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Occurrence of aflatoxin in *Colocynthis citrullus* L. (egusi) kernels in Southwestern Nigeria

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Abstract: *Egusi is an important soup condiment in many parts of Nigeria. It is infected with many storage fungi especially aflatoxigenic species of Aspergillus flavus which produces carcinogenic aflatoxins. However, information on AC in egusi is limited in Nigeria. Therefore, incidence of storage fungi, toxigenicity of A. flavus isolates and AC in Egusi kernels (EK) from southwestern Nigeria were investigated. One major Egusi market in each of the six southwestern states was purposively selected during the wet season (July-August). Traders in each market were listed and three traders were selected by simple random sampling. Egusi kernels (≈½kg, n=162) were purchased twice in two years from selected traders and taken to the laboratory for fungi isolation, identification and aflatoxin analysis. Aflatoxin concentration (µg kg^{-1}) in EK samples and Aspergillus isolates (n=1003) toxigenicity were determined using standard analytical procedures and quantified using scanning densitometer. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05.}$ Aspergillus (32.4±1.6%) was most predominant fungal species followed by Rhizopus spp (21.5±2.0%) in all states. Incidence of Aspergillus species was highest in Ekiti (62.1±6.5%) and lowest in Oyo (40.2±3.7%).Among Aspergillus species, 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. Aflatoxin-B (6.9-109.5 µg kg^{-1}) and aflatoxin-G (0.2-35.8 µg kg^{-1}) were detected in six states. Toxigenic A. flavus (77.7±0.9%) isolates were significantly higher than atoxigenics (22.3±0.5%). Toxigenic Aspergillus was most prevalent postharvest fungi on Egusi kernels in southwestern Nigeria. Egusi kernels were highly contaminated with aflatoxin at alarming levels.*

Key words*: Aspergillus flavus, toxigenicity, postharvest fungi, aflatoxin, egusi, aflatoxigenic*

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I. Introduction

Melon (egusi) is one of the crops produced in abundance in south west, south east and central parts of Nigeria. Melon is botanically known as *Colocynthis citrullus* L. (Van der Vossen *et al*., 2004). It is a member of the Cucurbitaceae family. Melon is in a group of cucurbit species that produce protein and oil rich seeds for which the crop is mainly grown (Achigan-Dako *et al.,* 2008). Melon (egusi) is one of the several members of the Cucurbitaceae cultivated widely within the smallholder traditional food crop production systems in Nigeria. The seed which is the economic portion is generally known as 'egusi.

In West Africa it is called 'egusi'. In Nigeria, it is cultivated as an increasingly important cash crop. Egusi is easy to grow in Nigeria's warm, arid climate (Brisibe *et al.,* 2011).*C. citrullus* originated from the western Kalahari region of Namibia and Botswana, where it still exists in the wild in a diversity of forms together with other *Citrullus* species. There are two major types of melon in this region; one has fruits that are generally bitter and mainly used for their seeds, called 'tsama melon', which is the probable ancestor of egusi melon. The other type has fruits that are mainly used as a source of water during periods of drought or as cooking melons, and may well represent the ancestral form of the watermelon and cooking melon (Van der Vossen *et al*., 2004).

The egusi fruit is a gourd which is mainly cultivated for the seeds because the flesh is neither sweet nor edible. Egusi fruits are extremely bitter and differ from the closely related watermelon (*Citrullus lanatus* ssp. *vulgaris*) by the white, bitter and inedible pulp; the seeds have soft testa that can be easily removed (Ayodele and Salami, 2006).

It is an important food crop in many sub-Saharan African countries. The seeds are rich in oils, which can be extracted for cooking purposes, and 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). The residue from oil extraction is made into balls that are fried to produce a local snack called 'robo' in Nigeria.

Egusi is not only valuable for local consumption, but as an export as well primarily to sell to people who have emigrated there from Africa.

