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Survey on the Post-Harvest Handling Practices of Some Selected Soup Condiments in Awka, Anambra State and Isolation of Fungi Responsible For Their Spoilage

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ABSTRACT

A survey on the post-harvest handling practices of *Citrullus colocynthis*, *Irvingia gabonensis* and *Brachystegia eurycoma* among traders in Awka was conducted from January to April, 2023 in Eke-Awka, Amaenyi and Ifite markets. A total of 180 respondents (sixty from each market) were randomly selected for the study. The well structured questionnaire captured social demographic information about the respondents while the second section focused on the post-harvest handling practices of soup thickeners. Most of the traders/respondents in the study area are within the Age range 31-35 and 41-45 with percentages of 16.67 and 16 respectively. While the Age groups with the lowest frequency is the age group 26-30 with 3 (1.67%) respondents. Majority of the respondents (80%) purchase these soup thickeners from wholesalers while 21 (11.67%) purchase directly from farmers. Most of the respondents (82.78%) dry soup thickeners, 30 (16.61%) sort them and 1 (0.56%) soak them in water as a post-harvest spoilage control practice. The most observed change in the seeds after 8 months in storage is change in colour (43.89%), the least observed change is the formation of mucor (0.56%) while 9 (5%) of the study population stated that there is no change after 8 months in storage. All the 180 respondents (100%) do not use chemical preservatives for preservation of these soup thickeners. For the microbial study, samples of Egusi, Ogbono and Achi collected from Eke-Awka, Amaenyi and Ifite markets were analyzed using standard mycological techniques. Fungi were isolated and identified based on their morphological and molecular characteristics. The results showed that all the samples were contaminated with fungi, with the highest level of contamination in Egusi seeds from Eke Awka with a mean occurrence of 41.33 ± 8.145. The most common fungal genera identified were *Aspergillus, Fusarium* and *Rhizopus.* A significant proportion of the isolates belonged to toxigenic species, which could potentially produce mycotoxins that pose a health risk to consumers. The findings also underscore the importance of proper handling, storage and processing of these seeds to minimize fungal contamination.

Keywords: Survey, Post-harvest, Fungi, Anambra state, Soup condiments, Markets

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INTRODUCTION

Stored foods travel through different stages before reaching the final consumer. In each of the pre-harvest and post-harvest operations, they encounter various barriers that affect the final quality of the stored food products. The deterioration of stored food quality is chiefly caused by fungal invasion. Stored food materials contaminated by microorganisms tend to develop foul odour, lose their nutritional quality, weight, flavor and color (Gulumbic and Kulik, 2012; Ahmed et al. 2016; Kumar, 2017). These fungal organisms produce highly toxic and carcinogenic metabolites or mycotoxins and also cause spoilage of agricultural produce (Tsehaye et al. 2017).

The major storage fungi of foods in the tropic region are *Aspergillus flavus, Aspergillus niger* and *Mucor* spp. These organisms causes easy deterioration of these seeds making it lose its taste and nutritional values. Some of these fungi also produce mycotoxins which are harmful to the human health when ingested especially in large quantities (Anukwuorji et al. 2012, 2013; Okigbo et al. 2012, 2014).

During storage, melon seeds foods are attacked by different storage fungi, some of which are from the genera *Aspergillus* and *Penicillium.* These fungi affect the quality and quantity of these stored foods causing a change in colour and decrease in their nutritional, economic and market value (Anukwuorji et al. 2020).

In most developing countries, fungi contaminate foods such as cereals, legumes, seeds, nuts, root and tuber crops, etc. The exposure occurs in towns and villages that produce their own food, hence regulatory measures to control exposure are largely ineffective (Wild and Gong, 2010). Unsafe food causes many acute and lifelong diseases ranging from diarrheal to various forms of cancer. The monitoring of contaminants in food provides important information on risks associated with the consumption of contaminated foods and on the efficiency of control measures that are in place. The safety of foods and feeds for human and animal consumption should be of topmost priority with regards to the regulation of agricultural and food industries so the markets are not compromised by the sale of low quality or unsafe food (Conway and Toenniessen, 2003). It is against the backdrop of the above that this research was conducted to ascertain the post harvest handling practices of soup condiments and to isolate the fungi responsible for their spoilage in storage.

