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EFFICACY OF THREE BOTANICALS ON POSTHARVEST FUNGAL CONTAMINANTS OF MELON (*Citrullus colocynthis*) KERNELS

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ABSTRACT

Melon (Citrullus colocynthis L.) is an important crop used mainly for soups in Nigeria. Egusi: as it is commonly called in Nigeria is contaminated by many fungal pathogens which reduce quality of seeds during storage. Use of botanicals can be a safe method to manage fungal contamination instead of chemicals which pose threat to human health. Therefore, efficacy of Piper guineense, Xylopia aethiopica and Ocimum gratissimum on fungi in shelled Egusi seed kernels (EK) were evaluated. One market in each of six South-western Nigerian states where Egusi is sold was purposively selected in 2012 and 2013. Egusi kernels (½kg, n = 162) were purchased from selected traders for fungi isolation, identification and incidence (%) determination. Clean EK treated with botanical powder (10, 20 and 40 g kg^{-1}) were inoculated with Aspergillus flavus, A. niger, A. tamarii, Rhizopus sp., Penicillium aurantiogriseum, P. citrinum and Fusarium solani bi-weekly for 14-week storage period to evaluate growth reduction (%). Control was inoculated with sterile distilled water. Aspergillus flavus, A. niger, A. tamarii, Penicillium citrinum, P. aurantiogriseum, Fusarium solani and Rhizopus sp. were frequently encountered in EK. Aspergillus (32.4±1.6%) was the most predominant fungus followed by Rhizopus (21.5±2.0%) in all States. Piper guineense (40 g kg^{-1}), X. aethiopica (20 g kg^{-1}) and O. gratissimum (10 g kg^{-1}) powders significantly reduced aflatoxin contamination by 42.5%, 56.5% and 45.0%, respectively; fungi growths were progressively reduced by P. guineense (5.5-90.0%), X. aethiopica (6.7-100.0%) and O. gratissimum (7.4-66.7%) up to 12 weeks of storage. Egusi kernels were highly infected with postharvest fungi especially Aspergillus species. Xylopia aethiopica at 20 g kg^{-1}, O. gratissimum (40.0%) and P. guineense (40.0%) reduced fungi growth on Egusi considerably and therefore could be used as a safe management option to mitigate storage fungi contamination in Egusi kernels.

Key words: storage fungi, botanical powder, Southwest Nigeria, fungi contamination

INTRODUCTION

Melon (*Citrullus colocynthis* L.) commonly known as Egusi is an important soup spice produced in abundance in different parts of Nigeria and many countries in sub-Saharan Africa (Van der Vossen *et al*., 2004). The oil rich seeds are the economic part of the crop. It is also rich in protein and contains good quantities of most of the essential amino acids (Ayodele and Salami, 2006).

The seeds can be used or processed by a variety of forms such as soaking, boiling, fermentation, grinding and molding into balls before use for soup preparation. Egusi is not only valuable as a staple for local consumption, but also as export commodity to be sold to people who have emigrated from Africa to other continents of the world (Van der Vossen *et al*., 2004).

Many groups of fungi are known to contaminate Egusi seeds during storage. They reduce seed storability, quality, export and marketability potentials; and above all deposit a large number of metabolites in the seeds; some of which are toxic to humans (Chiejina, 2006; Atehnkeng *et al*., 2008). Fungi species belonging to the genera *Rhizopus*, *Penicillium* amongst others have been reported as seed pathogens of Nigerian stored Egusi seed. Many storage fungi that have been variously implicated in the spoilage of fruits and vegetables have been isolated from Egusi seeds by various authors (Chiejina, 2006; Aboloma *et al.,* 2009; Aboloma *et al.,* 2012). Fungal deterioration of seeds occurs in form of rot, sclerotization of seed, and seed discolourization (Shetty 1992).

Prior to harvest of Egusi seeds, fungal infection may not be up to the level that can lead to economic damage. Small quantities of spores of storage fungi may be present on grain meant for storage or may be present on spilled grains present in storage equipment or structures. This small amount of inoculum can multiply rapidly leading to significant grain infection under poor storage conditions. Bankole (1993) reported as many as 13 storage fungal species in stored Egusi seeds. Also, Chiejina (2006) isolated thirteen different fungal species and

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two unidentified species from Egusi seed kernel. These fungi are widely distributed and almost always present on the seeds during storage. However, the development of these fungi are influenced by the moisture content, the temperature, the condition of the Egusi seed going into storage, the length of time the seed is stored and the amount of insect and mite activity in the stored seed.

Interestingly, nature supplies a reasonable number of plant products that have useful properties for crop protection, which are often neglected in favour of commercial products. These natural substances cause little disturbance to the natural balance between living organisms. They are cheap and can be produced by farmers from local sources. They are often harmless to humans and animals and are rarely toxic to plants when compared with artificial (Dusanee, 2011).

