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Fungi Contamination of Some Selected Brands of Sachet Water Marketed in Ahmadu Bello University, Zaria, Nigeria

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Abstract This study focuses on isolating and identifying fungi contaminants from sachet waters sold within the University's main campus, and determine the effect of these microbes on the quality of the sachet water. A sample size of 360 sachet water of 6 different brands was collected at random. From each brand of sachet water, ten sachets were purchased each month for the period of six months which was divided into two, halves of five sachet each, one half was stored at ambient temperature while the other half was refrigerated. Within 24 hours after collection, sample were analysed to assess the rate of contamination by fungi. Subsequently, each week single sachet was randomly picked from the stored samples for analysis within the month. The study utilizes the modified membrane filter technique using commercially prepared Potato Dextrose Agar as reagents for fungi isolation. This study revealed that, out of 360 sachet water, fungal species were isolated in 24 (6.66%) while 336 (93.33%) were devoid of fungi. Fungi were isolated in five brands while one was devoid of fungus. Among the fungi species lsolated, *Aspergillus*, *Penicllium* and *Fusarium* were the predominant isolates. The findings revealed the presence of pathogenic fungi species in the samples which indicate health risk involved in consumption of such products.

Keywords Fungi, Contamination, Sachet water, Zaria

1. Introduction

Water is indispensible for life considering the fact that it is an essential part of human nutrition. Water of good drinking quality is of basic important for man's continuous existence. It is also require for maintenance of personal hygiene and prevention of diseases. Lack of safe drinking water is a serious threat to national development and may have a negative impact on the health status of citizens. In many developing countries, availability of water has a critical and urgent problem and it is a matter of great concern to families and communities depending on non-public water supply system (Okonko *et al*., 2008).

The challenges associated with drinking water have encouraged the production of packaged drinking water by private enterprises that have little knowledge about manufacturing practices (Edema *et al*., 2011). The introduction of sachet water in Nigeria was a laudable idea, but studies suggested that this innovative idea is not risk free (Alli *et al.,* 2011). Today, the easy accessibility to drinking water in packaged forms has resulted in big thriving water industries with several millions of litres of these products consumed every year by Nigerians (Ogundipe, 2008). Most people in the cities and rural communities depend on sachet water as their ultimate source of drinking water. The integrity of sachet water is doubtful in fact, it has been reported that most of the vendors do not treat their sachet water before selling to the public and many engaged in the production, do not follow strictly the standard set by Federal Environmental Protection Agency and World Health Organisation (Oladipo *et al*., 2009; Okpako *et al*., 2009). The consumption of sachet water in Nigeria is on alarming rate and people are not mind full of the source, quality and possible consequences associated with sachet water consumption. Despite its popularity, studies conducted on the microbial quality of sachet water in some African cities have catalogued various levels of contamination (Adenkunle *et al*., 2004; Ifenyi *et al.,* 2006; WHO, 2011).

Fungi are widely distributed in nature and can occur as unicellular yeast or filamentous and, multicellular molds. Despite their wide occurrence, little attention has been given to their presence and significance in aquatic environments (Kirk *et al.,* 2001). In 1980s and 1990s, more cases of health problems caused by fungal contaminated drinking water were reported from Finland and Sweden (Muittari *et al.,* 1980; Aslund, 1984). Fungi have been reported from all

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types of water which included bottled and sachet drinking water (Mirian *et al*., 2007; Okpako *et al.,* 2009). The most commonly isolated genera from drinking water are *Penicillium*, *Cladosporium*, *Aspergillus*, *Phialophora* and *Acremonium* (DEFRA, 2011). They are involved in different forms of diseases, including allergies to fungal antigens, production of toxins, or direct invasion of hosts (McGinnis *et al*., 1996). Several species of fungi are capable of infecting healthy hosts and cause diseases ranging from mucosal to life threatening disseminated infections (Mirian *et al.,* 2007). The study aimed at assessing fungi which are capable of contaminating sachet water, Zaria, Nigeria.

