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FUNGAL CONTAMINANTS ASSOCIATED WITH SOME FOOD STUFF IN OWERRI, SOUTHEAST, NIGERIA

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ABSTRACT

This study aimed at isolating and identifying fungal contaminants of some selected food items (rice, maize, beans, groundnut, and peanut) in Owerri, Imo State Nigeria. An oral ethical approval was obtained from the Market Master before sample collection. Different fungal contaminants were isolated from all the selected raw and boiled food items that were directly seeded unto Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol, incubated at room temperature for 7 days, and were checked for growth daily. From the results obtained, a total of six (6) fungi were isolated and identified from all the samples cultured. The total of each mycotoxin-producing fungi isolated is *Aspergillus* spp. 8 (29.6%), *Penicillium* spp. 5 (18.5%), *Mucor* spp. 5 (18.5%), *Rhizopus* spp. 5 (18.5%), *Geotrichum* spp. 3 (11.1%) and *Botrytis* spp.1 (3.7%). Out of all the fungal isolates, a frequency of 3.3 (55.6%) was identified from the raw food samples and 2.7 (44.4%) from the boiled samples. The slight dissimilarity seen between the raw and boiled food fungal isolates is a result of the denaturation of the nutrient value of the grains during boiling and the ability of the fungal spores (that are thermoduric and heat-stable) to form a biofilm that can resist deactivation.

KEYWORDS

Food stuffs (cereals and legumes), fungal contaminants, Owerri, Nigeria.

INTRODUCTION

Food adds ardour to the essence of life as well as its vitality, yet majority of people give little concern to ensure that the food they consume is safe at all, without knowing that natural metabolites produced by microbes are potent toxins and carcinogens that can capably pose untold threat to food safety [26]. Therefore, food is any substance, of either plant or animal origin, consumed to provide nutritional support for an organism. The food must have to contain either some or all of the following essential nutrients: carbohydrates, proteins, fats, vitamins or minerals [8].

Foods are rich nutrient sources that will attract both bacterial and fungal colonizers, hence regarded as ecological resources. After successful colonization of the products, its nutritional properties are altered. When the nutritional value, structure, and taste of these foods and their products are thus negatively influenced by microbial colonization, this is called food spoilage[3]. It can be accompanied by the production of toxic secondary metabolites which often results in grave medical problems, and has been an issue that needs our continual awareness and concern for, with respect to food safety, human and livestock health[7]. Colonization of food is very diverse. Fungi that can spoil foods must be able to survive certain adverse conditions, manoeuvre protective compounds inside cells and possess heat-resistant structures[11]. Several books on food spoilage by fungi summarize many different aspects of fungi and food interaction. Samson[26]provided overviews on the taxonomic description and specificity of food spoilage fungi highlighting numerous aspects of food spoilage. Microbial contamination has been reported to be the cause of food spoilage and many of these microbes are fungi. Fungi are ubiquitous or cosmopolitan as explained by[12]. Generally, tropical conditions such as high temperatures and moisture, monsoons, unseasonal rains during harvest, and flash floods lead to fungal proliferation and production of mycotoxins. Poor harvesting practices, improper storage, and less than optimal conditions during transport and marketing can also contribute to fungal growth and increase the risk of mycotoxin production[7]. Fungal deterioration of grain is a dynamic ecological process which often involves a succession of activities by microorganisms; the breakdown of organic matter to yield CO_2 and H_2O, and the generation of heat resulting in nutrient loss[15].

They initially establish a fungus-host interface as a biotrophic fungus that can exhibit prolonged survival in a quiescent state, which is followed by a necrotrophic infection stage[28]. Following the initial stage of infection, the fungus resumes growth and develops from a biotrophic parasite towards a necrotrophic parasite. This entails their hemibiotrophic (a partly parasitic and then necrotrophic) lifestyle. Necrotrophic hyphae are developed and produce a variety of plant-cell-wall-degrading enzymes, reactive oxygen species or secondary metabolites[17]. The total number of fungal species involved in post-harvest diseases is much larger than can be completely covered in this review. Nevertheless, this paper focused on providing a list of mycotoxin-producing fungal species which are known to have caused, or are believed to have the potential to cause strongly immunosuppressive diseases in humans and livestock. Bankole[4] explained that these toxins are heat stable and durable (thermoduric), therefore can pave the way for subsequent bacterial infections, as well.