The use of benzoic acid, gamma-irradiation and fumigants to prevent postharvest fungal contamination have been emphasized and in use but they are not safe for crops meant for human consumption. Treatment with natural products from plants or herbs (botanicals) which are edible prior to storage is a safe option for grains meant for humans. The botanicals are mostly available locally and are not potential environmental and biological hazards. The possibilities of using botanical pesticides seem almost endless and their potentials can be fully exploited (Yallappa *et al*., 2012). The prevention of postharvest fungal contamination in Egusi is one of the best and most effective strategies to reduce yield loss and maximize income from harvested seeds; hence the need to assess the potentials of some medicinal plants for the control of storage fungi in stored Egusi seed kernels. The objective of this study therefore was to determine the effects of selected botanicals on fungal growth in treated Egusi seed kernels.

MATERIALS AND METHODS

Sample Collection

A total of 162 melon vendors were randomly selected in each of the six South-western Nigerian States (Sabo in Ondo, Sabo in Ekiti, Oto in Lagos, Sabo in Ogun, Oja Oba in Osun, Bodija in Oyo) in 2012 and 2013. Shelled melon seeds (kernel) were purchased directly from a major market where melon from the producing areas is unloaded in each of the six south-western states of Nigeria. For each market visited Simple random sampling was adopted for sample collection. From each trader, 0.5-1 kg of melon seed was purchased and taken to the laboratory for studies. Various melon seeds were purchased from three traders at different points within the same market, packed into different polythene bags and these served as the replicates. For each bag, sampled melon kernels were collected at different points in the bag to form a composite sample.

Preparation of Botanical Powder

The botanicals were purchased from the Ojoo market. The botanicals were thoroughly washed, air-dried under shade until they were properly dried; ground to fine powder using the Warring laboratory blender (Warring Commercial, Springfield, MO) and stored at 4°C until when needed.

Application of Plant Powders to Egusi Kernels and Fungal Contamination Test

The botanical powders were prepared as already described above; the botanical powders were used to dust 1 kg of clean (uninfected) Egusi kernels in plastic woven bags, mixed properly and placed on the shelve for a period of three and half months. Prior to treatment and storage, shelled melon kernels were dried to moisture content of 10% (determined with Pfeuffer helite moisture meter). The controls were not dusted with any botanical powder (Bankole and Joda, 2004). Subsamples of 50 g were collected from each 1 kg sample at 2nd, 4th, 6th, 8th 10th 12th and 14th week for fungal contamination test with *A. flavus* and other identified predominant fungal species (*A. niger, A. tamarii, Rhizopus* sp., *P. aurantiogriseum, P. citrinum* and *F. solani*). Inocula suspensions were prepared from fresh, mature (5-day-old) fungi cultures. Fungal colonies were covered with 5 ml of distilled sterile water containing 1% Tween 20 per 100 ml to enhance uniform spore dispersal for hydrophobic genus such as *Apergillus*. The final inoculum size was adjusted to a concentration of 1.0×10^6 spore/ml by microscopic enumeration with a cell-counting haemocytometer (Aberkane *et al*., 2002). The Egusi kernels treated with botanicals were washed in three changes of sterile distilled water and then twenty kernels were inoculated with 100 µL of spore suspension of each test fungi. Five kernels were plated on petri dishes containing solidified Potato Dextrose Agar (PDA) and incubated at room temperature for five days. The controls were plated on PDA without inoculation with any fungal species. Percentage kernel colonization was recorded after incubation.

Isolation and Identification of Fungi from Market Melon Samples

Egusi seed kernels from traders' shop were processed and fungi isolated done following the methods described by (Atehnkeng *et al.,* 2014) using PDA in which 0.05 ml of lactic acid had been added to suppress bacterial growth (Atehnkeng *et al.,* 2008). After incubation for 5 days at room temperature, the colony forming units (cfu ml^{-1}) of each fungal 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 the 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; Alexopoulos *et al.,* 2002; Samson *et al*., 2004) and the descriptions of Barnett and Hunter (1999). The experiments were carried out with treatment in triplicates laid out in completely randomized design.

Determination of Percentage Occurrence of the Fungal Isolates

This was done to determine the incidence of occurrence of the different fungal 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):

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

Data Analysis

Data on fungal incidence in melon grains were 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 means obtained from the different states or treatments.

RESULTS

Nine different fungal genera apart from *Aspergillus* were identified in the melon samples collected in 2012. They are *Fusarium, Penicillium, Rhizopus, Botryodiplodia, Trichoderma, Alternaria, Sclerotium, Cladosporium* and *Macrophomina*. Across the six states, *Aspergillus* species were the most predominant fungal species identified, followed by species belonging to the genera *Rhizopus, Fusarium, Alternaria,* and *Penicillium* while *Trichoderma, Botryodiplodia* and *Macrophomina* species were the least predominant. *Aspergillus* species had the highest fungal colonies per gram (cfu g^{-1}) and was significantly (p = 0.05) higher than all other fungal genera identified. The highest fungal colonies per gram (cfu g^{-1}) of *Aspergillus* species was recorded in samples from Osun State (11,920 cfu g^{-1}) while the least cfu g^{-1} was recorded in Lagos State and they were significantly different across the states. The highest cfu g^{-1} of *Fusarium, Botryodiplodia, Alternaria, Sclerotium* and *Cladosporium* were isolated from samples from Lagos State. The highest cfu g^{-1} of *Penicillium* and *Rhizopus* were found in Ondo (2193.3 cfu g^{-1}) and Ogun (766.7 cfu g^{-1}) States, respectively (Table 1).