2. Materials and Methods

Sample collections

A total of three hundred and sixty sachets from six brands of sachet water sold within Ahmadu Bello University, Zaria, Kaduna State, Nigeria. These were randomly collected from the production companies for six months. From each brand of sachet water, ten sachets were collected each month which were divided into two, halves of five sachet each. One half was stored at ambient temperature while the other half was refrigerated. Within 24 hours after collection, sample were analysed to assess the rate of contamination by fungi. Subsequently, each week single sachet was randomly picked from the stored samples for analysis within the month.

Preparation of Culture Medium for the Isolation of Fungi

Potato Dextrose Agar was used for the isolation, was prepared according to manufacturer's instruction. The molten medium was poured into conical flasks, plugged with aluminum foil. The medium was sterilized by autoclaving at 121°C at a pressure of 15 pound for 15 minutes. After sterilization, 15 ml of medium was aseptically dispensed into sterile petri dishes and allowed to solidify. The petri dishes were labeled accordingly.

Method for Isolation Fungi

Many methods for isolation of fungi have been mentioned in literatures; however, there is no standard method for isolation of fungi in water (DEFRA, 2011). Based on result obtained from pilot, Cheesbrough (2004) method was adopted by replacing the membrane filter with filter paper of 0.45*μm* pores. From each sample 100 ml was dispensed into two sterilized test tubes each and centrifuged. The centrifuged samples were filtered through a small disc of sterilized filter paper of 0.45*μm* pores and the membrane filter was placed on Potato Dextrose Agar plates in duplicates and labeled accordingly. All isolates from the medium were sub cultured into Potato Dextrose Agar (PDA) slant labeled appropriately and refrigerated for further assay.

Identification of Fungi

Isolated fungi were identified by examining both microscopic and macroscopic characters. The identification was aided by using identification keys of Barnett and Hunter (1972), Larone (2002), Klich (2002) and Samson *et al,* (2004).

3. Results

A total 9 of fungal species were isolated from five sachet water samples while one was devoid of any fungus even thought their distribution was not even (Table 1). The isolates were identified by studying their macroscopic and microscopic characters and were compared with already described species using identification keys by Barnnet and Hunter, (1972), Larone, (2002), Klich, (2002) and Samson *et al*., (2004) (Table 2). It is evident from Table 3 that *Aspergillus niger* was more prevalence out of the three *Aspergillus* species as it was isolated in three different samples with higher percentage of 12.50% followed by *Pencillium glabrum* and *Fusarium oxysporum* with 8-33% each while *Aspergillus flavus, Aspergillus fumigates, Cephalosporium curtipes, Odiodendron griseuum, Rhizoctonia solani* and *Trichoderma viride* had the same percentage of 4.17% each. There was no significant difference in the mean of the fungal colony count at (P□0.05) between after collection and when stored at ambient temperature and when refrigerated except OJT which was significantly different (P□0.05). It is evident that ambient temperature fovours the multiplication of fungi as the higher colony count was recorded at ambient temperature (Table 3).