Food spoilage is a major threat for our food stock and is responsible for the enormous losses worldwide, which makes this case study a research area that is very relevant, with respect to the increasing demand on food that will be encountered during the next decennia. Fungi are the main food degraders of the sturdy plant cell wall components[5], and are the major cause of post-harvest deterioration of cereals, legumes and oilseeds. Many groups of filamentous fungi such as *Penicillium, Aspergillus, Fusarium, Rhizopus, and Mucor spp.* have been successfully isolated from some street

vended foods[11]. There are some reports on post-harvest deterioration of starchy seeds (rice and maize), oil yielding seeds (groundnuts) and protein aceous seeds (beans and peanut). In one of the most recent investigations, gradual deterioration of the types of seeds mentioned above; namely, starchy (*Zea mays and Oryza sativa*), oil yielding (*Arachis hypogaea*) and proteinaceous (*Glycine max*), were studied[22,29,27]. In Nigeria, mycotoxin contamination of cereals and grains has raised a lot of concern for food safety[15] as these foods, especially rice and maize, are not only eaten directly, but also used in the production of various forms of indigenous foods like ogi, eko, tuwo, kunu, donkwa and many more.

Objectively, this study focused on isolating and identifying the mycotoxin-producing fungi associated with bio-deterioration of staple food samples collected from the major market location in the Owerri, Imo state of Nigeria. And, to find out if there is any similarities or differences in the fungi which contaminate the foods in their raw and boiled states.

Looking at the fungal food poisoning outbreaks in some countries as reported by[25], there is a pressing need to embark on this research in an attempt to highlight the health implications inherent in consuming foods contaminated with fungi in our locality. These fungi may produce certain secondary metabolites (mycotoxins) which are harmful carcinogens, mutagens, hepatotoxic, nephrotoxic and teratogenic agents to both humans and livestock. These reasons underscore the need for this study which could also form a basis for further research. Also, to bring to the awareness of the public (most importantly the inhabitants of Imo State and Nigeria at large) the health implications triggered by the ignorant consumption of fungal contaminated staple foods.

According to the National Population Commission (2015), Owerri metropolis has a human population of 715,800 with the larger fraction of the populace being civil servants and traders. It covers a land area of about 245 km² and lies on Latitude 5°32' N and Longitude 7°29'E with an elevated altitude of 152 meters. The average temperature is 26.2°C. The study area is known to be moderately active with human, commercial and recreational activities. Owerri sits in the rain forest with tropical wet climate and also a place where agricultural products like yam, cassava, maize, rubber, rice and palm products are produced and sold. Some products like beans, groundnut, etc, are brought in and sold in Owerri.

Materials and Methods:

The media were prepared according to the manufacturer's instruction and sterilized using an autoclave. A handful of the boiled food items were collected from each of the grain samples into dry, clean, transparent and covered plastic containers that were labeled. The samples were brought to the laboratory for processing.

Sample Processing:

The samples were processed and cultured according to the methodology described by[23]. Aseptically, a sterilized forceps was used to pick twenty (20) grains from each of the raw samples (rice, maize, groundnut, peanut and beans) and placed on the surface centre of the media that were labelled according to the respective food samples used for the study. The boiled samples were also aseptically seeded just as the raw samples. The preparation was incubated at room temperature for 7 days with daily check for growth.

Characterization and Identification: these were done based onb[19]. The isolates were identified based on the following:

Colonial Morphology; macroscopic examination of colonies was based on visible features such as: colour (both obverse and reverse sides), texture, and colonial morphology.