Therefore, the purpose of this study is to evaluate the quality of soup condiments sold in open markets in Awka, Anambra, and investigate the fungal species that contaminate the soup thickeners. The objectives of this study were to assess the post-harvest handling practices of ogbono (*Irvingia gabonensis*), egusi (*Citrullus colocynthis*) and achi (*Brachystegia eurycoma*) by local farmers in Awka, Anambra and to determine the level of fungal infestation of ogbono, egusi and achi seeds sold in markets within Awka.

MATERIALS AND METHODS

Study Area

This study was conducted in Awka, Anambra State. Awka is the capital of city of Anambra state It has an area of roughly 523.2km^2 and is located in a fertile tropical valley, however some portions of its rainforest has been lost to farm clearing and urbanization.

Ethno-study

Survey was conducted between December 2022 and March 2023 in three markets in Awka, Anambra State. The markets were Eke-Awka, Amaenyi and Ifite markets. A well-structured questionnaire on post-harvest handling practices of egusi (*Citrullus colocynthis*), ogbono (*Irvingia gabonensis*) and achi seeds (*Brachystegia eurycoma*) was designed and administered randomly to one hundred and eighty (sixty in each market) traders within the study area. The questionnaire was made up of two sections: the first section was made up of information about the respondents while the second section which focused on the post-harvest handling practices of soup thickeners.

Sample collection

The method of Adetunji et al. (2014) was adopted in sampling food materials; the food materials sampled were *I. gabonensis*, *C. colocynthis* and *B. eurycoma*. The objective of the sampling was to obtain a small quantity of food material that represents the whole. Using simple random sampling technique, a total of three (3) markets from Awka were sampled. The specimens were labeled, numbered and annotated with the date of collection and locality.

Sample Preparation

The samples were air-dried and were later weighed with a weighing machine and ground into a powdered form. Each sample was labeled, packaged in a polythene bag, and taken to the laboratory for analysis.

Isolation of Fungi from Stored Soup Thickeners

One gram (1g) from each sample was weighed on a sensitive meter scale. A test-tube plastic rack was arranged with 9ml of sterile test-tubes each containing 9 ml of sterile distilled water (SDW). A tenfold serial dilution (Fasole and Oso, 1988) was carried out by dispersing 1g sample into the first test-tube (10^{-1}) shaken together. One ml was again taken from (10^{-1}) dilution and transferred to the next test tube (10^{-2}). The dilutions continue to (10^{-9}). Each test tube was shaken vigorously before transfer. A pour plate method (Fasole and Oso, 1988; modified by Okigbo *et al.*, 2015) was used in plating all the samples. One mill (1 ml) from dilution (10^{-9}) was dispensed into a sterile Petri dish with a sterile pipette. A molten Potato Dextrose Agar was poured into the plates (about 10 ml). The plates were swirled for easy mix-up of the sample and the media. All plates were allowed solidification on the bench. Each plated sample was duplicated.

Sub culturing and Identification of purified cultures

A flamed surgical blade was used for sub-culturing the mycelia from Potato Dextrose Agar plates (PDA) into a newly prepared PDA plates for purification. All plates were incubated at 25^0C for 3-5days. Macroscopic examination was done by physical characteristics of the mycelia-like structure and color of the mycelia. Microscopic characteristics through the morphological structure according to (Mathur and Kongsdal, 2003) was employed. A wet mount method (Fasole and Oso, 1988) was done before viewing the isolates under ^40 compound microscope. The morphological structures viewed include septate or non-septate mycelia, presence of sporangiosphores, fruiting bodies and special organs like rhizoids. Each morphological structure of each isolates was matched with a mycology atlas for identification.

