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OCCURRENCE OF FUNGI IN FROZEN SHAWA Fish (*Clupea harangus*) SOLD IN SOME AREAS IN ILORIN METROPOLIS

Abubakar M.J.¹, Adeshina P.¹, Abdullahi A.M.¹, Hamza F.O.¹, Abdulraheem I.² and Musa A.M.³

¹Department of Aquaculture and Fisheries, Faculty of Agriculture, University of Ilorin, P.M.B. 1515.

²Department of Aquaculture and Fisheries Management Federal University of Agriculture, Abeokuta, Nigeria

³Department of Fisheries and Aquaculture, Faculty of Agriculture, Federal University, Gashua, Yobe State, Nigeria, P.M.B. 5006

Corresponding author: E-mail: a.midiog@yahoo.com, Tel: +2348036458262

ABSTRACT

A total number of 100 frozen fishes (*Clupea harangus*) 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. The frozen fish samples were transported in a sterile ice-packed 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 and samples 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 Shawa fishes. The isolated fungi were *Alternaria tenuis*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Neurospora crassa*, *Rhizopus stolonifer*, *Penicillium citrinum* and *Saccharomyces cerevisiae* with *Aspergillus spp* having the highest number. It was concluded that large occurrence of *Aspergillus spp* in the sampled frozen fish is risk to human health. The study therefore recommended proper processing and storage of frozen fish.

Keywords: *Clupea harangus*; fish; frozen; fungi and Occurrence

1.0 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 (Andrew, 2001). Fish is known to be rich in vitamins B₁₂ and B₆. It is

also a good source of fluorine and iodine which are needed for strong teeth development and for prevention of goiter 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

attract customers and to avoid the health risk associated with poor or spoiled 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 colonization and replication of microorganisms, decay occurs and the consumption becomes dangerous (Mal and Memon, 2011; Alparvian *et al.*, 2014). The quality and freshness of fish are rapidly deteriorated through microbial and biochemical mechanisms (Allaoui *et al.*, 2014).

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 (2003), most fish related food borne illness are traced to *Salmonella*, *Staphylococcus* spp., *Escherichia* spp., *Vibrio parahaemolyticus*, *Clostridium perfringens*, *Clostridium botulinum* E, and *Enteroviruses* (Yaqoub, 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 $< 10^{\circ}\text{C}$, thus they can multiply on fish even when refrigerated. Clostridia include all aerobic and facultative anaerobic gram negative non spore forming bacilli which ferment lactose with gas formation within 48 hours at 35°C . In the lab, *S. 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 193 validly described species (Euzebv, 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 causes 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.

Water availability is considered more important in reducing 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). 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 (*Clupea harengus*) sold in some areas in Ilorin metropolis.

2.0 MATERIALS AND METHODS

2.1 The Study Area

The study was carried out in Ilorin, the capital of Kwara State. Ilorin is located on latitude 8°30' and 8°50'N and longitude 4°20' and 4°35'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 °C, May 27.5 °C and September 22.5 °C (Kwara State Government, 2017).

