Effects of botanicals on *Aspergillus* and aflatoxin production in Egusi melon seeds

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Abstract: Aflatoxin contamination (AC) in egusi is usually a postharvest problem. The protective impact of the powders of Negro pepper (NP), *Xylopia aethiopica*, scent leaf (SC), *Ocimum gratissimum* and Ashanti pepper (AP), *Piper guineense* treatments before storage can help reduce on aflatoxin formation and *Aspergillus flavus* growth in stored egusi Kernels. However, information on the use of botanicals on and AC mitigation in egusi is limited in Nigeria. Therefore, effects of NP, *Xylopia aethiopica*, scent SC, *Ocimum gratissimum* and AP, *Piper guineense* on *A. flavus* and AC were evaluated in this study. Clean Egusi kernels treated with botanical powders (10, 20 and 40 g/kg) were inoculated with *A. flavus* bi-weekly for 12 weeks storage period at room temperature to evaluate AC and growth reductions (%) of *A. flavus* using standard laboratory procedures. *Piper guineense* (40 g/kg), *X. aethiopica* (20 g/kg) and *O. gratissimum* (10 g/kg) powders significantly reduced AC by 42.5%, 56.5% and 45.0%, respectively; *A. flavus* growth was progressively reduced by *P. guineense* (5.5-90.0%), *X. aethiopica* (6.7-100.0%) and *O. gratissimum* (7.4-66.7%) up to twelve weeks of storage. *Xylopia aethiopica* at 20 g/kg reduced aflatoxin and *A. flavus* growth considerably and therefore could be used to mitigate aflatoxin contamination during storage of egusi kernels.

1.   Introduction

It is established that there is a fundamental distinction between aflatoxin formations in crops before or immediately after harvest, and that occurring in stored commodities or foods. Aflatoxin contamination occurs in two distinct phases with the infection of the developing crop in the first phase and after maturation in the second phase (Cotty and Jaime-Garcia, 2007). *A. flavus* and *A. parasiticus* contamination infections during the first phase of susceptible crops are promoted by wounding of developing crops by birds, mammals, insects, mechanically or drought, terminal water stress prior to harvest and elevated temperatures (Guo *et al*., 2002). Crops such as peanuts, maize and cottonseed are associated with *A. flavus*, and *A. parasiticus,* so that invasion of plants and developing seed or nut may occur before harvest. In contrast, this affinity is lacking for other crops, so it is not normally present at harvest (IARC, 2002). In melon, aflatoxin contamination usually occurs at the postharvest stage. *Aspergillus* spp have not been reported as field disease of melon in Nigeria. Therefore, *Aspergillus* spp can be said to be a postharvest infection in melon production. Crop processing methods, transportation and storage in a warm climatic condition favour *Aspergillus* growth and enhance the production of aflatoxin. Postharvest contamination is also enhanced by delayed drying. Damage by insect or rats can also facilitate mould invasion and toxin production during storage (Melvin, 2012). The formation of aflatoxins therefore relies mainly on contamination after harvest, due to use of primitive and slow drying methods as well as poor storage practices (IARC, 2002).

Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials (Bittner *et al*., 2008). Drum stick (*Moringa oleifera*) is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses. It has antibacterial and antifungal activities with high nutritional value (Bouamama *et al*., 2006; Okigbo and Ogbonnaya, 2006). *M. oleifera* phytochemical screening revealed presence of flavonoids and saponins. *Moringa* inhibited the growth of *Mucor* spp and *Rhizopus* spp. Scent leaf (*Ocimum gratissimum)* has been reported to be used against human fungal infections. It is an established fact that the various extracts of *Ocimum gratissimum* have been tested *in vitro* and shown to be active against some bacterial and fungal isolatesi (Ijato, 2007). West African Pepper (*Piper guineense)* seeds contain significant proportion of myristicin, elemicin, safrole and dillapoil. Elemicin and myristicin are reported to exhibit significant fungicidal properties (http://martinslibrary.blogspot.com). Safrol reduced

the growth rates of *Rhizoctonia solani, Ceratocystis pilifera*, and *Fusarium oxysporum by* up to 50% (Bittner *et al.,* 2008). Negro pepper (*Xylopia aethiopica*) has a wide range of biological activities including insecticidal, anti-tumour, anti-asthmatic, anti-inflammatory, antimicrobial, hypotensive and coronary vasodilatory effects, and these were attributed to the wide variety of secondary metabolites in the plant Sun hemp (*Crotalaria* sp.) neem (*Azadirachta indica*), garlic (*Allium sativum),* ginger (*Zingiber officinale*), and species such as Derris (*Derris elliptica), Piper, Xylopia, Gongronema latifolium, Citrus, Bryophyllum pinnatum, Vernonia amygdalina, Chrysanthemum coccineum (*pyrethrum), etc have been reported to be promising species in crop protection, (Ijato, 2007; Hartman *et al*., 2003).

Botanicals contain biologically active chemical substances such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds which have plant protection properties. These complex chemical substances of different compositions are found as secondary plant metabolites in the medicinal plants (Kayode and Kayode, 2011). The control of pathogenic organisms in foods by the botanicals would reveal the potentials of these extracts as preservatives. The findings add impetus to the clarion call by consumers and authorities in food industries for the replacement of chemically-synthesized preservatives with "naturally derived" ones.