Determination of percentage of fungal occurrence

This was done to determine the frequency of occurrence of the different fungal isolates. Isolations were made from the three plant materials. The number of occurrence for each of the isolates in each of the samples (plant materials) were recorded and calculated as a ratio of the total number of occurrence and was then expressed as a percentage. It was given by the formula below;

Percentage occurrence= $^{x}/_{n}$ X $^{100}/_{1}$, where x= Total number of each organism in all the samples. n = Total number of the entire organism in all the samples screened.

Statistical Analysis

Descriptive statistics of frequency and percent counts were used to summarize the data collected from the survey. The data collected from the microbial analysis were subjected to analysis of variance (ANOVA) and means were separated using Duncan's Multiple Range Test (1955) at 0.05 probability level to determine significant differences among the samples obtained from the different locations and periods.

RESULTS

Socio-demographic characteristics of the respondents

Most traders/respondents in the study area are within the Age groups 31-35 and 41-45 with the frequency of 30 and 27 and percentages of 16.67 and 16 respectively. At the same time, the Age groups with the lowest frequency is the age group 26-30 with frequency of 3 (1.67%) (Fig. 1 and Table 1). Majority of the respondents were female with a frequency of 168 (93.33%). The result presented in the table also shows that 55.00% of the respondents were married, 27.78% were widowed and 17.22% were single. The highest level of education achieved by the respondents was SSCE, followed by OND/NCE, and FSLC with a frequency of 99, 45 and 21 and percentage of 55.00%, 25.00% and 11.67% respectively. Only 15 (8.33%) of the respondents had obtained a B.Sc./HND degree (Fig. 1 and Table 1).

Point of purchase, storage, and post-harvest spoilage control methods of soup thickeners

From Table 2 below, 144 (80%) respondents purchase these soup thickeners from wholesalers while 21 (11.67%) purchase soup thickeners directly from farmers and 15 (8.33%) purchase them from the farm gate. It was also observed that 123 (68.33%) of the respondents stored these soup thickeners for more

than 8 months before selling to customers, 54 (30.00%) stored them for 8 months before selling and 3 (1.67%) stored them for less than 8 months before selling to a customer. For storage materials, nylon bags were the most common storage materials used by the respondents to store these soup thickeners, with a frequency were 70 (38.89%). Hermetic bags were the least used storage materials, with a frequency of 9 (5.00%).

For post-harvest spoilage control, 149 (82.78%) of the respondents dry these soup thickeners, 30 (16.61%) sort them and 1 (0.56%) soak them in water. According to the respondents, the most observed change in the seeds after 8 months in storage is change in colour, with a frequency of 79 (43.89%), the least observed change is the formation of mucor 1 (0.56%) while 9 (5%) of the study population stated that there is no change. Before being stored, 78(43.33%) of the respondents displayed these soup thickeners on the bare floor, while 64(35.56%) placed them in baskets and 38 (21.11%) displayed them on aluminum tray (Table 2),

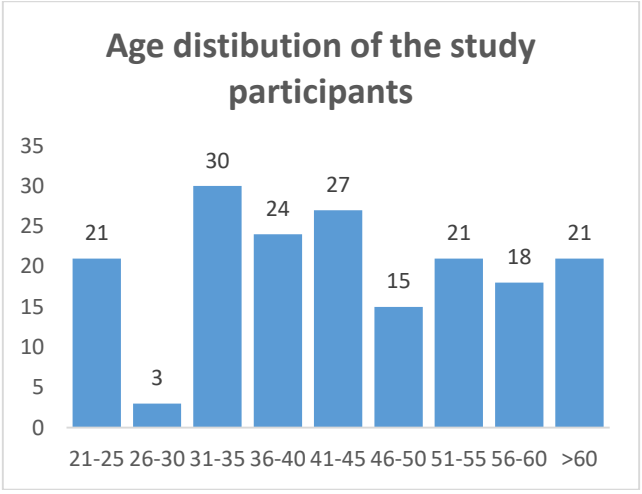

Fig 1: Age distribution of the participants involved in the study.