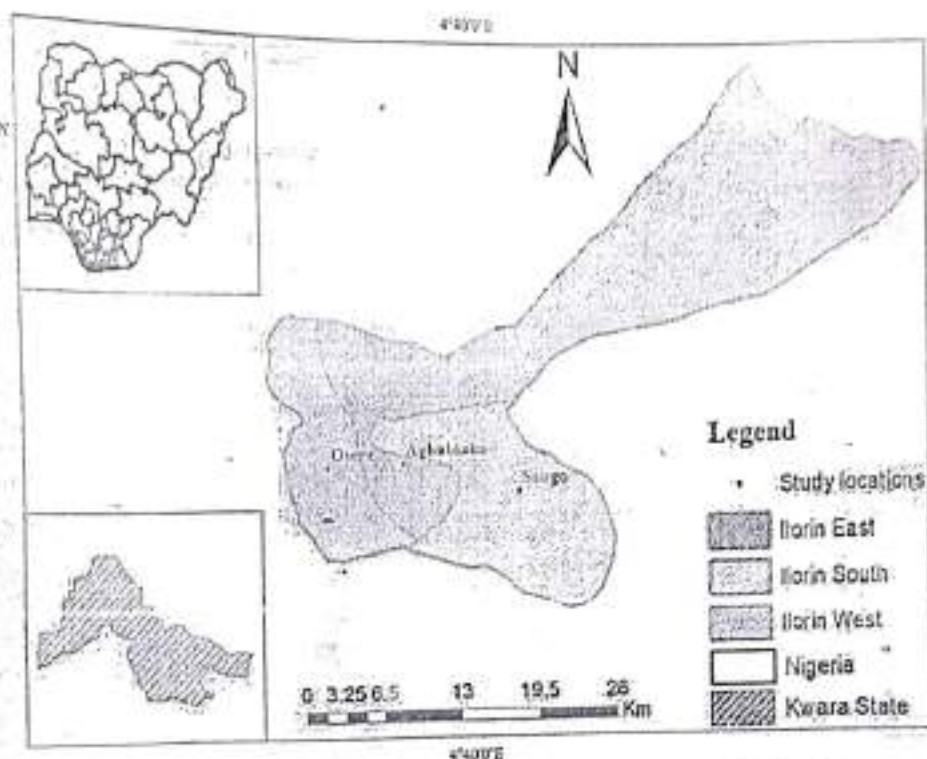


Figure 1.0 Map of Ilorin showing the site of fish collection (*)

2.1 Collection of the Fish Samples

The frozen Shawa fishes sold within Ilorin metropolis usually undergo proper inspection before being certified for human consumption. Samples of the Shawa 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 (Shawa: *Clupea harangus*) were purchased from three different locations (Osere, Agbabiaka and Sango). The plastic bags used in packing the frozen shawa fishes were sterilized using 70% ethanol. The frozen fish samples were transported in a sterile ice-packed 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.

2.2 Treatment of Samples

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

2.2.1 Sterilization of the material 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 (MIME-type) at 160°C for 1hour

2.3 Preparations of Culture Media

The media used for the analysis are

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

2.3.1 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°C before pouring into plates. This medium is very useful for growing large variety of fungi.

2.3.2 Yeast extract agar

23g 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 aluminum foil. The media was sterilized in the autoclave at a temperature 121°C for 15minutes and left to cool to about 45°C before pouring aseptically into sterile petri dishes (Chessbrough 2006).

2.4 Serial Dilution Technique

Serial dilution was used for the enumeration of fungi count. Using pipettes, serial dilution of the fungi isolates were 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 = 10^{-2} dilution

Tube 3: 1ml of tube 2 + 9ml sterile water = 10^{-3} dilution

Tube 4: 1ml of tube 3 + 9ml sterile water = 10^{-4} dilution

Tube 5: 1ml of tube 4 + 9ml sterile water = 10^{-5} dilution

Tube 6: 1ml of tube 5 + 9ml sterile water = 10^{-6} dilution

Tube 7: 1ml of tube 6 + 9ml sterile water = 10^{-7} dilution

Tube 8: 1ml of tube 7 + 9ml sterile water = 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 hemacytometer.

2.5 Identification and Characterization

The fungi isolated were identified and characterized based on their surface texture, pigmentation and undersurface texture using the manual of Noga (1995). Several sub-

Examination were carried out. After which the fungal isolates were examined under low power objective microscope (x40)

2.6 Statistical Analysis

Data collected were processed and analyzed on the computer using computer statistical package SPSS (version 16). Photomicrographs were processed and obtained using Digital Microscope with USB connected to a computer screen to record,

view and take the pictures of the fungi directly.