Microscopic Morphology; as described by[6], the definitive identification was based on the morphology of the:

Spores: depending on the type of spores, characteristic shape and arrangement of the spores along the hyphae,

Hyphae: based on important features like being septate or aseptate, and shape (whether long or branched in shape), and

Conidia: whether they are microconidia or macroconidia, and their arrangement along the hyphae that is either singly, in clusters or sessile.

Identification: using the tease mount method, a drop of lactophenol cotton blue was placed at the centre of a clean, grease free glass slide. Aseptically, a small portion of the fungal growth was picked, using sterile inoculating needles at halfway from the centre of the plates and placed in the drop of lactophenol cotton blue and gently teased to disperse the mycelial mass on the slide. This is to evenly spread out the fungal elements in the mounting medium. It was covered with a cover slip. The mount was pressed down slightly with the tip of the finger to expel any trapped air bubble and to further enhance observation. The slides were observed under low power (10X) magnification of a binocular microscope.

The isolates were identified according to the criteria of[6], and the results reported as follows:

***Aspergillis* species;**

Colonial morphology shows white, velvety and cottony surface.
Microscopic examination shows septate hyphae, long condiophores and conidia in chains.

***Penicillium* species;**

Colonial morphology shows brown and powdery colonies.
Microscopic examination shows septate hyphae, brush-like conidiophores with chains of conidia.

***Mucor* species;**

Colonial morphology shows yellowish-brown cottony colonies
Microscopic examination shows aseptate hyphae with many unbranched conidiophores bearing sporangia on columella.

***Rhizopus* species;**

Colonial morphology shows gray-cottony mycelia
Microscopic examination shows aseptate and branched hyphae with unbranched sporangiophores and clusters of rhizoids.

Geotrichum species;

Colonial morphology shows creamy-white cottony aerial mycelia
Microscopic examinat5ion shows septate hyphae with arthroconidia

Botrytis species;

Colonial morphology shows white colonies with fluffy surface
Microscopic examination shows septate hyphae with branched conidiophores and oval conidia.

The statistical analysis was carried out using statistical packages for social sciences (SPSS) version 21. With coefficient of significant difference, p value > 0.05.

RESULTS

Table 1: Cultured raw rice samples showing the identified fungal growth from Plates 1 and 2

FOOD SAMPLE	TYPE (raw)	NUMBER OF GRAINS SEEDED	FUNGAL SPECIES	ISOLATED (positive)	PERCENTAGE (%)
Rice	Plate 1	20	Aspergillus species	+	2.5%
			Rhizopus Species	+	2.5%
			Mucor Species	+	2.5%
	Plate 2	20	Aspergillus	+	2.5%
			species		
			Rhizopus Species	+	2.5%
			Mucor Species	+	2.5%
TOTAL		40	6		15%

Key:
+ = isolated
= not isolated

Table 2: Cultured boiled rice samples showing the identified fungal growth from Plates 3 and 4

FOOD SAMPLE	TYPE (boiled)	NUMBER OF GRAINS	FUNGAL SPECIES	ISOLATED (positive)	PERCENTAGE (%)
Rice	Plate 3	20	Aspergillus species	+	2.5%
			Penicillium Species	+	2.5%
			Rhizopus Species	+	2.5%
	Plate 4	20	Aspergillus species	+	2.5%
			Penicillium Species	+	2.5%
			Rhizopus Species	+	2.5%
TOTAL	4	0	6		15.0%

Key:
+ = isolated
= not isolated

Table 3: Cultured raw maize samples showing the identified fungal growth from Plates 5 and 6

FOOD SAMPLE	TYPE (raw)	NUMBER OF GRAINS	FUNGAL SPECIES	ISOLATED (positive)	PERCENTAGE (%)
Maize	Plate 5	20	Aspergillus species	+	2.5%
			Rhizopus Species	+	2.5%
	Plate 6	20	Aspergillus species	+	2.5%
			Rhizopus Species	+	2.5%
TOTAL		40	4		10.0%

