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OCCURRENCE OF FUNGI IN FROZEN TITUS FISH (*Scomber scombrus*) SOLD IN SOME AREAS IN ILORIN METROPOLIS

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ABSTRACT

A total number of 100 frozen fishes (*Scomber scrombus*) were purchased from three different locations in Ilorin metropolis (Osere, Agbabiaka and Sango). The plastic bags used in packing the frozen fishes were sterilized using 70% ethanol and transported in a sterile ice-parked cooler. Samples from the cutting slabs at each location were also collected using cotton swabs and they were soaked in a conical flask containing sterile distilled water. An Aluminum foil was used to cover the conical flask. The frozen fish samples and specimen from the cutting slabs at each location were transported to the Microbiology laboratory, University of Ilorin for fungi analysis. Results revealed high contamination of yeast and mould in the cutting slabs and the gills of the sampled frozen fish. The fungi isolated were *Alternaria tenuis, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Neurospora crazza, Rhizopus stolonifer, Penicillium citrinum* and *Saccharomyces cerevisae* with *Aspergillus spp* having the highest number. It was concluded that high occurrence of *Aspergillus spp* in the sampled frozen fish could be risk to human health. Recommendation was made for proper handling of processed and storage of frozen fish in sales outlets around Ilorin metropolis.

Keywords: Occurrence; fungi; frozen; fish; *Scomber scombrus*

INTRODUCTION

Fish is known to be a good source of protein. It contributes about 14% of all animal protein on a global basis (Abbas and Shier, 2010). In African countries, 17.50% of animal protein consumed comes from fish while over 50% of the animal protein intake comes from fish in Asian countries (Williams *et al*., 1988). Fish is known to be rich in vitamins B_{12} and B_{6}. It is also a good source of fluorine and iodine which are needed for strong teeth development and for prevention of goitre in man (Andrew, 2001). Due to its ever-increasing consumption demand, fish and fishery products also constitute an important item of trade and provide a source of income for large number of people (Harris, 2004). Therefore, it is

important to have quality fish to attract customers and to avoid the health risks associated with poor or spoilt fish.

Fish has a short shelf life and its spoilage is caused by microbial enzymes. Microbial load on the skin, gills and intestines of fish living in clear waters is usually high, while muscles are usually sterile (Pamuk *et al*., 2011). After contamination and replication of microorganisms, decay occurs and the consumption becomes dangerous (Mol and Tosun, 2011; Alparslan *et al*., 2014). The quality and freshness of fish are rapidly deteriorated through microbial and biochemical mechanisms (Aljasser *et al*., 2014).

Frozen fish are of great demand in Nigeria as a relatively cheaper source of animal protein (Ryder *et al*., 1993). Fish products are important not only from a nutritional point of view, but also as an item of international trade and or foreign exchange earner for a number of people worldwide (Yaqoub, 2009). Fish and shellfish are highly perishable, and prone to vast variations. Inequality due to feeding habits, differences in species and environmental habitats (Yaqoub, 2009).

According to the Center for Food Safety and Applied Nutrition in Washington (2001), most fish related food borne illness are traced to *Salmonella, Staphylococcus spp., Escherichia spp., Vibrio parahemolyticus, Clostridium perfringens, Clostridium botulinum E, and Enteroviruses* (Yagoub, 2009). Storage time and temperature affect the quality and shelf life of fish. Fishes are mostly contaminated by staphylococci, coliform, pseudomonas and some others. Staphylococcus are non-motile cocci that are catalase positive and facultative anaerobic, having both an oxidative and fermentative type of metabolism. Psychotropic type microbes have the ability to multiply at 3-10 °C thus they can multiply in fish even when refrigerated. Coliforms include all aerobic and facultitively anaerobic gram negative non spore forming bacilli which ferment lactose with gas formation within 48 hours at 35 °C. In the lab, *Staphylococcus aureus* produces white to golden colored colonies and is positive for the coagulase test. Pseudomonas is a genus of gammaproteobacteria, belonging to the family pseudomonadaceae containing 191 validly described species (Euzeby, 1997). The best studied species include *P. aeruginosa* in its role as an opportunistic human pathogen, the plant pathogen *P. syringae*, the soil bacterium *P. putida*, and the plant growth promoting *P. fluorescens* (Matthijs *et. al*., 2007). Fishes with microbes are dangerous to our health as they act as a source of various food borne diseases. Contamination may be caused by food borne pathogens which are naturally present in aquatic environments, such as *Vibrio spp* or derived from sewage contaminated water such as *Salmonella spp*. Consumption of these contaminated fish may cause infection when consumed.