Aspergillus, Fusarium, Penicillium, Rhizopus, Botryodiplodia, Paecilomyces, *Alternaria, Sclerotium* and *Macrophomina* were identified in the melon samples collected during 2013 sampling (Table 1). *Aspergillus* species were the most predominant fungal species identified across the six states, followed by species belonging to the genera *Rhizopus, Penicillium, Fusarium, Alternaria, Sclerotium*, while *Cladosporium, Botryodiplodia* and *Macrophomina* species were the least predominant. *Aspergillus* species had the highest fungal colonies per gram (cfu g^{-1}) and was significantly (p = 0.05) higher than all other fungal genera identified except in Ondo where the highest *Rhizopus* colonies was recorded (11990.7 cfu g^{-1}), and also in Ogun State (2466.7 cfu g^{-1}). The highest fungal colonies per gram (cfu g^{-1}) of *Aspergillus* species was recorded in samples from Oyo State (3250 cfu g^{-1}) while the least cfu g^{-1} was recorded in Ekiti State and they were significantly different across the states. The highest cfu g^{-1} of *Penicillium, Fusarium, Botryodiplodia, Alternaria,* and *Paecilomysis* were isolated from samples collected from Ogun State. The highest cfu g^{-1} of *Sclerotium* was recorded in Osun while the highest cfu g^{-1} of *Cladosporium* occurred in Lagos (22.2 cfu g^{-1}) and Ogun (22.2 cfu g^{-1}) States (Table 1).

Table 1: Mould content of Egusi kernels collected from the six states of South-western Nigeria in 2012 and 2013

		Fungal colonies (cfu g^{-1})									
Year	State	Asp	Rhz	Pen	Bot	Fus	Cld	Alt	Scl	Pec	Tri
2012	Ekiti	2406.7^{b}	444.4a^{b}	155.6^{c}	22.2b^{c}	22.2^{c}	22.2^{c}	22.2^{c}	22.2^{c}	0.0^{a}	0.0^{b}
	Lagos	2366.7^{b}	188.9^{c}	355.6^{c}	500.0^{a}	500.0^{a}	500.0^{a}	500.0^{a}	500.0^{a}	0.0^{a}	100.0^{a}
	Ogun	3128.3^{b}	766.7^{a}	513.3^{c}	0.0^{c}	20.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{a}	100.0^{a}
	Ondo	4600.0^{b}	122.2^{c}	2193.3^{a}	120.0a^{b}	120.0^{c}	500.0^{a}	120.0^{b}	120.0^{b}	0.0^{a}	0.0^{b}
	Osun	11920.0^{a}	322.2^{b}	1753.3b^{a}	158.0a^{b}	126.7ab^{c}	120.0^{b}	126.7^{b}	126.7^{b}	0.0^{a}	100.0^{a}
	Oyo	5794.7^{b}	133.3^{c}	1005.6a^{b}	94.4a^{b}	188.9^{ab}	88.9^{b}	89.0^{b}	88.9^{b}	0.0^{a}	0.0^{b}
2013	Ekiti	1511.1^{b}	383.3^{c}	372.2b	0.0^{b}	5.6^{c}	0.0^{b}	55.6^{b}	0.0^{c}	0.0^{b}	0.0^{a}
	Lagos	2116.7^{b}	225.0^{c}	8.3^{c}	0.0^{b}	5.6^{c}	22.2^{a}	25.0^{b}	0.0^{c}	0.0^{b}	0.0^{a}
	Ogun	2230.3^{ab}	2466.7^{ab}	2076.3^{a}	100.0^{a}	1868.7^{a}	6.7^{b}	880.0^{a}	126.7^{a}	266.7^{a}	0.0^{a}
	Ondo	2736.7^{ab}	11990.7a	1923.6^{ab}	100.0^{a}	873.6^{b}	22.2^{a}	116.7^{ab}	50.0^{b}	6.7^{b}	0.0^{a}
	Osun	2380.0^{ab}	1055.6^{b}	350.0^{b}	0.0^{b}	50.0^{c}	8.3^{b}	0.0^{b}	166.7^{a}	0.0^{b}	0.0^{a}
	Oyo	3250.0^{a}	2622.2^{ab}	566.7^{b}	0.0^{b}	50.0^{c}	0.0^{b}	0.0^{b}	150.0^{a}	111.1^{ab}	0.0^{a}

Note: Asp – *Aspergillus*; Pen – *Penicillium*; Fus –*Fusarium*; Rhz – *Rhozopus*; Bot – *Botryodiplodia*; Tri – *Trichodrma*; Cld *Cladosporium*; Alt – *Alternaria*; Scl – *Sclerotium*; Pec – *Paecilomysis*. For each column in each year, means with same letters are not significantly different.

Incidence of Fungal Species in Egusi Kernels

Aspergillus species had the highest percentage incidence across all the states, followed by *Penicillium* and *Fusarium* species while *Macrophomina* was the least in 2012 (Figure 1). In Ekiti and Ogun States, significant differences were not observed in the incidence of *Fusarium* and *Alternaria* species. The same trend was observed in *Fusarium* and *Penicillium* species in Lagos and Osun States. In 2013, *Aspergillus* species also had the highest percentage incidence across all the states, followed by *Rhizopus* and *Penicillium* species while *Paecilomyces* was the least. The incidence of *Aspergillus* species was significantly ($p = 0.05$) higher than all other fungal genera identified except in Osun State where *Aspergillus* and *Rhizopus* species were significantly ($p = 0.05$) higher than all other fungal genera identified in both 2012 and 2013 (Figures 1 and 2).

