

STORAGE FUNGI OCCURRENCE IN MELON (*Citrullus colocynthis*) IN UMUAHIA AND THEIR CONTROL USING SELECTED BOTANICALS

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SUMMARY

Storage fungi cause deterioration of stored melon (*Citrullus colocynthis*) kernels, leading to loss of quality and quantity of produce. This study was carried out to identify the fungal pathogens associated with postharvest storage rot of *C. colocynthis* kernel and to evaluate the efficacy of antifungal potentials of *Piper guineense*, *Xylopiia aethiopica* and a combination of both (*Piper* + *Xylopiia*) in the control of rot-causing pathogens. Isolation and identification of fungi were done using standard laboratory procedures. Hand and machine-peeled egusi samples (n=12) were collected from Ubani and Orie-ugha markets. Experimental design was completely randomized design with three replicates. Data were analyzed using analysis of variance at $\alpha_{0.05}$. *Rhizopus* sp; *Penicillium* sp, *Aspergillus niger*, *A. flavus*, *A. terreus* and *A. fumigatus* were encountered in *C. colocynthis* kernels. *Aspergillus flavus*, *A. niger* and *Rhizopus* sp were most prevalent and highly pathogenic species. Machine-peeled egusi kernels had higher 20.70% mould content than the hand-peeled < 14.00%. Botanical treatments had significant (p=0.05) effect on fungi growth in-vitro and in-vivo. *Aspergillus flavus* growth inhibition ranged from 37.84-100.00%, *A. niger* 65.47-100.00% and *Rhizopus* sp 27-94% across incubation period in in-vitro trial. For in-vivo trial, *X. aethiopica* inhibited *A. flavus* growth by 0.00 and 3.33 *A. niger* by 40 and 66.67%, *Rhizopus* by 50.00 and 66.67% before and after treatment respectively. *Piper guineense* reduced *A. flavus* growth by 63.33% and 13.33%, *A. niger* by 68.89% and 84.44%; *Rhizopus* sp by 90.00% and 63.33% before and after treatments, respectively. *Xylopiia* + *Piper* treatment reduced *A. niger* by 48.89% and 84.44% before and after treatment, respectively; while *Rhizopus* sp growth was reduced by 70.00% before and after treatment. Botanicals particularly *Piper guineense* consistently reduced fungi growth considerably and could be exploited in the management of storage fungi of egusi kernels.

Key words: *Colocynthis colocynthis* kernel, botanicals, hand and machine peeled, postharvest fungi

Melon (*Colocynthis citrullus*) L. Schrad is a widely cultivated and consumed oil seed crop in West Africa (4). The common names include: colocynth, bitter gourd, bitter apple, and bitter cucumber (8). In most parts of Nigeria, it is generally known as egusi. Some notable Nigerian egusi delicacies include Egusi soup, melon ball snacks and

ogiri (a local fermented condiment) (4;5).

One major problem that besets melon seeds is that they deteriorate quickly in storage due to fungal activities (6). Donli and Gulani (2011) reported that fungi are the major cause of spoilage of grains and seeds, and probably ranks second only to insects as spoilage organisms. Fungi of the genera

Aspergillus and *Penicillium* are widely distributed storage fungi of melon seeds, causing seed discolorations, decreased nutritive value, increase in free fatty acid and peroxide values, decreased seed germination, producing a number of toxic metabolites including mycotoxins; some of the fungi isolated from egusi are known producers of aflatoxins (5;13:).

The storage fungi reported to be associated with *C. citrullus* include: *Aspergillus* spp, *Penicillium* spp (15). Infection of stored *C. citrullus* seeds result in the deterioration of seeds, contamination of seeds with substances that are toxic to human and animals (mycotoxins), reduction of market value of nutrient composition of the seeds (15) and production of chemical substances that are toxic to human health. These storage fungi of *C. citrullus* have been reported in many parts of the Nigeria but there is limited report of the storage fungi associated with egusi in the Eastern part of Nigeria. The objectives of this study therefore, were: to isolate and identify storage fungi in *C. citrullus* kernels in Umuahia markets and their responses to selected botanicals.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Department of Plant Health Management Laboratory, College of Crop and Soil Sciences, Michael Okpara University of Agriculture, Umudike, Abia State. The area is located on 05 27' North, longitude 07 32' East with an attitude of 123m above sea level. Umuahia has an ambient temperature of 22-37°C with annual rainfall of 2,177mm and relative humidity of above 50-90% (NRCRI, 2016).

