IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 12, Issue 1 Ser. II (January 2019), PP 15-20
www.iosrjournals.org

Natural Occurrence of Aflatoxin in Different Egusi Types found in Nigeria

Obani, F. T[123]*., Atehnkeng, J. [2], Ikotun, B[1]. and Bandyopadhyay, R[2].
Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria.
International Institute of Tropical Agriculture, Ibadan, Nigeria. Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
Corresponding Author: Obani, F. T. [123]

Abstract: *Egusi is one of the crops of regional importance in West Africa because it is an important export commodity. It is mostly infected with aflatoxigenic species of Aspergillus flavus which produces carcinogenic aflatoxins. There are many close relatives of egusi which are characterized based on the seed type and seed coat colour. No studies have been conducted to compare the production of aflatoxins in different types of melon which are consumed in Nigeria. This study was undertaken to determine the occurrence of A. flavus and aflatoxins in different types of egusi kernels. Six types of egusi present in each Southwestern Nigerian state were sampled. Approximately ½k g of162 samples of the different egusi types were purchased twice in 2012 and 2013 from selected traders and taken to the laboratory for aflatoxin analysis, Aspergillus isolation and identification. Aflatoxin concentration (µg kg^{-1}) in egusi samples was determined using standard analytical procedures and quantified using scanning densitometer. Data collected were analysed using descriptive statistics and ANOVA at $\alpha_{0.05.}$ A. flavus (51.1±2.4%) was most prevalent followed by A. tamarii (31.8±1.9%), while A. ochraceus (0.3±0.2%) was the least in all egusi types. Incidence of Aspergillus species was highest in egusi Igbaa (61.7±9.6%) and lowest in egusi Itoo (43.5±0.2%). Aflatoxin-B (6.9 - 109.5 µg kg^{-1}), Aflatoxin-G (0.9 - 35.8 µg kg^{-1}) and A. flavus (7.0 –14.0%) were detected in all the six types of egusi. Aspergillus flavus was the most prevalent Aspergillus section Flavi on stored egusi kernels. Various egusi types are prone to aflatoxin contamination and were highly contaminated with aflatoxin at levels exceeding the permissible limits and hence, are potential sources of exposure to the harmful effects of aflatoxin when consumed.*

Key words*: Egusi (melon), aflatoxin contamination, Egusi types, Aspergillus species, southwest Nigeria.*

Date of Submission: 01-01-2019 Date of acceptance: 15-01-2019

I. Introduction

Egusi (*Colocynthis citrullus* L) is an important food crop in many sub-Saharan African countries. It is produced in abundance in different parts of Nigeria (Van der Vossen *et al*., 2004). It is a member of the Cucurbitaceae family. The seeds are rich in oils, which can be extracted for cooking purposes. The seeds can also be ground into a powder and used as a soup thickener or flavouring agent (Van der Vossen *et al*., 2004; Ayodele and Salami, 2006; Brisibe *et al.*, 2011).

In Nigeria, it is cultivated as an increasingly important cash crop. Egusi is easy to grow in Nigeria's warm climate during the beginning of the rainy season and harvested at the onset of the dry season (Van der Vossen *et al*., 2004; Brisibe *et al.*, 2011; http://www. allnigerianrecipes.com).

Egusi plants are cultivated for the seeds because the flesh is neither sweet nor edible and they differ from the closely related watermelon (*Citrullus lanatus*s sp. *vulgaris* Schrad) by the bitter and inedible white pulp and seeds, which have soft testa that can be easily removed by breaking the testa (Ayodele and Salami, 2006). The fruit is a berry and vary in shape and size depending on the type, usually globose to oblong or ellipsoid, sometimes ovoid, 5–70 cm long and weighing 0.1–2.5 kg. It is filled with a soft, white pulp, in which are imbedded numerous seed. The seeds are obovate to elliptical, flattened, 0.5–1.5 cm × 0.5–1 cm, smooth, yellow to brown or black, rarely white. One end of the seed is rounded while the other is tapered (Egunjobi and Adebisi, 2004; Van der Vossen *et al*., 2004; Ayodele and Salami, 2006; http://www. allnigerianrecipes.com).The seeds are marketed whole (unshelled) or as kernels (shelled). Shelling of egusi seeds can be by hand or by machine (Shittu *et al.*, 2002).