3.0 RESULTS

Results of the clinical examination and photomicrographs of different fungi isolated are explained herein: Identification and characterization of fungal isolates from frozen Shawa (*Clupea harangus*) are shown in Table 1 below:

Table 1.0 Identification and characterization of fungal isolates from frozen Shawa (*Clupea harangus*)

FUNGI ISOLATES	SURFACE TEXTURE	MACROCULTURE	UNDER-SURFACE TEXTURE	TENTATIVE IDENTIFICATION
F1	Leathery	Grayish black	Creamy	<i>Alternaria tenuis</i>
F2	Powdery	White-yellow	Creamy	<i>Aspergillus flavus</i>
F3	Powdery	Grey- green	Creamy	<i>Aspergillus fumigatus</i>
F4	Powdery	Black	Creamy	<i>Aspergillus niger</i>
F5	Powdery	Pink	Creamy	<i>Neurospora crassa</i>
F6	Powdery	Greenish-blue with narrow margin	Creamy	<i>Penicillium citrinum</i>
F7	Soft smooth	Creamy	Creamy	<i>Saccharomyces cerevisiae</i>

The fungi isolated and observed at the three locations are presented in Tables 2, 3, 4, 5, 6 and 7 respectively.

Table 2.0 Fungi isolated from the slab and Shawa obtained from Osere fish shop

FUNGI ISOLATE	GILLS	(%)	SKIN	(%)	FIN	TAIL
<i>Alternaria tenuis</i>	12	14%	0	0%	0	0
<i>Aspergillus flavus</i>	24	27%	1	0.1%	1	1
<i>Aspergillus fumigatus</i>	15	18%	5	0.8%	0	0
<i>Aspergillus niger</i>	27	33%	0	0%	0	2
<i>Neurospora crassa</i>	0	0%	0	0%	0	0
<i>Penicillium citrinum</i>	3	4%	0	0%	0	0
<i>Rhizopus stolonifer</i>	0	0%	0	0%	0	0
<i>Saccharomyces cerevisiae</i>	0	0%	00%		0	0

Tables 2 indicated that 14% *Alternaria tenuis* were present on the gills, none on the skin,

none on the fin and none on the tail. 27% *Aspergillus flavus* were found on the gills,



0.1% on the skin, 1 on the fin and 1 on the tail. 18% *Aspergillus fumigatus* isolates were present on the gills, 0.8% on the skin, none on the fin and none on the tail. 33% *Aspergillus niger* were found on the gills, none on the fin and tail. No *Neurospora*

crazza isolate was present in the fish's body parts. There were absence of *Rhizopus stolonifer* and *Saccharomyces cerevisiae* isolates on the fish.

Table 3: Fungi isolated from the Slab and Shawa obtained from Agbabiaka Market

Table 3.0 Shows that no *Alternaria tenuis* isolate was present on the slab, but 0.03% was found on

FUNGI ISOLATE	SLAB	(%)	GILLS	(%)	SKIN	(%)	FIN	TAIL
<i>Alternaria tenuis</i>	0	0%	3	0.03%	0	0%	0	0
<i>Aspergillus flavus</i>	13	30%	21	26%	3	0.3%	1	1
<i>Aspergillus fumigatus</i>	10	23%	27	34%	0	0%	0	0
<i>Aspergillus niger</i>	0	0%	18	23%	1	0.1%	0	0
<i>Neurospora crassa</i>	8	18%	0	0%	0	0%	0	0
<i>Penicillium citrinum</i>	2	0.04%	0	0%	0	0%	0	0
<i>Rhizopus stolonifer</i>	4	0.1%	0	0%	0	0%	0	0
<i>Saccharomyces cerevisiae</i>	6	13%	9	11%	5	0.5%	0	0

On the gills, none on the skin, none on fin and none on tail. 30% *Aspergillus flavus* were found on the slab, 26% on the gills, 0.3% on the skin and 1 each on the fin and tail. 23% *Aspergillus fumigatus* were present on the slab, 34% on the gill, none on the skin,

none on the fin and none on the tail. About 23% *Aspergillus niger* was found in the gills. 18% *Neurospora crassa* isolates were present on the slab, 13% *Saccharomyces cerevisiae* were found on the slab, 11% from the gill, 0.5% on the skin and none on the fin and tail.