Source of egusi samples

Shelled egusi kernels (diseased and clean) *C. citrullus* were purchased directly from

two major markets in Umuahia namely: Orié Ugba (5°32N 7°30E) and Ubani (5°35N 7°30E) and purchased samples were bagged in a polythene bag taken to the Laboratory for isolation and identification of associated fungi.

Collection and preparation of botanical powders

The botanicals were purchased from Orié Ugba market in Umuahia. The botanicals that were used are Negro pepper (*Xylopiæ aethiopica*) and Ashanti pepper seed (*Piper guineense*) locally known as Uda and Uziza, respectively. The botanicals were thoroughly washed, air dried under shade until properly dried, and ground to fine powder using blender before use.

Isolation and identification of fungal pathogen

One gram (1g) of each egusi sample was milled and suspended separately in 10 ml of sterile distilled water, and thoroughly shaken. One hundred micro liter (100µl) of each of the suspension was dispensed into 15 cm Petri dish containing sterile potato dextrose agar (PDA) in which 0.05 ml of lactic acid was added to suppress bacterial growth. These were incubated on the bench at 25±2°C for 3-5 days. The colony forming units (CFU/ML) of each fungi species that emerged were determined by counting the number of colonies formed. Axenic culture of each of the isolates was obtained by subculturing on fresh PDA plates. Identification of the isolated fungi was done based on colony morphology and microscopic examination. Observations were recorded on colony colour, structure, shape, size, pigment and structure of mycelium, its branching, presence of Conidiophores, Sclerotia and shape were compared with literatures. Slides were prepared from fungi colonies for identification of organisms using mycological reference books, research

books and research articles (2;15). Identification of the isolated fungi was carried out following the description of Alexopoulos *et al.*, (2) and that reported by Obani *et al.*, (15).

Determination of percentage incidence of the fungal isolates

This was done to determine the percentage occurrence of the different fungi isolates. The total number of each isolate in all samples were obtained against the total number of the isolates in all the samples screened. Frequency of occurrence was determined using the method reported by Nwokocha and Opara (14).

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

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Pathogenicity test

Healthy *C. citrullus* seeds were surface sterilized using (10%) sodium hypochlorite and washing in three changes of sterile distilled water, seeds were then dried with sterile paper towel and inoculated with spore suspension of identified fungi. The inoculated seeds were placed on layers of moistened filtered paper and incubated for 7 days. The effects of mould on *C. citrullus* seed were observed and recorded. egusi kernels that showed sign of rot were re-isolated as described above according to Koch's principle. The control was inoculated with sterile water. This was done in triplicates.

Assessment of effect of botanicals on growth of isolated fungal pathogens in culture

The method of Okigbo *et al.*, (19) was adopted for extract preparation. Twenty-five grams (25 g) of each botanical powder in 100 ml of sterilized distilled water was allowed to soak for 6 hours and then filtered

before use. This experiment consists of three treatments: *Xylopia aethiopica*, *Piper guineense* and *Xylopia aethiopica* + *Piper guineense*. The autoclaved PDA medium was amended with individual extracts using the modifications of the method described by Begum and Bhuiyan (7). Plant extract (2 ml) was added to autoclaved PDA medium approximately 15 ml in 9cm Petri dishes. After thorough mixing, the mixture of PDA and extracts, they were allowed to solidify; the plates were inoculated by placing 5 mm discs of 5 days old culture of the fungal isolates at the centre of the Petri dishes. Inoculated plates were incubated at $25 \pm 2^\circ\text{C}$ and growth of the test organisms in each of the test extracts were recorded from 24hrs after inoculation for up to 6 days after inoculation. Each treatment was replicated three times and the mean of the radial growth were determined for the organism per test extract. Control experiments were set up without addition of botanicals. Fungitoxicity was recorded in terms of percentage colony inhibition (15) and was calculated using the formula stated by Sundar *et al.*, (1995).