Egusi exist in a variety of forms. There are six types of egusi in Nigeria; however, there are two major medium sized seed types, which can be differentiated by the presence or absence of a seed edge. The two major types are referred to as 'bara' (with prominent thick seed edge) and 'serewe' (without pronounced seed edge) (Kehinde, 2011; Bankole and Joda, 2004; Van der Vossen *et al*., 2004; Bankole *et al.*, 2005). The other four are close relatives of egusi (Egunjobi and Adebisi, 2004) which are used for cooking just like the two

major/common types. The close relatives of egusi are characterized based on the seed type, size and seed coat colour (Ayodele and Salami, 2006; Chiejina, 2006; Achigan-Dako *et al*., 2008).The small seeds designated 'N' have uniform brown colour, while large seeds designated 'E' have white edges. 'E' and 'N' are morphotypes of the 'Serewe' and 'Bara' respectively (Ayodele and Salami, 2006). The medium sized type is classified as *C. citrullus*, the large seeds, that is, 'E' is classified as *C. vulgaris* while the small seeds, i.e. 'N' are classified as *C. lanatus* (Thunb). The seeds of *C. vulgaris* are the largest in size followed by those of *C. citrullus* and the least are those of *C. lanatus*. *C. lanatus* is less than 1/6 the size of *C. vulgaris* and about ¼ that of *C. citrullus* (Chiejina, 2006). *Cucumeropsis mannii* Naudin (syn. *Cucumeropsis edulis* (Hook.f.) Cogn.), said to be the true egusi is also used as egusi (Achigan-Dako *et al*., 2008). However, the production of *C. mannii* is strongly declining nowadays and is being continuously replaced by other egusi species; this could be attributed to the long cropping cycle of this species which covers seven to eight months (Egunjobi and Adebisi, 2004).

Many groups of fungi are known to contaminate egusi seeds during storage, reduce seed storability, quality and above all deposit toxic metabolites in the seeds. Seed-borne fungi are also responsible for deterioration of food reserves in the seeds. Fungal deterioration of seeds occurs in form of seed rot, sclerotization of seeds and seed discoloration (Shetty, 1992).The most common storage fungi are species of *Aspergillus* and *Penicillium.* These fungi are widely distributed and almost always present. Most storage fungi are also known to produce a large number of metabolites in seeds, some of which are toxic to humans. Various studies have shown that egusi is contaminated with many pathogens of fungal origin during storage.

The two major types of egusi 'bara' and 'serewe' have been reported to be contaminated with many postharvest fungal pathogens including species of *Aspergillus* that produce aflatoxins in food crops (Somorin and Bankole, 2010). Aflatoxins are produced mainly by many species of the fungus *Aspergillus*, most notably *Aspergillus flavus* (Link) and *Aspergillus parasiticus* (Speare), as a result of their metabolic activities in infected crop species under favourable conditions for fungal growth (Othman and AL-Delamiy, 2012). Aflatoxins are the most carcinogenic substances known to cause hepatotoxicity. In addition to cancer, aflatoxins also affect child growth and development and cause immune depression (Wild, 2007). Aflatoxins are not easily denatured and are quite stable in many food processes, resistant to degradation and are not destroyed under normal cooking conditions (https://ntp.niehs.nih.gov/ntp/roc/content/profiles/aflatoxins.pdf). Aflatoxin contaminations culminate in reduced crop value and diminished health of humans that consume the contaminated crops (Wu and Khlangwiset, 2010).

Aflatoxin is a dreaded human carcinogen and is now well recognized as a public health hazard that is a subject of regulation worldwide. Therefore, the monitoring of aflatoxins in agricultural products should continue to receive high priority (FAO, 2004;Strosnider*et al*., 2006; Reddy *et al*., 2010).Egusi is among the commodities that have received international attention most recently due to unsafe aflatoxin levels (ABT Associates, 2012; Njonga, 2014). EU alerts of import rejections of Nigeria-originating commodities due to aflatoxin contaminations between 2007 and 2012is well documented (ABT, Associates, 2012).

Presently, no studies have been conducted to compare the production of aflatoxins in different types of melon which are present and are being consumed in Nigeria. This study was undertaken to determine the levels of aflatoxins contamination in six different types of egusi in order to assess consumer exposure to this toxin on the basis of current regulations on aflatoxins. The objective of this study therefore is to determine the occurrence of *A. flavus* and aflatoxins in different types of egusi kernels.