Table 4.0 Fungi isolate from Shawa obtained from Sango Fish shop

FUNGI ISOLATE	GILLS	(%)	SKIN	(%)	FIN	TAIL
<i>Alternaria tenuis</i>	0	0%	0	0%	0	0
<i>Aspergillus flavus</i>	26	36%	4 0.2%		0	0
<i>Aspergillus fumigatus</i>	0	0%	0	0%	0	0
<i>Aspergillus niger</i>	30	41%	5	0.3%	0	0
<i>Neurospora crassa</i>	0	0%	0	0%	0	1
<i>Penicillium citrinum</i>	5	7%	0	0%	0	0
<i>Rhizopus stolonifer</i>	6	8%	5	0.3%	0	0
<i>Saccharomyces cerevisiae</i>	5	7%	0	0%	0	1

Table 4 Revealed that *Alternaria tenuis* isolate was absent in all of the fish's body parts. 36% *Aspergillus flavus* were present on the gills, 0.2% on the skin, none on the fin and none on the tail. 41% *Aspergillus niger* were found on the gill, 0.3% on the skin, none on the fin and none on the tail too. About 7% *Saccharomyces cerevisiae* isolates

were present on the gills, none on the skin, none on the fin and 1 on the tail. 8% *Rhizopus stolonifer*, 0.3% was on the skin, none on the fin and none on the tail. 7% *Penicillium citrinum* isolates were also found on the gills, none on skin, none on the fin and none on the tail.



Table 5.0 Comparison of fungi isolated from the slabs in the three locations

FUNGI ISOLATE	SLAB A (OSERE FISH SHOP)	SLAB B (AGBABLAKE MARKET)	SLAB C (SANGO FISH SHOP)
<i>Alternaria tenuis</i>	5%	0%	0%
<i>Aspergillus flavus</i>	30	13	23
<i>Aspergillus fumigatus</i>	0	10	15
<i>Aspergillus niger</i>	23	0	10
<i>Neurospora crassa</i>	5	8	15
<i>Penicillium citrinum</i>	8	2	0
<i>Rhizopus stolonifer</i>	0	4	2
<i>Saccharomyces cerevisiae</i>	5	6	0

Table 5 shows that slab A has 5% *Alternaria tenuis*, 40% *Aspergillus flavus*, 35% *Aspergillus niger*, 7% *Neurospora crassa*, 11% *Penicillium citrinum* and 7% *Saccharomyces cerevisiae*. Slab B has 30% *Aspergillus flavus*, 23% *Aspergillus fumigatus*, 18% *Neurospora crassa*,

0.04% *Penicillium citrinum*, 0.1% *Rhizopus stolonifer* and 13% *Saccharomyces cerevisiae*. Slab C has 35% *Aspergillus flavus*, 23% *Aspergillus fumigatus*, 15% *Aspergillus niger*, 13% *Neurospora crassa* and 3% *Rhizopus stolonifer*.

Table 6.0 comparison of fungi isolates from the gills of Shawa from the three locations

FUNGI ISOLATES	LOCATION A (%)	LOCATION B (%)	LOCATION C (%)
<i>Alternaria tenuis</i>	12	0	14%
<i>Aspergillus flavus</i>	24	26	5%
<i>Aspergillus fumigatus</i>	15	0	22%
<i>Aspergillus niger</i>	27	30	23%
<i>Neurospora crassa</i>	0	0	16%
<i>Penicillium citrinum</i>	3	5	5%
<i>Rhizopus stolonifer</i>	0	6	13%
<i>Saccharomyces cerevisiae</i>	0	5	2%

