Microbial Analysis of Two Common Processed Fish Sold in Two Local Government Areas of Nasarawa State, Nigeria

1.M.U Bahago; 2.H.A Adedeji; 3.A.A Adam; 4.S. Idris
5.Y .Chiroma; 6. N.I Agani; 7.N. A.Agbason

[1]Department of Aquaculture and Fisheries Management, Nasarawa State University Keffi
[2]Department of Fisheries, Modibbo Adamawa State University, Yola
[3]Department of Fisheries Technology, Binyaminu Usman Polytechnic Hadejia, Jigawa State.
[4]Department of Fisheries Technology, College of Agriculture, Science and Technology Lafia

Abstract

The study on microbial analysis of two common processed fish sold in two Local Government Areas of Nasarawa State, Nigeria. The samples of both processed African catfish (*Clarias gariepinus*) and *Tilapia zillii* were sourced from markets in Doma and Obi Local Government Areas. The samples of *Clarias gariepinus* and *Tilapia zillii* were both collected from three different locations in the both markets of the study areas and transported to the laboratory Fish samples of *Tilapia zillii* and *Clarias gariepinus* were purchased from Doma and Obi respectively. The fish were milled into powdered and were packaged according to location and taken to the laboratory for analysis. Data were subjected to statistical analysis using analysis of variance (ANOVA) at a 5% significance level, and significantly different means were separated using Fisher's LSD. The results of the study showed that high microbial activities of fish was obtained from Obi. Tilapia fish from Doma had more of total heterotrophic fungal count, and total coli form counts except for total heterotrophic bacterial count. Doma had the highest percentage for bacteria contamination particularly in *Clarias gariepinus.* Seven organisms which are *Micrococcus spp, Salmonella spp, E. coli, Proteus spp, Streptococcus agalactiae, Serratiamarcescens,* and *Staphylococcus epidermidis* were isolated from fish in Doma, and nine organisms which were *Bacillus spp, Candida albicans, E. coli, Enterococcus spp, Klebsiellaspp,Micrococcusspp,Pseudomona aeruginosa,Salmonella spp, Staphylococcus aureus* were isolated from Obi. Three species of fungi including *Aspergillus niger, Candida albicans,* and *Aspergillus fumigatus*in *Clarias gariepinus* isolated carried the highest percentage compared to Tilapia. There should be a holistic strategy to improved sanitary methods, increased storage conditions, and consumer education on food safety in the study area. Individuals in the study area should be sensitized on

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aquaculture production and fisheries to improve food security in the area.

Keyword: Microbial, preservation, fish, Bacteria, Fungi

Introduction

Fish form a very essential component of diet for man, and often provide required nutrient needed for a healthy being. Fish constitutes an important source of protein intake of many people in the world which is very affordable compare to other protein food (Fawole *et al.*, 2007). In Nasarawa State, about 41% of the total animal protein intake is obtained from fishery products, with a total fish consumption rate of about 2.66 million metric tons annually (Omeje *et al*., 2020) in Nigeria. Fish is a major source of animal protein and essential food item in the diet of Nigerians because of its cheap characteristics than meat; Fish protein is now ahead of other animal origin, and compares favorably with that of egg, meat and milk and its amino acid composition (Idris *el al.,* 2010).

Despite the value of fish, fish is a perishable food material that deteriorates soon after harvest at high ambient temperature therefore, needs immediate preservation. Microbial action plays a large part in the spoilage of fish and fish products (Zanin *et al.,* 2015). The exposure of fish to dust, microbial, and other environmental contaminants result in spoilage. Enzymatic tissue breakdown initiates the deterioration of fish products, and microorganisms proliferate and penetrate the fish flesh as the process continues (Garba *et al.,* 2021). Many scientists have reported microbial load associated with smoked fish. *S. aureus, Pseudomonas, Streptococcus, Bacillus,* (Bacteria) and *Yeast, Aspergillus niger* and *Penicillium* spp. (fungi) were reported to be isolated (Daniel *et al.,* 2013) on microbial diversity of smoked fish sold in Benin City, Nigeria. Akise *et al.* (2013) reported major fungi species isolated from smoke-dried fish under storage including *P. italicum, Penicillium oxalicum, Mucor, Saccharomyces, Rhodotorula spp.* and *Aspergillus spp*. Majority of the fungi produce mycotoxins. *Aspegillus* species are known to produce mycotoxins such as aflatoxins, ochratoxins and sterigmatocystin (Hashem, 2011). On this account, the research aimed determining the qualitative and quantitative analysis of microbial load of two common processed fish sold in two Local Government Areas of Nasarawa State, Nigeria.