II. Materials And Methods

2.1 Sample collection

Six markets in the southwestern part of Nigeria were visited and as many as the different types of egusi in each market were randomly selected and sampled. A total of 162 samples of the different egusi types were purchased from the traders interviewed for aflatoxin analysis and mould isolation. Information sought from the traders included: the type of melon, source of melon (locally or from other states) and melon local names. Approximately 0.5 kg of melon kernel was purchased and taken to the laboratory for studies. Each type of melon kernel was purchased from three traders at different points within the same market and packed into different polythene. For each bag sampled melon kernels were collected at different points in the bag to form a composite sample. In all, six different types of melon seeds from different sources found in various markets were purchased.

2.2 Isolation and Identification of Aspergillus species

Melon kernels were processed and fungi isolated following the methods described by Atehnkeng *et al*.(2008b) using potato dextrose agar (PDA) in which 0.05 ml of lactic acid had been added to suppress bacterial growth (Atehnkeng *et al*., 2008b). After incubation at 25 ±2° C for 5 days, the colony forming units (cfu/ml) of *Aspergillus* species identified was determined by counting the number of colonies formed. Axenic culture of each isolate was obtained by subculturing on fresh PDA plates. Identification of the

isolated *Aspergillus* species were done based on colony morphology and microscopic examination which were compared with literature. Slides were prepared from fungal colonies produced on the medium for identification of the organisms using mycological reference books and research articles (Barnett and Hunter, 1999; Alexopoulus e*t al.*, 2002; Samson *et al*., 2004) and the descriptions of Alexopoulus *et al*. (1996), Barnett and Hunter (1999).

2.3 Determination of Percentage occurrence of Aspergillus isolates

This was done to determine the incidence of occurrence of the different *A. flavus* isolates. The total number of each isolate in all samples was obtained against the total number of all the isolates in all the samples screened. Frequency of occurrence was determined using the method described by Giridher and Ready (1997).

$$\text{Percentage of frequency} = \frac{\text{No of observations in which a species appeared}}{\text{Total no. of observations}} \times 100$$

2.4 Extraction and Quantification of Aflatoxins from Dry Melon kernels

Egusi kernels were ground using a high speed Warring laboratory blender (Warring Commercial, Springfield, MO) and 20 g sub-sample was weighed out from the bulk sample after thorough mixing. Aflatoxin extraction was done using the modifications of Bankole *et al*. (2004), Countryman *et al*. (2009), Odoemelam and Osu (2009) and Kimani (2012). Samples were extracted with 80% methanol at the ratio of 5 ml: 1g and 2% of sodium chloride using the high-speed blender. The mixture was shaken with the orbital shaker for 30 minutes and filtered using Whatman paper No. 1. The solution was extracted twice; first with 25 ml n-hexane and then 25 ml chloroform. The chloroform layer was collected and evaporated to dryness leaving the toxin extracts. The extracts were dissolved with 1 ml of chloroform and 4 µL spotted on TLC plates (silica gel 60,250 µm)with aflatoxin standard and separated on thin-layer chromatography (TLC) plates (silica gel 60,250 µm) using a solution of chloroform, acetone and isopropanol at the ratio of 90:10:1.The plates were scanned using the densitometer CAMAG TLC Scanner 3 with win CATS 1.4.2 software *(*Camag AG, Muttenz, Switzerland) to quantify the aflatoxin extracted from the melon kernels*(*Aquino *et al*., 2005; Suhagia *et al*., 2006*;;* Atehnkeng *et al.*, 2008b*;* Leslie *et al*., 2008).

2.6 Data analysis

Data on fungal incidence and aflatoxin levels in egusi grains were summarized and analyzed using SAS (version 9.4, SAS Institute Inc., Cary, NC). The means were separated using Fisher's protected least significant difference (LSD) test to determine significant differences among the samples obtained from the different states. Prior to analysis, aflatoxin concentration data were transformed by the equation $y = \log_{10}(1 + \text{ng of aflatoxin per gram of ground egusi})$ to homogenize the variances.

III. Results

There were six different types of melon seeds sold in the markets namely: wewe, bara, serewe, bojuri, igbaa, and itoo. However, the "bara" and "serewe" types were the most common types of melon found in all the markets visited (Table 1).