Microbes are organisms that cannot be seen with the naked eyes except by the use of microscope. Microorganisms include fungi, algae, bacteria, virus and protozoa. Most of these organisms are unicellular and all the life processes are processed by a single cell.

Fungal growth is assisted by moisture and oxygen (Dada, 1997). However, water availability is considered more important as controlling it has been shown to reduce the growth of fungi (Krska *et al*., 2008). There is high risk of mould infestation in areas with high amount of water in the environment (Okoye, 1993). Indoor air in areas with high relative humidity has been shown to have a higher concentration of fungi when compared to air within low relative humidity area (Makun *et al*., 2007). Fungi growth in damp places is believed to be an increasing problem globally due to the health and financial implications which comes from it (Andersen *et al*., 2011). A wide range of fungi have been isolated from indoor air of houses in flood prone areas and the sources of these organisms are suggested to vary from soil, plant wastes, animal, human and accumulated dust which find their way into indoor air

as aerosols and air which moves from external surrounding (Bullerman, 2007). Fungi are isolated from fish substrates by techniques that vary in complexity, media are used to culture fungi meant for isolation and identification (Abubakar, 2016).Fungal contamination of fish is considered as one of the main cause of spoilage in fish which is usually associated with off flavor and unpalatable taste, which may cause health problems as well as many economic losses (Adejumo *et al*., 2012). The presence of fungi in fish might make the consumption dangerous to human health as they might contain metabolites produced by the fungi (Adebajo *et al*., 1994). Disease outbreaks due to consumption of contaminated food are recurring problem worldwide. The major factor contributing to this contamination is fungi, which produces low molecular-weight compounds as secondary metabolites with toxic properties referred to as mycotoxins, several mycotoxins reported till date are cosmopolitan in distribution and incur several health associated problem including cancer and neurological disorders (Bhat *et al*., 2010).

The aim of this study was to determine the occurrence of fungi in frozen fish (*Scomber scombrus* and *Clupea harengus*) sold in some areas in Ilorin metropolis.

MATERIALS AND METHODS

The Study Area

The study was carried out in Ilorin, the capital of Kwara State. Ilorin is located between latitude 8º30' to 8º50'N and longitude 4º20' to 4º35'E of the equator. Ilorin city occupies an area of about 468sqkm and it is situated in the transitional zone within the forest and the guinea savannah regions of Nigeria. It is about 300 kilometers away from Lagos and 500 kilometers away from Abuja the federal capital of Nigeria. The climate of Ilorin is tropical under the influence of the two trade winds prevailing over the country. Ilorin metropolis experiences two climatic seasons i.e. rainy and dry season. The rainy season is between March and November and the annual rainfall varies from 1000 mm to 1500 mm, with the peak between September and early October. Also, the mean monthly temperature is generally high throughout the year. The daily average temperatures are in January with 25 ºC, May 27.5 ºC and September 22.5 ºC (Kwara State Government, 2017).

Collection of the Fish Samples

The frozen fishes sold within Ilorin metropolis usually undergo proper inspection before being certified for human consumption. Samples of the Titus frozen fish sold in Ilorin metropolis of Kwara State were obtained from three different locations for this study. A total number of 100 frozen fishes (Titus: *Scomber scrombus*) were purchased from the three different locations (Osere, Agbabiaka and Sango) that people patronize for frozen fishes in Ilorin metropolis. The plastic bags used in packing the frozen fishes were sterilized using 70% ethanol. The frozen fish samples were transported in a sterile ice-parked cooler. Samples from the cutting slabs at each location were also collected using cotton swabs to pick specimen and were soaked in a conical flask containing sterile distilled water. An Aluminum foil was used to cover the conical flask. The frozen fish samples and specimen samples from the cutting slabs at each location were transported down to the Microbiology laboratory, University of Ilorin for fungi analysis.