Traders perception on the post-harvest handling procedure for egusi, ogbono and achi

From table 3 below, all the respondents 20 (13.4%) in Eke-Awka market dry their Ogbono seeds, 5 (16.7%)sort their Achi seeds while none of the respondents soak any of these soup thickeners as a post-harvest handling technique to prevent spoilage. However, only one of the respondents from the three markets soak their Achi seeds in water. In Nkwo-Amaenyi, only 2 (6.7%) of the food handlers sort Egusi seeds. From table 3, it can be deduced that drying is the most common postharvest handling procedure with a frequency of 149 while soaking in water is the least common with a frequency of 1.

Occurrence of Fungi Pathogens on Stored Food Samples from Different Locations in Awka

Based on the growth of the fungi on the cultured soup thickeners, Table 1 reveals the presence of *Aternaria alternata, Rhizopus stolonifer, Fusarium oxysporium, Aspergillus niger* and *Aspergillus flavus*. *Aspergillus niger* was observed in the samples from Achi and Ogbono from Eke Awka and Ifite respectively, *Aspergillus flavus* was present in Ogbono samples from Eke Awka and from Egusi and Ogbono samples from Ifite while *Alternaria alternata* wasseen in Egusi samples from all locations and also from Ogbono sample in Nkwo Amaenyi. However, *Fusarium oxysporium* was isolated in Achi collected from Ifite and Amaenyi, it was also isolated from Ogbono from sampled from Eke Awka. *Rhizopus stolonifer* was seen only in Achi samples from Eke Awka (Table 4).

Identification of Fungi Isolates on Stored Food Samples Analyzed

Table 5 shows the fungal species isolated from the samples. These isolated organisms were characterized and identified based on physical and microscopic observations of their growth, hyphae, fruiting bodies, and the types of resting spores.

Mean Occurrence of Fungi Pathogens on Stored Food Samples from Different Locations in Awka

Result on the mean occurrence of fungi isolates from the cultured stored food samples showed that *Alternaria alternata* was higher in Egusi sampled from Eke Awka market (41.33±8.145) but lower in the Egusi samples purchased from Nkwo Amaenyi market (37.33±5.508) (Table6). The fungi isolates of *Fusarium oxysporium* was higher in Ifite (41.00±8.718) than in Nkwo Amaenyi (36.00±7.937) (Table 7). The mean percentage occurrence of *Aspergillus flavus* was significantly higher ($p<0.05$) in stored Ogbono samples collected in Eke Awka (41.00±4.000) than that of Ifite (33.00±3.606) (Table 8). There was a significant difference in the percentage occurrence of fungi isolates of stored food samples from the different locations ($p<0.05$).

Table 1: Socio-demographic characteristics of the respondents

Socio-demographic characteristics	Frequency (n=180)	Percentage (%)
Age group (years)		
21-25	21	11.67
26-30	3	1.67
31-35	30	16.67
36-40	24	13.33
41-45	27	15.00
46-50	15	8.33
51-55	21	11.67
56-60	18	10.00
>60	21	11.67
Sex		
Female	168	93.33
Male	12	6.67
Marital status		
Married	99	55.00
Single	31	17.22
Widow	50	27.78
Highest level of education		
B.SC/HND	15	8.33
FSLC	21	11.67
OND/NCE	45	25.00
SSCE	99	55.00
Total	**180**	**100.0**

Table 2: Point of purchase, storage, and control of post-harvest spoilage of soup thickeners

Variables	Frequency (n=180)	Percentage (%)
Point of Purchase		
Farm gate	15	8.33
Farmer	21	11.67
Wholesalers	144	80.00
Average storage time before selling to customers		
Less than 8 months	3	1.67
8 months	54	30.00
More than 8 months	123	68.33
Storage Materials used by the respondents		
Hermetic Bags	9	5.00
Metal Buckets	18	10.00
Moulded plastic boxes	26	14.44
Nylon bags	70	38.89
Synthetic fibre sack	57	31.67
Post-harvest spoilage control		
Drying	149	82.78
Soaking in water	1	0.56
Sorting	30	16.67
Changes in seed after 8 months in storage		
Change in colour	79	43.89