Effect of Selected Botanicals on Growth of Fungal Contaminants in Treated Egusi kernels

Storing Egusi kernels together with botanical powders significantly reduced fungal growth in the stored Egusi kernels. All the botanicals tested differed from each other in their ability to reduce fungal growth on the seeds. The result presented in Table 2 shows the percentage reduction of fungal growth in Egusi seeds treated with different concentrations of *P. guineense*. Forty-gram (40 g kg^{-1}) treatment generally had the highest growth reduction for the various fungal isolates except for *F. solani* where 10 and 20 g kg^{-1} treatment gave the highest growth reduction of 60.0% while 40 g kg^{-1} had the least growth reduction (10.0%). Percentage growth reduction declined gradually as the storage period increased. One month after treatment, the botanicals were not effective in inhibiting the growth of *Rhizopus* sp. The growth of *P. aurantiogriseum* was inhibited effectively up to 8th week, 10th week and 12th week after storage at 10, 20 and 40 g kg^{-1} treatment, respectively. *P. citrinum* growth was effectively reduced by more than 20.0% by the various levels of the botanical treatments throughout the 14 weeks of Egusi storage. The second experiment gave similar results. Treatment at 40 g kg^{-1} generally had the highest growth reduction for the various fungal isolates except for *F. solani* where 10 and 20 g kg^{-1} treatment gave the higher growth reductions of 55.0% and 65.0%, respectively than 40 g kg^{-1} which had the least growth reduction of 15.0%. *P. citrinum* growth was still effectively reduced by more than 20.0% by the various levels of the botanical treatments throughout the period of 14 weeks of Egusi storage (Table 2).

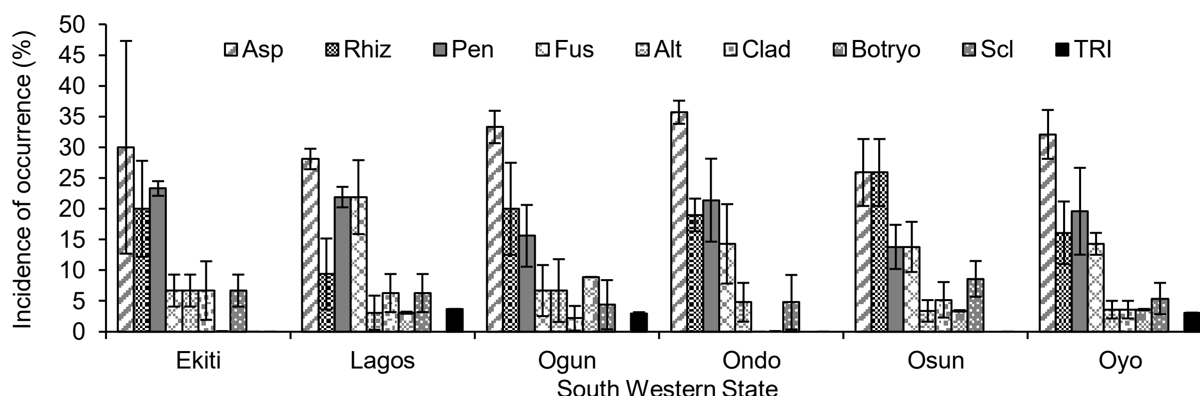

Figure 1: Percentage incidence of fungal species isolated from Egusi kernels collected from six states in South-western Nigeria in 2012. Asp – *Aspergillus*; Pen – *Penicillium*; Fus – *Fusarium*; Rhiz – *Rhozopus*; Botryo – *Botryodiplodia*; Tri – *Trichoderma*; Clad – *Cladosporium*; Alt – *Alternaria*; Scl - *Sclerotium.* For each bar, the vertical line represents the standard error of the means.