Table 1: List of egusi types from Southwestern States of Nigeria: their sources, local names and description

Source of seeds	Size of seeds	Colour of unshelled seeds	Local name	No. of samples collected	Acronym
Adamawa	Small seeded	Yellow	Wewe	18	NGU
Niger	Medium seeded	Yellow/ brown with thick black edge	Bara	36	NIG
Nassarawa	Medium seeded	Yellow with thin white edge	Serewe	36	NAS
Kebbi	large seeded	Light brown	Bojuri	30	KEB
Oyo	large seeded	Yellow with brown patch at the seed base	Igbaa	24	NGB
Oyo	Large seeded	White	Itoo	18	ITO

NGB = Igbaa, KEB = Kebbi, NAS = Nassarawa, NIG = Niger, NGU= Nguru, ITO = Itoo.

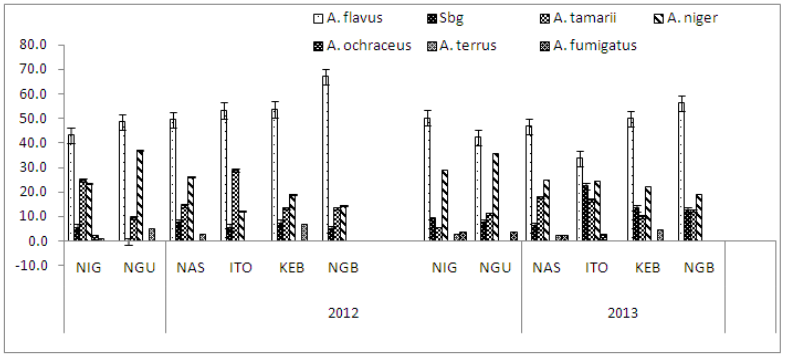

Figure1. Incidence of *Aspergillus* species isolated from various egusi kernel types collected from Southwestern in Nigeria in 2012 and 2013.

NB: NIG = Bara, NGU = Nguru, NAS = Serewe, ITO = Itoo, KEB = Bojuri,NGB = Igbaa.

The *Aspergillus* species isolated from the `egusi kernels were *A. flavus*, *A. tamarii*, *A. niger*, *A. terreus*, *A. ochraceus*, *A. parasiticus*, *A. fumigatus* and the unamed taxon S_{BG}. *Aspergillus flavus* with mean incidence of 52.0 % was the most commonly isolated species across the egusi types inboth years, followed by *A. niger*with mean incidence of approximately 22.0 % *while A. ochraceus* (0.3%) had the least in 2012.The highest percentage incidence of occurrence of *A. flavus* was recorded in egusi Igbaa (67.0%) followed by Bojuri (54.0 %) while Bara had the lowest incidence of 43.0 % (Figure 1). Strain S_{BG} was isolated in samples from all other egusi except Nguru with overall mean incidence of 5.0 %.Significant differences were observed in percentage incidence of all the *Aspergillus* species in the egusi types (Figure 1).

In 2013, the highest percentage incidence of occurrence of *A. flavus* was recorded in egusi Igbaa state (67%) followed by Bojuri and Bara (50.0 % each) while Itoo had the lowest incidence of 34 % (Figure 1). *A. tamarii* was isolated from all the egusi types in 2013. Strain S_{BG} was isolated in samples from all egusi types with overall mean incidence of 12.0 %.Significant differences were observed in percentage incidence of all the *Aspergillus* species in across the egusi types (Figure 1).

In general, *A. flavus* with mean incidence of 47.0 % was the most commonly isolated species across the egusi types in both years, followed by *A. niger* with mean incidence of approximately 27.0 % *while A. ochraceus*(0.2 %) had the least.

Table2. Aflatoxin concentration different Egusi types from Southwestern Nigeria in 2012 and 2013.

	Aflatoxin concentration (ng/g)				
	2012			2013	
Type	B	G		B	G
Igbaa	**32.2±0.1**	**2.1±0.9**		**65.5±0.1**	**35.8±16.5**
Nguru	**24.2±6.7**	**2.8±0.4**		**19.2±4.2**	**8.3±2.8**
Itoo	**109.5±34.2**	**0.9±0.5**		**6.9±4.1**	**7.4±1.3**
Bojuri	**81.2±27.5**	**2.7±0.4**		**55.1±16.7**	**6.3±1.6**
Serewe	**34.3±8.8**	**2.5±0.7**		**46.5±17.9**	**0.6±0.3**
Bara	**14.1±1.6**	**2.6±0.6**		**23.4±7.8**	**2.2±0.9**

3.1 Aflatoxin concentration in different types of melon

Both aflatoxin B and G were recorded in all the different types of melon at varying levels in 2012. The highest level of aflatoxin contamination was detected in the ITOO (ITO) type (109.5 ng/g) while the Melon samples from Niger state origin (NIG) (14.1 ng/g) recorded the least level of contamination. There were significant differences (P= 0.05) in the means of various types of melon. Aflatoxin G was also detected in the different types of egusi- melon although at levels lower than the FDA limit. The highest level of aflatoxin G contamination was detected in NGU (2.8 ng/g) while ITO had the least contamination (0.9 ng/g) and they differed significantly (Table 2).