The melon seeds have been reported to be contaminated at postharvest stage by species of *Aspergillus* which produce aflatoxin in food crops. Attendant with the presence of toxin-producing species is a potential risk of mycotoxin contamination in melon seeds (Somorin and Bankole, 2010). Aflatoxins are naturally occurring mycotoxins which are produced by many species of the fungus *Aspergillus*, most notably *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are toxic and are among the most carcinogenic substances known. In addition to being potent hepatotoxic and carcinogenic metabolites, aflatoxins also impact on child growth and development and adversely affect immune status of people (Wild, 2007).

Aflatoxin has been classified as a human carcinogen and is the subject of regulation world-wide. Aflatoxin contamination in food grains is now well recognized as a public health hazard. Therefore the monitoring of aflatoxins in agricultural products should continue to receive high priority(FAO, 2004;Strosnider*et al*., 2006).In Nigeria, mould and aflatoxin contamination of melon have been reported(Bankole *et al*.,2004); but gaps in information exist on exposure of consumers to aflatoxins in the melon seeds on the basis of current regulations on aflatoxins. Also, no studies have been conducted to determine the toxigenicity of *Aspergillus* section *Flavi* associated with melon in in Nigeria. There is therefore the need to assess the possible exposure of consumers to aflatoxins through the consumption of melon on the basis of current regulations on aflatoxins. The objective of this study is to determine *A. flavus* contamination and the concentration of aflatoxins in melon kernels in Southwestern Nigerian markets.

II. Materials And Methods

2.1 *Sample collection*

Purposive Sampling method was adopted because the main interest was to focus on traders that were dealing in the sale of melons on wholesale basis (Mutegi *et al*., 2010); using the method of USDA Grain Inspection, Packers, and Stockyards Administration (GIPSA). Briefly, multiple samples from were collected from different points and depths in the grain mass and mixed well to form a composite sample (http://www.gipsa.usda.gov/publications/fgis/handbooks/afl_insphh.html). A total of 162 samples were purchased from the vendors interviewed for aflatoxin analysis and mould isolation. Information sought from the traders included: packaging material used for melon kernels (whether it was jute bags, Woven plastic bags), source of melon (locally or form other states) and melon local names.

Shelled melon kernels were purchased directly from a major market where melon from the producing areas is unloaded in each of the six southwestern states of Nigeria. For each market visited simple random sampling was adopted for sample collection. Briefly, a list of the traders was made and those from which the samples were collected and interviewed were selected randomly. From each trader ≈0.5 kg of melon kernels was purchased and taken to the laboratory for studies. Melon kernels were purchased from three traders at different points within the same market, packed into different polythene bag. For each bag sampled melon kernels were collected at different points in the bag to form a composite sample.

2.2 Isolation, Identification and storage of Aspergillus species

Melon kernels were processed and fungi isolated 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 sub culturing on fresh PDA plates. Identification of the isolated fungi was 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 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) and Barnett and Hunter (1999). Isolates were stored by transferring 3 ager plugs (5 mm) of the isolates into 2 ml sterile distilled water in 4 ml vials until used for further studies.

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 Egusi kernels

Each melon sample was 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) and Odoemelam and Osu (2009). 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 for 30 minutes and filtered using Whatman paper No. 1. The solution was extracted twice; first with 25 ml n-hexane and then 35 ml chloroform. After separation, the chloroform layer which contains the toxin was filtered through anhydrous sodium sulphate into polypropylene cups and allowed to evaporate to dryness. The extracts were dissolved using 1 ml of chloroform and spotted with a*flatoxin standard (*4 µL) on TLC plates (silica gel 60,250 µm) using the capillary tube. The spotted extracts were *separated on thin-layer chromatography (TLC) plates (silica gel 60,250 µm)* and developed using chloroform, acetone and isopropanol (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).