Considering the great values of melon as major soup constituent, losses in quality of seeds particularly due to seed-borne pathogens must be minimized if not totally eliminated. Often no single method can provide sufficient control of seed-borne pathogens. Many control methods namely chemical, biological etc aimed at eradicating or reducing the amount of pathogens present in seeds are available (Khare, 2008). However, alternative control measures should be compared for cost, efficacy and safety. The use of medicinal botanicals as seed dusts can be one of the promising methods to control fungal deterioration of stored melon seeds that will jointly ensure cost effectiveness, efficacy and safety. In many areas of Africa and Asia locally available botanicals are being widely used to protect stored products against damage by storage pests, as alternatives to chemical pesticides (Suleiman and Yusuf, 2011).

In view of the harmful effects of aflatoxin production by *Aspergillus* species an adequate control measure is necessary to be put in place to reduce or if possible prevent aflatoxin in melon seeds (Chiejina, 2006). The use of botanicals can be one of the promising methods to alleviate the problem of egusi contamination by aflatoxins. The prevention of postharvest aflatoxin contamination in melon is one of the best and most effective strategies; hence the need

to assess the potential of some botanicals for the control of aflatoxin production in melon seeds.

2.0. Materials And Methods
2.1. Botanicals and Maize Samples
The botanicals were purchased from a local market in Ojoo area of Ibadan, Oyo State; washed botanicals thoroughly, air dried under shade and ground to fine powder using the Warring laboratory blender (Warring Commercial, Springfield, MO). The egusi seeds were purchased from farmers within 3 days of harvest and drying. A highly toxigenic *A. flavus* isolated from egusi was used as inoculum in this study.

2.2. Laboratory Screening of Effects of Plant Extracts on Growth of *Aspergillus flavus*
The method of Okigbo *et al.* (2005) was adopted by putting 25g of each plant material powder in 100 ml of sterilized distilled water and allowed to stand for 12 hours and then filtered. Autoclaved PDA medium was amended with individual extract at 10, 20 and 40% (v/v) as described by Begum and Bhuiyan (2006). Requisite quantity of individual plant extract was added to the 250 ml conical flask containing PDA medium to have concentrations of 10, 20 and 40% (v/v). After thorough mixing with plant extracts, approximately 15 ml of the melted PDA and the plant extracts mixture was dispensed into each 9 cm Petri dish. After gelling, the plates were inoculated by placing 5 mm discs of 5 days old PDA cultures of *A. flavus* at the centre of the Petri dishes. Inoculated plates were incubated at $25 \pm 2^{o}C$ and growth measured along perpendicular lines drawn under the plates. Daily radial growth on each of the test extracts was recorded for 10 days. Each treatment was replicated three times and the mean radial growth determined per test extract. Control experiment was set up without addition of any botanical.

Fungitoxicity was recorded in terms of percentage colony inhibition and calculated using the formula stated by Sundar *et al*. (1995):

$$\text{Growth inhibition (\%)} = \frac{DC - DT}{DC} \times \frac{100}{1}$$

Where: DC -Average Diameter of control and
DT -Average diameter of fungal colony with treatment.

2.3. Effects of Plant Extracts on Aflatoxin Production and growth of *A. flavus* in stored egusi
The botanical powders 10g, 20g, and 40g were applied to 1kg of clean (unifected) egusi seeds and kernels for a period of 3 months. The controls were not dusted with any botanical powder (Bankole and Joda, 2004; Krishnamurthy and Shashikala, 2006). Subsamples of 50g were collected from each 1kg

sample at 2nd, 4th, 6th, 8th 10th and 12th week for contamination with a highly toxigenic *A. flavus*.

2.4. Fungal inoculation of treated egusi seeds

Inoculum suspension was prepared from fresh, mature (5-day-old) *A. flavus* culture. Fungal colonies were covered with 5 ml of distilled sterile water containing 1% Tween 20 per 100ml to enhance uniform spore concentration because the genus *Apergillus* is hydrophobic. The final inoculum size was adjusted to a concentration of 1.0×10^6 spores/ ml by microscopic enumeration with a cell-counting haemocytometer. The egusi seeds treated with botanicals were washed in three changes of sterile distilled water and then inoculated with 100 µL of spore suspension of *A. flavus* per twenty seeds which were plated on Petri dishes containing solidified PDA and incubated at room temperature for five days. Percentage seed colonization was recorded after incubation.

Percentage growth reduction was calculated using the formula:

Percentage reduction = 100 - (Treated x 100) / Control

After incubation of the inoculated seeds, aflatoxin extraction was done using the modifications of Bankole *et al.* (2004) and Odoemelam and Osu (2009). Each gram of the samples was extracted with 5 ml of 80% methanol and 2% of sodium chloride using the high-speed blender. The mixture was filtered using Whatman paper No. 1, in a funnel and the extracts cleaned up with 25 ml n-hexane and then 25 ml chloroform. After separation, the chloroform layer which contains the toxin was filtered through anhydrous sodium sulphate to remove residual water. Further toxin analysis was done using the methods described by (Aquino *et al.*, 2005; Atehnkeng *et al.,* 2008*;* Leslie *et al.*, 2008).

Percentage aflatoxin reduction was calculated using the formula:

Percentage reduction = 100 - (Treated x 100) / Control

l2.5. Data analysis

All data generated were analyzed using the SAS (version 9.1, SAS Institute, Cary, NC). Data on aflatoxin levels were summarized and analyzed using analysis of the SAS (version 9.1, SAS Institute, Cary, NC). Means were compared using Least significant difference Test (LSD) at $P = 0.05$ procedure in SAS (SAS Institute Inc., Cary, NC, USA) to compare the differences among the results obtained from different treatments.