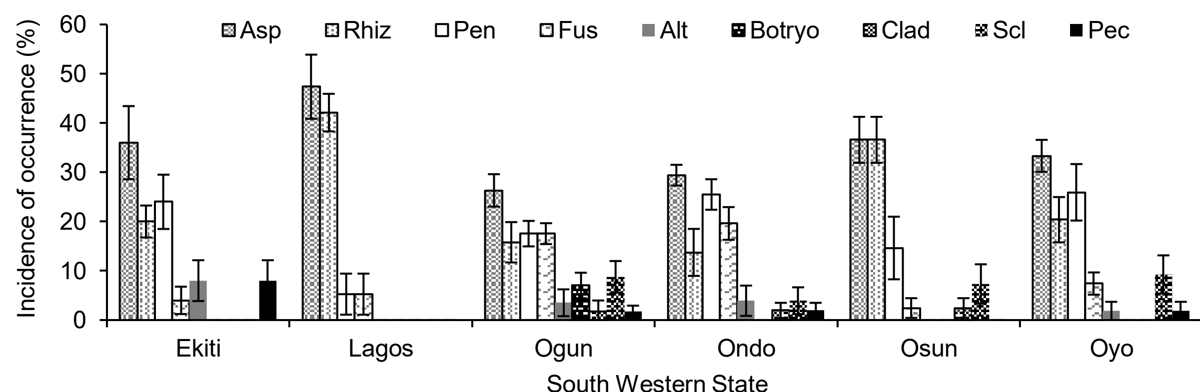

Figure 2: Percentage incidence of fungal species isolated from Egusi kernels collected from six states in South-western Nigeria in 2013. Asp – *Aspergillus*; Pen – *Penicillium*; Fus – *Fusarium*; Rhiz – *Rhozopus*; Botryo – *Botryodiplodia*; Tri – *Trichoderma*; Clad – *Cladosporium*; Alt – *Alternaria*; Scl – *Sclerotium*; Pec – *Paecilomysis.* For each bar, the vertical line represents the standard error of the means.

Table 2: Effect of different concentrations of *P. guineense* on fungal contaminants of stored Egusi kernels at different weeks after storage

Weeks	Conc. (g kg^{-1})	Percentage growth reduction													
		First experiment (2012)							Second experiment (2013)						
		FLV	NIG	PUR	RHZ	SOL	TAM	YEL	FLV	NIG	PUR	RHZ	SOL	TAM	YEL
2	10	25.0b	20.0a	70.0b	20.0c	60.0a	25.0b	90.0a	25.0b	25.0a	85.0b	20.0b	55.0a	40.0a	90.0b
	20	55.0a	50.0a	100.0a	30.0b	60.0a	50.0a	100.0a	60.0a	40.0a	100.0a	30.0b	65.0a	40.0a	100.0a
	40	50.0a	40.0a	90.0a	60.0a	10.0b	45.0a	100.0a	60.0a	35.0a	95.0ab	60.0a	15.0b	35.0a	100.0a
4	10	25.0b	35.0b	75.0a	0.0a	15.0a	50.0a	95.0c	20.0a	30.0b	88.9a	0.0a	10.0a	40.0a	80.0b
	20	30.0a	25.0b	95.0a	0.0a	10.0a	35.0a	95.0b	40.0a	35.0ab	95.0a	0.0a	25.0a	25.0a	80.0b
	40	50.0a	65.0a	100.0a	5.0a	25.0a	45.0a	100.0a	40.0a	65.0a	100.0a	5.0a	40.0a	45.0a	100.0a
6	10	0.0a	15.0a	40.0a	0.0a	10.0a	10.0a	80.0b	5.0a	30.0a	35.0b	0.0a	5.0a	15.0a	80.0b
	20	20.0a	35.0a	58.3a	0.0a	5.0b	0.0b	80.0b	10.0a	30.0a	58.3a	0.0a	0.0a	0.0a	80.0b
	40	10.0a	25.0a	65.0a	0.0a	5.0b	5.0a	90.0a	10.0a	40.0a	60.0a	0.0a	5.0a	5.0a	90.0a
8	10	0.0b	15.0b	35.0b	0.0a	5.0a	5.0a	65.0b	5.0a	15.0a	30.0b	0.0a	10.0a	10.0a	70.0b
	20	10.0ab	10.0ab	45.0ab	0.0a	0.0a	0.0a	85.0ab	10.0a	5.0a	40.0b	0.0a	0.0a	0.0a	85.0ab
	40	15.0a	25.0a	70.0a	0.0a	5.0a	0.0a	90.0a	15.0a	30.0a	64.3a	0.0a	10.0a	0.0a	90.0a
10	10	0.0a	15.0a	15.0c	0.0a	5.0a	0.0a	65.0b	0.0a	5.0a	15.0b	0.0a	5.0a	5.0a	70.0b
	20	0.0a	6.7a	40.0b	0.0a	0.0a	5.0a	85.0a	0.0a	0.0a	40.0ab	0.0a	0.0a	0.0a	90.0a
	40	5.0a	25.0a	55.0a	0.0a	5.0a	0.0a	85.0a	5.0a	5.0a	55.0a	0.0a	10.0a	0.0a	85.0ab
12	10	0.0b	14.3a	12.5b	0.0a	10.0a	7.5a	65.0a	0.0b	15.0a	13.3b	0.0a	0.0a	0.0b	65.0c
	20	0.0b	7.7a	13.3b	0.0a	10.0a	5.6a	80.0a	0.0b	5.9a	26.7ab	0.0a	0.0a	0.0a	80.0b
	40	30.8a	42.9a	50.0a	0.0a	15.0a	5.9a	90.0a	5.0b	30.0a	56.3a	0.0a	0.0a	0.0a	90.0a
14	10	0.0b	5.0a	6.7a	0.0a	5.0a	5.0a	35.0b	0.0a	7.1a	7.0a	0.0a	5.0a	5.0a	40.0b
	20	0.0b	5.6a	7.1a	0.0a	0.0a	0.0a	50.0a	0.0a	9.1a	14.0a	0.0a	0.0a	0.0a	45.0b
	40	25.0a	10.0a	15.4a	0.0a	10.0a	0.0a	47.0a	11.1b	12.5a	19.0a	0.0a	7.5a	0.0a	50.0a

Conc. - concentration; FLV - *Aspergillus flavus*; NIG - *Aspergillus niger*; PUR - *Penicillium aurantiogriseum*; RHZ- *Rhizopus* sp.; SOL - *Fusarium solani*; TAM - *Aspergillus tamari*; YEL - *Penicillium citrinum.* For each week, means with same letter in each column are not significantly different.