Dusty	31	17.22
Formation of mucor	1	0.56
Moist	19	10.56
Shrinking/reduction in size	41	22.78
None	9	5.00
Mode of display before storage		
Aluminum tray	38	21.11
Bare floor	78	43.33
Basket	64	35.56
Use of synthetic chemical preservatives		
No	180	100.0

Table 3: Post-harvest handling procedure for Egusi, Ogbono and Achị by traders in Eke-Awka, Nkwo-Amaenyi and Ifite Second market

Markets	Post-harvest handling procedure			Total (%)
	Drying (n=149)	Soaking in water (n=1)	Sorting (n=30)	
Eke Awka-Ogbono	20 (13.4)	-	-	20 (11.1)
Eke- Awka –Achi	15 (10.1)	-	5 (16.7)	20 (11.1)
Eke- Awka-Egusi	16 (10.7)	-	4 (13.3)	20 (11.1)
Ifite- Achi	15 (10.1)	1 (100)	4 (13.3)	20 (11.1)
Ifite-Egusi	17 (11.4)	-	3 (10.0)	20 (11.1)
Ifite-Ogbono	14 (9.4)	-	6 (20.0)	20 (11.1)
NkwoAmaenyi –Achi	20 (13.4)	-	-	20 (11.1)
NkwoAmaenyi-Egusi	18 (12.1)	-	2 (6.7)	20 (11.1)
NkwoAmaenyi-Ogbono	14 (9.4)	-	6 (20.0)	20 (11.1)
Total	149 (100.0)	1 (100)	30 (100)	180 (100.0)

