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Fungi of traditionally processed Nile fish in Sudan

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Abstract
Yeast extract agar media, Potato dextrose agar media, Plate count agar, Plate count agar+, Mannitol salt agar and Eosin Methylene Blue agar were prepared, cultured, incubated and stored following standard methods. The fungal inoculations included 4 replicates, 19 samples, 46 agar media and 7 dilutions form. *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stonloifara*, *Alternaria* sp. and *Pencillim* sp. were detected. The highest fungal count was in wet salted *Hydrocynus* spp. with 49% moisture and 27% organic content, followed by *Alestes* spp. with 48% moisture and 32 % organic content. The least fungal count was in dry salted *Oreochromis niloticus* with 44% moisture and 30 % organic content. No consistent correlates were observed between moisture and organic content, and the fungal isolates or species or the processed fish species.

Keywords: Fessikh, maloha, mendishi, kajjake, fungi, fish, Sudan

Introduction
Although some fungi are useful as a source of food, antimicrobial agents, medicine, decomposers, bio-fertilizers and biocontrol agents, yet some species are pathogenic causing disease in living organisms and spoilage of food (Webster and Weber [1]). Fish spoils more rapidly than other animal foods, particularly when mishandled (El Hag *et al.* [2]; Ahmed *et al.* [3]). Spoilage leads to about 30% loss of captured fish (Chauhan *et al.* [4]) if rigor mortis is not prevented. High fish quality for processing requires identification of spoiling agents and their mechanism of action for appropriate intervention. This is due to the growing interest in quality assurance of fish and its products. A large portion of alestids, clarids among few other fish species from freshwater bodies of Sudan are variously processed to meet local and export demands.

Several studies related quality deterioration of traditionally processed fish to bacteria attack (Essuman [5]; Dirar [6]; Lnovotny [7]; Herrera [8]; Kasozi *et al.* [9]; Ahmed *et al.* [3]) and/or to fungal attack (Edema and Agbon [10]; Chauhan *et al.* [4]; Shamsan and Al-Jobory [11]). The fungi of Nile fishes were studied from different standpoints. In Sudan the fungi of sun dried 'Kajake fish' was investigated by Suleiman *et al.* [12]. In Egypt the fungi work of Ammar[13] on salted fish; Youssef *et al.* [14] on salted Fish "Moloha", El-Ahl [15] on some fish species and Hassan *et al.* [16] in *Tilapia nilotica*, are examples. In Ethiopia Melaku *et al.* [17] worked on fungi from *Clarias gariepinus* eggs and adults.

The present work investigated the fungi of traditionally processed *Alestes* spp., *Hydrocynus* spp., *Labeo* spp., *Claris* spp., *Synodontis* spp., and *Oreochromis niloticus*.

Materials and Methods
Fish samples
The state and source of 19 randomly selected processed fish is given in Table 1. Tissue samples were collected using sterile tools and kept in sterile jars at 4^0C till fungal examination.

Table 1: Source of salted fish samples

Code	Sample source	Processing state
Alestes spp.		
F5	Huda fish foundation	8 days old wet salted.
F15	Huda fish foundation	9 days old wet salted.
F7	Ismail fish foundation	10 days old wet salted.
F1	Huda fish foundation	13 days old wet salted
F3	Huda fish foundation	25 days old wet salted.
F4 and F18	Huda fish foundation	Mature wet salted.
Hydrocynus spp.		
F6	Ismail fish foundation	8 days old wet salted.
F14	Khartoum Fish market	12 days old wet salted.
F8, F9 and F13	Khartoum Fish market	Mature wet salted.
Labeo spp.		
F10	Huda fish foundation	Mature wet salted.
"Maloha" a mixture of *Alestes* and *Hydrocynus* spp.		
F12 and 19	Khartoum Fish market	Mature watery salted "Maloha"
"Mandeshe"a mixture of *Synodontis* spp.		
F2 and F11	Khartoum Fish market	Mature fermented "Mandeshe".
"Kajake" dry salted		
F16	Khartoum Fish market	Dry salted *Oreochromis niloticus.*
F17	Khartoum Fish market	Dry salted *Clarias* spp.