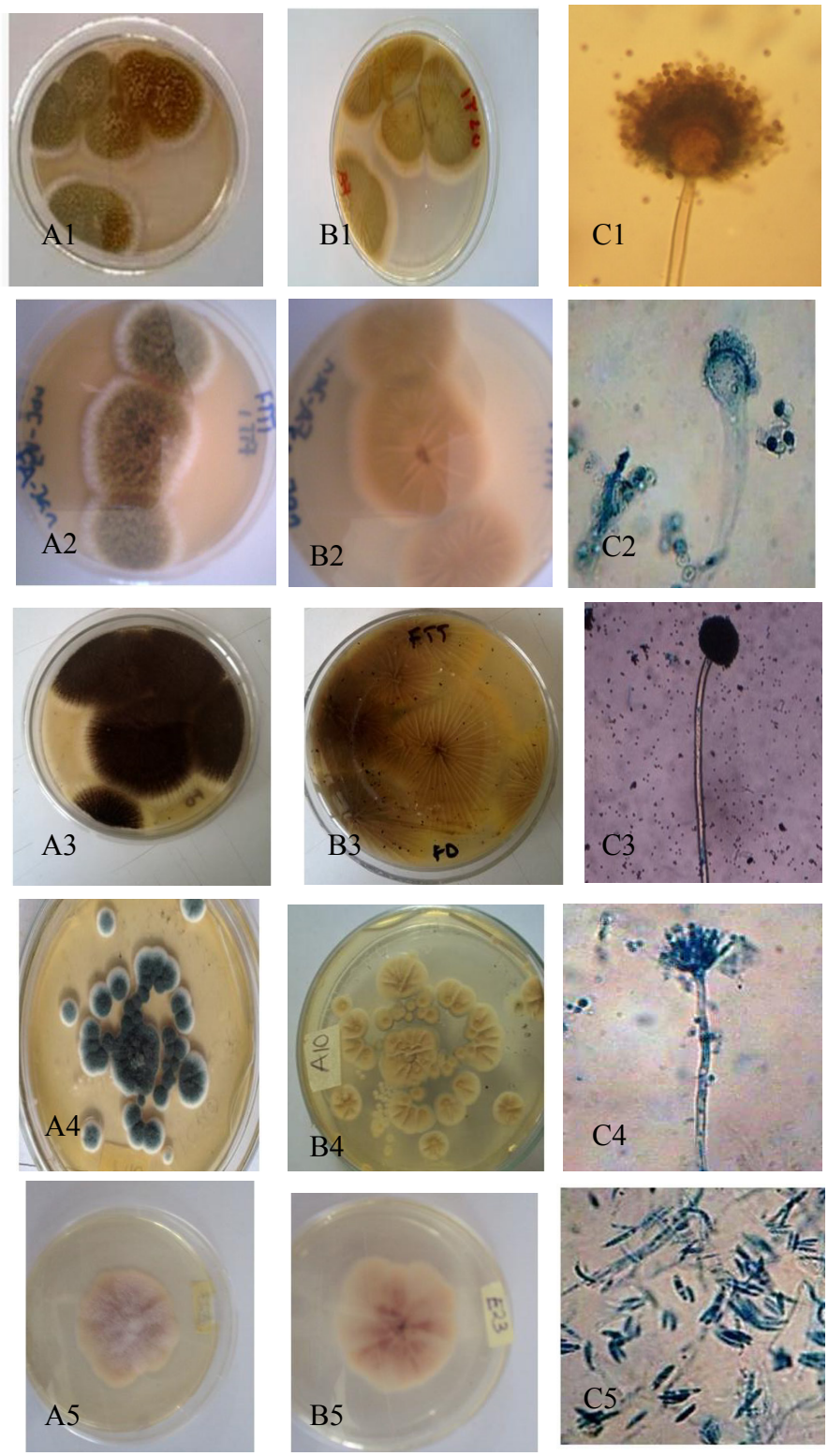
Table 1. Fungi Species Isolated and their Distribution from Six Different Brands of Sachet Water

Fungi Species Isolated	Brands of Sachet Water					
	FTT[x]	OJT[x]	BST[x]	BRT[x]	LST[x]	DFT[x]
Aspergillus flavus	-	-	✓	-	-	-
Aspergillus fumigatus	✓	-	-	-	-	-
Aspergillus niger	✓	✓	✓	-	-	-
Penicillium glabrum	-	✓	-	-	-	-
Fusarium oxysporum	-	✓	✓	-	-	-
Cephalosporium curtipes	-	-	-	-	-	✓
Oidiodendron griseum	-	✓	✓	-	-	✓
Rhizoctonia solani	-	✓	-	-	-	✓
Trichoderma viride	-	✓	✓	-	✓	✓

