



STUDY OF FUNGAL ORGANISMS ASSOCIATED WITH SMOKED AND DRIED FISH SOLD IN AWKA MARKETS, ANAMBRA STATE

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ABSTRACT: *Fungal contamination of fish is considered as the major cause of spoilage and it constitutes a significant public health problem. This study was carried out to determine the fungal organisms associated with smoked and dried fish samples: *Scomber scombrus* (Mackerel), *Sardinella longiceps* (Sardine), *Oreochromis niloticus* (Tilapia), *Clarias gariepinus* (catfish), *Gadus morhua* (Stock fish), *Ethmalosa fimbriata* (Bonga fish), and *Citharinus citharus* (Mangala) sold in Awka markets, Anambra State, Nigeria. A total of fifty (50) (25 smoked and 25 dried) fishes were obtained from three markets in Awka and examined microbiologically for viable fungal infestation using Potato Dextrose Agar (PDA) media. A total of 121 fungal organisms were isolated from 25 smoked and 25 dried fish samples and identified. The isolated fungi were *Mucor* spp, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* spp, *Fusarium* spp and *Rhizopus* spp. It was observed that all the fish samples harboured multiple fungal organisms. *Aspergillus niger* was the fungus with the highest mean fungal count of 9.00 ± 5.568 , followed by *Mucor* spp (8.00 ± 1.732), while *Aspergillus flavus* was the lowest (3.67 ± 1.528). In the dried fish samples, *Gardus morhua* (stock fish) had the highest infestation with fungi (1.08 ± 1.083), while in smoked fish, *Clarias gariepinus* (catfish) had the highest infestation. The high level of fungal contamination can be attributed to poor handling of fish and the environment. Considering the danger to public health, food and safety authorities should intensify their monitoring efforts towards controlling fish contamination, and fish sellers should also be sensitized on hygienic fish handling techniques.*

KEYWORDS: Fungi, Smoked fish, Dried fish, Spoilage, PDA Media, Awka, Anambra.



INTRODUCTION

Fish is a highly nutritious food and an important source of high-quality protein; it constitutes a vital portion of man's diet especially in low-income regions of the world where it represents 15-20% of all animal protein (Benedict *et al.*, 2023; Ojutiku *et al.*, 2009). In Nigeria, fish has an edge over other meat sources due to its affordability and relative abundance. In West Africa, fish has been reported to provide 40-70% of the protein intake of the population and is a critical source of dietary protein that is not readily available in carbohydrate based staple foods (Ikutegbe & Sikoki, 2014). Fish is a rich source of lysine, vitamins A, B, and E, oils containing polyunsaturated fatty acids and iodine (Ike *et al.*, 2020).

According to FAO (2016b), worldwide fish consumption increased from 9.9 kg per capita to 20.5 kg per capita between 1960 and 2018. However, the very high perishability of fish, due to high ambient temperature, requires preservation immediately after harvest. Fish products are one of the globe's most perishable food items due to microbial contamination. It has been reported that about one third of the world's food production is lost yearly due to microbial spoilage (Daramola *et al.*, 2020). Over the years, several post-harvest processing and preservation techniques such as drying, salting, smoking, freezing, and canning are common scenery and have been utilized both in Nigeria and different parts of the world. In Nigeria, drying and smoking are the most popular methods and are famed for their ability to give the fish a delicious aroma, taste, lowered pH value and a longer shelf life through its anti-bacterial and oxidative effect, influencing desired colouration as well as accelerating the drying process and serving as an antagonist to spoilage agents (Ngo-oum *et al.*, 2021). Smoking is the traditional fish processing technique since more advanced preservation methods, such as refrigeration, are mostly not affordable in remote fishing communities where the majority of the fishing activities are carried out. Improper smoking and drying of fish greatly impacts consumers' health negatively. It has been observed that the majority of post processing microbial contaminants arise from poor handling and processing practices, while some could be from the air, water bodies or other degrading substances (Ayuba *et al.*, 2013; Oparaku & Mgbemena 2012). Very dry smoked fish having medium to high moisture contents in humid tropical conditions is predisposed to both fungal and bacterial contaminations (Akwuobu *et al.*, 2019). Spoilage of fresh and highly preserved fish products is mostly caused by microbes such as fungi (Uzugbu & Eke 2000).

