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(RESEARCH ARTICLE)

Assessment of fungi incidence, seed germination and aflatoxin contamination of groundnut (*Arachis hypogaea* L.) from Lagos, Nigeria.

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Abstract

Groundnut (*Arachis hypogaea* L.) is a legume of high nutritive and market value usually contaminated by aflatoxin. The evaluation of aflatoxin contamination of groundnut entering the food system through the markets is necessary to ascertain the quality of the groundnut sold and subsequently advice groundnut consumers. The study assessed the aflatoxin B_1 (AfB_1) contamination levels in groundnut sold in some markets in Lagos, Nigeria. Groundnut seeds were sampled from five Local Government Areas (L. G.A) of Lagos State namely: Agege, Eti-Osa, Ifako Ijaye, Ketu and Oshodi. The samples were packaged in envelopes and transported to the Mycotoxin laboratory of the Federal University of Agriculture, Makurdi, Nigeria for assessment of the fungi infections, seed germination and aflatoxin contamination levels. The experiment for the assessment of fungi infecting and germination of groundnut seeds was laid out in a completely randomized design in ten replicates. Detection of AfB_1 present in the groundnut samples was done using Enzyme Linked Immunesorbent Assay (ELISA) method in three replicates. Result showed that *Aspergillus flavus* Link was the most frequently isolated fungi with 46 % incidence, *Lasiodiplodia theobromae* Pat. had 20 % incidence, *Aspergillus niger* van Tiegh with 11 % and *Fusarium verticollioides* Sacc. 7 % incidence. The moisture content of groundnut seeds ranged between 7.20 % and 6.53 %. Aflatoxin B_1 concentrations in groundnut samples was highest in Eti Osa LGA (5.11 µg/kg) while Oshodi LGA had the least concentration (3.09 µg/kg). Groundnut samples from the five Local Government Areas of Lagos State were contaminated with AfB_1 at concentration levels below the safe limits for human consumption and health of 20µg/kg. Management of fungi infection on the field is recommended to prevent field to store contamination of groundnut seeds.

Keywords: Groundnut; Aflatoxin; Fungi infections; Market; Contamination; Incidence.

1. Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed and cash crop rich in proteins, fats and minerals. In Nigeria, groundnut is consumed both locally and as export crop to other countries.

It is used for domestic and industrial purposes, cooking, as confectionery product, as snacks together with popcorn popularly called 'guguru ati epa' among the Yorubas of Lagos State, Nigeria. Roasted groundnut is also used for groundnut cakes known as 'kuli kuli', production of groundnut oil used in cooking, for soap production and as body cream [1].

Nigeria is the largest producer of groundnut in West Africa with about 10 % of the global groundnut production [2].Groundnut consumption contributes about ten percent of the calorie intake of Nigerian diets and is also a source of income for farmers in North Central Nigeria [3, 4].

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Groundnuts are regarded as a good substrate for aflatoxin contamination especially when the seeds are not properly dried to moisture content below 10 % and temperature below 25°C before storage[5]. The fungal species causing aflatoxin contamination could penetrate the hard shell of the groundnut to commence biodeterioration processes without necessarily showing any form of mouldiness [6]. This results in microbial biodeterioration of food crops leading to food and economic losses, thereby reducing the export value of crops[6, 7]. Aflatoxin contamination was responsible for the import ban of five commodities including groundnut by the European Union resulting in loss of ₦671.1 billion in import revenue in Nigeria. At a prevalence rate of 20 µg/Kg, the monetized burden of aflatoxin contamination was between $112 and $942 million which was about 0.5 % of Nigeria's GDP in 2010 [3]. Similarly, Nigeria experienced export sanctions as a result of aflatoxin contamination of groundnut and some other commodities in 2016 [8].

Aflatoxin B_1 which is the most common and most toxic mycotoxin hazardous to humans and animals worldwide is mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus*[8]. Aflatoxins in human diet may lead to cancer, liver diseases, hepatocarcinogenicity, hepatitis B viral infection, immunosuppression interference, stunted growth and may lead to death [9, 10, and 11]. Aflatoxin contamination is responsible for the suppression of human and animal immune system and can lead to increase in the viral load of HIV and AIDS patients [4]. Fatal outbreaks of toxicities resulting from aflatoxins have been reported in Nigeria and elsewhere in Africa [12, 13].