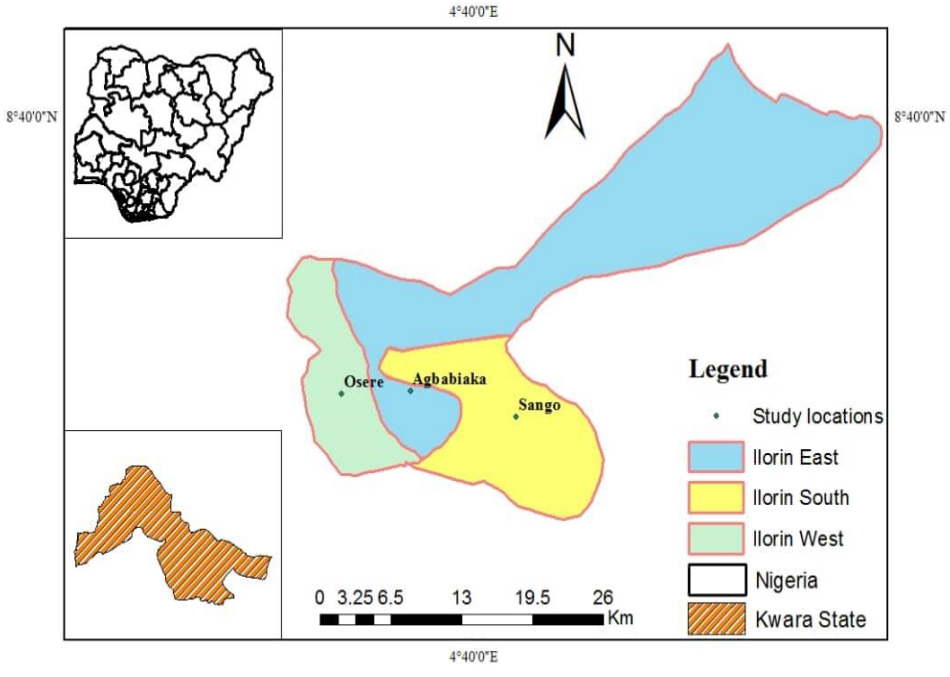

Figure 1: Map of Ilorin showing the site of fish collection (•)

Treatment of Samples

Sterile scalpel was used to remove suspected body parts needed for the analysis, 1gram of each part was weighed and homogenized using 9ml sterile distilled water

Sterilization of the Materials Used

All the glassware used were thoroughly washed with detergent solution, sterile distilled water was used to rinse the glasses and were allowed to dry and sterilized in an oven at 160^0C for 1hour.

Preparation of Culture Media

The media used for the analysis are:

- Potato Dextrose agar (PDA)
- Yeast extract agar (YEA)

Potato Dextrose Agar

Potato Dextrose Agar was prepared using the method of Abubakar (2016) as follows:

Peeled, sliced potatoes	200g
Dextrose	10-20g
Agar	12-15g
Distilled water	1 litre

Sliced potatoes were added and allowed to simmer for 30-60 minutes after which it was filtered through layers of chesses-cloth. Agar and other ingredients were added to the filtrate before auto cleaving. To prevent bacterial contamination, 100mg of streptomycin sulphate, prepared as stock solution in sterile distilled water was added to 1 litre of autoclaved medium under sterile hood at 45^0C before pouring into plates. This medium is very useful for growing large variety of fungi.

Yeast Extract Agar

Twenty-three (23) g of the yeast extract agar powder was weighed and suspended into 1litre of distilled water; it was then shaken to mix properly and heated to dissolve the powder completely. After heating, the mouth of the flask was covered with cotton wool and wrapped with aluminium foil. The media was sterilized in the autoclave at a temperature 121^0C for 15minutes and left to cool to about 45^0C before pouring aseptically into sterile petri dishes (Chessbrough, 2006).

Serial Dilution Technique

Serial dilution was used for the enumeration of fungi count: Using pipettes, serial dilution of the fungi isolates was taken as follows:
Tube 1: 1ml of original broth culture + 9ml sterile water = 1 in 10 dilution
Tube 2: 1ml of tube 1 + 9ml sterile water =1: 10^2 dilution
Tube 3: 1ml of tube 2 + 9ml sterile water =1: 10^3 dilution
Tube 4: 1ml of tube 3 + 9ml sterile water =1: 10^4 dilution
Tube 5: 1ml of tube 4 + 9ml sterile water =1: 10^5 dilution
Tube 6: 1ml of tube 5 + 9ml sterile water =1: 10^6 dilution
Tube 7: 1ml of tube 6 + 9ml sterile water =1: 10^7 dilution
Tube 8: 1ml of tube 7 + 9ml sterile water =1: 10^8 dilution

The product of serial dilution was poured into sterile Petri dishes and was shaken clockwise and anticlockwise so as to make it gel together. After gelling, each of the plates was incubated at room temperature for 72hours. After incubation period, each of the plates was examined for fungi growth. Counting of fungi growth was done using heamatocytometer.