Fungal contamination is a major problem confronting fish farmers and consumers alike in Nigeria and Africa in general. The numerous fungi species implicated in fish spoilage are also a serious threat to consumers' health due to their ability to produce mycotoxins. Mycotoxins, particularly aflatoxins, are major food contaminants globally (John *et al.*, 2020; Fallah *et al.*, 2014). Aflatoxins are secondary metabolites which are highly toxic, mutagenic and carcinogenic, and they possess the ability to survive from a heating period. Many fungi species such as *Aspergillus flavus*, *Aspergillus terens*, *Aspergillus fumigatus*, *Aspergillus niger*, *Mucor* sp, *Penicillium* sp, *Cladosporium* sp, *Candida tropicalis* and *Fusarium moniliformis* have been reported in smoked fish sold in Nigeria (Fafioye *et al.*, 2002). In Awka, the handling, processing, transportation, storage and poor sanitary conditions of the markets have exposed fish and fish products to various contaminants resulting in spoilage. Despite the series of reports on fungal spoilage of smoked and dried fish in Nigeria and its public health implications, mycological studies of smoked and dried fish in Awka remains grossly under-reported. Hence, this study was carried out to ascertain the different types of fungal pathogens associated with



spoilage of smoked and dried fish sold in open markets in Awka, Anambra State, Southeast Nigeria.

MATERIALS AND METHOD

Study Area

The study was conducted in three (3) different markets situated in Awka metropolis. Awka is the capital of Anambra State and has a population of 371,038 inhabitants as of the 2006 population census. Located in the Southeast geopolitical zone in Nigeria, the city lies within latitude 6° 12'45.68" N and longitude 7° 04'19.16" E. The area is characterized by eight (8) months of rainfall usually from March to October and four (4) months of dryness from November to February yearly. The city experiences varying temperature degrees of 27-30°C between June and December and 32-34°C between January annually (Ishar *et al.*, 2024).

Sample Collection

Twenty-five (25) smoked and twenty-five (25) dried fish samples were randomly purchased from market sellers situated in Eke Awka, Nkwo Amaenyi and Ifite first markets respectively and kept inside a sterile nylon and transported to the Zoology laboratory of Nnamdi Azikiwe University, Awka for microbiological analysis.

Media Preparation

All fish samples were surface sterilized with 70% ethanol. About 10 g tissue portion of each fish species was scraped from the abdominal region, head and tail with a sterile forcep and mixed with 200 ml of distilled water. Potato dextrose agar (PDA) supplemented with streptomycin to inhibit bacterial growth was then used as medium for the enumeration and isolation of fungi. The media was autoclaved at 121°C for 15 minutes and allowed to cool. About 20 ml of the cooled media was poured into different sterilized petri dishes and labelled as S1-S25 and D1-D25.

Serial Dilutions

Ten folds of serial dilution were carried out. About 1 ml of the fish tissue sample was dispensed into 9 ml of distilled water and mixed thoroughly. From each sample, 0.1 ml of the dilution was used to inoculate the solid media and the plates were incubated at 37°C for 7 days. The cultures were examined for growth at regular intervals. All observed colonies after 7 days were subcultured to obtain pure cultures. The pure cultures were then isolated and identified using morphological characteristics like spore formation, production of fruiting bodies and biochemical reactions. Mycology atlas was used to assist in the identification of colonies.

Identification of Fungi Organisms

For easier identification of the fungal organisms, Lactophenol Cotton Blue Staining (Needle Mounts Preparation) was done. A drop of lactophenol cotton blue stain was placed on a clean grease-free glass slide. With the aid of a sterile straight wire, a small fragment of colony was picked at mid-point of culture and teased in the stain until a homogenous blue mixture of stain and culture was obtained. The slides were then covered with a cover slip and examined using



x10 and x40 objectives. With the aid of a wire loop, a thin smear of the isolates was collected and stored into bottle slants or Bijou bottles for storage and future use.