3.0. Results

3.1. Effect of Botanicals on mycelial growth of *A. flavus*

The results showed that the botanicals had varying degrees of inhibition on *A. flavus* growth at different concentrations of the extract *in vitro* and *in vivo*. The aqueous extracts of *P. guineense, O. gratissimum* and *X. aethiopica* at 10%, 20% and 40% concentrations reduced the growth of *A. flavus* at varying percentages. Aqueous extract of the fruit of *P. guineense* significantly had antifungal activity against *A. flavus*. At 10%, 20% and 40% concentrations, there was reduced growth of the fungus when compared with the control. The mycelial growth of the fungus was observed to decrease with increase in extract concentration. At 10% concentration the mean growth reduction was 5.7; however, it did not differ significantly from the mycelial growth recorded at 20% (5.1 cm). The highest mycelial growth reduction was recorded at 40% (3.8 cm) concentration which was significantly differently from 10% and 20% concentrations of the extract and the control (9.0 cm). The results followed the same trend in the second experiment. There were significant differences between the control and the different concentrations of *P. guineense*. Significant differences were not observed between 10% and 20% concentrations but there were significant differences between 40% (3.8 cm) which had the highest growth reduction and 10% (5.1 cm) and 20% (4.9 cm) concentrations of the extract and the control which had 9.0 cm mycelia growth (Table 1). Aqueous extract of *P. guineense* at 10% reduced the growth of *A. flavus* by ~ 37.0%, 20% by 43.0 and 40% by 58.0%. In the second experiment, 40% also gave the highest percentage growth reduction of ~ 58 %, and this differed significantly from 10% and 20% concentrations which reduced *A. flavus* growth by 46% and 43 %, respectively (Table 2).

The mycelial growth of the *A. flavus* was decreased by the different concentrations of *O. gratissimum* extract. Significant differences were not observed between 10%, 20% and 40% concentrations but there were significant differences between the growths of *A. flavus* on the amended media and the control which had the highest mycelia growth. However, the least mycelial growth was recorded at 20% concentration (5.1 cm). In the second experiment, the results followed almost the same trend. There were significant differences between the control and the different concentrations of *O. gratissimum* extract but not there was no significant difference between the different concentrations of the extract even though 10% concentration had the least mycelial growth (5.4 cm) compared to 20 5 and 40 % concentrations which both had 5.5 cm mean mycelia growth (Table 1). Percentage growth reduction of *A. flavus* by *O.*

gratissimum was ~ 37.0% at 10%, 43.0 % at 20% and 37.0% at 40.0%. In the second experiment, 10% also gave the highest percentage growth reduction of ~ 40.0 %, and this did not differ significantly from 20% and 40% concentrations which both reduced *A. flavus* growth by ~ 39 % (Table 2).

Table 1. Effect of botanical extracts on mycelial growth of *A. flavus*

Growth reduction (%)

Conc.	*P. guineense*		*O. gratissimum*		*X. aethiopica*	
	EXPT 1	EXPT 2	EXPT 1	EXPT 2	EXPT 1	EXPT 2
10	5.7	4.9	5.7	5.4	6.7	6.3
20	5.1	5.1	5.1	5.5	5.0	5.0
40	3.8	3.8	5.6	5.5	5.1	5.3
Control	9.0	9.0	9.0	9.0	9.0	9.0
LSD	0.6	0.8	0.8	0.6	1.1	1.0
CV	5.5	7.4	6.5	4.6	8.7	8.5

*EXPT = Experiment

Aqueous extract of the fruit of *X. aethiopica*, significantly had antifungal activity against *A. flavus*. At 10%, 20% and 40% concentrations, the growth of *A. flavus* was significantly ($P= 0.05$) reduced compared to the control which had 9.0 cm mycelia growth. Twenty percent (5.0 cm) reduced the mycelia growth of *A. flavus* better than 10% (6.7 cm) and 40% (5.1); although there was no significant difference between 20 % and 40% concentrations. The results obtained in the second experiment followed the same trend. The different concentrations of *X. aethiopica* extract significantly ($P= 0.05$) reduced the growth of *A. flavus* compared to the control. *Xylopia aethiopica* *reduced* the growth of *A. flavus* at percentages which ranged from 25.6 to 44.4 %. The highest growth reduction was recorded at 20% treatment (44.4%), however, there was no significant difference between 20% with the highest growth reduction and 40% concentration of *X. aethiopica* which reduced *A. flavus* growth by 43%. In the second experiment, 20% also gave the highest percentage growth reduction of ~ 44 %, and this did not differ significantly from 40% concentrations which reduced *A. flavus* growth by 41 % (Table 2).