Table 3 shows the percentage reduction of fungal growth in Egusi kernels treated with different concentrations of *X. aethiopica. A. tamarii* and *A. niger* had maximum growth reduction at 40 g kg^{-1} treatment while *A. flavus* recorded the highest growth reduction of 50% at 20 and 40 g kg^{-1} treatment. For *F. solani,* 20 g kg^{-1} treatment gave the highest growth reduction of 75.0%, followed by 10 g kg^{-1} treatment (70% reduction) while 40 g kg^{-1} had the least growth reduction (30%) after 2 weeks of treatment. Percentage growth reduction gradually declined as the storage period increased. *X. aethiopica* at various concentrations only had effect on *Rhizopus* sp. growth after 2 weeks of treatment. The growth of *P. aurantiogriseum* was effectively inhibited up to 14th week after storage at all treatment levels because up to 20% growth reduction was recorded at 10 g kg^{-1} treatment even at the 14th week. *P. citrinum* growth was also effectively reduced by more than 20% by the various levels of the botanical treatments throughout the 14 weeks of Egusi storage (Table 3). The second experiment gave similar results. *A. tamarii* had maximum growth reduction at 40 g kg^{-1} treatment while *A. flavus* growth reduction of 66.7% was recorded at 20 g kg^{-1}; although not significantly different from 40 g kg^{-1} treatment which had 55.6% growth reduction at two weeks after storage. However, at 4th week after storage 40 g kg^{-1} treatment recorded a growth reduction of 61.1% while 20 g kg^{-1} had 66.7% growth reduction. Percentage growth reduction declined gradually as the storage period increased. After one month of treatment *O. gratissimum* was not effective in inhibiting the growth of *Rhizopus* sp. The growth of *P. aurantiogriseum* was effectively inhibited up to 8th week, 10th week and 12th week after storage at 10, 20 and 40 g kg^{-1} treatment, respectively. Growth of *P. citrinum* was effectively reduced by up to 20% by the various levels of the botanical treatments throughout the 14 weeks of Egusi storage. The second experiment gave similar results. Forty (40) g kg^{-1} treatment generally had the highest growth reduction for the various fungal isolates except for *A. flavus,* where 20 g kg^{-1} treatment gave the highest growth reduction of 80%. This was followed by 40 g kg^{-1} treatment which had 65.0% growth reduction *P. citrinum* growth was still effectively reduced by up to 20.0% and above by the various levels of the botanical treatments throughout the 14 weeks of Egusi storage except at 2.0% treatment in the 14th week after storage where only 10.0% reduction was recorded (Table 4).

Table 3: Effect of different concentrations of *X. aethiopica* on fungal contaminants of stored Egusi kernels at different weeks after storage

Weeks	Conc.(g kg^{-1})	Percentage growth reduction													
		First experiment (2012)							Second experiment (2013)						
		FLV	NIG	PUR	RHZ	SOL	TAM	YEL	FLV	NIG	PUR	RHZ	SOL	TAM	YEL
2	10	16.7b	35.0b	100.0a	20.0c	70.0a	15.0b	100.0a	5.6b	25.0b	100.0a	20.0c	55.0b	20.0c	100.0a
	20	50.0a	50.0ab	100.0a	30.0b	75.0a	45.0a	100.0a	66.7a	65.0a	100.0a	30.0b	70.0ab	40.0b	100.0a
	40	50.0a	65.0a	100.0a	40.0a	30.0b	55.0a	100.0a	55.6a	55.0a	100.0a	40.0a	25.0b	60.0a	100.0a
4	10	22.2b	25.0b	85.0a	0.0b	30.0b	15.0b	90.0b	22.2b	25.0b	95.0b	5.0a	35.0b	20.0a	100.0a
	20	50.0a	31.3ab	97.5a	10.0b	70.0a	30.0b	100.0a	66.7a	56.3ab	100.0a	5.0a	75.0ab	40.0a	100.0a
	40	38.9a	65.0a	100.0a	25.0a	25.0b	65.0a	100.0a	61.1a	66.7a	100.0a	15.0a	15.0b	65.0a	100.0a
6	10	5.6a	20.0b	43.0b	0.0a	20.0ab	15.0a	80.0b	5.6a	25.0a	65.0b	0.0a	20.0a	10.0a	80.0b
	20	10.0a	35.0b	60.0b	0.0a	15.0b	10.0a	90.0a	10.0a	30.0a	60.0b	0.0a	20.0a	15.0a	90.0a
	40	16.7a	66.7a	100.0a	0.0a	30.0a	20.0a	92.5a	16.7a	44.4a	100.0a	0.0a	20.0a	15.0a	87.5a
8	10	5.6a	25.0b	35.0b	0.0a	5.0b	15.0a	60.0c	11.1a	12.5a	40.0b	0.0a	5.0a	10.0a	60.0c
	20	10.0a	15.0ab	45.0b	0.0a	10.0b	10.0a	70.0b	11.1a	30.0a	55.0b	0.0a	5.0a	15.0a	75.0b
	40	15.0a	50.0a	100.0a	0.0a	35.0a	15.0a	90.0a	20.0a	40.0a	100.0a	0.0a	25.0a	10.0a	90.0a
10	10	5.0a	25.0a	15.0bc	0.0a	5.0b	10.0a	40.0c	5.0a	12.5a	15.0b	0.0a	0.0b	5.0a	70.0b
	20	15.0a	18.8a	25.0b	0.0a	10.0b	10.0a	82.5b	15.0a	18.8a	25.0b	0.0a	10.0a	7.5a	80.0aa
	40	15.0a	20.5a	100.0a	0.0aa	30.0a	5.0a	80.0a	15.0a	30.0a	100.0a	0.0a	10.0a	10.0a	80.0a
12	10	6.7a	20.0a	20.0c	0.0a	8.3b	7.5a	25.0c	7.1ab	10.5b	20.0c	0.0a	0.0b	5.0b	40.0b
	20	21.4a	21.1a	23.1b	0.0a	10.0b	15.0a	60.0b	21.4ab	12.5ab	23.1b	0.0a	10.0a	20.0ab	50.0a
	40	10.0a	16.7b	100.0a	0.0a	30.0a	6.3a	68.8a	20.0a	16.7a	100.0a	0.0a	20.0a	7.1b	75.0a
14	10	4.4a	10.0a	10.0b	0.0a	6.3b	5.3a	18.8b	0.0a	0.0a	8.3c	0.0a	0.0c	5.0b	35.0b
	20	0.0a	6.3ab	20.0b	0.0a	7.7ab	15.0a	56.3b	0.0a	5.6a	12.5b	0.0a	7.7b	10.0ab	50.0ab
	40	4.4a	7.1b	85.0a	0.0a	21.4a	5.9a	68.8a	10.0a	10.0a	90.0a	0.0a	11.1a	5.9b	62.5a