Although groundnut is not commonly cultivated in Lagos State Nigeria, it is on popular demand and it is usually consumed together with popcorn as a delicacy and snack. There is low awareness and paucity of information on aflatoxin B_1 contamination of groundnut sold in some local markets in Lagos, Nigeria. This has necessitated the assessment of fungal and aflatoxin contamination of locally consumed groundnut to ascertain its safety for consumption. This study was therefore aimed at assessing the fungi incidence, seed germination and aflatoxin B_1 contamination levels in groundnut sold in some markets in five LGAs of Lagos State, Nigeria.

2. Material and methods

2.1. The study area

The study was conducted in selected markets in five out of the twenty LGAs of Lagos State where groundnut is sold namely: Agege (Agege market), Eti Osa (Ajah market), Ifako Ijaye (Abule Egba market), Ketu (Mile 12 International market), Oshodi (Arena market). Lagos State is located in the South West of Nigeria between longitude 3°23'- 3°39' E and latitude 6° 27'- 6° 45' N 11m above sea level. Lagos State shares borders with Ogun State on the North and East, the Atlantic Ocean in the South and the Republic of Benin on the West.

2.2. Sample collection and preparation for analysis

Forty five ~~groundnut~~ shelled groundnut seed samples were collected from nine marketers in each of the five LGAs of Lagos State in June 2018.The samples were packaged in envelopes and transported to the Mycotoxin laboratory of the Federal University of Agriculture, Makurdi, Nigeria for assessment of the seed germination, fungi incidence and aflatoxin contamination levels.

2.3. Seed health testing and germination

Seed health testing was done by blotter method. The experiment was a completely randomized design (CRD) with ten replicates. The groundnut seeds were sterilized in 10% sodium hypochlorite solution for one minute. One hundred seeds were used for each LGA giving a total of 500 seeds. Ten seeds were placed in a 9cm 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 organisms were identified by preparing slides and viewing spores under compound microscope (x40 magnifications) for the presence of fungi. Isolated fungi were identified using reference manual [14]. The number of germinated and infected seeds was recorded for each LGA.

2.4. Detection of Aflatoxin B_1 on groundnut seeds

Detection of Aflatoxin (AfB_1) quantity present in the groundnut samples was done using the ELISA method at the Aflatoxin laboratory of the Department of Crop and Environmental Protection of the Federal University of Agriculture, Makurdi, Nigeria. The experiment was laid out in a completely randomized design with three replications.

2.5. Detection and quantification of Aflatoxin (AfB_1) from groundnut seed samples.

Detection of Aflatoxin (AfB_1) quantity present in the groundnut samples collected from the study area was done using the Enzyme Linked Immunosorbent Assay (ELISA) method.

Twenty grams (20g) out of one hundred grams (100 g) of blended groundnut seed samples obtained from each LGA were triturated with 100 ml of 70% methanol containing 5g Potassium Chloride in Waring Commercial blender to make a homogeneous mixture. The resulting extract was transferred into a 250 ml conical flask and shaken on an orbital (Model ORBI-Shaker) shaker for 30 minutes and filtered using Whatman No 1 filter paper. It was then diluted in 1:10 phosphate buffer saline in Tween-20 (1 ml of extract and 9 ml of buffer) and left to stand for 10 hours and analyzed.

The determination of aflatoxin contamination of groundnut samples was done by adding 1.5 µl of AfB_1-BSA congugate to 15ml of carbonate Buffer, which was vortexed 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 AfB_1 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 pipette 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 1hour for 37 °C. One hundred and fifty microlitre (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 twenty minutes and AfB_1 levels quantified using a spectrophotometer.

2.6. Data Collection

Aflatoxin contamination was quantified in microgram per kilogram (µg/Kg) using a spectrophotometer. Data on percentage germination was recorded as the number of germinated seeds relative to the total number of seeds plated expressed as a percentage at 5, 6 ,7 and 10 days after incubation (DAI). Percentage infection was recorded as the number of infected seeds relative to the total number of seeds incubated expressed as a percentage.

2.7. Data Analysis

Data were subjected to analysis of variance using GenStat version 9.0 statistical software. Significant means were separated using Fishers least significant difference (F-LSD) at 5% level of probability.

3. Results

Table 1 shows the aflatoxin B_1 concentration and moisture content of groundnut samples from five LGAs of Lagos State, Nigeria. There was no significant difference ($P > 0.05$) in the moisture content of groundnut samples from the five LGAs with the moisture content of groundnut samples ranging between 6.53 and 7.20% across the LGAs tested. The concentration of AfB_1 detected in groundnut seeds was significantly higher ($P < 0.05$) in Eti osa LGA with 5.11 µg/Kg and Ketu LGA with 5.09 µg/Kg compared with Ifako (4.22 µg/Kg), Agege (4.12 µg/Kg) and Oshodi with the lowest concentration of 3.09 µg/Kg.