In 2013, all the egusi types were also contaminated with aflatoxin B and G. The highest level of aflatoxin was recorded in the NGB (Igbaa) type (65.5 ng/) while the least was recorded in ITO (6.9 ng/g). NGB, KEB, and NAS consistently had aflatoxin B contamination levels which exceeded the FDA limit in 2012 and 2013 (Table 2). Aflatoxin G was also detected in the different types of egusi although at levels lower than the FDA limit except the NGB in which up to 35.8 ng/g of aflatoxin B was recorded. The least level of aflatoxin G contamination was detected in NAS (0.6 ng/g) and this differed significantly from the mean aflatoxin G contamination in the other types of melon analyzed.

IV. Discussion

Aspergillus flavus are the prevalent species of the genus *Aspergillus* isolated in this study. The high frequency of *A. flavus* compared to other members of *Aspergillus* section Flavi have been explained by the reports of Horn and Dorner (1999); Nesci and Etcheverry (2002); Jaime-Garcia and Cotty (2004). S-type *A. flavus* that produce only B-aflatoxins and have the S-type morphology were not isolated in this study (Cardwell and Cotty, 2002), this study. However, only low percentage of highly toxigenic unnamed taxon S_{BG} was seen in this study. Unlike in the present study, did not the strain S_{BG} was not observed on egusi seeds (Bankole, 1993; Bankole *et al.*, 2004, 2006 and Chiejina, 2006).The different egusi types were positive to aflatoxin contamination. In either of the years or both years, aflatoxin levels recorded were all higher than those permitted by the different aflatoxin regulatory bodies around the world. For instance, <4 ng/g is the EU/Nestlé acceptable limit; <10 ng/g is the World Food Program acceptable limit; <20 ng/g is the United States Food and Drugs Administration regulation limit; >20 ng/g is an unacceptable level of aflatoxin. Furthermore, all the samples recorded higher aflatoxin concentrations than those recorded in a particular egusi type in Nigeria (Bankole and Adebanjo, 2003a, 2003b). It is well established from this study that all egusi types represent a significant source of exposure to aflatoxin. The predominance of *A. flavus* over the other *Aspergillus* spp, as well as other fungal species in the various egusi types contributes to the validity of the aflatoxins contamination levels recorded (Bankole and Mabekoje, 2004; Bankole, 2006; Chiejina, 2006). The study shows that aflatoxin contamination is occurring at unacceptable levels in a greater percentage of egusi types consumed in Nigerian States by both Nigerian and international standards; because Nigeria regulates aflatoxin B1 at 20 ppb (= ng/g, µg/g) in all foods, (FAO, 1997; Ntare *et al.*, 2005).

V. Conclusion

All egusi types are produced exclusively for human consumption, occurrence of different types of aflatoxin in all types of melon kernels at unsafe levels implies that their consumption is a potential means of exposure to the harmful effects of aflatoxin. Aflatoxin control measures should be targeted towards all the egusi types and not just on the two common ones

References

[1]. Abt Associates Incorporated 2012. Aflatoxin Country Assessments-A Progress Report. Accessed fromhttp://abtassociates.com/AbtAssociates/files/fa/facafce3-af77-4c5a-a3d5-a27198d619f1.pdf

[2]. Achigan-Dako, E. G., Fagbemissi, R., Avohou, H. T., Vodouhe, R. S., Coulibaly, O. and Ahanchede, A. (2008). Importance and practices of Egusi crops (*Citrullus lanatus* (Thunb.) Matsum and Nakai, *Cucumeropsis mannii* Naudin and *Lagenaria siceraria* (Molina) Standl. cv. ' Aklamkpa) in sociolinguistic areas in Benin», *Biotechnol. Agron. Soc. Environ.* 12(4):393-403. Retrieved fromhttp://popups.ulg.ac.be/Base/document.php?id=3254.