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

Where: DC = Average Diameter of control and

DT = Average diameter of fungal colony with treatment

Influence of plant extracts on fungi growth on egusi kernels before and after treatment *in vivo*

This experiment also consists of three treatments: *Xylopia aethiopica*, *Piper guineense* and *Xylopia aethiopica* + *Piper guineense*. The extracts were prepared as described above. Inocula suspensions of *A. niger*, *A. flavus* and *Rhizopus* sp were prepared from fresh (5 day-old) fungi cultures. Fungi colonies were covered with 5 ml of distilled sterile water and shaken

vigorously to enhance uniform spore dispersal. The final inoculum size was adjusted to a concentration of 1.0×10^6 spore/ml by microscopic enumeration with a hemocytometer (1). Collected egusi kernels egusi kernels were washed in three changes of sterile distilled water and dried with sterile paper towel. Five kernels were inoculated with 100 μ L of spore suspension of each test fungus and allowed to stand for 30 minutes before treatment with botanical extracts and then incubation on filter paper blotter in sterile Petri dishes. For kernels treated before inoculation, five egusi kernels were treated with crude botanical extracts and wrapped with sterile paper towel and allowed for 30 mins; then inoculated with test fungi and plated on Petri dishes containing moistened filter paper. Both samples inoculated before and after treatments were incubated at room temperature for seven days. Percentage fungi colonization was recorded after incubation. The experiment was laid out in completely randomize design (CRD) with three replicates. The control was inoculated with test fungi without treatment with

botanicals.

Data analysis

Data collected on fungi colony forming units, percentage growth and percentage fungi colonization were analyzed using Statistical Package and Service solutions (SPSS) version 23. Means were compared and separated using least significant difference (F-LSD) and at 5% probability level.

RESULTS

Pathogenicity Test

Table 1 shows the different fungi isolated from hand peeled and machine peeled *Colocynthis citrullus*, kernels, their symptoms and their pathogenicity (%) to egusi kernels.

Aspergillus species (*A. tamaraii*, *A. niger*, *A. flavus*, *A. terreus* and *A. fumigatus*), *Rhizopus sp*, and *Penicillium species* were isolated from *C. citrullus* kernels (Plate 1).

The pathogenicity test showed that *A. niger*, *A. flavus*, and *Rhizopus sp* were pathogenic to *C. citrullus* kernels compared to other fungi encountered and were severely used for further studies.

Table 1: The pathogenicity of fungi isolated from *Colocynthis citrullus* kernels.

Symptoms/colorations	Fungi isolated	Pathogenicity (%)
Softened and yellowish	<i>Rhizopus sp</i>	100.00
Light brown	<i>Penicillium sp</i>	10.00
White mycelial mass	<i>Aspergillus tamaraii</i>	50.00
Yellow	<i>Aspergillus niger</i>	100.00
White mycelial mass	<i>Aspergillus flavus</i>	90.00
White mycelial mass	<i>Aspergillus terreus</i>	10.00
White mycelial mass	<i>Aspergillus fumigatus</i>	10.00
	Control	0.00

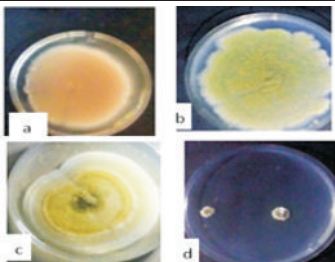


Plate 1: From top left: (a) *Aspergillus terreus*, (b) *A. flavus*, (c) *A. tamaraii* (d) *Penicillium sp* isolated from egusi kernels

Mould content in *Colocynthis citrullus* kernels collected from Ubani and Orié Ugba markets

Figure 1 shows the mould content of both hand peeled and machine peeled melon kernels collected from Orié Ugba and Ubani markets. Machine peeled melon kernels collected from both Orié Ugba and Ubani markets had the highest mould content with *A. niger* (383,400 cfu/ml) recording the highest mould content followed by *Penicillium* sp (217,267 cfu/ml), *A. flavus* (300,117 cfu/ml) and *Rhizopus* sp (130,67 cfu/ml) while *A. fumigatus* (0.0, 0.0 cfu/ml) was the least. Also, hand peeled melon kernels collected from both markets were lowest in mould content compared to the machine peeled except for *Rhizopus* sp (200 cfu/ml) which had higher mould content in hand peeled egusi kernels from Ubani market. The study shows that machine and hand peeled egusi kernels sourced from Ubani and Orié Ugba markets were high in mould content

of *Rhizopus* sp, followed by *Penicillium* sp and *A. tamarii* respectively.