Key:
+ = isolated
= not isolated

Table 4: Cultured boiled maize sample showing the identified fungal growth from Plates 7 and 8

FOOD SAMPLE	TYPE (boiled)	NUMBER OF GRAINS	FUNGAL SPECIES	ISOLATED (positive)	PERCENTAGE (%)
Maize	Plate 7	20	Aspergillus species	+	2.5%
			Geotrichum species	+	2.5%
			Rhizopus Species	+	2.5%
	Plate 8	20	Aspergillus Species	+	2.5%
			Geotrichum Species	+	2.5%
			Rhizopus Species	+	2.5%
TOTAL		40	6		15.0%

Key: + = isolated
- = not isolated

Table 5: Cultured raw groundnut samples showing the identified fungal growth from Plates 9 and 10

FOOD SAMPLE	TYPE (Raw)	NUMBER OF GRAINS	FUNGAL SPECIES	ISOLATED (positive)	PERCENTAGE (%)
Groundnut	Plate 9	20	Aspergillus species	+	2.5%
			Geotrichum species	+	2.5%
			Mucor species	+	2.5%
			Penicillium Species	+	2.5%
	Plate 1020		Aspergillus Species	+	2.5%
			Geotrichum Species	+	2.5%
			Mucor Species	+	2.5%
			Penicillium Species	+	2.5%
TOTAL		40	6		20.0%

Key:
+ = isolated
- = not isolated

Table 6: Cultured boiled groundnut samples showing the identified fungal growth from Plates 11 & 12

FOOD SAMPLE	TYPE (boiled)	NUMBER OF GRAINS	FUNGAL SPECIES	ISOLATED (positive)	PERCENTAGE (%)
Groundnut	Plate 11	2 0	Aspergillus species	+	2 . 5 %
			Penicillium species	+	2 . 5 %
			Mucor Species	+	2 . 5 %
	Plate 12	20	Aspergillus Species	+	2.5%
			Penicillium Species	+	2.5%
			Mucor Species	+	2.5%
TOTAL		40	6		1 5 . 0 %

Key:
+ = isolated
= not isolated

Table 7: Cultured raw peanut samples showing the identified fungal growth from Plates 13 and 14

FOOD SAMPLE	TYPE (Raw)	NUMBER OF GRAINS	FUNGAL SPECIES	ISOLATED (positive)	PERCENTAGE (%)
Peanut	Plate 13	20	Aspergillus species	+	2.5%
			Rhizopus species	+	2.5%
			Botrytis Species	+	2.5%
	Plate 14	20	Aspergillus Species	+	2.5%
			Rhizopus Species	+	2.5%
			Botrytis Species	+	2.5%
TOTAL		40	6		15.0%

Key:

+ = isolated

= not isolated

Table 8: Cultured boiled peanut samples showing the identified fungal growth from Plates 15 and 16

FOOD SAMPLE	TYPE (boiled)	NUMBER OF GRAINS PI	FUNGAL SPECIES	ISOLATED (positive)	PERCENTAGE (%)
Peanut	Plate 15	20	Penicillium Species	+	2.5%
	Plate 16	20	Penicillium Species	+	2.5%
TOTAL		40	2		5.0%

Key:

+ = isolated

= not isolated

Table 9: Cultured raw beans samples showing the identified fungal growth from Plates 17 and 18

FOOD SAMPLE	TYPE (raw)	NUMBER OF GRAINS	FUNGAL SPECIES	ISOLATED (positive)	PERCENTAGE (%)
Beans	Plate 17	20	Aspergillus species	+	2.5%
			Penicillium species	+	2.5%
			Mucor Species	+	2.5%
	Plate 18	20	Aspergillus Species	+	2.5%
			Penicillium Species	+	2.5%
			Mucor Species	+	2.5%
TOTAL		40	6		15.0%