Table 2. Percentage growth reduction of mycelial growth of *A. flavus*

Mycelial growth reduction (%)

Conc.	*P. Guineense*		*O. Gratissimum*		*X. aethiopica*	
	EXPT 1	EXPT 2	EXPT 1	EXPT 2	EXPT 1	EXPT 2
10	36.7	45.6	36.7	40.0	25.6	30.0
20	43.3	43.3	43.3	38.9	44.4	44.4
40	57.8	57.8	37.8	38.9	43.3	41.1

*EXPT = Experiment

3.2. Effect of Botanicals on Growth of fungus *A. flavus* on egusi-melon seeds

Co-storing egusi-melon seeds/kernels with botanical powders significantly reduced *A. flavus* growth during storage. All the botanicals tested differed from each other in their ability to reduce *A. flavus* growth on egusi. The result presented in Table 3 shows the percentage reduction of *A. flavus* growth in egusi treated with different concentrations of *P. guineense*. Forty percent treatment generally had the highest growth reduction except at two weeks after treatment where 20% gave the highest growth reduction 55% followed by 40% treatment which recorded 50% although they were not significantly different (p=0.05) from each other. Percentage growth reduction declined gradually as the storage period increased. The second experiment gave similar results. Forty and twenty percent treatments had same percentage growth reduction. However, subsequently, 40% had the highest growth reduction of *A. flavus* 15% after 8 weeks of treatment. *X. aethiopica* had maximum growth reduction of 50% at 20% and 40% treatment while 10% had the least growth reduction (16%) after 2 weeks of treatment. *X. aethiopica* at 20% and 40% treatments did not differ significantly in reduction of *A. flavus* growth. Percentage growth reduction gradually declined as the storage period increased. After four weeks of treatment, there were no significant differences between the different levels of treatment. The second experiment gave similar results *A. flavus* recorded the highest growth reduction of 66% at 20% treatment. However, the second

experiment was better because on the average the percentage reduction was higher than those recorded in the first experiment in the first 4 weeks after treatment (Table 3).

For O. gratissimum treated samples 20% application generally had the highest growth reduction of *A. flavus* growth; although not significantly different from 40% treatment at seven days after storage. On the average, 40% recorded the highest

growth reduction but the differences did not differ significantly (P = 0.05). Percentage growth reduction declined gradually as the storage period increased. The second experiment gave similar results. Twenty percent treatment had the highest growth reduction of *A. flavus* followed by 40% treatment and then 10% up to 4weeks after treatment; thereafter 40% gave the best growth reduction (Table3).

Table 3. Effect of botanical powders on aflatoxin production by *A. flavus* in stored egusi-melon seeds

		Percentage growth Reduction					
		P. guineense		*O. gratissimum*		*X. aethiopica*	
Weeks	conc.	Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2
2	10	25.0b	25.0b	25.0b	35.0b	16.7b	5.6b
	20	55.0a	60.0a	65.0a	80.0a	50.0a	66.7a
	40	50.0a	60.0a	60.0a	65.0a	50.0a	55.6a
4	10	25.0b	20.0a	45.0b	60.0a	22.2b	22.2b
	20	30.0a	40.0a	65.0a	70.0a	50.0a	66.7a
	40	50.0a	40.0a	70.0a	65.0a	38.9a	61.1a
6	10	0.0a	5.0a	15.0a	10.0a	5.6a	5.6a
	20	20.0a	10.0a	15.0a	20.0a	10.0a	10.0a
	40	10.0a	10.0a	40.0a	35.0a	16.7a	16.7a
8	10	0.0b	5.0a	10.0b	5.0a	5.6a	11.1a
	20	10.0ab	10.0a	15.0ab	10.0a	10.0a	11.1a
	40	15.0a	15.0a	20.0a	30.0a	15.0a	20.0a
10	10	0.0a	0.0a	5.0a	10.0a	5.0a	5.0a
	20	0.0a	0.0a	11.8a	10.0a	15.0a	15.0a
	40	5.0a	5.0a	16.7a	20.0a	15.0a	15.0a
12	10	0.0b	0.0b	10.0a	11.1a	6.7a	7.1ab
	20	0.0b	0.0b	12.5a	14.3a	21.4a	21.4ab
	40	30.8a	5.0b	12.5a	16.7a	10.0a	20.0a
14	10	0.0b	0.0ba	0.0b	8.3a	4.4a	0.0a
	20	0.0b	0.0b	8.3ab	0.0a	0.0a	0.0a
	40	25.0a	11.1a	12.5a	12.5a	4.4a	10.0a

Conc. = concentration, Expt 1 = experiment 1, Expt 2 = experiment 2
For each week, means with same letter in eeach column are not significantly different.

3.3. Effect of botanicals on aflatoxin production in stored egusi seeds

The data presented in Figures 1 to 3 shows that the 3 botanicals tested reduced aflatoxin production in egusi-melon seeds treated with the test (botanicals) plant powders from two weeks prior to inoculation at significant levels when compared with the control. Up to 73% reduction in aflatoxin concentration was observed in *X. aethiopica* at 20% concentration after two weeks of treatment (Figure 1a). Twenty percent treatment gave significantly reduced aflatoxin reduction concentration more than 40% and 10% which reduced the amount of toxin by 48% and 30% respectively. *O. gratissimum* recorded the best percentage reduction of aflatoxin concentration of 67% at 10% concentration after two weeks of

treatment; 20% and 40% respectively reduced aflatoxin by 56% and 59% (Figure 2a). *P. guineense* had maximum reduction in aflatoxin concentration of 74% at 40% level of treatment (Figure 3a). The second experiment followed the same trend; *X. aethiopica* at 20% level of treatment gave 61% reduction than 40% and 10% which had 39% and 29% aflatoxin reduction respectively after two weeks of treatment (Figure 1b). *O. gratissimum* recorded the best percentage reduction of aflatoxin concentration of 66% at 10% concentration after two weeks of treatment; 20% and 40% respectively reduced aflatoxin by 60% and 61% (Figure 2b); even though no significant differences were observed between the treatment levels. *P. guineense* had maximum reduction in aflatoxin concentration of 75% at 40% level of treatment and