Table 1 Aflatoxin contamination and moisture content of groundnut samples from five LGAs of Lagos State, Nigeria

LGA	Aflatoxin B_1 (µg/Kg)	Moisture Content (%)
Agege	4.12	6.97
Eti osa	5.11	6.87
Ifako	4.22	6.53
Ketu	5.09	6.83
Oshodi	3.09	7.20
LSD (0.05)	0.02	NS

NS= Not significant

The interactive effect of incidence of fungi and the five LGAs of Lagos State from which they were isolated is presented in Table 2. The incidence of *Aspergillus flavus* was significantly ($P < 0.05$) higher with a mean incidence of 46.00 % compared with the incidence of *L. theobromae* (20.00 %)*, A. niger* (11.00 %)and *F. verticollioides* with the least mean incidence of 7.00 %. Although location had no significant ($P >0.05$) effect on the incidence of fungi on groundnut seeds, Agege LGA had the highest mean fungi incidence of 25.00 % followed by Oshodi 23.75 %, Eti Osa 21.00 %, Ketu 18.00 % and Ifako Ijaye with the least mean incidence of 17.25 %.

Table 2 Interactive effect of fungi incidence on groundnut seeds and the five Local Government Areas of Lagos State sampled.

Location/Fungi	*A. flavus*	*A. niger*	*L. theobromae*	*F. verticollioides*	Location mean
Agege	43.00	15.00	29.00	13.00	25.00
Eti Osa	24.00	15.00	36.00	9.00	21.00
Ifako Ijaye	41.00	15.00	11.00	2.00	17.25
Ketu	54.00	8.00	5.00	5.00	18.00
Oshodi	67.00	0.00	21.00	7.00	23.75
Fungi Mean	46.00	11.00	20.00	7.00	

LSD (0.05); Location = NS; Fungi = 7.4; Location x Fungi = 16.5

The percentage infection of groundnut seeds from the five sampled locations is shown in Figure 1. The fungal infection of groundnut seeds was not significantly different($P > 0.05$) at 4 and 5 days after incubation across the five LGAs with groundnut samples from Ifako Ijaye recording the least fungal infection of 20.00 % and 34.6 % infection respectively for the two days.

At 6 and 7 days after incubation (DAI) the groundnut seeds, groundnut samples from Ifako Ijaye had significantly ($P < 0.05$) lower infection (37.9 %) compared with the groundnut samples from all other locations which were statistically similar.At 10 DAI, the percentage infection of groundnut samples was not significantly different($P > 0.05$) however samples from Agege LGA had the least percentage infection of 44.20 % followed by samples from Ifako Ijaye with 50.00 % infection, Ketu recorded 84.60 % infection, Eti Osa recorded 90.80 % infection while Oshodi LGA recorded the highest fungal infection of 95.40 %.

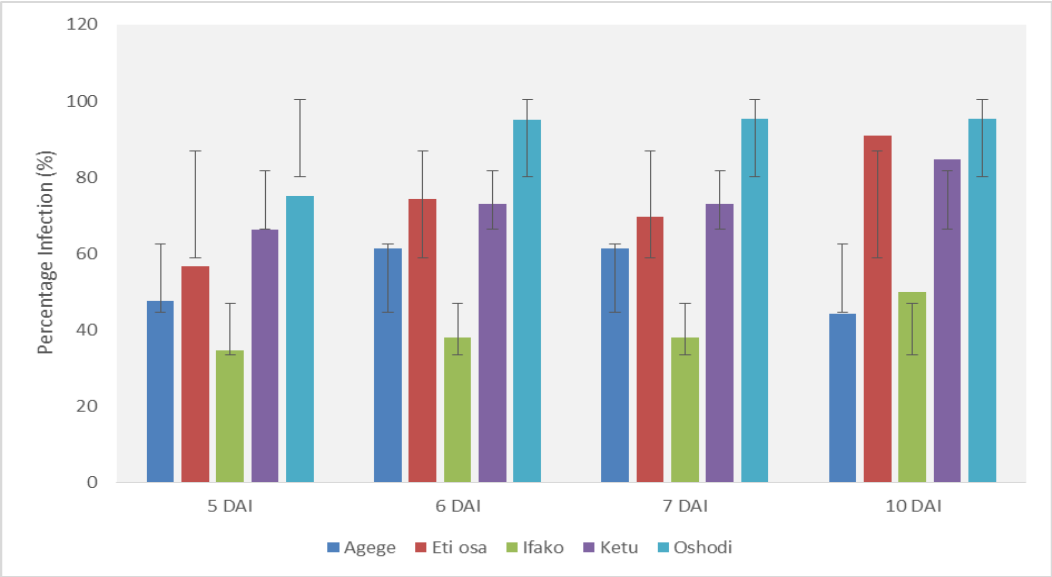

Figure 1 Percentage infection of groundnut seeds from five LGAs of Lagos State.