[3]. Adebayo-Tayo, B.C., Onilude, A. A., Ogunjobi, A. A., Gbolagade, J. S., and Oladapo, M. O.(2006). Detection of fungi and aflatoxin in shelved bush mango seeds (*Irvingia* spp.) stored for sale in Uyo, Eastern Nigeria. *Electronic Journal of Environmental, Agricultural and Food Chemistry* 5(5):1,569-74.

[4]. Alexopoulos, C. J. Mims, C.W. and Blackwell, M. (1996). Introductory mycology: 4th Edition. John Wiley and Sons, INC. 869pp.

[5]. Aquino, S., Ferreira, F., Ribeiro, D. H. B., Corrêa, B., Greiner, R., and Villavicencio, A. L. C. H. (2005). Evaluation of Viability of *Aspergillus flavus* and Aflatoxins Degradation in Irradiated Samples of Maize. *Brazilian Journal of Microbiology* 36: 352-356.

[6]. Atehnkeng, J., Ojiambo, P. S., Ikotun, T., Sikora, R. A., Cotty, P. J. and Bandyopadhyay, R. (2008a). Evaluation of atoxigenic isolates of Aspergillus *flavus* as potential biocontrol agents for aflatoxin in maize. *Food Addit. Contam.* 25:1264–1271.

[7]. Atehnkeng, J., Ojiambo, P. S., Donner, M., Ikotun, T., Sikora, R. A., Cotty, P. J., Bandyopadhyay, R. (2008b). Distribution and toxigenicity of *Aspergillus* species isolated from maize kernels from three agro-ecological zones in Nigeria. *International Journal of Food Microbiology* 122(1-2):74-84.

[8]. Ayodele, O. J. and Salami, A. E. (2006). Physiological response of two variants of egusi melon (*Citrullus lanatus*) to plant population density in a humid environment .*Journal of Food, Agriculture & Environment* 4 (3and 4):110-113. Retrieved from www.world-food.net.

[9]. Bankole, S. A. (1993). Moisture content, mould invasion and seed germinability of stored melon. *Mycopathologia* 122:123-126.

[10]. Bankole, S.A. (2006) Mycotoxins in food systems in Sub-Saharan Africa: a review. *Mycotoxin Research* 22:163–169.

[11]. Bankole, S.A. and Adebanjo, A. (2003a). Mycotoxins in food in West Africa: current situation and possibilities of controlling it. *African Journal of Biotechnology* 2(9):254–263.

[12]. Bankole, S. A and Adebanjo, A. (2003b). Aflatoxin contamination of dried yam chips marketed in Nigeria. Tropical Science 43:201-203.

[13]. Bankole, S.A. and Joda, A.O. (2004). Effect of lemon grass (*Cymbopogon citratus*) powder and essential oil on mould deterioration and aflatoxin contamination of melon seeds (*Colocynthis citrullus* L.). *African Journal of Biotechnology* 3(1):52-59.

[14]. Bankole, S. A. and Mabekoje, O. O. (2004). Mycoflora and occurrence of aflatoxin B-1 in dried yam chips from markets in Ogun and Oyo States, Nigeria.*Mycopathologia* 15: 111-115.

[15]. Bankole, S. A. Ogunsanwo, B. M. and Mabekoje, O. O. (2004). Natural occurrence of moulds and aflatoxin B1 in melon seeds from markets in Nigeria. *Food and Chemical Toxicology* 42:1309-1314.

[16]. Bankole, S. A., Osho, A., Joda, A. O. and Enikuomehin, O. A. (2005). Effect of drying method on the quality and storability of 'egusi' melon seeds (*Colocynthis citrullus* L.). *African Journal of Biotechnology* 4(8):799-803.

[17]. Bankole, S.A., Schollenberger, M. and Drochner, W., (2006). Mycotoxin contamination in food systems in sub-Saharan Africa: A review. *Mycotoxin Research*22: 163-169.

[18]. Bankole, S. A., Adenusi, A. A., Lawal, O. S. and Adesanya, O. O. (2010). Occurrence of aflatoxin B1 in food products derivable from 'egusi' melon seeds consumed in Southwestern Nigeria. *Food Control* 21:974-976.

[19]. Barnett, H. L. and Hunter, B. B. 1999.*Illustrated Genera of Imperfect Fungi*: 4th Edition.The American Phytopathological Society St. Paul, Minnesota.218 pp.