✓ = Present, - =Absent, [x] - Codes representing the trade names of sachet water

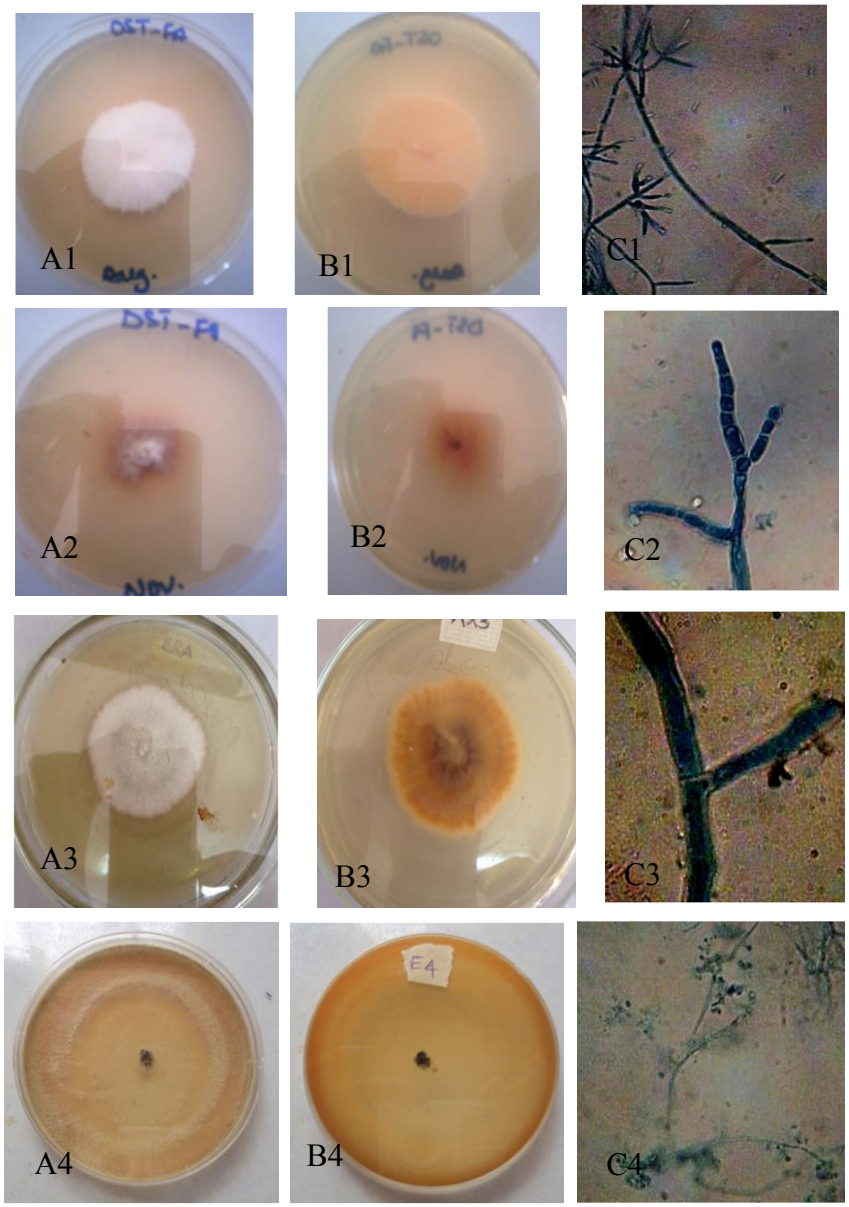
Table 2. Description of the Macroscopic and Microscopic Characteristics Fungi Species Isolated