Material And Methods

Description of the study area

Doma and Obi Local Government Areas (LGAs) are two administrative regions located in the southern part of Nasarawa State, North-Central Nigeria. Both LGAs share socio-economic and geographical characteristics typical of the Guinea Savannah region and play significant roles in the state's agricultural and cultural landscape.

The analysis was carried out at the Faculty of Agriculture Shabu, Lafia Campus Nasarawa State University Keffi, Nigeria. The region is situated between latitude 08^{o} 33N and longitude 08^{0} 32E in Nigeria, and it is a part of the southern guinea savanna zone. Typically, rain falls between March and October, with an average monthly total of 40 to 350 millimetres (NIMET, 2016).

Map of Nasarawa State

Sample Collection

The samples of both processed African catfish (*Clarias gariepinus*) and *Tilapia zillii* were sourced from markets in Doma and Obi Local Government Areas. The samples of *Clarias gariepinus* and *Tilapia zillii* were both collected from three different locations in the both

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markets of the study areas and transported to the laboratory.

Determination of Microbial Load of Smoked Tilapia and *Clarias gariepinus*

Fish samples of *Tilapia zillii* and *Clarias gariepinus* were purchased from Doma and Obi respectively. The fish were milled into powdered and were packaged according to location and taken to the laboratory for analysis.

Procedure

Bacterial contamination of smoked fish sourced from Doma and Obi market samples was examined. The samples were aseptically packaged for microbial load in sterile plastic bags. The tissues from each treatment were cut and prepared by crushing fish muscle in a sterile mortar. 1 gram of the sample was taking to estimate the number of living organisms per gram. All apparatus used were autoclave for at 121^0c

Culture Media

28g of nutrient Agar was measured at 100ml in conical flask and corked after the addition of 1000ml of distilled water. 9ml of distilled water was pipette into various test-tubes and corked as well. The tube and the media were all autoclave at a temperature of 121^OC

Preparing the dilution

Sterilized pipette was used to blow the tube sample by holding it vertically to introduce its tips not more than 3cm below the surface and sucked up and down ten times into the sample medium. Then 1ml was withdrawn from the sample by touching the tips of the pipette against the neck of the tube and the excess liquid adhering to the outsider of the pipette was removed. The pipette was transferred to the first test tube set 1-5 of the dilution series with the tips of the pipette touching the side of the tube 2-3cm above the level of the diluent. The pipette was discarded and the dilution tubes were label 10^{-2} dilution for each part in location.

Preparing the Dilution

sterilized pipette was used to blow the tube sample by holding it vertically to introduce its tips not more than 3cm below the surface and sucked up and down ten times to the 1ml mark to allow even distribution of the organism in the sample medium. Then 1ml was withdrawn from the sample by touching the tips of the pipette against the neck of the tube and the excess liquid adhering to the outsider of the pipette was removed. The pipette was transferred to the first test tube set 1-5 of the dilution series with the tip to the pipette touching the side of the tube 2-3 above the diluents. The pipette used was discarded and the dilution tubes were labeled 10^{-2} for each part in each location.

The fresh sterile pipette was taken to mix the content in the 10^{-2} dilution tubes by sucking up and down to the 1ml mark ten times again and sometimes rotated between the hand with the tip of the pipette not more than 2-3cm below the surface of the dilution to allow even distribution of the organisms. Then, 1ml was withdrawn from the 10^{-2} tube and transferred into the third tubs and the content in the pipette was expelled as described above. The pipette used was again discarded and the dilution tubes was labeled 10^{-3}. Further dilution of the remaining tube prepared in the same way to the 10^{-5} tube as required. 1ml was withdrawn from the last tubes and discarded out to give equal measurement and accurate counting of the organisms.