Key:
+ = isolated
= not isolated

Table 10: Cultured boiled beans samples showing the identified fungal growth from Plates 19 and 20

FOOD SAMPLE	TYPE (boiled)	NUMBER OF GRAINS PI	FUNGAL SPECIES	ISOLATED (positive)	PERCENTAGE (%)
Beans	Plate 19	20	Geotrichum species	+	2.5%
			Mucor Species	+	2.5%
	Plate 20	20	Geotrichum Species	+	2.5%
			Mucor Species	+	2.5%
TOTAL		40	4		10.0%

Key:
+ = isolated
= not isolated

Table 11: Shows all the fungi isolated from the raw and the boiled food samples collected from the Eke-Ukwu Owere market in Owerri, Imo State of Nigeria

S/N	ISOLATES	FREQUENCY IN RAW SAMPLES	PERCENTAGE (%)	FREQUENCY IN BOILED SAMPLES	PERCENTAGE (%)
1.	Aspergillus Species	5	18.5%	3	11.1%
2.	Penicillium Species	2	7.4%	3	11.1%
3.	Rhizopus species	3	11.1%	2	7.4%
4.	Mucor species	3	11.1%	2	7.4%
5.	Geotrichum species	1	3.7%	2	7.4%
6.	Botrytis species	1	3.7%	-	-
TOTAL		15	55.6%	11	44.4%

Key:
+ = isolated
= not isolated

Figure 1: Pie chart showing the percentage of each of the isolates from the raw staple food samples