Identification and Characterization

The fungi isolated were identified and characterized based on their surface texture, pigmentation and under surface texture using the manual of Noga (1995). Several sub-culturing was carried out. After which the fungi isolates were examined under low power objective microscope (x40)

Statistical Analysis

Data collected were processed and analysed on the computer using computer statistical package SPSS (version 16). Photomicrographs were processed and obtained using electron microscope.

RESULTS

Results of the clinical examination and photomicrographs of different fungi isolated from fish species are explained herein:

Identification and characterization of fungal isolates from frozen Titus (*Scomber scrombus*) are shown in Table 1 below:

Table 1: Identification and characterization of fungal isolates from frozen Titus

Fungi isolates	Surface texture	Macroculture	Under-surface texture	Tentative identification
F1	Leathery	Greyish black	Creamy	*Alternaria tenuis*
F2	Powdery	White-yellow	Creamy	*Aspergillus flavus*
F3	Powdery	Grey- green	Creamy	*Aspergillus fumigatus*
F4	Powdery	Black	Creamy	*Aspergillus niger*
F5	Powdery	Pink	Creamy	*Neurospora crazza*
F6	Powdery	Greenish-blue with narrow margin	Creamy	*Penicillium citrinum*
F7	Soft smooth	Creamy	Creamy	*Saccharomyces cerevisae*

Table 2 revealed the presence of *Alternaria tenuis* on the slabs from the three locations (Osere, Agbabiaka and Sango) in the order of four (5%), Zero (0%) and Zero (0%) respectively. For the gills, the order of occurrence was eight (9%), three (0.03%) and two (2%) respectively. The order of occurrence on the skin were two (2%), zero (0%) and zero (0%) respectively. None were isolated from their fins and caudal peduncle respectively. *Aspergillus flavus* isolated from the slabs of the three locations were thirty (40%), 13 (30%) and 23 (35%) respectively. For the gills, the order of occurrence was 27 (30%), 21 (26%) and 26 (28%) respectively. The order of occurrence on the skin were five (5%), three (0.3%) and zero (0%) respectively. The order of occurrence on the fins were zero (0%), one (1%) and zero (0%) respectively. Caudal peduncle had zero (0%), one (1%) and two (2%) respectively.

Aspergillus fumigatus isolated from the slabs of the three locations were zero (0%), ten (23%) and fifteen (23%) respectively. For the gills, the order of occurrence was 13 (15%), 27 (34%) and 20 (22%) respectively. The order of occurrence on the skin was zero (0%), zero (0%) and one (1%). None were found on their fins and caudal peduncle respectively. *Aspergillus niger* isolated from the slabs of the three locations were 23 (31%), zero (0%) and ten (15%) respectively. For the gills, the order of occurrence was 10 (11%), 18 (23%) and 23 (25%) respectively. The order of occurrence on the skin was zero (0%), one (0.1%) and two (0.3%) respectively. None were isolated from their fins respectively. *Neurospora crazza* found on the slabs from the three locations were in the order of five (7%), eight (18%) and fifteen (23%) respectively. For the gills, the order of occurrence was 0(0%), 0(0%) and 21(28%) respectively. The orders of occurrence on the skin were zero (0%), zero (0%) and

five (0.6%) respectively. None were isolated from the fins and caudal peduncle repectively. *Penicillium citrinum* found on the slabs from the three locations were eight (11%), two (0.04%) and zero (0%) respectively. For the gills, the order of occurrence was twenty-five (28%), zero (0%) and zero (0%) respectively. The orders of occurrence on the skin were zero (0%), zero (0%) and zero (0%) respectively. None were isolated from their fins and caudal peduncle respectively. *Rhizopus stolonifer* isolated from the slabs of the three locations were zero (0%), four (0.1%) and two (3%) respectively. For the gills, the order of occurrence was five (6%), zero (0%) and zero (0%) respectively. None were found on their skin, fins and caudal peduncle respectively. *Saccharomyces cerevisae* isolated from the slabs of the three locations were five (7%), six (13%) and zero (0%) respectively. For the gills, the order of occurrence was zero (0%), nine (11%) and zero (0%) respectively. The orders of occurrence on their skin were zero (0%), five (0.5%) and zero (0%) respectively. None were found on their skin, fins and caudal peduncle respectively.