Figure 2 shows the percentage germination of groundnut seeds from the five LGAs of Lagos State. The figure shows a decline in seed germination across the LGAs during the period of incubation. Seed germination was highest in seed samples from Ifako Ijaye LGA which had 77.5 % of seeds germinated 4 DAI and this reduced to 50.0 % at 10 DAI while the least germination of groundnut seeds were recorded in seeds from Oshodi LGA with 36.2 % reducing to 4.6 % 10 DAI.

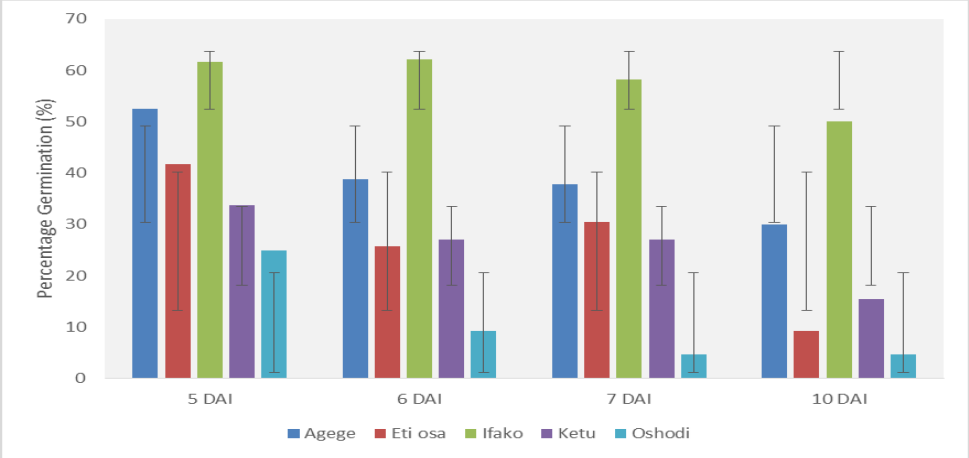

Figure 2 Percentage germination of groundnut seeds from five LGAs of Lagos State.

4. Discussion

The present study recorded moderate fungi incidence and low AfB_1 concentration with *Aspergillus flavus* recording the highest incidence. This finding agrees with the report of [15, 16] which identified *Aspergillus* genera as the prevalent fungal genera infecting groundnut seeds. The report of [17] observed that infection and contamination of groundnut seeds by *Aspergillus* can occur either on the field or at post-harvest. [18] reported the susceptibility of seeds stored at high temperature and relative humidity to fungal infection and mycotoxin contamination. Similarly [12] reported *Aspergillus* to be typical storage fungi that grow in relatively dry conditions. [19] reported that *Aspergillus* and *Fusarium* apart from being toxic to humans and animals, primarily affects seed quality, viability, germination and seedling vigour. The presence of these fungi may result in the development of plant disease symptoms when infected seeds are planted. Continuous cropping of groundnut on a piece of land for many seasons is also reported to affect the incidence of *A. flavus* and consequently the amount of aflatoxin produced on groundnut harvested from such plots [12].

Aspergillus flavus is known to be the main producer of aflatoxin which are carcinogenic [20]. The fungus has the ability to produce a number of enzymes which enables the fungus to develop and produce toxin on a wide variety of stored grains such as wheat, peanuts, soybeans, corn, groundnut and oil foods [21]. *Aspergillus flavus* isolates is reported to produce aflatoxins B_1, B_2 and cyclopiazonic acid (CPA), with some strains producing G_1 and G_2 aflatoxin [22]. Aflatoxins in foods are converted to aflatoxin-8, 9-epoxide metabolite in the liver which may be responsible for many of the toxic effects in the body [23].