Conc. – concentration; FLV - *Aspergillus flavus*; NIG - *Aspergillus niger*; PUR - *Penicillium aurantiogriseum*; RHZ- *Rhizopus* sp.; SOL - *Fusarium solani*; TAM - *Aspergillus tamari*; YEL - *Penicillium citrinum.* For each week, means with same letter in each column are not significantly different.

Table 4: Effect of different concentrations of *O. gratissimum* on fungal contaminants of stored Egusi kernels at different weeks after storage

Weeks	Conc.(g kg^{-1})	Percentage growth reduction													
		First experiment (2012)							Second experiment (2013)						
		FLV	NIG	PUR	RHZ	SOL	TAM	YEL	FLV	NIG	PUR	RHZ	SOL	TAM	YEL
2	10	25.0b	30.0a	75.0b	20.0c	50.0b	10.0b	100.0a	35.0b	25.0a	70.0b	20.0b	60.0a	20.0b	85.0a
	20	65.0a	40.0a	100.0a	40.0b	65.0a	50.0a	100.0a	80.0a	35.0a	100.0a	40.0a	60.0a	65.0a	100.0a
	40	60.0a	50.0a	100.0a	70.0a	75.0a	50.0a	100.0a	65.0a	40.0a	100.0a	70.0a	75.0a	65.0a	100.0a
4	10	45.0b	35.0b	75.0b	0.0b	40.0b	30.0a	80.0b	60.0a	55.0a	80.0b	5.0a	25.0b	50.0b	75.0b
	20	65.0a	35.0b	95.0a	25.0a	50.0a	30.0a	92.5a	70.0a	65.0a	100.0a	5.0a	60.0b	50.0b	95.0b
	40	70.0a	65.0a	95.0a	55.0a	65.0a	50.0a	100.0a	65.0a	65.0a	100.0a	5.0a	75.0a	65.0a	100.0a
6	10	15.0a	25.0a	45.0a	0.0a	10.0a	15.0b	70.0b	10.0a	25.0a	40.0c	0.0a	15.0a	20.0a	70.0b
	20	15.0a	40.0a	45.0a	0.0a	25.0a	10.0a	70.0b	20.0a	35.0a	50.0b	0.0a	25.0a	10.0b	70.0b
	40	40.0a	55.0a	60.0a	0.0a	20.0a	10.0a	90.0a	35.0a	40.0a	60.0a	0.0a	20.0a	10.0a	92.5a
8	10	10.0b	25.0b	35.0a	0.0a	10.0b	5.6a	60.0b	5.0a	30.0a	40.0a	0.0a	10.0a	10.0a	65.0a
	20	15.0ab	40.0ab	40.0a	0.0a	15.0ab	15.0a	70.0b	10.0a	45.0a	30.0a	0.0a	25.0a	15.0a	75.0a
	40	20.0a	45.0a	60.0a	0.0a	20.0a	11.1a	85.0a	30.0a	35.0a	60.0a	0.0a	5.0a	5.6a	80.0a
10	10	5.0a	20.0a	25.0c	0.0a	10.0a	15.0a	70.0b	10.0a	20.0a	30.0c	0.0a	10.0a	10.0a	70.0b
	20	11.8a	20.0a	55.0b	0.0a	10.0a	5.0a	66.7a	10.0a	30.0a	65.0b	0.0a	15.0a	0.0b	66.7b
	40	16.7a	25.0a	90.0a	0.0a	12.5a	5.0a	92.5a	20.0a	30.0a	100.0a	0.0a	6.3a	5.6b	90.0a
12	10	10.0a	25.0a	16.7b	0.0a	6.7a	0.0a	60.0b	11.1a	20.0a	25.0b	0.0a	20.0a	20.0a	60.0b
	20	12.5a	18.8a	25.0ab	0.0a	8.3a	0.0a	40.b	14.3a	6.3a	12.5b	0.0a	12.5a	0.0a	50.0b
	40	12.5a	18.8a	66.7a	0.0a	10.0a	6.7a	82.5a	16.7a	25.0a	66.7a	0.0a	8.3a	7.7a	85.0a
14	10	0.0b	11.1a	9.1b	0.0a	1 6.3b	0.0a	50.0ab	8.3a	17.5a	14.3a	0.0a	15.0a	0.0a	45.0a
	20	8.3ab	12.5a	16.7b	0.0a	7.7ab	0.0a	20.0b	0.0a	6.3a	8.3a	0.0a	6.3b	0.0a	10.0a
	40	12.5a	11.1a	36.4a	0.0a	10.0a	6.3a	54.5a	12.5a	16.7a	36.4a	0.0a	7.7b	0.0a	45.5a