Table 6 revealed that the gills of Shawa from location A have 14% *Alternaria tenuis*, 27% *Aspergillus flavus*, 18% *Aspergillus fumigatus*, 33% *Aspergillus niger*, zero *Neurospora crassa*, 4% *Penicillium citrinum*,

zero *Rhizopus stolonifer* and zero *Saccharomyces cerevisiae*. Gills of Shawa from location B have zero *Alternaria tenuis*, 36% *Aspergillus flavus*, zero *Aspergillus fumigatus*, 41% *Aspergillus niger*

zero *Neurospora crassa*, 7% *Penicillium citrinum*, 8% *Rhizopus stolonifer* and 2% *Saccharomyces cerevisiae*. Gills of Shawa from location C have 14% *Alternaria tenuis*, 5% *Aspergillus flavus*, 22% *Aspergillus*

fumigatus, 23% *Aspergillus niger*, 16% *Neurospora crassa*, 5% *Penicillium citrinum*, 2% *Saccharomyces cerevisiae* and 13% *Rhizopus stolonifer*.

Table 7.0 Comparison of fungi isolates from the skin of Shawa from the three locations

FUNGI ISOLATES	LOCATION A		LOCATION B	LOCATION C
	(%)	(%)	(%)	(%)
<i>Alternaria tenuis</i>	2%	0%	0%	0%
<i>Aspergillus flavus</i>	5%	0.3%	0%	0%
<i>Aspergillus fumigatus</i>	0	0%	0%	0.1%
<i>Aspergillus niger</i>	0	0.1%	0%	0.3%
<i>Neurospora crassa</i>	0	0%	0%	0.6%
<i>Penicillium citrinum</i>	0	0%	0%	0%
<i>Rhizopus stolonifer</i>	0	0%	0%	0%
<i>Saccharomyces cerevisiae</i>	0	0.5%	0%	0%

Table 7 revealed that the skin of Shawa from location A has 2 *Alternaria tenuis*, 5% *Aspergillus flavus* while none were recorded for *Aspergillus fumigatus*, *Aspergillus niger*, *Neurospora crassa*, *Penicillium citrinum*, *Rhizopus stolonifer* *Saccharomyces cerevisiae* respectively. Skin of Shawa from location B, had none *Alternaria tenuis*, 0.3% *Aspergillus flavus*, zero *Aspergillus fumigatus*, 0.1% *Aspergillus*

niger, zero *Neurospora crassa*, zero *Penicillium citrinum*, zero *Rhizopus stolonifer* and 0.5% *Saccharomyces cerevisiae* respectively. Skin of Shawa from location C had none *Alternaria tenuis*, zero *Aspergillus flavus*, 0.1% *Aspergillus fumigatus*, 0.3% *Aspergillus niger*, 0.6% *Neurospora crassa*, zero *Penicillium citrinum*, zero *Saccharomyces cerevisiae* and zero *Rhizopus stolonifer* respectively.



Neurospora crassa, zero *Penicillium citrinum*, zero *Rhizopus stolonifer* and zero *Saccharomyces cerevisiae*. Tail of Shawna from location B have zero *Alternaria tenuis*, 1 *Aspergillus flavus*, zero *Aspergillus fumigatus*, zero *Aspergillus niger*, 1 *Neurospora crassa*, zero *Penicillium citrinum*, zero *Rhizopus stolonifer* and 1

Saccharomyces cerevisiae. Tail of Shawna from location C has zero *Alternaria tenuis*, 2 *Aspergillus flavus*, zero *Aspergillus fumigatus*, 3 *Aspergillus niger*, 8 *Neurospora crassa*, zero *Penicillium citrinum*, zero *Saccharomyces cerevisiae* and zero *Rhizopus stolonifer*.