Statistical Analysis

Data obtained from the study was analyzed using SPSS version 21. Paired T-test was used to compare percentage occurrence between the study locations. ANOVA was also used to determine the difference between means.

RESULTS

The results of the abundance of fungal organisms in the study locations revealed the presence of six (6) fungal organisms, namely *Mucor spp*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium spp*, *Fusarium spp* and *Rhizopus spp*. From these, *Mucor spp* (10), *A. niger* (15), *Penicillium spp* (8) and *Fusarium spp* (9) were the most abundant in Eke Awka market while *A. flavus* were the most abundant in Nkwo Amaenyi market (Table 1). The results of the mean fungal count of smoked and dried fish sampled in three markets in Awka showed that *Aspergillus niger* had the highest mean fungal count (9.00 ± 5.568) followed by *Mucor sp* (8.00 ± 1.732), the least being *Aspergillus flavus* (3.67 ± 1.528). There was no significant difference ($P > 0.05$) in the mean fungal count of the smoked and dried fish in the three markets sampled (Table 2). In Table 3 presented below, the result revealed that mean fungal count was highest in Eke Awka (7.67 ± 5.164) followed by Nkwo Amaenyi (6.67 ± 2.582) and lastly, Ifite first market (5.83 ± 2.639). There was no significant difference in the mean fungal count between the three locations sampled ($P > 0.05$).

Table 1: Abundance of fungal organisms in smoked and dried fish sampled in the three markets in Awka

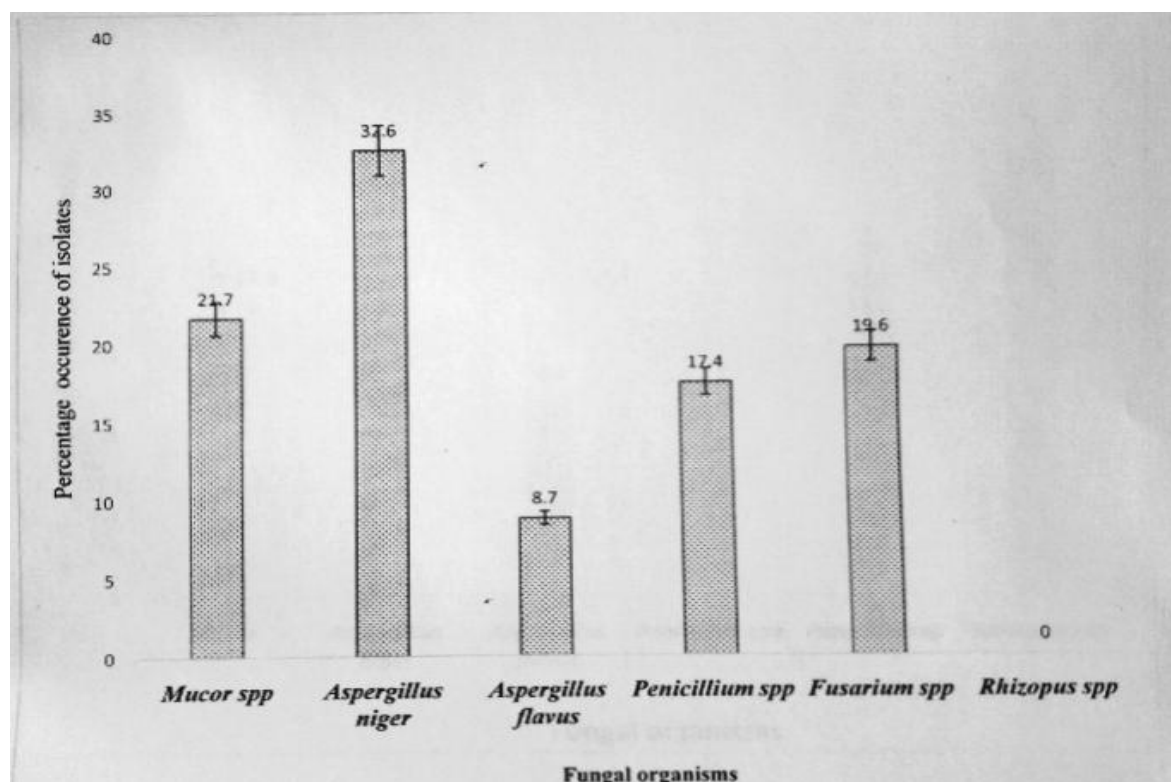
Organisms	Eke Awka	Nkwo Amaenyi	Ifite market	first	Total
<i>Mucor spp</i>	10	7	7		24
<i>Aspergillus niger</i>	15	4	8		27
<i>Aspergillus flavus</i>	4	5	2		11
<i>Penicillium spp</i>	8	5	5		18
<i>Fusarium spp</i>	9	8	4		21
<i>Rhizopus spp</i>	0	11	9		20
Total	46	40	35		121