Table2: Fungi isolated from slabs, gills, skins, fins and caudal peduncle of Titus (*Scomber scrombus)* obtained from Osere Fish Shop, Agbabiaka Fish Market and Sango Fish Shop

Fungi specie	Slab (% of fungi)			Gill (% of fungi)			Skin (% of fungi)			Fin (% of fungi)			Caudal peduncle (% of fungi)		
	Osere	Agbabiaka	Sango	Osere	Agbabiaka	Sango	Osere	Agbabiaka	Sango	Osere	Agbabiaka	Sango	Osere	Agbabiaka	Sango
Alternaria tenuis	4 (5%)	0 (0%)	0 (0%)	8 (9%)	3 (0.03%)	0 (0%)	2 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Aspergillus flavus	30 (40%)	13 (30%)	23 (35%)	27 (30%)	21 (26%)	26 (28%)	5 (5%)	3 (0.3%)	0 (0%)	0 (0%)	1 (1%)	0 (0%)	0 (0%)	1 (1%)	2 (2%)
Aspergillus fumigatus	0 (0%)	10 (23%)	15 (23%)	13 (15%)	27 (34%)	20 (22%)	0 (0%)	0 (0%)	1 (0.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Aspergillus niger	23 (31%)	0 (0%)	10 (15%)	10 (11%)	18 (23%)	23 (25%)	0 (0%)	1 (0.1%)	2 (0.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)
Neurospora crazza	5 (7%)	8 (18%)	15 (23%)	0 (0%)	0 (0%)	21 (23%)	0 (0%)	0 (0%)	5 (0.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Penicillium citrinum	8 (11%)	2 (0.04%)	0 (0%)	25 (28%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Rhizopus stolonifer	0 (0%)	4 (0.1%)	2 (3%)	5 (6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Saccharomyces cerevisae	5 (7%)	6 (13%)	0 (0%)	0 (0%)	9 (11%)	0 (0%)	0 (0%)	5 (0.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Photomicrographs of fungi isolated from the frozen Titus fish (*Scomber scombrus)*

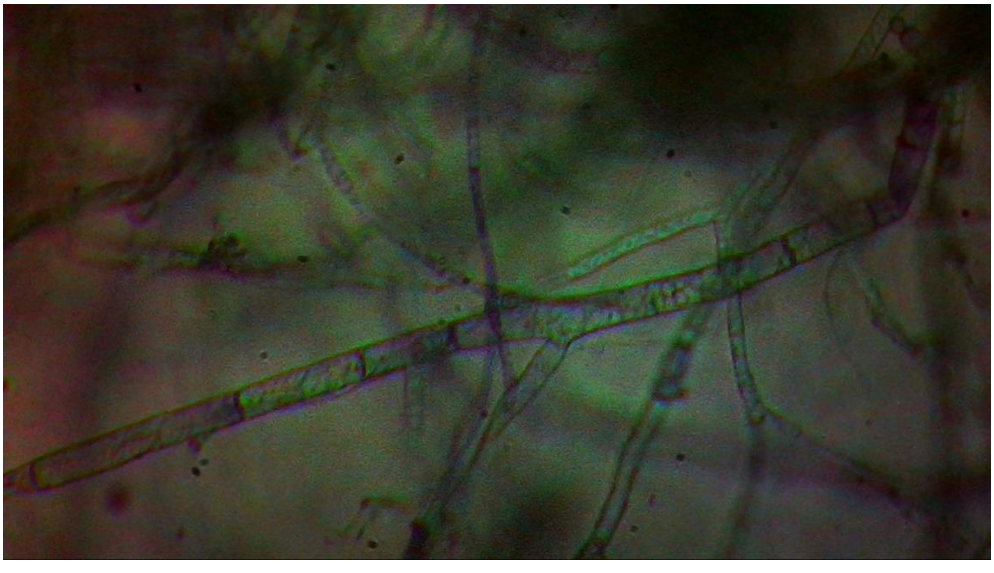

Plate 1: Photomicrograph of *Neurospora crazza* isolated from Titus (MG X100)