Fungi species isolated	Macroscopic Characteristics	Microscopic Characteristics
Aspergillus flavus	The upper surface of colonies was olive green with white edge, granular surface and green coloration on the reverse side on Potato Dextrose Agar. The Colonies were ovoid in shape and varied in size (ranged) from 2.5-3.7 cm with an average of 3.1 cm in diameter within 5 days at room temperature	The conidiophore was thick walled, hyaline and slightly roughened, erect, long, aseptate with a vesicle at the top with phialides and with short conidial chains. The length of conidiophore measured ranged between 110-320µm with an average length of 290.20 µm. The width of the conidiophore near the vesicles measured 21-45 µm, 22-44 µm at the middle and 23-45 µm at the base. The vesicles measured 57-103 µm in diameter with an average 80 µm. The conidia measured 2-4 µm in diameter with an average 3.9 µm
Aspergillus fumigatus	The colony was widely spread, dark green with smooth white edges and spongy surface and brown on the reverse side on Potato Dextrose Agar. The colonies were round in shape and measured 7 cm in diameter within 5 days at room temperature	The conidiophore was long, narrow at the base and broad near the vesicle, smooth walled hyaline. The length of conidiophore ranged 100-120µm, width near the vesicle and measured 9-12 µm, 7-9 µm at the middle and 7-8 µm at the base. The vesicle measured 30-35 µm with an average of 30 µm. The conidial head is grayish near the apices with an irregular in shape. Conidia measured 4-6 µm with an average of 4.2 µm in diameter.
Aspergillus niger	The colonies were widely spread, black, with smooth white edges and spongy surface densely packed and brown on the reverse side on Potato Dextrose Agar. The colonies were round in shape and measured 2-4.5 cm with an average of 3 cm within 5 days at room temperature.	The conidiophore was long, erected from the base to the vesicle, smooth walled, hyaline with globes conidial head. The length of the conidiophore ranged 100-102 µm with an average 100 µm. The width of the conidiophore near the vesicle measured 10 µm, at the middle measure 10 µm and 9 µm at the base. The conidial head was blackish in colour and measured 4-6 µm in diameter with an average 5 µm.
Penicillium glabrum	The colonies were green with cottony upper surface, white border and brown on the reverse side on Potato Dextrose Agar. The colonies were round in shape and varied in size, measured 1-1.5 cm in with an average of 2 cm within 5 days at room temperature.	The conidiophore was long, thick walled and hyaline. The conidiophore ranged 100-125 µm in diameter with an average of 100 µm, near the vesicle measured 12 µm, 11 µm at the middle and 12 µm at the base. The conidial head was bluish near the apices with an irregular shape and the conidia measured 5-6 µm in diameter with an average of 4µm.
Fusarium oxysporum	The colony was pink with white patch on the surface and on the reverse side was brown in coloration on Potato Dextrose Agar. The colony was round in shape and measured 4 cm in diameter within 5 days at room temperature.	The macroconidia are canoe shaped, multiseptate which contain 3-6 septations and slightly pointed at the end. Measured 29-100 µm in length with an average of 75 µm. The width measured 20-30 µm with an average of 35 µm. The microconidia is ovoid or oblong borne single with no septation and single celled. The conidia measured range between 20-40 µm in length with an average length of 30 µm. The width measured between 9-18 µm with an average of 18 µm.
Cephalosporium curtipes	The colony is white with smooth edge and tuft surface and on the reverse side is brown in colour growing on Potato Dextrose Agar. The colony is round in shape and measured 4 cm in diameter within 5 days at room temperature	The hyphae are hyaline, branched, measured 20-30µmin diameter and bear the conidiophores on aerial branches. The conidiophore ranged 25-50 µm, with 3-4 side branches. Conidia are developed at the tip of the conidiophore form a globose head and measured 4-8 µm with an average of 4 µm.
Oidiodendron griseum	The colony was white with irregular edge and spongy surface and brown in colour on the reverse side on Potato Dextrose Agar. It measured 3 cm in length within 5 days at room temperature	The conidiophore was irregularly branched at the upper portion and the branches segmented into rod-shaped conidia whichwere in chains. The conidiophore measured 80-120 µm in length with an average of 80 µm. The conidia measured 30-40 µm with an average of 30 µm.
Rhizoctonia solani	The colony was white with cottony surface and on the reverse side is brown in coloration on Potato Dextrose Agar. It measured 4 cm within 5 days at room temperature	Dark mycelium hyaline, long mycelium cell and branched at the upper part, no septation of branches set off from the main hyphae. The mycelium measured 100-250 µm in length with an average of 160 µm
Trichoderma viride	White colony with folded cottony upper surface, creamy colored spores and a yellowish brown on the reverse side growing on Potato Dextrose Agar. It measured 7 cm within 7 days at room temperature	The conidiophores hyaline was irregularly branched. Lateral branch below with short branches near the apex. Phialides are in group of 2-3. The conidia are round, clustered at the end of phialide and measured 3-4 µm with an average of 2.5 µm. The conidiophore measured 80-110 µm in length with an average of 90 µm.