Samples culturing

Yeast extract agar media (YEA), Potato dextrose agar media (PDA), Plate count agar (PCA), Plate count agar+ (PCA+), Mannitol salt agar (MSA) and Eosin Methylene Blue agar (EMBA) were prepared, cultured, incubated and stored at 4ºC according to Waksman [18] and Tournas *et al*. [19]. Four samples of each of the 19 fish products were investigated for their fungal infections.

Colony colour and species identification

Petri-dish containing the pure individual colonies were examined visually or under a Stereo-microscope to determine the colony colour.

Slides of different fungi colonies were prepared by using a flamed inoculating needle. Small amount from edge of each colony was picked-up and placed onto a drop of cotton blue stain; covered with a cover slip and examined under the microscope. Fungi identification followed Webster and Weber [1] and count was according to (Surendran *et al*. [20]).

Chemical analysis

The gross chemical composition of fish (moisture, protein, fat and ash) were determined using the standard methods of the Association of Official Analytical Chemists (AOAC [21]).

Results

Growth colony morphology and microscopic characteristics of some of the isolated fugal genera are given in Plates 1-8 and Table 3. These were *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stonloifara*, *Alternaria* sp. and *Pencillim* sp.

Plate 1: Green *Aspergillus niger* culture

Plate 2: Green *Aspergillus flavus* culture

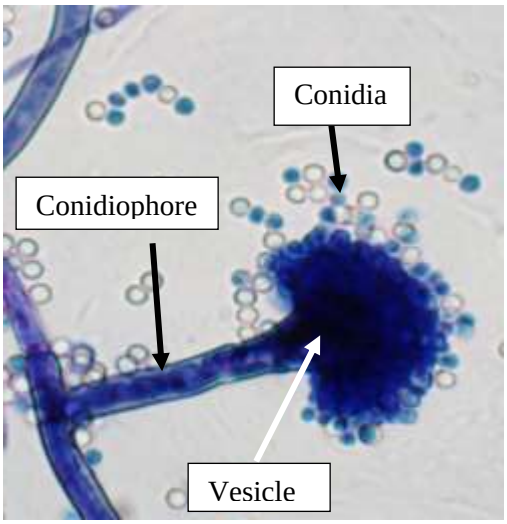

Plate 3: *Aspergillus niger*

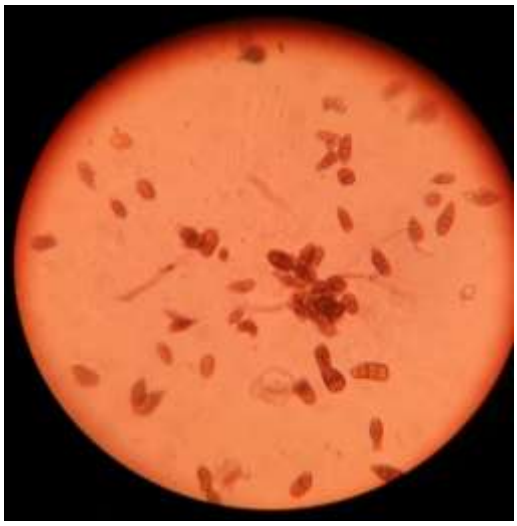

Plate 4: *Alternaria* sp.