Incidence of Fungal species in egusi kernels from Ubani and Orié Ugba markets

Figure 2 shows the percentage incidence of the fungal species isolated from egusi kernel. Hand peeled egusi kernel collected from Orié Ugba market had the highest occurrence of the following fungi species; *Rhizopus* sp (20.5%), *Penicillium* sp (14.5%), followed by *Aspergillus niger* (9.5%) while *A. tamarii*, *A. flavus* and *A. fumigatus* were not recorded. For Machine peeled melon samples collected from Ubani market, the highest incidence was recorded in *Aspergillus niger* (20.5%), followed by *Penicillium* sp (14.00%) and *A. flavus* (5.00%) while *A. fumigatus* (0.00%) and *A. tamarii* (1.0%) had the least diseases incidences. Hand peeled egusi kernel sourced from Ubani markets had the least diseases incidences of the seven fungal pathogens with *Aspergillus* sp having the lowest occurrence amongst others.

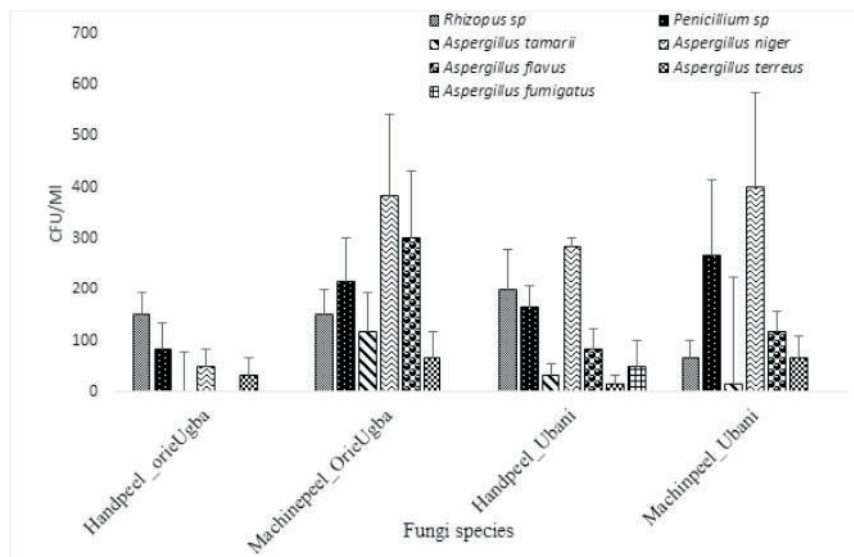


Figure 1: Mould content of egusi kernels collected from Ubani and Orié Ugba markets in 2022

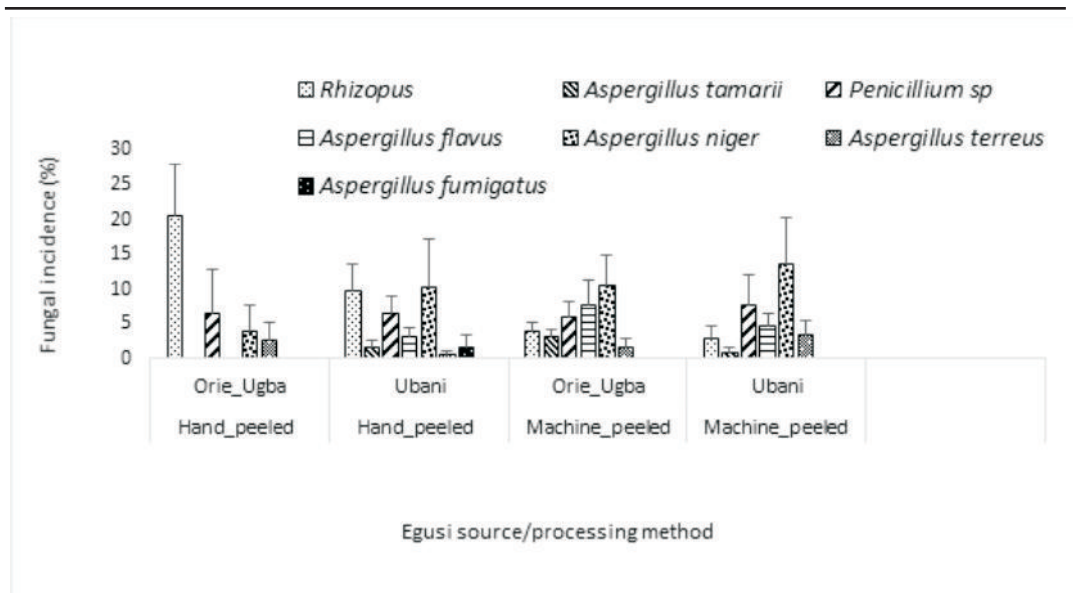


Figure 2: Percentage incidence of fungal species isolated from egusi kernels Ubani and Ori Ugba markets in (2022)