Serial Dilution

A liquid medium was prepared of 9ml of distilled water in the test-tubes for each sample of the smoked dried fish. 1gm of smoked dried

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fish samples was weighed using a weighing balance and put into the test-tube label and plug immediately with compressed cotton wool and aluminum foil paper to prevent further contamination with microbes. Then 1-5 sets of test tubes with 9ml of distilled water each were arrange for further serial dilution of the smoked dried fish sample. The sample tube vigorously shaken 15 times to allow even distribution of the organism in the medium and label.

Pouring the Plate

1 ml of the diluted sample was taken and dispensed into the sterile Petri-dish and about 20ml of the molten agar was poured into the Petri-dish and rock shifted gently for homogeneity. The culture plate was allowed to solidify and then transfer into the incubator. The culture plate containing the Nutrient gar was culture at 37^0C for 24hrs. The resulting growth of the culture was the colony forming unit per mil (CFU/ ml)

Total viable Plate Count

The incubation lasted for 24hours for total bacterial count at 37^0C. The colonies on each plate were counted including those on the agar surface and those grown within. Electronic Colony counter was used to do the counting. The plate was placed in the inverted position of the counter light surface, and the colonies was count by marking their positions on the back of the Petri-dish with a marking pen. This aided in keeping track of those colonies previously counted and to avoid recounting of the colonies. However, for each Colony Forming Unit (CFU) the number of bacteria and fungi cell in the original sample were determine by multiplying the dilution factor.

Statistical Analysis

Data were subjected to statistical analysis using analysis of variance (ANOVA) at a 5% significance level, and significantly different means were separated using Fisher's LSD.

Results

Microbial Analysis on Smoked Catfish from Doma and Obi Local Government Areas

The result in Figure 1 showed the microbial analysis of smoked catfish from Doma and Obi LGAs. The results indicated that Obi LGA was found with more Total Heterotrophic Fungal Count than Doma. The values of total coliform count were higher in Obi LGA than Doma LGA. Similar observation was recorded for total heterotrophic bacterial count whereby Doma LGA was recorded higher than Obi LGA. There were significant differences ($P<0.05$) in the microbial count of smoked *Clarias gariepinus* from the two locations.

Figure 1: Microbial Analysis obtained from Catfish (*Clarias gariepinus*) from Doma and Obi Local Government Areas

THFC = Total Heterotrophic Fungal Count; TCC= Total Coliform Count; THBC = Total Heterotrophic Bacterial Count

Microbial Analysis on Smoked Tilapia from Doma and Obi Local Government Areas

The result of microbial count of smoked tilapia from Doma and Obi is presented in figure 2 below. The analysis showed that Obi was recorded higher for THFC (1.40±0.10), TCC (2.70±1.16) except THBC (4.53±0.38) than Doma in THFC (1.30±0.10), TCC (2.27±0.95), and THBC (5.37±0.38) from smoked Tilapia respectively indicating significant differences ($P<0.05$) from each other.

Figure 2: Microbial Analysis obtained from Tilapia from Doma and Obi Local Government Areas

THFC = Total Heterotrophic Fungal Count; TCC= Total Coliform Count; THBC = Total Heterotrophic Bacterial Count

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Bacteria Isolates in Fish Obtained from Doma and Obi Local Government Areas

Table 1 showed the results of bacteria isolates from fish in Doma and Obi. The bacteria isolates from fish in the two locations showed that, seven organisms (*Micrococcus spp* , *Samonellaspp* , *E. coli* , *Proteus spp* , *Streptococcus agalactiae* , *Serratiamarcescens* , and *Staphylococcus epidermidis*) were isolated from fish in Doma. Among the organism isolated in Doma *Micrococcus spp* was recorded with the highest (11.11%) followed by *Samonella spp* (8.33%), *E. coli* (8.33%), *Proteus spp* (8.33%), *Streptococcus agalactiae* (8.33%) *Serratia marcescens* (5.56%) while *Staphylococcus epidermidis* (2.78%) was the lowest respectively. In Obi, nine organisms which are *Bacillus spp, Candida albicans*, *E. coli*, *Enterococcus spp, Klebsiella spp* , *Micrococcus spp, Pseudomonas aeruginosa, Samonella spp, Staphylococcus aureus* were isolated from the fish. Among the isolated organism from fish obtained in Obi, *Candida albicans* (8.33%), *Pseudomonas aeruginosa* (8.33%), *Staphylococcus aureus* (8.33%) were the highest followed by *E. coli* (5.56%), *Enterococcus spp* (5.56%) while *Bacillus spp*(2.78%), *Klebsiellaspp* (2.78%), *Micrococcus spp* (2.78%), *Samonellaspp* (2.78%) were the lowest respectively.