2.5 Toxigenicity of A. flavus Isolates from Egusi kernels

Clean maize grains (5g) were weighed into 40 ml Pyrex glass vial and the moisture content was adjusted to about 25% by washing the grains in five changes of tap water and then soaked for four hours and the water drained. The grains were autoclaved for 30 minutes at 121^0C and allowed to cool. Then 500µl of water containing 1ml (1000µl) of Tween 20 per 1000mls of sterile distilled water was dispensed into each of the vials containing sterilized grains. Each of the stored isolates was inoculated by dispensing 500µl of spore suspension into the vial containing sterilized grains with corresponding sample code as the stored isolate, shaken to properly coat the grains with fungal spores. The vials were placed in slanting position with their caps loosened and incubated for seven (7) days. After 7 days the visual colonization scoring of grains was carried out and the grains were analyzed for aflatoxin contamination using slight modifications of the methods described above *(*Atehnkeng *et al., 2008b;* Leslie *et al*., 2008; Dehghan *et al.,* 2008; Yazdani *et al*., 2010).

2.6 Data analysis

Data on fungal incidence and aflatoxin levels in melon grains were summarised and analyzed using SAS (version 9.2, 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 $y = \log_{10}(1 + \text{ng of aflatoxin per g of ground maize})$ to homogenize the variances.

III. Results

Incidence of Aspergillus species in egusi kernels

The moisture content of the kernels ranged from 10.9 - 12.4 % in 2012 and 9.5 – 20.4% in 2013 (data not shown). The average moisture content of the kernels was 13.9% in 2012 and 15.1% in 2013. A total of 477 and 526 isolates of *Aspergillus* section *Flavi* were isolated from 100% of the melon samples in 2012 and 2013 respectively. *A. flavus* L-strain was the most commonly isolated group of *Aspergillus* across the states with overall mean incidence ranging from 40 – 59.9% in 2012 (Table 1) and 40 – 62 % in 2013 (Table 1) while *A. terreus* and *A. ochraceus* had the least overall mean incidence of 3.3 % and 0.4 in 2012 respectively.

Within *Aspergillus* section Flavi, *A. flavus* with mean incidence 53 % was the most commonly isolated species across states in 2012. S_{BG} unnamed taxon was only isolated from Osun, Oyo and Ogun with overall mean incidence of 5 %. The highest percentage incidence of occurrence of *A. flavus* was recorded in Ogun state (59%) followed by Ekiti state (62%) while the lowest incidence occurred in Oyo state (40 %). *A. tamarii* was isolated from samples in all the six states (Table 1) in 2012. Significant differences (P= 0.05) were observed in incidence of *A. flavus*, *A. tamari*, *A. niger*, *A. a*nd strain S_{BG} . No significant difference was observed between *A. ochraceus*, and *Aterreus.*

The *Aspergillus* species isolated from the `egusi kernels in 2013 were *A. flavus* Link, *A. tamarii, A. niger, A. terreus, A. ochraceus, A. parasiticus, A. fumigatus* and the unamed taxon S_{BG}. *A. flavus* with mean incidence of 50 % was the most commonly isolated species across the states in 2013, followed by *A. niger* with mean incidence of approximately 25 % *while A. ochraceus* (0.3%) had the least.

Table 1: Percentage incidence of *Aspergillus* species isolated from egusi kernels collected from the six states in South Western in Nigeria in 2012 and 2013.