this significantly differed from 10% and 20% treatment which reduced aflatoxin concentration by 36% and 33% respectively (Figure 3b). Percentage aflatoxin reduction declined as the week progressed. On the average however, Negro pepper (*X. aethiopica*) appears to be more effective in reducing aflatoxin production because at the 12th week after treatment over 50% aflatoxin reduction was still recorded in egusi-melon seeds treated with 20g of plant powder per kg.
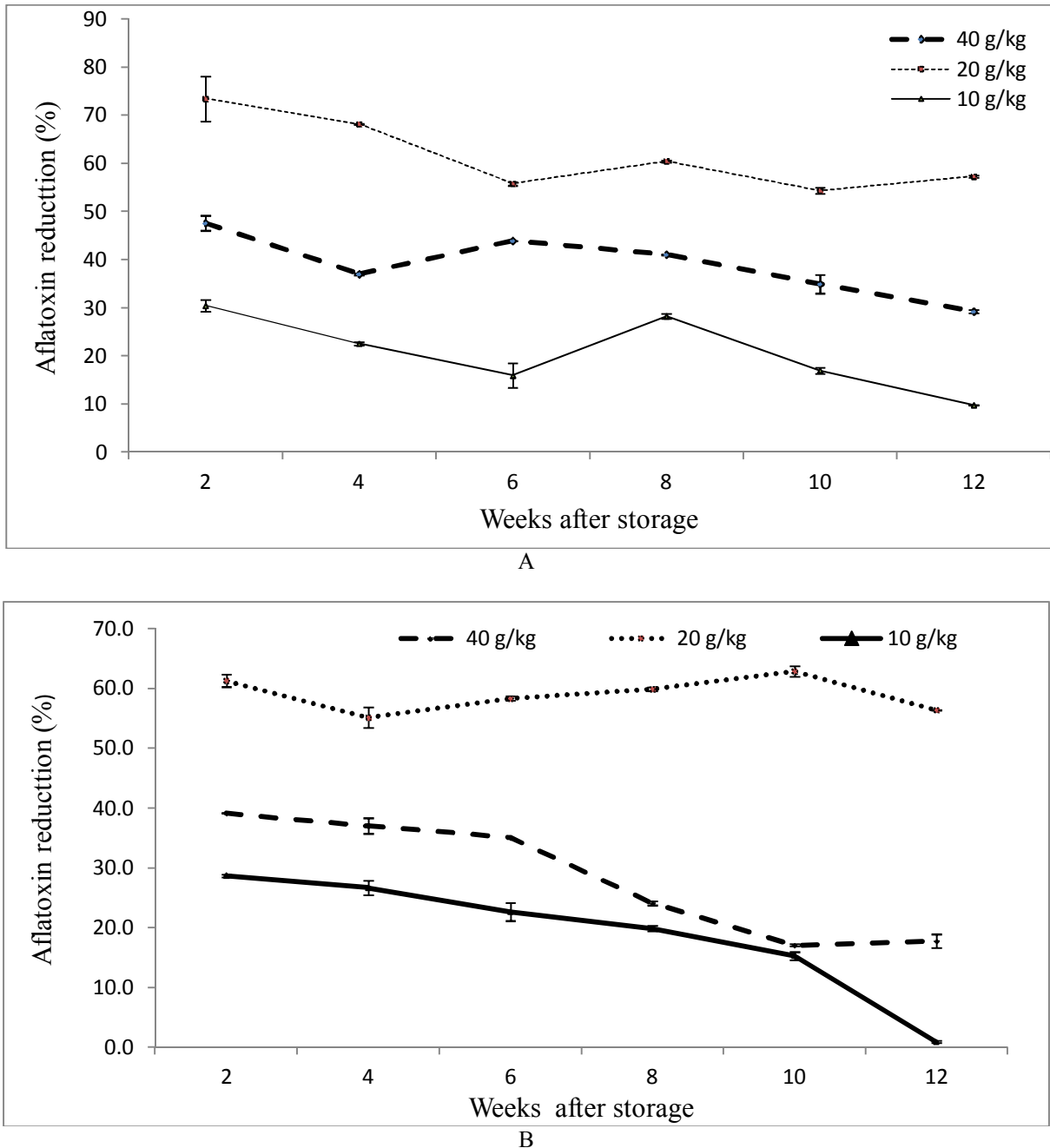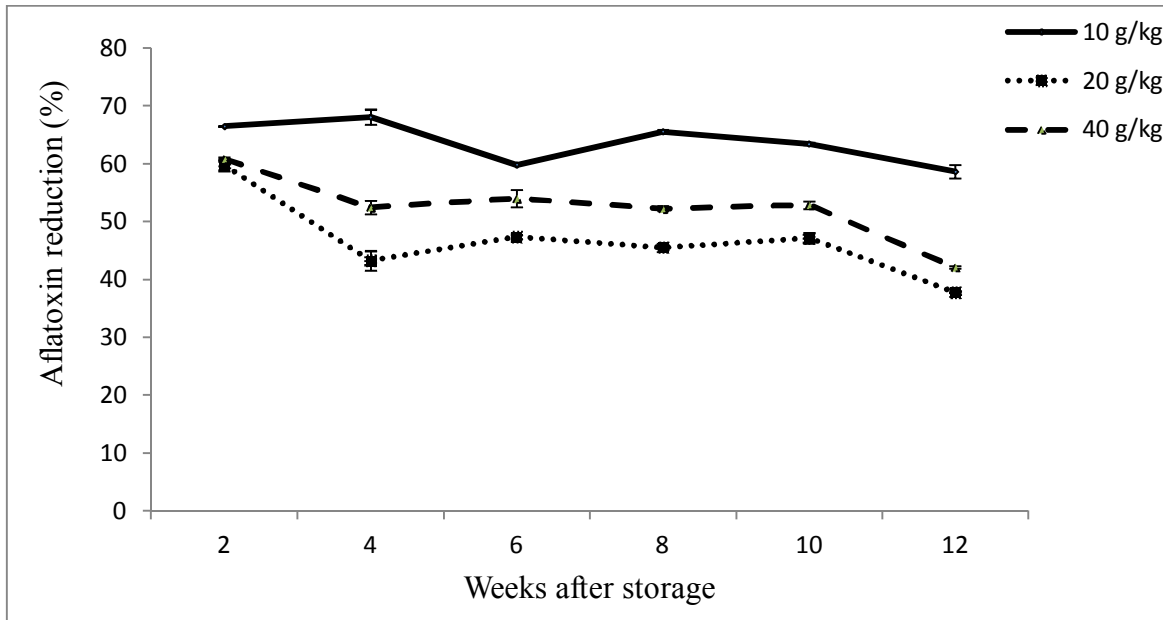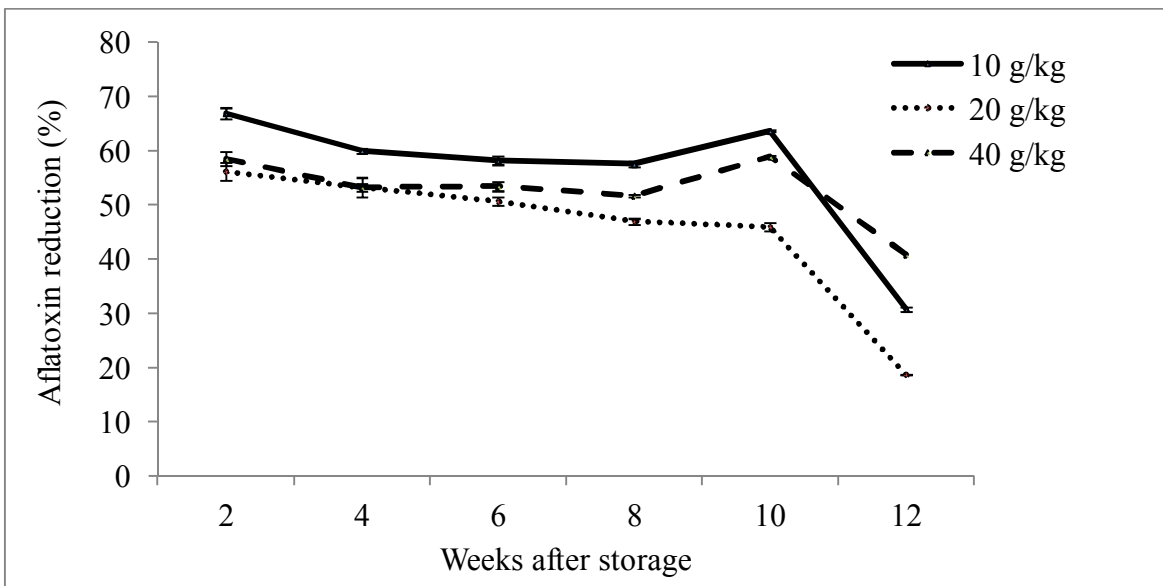