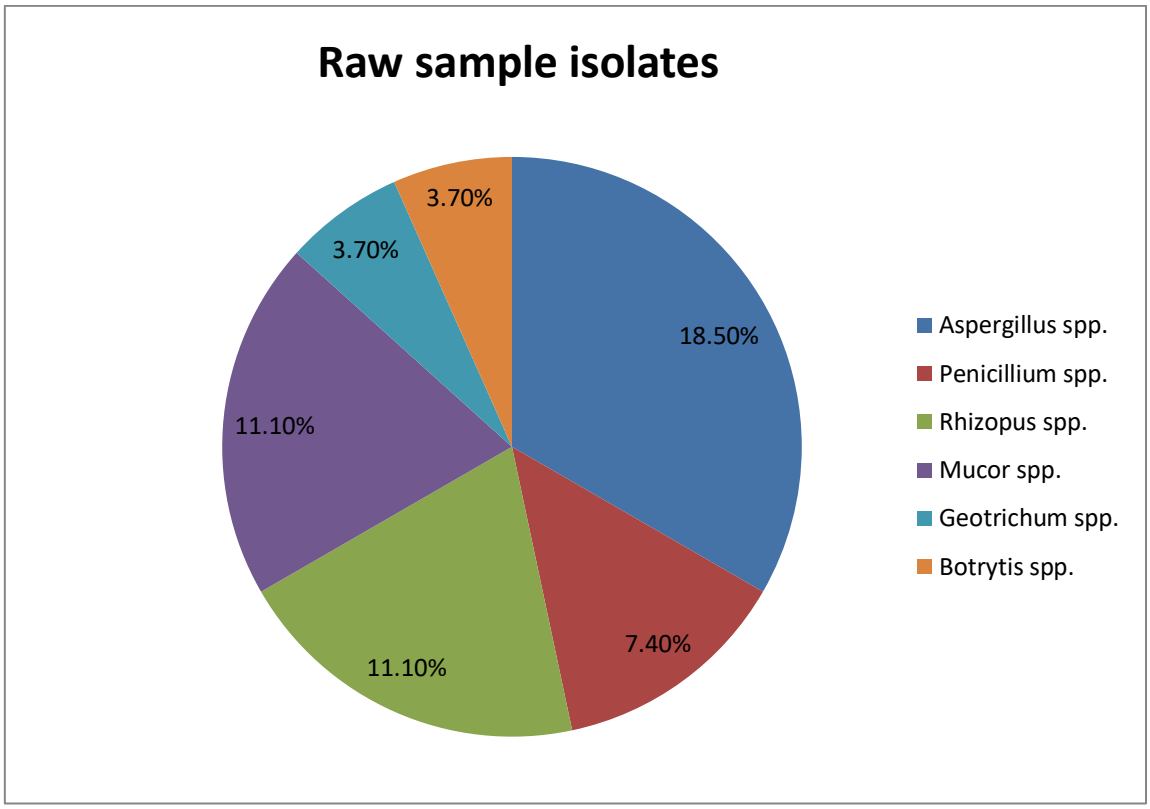
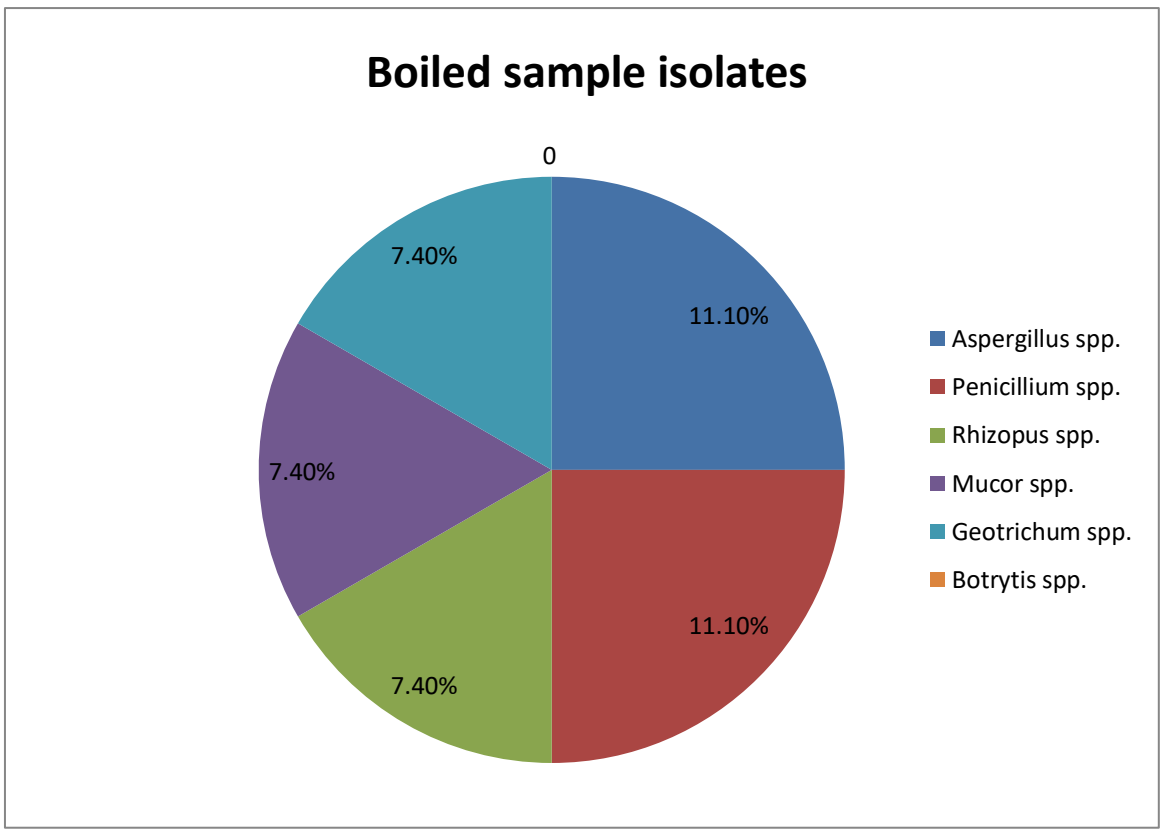