Plate 5: *Rhizopus stolonifera* culture

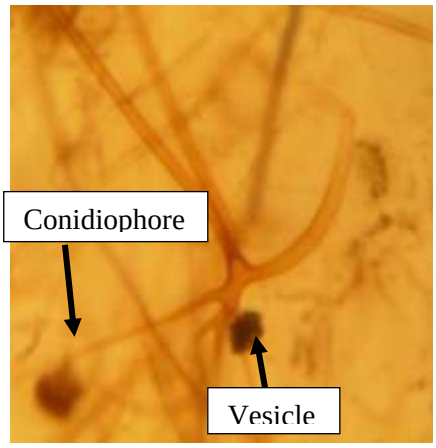

Plate 6: *Rhizopus stolonifera*

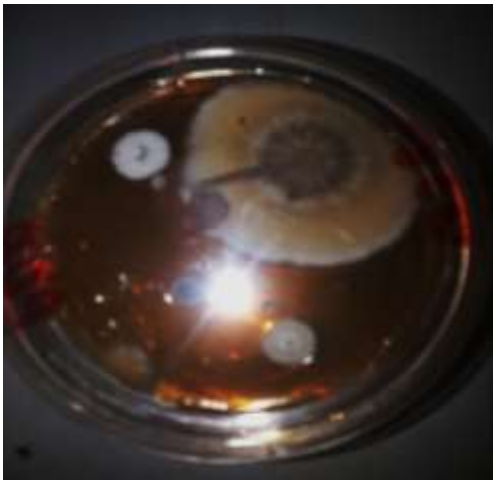

Plate 7: White *Pencillim* sp. culture

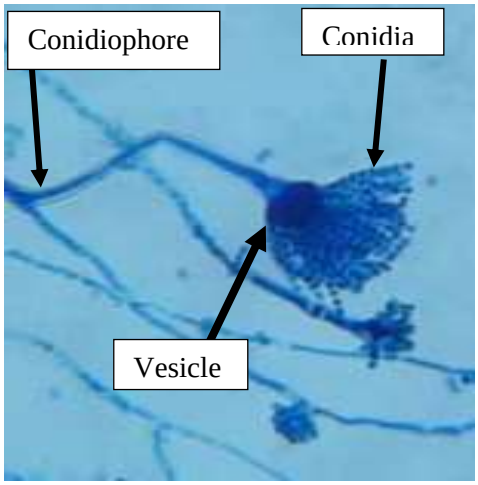

Plate 8: *Pencillim* sp.

Mature wet salted *Hydrocynus* spp. samples (F8 Table 1) showed no fungal growth in all culture media (Table 2). On the other hand, PEMBA culture media supported no fungal growth in all dilutions. In 8-day old wet salted *Alestes* spp., *A. flavus*, *R. stonloifara*, *Alternaria* sp. and *Pencillim* sp. were encountered. In the rest of the samples single or bi-occurrence of fungi was observed (Table 3).

Fungi species isolates were 30 *A. niger*, 13 *R. stonloifara*, 3 *A. flavus*, 2 *Alternaria* sp. and 2 *Pencillim* sp. totaling 50 isolates. Uncountable fungi colonies were mostly from 10^{-1} and 10^{-2} dilution.

Out of the 114 fungi growth media, DPA, YEA, PCA+, MSA and PCA supported 34, 13, 5, 2 and 1 colony growth, respectively.

In DPA media mature wet salted *Hydrocynus* spp. (F13, Table 1) and mature *Alestes* spp. recoded uncountable fungi in 7 and 5 tested medium (F13 and F18, Table 1), respectively.

Pencillim sp. showed white colony coloration. In *Alternaria* sp.one colony was white and the other was grey. *Aspergillus niger* showed 28 black and 2 green colonies. The colonies of *A. flavus* were green. Eight grey, 4 white and 1 black colonies were encountered in *R. stonloifara* (Table 3).

Table 2: Fish samples fungi colony count using different media types, UNF=Uncountable fungi