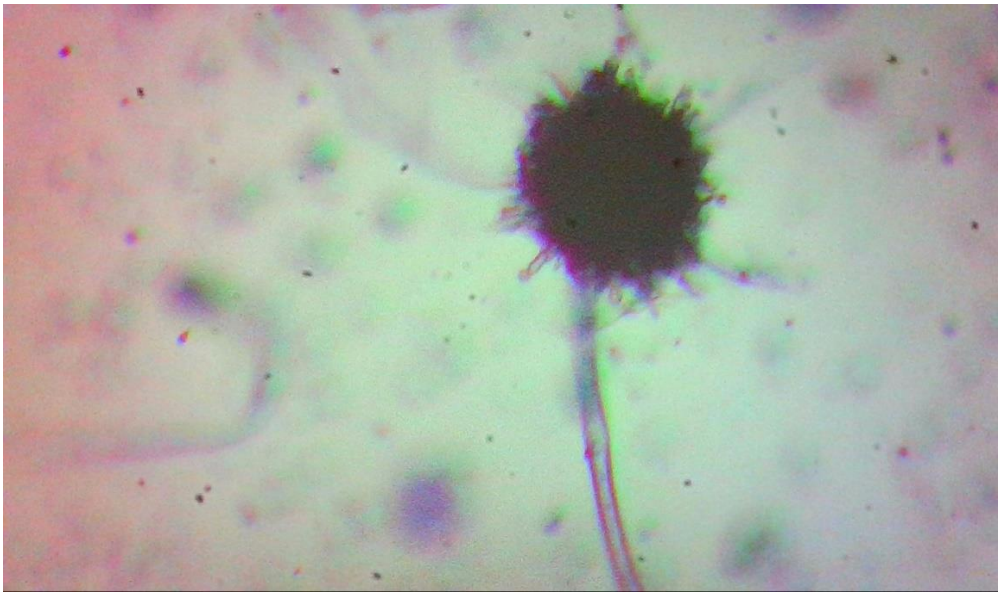

Plate 2: Photomicrograph of *Aspergillus niger* isolated from Titus (MG X 100)

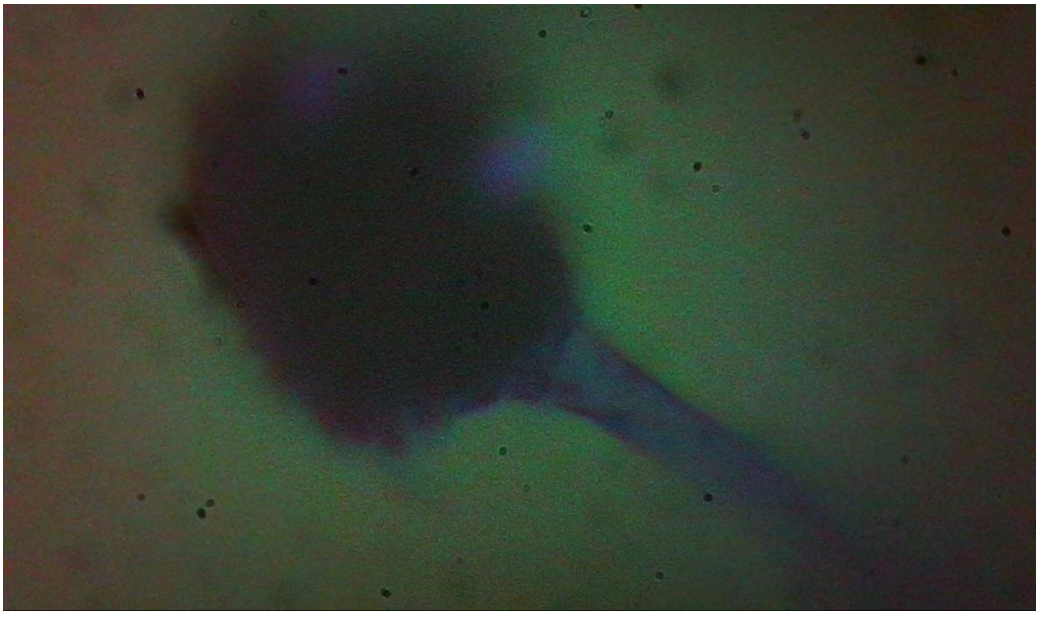

Plate 3: Photomicrograph of *Penicillium citrinum* isolated from Titus (MG X 100)