			Incidence of Occurrence (%)							
Year	State	N	*A. flavus*	Sbg	*A. tamarii*	*A. parasiticus*	*A. niger*	*A. ochraceus*	*A. Terrus*	*A. fumigatus*
2012	Ekiti	123	61.7±7.3	5.6±5.6	6.7±6.7	-	15.0±7.6	0.0±0.0	11.1±11.1	-
	Lagos	90	47.6±2.4	0.0±0.0	26.2±5.0	-	20.6±4.0	0.0±0.0	5.6±5.6	-
	Ogun	102	59.9±13.4	4.3±2.9	24.3±2.9	-	31.6±10.5	0.0±0.0	0.0±0.0	-
	Ondo	189	53.7±6.8	0.0±0.0	22.3±6.1	-	24.0±8.4	0.0±0.0	0.0±0.0	-
	Osun	297	52.0±7.1	9.0±3.9	17.5±9.4	-	19.0±6.3	2.5±2.5	0.0±0.0	-
	Oyo	185	40.4±4.7	11.3±5.2	25.2±5.5	-	19.6±7.6	0.0±0.0	3.4±2.9	-
2013	Ekiti	61	62.5±19.1	0.0±0.0	0.0±0.0	0.0±0.0	29.2±15.0	0.0±0.0	4.3±4.2	4.2±4.2
	Lagos	123	51.0±5.0	5.6±5.6	9.5±9.5	0.0±0.0	34.0±3.3	0.0±0.0	0.0±0.0	0.0±0.0
	Ogun	180	53.3±9.4	4.4±4.4	15.0±4.4	0.0±0.0	19.4±5.6	0.0±0.0	5.7±3.5	2.2±2.2
	Ondo	180	42.5±8.3	6.7±4.1	25.8±6.7	0.0±0.0	20.0±5.7	1.7±1.7	3.3±0.0	0.0±0.0
	Osun	177	48.3±4.1	4.4±2.7	11.1±3.0	2.2±2.2	31.7±0.0	0.0±0.0	2.2±2.2	0.0±4.4
	Oyo	210	40.0±2.7	18.7±4.2	8.47±2.7	11.9±5.0	21.0±3.0	0.0±0.0	0.0±0.0	0.0±0.0

The highest percentage incidence of occurrence of *A. flavus* was recorded in Ekiti state (63%) followed by Ogun state (53 %) while Oyo state had the lowest incidence of 40 % (Table 1). *A. tamarii* was isolated from only in 50% of the samples in 2013. Strain S_{BG} was isolated in samples from all other states except Ekiti with overall mean incidence of 7 %.Significant differences (P= 0.05) were observed in percentage incidence of all the *Aspergillus* species in across the states (Table 1).

Toxigenicity of Aspergillus flavus isolates

The relative proportions of toxigenic and atoxigenic strains of *A. flavus* varied among the states sampled (Figure 1). Generally, there were more toxigenic (89%) *A. flavus* isolates than atoxigenic isolates (21%). The incidence of toxigenic strains was significantly (P 0.05) higher than that of atoxigenic aflatoxin B producing strains in all the states except in Ekiti State. Strains producing G aflatoxin (S_{BG}) was observed in Osun, Ogun and Oyo states in 2012. In 2013, the G aflatoxin producing strains (S_{BG} and *A. parasiticus*) were recorded in Lagos, Ondo, Osun, Ogun and Oyo states. However, the non-toxigenic strains were significantly higher in these states. Across states, the lowest and highest incidence of toxigenic strains was observed in Ekiti (31%) and Ondo (48%), respectively (Fig 1) in 2012. However, in 2013 both Osun and Ogun had the least toxigenic strains (35%) while Lagos had the highest toxigenic strain (40%) (Figure 1) *A. flavus*, both the L-type and the unamed taxon S_{BG} were isolated in both years. All isolates with S-type sclerotia also produced both B and G aflatoxins and as a result were classified as the unnamed taxon, strain S_{BG}.

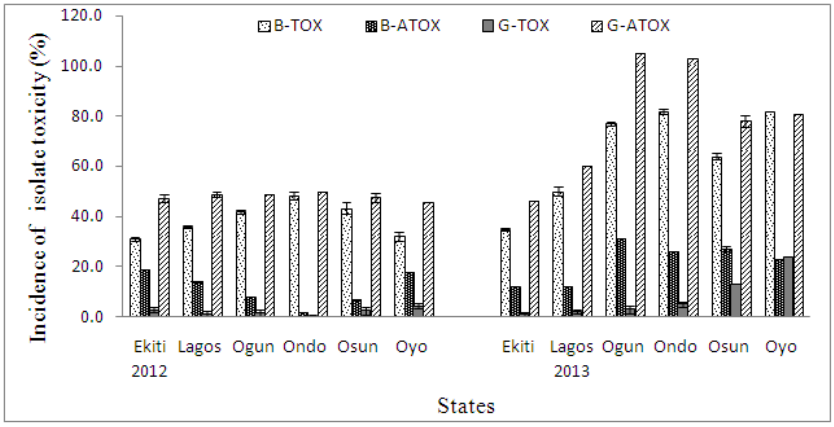

Figure 1: Percentage incidence of toxigenic and atoxigenic strains of *Aspergillus* section *flavi* isolated from melon kernels in 2012 and 2013.