Code	Media	Colony Count at different Dilutions						
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
F1	YEA	UnF	UnF	0	0	0	0	0
	PCA+	0	UnF	1	0	0	0	0
	PDA	0	UnF	0	0	0	0	0
F2	YEA	UnF	UnF	0	0	UnF	0	0
	PDA	UnF	0	0	0	0	0	0
F3	PDA	UnF	UnF	0	0	0	0	0
F4	PDA	UnF	0	0	0	0	0	0
F5	YEA	UnF	UnF	0	0	0	0	0
	PDA	UnF	0	0	0	0	0	0
F6	YEA	UnF	UnF	0	0	0	0	0
	PDA	UnF	UnF	0	0	0	0	0
F7	PDA	UnF	UnF	0	0	0	0	0
F8	All media showed no fungal growth							
F9	PDA	UnF	0	0	0	0	0	0
F10	YEA	0	0	0	0	0	UnF	UnF
	PDA	UnF	0	0	0	0	0	0
F11	PDA	UnF	0	0	0	0	0	0
F12	PDA	UnF	UnF	UnF	0	0	0	0
F13	PDA	UnF	UnF	UnF	UnF	UnF	UnF	UnF
F14	YEA	UnF	0	0	0	0	0	0
	PDA	1 F	0	0	0	0	0	0
F15	YEA	UnF	0	0	0	0	0	0
	PDA	0	0	0	0	UnF	UnF	UnF
F16	PDA	3 F	I F	0	0	0	0	0
F17	PCA	4	0	0	0	0	0	0
	PCA+	UnB	UnB	0	0	1	0	0
	MSA	3	0	0	0	1	0	0
F18	YEA	UnF	0	0	0	0	0	0
	PDA	UnF	UnF	UnF	UnF	UnF	0	0
F19	PDA	1 F	0	0	0	0	0	0

Table 3: Identified fungi species and colony colour.

Code	No. of Samples	Medium	Colour	Fungi species
F_1	R_1 and R_2	YEA	Black	*Aspergillus niger*
	R_3	PDA	Green	*Aspergillus flavus*
	R_4	PDA	Black	*A. niger*
F_2	R_5	YEA	Black	*A. niger*
	R_6	YEA	Grey	*Rhizopus stonloifara*
	R_7	YEA	Grey	*R. stonloifara*
	R_8	PDA	White	*R. stonloifara*
F_3	R_9	PDA	Black	*A. niger*

	R_{10}	PDA	Black	*Alternaria* sp.
F_4	R_{11}	PDA	Black	*A. niger*
F_5	R_{12}	YEA	Black	*R. stonloifara*
	R_{13}	YEA	White	*Pencillim* sp.
	R_{14}	YEA	Black	*Alternaria sp.*
	R_{15}	YEA	Green	*A. Flavus*
F_6	R_{16}	PDA	Black	*A. niger*
	R_{17}	PDA	Grey	*R. stonloifara*
F_7	R_{18} and R_{19}	PDA	Black	*A. niger*
F_9	R_{20}	PDA	Grey	*R. stonloifara*
F_{10}	R_{21} and R_{22} and R_{23}	YEA	Black	*A. niger*
F_{11}	R_{24}	PDA	White	*R. stonloifara*
F_{12}	R_{25}	PDA	Black	*A. niger*
	R_{26}	PDA	Grey	*R. stonloifara*
	R_{27}	PDA	White	*R. stonloifara*
F_{13}	R_{28} till R_{32}	PDA	Black	*A. niger*
	R_{33}	PDA	White	*Pencillim sp.*
F_{14}	R_{34}	YEA	White	*R. stonloifara*
	R_{35}	PDA	Black	*A. niger*
F_{15}	R_{36} and R_{38} and R_{39}	YEA	Black	*Niger*
	R_{37}	PDA	Green	*A. niger*
F_{16}	R_{40}	PDA	Grey	*R. stonloifara*
	R_{41}	PDA	Black	*A. niger*
F_{17}	R_{42} and R_{43} and R_{45}	YEA	Black	*A. niger*
	R_{44}	PDA	Green	*A. Flavus*
F_{18}	R_{46} and R_{49}	PDA	Green	*A. niger*
	R_{47} and R_{48}	PDA	Grey	*R. stonloifara*
F_{19}	R_{50}	PDA	Black	*A. niger*

The highest fungal count in mature wet salted fish was in *Hydrocynus* spp. with 49% moisture and 27% organic content, followed by *Alestes* spp. with 48% moisture and 32 % organic content. The least fungal count was in dry salted *O. niloticus* with 44% moisture and 30 % organic content. No correlates were observed between moisture and organic content, and the fungal isolates or species or the processed fish species.