Effect of crude extracts of botanicals on reduction (%) of mycelial growth of fungi *in-vitro*

The results in Table 2 showed that the botanicals had varying degrees of inhibition on *A. flavus*, *A. niger* and *Rhizopus* sp growth of the extract *in vitro*. The aqueous extracts of *P. guineense*, *X. aethiopica* and combination of both (*P. guineense* + *X. aethiopica*) reduced the growth of *A. flavus*, *A. niger* and *Rhizopus* sp at varying percentages. Aqueous extracts reduced fungi growth by 48.53-94.95%, *P. guineense* by 30.93-55.89% while *Xylopi*a + piper at day 1 after treatment, the treatment reduced the mycelia growth of *A. flavus* by 100%. Mycelial growth reduction ranged from 43.67-100% for *A. aethiopica*, 65.08-100% for *P. guineense* and 37.84-100% for *Xylopi*a + *Piper* across the 6 days of incubation. They were significant ($p=0.05$) mycelia growth reduction between the treatment and the control. Among the three botanical treatments *Xylopi*a + *Piper* reduced the mycelia

growth of *A. flavus* better than treatment with *X. aethiopica* and *P. guineense* alone up to the days after treatment. For *A. niger* the botanicals reduced the growth of fungi within the range of 77.40-100% for *X. aethiopica*, 67.88 -100% for *P. guineense* and 65.75-100% for *Xylopi*a + *Piper* and were significantly different from the control. *Xylopi*a + *Piper* performed better than the other treatments up to day after treatment, except in day 5 where *X. aethiopica* had the highest growth reduction of 84.63%, followed by *P. guineense* which had 65.77%. For *Rhizopus*, *X. aethiopica* had the highest growth reduction at day 4 (52.78%), followed by (*P. guineense* 30.30%) (Plate 2) and then combination of *Xylopi*a + *Piper* which had the least (27.41%).

In-vivo inhibitory effects of crude extracts of botanicals on mycelial growth of fungi

The result presented in Table 3 shows the percentage growth inhibition of *A. flavus*, *A. niger* and *Rhizopus* sp growth in egusi

treated before and after treatment with the aqueous extracts of *X. aethiopica*, *Piper guineense*, and *X. aethiopica* + *Piper guineense*. Treatment with *X. aethiopica* reduced growth of *A. flavus* by (0.00 and 33.33%) before and after treatment, respectively when compared with control (0.00%).

Treatment with *Piper guineense*, after application of fungal spore suspension generally reduced the growth of *A. flavus* (63.33%) While 13.33% fungal growth reduction was recorded after treatment. Also, the application of *X. aethiopica* + *Piper guineense* reduced *A. flavus* growth by (3.33%) before treatment and (16.67%) after treatment.

For *A. niger*; *X. aethiopica* (40.00, 66.67 %), *P. guineense* (68.89, 84.44 %), and *Xylopi* + *Piper* (48.89, 84.4%)

respectively reduced the growth of *A. niger* before and after treatment. For the result, inoculation of *A. niger* after botanical extracts appeared to be more effective in *A. niger* growth reduction than before treatment.

For *Rhizopus* sp, *X. aethiopica* reduced fungal growth by 50.00% and 66.67% before and after treatment respectively. *P. guineense* reduced *Rhizopus* growth by 90.00% and 63.33% before and after treatment, respectively.

Xylopi + *Piper* reduced *Rhizopus* growth by 70.00% both before and after treatment. The botanical treatment has significant effect on fungi growth.

Aqueous extract of *X. aethiopica* reduced the growth of *A. niger* (66.67cm) significantly while *Piper guineense* did not differ significantly ($p=0.05$) from *X. aethiopica* + *Piper guineense* (84.44 cm).