The results from the toxin analysis as presented in Table below show that the four aflatoxins,B1, B2, G1 and G2 were detected in melon even though with differences among the states. B and G were detected in most of the samples. The samples had aflatoxin B and the levels ranged from 0.1 –759.3 ng/g with a mean of 45.4 ng/g in samples where they were detected in 2012. Aflatoxin G was found in only 42% of the samples at concentrations ranging 0.4 - 23.8 ng/g with a mean 0f 2.2 ng/g.

On state basis, the highest level of aflatoxin contamination was detected in Osun (100.4 ng/g) while samples from Ogun (12.9 ng/g) were the least contaminated (Table 2). G aflatoxins were also detected in

samples from all the states. The mean aflatoxin level in the different states exceeded the FDA Limit (20 ng/g) except in Ogun state in 2012. There were significant differences (P= 0.05) in the means of aflatoxin B contamination of the various states. The mean aflatoxin contamination in the different states was 45.4 ng/g. The aflatoxin content ranged from 0.0 - 759.3 ng/g. Aflatoxin G was also detected in the different states even though they were all lower than the FDA limit. Ondo state had the highest level of aflatoxin G contamination (3.4 ng/g) while Osun had the least contamination (0.2 ng/g) and this differed significantly from that of Ondo (Table 2).

In 2013, both aflatoxin B and G were also recorded in all the states at varying levels. The mean aflatoxin contamination in the different states exceeded the FDA Limit (20 ng/g) except in Lagos and Ekiti states. The highest level of aflatoxin contamination was detected in Ondo (101.4 ng/g) while samples from Ekiti (9.0 ng/g) were the least contaminated (Table 2). The mean aflatoxin content of the different states was 38.33ng/g. The aflatoxin content ranged from 0.0-636.02 ng/g. Aflatoxin G was also detected in the different states even at varying levels. Similarly, Ondo state had the highest level of aflatoxin G contamination (23.2 ng/g) which was higher than the FDA limit while Ekiti had the least contamination (0.2 ng/g). The aflatoxin G means from the other states differed significantly from that of Ondo (Table 2). In 2013, Ekiti state recorded the lowest level of both aflatoxins B and G contamination.

Table 2. Aflatoxin concentration in melon from different States in Southwestern Nigeria in 2012 and 2013.

	Aflatoxin concentration (ng/g)			
	2012		2013	
	B	G	B	G
Ondo	77.7	3.4	101.4	23.1
Osun	100.4	0.2	28.6	8.6
Ogun	12.9	2.1	32.8	3.9
Oyo	19.7	2.7	26.9	8.6
Ekiti	29.5	2.6	9.0	0.2
Lagos	21.4	2.2	10.9	0.5
LSD($p \leq 0.05$)	47.8	1.9	38.5	17.8
CV (%)	26	10	29.5	22.9

For each bar the vertical line represents the standard error of the means.

IV. Discussion

This study provides an initial broad report of the record of the distribution of fungi and toxigenicity of *Aspergillus* species within *Aspergillus* section Flavi infecting stored egusi kernels in Southwestern markets of Nigeria. Aflatoxin concentration levels associated with the melon kernels collected after months of postharvest szdxsdfxededr3estorage is also documented and differed across states.

Moisture content of the melon kernels sampled were higher than the recommended safe moisture level(<10.5%) for oil seeds(Shukla *et al.,* 1992; Leslie *et al.,* 2008), and were also much higher than those reported in previous studies on melon in Nigeria (Bankole and Mabekoje, 2004).This may also be due to high relative humidity during the storage period.