Discussion

The present study on wet salted and sun-dried processed fish showed that 50 isolates of fungi were obtained from the 19 samples (Table 2). In 8-day old wet salted *Alestes* spp., *A. flavus*, *R. stonloifara*, *Alternaria* sp. and *Pencillim* sp. were encountered. In the rest of the samples single or bi-occurrence of fungi was observed (Table 3).The fungal species occurrence in a descending order was *A.* niger (60%), *R. stonloifara* (26%) *A. flavus* (6%), *Alternaria* sp. (4%) and *Pencillim* sp. (4%). According to Chauhan *et al.* [4] the worldwide distributed fungi is *A. niger* which is responsible for post-harvest decay. Chauhan *et al.* [4] stated that in recent years Aspergillus infections have increased in fresh water fishes. They isolated A. fumigatus, A. niger and A. sydowii from 9 different species of freshwater fishes. According to Youssef *et al.* [14] and Junaid *et al.* [22] most of *Aspergillus* spp., *Penicillium* spp., *Eurotium* spp., *Mucor* spp., and other species obtained during their studies, had been identified before from salted, smoked and sun-dried fish. They stated that except for *Eurotium* spp., all the species they mentioned were known as pathogenic to human beings causing food spoilage. El-Ahl [15] isolated *A. flavus* from most of the Nile fish samples examined.

Fafioye *et al.* [23] reported that fungi of samples of traditionally smoke-dried fishlike *Clarias* spp. and *Heterobranchus* spp, in Ago-Iwoye, in Nigeria included *Mucor* sp., *Aspergillus* spp., *Rhizopus* spp. and *Fusarium* spp. *Aspergillus* spp. among other fungi were reported from smoked-dried fish by (Adebayo-Tayo *et al.* [24]). Shamsan and Al-Jobory [11] studied the fungi of sun-dried fish locally named 'Wazef' from Yemin. They found 26 fungal species including *Aspergillus*, *Rhizopus* and *Penicillium*.

Wheeler *et al.* [25] studied the mycoflora of dried salted fish from Indonesia and reported *Polypaecilum pisce*, 3 *Eurotium* spp, 5 *Aspergillus* spp. and a variety of *Penicillium* spp. Atapattu and Samarajeewa [26] in their study of fungi of dried fish in Sri Lanka, reported *Aspergillus flavus*, *A. fumigatus*, *A. glaucus*, *A. restrictus*, *Aureobasidium* spp. *Basipetospora halophila* (a genuinely halophilic fungus) *Cladosporium herbarum*, *Gliomastix*, spp., *Penicillium chalybeum* and *Penicillium expansum*. Strong xerophilic moulds isolates from salted and unsalted dried fish from traditional markets in Jakarta belong to *Aspergillus awainori*, *A. carbonarius*, *A. glaucus*, *A. tamarii*, and *Eurotium glaucus* (Santoso *et al.* [27]). Ammar [13] isolated *Aspergillus* spp., *Penicillium* spp. and *Rhizopus* spp. from some salted Nile fish samples. Suleiman *et al.* [12] isolated *Aspergillus niger*, *Alternaria* sp, and *Penicillium* from Kejeik samples (probably *Clarias* spp.).

Occurrence of *Penicillium* spp, in some samples during this investigation is in agreement with Youssef *et al.* [14] work on Maloha and Shamsan and Al-Jobory [11] on sun-dried fish.

In the present study the least isolated fungi species was *Alternaria* spp. It was found in 8 and 25-old wet salted *Alestes* spp. as well as in mature wet salted *Hydrocynus* spp. Nyamwaka[28] found that *Alternaria* sp., was the least isolated fungus species from the samples of sun dried *Rastrineobola argentea* in Gucha South, Kenya. Similar findings were reported by Bassey and Effiong[29] in their study of fungi of dried *Clarias gariepinus* sold in some markets in Nigeria. They reported that *Alternaria* sp., was the least prevailing fungus. On the contrary Hassan *et al.* [16] found that most commonly isolated mold species in the examined *Tilapia nilotica* fish samples were *Alternaria* sp. (90%),

Junaid *et a*l. [22] found that the samples that had moisture content above 15 % recorded the highest fungal count.