A-Upper surface
B-Reverse side
C1, C2, C3, C4 = conidiophore bearing condia, C5 = macroconidia and microcondia

Plate 1. Pure Culture of **1.** Aspergillus flavus **2.** Aspergillus fumigates **3.** Aspergillus niger **4.** Penicillium glabrum **5.** Fusarium oxysporum



A-Upper surface
B-Reverse side
C1, C2, C3, C4 = conidiophore bearing condia,

Plate 2. Pure Culture of **1.** Cephalosporium curtipes **2.** Oidiodendron griseum **3.** Rhizoctonia solani **4.** Rhizoctonia solani

Table 3. Fungi Species Isolated from Six Brands of Sachet Water and their Percentage Occurrence in Parenthesis

	Brands of Sachet Water					
Fungi Species Isolated	**FTT[x]**	**OJT[x]**	**BST[x]**	**BRT[x]**	**LST[x]**	**DFT[x]**
Aspergillus flavus	0(0.00)	0(0.00)	1(4.17)	0(0.00)	0(0.00)	0(0.00)
Aspergillus fumigatus	1(4.17)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Aspergillus niger	2(8.33)	2(8.33)	3(12.50)	0(0.00)	0(0.00)	0(0.00)
Penicillium glabrum	0(0.00)	2(8.33)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Fusarium oxysporum	0(0.00)	2(8.33)	1(4.17)	0(0.00)	0(0.00)	0(0.00)
Cephalosporium curtipes	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(4.17)
Oidiodendron griseum	0(0.00)	1(4.17)	1(4.17)	0(0.00)	0(0.00)	1(4.17)
Rhizoctonia solani	0(0.00)	1(4.17)	0(0.00)	0(0.00)	0(0.00)	1(4.17)
Trichoderma viride	0(0.00)	1(4.17)	1(4.17)	0(0.00)	1(4.17)	1(4.17)

[x] = Codes representing trade names of sachet water, Values outside parenthesis represent occurrence of fungi

Table 4. Mean (±S.E.) Value of Fungi Colony Count of Six Brands of Sachet Water

Brands of Sachet Water	After Collection	Storage Modes	
		Ambient Temperature	Refrigerated
FTT[x]	0.33±0.33[a] (0.00-2.00)	0.17±0.10[a] (0.00-2.00)	0.00±0.00[a] (0.00-0.00)
OJT[x]	1.17±0.48[a] (0.00-3.00)	0.29±0.14[b] (0.00-2.00)	0.00±0.00[b] (0.00-0.00)
BST[x]	0.33±0.33[a] (0.00-2.00)	0.33±0.19[a] (0.00-3.00)	0.00±0.00[a] (0.00-0.00)
BRT[x]	0.00±0.00[a] (0.00-0.00)	0.00±0.00[a] (0.00-0.00)	0.00±0.00[a] (0.00-0.00)
LST[x]	0.00±0.00[a] (0.00-0.00)	0.08±0.08[a] (0.00-2.00)	0.04±0.04[a] (0.00-0.00)
DFT[x]	0.17±0.00[a] (0.00-1.00)	0.08±0.08[a] (0.00-2.00)	0.00±0.00[a] (0.00-0.00)

Means with different superscript across the rows are significantly different (P□0.05), [x] = Codes representing trade names of sachet water, Values in parenthesis represent the ranges of colony count