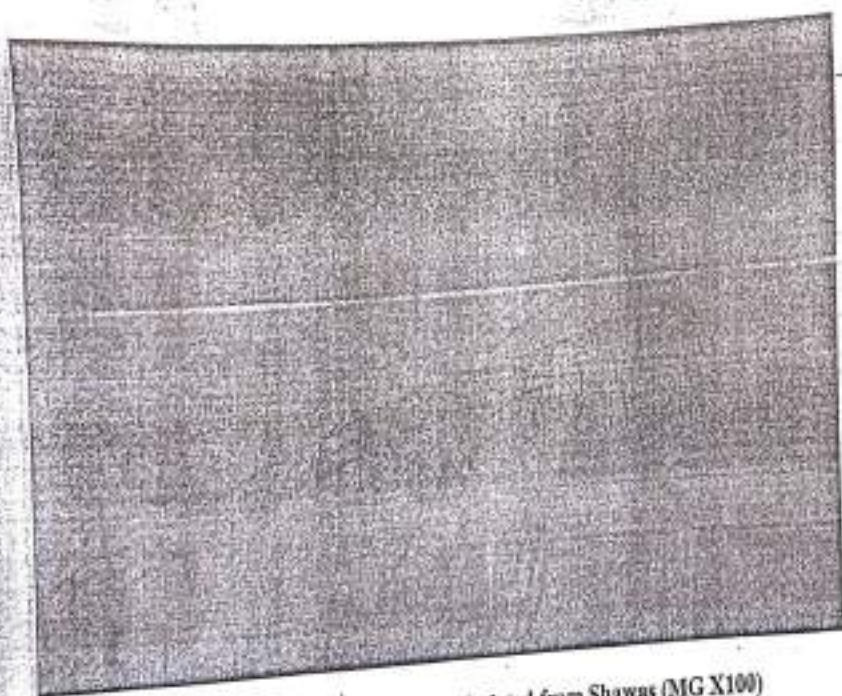


Plate 1.0 Photomicrograph of *Neurospora crassa* isolated from Shawna (MG X100)

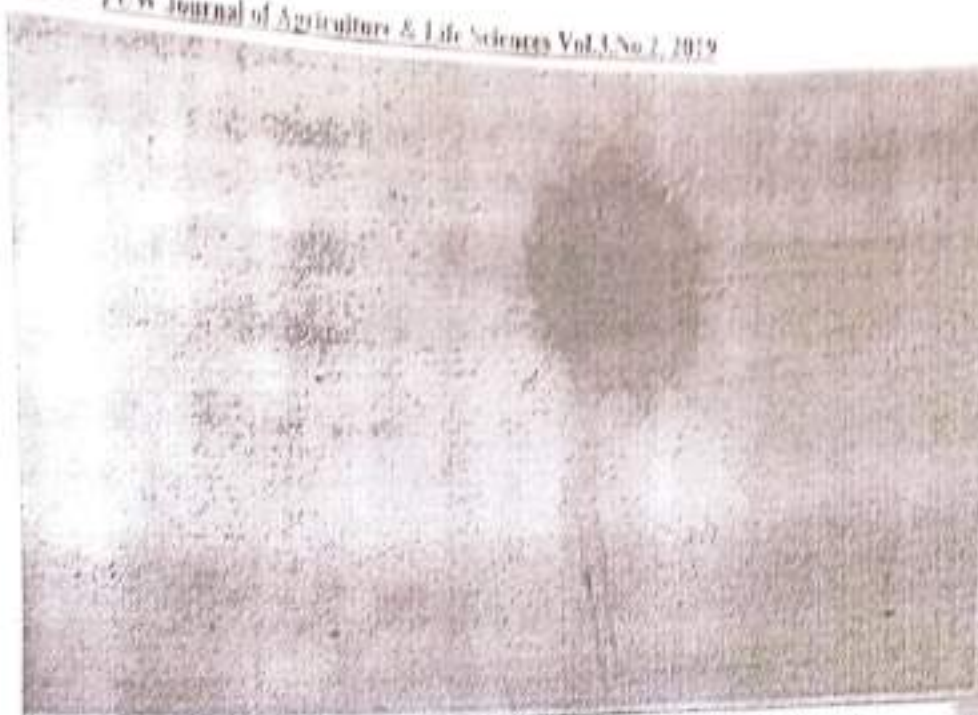


Plate 2.0 Photomicrograph of *Aspergillus niger* isolated from Shawa (MG X 100)