A

B

Figure 1. Effect of different concentrations of *Xylopia aethiopica* pod powder on aflatoxin contamination in stored egusi kernels at different weeks after storage

The vertical bars represent the standard error of the means.

A

B

Figure 2. Effect of different concentrations of *Ocimum gratissimum* leaf powder on aflatoxin contamination in stored egusi kernels at different weeks after storage

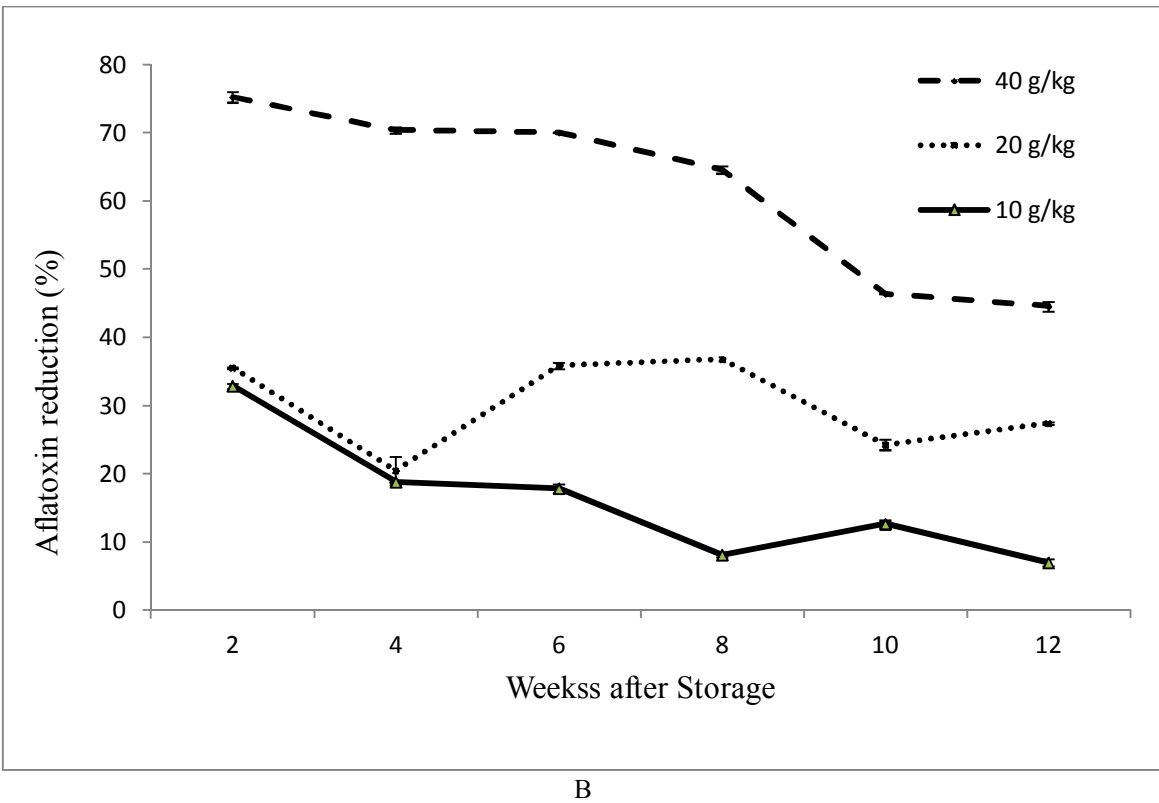The vertical bars represent the standard error of the means

Figure 3. Effect of different concentrations of *Piper guineense* fruit powder on aflatoxin contamination in stored egusi kernels at different weeks after storage

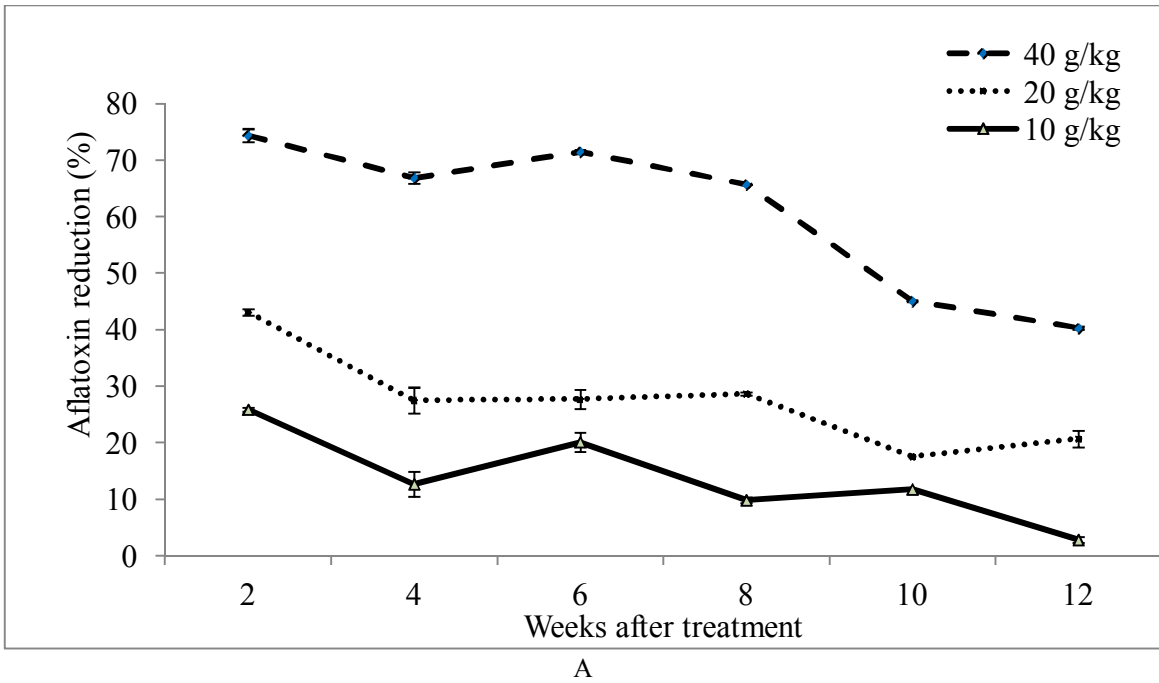The vertical bars represent the standard error of the means.

4.0. Discussion

In this study, the crude aqueous extracts of the 3 selected plants (*O. gratissimum, P. guineense* and *X. aethiopica*) tested showed varied antifungal potential on *A. flavus* isolated from egusi-melon. Among the three plant extracts studied, *P. guineense* was found to be more effective against *A. flavus*. The antimicrobial activities of the extracts varied widely. This was in agreement with the findings of Pundir and Jain (2010) that antimicrobial activity varies widely depending on the type of spice and organism involved. Many workers have reported antifungal activities of different plant species and stressed the importance of plants as possible sources of natural fungicides (Ogbebor *et al.,* 2005; Ogbebor *et al.,* 2007; Ogbebor and Adekunle*,* 2008; Oyewole and Abalaka, 2012). This study reveals that, all the concentrations used showed antifungal activity and can be used in control of *A. flavus* contamination in stored egusi-melon. This is in agreement with the findings of Kuri *et al. (*2010). Ogbebor *et al.* (2007) reported that extracts of *A. sativum* and *O. basilicum* demonstrated good inhibitory effect on the pathogens tested.