Table 1: Bacteria isolates in *Clarias gariepinus* Obtained from Doma and Obi LGAs of Nasarawa State

Location	Organism	Freq.	%
Doma	**Micrococcus spp**	**4**	**11.11**
	Samonella spp	**3**	**8.33**
	E. coli	**3**	**8.33**
	Proteus spp	**3**	**8.33**
	Streptococcus agalactiae	**3**	**8.33**
	Serratia marcescens	**2**	**5.56**
	Staphylococcus epidermidis	**1**	**2.78**
	Sub Total	**19**	**52.78**
Obi	**Bacillus spp**	**1**	**2.78**
	Candida albicans	**3**	**8.33**
	E. coli	**2**	**5.56**
	Enterococcus spp	**2**	**5.56**
	Klebsiella spp	**1**	**2.78**
	Micrococcus spp	**1**	**2.78**
	Pseudomonas aeruginosa	**3**	**8.33**
	Samonella spp	**1**	**2.78**
	Staphylococcus aureus	**3**	**8.33**
	Sub Total	**17**	**47.22**
	Grand Total	**36**	**100**

Fungi Isolate from Clarias gariepinus obtained from Doma and Obi

The results showed in Table 2 indicated the fungal isolates in Clarias gariepinus from Doma and Obi. The results showed that Doma was observed with more fungi than Obi. However, Doma was recorded with two fungi (Aspergillus niger and Candida albicans)

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whereby 35.71% of the fungi was recorded in Aspergillus niger while 21.43% in Candida albicans respectively. In Obi, only one species of fungi (Aspergillus fumigatus) was recorded having 42.86%.

Table 2: Fungi Isolate on Catfish

Location	Organism	Freq.	%
Doma	Aspergillus niger	5	35.71
	Candida albicans	3	21.43
	Sub total	8	57.14
Obi	Aspergillus fumigatus	6	42.86
	Sub total	6	42.86
	Grand Total	14	100

Bacteria isolate on Tilapia obtained from Doma and Obi Local Government Areas

Table 3 showed the results of bacteria isolated from tilapia obtained from Doma and Obi. The resultsshowedthatten organisms (*Pseudomonas aeruginosa, Staphylococcus aureus, Samonella spp, E. coli, Rhizopus spp, Micrococcus spp, Shigella spp, Klebsiella spp, Proteus spp, and Bacillus spp*) were identified from Tilapia fish in Obi while seven organisms (*Staphylococcus aureus, E. coli, Samonella spp, Shigella spp, Rhizopus spp, Micrococcus spp*, and *Proteus spp*) were identified from Doma. The percentage occurrence of fungi in Doma and Obi were 50% and 50% respectively.

Table 3: Bacteria isolate on Tilapia

Location	Organism	Freq.	%
Doma	**Staphylococcus aureus**	**4**	**11.11**
	E. coli	**3**	**8.33**
	Samonella spp	**3**	**8.33**
	Shigella spp	**1**	**2.78**
	Rhizopus spp	**3**	**8.33**
	Micrococcus spp	**2**	**5.56**
	Proteus spp	**2**	**5.56**
	Sub Total	**18**	**50.00**
Obi	**Pseudomonas aeruginosa**	**1**	**2.78**
	Staphylococcus aureus	**3**	**8.33**
	Samonella spp	**3**	**8.33**
	E. coli	**2**	**5.56**
	Rhizopus spp	**3**	**8.33**
	Micrococcus spp	**1**	**2.78**
	Shigella spp.	**2**	**5.56**
	Klebsiella spp	**1**	**2.78**
	Proteus spp	**1**	**2.78**
	Bacillus spp	**1**	**2.78**

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	Sub Total	**18**	**50.00**
	Grand Total	**36**	**100**

Fungi Isolate on Tilapia

Table 4 showed the number of fungi isolated from Tilapia fish in Doma and Obi. The Tilapia fish obtained from Doma were recorded with four (4) fungi among which includes; *Saprolegina spp* (2.63%), *Fusarium solani* (7.89%), *Penicillium spp* (7.89%), and *Aspergillus niger* (5.26%) respectively. The tilapia fish obtained from Obi were three (3) which are; *Fusarium solani* (7.89%), *Penicillium spp* (7.89%), and *Aspergillus niger* (7.89%) respectively. The highest percentage of occurrence was recorded in Obi (71.05%) than Doma (23.68%) respectively.