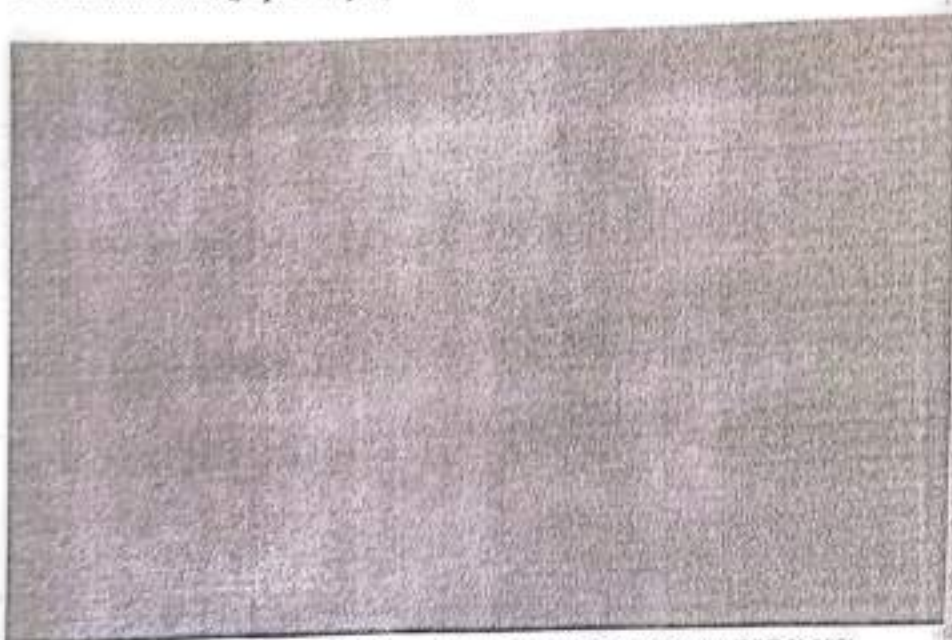


Plate 3: Photomicrograph of *Penicillium citrinum* isolated from Shawa (MG X 100)