The isolation of *A. flavus* and *A. niger* in this study is in line with the report of [24] in which the two *Aspergillus* spp had the highest rate of occurrence among the isolated fungi. Also [25] reported *A. flavus* as the most dominant fungal species occurring on okra, tomato and pepper fruits from Accra. Similarly [26] and [27] also identified *A. flavus* as the most frequently isolated fungal species in spices and spice product. Furthermore, [8] identified *A. flavus* as a toxigenic strain of the genera *Aspergillus* that contaminates groundnut by the production of aflatoxin. Conversely, *Aspergillus niger* was reported as a secondary invader on eggplant [28]. *Aspergillus niger* is also reported to biosynthesize some types of fumonisins [13]. In addition [11] noted that the isolation of a potentially toxigenic fungi does not imply the presence of mycotoxins since some mycotoxins are produced by more than one fungal specie. Although [12] reported *Fusarium* as a field fungus, its isolation from the present study shows the possibility of field to store transmission. [29] reported *F. verticillioides* as an important pathogen frequently detected in rice seed and further noted a significant positive correlation between germination and *F. verticillioides* resulting in reduced germination leading to about 42 % population reduction.

The studies of [2] and [12] agreed 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. The moisture content of groundnut seeds in this study ranged between 6.53 % to 7.20 % and this was below 10 % which is the moisture content below which fungal growth is inhibited and seed viability is preserved. The report of [29] also showed a correlation between moisture content and the incidence of some *Fusarium* and *Aspergillus* species in rice seed. Although high seed moisture content between 12-14 % is known to favour fungal infection, [12] and [30] reported the possibility of *A. flavus* growing on grains with lower moisture content.

The incidence of *A. flavus* isolated from Oshodi LGA was highest (67.00 %) the LGA recorded the lowest aflatoxin concentration of 3.09 µg/Kg. This finding corroborates the report of [27] which noted high fungi incidence and low aflatoxin B_1 in West African pepper. This indicates the possibility of the presence of some atoxigenic strains of *A. flavus* in the groundnut sample from Oshodi LGA. Agbetiameh et al [31] reported that the atoxigenic L morphotype of *A. flavus* was common in groundnut samples and that the L morphotype could be utilized in the biocontrol of aflatoxin contamination in groundnut fields. Furthermore [32] isolated toxigenic *A. flavus* L strain producing aflatoxin from retailed raw and roasted groundnuts from South Western Nigeria with all samples exceeding the 20 µg/Kg limit for AfB_1 recommended by the National Agency for Food and Drug Administration Control in Nigeria and 79 % of samples recording AfB_1 above the European Union limits of 4 µg/Kg. The study of [33] noted that aflatoxin contamination of kuli kuli a snack made from groundnut in northern Nigeria was above the aflatoxin safe levels of 20 µg/Kg recommended in Nigeria. Similarly, [4] reported that 30 % of groundnut seeds sold in local markets in Nigeria were contaminated with aflatoxins with 25-83 % exceeding the permissible levels in Nigeria with higher aflatoxin levels in local varieties than in the improved varieties. The current study shows a decline in the aflatoxin levels in groundnut sold in some popular markets in Lagos, Nigeria.

The present study recorded reduction of seed germination count as a result of the increase in percentage fungi infection over time. This decrease could be attributed to damping off of seedlings leading to a reduction in the germination count as the experiment progressed. The report of [34] noted that fungi infection on stored seeds such as groundnut may reduce seed germination. The infection of seeds by fungi is reported to result in the production of mycotoxins and loss of seed quality [35]. According to [36] mycotoxins produced by fungal species affect germination and seedling growth. Hasan [37] also reported 50 % reduction in seedling viability of wheat, barley and sorghum by aflatoxin noting that aflatoxin at concentrations higher than 10 µgm L-1 resulted in the reduction of growth rate of hypocotyls after germination. Similarly, [30] reported a significant correlation between the presence of *Fusarium verticillioides* and poor germination in rice seed.

Atanda et al.[12] noted that total aflatoxin of which aflatoxin B_1 is a constituent is a basis for the classification of product quality into high, medium and low quality produce and this classification was used for pricing of premium (high quality) and discount (poor quality) crop. Milicevic et al. [11] also agreed that the evaluation of mycotoxin and mycotoxingenic fungi aids in the determination of the quality of food and feed while [34] reported a relationship between seed borne pathogens and deterioration in the seed quality of groundnut seeds.

5. Conclusion

Seeds of groundnut from the five Local Government Areas of Lagos State, Nigeria were contaminated with AfB_1 at concentration levels below 20µg/ Kg which is the safe limits for human consumption in Nigeria. *Aspergillus flavus* recorded the highest percentage incidence followed by *Lasiodioplodia theobromae, Aspergillus niger* and *Fusarium verticillioides*. The presence of these fungi reduced germination percentage of groundnut seeds. Farm and store sanitation should be encouraged to reduce and further eliminate the incidence of aflatoxin contamination on groundnut sold in local markets in the study area.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared no conflict of interest.

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