Table 4: Fungi Isolate on Tilapia

Location	Organism	Freq.	%
Doma	**Saprolegina spp**	**1**	**2.63**
	Fusarium solani	**3**	**7.89**
	Penicillium spp	**3**	**7.89**
	Aspergillus niger	**2**	**5.26**
	Sub Total	**9**	**23.68**
Obi	**Fusarium solani**	**3**	**7.89**
	Penicillium spp	**3**	**7.89**
	Aspergillus niger	**3**	**7.89**
	Sub Total	**27**	**71.05**
	Grand Total	**38**	**100**

Percentage Composition of Bacteria Isolated from Doma and Obi Local Government Areas

The figure 3 below showed the percentage composition of bacteria isolated from Catfish and Tilapia in Doma and Obi. The study showed that catfish accumulated more of bacteria in Doma (52.78%) than Obi (50.00%) with slight significant differences ($P<0.05$) from each other respectively. The Tilapia fish obtained from the two locations showed Doma (57.14%) having the highest accumulation of bacteria than Obi (23.68%) respectively. The study showed that Doma had more of bacteria isolated.

Figure 3: Percentage Composition of Bacteria Isolated from Fish by Location

Percentage Composition of Fungi Isolated from Doma and Obi Local Government Areas

The percentage composition of fungi isolated from Doma and Obi is presented in Figure 4. In catfish, Obi (50%) showed higher percentage of fungi than Doma (47.22%) respectively. In tilapia fish, Obi (71.05%) also showed higher percentage of fungi than Doma (42.86%). The fish species studied from the two locations showed that tilapia was recorded as the highest percentage respectively.

Figure 4: Percentage Composition of Fungi Isolated from Fish by Location

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Discussion

Microbial Contamination of Fish Samples

Microbial activities on the fish species from the two locations showed that fish obtained from Obi had more contaminated organism than those obtained from Doma. It was observed in the study that, the level of fish consumption in Doma is higher than Obi therefore fish seller tends to sell out their fish without storing them for long period of time. In the case of Obi, weather condition and long storage of smoked fish by seller awaiting buyers could encourage bacteria multiplication and growth of fungi in product. Generally, the total heterotrophic fungal count, total coliform counts and total heterotrophic bacterial count were high in *Clarias gariepinus* particularly in Doma compared to Obi. Similar observation was recorded in tilapia fish obtained in the two locations. Tilapia fish from Doma had more of total heterotrophic fungal count, and total coliform counts except for total heterotrophic bacterial count. Fish perishability is aggravated by its intrinsic properties, such as high water activity, near-neutral pH, and high digestible protein content, all of which provide conducive conditions for microbial proliferation (Ghaly 2010). Many spoilage microorganisms, known to be opportunistic pathogens, including *Pseudomonasspp*. and *Proteus spp*., have also been associated with fish (Ikutegbe and Sikoki 2014). The measured microbial load (1.40-5.97 CFU/g) in this study exceeds the values reported by Yusuf and Hamid (2017) in Bauchi Metropolis, demonstrating regional disparities in hygiene practices and environmental factors. Contamination of fish is likely to take place along the supply chain, including processing, storage, and market display. Traditional smoking procedures which were the most used method of processing in this study may introduce germs and fungi into the fish through open fires and exposure to ambient contaminants. Additionally, the practice of exposing fish publicly at marketplaces exposes them to dust, insects, and other sources of infection. (Ibrahim *et al.*, 2022).