[20]. Cardwell, K.F. and Cotty, P.J. (2002).Distribution of *Aspergillus flavus* section Flavi among soils from the four agroecological zones of the Republic of Bénin, West Africa. *Plant Disease* 86:434–439.

[21]. Chiejina, Nneka V. (2006). Studies on Seed-Borne Pathogens of some Nigerian Melons. *Journal of Agriculture, Food, Environment and Extension* 5(1): 13-16. URL: http://www.agrosciencejournal.co

[22]. Cotty, P. J. (1997).Aflatoxin-producing potential of communities of *Aspergillus* section *Flavi* from cotton producing areas in the United States. *Mycological Research* 101:698–704.

[23]. Cotty, P. J. and Cardwell, K. F (1999).Divergence of West African and North American communities of *Aspergillus* section Flavi. *Applied and Environmental Microbiology* 65:2264-2266.

[24]. Countryman, S., Huq, S. and Mathews, T. (2009). Rapid, High Resolution Analysis of Aflatoxin Extracts from Peanut Butter Using Kinetex™ Core-Shell Technology and Strata® SPE. 411 Madrid Ave., Torrance, CA 90501 USA: Phenomenex, Inc., Retrieved from www.phenomenex.com

[25]. Egunjobi, J. K. and Adebisi, A. A. (2004).*Cucumeropsis mannii* Naudin In: Grubben, G.J. H. and Denton, O.A. (Editors). PROTA 2: Vegetables/Légumes. [CD-Rom].PROTA, Wageningen, Netherlands.

[26]. FAO. 1997. *Worldwide regulations for mycotoxins 1995 - a compendium.* FAO Food and Nutrition Paper No. 64. Rome. Retrieved from http://www.fao.org/docrep/w9474t/w9474t06.htm

[27]. FAO. (2004).Worldwide regulations for mycotoxins in food and feed in 2003. Rome: Food and Agriculture Organization. *Food and Nutrition papers* 81:170 pp. ISBN 92-5-105162-3.

[28]. Gbolagade, J., Ajayi, I. and Omitade, Y. (2011). Nutritional compositions, fungi and aflatoxins detection in stored 'gbodo' fermented (*Dioscorea rotundata*) and 'eluboogede' fermented (*Musa parasidiaca*) from south western Nigeria. *African Journal of food science* 5(2): 105-110.

[29]. Giridher, P. and Ready, S. M.P. (1997). Incidences of mycotoxin producers on spices from Andhra Pradesh. *J. Indian Bot. Soc.* 76:161-164.

[30]. Horn, B. W. and Dorner, J. W. (1998). Soil populations of *Aspergillus* species from section *Flavi* along a transect through peanut-growing regions of the United States. *Mycologia* 90:767–776.

[31]. Horn , B. W. and Dorner, J. W. (1999). Regional differences in production of aflatotxin B_1 and cyclopiazonic acid by soil isolates of *Aspergillus flavus* along a transect within the United States. *Applied Environmental Microbiology* 65:1444-1449.

[32]. Jaime-Garcia, R., Cotty, P.J., (2004).*Aspergillus flavus* in soils and corncobs in south Texas: implications for management of aflatoxins in corn-cotton rotations. *Plant Disease* 88:1366–1371.

[33]. Kehinde, I. A. 2011. Response of melon cultivars to natural infection by diseases in South Western Nigeria. *International Journal of Biology* 3(4): 47-55.

[34]. Kimani, P. M. and Ngeranwa, J. J. N. (2012). Determination of aflatoxin levels in stored white maize (Zea mays L.) and flour in Kitui, Mwingi and Makueni Districts in Kenya. Retrieved from http://ir-library.ku.ac.ke/handle/123456789/3493

[35]. Leslie, John F., Bandyopadhyay, R., and Visconti, A. (Eds), (2008). Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. Wallingford: CAB International. Pp 496.

[36]. Nesci, A. and Etcheverry, M. (2002. Aspergillus section Flavi populations from field maize in Argentina. Letters in Applied Microbiology 34: 343-348.

[37]. Njonga, B. 2014.The dangers of aflatoxins in maize, groundnuts under review in the Farmer's voice .Monthly magazine of the rural contractor 28 May, 2014. Accessed from http://www.thefarmersvoice.org/uk/developement-news.php?