Conc. concentration, FLV - *Aspergillus flavus*; NIG - *Aspergillus niger*; PUR - *Penicillium aurantiogriseum*; RHZ- *Rhizopus* sp.; SOL - *Fusarium solani*; TAM - *Aspergillus tamari*; YEL - *Penicillium citrinum.* For each week, means with same letter in each column are not significantly different.

DISCUSSION

Fungal species belonging to nine genera were isolated and identified in Egusi kernels from the six states in South-western region of Nigeria and *Aspergillus* species were the most predominant. High levels of *Aspergillus* species population have previously been reported in Nigeria in post-harvest maize (Atehnkeng *et al*., 2008). The *Aspergillus* species recorded in the present study had previously been reported in high frequencies in Egusi (Bankole, 1993) and groundnut, another Nigerian oil seed (Ogundero, 1980).

Besides *Aspergillus*, species of *Penicillium*, *Fusarium*, *Trichoderma*, *Paecilomyces*, *Aternaria*, *Cladosporium*, *Sclerotium* and *Botryodiplodia* were associated with melon seeds in Nigeria (Bankole and Joda, 2004; Chiejina, 2006; Aboloma and Ogunbusola, 2012). The bio deteriorating and aflatoxigenic fungal species spores that colonized melon must have been present in the atmosphere during sun drying and storage of the seeds. The fungi could have been introduced during exposure and direct contact of' the seeds in the market (Okigbo, 2003; Gbolagade *et al*., 2011).

This study reveals that, all the concentrations used showed antifungal activity. Thus, they can be useful in the control of the fungi associated with stored Egusi. This agrees with the findings of Kuri *et al*. (2011). Ogbebor and Adekunle (2005) and Ogbebor *et al.* (2007) reported that extracts of *A. sativum* and *O. basilicum* demonstrated good inhibitory effect on the pathogens tested. Many workers have reported antifungal activities of different plant species and stressed the importance of plants as possible sources of natural fungicides (Ogbebor and Adekunle, 2005; Ogbebor *et al.,* 2005; Ogbebor *et al.,* 2007; Ogbebor and Adekunle, 2008; Shovan *et al*., 2008; Oyewole and Abalaka, 2012).

Many reports exist on the use of botanicals against the plant pathogenic fungi. For example, *O. basilicum* and *A. sativum* on *Colletotrichum gloeosporioides* (Penz). *Allium cepa* L., against *Alternaria tenuis* and *Curvularia lunata* Wakker, *X.* against *Proteus mirabilis* Hauser*, Candida albicans* Berkh and *Staphylococcus aureus* (Misra and Dixit, 1976; Okigbo *et al.,* 2005; Ogbebor *et al.,* 2007). This shows that these botanicals contain bioactive ingredients that are inhibitory to the growth of these pathogens. The antifungal activities of botanicals were supported by many other investigators; neem oil, betel (*Piper betel* L.) leaf extract, *Psidium guajava* L. (Hema *et al.,* 2009), *Thymus vulgaris* (LINN.)*, Zingiber officinale, Cymbopogon citrates* Stapf (lemon grass) (Zeringue *et al*., 2001; Chalfoun *et al.,* 2004; Neguefact *et al.,* 2004; Faria *et al*., 2006; Kumar *et al.,* 2007; Srichana *et al*., 2009; Bahraminejad, 2012). The presence of anti-nutritional factors in the botanical species is responsible for their broad antimicrobial activity. The antifungal activity of *X. aethiopica* and *O. gratissimum* may be due to the presence of anti-nutritional factors in them.

CONCLUSION

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 postharvest/storage fungi. 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 botanicals, which are eco-friendly for the management of the important seed borne fungi. Therefore, 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. This can also be usefully exploited in the protection of foods from mycotoxin contamination.

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