Bacteria Isolate from the Fish

Seven organisms (*Micrococcus spp*, *Salmonella spp*, *E. coli*, *Proteus spp*, *Streptococcus agalactiae*, *Serratia marcescens*, and *Staphylococcus epidermidis*) were isolated from fish in Doma, while in Obi nine organisms which are *Bacillus spp*, *Candida albicans*, *E. coli*, *Enterococcus spp*, *Klebsiella spp*, *Micrococcus spp*, *Pseudomonas aeruginosa*, *Salmonella spp*, *Staphylococcus aureus.* Similar observation was recorded by Mu'azu *et al.* (2024) in Monai, Southern Basin of Kainji Lake, New Bussa, Niger State, Nigeria. From the study, Doma had the highest percentage for bacteria contamination in all the fish species particularly *Clarias gariepinus* was contaminated with microbes than tilapia fish and this observation could be linked to differences in their sizes, environment where they are captured and storage conditions. One of the factors that promote the growth of spoilage bacteria in fish is the temperature which is regarded as the most important factor that encourages bacterial multiplication (Abraha*et al.*, 2018). Foodborne bacteria such as *Escherichia*, *Bacillus*, *Clostridium*, *Micrococcus*, *Proteus*, and others grow mostly at room temperature and are mostly dominated in fish (Coimbra *et al.*, 2022). The presence of Escherichia coli and Salmonella sp. is particularly problematic due to their link with food borne diseases. These bacteria cause serious gastrointestinal illnesses through toxin production. Salmonella sp., including S. Enteritidis and S. Typhi, offers considerable dangers, with S. Typhi producing typhoid fever and non-typhoidal Salmonella contributing to gastroenteritis (Joseph *et al.*, 2020). The presence of these pathogens in smoked fish indicates probable fecal contamination, possibly introduced during handling or

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processing and also lack of degutting of fish before smoking. Akinwumi and Adegbehingbe (2015) similarly observed E. coli and Salmonella sp. in smoked fish from Ondo State, Nigeria, attributing contamination to unsanitary methods and environmental exposure. The presences of *Pseudomonas aeruginosa* and *Staphylococcus aureus* and others suggests post-processing contamination, likely due to inappropriate handling and inadequate storage conditions (Quintieri*et al.*, 2021). Ibrahim *et al.* (2022) identified comparable bacterial profiles in smoked catfish marketed in Bida, Nigeria, attributing contamination to inadequate market cleanliness.

Fungi Isolate of Fish

This study isolated three species of fungi including *Aspergillus niger, Candida albicans,* and *Aspergillus fumigatus* in *Clarias gariepinus* while in Tilapia four species including *Saprolegina spp, Fusarium solani, Penicillium spp,* and *Aspergillus niger* were isolated. Despite the frequency of occurrence, fungi isolated from *Clarias gariepinus* carried the highest percentage. The prevalence of *Aspergillus species* is similar to Nwachukwu and Madubuko (2010), who observed substantial fungal contamination in smoked fish stored under suboptimal conditions. *Aspergillus species* which produces aflatoxins, carcinogenic chemicals associated with liver cancer, especially in locations with heavy fish consumption (Benkerroum, 2020). The discovery of Penicillium sp. also implies poor storage conditions, as these fungi flourish in moist environments (Smiri *et al.*, 2021).

Conclusion

Microbial activities of fish obtained from Obi showed more contaminated organism than those obtained from Doma. Seven organisms (*Micrococcus spp, Salmonella spp, E. coli, Proteus spp, Streptococcus agalactiae, Serratia marcescens,* and *Staphylococcus epidermidis*) were isolated from fish in Doma, and nine organisms which are *Bacillus spp, Candida albicans, E. coli, Enterococcus spp, Klebsiella spp, Micrococcus spp, Pseudomonas aeruginosa, Salmonella spp, Staphylococcus aureus* were isolated from Obi. Total heterotrophic fungal count, total coliform counts and total heterotrophic bacterial count were high in *Clarias gariepinus* than Tilapia which showed that catfish fish are prone to bacterial contamination than Tilapia. Three species of fungi including *Aspergillus niger, Candida albicans,* and *Aspergillus fumigatus* in *Clarias gariepinus* isolated carried the highest percentage compared to Tilapia.

Recommendation

There should be a holistic strategy to improved sanitary methods, increased storage conditions, and consumer education on food safety in the study area.

Individuals in the study area should be sensitized on aquaculture production and fisheries to improve food security in the area.

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