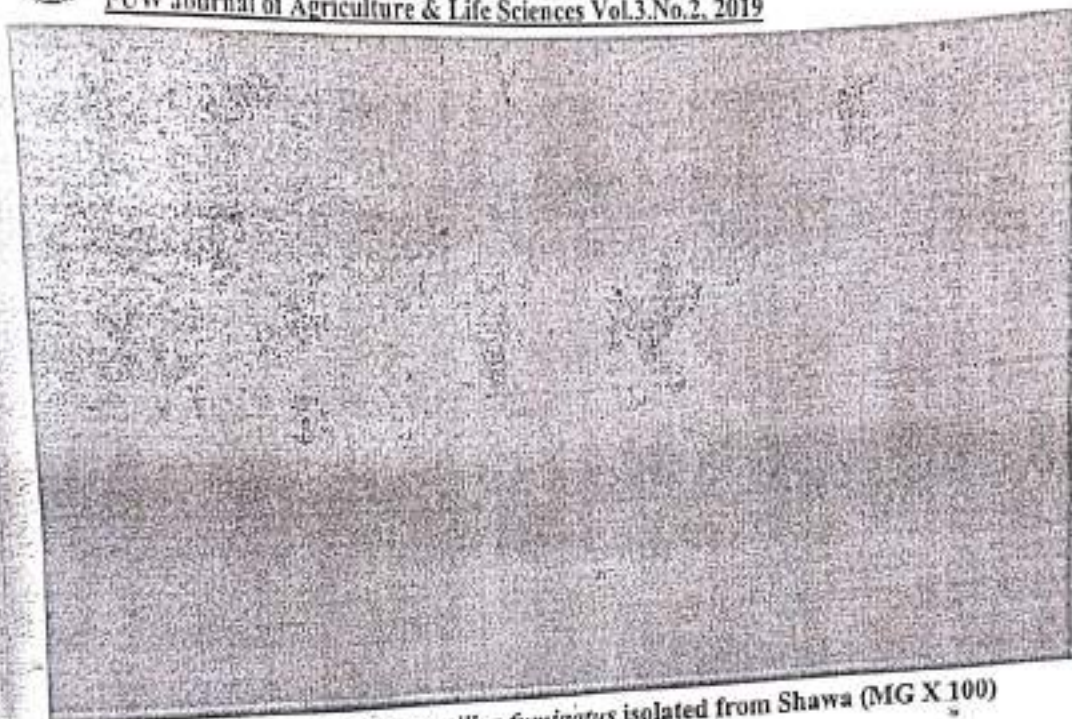


Plate 4.0 Photomicrograph of *Aspergillus fumigatus* isolated from Shawa (MG X 100)

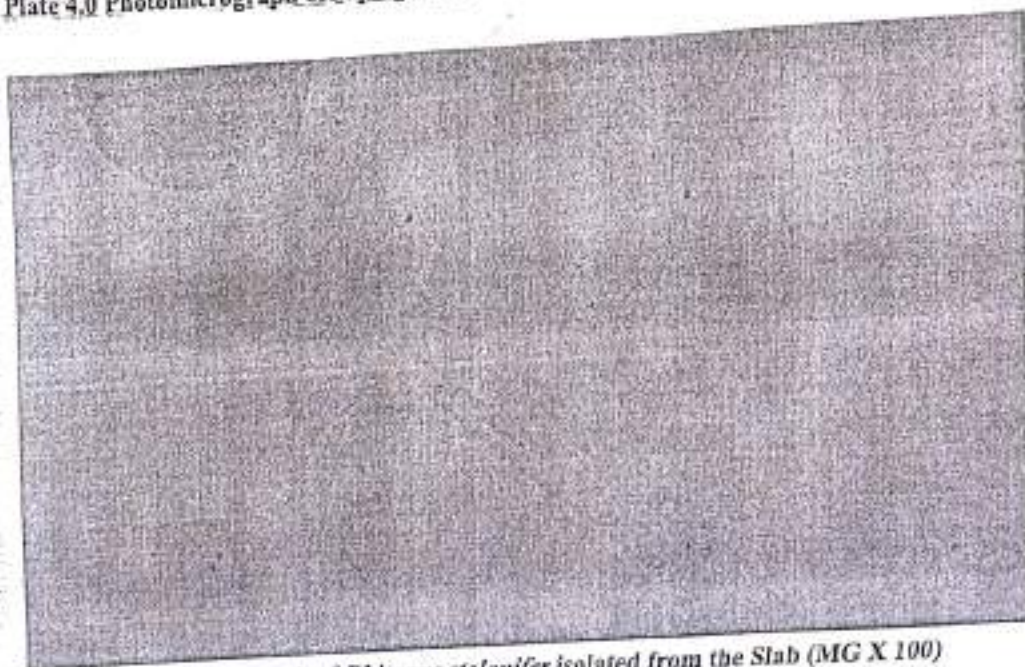


Plate 5.0 Photomicrograph of *Rhizopus stolonifer* isolated from the Slab (MG X 100)



3.1 DISCUSSION

The fungi isolated include *Alternaria tenuis*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Neurospora crassa*, *Penicillium citrinum*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae* 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. This is also in agreement with the findings of Kraka *et al.*, (2008) who stated that moulds have the ability to survive in harsh conditions with low moisture content. This study also showed 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 *Aspergillus 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 *Aspergillus niger* 9 (36%), *Aspergillus fumigatus* 7 (28%), *Fusarium* spp. 6 (24%), *Mucor* spp. 5 (20%), *Alternaria alternate* 4 (16%), *Aspergillus fumigatus*, *Aspergillus terrus* 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* (Ismail 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; Wignmann *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

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).

4.0 CONCLUSION

This study showed 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 called for improved sanitary, processing and storage facilities from frozen fish sellers and customers to prevent impending danger of ingesting toxins of fungi. The study recommended:

- 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.

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