Int. Arachis News. 22: 42-44.

Lee, Y. I. K., Lee, S., Das, G. C., Park, U. S., Park, S.M. and Lee, Y. I. (2000). Activation of the insulin-like growth factor II transcription by aflatoxin B1 induced P53 mutant 249 is caused by activation of transcription complexes; implications for a gain- of – function during the formation of heptatocellular carcinoma. Onco. 19: 3717-3726.

Lee, N. A., Wang, S., Allan, R. D. and Kennedy, I. R. (2004). A rapid aflatoxin B1 ELISA: Development and validation with reduced matrix effects for peanuts, corn, pistachio, and soybeans. J. of Agr. and Food Chem. 52: 2746–2755.

Mensah, J. K. and Owusu, E. (2012). Capsicum annum L. (pepper), *Abelmoschus esculentus* L. Moench (Okra), and *Lycopersicon esculentum* Mill. (tomato) from Accra metropolis. Afr. J. of Food Sci. 6(1):1-7.

Muhammad, S., Shehu, K., Amusa, N. A. (2004). Survey of the market diseases and aflatoxin contamination of tomato (Mill.) fruits in Sokoto, North Western Nigeria. Nutr. and Food Sci. 34: 72-76.

Oyebanji, I. A. O, and Efiuwevwere, B. J. O. (1999). Growth of spoilage mould and aflatoxin B production in naturally contaminated or artificially inoculated maize as influenced by moisture content under ambient tropical condition. Biodegra. 44: 209-217.

Ousman, S. D. (2015). Studies of *Aspergillus flavus* and aflatoxin contaminantion of groundnut (*Arachis hypogaea* L.) from six markets in the central region, Ghana. Master of Philosophy thesis in seed science and technology, University of Cape Coast, Ghana.124pp.

Richard, J. L., Bennett, G. A., Ross, P. F. and Nelson, P. E. (1993). Analysis of naturally occurring mycotoxins in feedstuffs and food. J. of Ani. Sci. 71: 2563- 2574.

Rijsberman, F. (2014). Partnership for aflatoxin control in Africa (PACA) presentation at the global forum for innovations: Middle East and Africa focus (Meridian Institute Washington, DC), Aflatoxin partnership newsletter Vol. II (Assessed August 2018).

Rodrigues, P., Soares C., Kozakiewicz, Z., Paterson, R. R. M., Lima, N., and Venâncio, A. (2007). Identification and characterization of *Aspergillus flavus* and Aflatoxins. Comm. Curr. Res. and Edu. Top. and Trends in Appl. Micro. 527- 534.

Samson, R. A. and Van Reenen-Hoekstra, E. S. (1988). Introduction to Food Borne Fungi. 3rd Ed. Centraalbureau voor Schimmel cultures. Baarn Institute of the Royal Netherlands Academy of Arts and Sciences.

SAS, (2009). Institute Inc. SAS/STAT Guide for personal computers, Version 9.2 edition. SAS Institute Cary, NC. USA.

Smith, J. E. and Moss, M. O. (1985). Mycotoxins: formation, analysis and significance. John Wiley and Sons, New York. p148.

Sweeney, M. J. and Dobson, A. D. W. (1998) "Mycotoxin Production by Aspergillus, Fusarium and Penicillium Species," Int. J. of Food Micro. 43(3): 141-158.

Vabi, M. B., Eche, C. O., Mukhtar, A. A. , Kunihya, A., Alabi, O., Adobe, K., Ekefan, E. J., Haruna, S. G., Motagi, B. N. and Ajeigb, H. A. (2016). Understanding and Managing Aflatoxin Contamination in the Groundnut Value Chain in Nigeria. International Crops Research Institute for the Semi-Arid Tropics. p 9.

Villers, P. (2014). Aflatoxins and safe storage. Frontiers in Micro 5: 158. Watanabe, T. (2010). Pictorial atlas of soil and seed fungi: Morphologies of cultured fungi and key to species, 3rd edition. American Phytopathology Society press. p 440.

Williams, J. H. (2011). Aflatoxin as a public health factor in developing countries and its influence on HIV and other diseases. Peanut collaborative Research support program, University of Georgia, World Bank Report No 60371- AFR, 1- 95.