Table 2: Percentage inhibition of fungi growth by crude extracts of botanicals *in-vitro*

Treatment	Fungi	Percentage growth reduction (%)					
		1	2	3	4	5	6
<i>Xylopi</i> <i>aethiopica</i>	<i>A. flavus</i>	100.00	88.83	82.67	79.34	79.72	43.67
<i>Piper guineense</i>		100.00	78.93	65.47	65.75	65.08	71.05
<i>Xylopi</i> + <i>Piper</i>		100.00	92.98	83.95	87.94	83.66	37.84
Control		0.00	0.00	0.00	0.00	0.00	0.00
LSD($p=0.05$)		0.82	2.55	5.43	5.46	5.11	7.23
<i>Xylopi</i> <i>aethiopica</i>	<i>A. niger</i>	100.00	77.40	85.36	87.53	84.63	82.46
<i>Piper guineense</i>		100.00	67.88	74.44	65.47	65.77	72.44
<i>Xylopi</i> + <i>Piper</i>		100.00	83.05	86.53	82.57	68.75	83.66
Control		0.00	0.00	0.00	0.00	0.00	0.00
LSD($p=0.05$)		0.66	7.39	4.92	10.95	15.62	11.47
<i>Xylopi</i> <i>aethiopica</i>	<i>Rhizopus</i> sp	94.95	48.53	55.63	52.78	.	.
<i>Piper guineense</i>		51.19	55.89	42.86	30.93	.	.
<i>Xylopi</i> + <i>Piper</i>		75.94	50.62	40.10	27.41	.	.
Control		0.00	0.00	0.00	0.00	.	.
LSD($p=0.05$)		21.40	16.04	19.04	1.98		

Percentage growth reduction declined gradually as the storage period increased. After six weeks of treatment, there were no significant difference between the different levels of treatment.

Application of the plant extracts before and

after inoculation of the pathogens reduced disease incidence significantly ($p \leq 0.05$) when compared with control. The highest disease incidence was observed in control experiment treated with water alone.

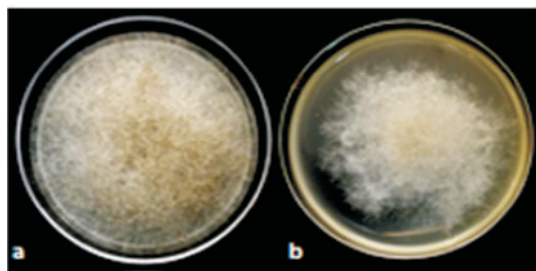


Plate 2. (a) Control (b) radial growth of *Rhizopus Piper guineense* amended PDA at four days after inoculation

Table 3: Percentage fungi growth inhibition by botanicals before and after treatment *in-vivo* (n prophylactic and curative)

Treatment	Fungi	Percentage reduction (%)	
		Before	After
<i>Xylopi</i>		0.00	33.33
<i>aethiopica</i>			
<i>Piper</i>		63.33	13.33
<i>guineense</i>			
<i>Xylopi</i> + <i>Piper</i>	<i>A. flavus</i>	3.33	16.67
Control		0.00	0.00
LSD(p=0.05)		38.01	21.60
<i>Xylopi</i> <i>aethiopica</i>		40.00	66.67
<i>Piper guineense</i>		68.89	84.44
<i>Xylopi</i> + <i>Piper</i>		48.89	84.44
Control		0.00	0.00
	<i>A. niger</i>		
LSD(p=0.05)		24.49	19.44
<i>Xylopi</i> <i>aethiopica</i>		50.00	66.67
<i>Piper guineense</i>		90.00	63.33
<i>Xylopi</i> + <i>Piper</i>		70.00	70.00
Control	<i>Rhizopus</i> sp	0.00	0.00
LSD(p=0.05)		27.58	19.75