4. Discussion

Five out of the six brands of sachet water were to be contaminated with varied number of fungi species (Table 1). The occurrence of varied number of fungal species in different sachet water indicates the status of the treatment rendered to the water during production. The presence of fungi species in any sample indicates inadequate treatment while the absences of fungus in BRT imply the treatment was probably effective (Table 1). *Aspergillus niger* had the higher percentage of occurrence of 3 (12.50%) followed by *Pencillium glabrum* and *Fusarium oxysporum* which recorded 2 (8.33%) each (Table 2). The higher percentage of occurrence by *Aspergillus niger, Pencillium glabrum* and *Fusarium oxysporum* could be attributed to its ability to secrete pigment called melanin which provides protection against a range of stresses making them resistant to water treatment coupled with inadequate treatment (Waipara, 1998; Langfelder *et al*., 2003). *Aspergillus flavus, Aspergillus fumigatus, Cephalosporium, Odiodendron griseuum, Rhizoctonia solani* and *Trichoderma viride* had the low percentage of occurrence of 1 (4.17%) each (Table 2). The low percentage of occurrence could be adduced to effective water treatment methods such as ozone and chlorine dioxide against the fungal spores as reported by Kelley *et al.,* (2003).

Mean of the colony count also varied even though it was not significantly different (P□0.05) except OJT which was significantly different (P□0.05). OJT had the highest mean of colony counts than other samples which indicated high level of contamination This varied percentage of occurrence and mean of colony count could be attributed to variation in level of treatment, poor hygiene practices, poor storage of treated water and poor management of storage facilities, unhygienic working environment, use of unsterilized materials, difference in sources of water used for production coupled with inadequate treatment. Similar observation was made by Hageskal (2007); Okpako *et al.,* (2009); Tanveer *et al*., (2011).

The occurrence of these fungi may cause diverse effects on human health as they have the potential of producing mycotoxins. The concentrations of these substances may increase during storage of water due increase in the population of the fungi species, hence, daily intake of such water containing mycotoxin could result in bioaccumulation in the body which could be hazardous to human health. *Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus niger* are known to produce aflatoxins, ochratoxins and fumitremoigin which are carcinogenic and are capable of causing kidney and liver disorders, invasive and non invasive aspergillosis, allergic and sinusitis (Bryce, 1999; Bennett and Klich, 2003 and Samson *et al.*, 2004). *Penicillium* glabrum produce citromycetin which is known to cause allergy, asthma and some respiratory problems (Cooley *et al.,* 1998; Frisvad *et al.,* 1998; Samson *et al*., 2004 and Gunhild *et al.,* 2006). *Fusarium oxysporum* is capable of producing fusaric acid, monilifornun, gibepyrones and naphthoquinone pigment which have been recognized as agents of superficial infections (Guarro and Gene, 1995 and Samson *et al.*, 2004). *Trichoderma virides* produces trichothecenes, alamethicins, emodin, trichotoxin and suzukacillin and have been reported to cause mycosis and allergy in humans (Jaakkola *et al.,* 2002; Tang *et al.,* 2003 and Samson *et al.*, 2004).

5. Conclusions

The findings revealed the presence of pathogenic fungi species in the samples which indicate risk involved in consumption of such products and are therefore could be hazardous to human health. Based on the results obtained in this study, it could be concluded that sachet water are contaminated with fungi. The predominant fungi genera associated with the sachet water are *Aspergillus*, *Penicllium* and *Fusarium.* The presence of those microbes in sachet water has been traced to inadequate treatment and poor hygiene practices during production which has significantly affected the quality of the sachet water making the water unfit for human consumption. It also revealed that ambient temperature favours multiplication of fungi. It revealed that storage has significant effect on the microbial quality of sachet water if the treatment is inadequate during production. It also revealed that the quality of sachet water can be deteriorated within the shelf life if the water produced is not adequately treated.

6. Recommendations

It is recommended that all manufacturing industries must adhere to National Agency for Food and Drug Administration and Control (NAFDAC) guidelines and all the existing laws should be enforced considering the high patronage of sachet water in the area of study. In addition, there is need for awareness programs in order to educate the general public on the potential health implication associated with consumption of such products. Furthermore, to safeguard the health of the people there is need for regular monitoring of the quality of the water and the environment by National Agency for Food and Drug Administration and Control (NAFDAC).

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