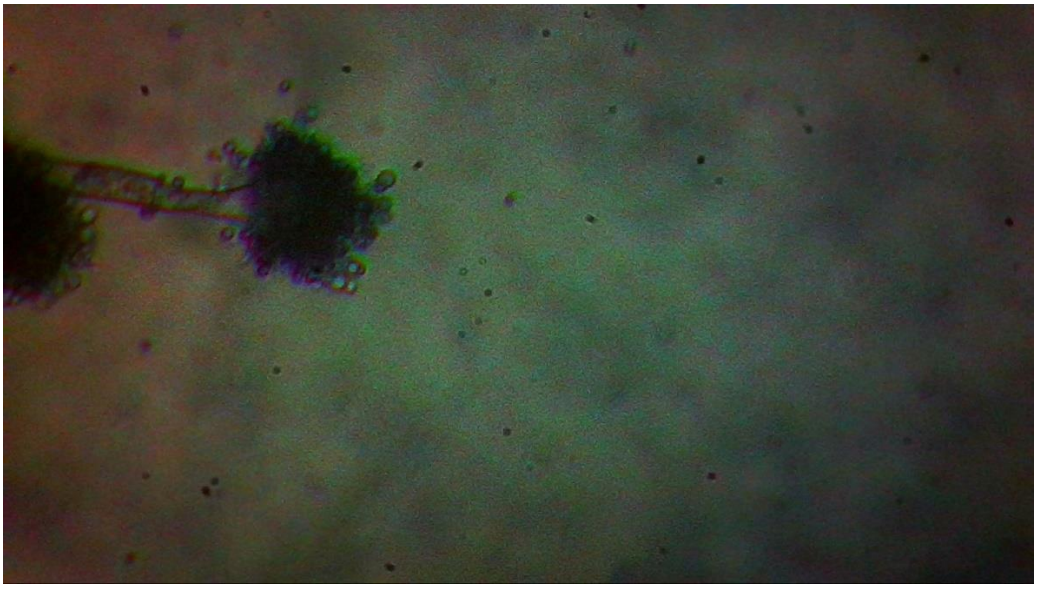

Plate 4: Photomicrograph of *Aspergillus fumigatus* isolated from Titus (MG X 100)

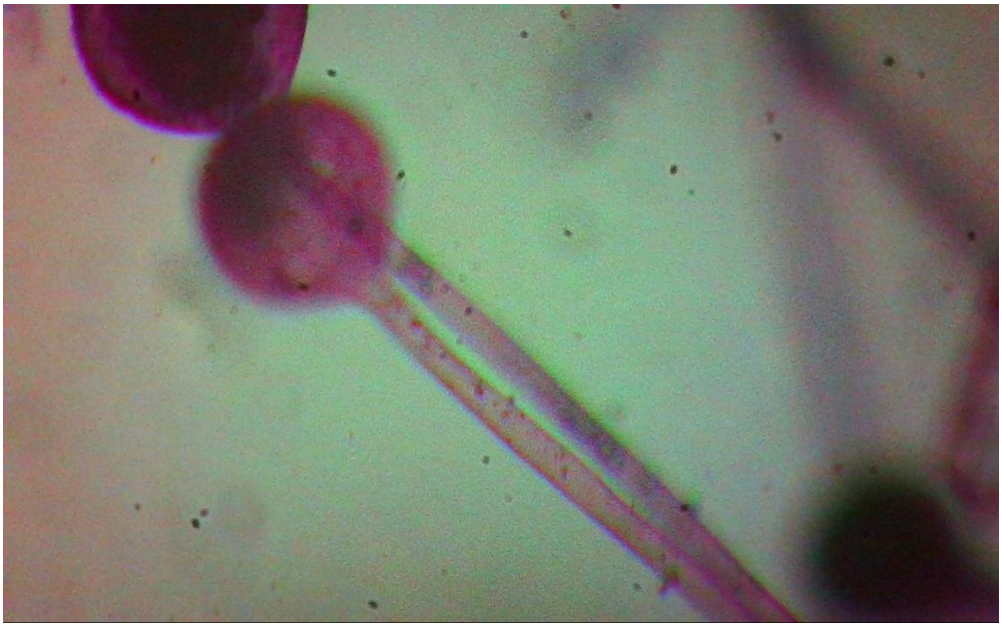

Plate 5: Photomicrograph of *Rhizopus stolonifer* isolated from the Slab (MG X 100)