[38]. Ntare, B. R., Waliyar, F., Ramouch, M., Masters, E. and Ndjeunga, J. (eds.). 2005. Market

[39]. prospects for Groundnut in West Africa (Eng/Fr). CFC Technical Paper No. 39 PO Box 74656, 1070 BR Amsterdam. The Netherlands: Common Fund for Commodities; and Patancheru, India: International Crops Research Institute for the Semi-Arid Tropics. 252 pp.

[40]. Odoemelam, S. A. and Osu, C. I. (2009). Aflatoxin B1 Contamination of Some Edible Grains Marketed in Nigeria. *E-Journal of Chemistry* 6(2):308-314. Retrieved from http://www.e-journals.net

[41]. Othman, G. A. and Al-Delamiy, K. S. 2012.Aflatoxin b1 production by *Aspergillus flavus* in different media and containers and the antifungal activity of garlic and black cumin. *Research Journal in Engineering and Applied Sciences* 1(2):117-121.

[42]. Reddy, K. R. N., Salleh, B., Saad, B., Abbas, H. K., Abel, C. A., and Shier, W. T. 2010. An overview of mycotoxin contamination in foods and its implications for human health. *Toxin Reviews* 29(1):3–26

[43]. Samson R. A., Hoekstra E. S, Frisvad J. C. 2004.Introduction to food-borne fungi.7th Edition. CentraalbureauvoorSchimmelcultures.389 pp.

[44]. Shetty, H. S. 1992. Different types of damages in seeds caused by seed-borne fungi. 53-62 pp., in: Seed pathology. Proc. of Technical Center for Agricultural and Rural Cooperation Seminar held in Copenhagen Denmark. Eds. Mathur, S. B and Jorgensen, J. Technical Center for Agricultural and Rural Cooperation, Wageningen, Netherlands, 412 pp.

[45]. Shittu, G. A., Umari, D. and Bawa, M. 2002. Mycoflora associated with two varieties of groundnut (*Arachis hypogea* L.). *Nigerian Journal of Botany* 15: 68-73.

[46]. Somorin, Y. M. and Bankole, S. A. (2010). Mycoflora of stored "Ofada" and "Abakaliki" rice in Lagos and Ogun States, Southwestern Nigeria.*African Journal of Microbiology Research*4(16): 1724-1726. http://www.academicjournals.org/ajmr

[47]. Strosnider, H., Azziz-Baumgartner, E., Banziger, M., Bhat, R. V., Breiman, R., Brune, m., DeCock, K., Dilley, A., Groopman, J., Hell, K., Henry, S. H., Jeffers, D., Jolly, C., Jolly, P., Kibata, G. N., Lewis, L., Liu, X., Luber, G., McCoy, L., Mensah, P., Miraglia, M., Misore, A., Njapau, H., Ong, C., Onsongo, M. T.K., . Page, S. W., Park, D., Patel, M., Phillips, T., Pineiro, M., Pronczuk, J., Rogers, H. S., Rubin, C., Sabino, M., Schaafsma, A., Shephard, G., Stroka, J., Wild, C., Williams, J. T, and Wilson, D. (2006). Workgroup Report: Public Health Strategies for Reducing Aflatoxin Exposure in Developing Countries. *Environ Health Perspect* 114(12):1898-1903.

[48]. Suhagia, B. N., Shah, S .A., Rathod, I. S., Patel, H.M., Shah, D.R., Marolia, B. P., 2006. Determination of gatifloxacin and ornidazole in tablet dosage forms by high-performance thin-layer chromatography. Analytical Sciences 22:743-745.

[49]. Van der Vossen, H.A.M. and Denton, O.A. and El Tahir, I.M., (2004). *Citrullus lanatus* (Thunb) Matsum.and Nakai. [Internet] Record from Protabase. Grubben, G.J.H. and Denton, O.A. (Editors).PROTA (Plant Resources of Tropical Africa / Ressources végétables de l'Afriquetropicale), Wageningen, Netherlands pp 737.

[50]. Wild C. P. (2007). Aflatoxin exposure in developing countries: the critical interface of agriculture and health. *Food NutrBull*.28: S372-S380.

[51]. Wu, F., and Khlangwiset, P. 2010. Health economic impacts and cost-effectiveness of aflatoxin reduction strategies in Africa: case studies in biocontrol and postharvest interventions. *Food Additives and Contaminants* 27:496-509.

Obani, F. T. "Natural Occurrence of Aflatoxin in Different Egusi Types found in Nigeria. "IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 12.1 (2019): PP- 15-20.