According to Nyamwaka [28] the moisture content ranged from 12.24 to 23.54% in sun dried *R. argentea*. The present study found now clear cut correlation between moisture content and fungal count. It recorded highest fungal count in mature wet salted *Hydrocynus* spp. with 49% and *Alestes* spp. with 48% moisture content. The least fungal count was in dry salted *O niloticus* with 44% moisture content.

The presence of fungi species in the wet salted and sun-dried fish studied could be attributed to mal-hygienic practices along the market chain from fisher to the consumer. Fungal attack is encouraged by unhygienic methods by fishers, mongers, processors and sellers as stated by Eyo [30]. Mmycotic contamination of stock fish was reported in Jos Metropolis in Nigeria by Junaid *et al*. [22] and from sun dried *R. argentea* sold in South Gucha, Kenya by Nyamwaka [28]. The presence of these fungi in foods is of great concern in human heath because *Aspergillus* spp., and *Penicillium* spp. produce aflatoxin (Nyamwaka [28]). This should be considered seriously in Sudan as *Aspergillus* spp., and *Penicillium* spp. constituted 66% and 4% of isolates, respectively.

Conclusions

The presence of these fungi is of a great significance in view of fish food safety making consumption of poorly cooked fish hazardous to health. From a nutritional value and food safety perspectives, it is recommended to test the encountered fungi species for aflatoxin to determine the quality of the fish commodity offered in the market.

Conflict of Interests

The authors declared no conflict of interests.