All the botanical extracts were effective in controlling the mycelial growth of *A. flavus*. The results from this study strongly suggest that *A. flavus* can be fairly managed by using these botanical extracts. The result has an agreement with the findings of (Reddy *et al.*, 2005**).** They found that the plant extracts showed complete inhibition of *Aspergillus flavus* growth. In poisoned feed technique all plant extract showed more than 20% mycelia growth inhibition at the least concentration (10%).

Results obtained with the botanicals in this study confirmed the importance of these plant species as exhibiting antifungal properties both in the i*n vitro* and *in vivo* experiments. The present investigation is an important step in preventing contamination of seeds with seed protectants which are eco-friendly for the management of this important seed borne fungi. So exploitation of naturally available chemicals from plant protection will play a prominent role in development of future commercial pesticides for crop protection strategies, with special reference to the management of plant diseases.

Much work has been done on the use of plant extracts against the plant-pathogenic fungi. Ogbebor *et al.* (2007) demonstrated high antifungal properties of aqueous extracts of *O. basilicum* and *A. sativum* on *Colletotrichum gloeosporioides*. Okigbo *et al.* (2005) reported that *X. aethiopica* exhibited antimicrobial activity against *Proteus mirabilis, Candida albicans* and *Staphylococcus aureus*. This shows that these botanicals contain bioactive ingredients that are inhibitory to the growth of these pathogens. These compounds are reported to exhibit physiological activity against most microorganisms such as *F. verticiloides* and *A. flavus* (Fleischer *et al.*, 2008). *O. gratissimum* was effective in inhibiting the growth of *F. solani* however, higher concentrations of the extract promoted mycelial growth. This also agrees with the finding of Ogbebor *et al.,* (2007). Mondali *et al.* (2009) reported that the growth of *A. flavus* was inhibited significantly and controlled with both alcoholic and water extracts extracts of neem leaf. The antifungal activities of plant extracts were supported by many other investigators; betel leaf extract (Srichana *et al.*, 2009), *Thymus vulgaris, Zingiber officinale, Cymbopogon citratus* (lemon grass) (Kumar *et al.,* 2007)*.* The antimicrobial activity of the botanical species was shown to be related to the presence of tannins and phytate. The antifungal activity of *X. aethiopica* and *O. gratissimum* probably may be due to presence of tannins in their content. For example, proximate composition analysis of *X. aethiopica* revealed a high content of protein (11.2 %) and ash 38.6 5) compared to 5.6% and 0.33 % respectively in *P. guineense*. This explains the reason behind the trends obtained in their antifungal activity. While *X. aethiopica* accelerated growth of most of the fungal isolates at higher concentration *P. guineense* reduced fungal activity with increase in concentration. Furthermore, *X. aethiopica* and *O. gratissimum* had very high tannin content of 36.7 and 31.3 mg/g respectively compared to 4.6 mg/g in *P. guineense*. Their phytate content was respectively 4.4, 4.0 and 3.7 mg/g. The mechanisms of action of these botanicals and their antinutritional compounds are yet to be proven and needs to be worked on. Although all of the plant extracts showed antifungal activity, the order of their activity *in vivo* experiments was somehow different from *in vitro* experiments; although *X. aethiopica* and *O. gratissimum* showed very close order of activity. The differences between results of the two experiments could be due to the nature of the plant compounds and their interaction with seed constituents (Bahraminejad, 2012).

It is clear from the above observations that all the botanicals (*O. gratissimum, P. guineense* and *X. aethiopica*) investigated proved to be useful in the management of aflatoxin problem and can be exploited in the protection of foods from mycotoxin contamination with repeated applications for at least 10 weeks intervals after which the potency of the botanicals reduces considerably. The decrease in the potency of the botanicals could have facilitated by the loss of the volatile compounds which decrease with increase in storage time (Ezekiel et al., 2014).

Egusi in various forms is an important constituent of different types of soup in many parts of Nigeria. The protection against fungal contamination

and their corresponding mycotoxins production during storage is very critical point for the safety of this agro-food product because of its role in human nutrition. This has placed a strong interest on variable alternative to use of chemicals for food preservation. The results from this study suggest that the postharvest seed pathogen can be considerably managed using these botanicals (*P. guineense, X. aethiopica* and *O. gratissimum*). The extracts of several other wild and medicinal plants have been tested for inhibition against aflatoxin producing fungi (Chulze, 2010).

A large number of medicinal plants have been tested for inhibition against mycotoxin producing fungi. *Mentha viridis* (Gabriel *et al.,* 2011), *Cymbopogon citratus* (lemon grass) (Bankole *et al.,* 2005; Dambolena *et al.*, 2010), *Mentha piperita* (peppermint), *Syzygium aromaticum* (clove) and *Pimpinella anisum* and *Hedeoma multiflora* (Bluma and Etcheverry, 2008) were reported to inhibit mycotoxin production in inoculated maize.

This study has shown that *X. aethiopica* (20 g/kg), *O. gratissimum* (10 g/kg), and *P. guineense* (20 g/kg) reduced aflatoxin and *A. flavus* growth considerably and therefore could be used to mitigate aflatoxin production in Egusi kernels during storage which is the critical period of aflatoxin contamination. These botanicals are also abundantly available in the local areas and therefore needs technological development to a form that will be easily accessible to farmers and traders for egusi protection against aflatoxin contamination.

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