Aspergillus species particularly L-type *A. flavus* was the major *Aspergillus* section Flavi found in egusi kernels in all the examined Southwestern states of Nigeria in this study. The high incidence of *A. flavus* in comparison to other members of *Aspergillus* section Flavi could be explained by the occurrence of high levels of *Aspergillus* section Flavi found in the soil , insects, air and plant debris which acts as inoculum reservoir for infection of melon kernels if not handled properly during processing and storage(Nesci and Etcheverry, 2002; Jaime-Garcia and Cotty, 2004). Similar results have been reported from other parts of West and the United States (Cotty, 1997; Horn and Dorner, 1998). In neighbouring Benin as reported by Cardwell and Cotty (2002). *Aspergillus flavus* stains that produce only B-aflatoxins but have the S-type morphology were also not encountered in this study. Contrary to the Benin studies, only low frequencies (<20 %) of the unnamed taxon S_{BG} were detected in this study. Unlike in the present study, Bankole (1993), Bankole *et al.* 2004, Bankole *et al.* 2006 and Chiejina (2006) did not report S_{BG} in their studies on storage fungi in melon seeds. In this study, our isolation technique, dilution plating could have enabled them to be spotted and identified. Cardwell and Cotty (2002) that also detected strain S_{BG} in their studies also adopted dilution plate technique in their soil mycoflora studies. All isolates of strain S_{BG} isolated were highly toxigenic and could significantly contaminate egusi with aflatoxin under (2002) and Atehnkeng *et al*. (2008a).Therefore, very low incidences of infection may pose a serious problem of aflatoxin contamination in egusi.

Contrary to the study of Atehnkeng *et al*. (2008a) in maize were the majority of *A. flavus* isolates were atoxigenics, there were more toxigenic *A. flavus* than the atoxigenics. In previous studies, the average aflatoxin producing potentials of fungal communities are highly variable. For example, *A. flavus* isolates in southern USA widely produce aflatoxins, while a lower percentage (only 29%) in Argentina is toxigenic (Vaamonde *et al.,*

2003). This implies that the toxigenic potential of *A. flavus* isolates differ in locations. In contrast to the reports of Cotty (1997), (Ehrlich *et al*., 2007), (Nesci and Etcheverry, 2002), (Giorni *et al.*, 2007) and the (Probst *et al*., 2007), less than 2% of the *A. flavus* isolates were S-type. This is in agreement with the study of Atehnkeng *et al* (2008a) in a similar study on maize in Nigeria.

The incidence of toxigenic strains was higher than atoxigenic strains across all the six states studied. Highly toxigenic isolates of the unnamed taxon S_{BG} and *A. parasiticus* were also isolated from the melon kernels. However, it did not exceed 10% and 20% of the isolates obtained from the locations where they occurred in 2012 and 2013 respectively. The L-type *A. flavus* appears to be the most important causal agent of melon aflatoxin contamination in Nigeria and therefore management strategies to reduce aflatoxin contamination should be directed at this species.

The reports of the authors cited above were in support of the findings of this study, which showed that high *Aspergillus* section Flavi population and moisture content may be the major factor that contributed to the high level of aflatoxin detected in melon (Gbolagade *et al.*, 2011; Kimani and Ngeranwa, 2012; Bhushan *et al.*, 2014) especially during prolonged storage (Abulude and Ojediran, 2006). In addition, the study of Bhushan *et al*. (2014) clearly indicated that when the seeds were stored at high moisture content, the activity of aspergilli was found to be greater and this releases toxic metabolites into seeds which alter the quality of the seeds and make them unsafe for consumption.