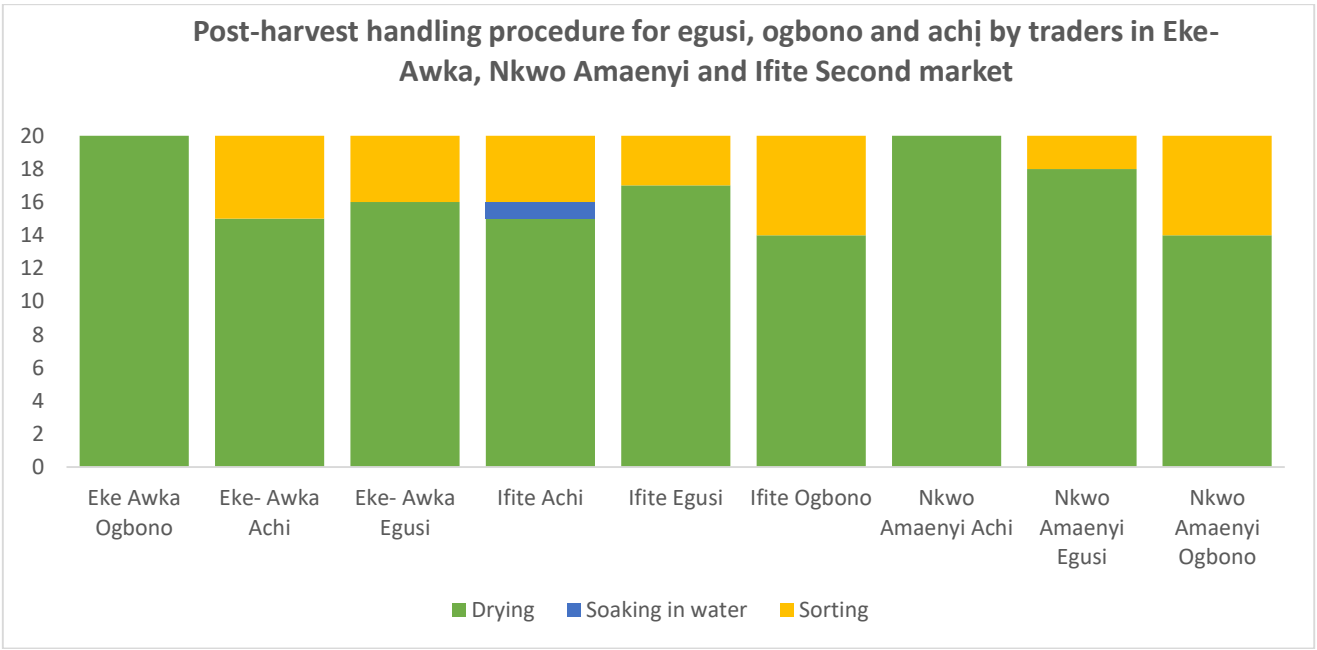

Fig 2: Stacked bar chart showing the Post-harvest handling procedure for egusi, ogbono and achị by traders in Eke-Awka, NkwoAmaenyi, and Ifite Second markets

Table 4: Occurrence of Fungi Pathogens on Stored Food from Different Locations in Awka

	Locations								
	Eke-Awka			Nkwo-Amaenyi			Ifite		
Fungi Isolates	Food Samples			Food Sample			Food Samples		
	Egusi	Achi	Ogbono	Egusi	Achi	Ogbono	Egusi	Achi	Ogbono
Aspergillus niger	-	+	-	-	-	-	-	-	+
Aspergillus flavus	-	-	+	-	-	-	+	-	+
Alternaria alternata	+	-	-	+	-	+	+	-	-
F. oxysporium	-	-	+	-	+	-	-	+	-
R. stolonifer	-	+	-	-	-	-	-	-	-

Table 5: Identification of Fungal Isolates on Stored Food Samples Analyzed

MACROSCOPY	MICROSCOPY	ORGANISM
Black colony, powder with diffused hyphae in media	Smooth-walled stipe, conidiophores radiate and terminate in vesicle.	*A. niger*
Light green and powdery colonies	Rough and coarse aerial hyphae present with simple sporangiophore which are shaped globose.	*A. flavus*
Texture deeply cottony; White becoming gray-brown on surface	Angular, subglobose or ellipsoidal sporangia, aerial erect hyphae.	*R. stolonifer*
Whitish colonies later turn brownish	Brown-black, globose sporangia, rhizoids also present, and zygospores.	*A. alternata*
White cottony colonies and dark-purple undersurface	Mesoconidia, and microconidia arranged in false heads present.Intercalary chlamydospores.	*F. oxysporium*

Table 6: Mean Occurrence of Fungi Pathogens on Stored Egusi Samples from Different Locations

Fungi Isolates	Eke Awka	Nkwo Amaenyi	Ifite
A. niger	-	-	
A. flavus	-	-	41.33±16.073
R. stolonifer	-	-	-
A. alternate	41.33^{a}±8.145	37.33^{a}±5.508	38.00^{b}±3.306
F. oxysporium	-	-	-

Values are mean ± standard deviation of triplicate determination. Means on the same row with different superscripts are significantly different at ($p<0.05$).- = Absent

Table 7: Mean Occurrence of Fungi Pathogens on Stored Achi Samples from Different Locations

Fungi Isolates	Eke Awka	Nkwo Amaenyi	Ifite
A. niger	38.67±3.512	-	-
A. flavus	-	-	-
R. stolonifer	178.33±8.505	-	-
A. alternata	-	-	-
F. oxysporium	-	36.00^{a}±7.937	41.00^{a}±8.718

Values are mean ± standard deviation of triplicate determination. Means on the same row with different superscript are significantly different at ($p<0.05$). - = Absent

Table 8: Mean Occurrence of Fungi Pathogens on Stored Ogbono Samples from Different Locations