Figure 2: Pie chart showing the percentage of the isolates from the boiled staple food samples



DISCUSSION:

The need for the safety of staple foods in Owerri is very vital and a matter of public health interest. Hence, this study was carried out to isolate and identify the fungal contaminants from the staple foods consumed in Owerri, Imo State of Nigeria. The choice of the study area is based on the fact that it is the central point where most of the foods consumed in the state are sold both in wholesale and retail scales. The isolation of the fungal organisms like the *Aspergillus spp., Penicillium spp., Mucor spp.*, and *Rhizopus spp.* from the raw and boiled food samples indicates that the contamination could be at the post-harvest (from the retail to the table) stage. This is in accordance with the findings of[29] who noted that fungal contaminants, like *Botrytis spp.* and *Geotrichumspp.*, are field fungi and are known to be mycotoxin-producers.

In this study, all the examined ten (10) food samples yielded growths justifying the fact that all the samples had fungal contaminants. A total of six (6) fungal contaminants were identified, out of which a statistical value of 3.3 (55.6%) were from the raw samples and 2.7 (44.4%) were isolated from the boiled food items. This report holds slight similarity with the findings of Bankole *et al., (2008);* which stated that raw food items yielded more fungal contaminants than boiled ones. *Aspergillus* species were isolated from all the raw and boiled food samples. It has the highest frequency of 8 (29.6%) and *Botrytis* species were isolated from one (1) raw food only, with the least frequency of 1 (3.7%). *Penicillium* species isolated from two (2) raw samples and three (3) of the boiled samples with a frequency of 5 (18.5%). *Rhizopus* and *Mucor* species were isolated from three (3) of the raw samples and two (2) boiled samples, with the frequency of 5 (18.5%) each. *Geotrichum* species was isolated from one (1) raw food sample and two (2) boiled samples with the total frequency of 3 (11.1%).

From the results, the raw and boiled rice samples had slight disparity in the fungal isolates identified. This means that most of the isolates were not affected by any change in the nutrient value of the grains caused by boiling. This aligns with the findings of[1] which states that the spores are abundantly airborne, ubiquitous and can form heat stable surface layers on the foods. There were more isolates from the boiled maize food items than the raw ones. This may be due to breakdown in the integrity of the seed coat by heat, which may have allowed entry of the fungi into the foods. This slightly disagrees with the findings of[7] which observed that the isolates decreased in the boiled samples. The raw groundnut food items had more fungal growths than the boiled. This may be due to the light seed coat, and the fact that the boiled samples had less food nutrients due to boiling. This explains the fact that boiling affected much of the nutrients of the nuts which some of the fungi need for colonization. This is in line with the records of[15] which found that groundnut proteins are denatured by heating above 80^0C for more than twenty minutes. And these legumes are boiled for more than 80^0C for them to be well cooked. A similar occurrence was seen with the peanut samples. The isolates from the beans samples differed quite much; the boiled samples had less but different fungal contaminants. [2] reported that the isolation of these fungal species from the boiled sample explains the fact that they are heat stable; that is, they were not completely destroyed by the heat during boiling. The slight dissimilarity in the fungal isolates seen between the raw and boiled food items, agrees with the findings of[18] which records that the fungal spores resist and remains viable to form biofilms after heating for a period of time.

CONCLUSION

From the study, it was found out those fungi such as: *Aspergillus, Penicillium, Mucor, Rhizopus, Geotrichum* and *Botrytis spp.* were isolated from both the raw and boiled legumes and cereals studied. In other words, all the chosen food samples are prone to post-harvest fungal contamination. Therefore, care should be taken in consuming raw foods and poorly cooked food items especially cereals and legumes by the populace. This is because fungal organisms inhabit our food stuffs both in raw and cooked states. They cause diseases to man and livestock due to their secondary metabolites (mycotoxins) such as aflatoxins, ochratoxins, fumonisins, etc, which are known to have adverse health implication upon consumption in high doses.

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