Egusi kernels were positive to aflatoxin contamination as previously reported. This is in contrast to reports from Benin, Mali and Togo where no aflatoxin was detected in shelled melon (Hell *et al.*, 2009). However, total levels of aflatoxin reported in the current study were much higher than those reported in the previous studies in Nigeria (Bankole *et al.*, 2004, 2006,and 2010). Over 80% of the samples tested positive to aflatoxin contamination. Contrary to the previous studies on melon, results of this study indicated that the levels of aflatoxin were comparable to those of other Benin, Kenyan and Nigerian food crops (maize, dried yam chips, plantain and yam flours, bush mango and groundnut) which are prone to aflatoxin contamination (Bankole and Mabekoje, 2004; Bankole and Adebanjo, 2003a, 2003b; Lewis *et al*., 2005; Adebayo-Tayo *et al*. 2006; Atehnkeng *et al.*, 2008b). Thirty eight percent of the analysed melon samples had aflatoxin level above the maximum residue limit of 20ng/g permitted by the FDA and that permitted in Nigerian foods, while previous studies reported only 3.5% of analysed melon samples to have aflatoxin level above the 20 ppb limit (Bankole *et al.*, 2004).

The contamination levels recorded in the melon kernels is not surprising because *A. flavus* clearly dominated other fungi in the present study and several studies on melon by Bankole and Mabekoje (2004), Bankole (2006) and Chiejina (2006).Also, melon is one of the oilseeds reported as common hosts for this aflatoxin-producing species (Duran *et al.*, 2009).Aflatoxin G that was not reported in previous studies on melon was also detected in 41% of the melon kernels in 2012 and 22% of the samples in 2013. The presence of G-aflatoxins in the samples is another line of evidence suggesting the isolation technique was effective for the isolation of strain S_{BG} and *A. parasiticus* both of which produce aflatoxin G and were detected. High incidence of toxigenic *A. flavus* with very low aflatoxin concentration in some samples could be as a result of unfavourable environmental conditions for development on the substrate. In addition, the genotype of each species, biological and chemical factors determine the amount of aflatoxin that is produced. The principal factors for aflatoxin production are the aflatoxigenic fungus, the substrate, environmental and substrate humidity, temperature, oxygen of the storage atmosphere and the time of storage (Carvajal and Castilo, 2002).

The European Union's imposition of a new and more strict aflatoxin regulation of 2ppb aflatoxin B_1 and 4ppb total aflatoxins in foods for human consumption than 20 ppb total aflatoxins (Dimanchie, 2001) proposed by the Codex Alimentarius Commission (Ntare *et al*. 2005) and Nigerian regulatory limit (FAO, 1997 indicates that aflatoxin contamination is occurring at unacceptable levels in melons consumed in Southwestern Nigerian States by both Nigerian and international standards. Hence, Nigerian melons will never make it to foreign markets and is forced to be consumed locally. From the foregoing, egusi consumption is a potential means of exposure to the harmful effects of aflatoxin and steps must be taken to minimize contamination levels. This is necessary, because the expanding knowledge of the harmful effects of aflatoxin, the current strict tolerance standards by foreign bodies such as World Food Program (10 ppb) and European Union (4ppb); and the fact that melon soup (melon soup) is widely consumed in Nigeria calls for the revision of the current tolerance level in foodstuffs. NAFDAC which is a Nigerian agency in charge of safe guiding the public health of the nation may need to further reduce the regulatory limit to <20 ng/g for national safety, as well as to meet these international standards and enhance the export potentials of melon to other countries.

V. Conclusion

The occurrence of aflatoxin in egusi at alarming levels is well established from the analysis of the kernels sampled in 2012 and 2013. *Aspergillus* species particularly the L-strains were the most predominant fungal species identified, followed by species belonging to the genera *Penicilium and Rhizopus.* The toxigenics had

higher incidence than the atoxigenics in all the six states in both years indicating a greater risk of aflatoxin contamination, if melon is handled poorly during processing and storage. The occurrence of *Aspergillus* species in egusi which deposits toxins in them and expose the consumers to the risk of cancer due to carcinogenic effects of aflatoxin required an accessible and safe management strategy, which will not be harsh to the environment and the consumers of egusi.

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