Fungi Isolates	Eke Awka	Nkwo Amaenyi	Ifite
A. niger			49.00±7.211
A. flavus	41.00^{a}±4.000	-	33.00^{b}±3.606
R. stolonifer	-	-	-
A. alternate	-	36.00±7.810	-
F. oxysporium	106.00±12.288	-	-

Values are mean ± standard deviation of triplicate determination. Means on the same row with different superscript are significantly different at ($p<0.05$). - = Absent

DISCUSSION

The survey carried out showed that the food handlers/respondents across Awka had fairly uniform ways of handling soup thickeners. In this study, storage of food materials before marketing was reported to last as long as 8 months, such an extended storage period promotes insects/rodents and mold infestation and can lead to discoloration and change in flavor (Kaaya and Eboku, 2010). Bankole et al. (2005) reported that one major problem that besets food materials especially *C. colocynthis* is that it deteriorates quickly in storage due to fungal infestation. Storage of food materials for a long period of time decreases its nutritive value. On the same hand, the results of a study conducted by Kolapo and Sanni (2006) confirmed that prolonged storage negatively affect the overall quality of foodstuffs such as its proximate composition.

This study also confirmed that food handlers in Awka do not use chemical preservatives to prevent spoilage which is likely to be one of the factors that predisposes these soup thickeners to fungal infestation. In Awka, the manner in which food materials are processed and handled increases their vulnerability to contaminants. This is due to the disregard and neglect of several drying and storage factors that could otherwise reduce contaminants in stored food materials. These factors include the need for proper drying of seeds to eliminate moisture, controlling storage insect pests by separating damaged parts from undamaged ones, employing safe and appropriate preservatives, and raising awareness of the risk posed by pests and contaminants in stored foodstuffs (Bankole et al., 2005).

Fungi isolated from these stored soup thickeners are *Aternaria alternata, Rhizopus stolonifer, Fusarium oxysporium, Aspergillus niger* and *Aspergillus flavus*. Most of the organisms isolated from this research work are storage fungi whose spores may have attached to the seeds during storage/processing or handling and have been at various times implicated in the spoilage of fruits and vegetables. This is in tandem with the result of a similar experiment conducted by Bankole abd Adebanjo (2003) but differs with the report of Oyedele and Fatoki (2017) who in addition to *Aspergillus* species also isolated *Mucor racemosus* and *Saccharomyces cerevisiae* from various food materials sold in various markets in Bayelsa state, Nigeria. Members of the fungal group micromycetes which include all the fungi isolated from the present research work have at various times implicated as being responsible for the spoilage of stored fruits of the family Cucurbitaceae to which *C. colocynthis* belongs. The high occurrence of *Aspergillus* species in the food samples agrees with the report of so many workers who reported *Aspergillus* species as one of the most frequent organisms associated with seed rot of melon.

CONCLUSION

Complete elimination of stored fungi detected in the soup condiments is not feasible even through thermal -processing. The stored fungi can cause irreparable damage to these seeds. Therefore, devising a sustainable strategy to prevent contamination and proliferation of fungi in stored foodstuffs as a whole is of utmost importance. It is necessary to control the factors that are responsible for fungi contamination and growth, the most important being moisture content. Insect infestation should be avoided and proper hygienic practices should be maintained throughout the supply chain.

RECOMMENDATION

➢There is need for consistent sensitization of the masses in Awka and awareness campaign on dangers of microbial infestation in food materials sold in the open markets. In addition to this, food handlers should also be sensitized on good food handling/processing procedures with respect to storage and drying techniques/duration.

➢Government should provide good storage facilities to prevent post-harvest losses and contamination of these food materials.

➢Information concerning the level of microbial infestation of foodstuffs sold in the open market should be made available to the masses by relevant agencies such as the Research institutes and the Federal Ministry of Health, this information should be disseminated at all levels of society in the country by extension workers.

➢Government should prioritize research and make available funds for the development of relatively cheaper biological control and plant based agents. There is no doubt that the prospect of Nigeria becoming food secured and is tied to the above recommendations.

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