References

1. Webster J, Weber R, eds. Introduction to Fungi. Cambridge University Press, 2007, 841 pp.
2. El-Hag GA, Abu Gideiri YB, Ali ME, Abu Zied IM. Nutritive value and microflora of salted Kawara (Alestes spp.) during storage. Researcher 2012;4(2):69-76.
3. Ahmed RM, Hammad NMH, Mahmoud ZN. Bacteria of some Traditionally Processed Freshwater Fish in Sudan. International Journal of Agriculture and Environmental Research 2019;5(5):684-698. ISSN: 2455-6939.
4. Chauhan R, Lone S, Beigh AH. Pathogenecity of three species of Aspergillus (*A. fumigatus*, *A. niger* and A. *sydowii*) on some freshwater fishes. Life Sciences 2014;48:65-72.
5. Essuman KM. Fermented fish in Africa: A Study on Processing, Marketing, and Consumption. FAO Fisheries Technical Paper 1992, 329.
6. Dirar HA. Flavours and subtitles. The Indigenous Fermented Foods of the Sudan: A Study in African Food and Nutrition. Ed. Ox son Cab International, UK, Wallingford 1993, 411-476.
7. Lnovotny L, Dvorska l, Lorecova A, Beran V, Pavlik, I. Fish: a potential source of bacterial pathogens for human beings. Vet. Med.–Czech 2004;49(9):343-358.
8. Herrera FC, Santos JA, Otero A, García-López ML. Occurrence of foodborne pathogenic bacteria in retail prepackaged portions of marine fish in Spain. Journal of Applied Microbiology 2006;100(3):527-536.
9. Kasozi N, Namulawa VT, Degu GI, Kato CD, Mukalazi J. Bacteriological and physicochemical qualities of traditionally dry-salted Pebbly fish (*Alestes baremoze*) sold in different markets of West Nile Region, Uganda. African Journal of Microbiology Research 2016;10(27):1024-1030.
10. Edema MO, Agbon AO. Significance of fungi associated with smoke cured *Ethmalosa fimbriata* and *Clarias gariepinus*. Journal of Fish Processing and Preservation 2010;34(1):355-363.
11. Shamsan FF, Al-Jobory HJ. Microbial Status of Sun-Dried Fish (Wazef) Sold in Different Yemeni Markets. PSM Biological Research 2018;3(1):1-8.
12. Suleiman A, Hassan Z, Al-Khalifa E. Microbial Safety of Dried Fish Meat (Kejeik) Produced in Sudan. Food and Nutrition Sciences 2014;5(7):606-613.
13. Ammar MAM. Sanitary assessment of some common fresh water fish in Assiut. M.V.Sc. Thesis, Faculty of Veterinary Medicine, Assuit University, Egypt 2001.
14. Youssef MS, Abo-Dahab NF, Farghaly RM. Studies on Mycological Status of Salted Fish "Moloha" in Upper Egypt. Mycobiology 2003;31(3):166-172.
15. El-Ahl RS. Studies on fungi in fish and fish products and their control. Ph. D. Thesis, Department of Microbiology, Faculty of Science, University of Cairo, Egypt 2010.
16. Hassan AA, Hassan AM, El-Shafei HM, El-Ahl RS, Abd El-Dayem RH. Detection of aflatoxigenic molds isolated from fish and their products and its public health significance. Nature and Science 2011;9:106-114.
17. Melaku H, Lakew M, Alemayehu E, Wubie A, Chane M. Isolation and Identification of Pathogenic Fungus from African Catfish (*Clarias gariepinus*) Eggs and Adults in National Fishery and Aquatic Life Research Center Hatchery, Ethiopia. Fisheries and Aquaculture Journal 2017;8:213. Doi: 10.4172/2150-3508.1000213.
18. Waksman SA. The Actinomycetes-Classification, Identification and Description of Genera and Species. The Williams and Wilkins Co., Baltimore 1961, 363 pp.
19. Tournas V, Stack ME, Mislivec PB, Koch HA, Bandler R. Bacteriological Analytical Manual Chapter 18 Yeasts, Molds and Mycotoxins 2001, https://www.fda.gov/food/laboratory-methods-food/bam-chapter-18-yeasts-molds-and-mycotoxins
20. Surendran P, Nirmala T, Narayanannambiar KV, Lalitha KV. Laboratory manual on microbiological examination of seafood, (URI), 2005. http://hdl.handle.net/123456789/1792
21. AOAC. Association of Official Analytical Chemists. 20th Edition, 2016.
22. Junaid SA, Olarubofin F, Olabode AO. Mycotic contamination of stock fish sold in Jos, Nigeria. Journal of Yeast and Fungal Research 2016;1(7):136-141.
23. Fafioye O, Efuntoye M, Osho A. Studies on the fungal infestation of five traditionally smoke-dried freshwater fish in Ago-Iwoye, Nigeria. *Mycopathologia* 2002;154:177-179.
24. Adebayo-Tayo BC, Onilude AA, Patrick UG. Mycofloral of smoked-dried fishes sold in Uyo, Eastern Nigeria. World Journal of Agricultural Sciences 2008;4:346-350.
25. Wheeler K, Hocking AD, Pitt JH, Anggawati AM. Fungi associated with Indonesian dried fish. Microbiology 1986;3(4):351-357.
26. Atapattu R, Samarajeewa U. Fungi associated with dried fish in Sri Lanka. Mycopathologia 1990;111:55-59.
27. Santoso I, Gandjar I, Sari RD, Sembiring ND. Xerophilic Moulds Isolated from Salted and Unsalted Dried Fish from Traditional Markets in Jakarta. Indonesian Food and Nutrition Progress 1999;6(2):55-58.

28. Nyamwaka IS. Determination of Fungi and Factors Associated with their Growth on Sun Dried *Rastrineobola argentea* in Gucha South, Kisii County, Kenya. M. Sc. (Microbiology) in the School of Pure and Applied Sciences of Kenyatta University 2014.
29. Bassey IN, Effiong MU. Fungi associated with post-harvest deterioration of dried *Clarias gariepinus* vended in some markets in Uyo, Akwa Ibom State, Nigeria. Journal of Aquatic Sciences 2016;31(2B):409.
30. Eyo AA. Fish Processing Technology in the Tropics. University of Horin Press 2001, 403pp.