Table 2: Mean fungal count of the isolates obtained in the three locations

Organisms	Mean fungal count \pm SD
<i>Mucor spp</i>	8.00 \pm 1.732
<i>Aspergillus niger</i>	9.00 \pm 5.568
<i>Aspergillus flavus</i>	3.67 \pm 1.528
<i>Penicillium spp</i>	6.00 \pm 1.732
<i>Fusarium spp</i>	7.00 \pm 2.646
<i>Rhizopus spp</i>	6.67 \pm 5.859

Table 3: Mean fungal count among the three study locations sampled

Location	Mean fungal count
<i>Eke Awka</i>	7.67 \pm 5.164
<i>Nkwo Amaenyi</i>	6.67 \pm 2.582
<i>Ifite first market</i>	5.83 \pm 2.639

**Figure 1: Percentage occurrence of fungal in Eke Awka market**

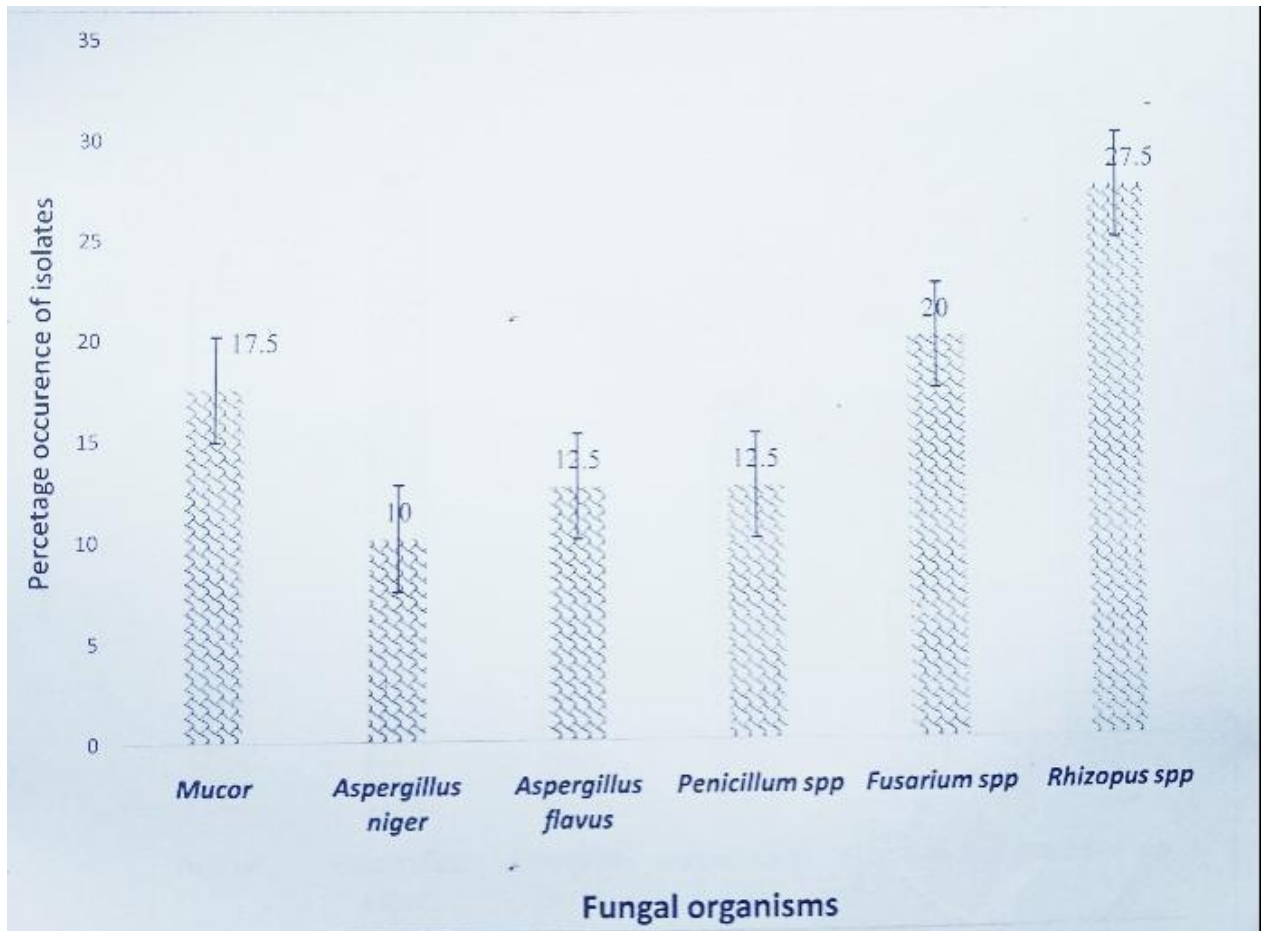


Figure 2: Percentage occurrence of fungal organisms in Nkwo Amaenyi market

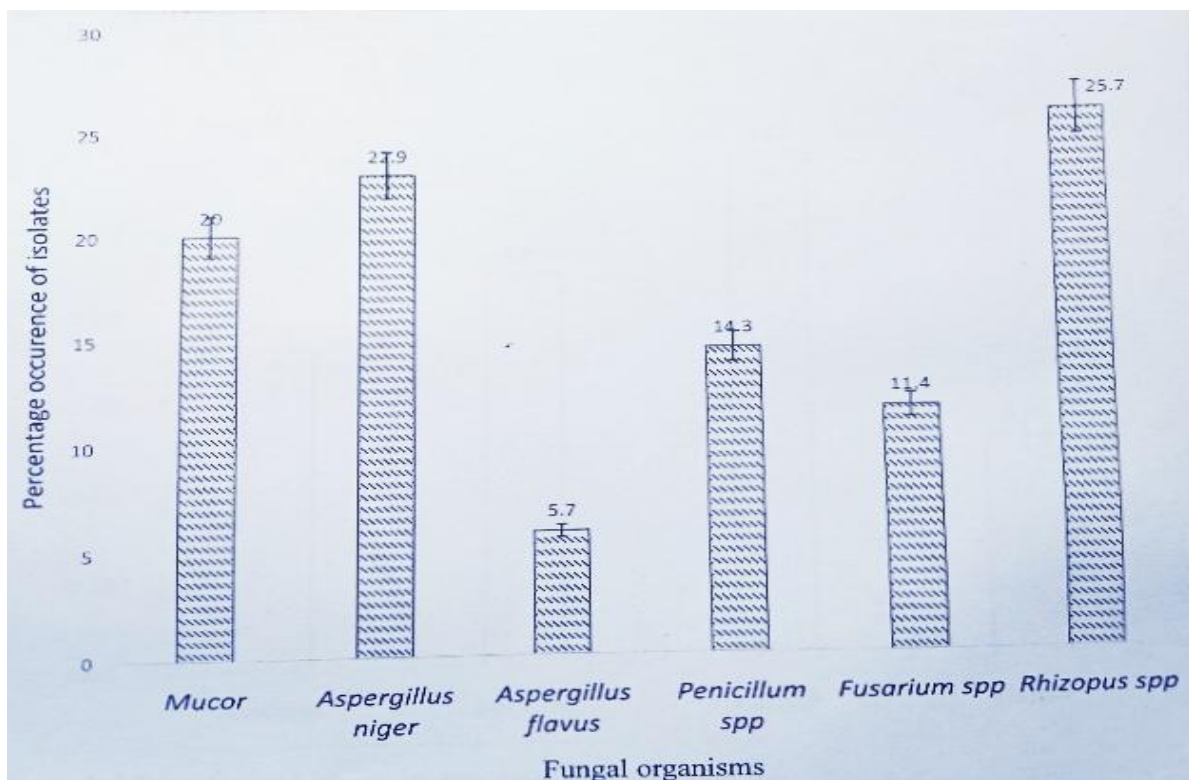


Figure 3: Percentage occurrence of fungi in Ifite first market

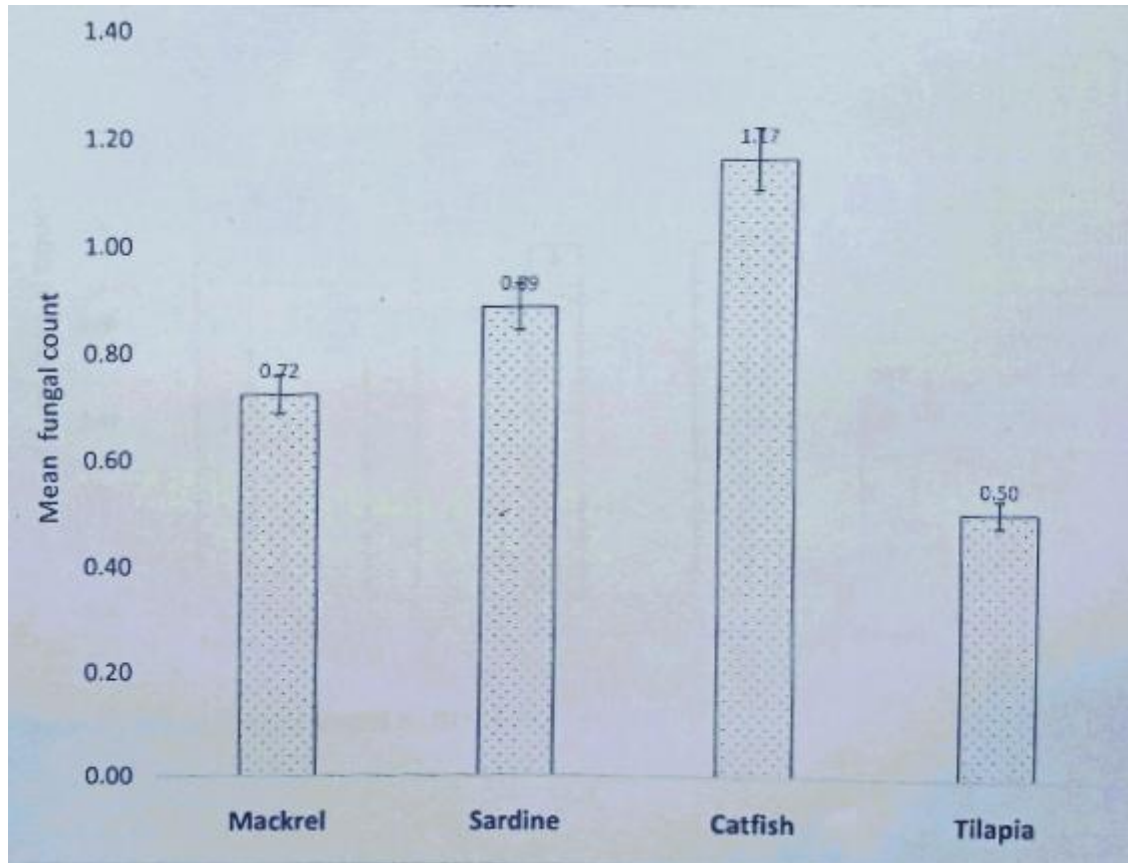


Figure 4: Mean fungal count in smoked fish species

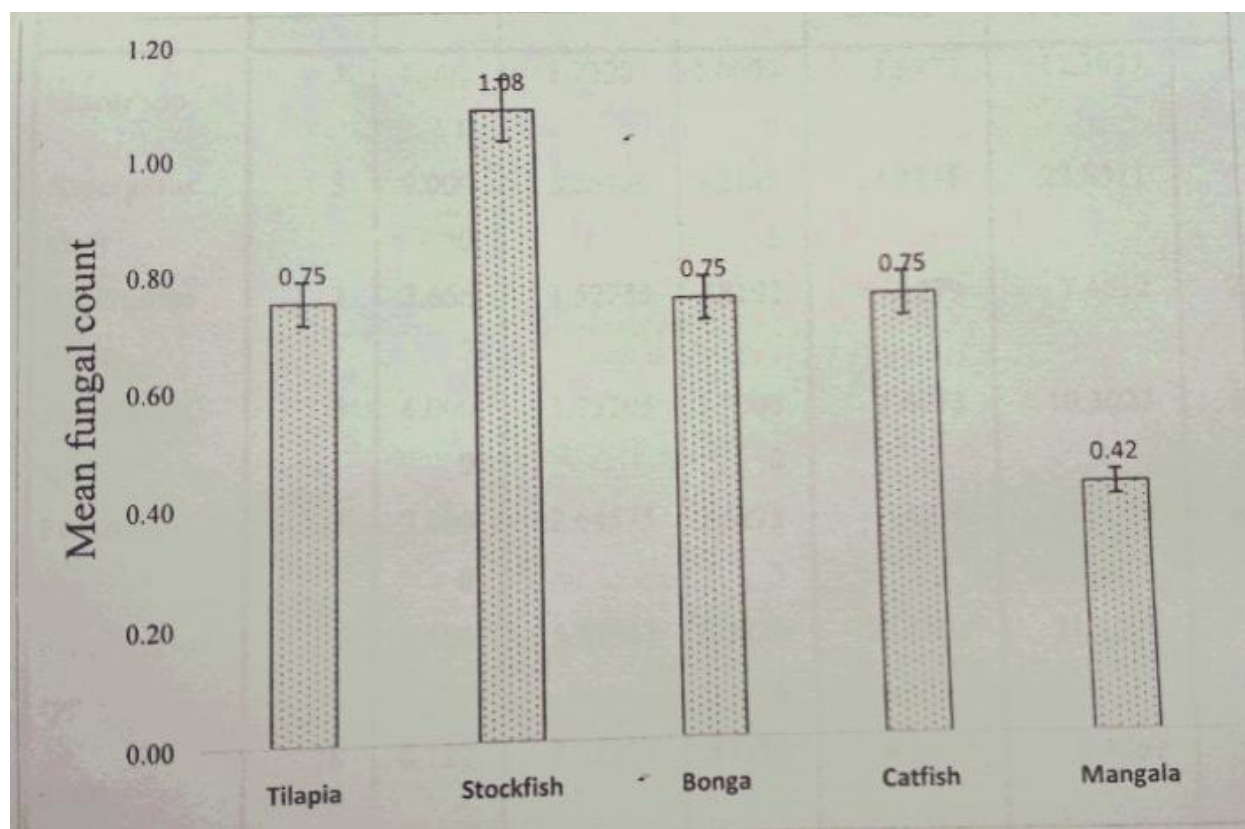


Figure 5: Mean fungal count in dried fish species

DISCUSSION

The result of our study showed that *Aspergillus niger* was the most abundant fungi organism 27 (22.31%) isolated from the fish samples in the three markets studied. This finding is in contrast to the work of Ngo-oum *et al.* (2021) and John *et al.* (2020) that reported *Aspergillus flavus* as the most frequently occurring fungi organism 23 (25.3%) and 32.88% in four different markets in Cameroon and three markets in northern Nigeria respectively. This result also contradicts the work of Akwuobu *et al.* (2019) who reported *Mucor spp* as the most abundant fungal organism, 16 (20.8%), in Benue State. The findings of our study show that *Aspergillus niger* had the highest mean fungal count 9.00 ± 5.568 in the three sampled locations. This is in line with the work of Ike *et al.* (2020) who also reported *Aspergillus niger* as the most common and easily identifiable species of the genus *Aspergillus* in Abia State, Southeast, Nigeria. A similar microorganism was also reported by Abolagba and Igbineybo (2010) in smoked fish (*Claria spp*) sold in Benin metropolis.