DISCUSSION

The fungi isolated were *Alternaria tenuis, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Neurospora crazza, Penicillium citrinum, Rhizopus stolonifer and Saccharomyces cerevisae* with the gills carrying the highest number of fungi contamination followed by the slab. The skin, tail and fin carried the lowest number of fungi species. Samaha *et al*., (2015) reported higher yeast from Baca (64%) and Barbone (64%) respectively which is also similar to Kubo (2012) who recorded presence of fungi in 100% fish stock. The high percentage of fungi might be due low moisture content which makes it prone to fungi action (Holdsworth, 1971). This is also in agreement with the findings of Krska *et al*., (2008) who stated that moulds have the ability to survive in harsh conditions with low moisture content. This study also shows that among the eight species isolated:*Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Penicillium citrinum* were the dominant species. This result is in agreement with those recorded by Ibrahim, (2000) and Nasser, (2002) who isolated *Aspergillus niger* from most of their fish samples. Samaha *et al*., (2015) also reported Penicillium spp (48%) as predominant genera of mould from imported frozen barbone followed by *Cladosporium spp*. 8 (32%), *Aspergillus niger* 6 (24%), *Aspergillus flavus* 5 (20%), *Aspergillus fumigatus* 4(16%), *Fusarium and Alternaria alternate* 3 (12%), *Nigrosporium spp*.,*Rhizopus spp*. 2 (8%) and *Mucor spp*. 1(4%) . Samaha *et al*., (2015) also reported the predominance of *Penicillium spp* in frozen sardine as 11 (44%) followed by *Aspergillus niger* 8 (32%), *Aspergillus* flavus and *Nigrosporium spp*. 6 (24%), *Aspergillus fumigatus* 4 (16%), *Cladosporium spp*. and *Aspergellus Terrus* 2 (8%) and *Mucor spp*. 1 (4%). The predominant genera of the isolated mould from imported frozen Baca were *Penicillium spp*. 14 (56%) followed by *Aspergellus niger* 9 (36%), *Aspergillus flavus* 7

(28%), *Fusarium spp*. 6 (24%).*Mucor spp*. 5 (20%), *Alternaria alternate* 4 (16%),*Aspergillus fumigatus*, *Aspergillus terreus* and *Rhizopus spp*. 3 (12%), *Cladosporium spp*. and *Paecilomyces spp*. 2 (8%), *Aspergillus ochraceus* and *Nigrosporium spp*. 1 (4%). Sule *et al*., (2015) said that some fungi under certain environmental conditions release secondary metabolites known as mycotoxins. Mycotoxins are known to cause serious problems in humans and animals. Some of the major mycotoxigenic fungi are distributed among the genera Aspergillus and penicillium (Ismaiel and Papenbrock, 2015). Continuous exposure to food contaminated by mycotoxins can cause severe health hazards such as cancer, allergic reactions and organ damages (Zukiewicz *et al*., 2015; Wigmann *et al*., 2015). Most of the moulds and yeasts are capable of hydrolyzing a wide range of proteinaceous materials (El-Ahl, 2010; Awaad *et al*., 2011). Several strains of moulds particularly *Aspergillus flavus* isolated from different types of fish and fish feed were able to produce aflatoxins on Yeast Extract Sucrose medium, under the ideal experiment condition.

An increase in the frequency of fungal infections is related with progress in mycology and decreased susceptibility of fungal strains to commonly used antifungal agents as reported by Nowakowska *et al*., (2009). Moreover , the changes in treatment strategies and the increased use of antifungal prophylaxis (Lass- Flörl, 2009) and the lack of an effective fungicidal regimen as well as the development of antifungal resistant strains suggest that continued investigation is necessary to devise immuno therapeutic strategies and or drug targets to combat fungal infection (Wormly and Perfect , 2005) . Accordingly, alternatives to conventional antimicrobial therapy are needed due to the emergence of multi-drug resistance as reported by Van Vuuren *et al*., (2009). The initial quality of sea foods on board is affected by the species characteristics, the seasonal biological changes in the gonads and muscles, the culture conditions and fishing techniques. Until fish reaches the consumer, its quality attributes are prone to change under the impact of post- harvest handling, standard of hygiene during handling, storage and processing, environmental factors and parameters of applied preservation treatments (Nowakowska *et al*., (2009). Studies have shown that relatively high temperature and humid conditions majorly favors fungal to multiply and secretes mycotoxins (Atanda *et al*., 2007). Idi-Ogede and Tsadu (2003) isolated seven fungi species from gills and fins of *Clarias gariepinus* obtained from ponds and fish market in Minna, Niger State. Adedeji *et al*., (2012) isolated *Aspergillus fumigates, Aspergillus niger* and *Fusarium spp* respectively from fish *spp*

CONCLUSION

This study shows that frozen fish and cutting slab samples were highly contaminated with different species of mould and yeast. The detection of relatively high number of potential mycotoxigenic fungi in this study calls for improved sanitary, processing and storage facilities from frozen fish sellers and customers to prevent impending danger of ingesting toxins of fungi.

Regular environmental sanitation, good handling practices, proper storage temperatures and adequate thermal treatment of frozen fish should always be observed. Frozen fish processors/sellers should apply strict hygienic measures so as to prevent contamination of these frozen fish products. The cutting slabs should also be washed using sterile distilled water in order to prevent cross-contamination. Frozen fish processors should be educated on the adverse effect of using untreated water for processing as this could serve as sources of fungal growth. Importation of frozen fish should be disbanded by government.

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