DISCUSSION

Aspergillus species (*A. tamar*, *A. niger*, *A. flavus*, *A. terreus* and *A. fumigatus*), *Rhizopus* sp, and *Penicillium* species were isolated from hand-peeled and machine-peeled melon kernels. The pathogenic organisms of egusi kernels after

pathogenicity test include *A. niger*, *A. flavus*, and *Rhizopus* sp. In this study, the crude aqueous extracts of the two selected plants (*P. guineense* and *X. aethiopica*) and a combination of both (*Xylopi* + *Piper*) tested showed varied antifungal potential on *A. flavus*, *A. niger* and *Rhizopus* sp

isolated from hand peeled and machine peeled egusi kernels. Among the extracts studied, combination of *P. guineense* and *X. aethiopica* was found to be more effective against *A. flavus* and *Rhizopus* sp while *Piper guineense* was more effective on *A. niger*. The antimicrobial activities of the extracts of *P. guineense* and *X. aethiopica* varied widely. This was in agreement with the findings of Pundir and Jain (21) that antimicrobial activity varies widely depending on the type of spice and organism involved. Antifungal activities of different plant species and importance of plants as possible sources of natural fungicides were reported by various researchers (18;20;17).

This study reports that, application of botanicals before and after fungal infection showed antifungal activity and can be used in control of *A. flavus*, *A. niger* and *Rhizopus* sp contamination in egusi kernels. This is in agreement with the findings of Kuri *et al.* (12).

The botanicals that were tested in this study, were effective in controlling the mycelial growth of *A. flavus*, *A. niger* and *Rhizopus* sp. The results gathered from this research indicated that *A. flavus*, *A. niger* and *Rhizopus* sp can be fairly managed by using *P. guineense*, *X. aethiopica* and also a combination of *Xylopi* with *Piper*. The result has an agreement with the findings of (22;15). The plant extracts showed considerable inhibition of the growth of *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus* sp.

The results from this study affirms that the importance of these plant species as exhibiting antifungal properties both in the *in 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 research studies have been done on the use of plant extracts against the plant-pathogenic fungi. Okigbo *et al.* (19) 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. verticilloides* and *A. flavus* (10). Obani *et al.*, (15) reported that *Piper guineense* treatments before storage can help reduce on aflatoxin formation and *Aspergillus* sp growth in stored egusi kernels and it was also effective in inhibiting the growth of the fungus. The antifungal activities of botanicals were supported by many researchers as pesticide alternatives; betel leaf extract (23), *Thymus vulgaris*, *Zingiber officinale*, *Cymbopogon citratus* (lemon grass) (11). The antimicrobial activity of the botanical species was shown to be due to their content of tannins and phytate. The antifungal activity of *P. guineense* and *X. aethiopica*, may be due to presence of tannins in their properties (171).

The differences between results of the three treatments could be due to the nature of the plant compounds and their interactions with seed constituents (3).

From the observation above, *P. guineense* + *X. aethiopica*, *P. guineense* and *X. aethiopica* investigated proved to be useful in the management storage fungi associated with *Citrullus colocynthis* 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 (9).

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.

CONCLUSION

In this study *Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus sp* were identified as the fungal pathogens causing postharvest storage rot of egusi kernel obtained from Orié Ugba and Ubani markets both in Umuahia. Pathogenicity test confirmed that these pathogens actually caused storage rot of egusi kernel. The efficacy of the crude plant extracts (*Piper guineense*, *Xylopia aethiopica* and a combination of both *P. guineense* + *X. aethiopica*) against storage rot fungi were tested *in vitro* and *in vivo*. The *in vitro* results showed that the extracts significantly inhibited the mycelia growth of the fungal pathogens when compared with the control. However, in the *in vivo* assay, the crude plant extracts significantly ($p \leq 0.05$) inhibited the mycelial growth of the fungal isolates when applied before and after inoculation of fungal isolates and the rate of inhibition differed from each other. It is clear from the above observations that all the botanicals investigated proved to be useful in the management of postharvest/storage of egusi melon (*Citrullus colocynthis*). Results obtained with the botanicals in this study confirmed

the importance of these plant species as exhibiting antifungal properties both in the *in vitro* and *in vivo* experiments.

RECOMMENDATIONS

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 stored egusi protection strategies, with special reference to the management of postharvest diseases of egusi. These botanicals are also abundantly and readily available in the local areas and therefore needs technological development to a form that will be easily accessible to farmers for egusi protection against storage fungi. This can also be usefully exploited in the protection of foods from mycotoxin contamination. Based on the findings in this study, the use of aqueous extracts of *Piper guineense* and *Xylopia aethiopica* combined together could be a substitute to synthetic fungicide in the control of fungal pathogen associated with storage rot of egusi melon (*Citrullus colocynthis*).

LITERATURE CITED

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