The present study revealed the presence of fungi organisms in all the three markets sampled in the study area. Our finding correlates with the studies by Akwuobu *et al.* (2019) that also reported fungi contamination of fish in all three study markets in Makurdi, Benue State. This could be attributed to the similar atmospheric conditions of the study area. Adverse similarity in the optimal atmospheric conditions of an environment triggers favourable conditions for microorganisms to thrive, which reduces the quality of fish and its potential time leading to fish loss. *Aspergillus flavus* was the least isolated fungi organism in this study probably because the fish has no contact with soil. Previous studies by David *et al.* (2007) reported that



Aspergillus flavus normally occurs as a saprophyte in soil and on many kinds of decaying organic matter. The result of this study revealed that fungal organisms were isolated from all the fish samples in the three markets sampled. The present study showed that the fungal genera colonizing the smoked and dry fish samples sold in the three local markets in Awka were *Mucor*, *Rhizopus*, *Aspergillus*, *Fusarium*, and *Penicillium*. This finding is in line with earlier studies conducted by Ngo-oum *et al.* (2021), Nurhayati *et al.* (2020) and Sani *et al.* (2016) that also reported similar fungi organisms to those in this study in Cameroon, India and Nigeria respectively. However, our study differs slightly from the work of John *et al.* (2020) who aside from *Aspergillus*, *Penicillium* and *Rhizopus spp* also reported other fungi pathogens such as *Candida spp* and *Acremonium butyric*.

The fungi organisms isolated in this study have been documented as toxigenic agents of medical importance. For instance, some species of *Penicillium* and *Aspergillus* increase the risk of mycotoxins production which could induce gastrointestinal and metabolic disturbances when contaminated food products like fish are consumed. Depending on the exposure, contamination of fish by aflatoxins can induce adverse health consequences in fish such as poor growth rate and presence of gross and microscopic lesions. These result in economic losses due to low production, morbidities, mortalities and poor quality of fish (Mahfouz & Sherif, 2015). The presence of these fungi pathogens may be related to the population and degree of filthiness in the different locations (Amadi *et al.*, 2014). Other reasons for fungal contamination include the fact that retailers most times display the smoked and dried fish samples in open trays beside refuse heaps and this encourages fungal attack through air droplets (Akande & Tobor, 2012).

However, the mean fungal count was highest in Eke Awka followed by Nkwo Amaenyi and lastly Ifite first market. Eke Awka is the biggest market in Awka, with immense high commercial activity and with people from neighboring towns and villages bringing their products for sale. This situation is responsible for the high human and vehicular traffic in this location with its attendant effect on the environment. The high infestation of fish sold in this market can be traced to the dusty atmosphere usually prevalent in this location. This is in line with the observation made by Amadi *et al.* (2014) who also reported that Eke Awka had mean total fungal count significantly higher than other locations they studied within Awka, although there was no significant difference between the locations with respect to fungal load. However, the result could not establish whether contamination occurred before or after smoking. The implication of this finding is that such dust will continue to contaminate the environment, hence fishes and even other food items sold openly within the vicinity, leading to constant fish spoilage, detrimental effect to human health who are the consumers and other infectious diseases within the area, and thus becoming a major public health concern that requires urgent attention to prevent epidemics or even pandemics. Venugapol (2002) established that contamination of fish, particularly by pathogens, may occur prior to harvest during capture, processing, distribution and or storage. The occurrence of *Aspergillus*, *Rhizopus* and *Penicillium* species could be due to absorption of moisture during storage and sampling. The sampled fish might have reabsorbed moisture from the environment which then supported the growth of microorganisms in addition to the contamination during processing, handling and display on the market stall (Ayolabi & Fagade, 2010).



CONCLUSION

Several factors affect the growth of organisms on fish including many fungal organisms, which contributes to reduction in shelf lives of fishes. Microbial contamination by some fungal organisms may be considered as an important warning signal for human consumption. The result of this study showed that dried stockfish species had the highest mean fungal count. Also, of all the fungal isolates, *Aspergillus niger* had the highest mean fungal count during the study. In this study, Eke Awka market had the highest mean fungal count. It was observed that smoked catfish and species and dried stock fish species had the highest mean fungal count.

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COMPETING INTERESTS

The authors declare no conflict of interest regarding the publication of this paper.

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