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 stolonifera, Alternaria sp. and Pencilium 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]). 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 ‘Kajjake fish’ was investigated by Suleiman et al. [12]. In Egypt the fungi work of Ammar et al. [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°C till fungal examination.
Table 1: Source of salted fish samples

| Code       | Sample source            | Processing state         |
|------------|--------------------------|--------------------------|
| 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.       |
|            | "Alestes" 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.       |
|            | "Hydrocynus" spp.        |                          |
|            |                          |                          |
| F10        | Huda fish foundation     | Mature wet salted.       |
|            | "Maloha" a mixture of "Alestes" and "Hydrocynus" spp. | |
| F12 and 19 | Khartoum Fish market     | Mature wet 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 4°C 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 [11] 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 stolonifera, Alternaria sp. and Pencillium sp.

Plate 1: Green Aspergillus niger culture
Plate 2: Green Aspergillus flavus culture

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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. stolonifera*, *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. stolonifera*, 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. recorded 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. stolonifera* (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   | Un F       | Un F       | 0         | 0         | 0         | 0         | 0         |
|      | PCA+  | 0          | Un F       | 1         | 0         | 0         | 0         | 0         |
|      | PDA   | 0          | Un F       | 0         | 0         | 0         | 0         | 0         |
| F2   | YEA   | Un F       | Un F       | 0         | 0         | Un F      | 0         | 0         |
|      | PDA   | 0          | Un F       | 0         | 0         | 0         | 0         | 0         |
| F3   | PDA   | Un F       | Un F       | 0         | 0         | 0         | 0         | 0         |
| F4   | PDA   | Un F       | Un F       | 0         | 0         | 0         | 0         | 0         |
| F5   | YEA   | Un F       | Un F       | 0         | 0         | 0         | 0         | 0         |
|      | PDA   | 0          | Un F       | 0         | 0         | 0         | 0         | 0         |
| F6   | YEA   | Un F       | Un F       | 0         | 0         | 0         | 0         | 0         |
|      | PDA   | Un F       | Un F       | 0         | 0         | 0         | 0         | 0         |
| F7   | PDA   | Un F       | Un F       | 0         | 0         | 0         | 0         | 0         |
| F8   | All media showed no fungal growth |
| F9   | PDA   | Un F       | 0          | 0         | 0         | 0         | 0         | 0         |
| F10  | YEA   | 0          | 0          | 0         | 0         | 0         | Un F      | Un F      |
|      | PDA   | 0          | 0          | 0         | 0         | 0         | 0         | 0         |
| F11  | PDA   | Un F       | 0          | 0         | 0         | 0         | 0         | 0         |
| F12  | PDA   | Un F       | Un F       | 0         | 0         | 0         | 0         | 0         |
| F13  | PDA   | Un F       | Un F       | Un F      | Un F      | Un F      | Un F      | Un F      |
| F14  | YEA   | Un F       | 0          | 0         | 0         | 0         | 0         | 0         |
|      | PDA   | 1 F        | 0          | 0         | 0         | 0         | 0         | 0         |
| F15  | YEA   | Un F       | 0          | 0         | 0         | 0         | 0         | 0         |
|      | PDA   | 0          | 0          | 0         | 0         | Un F      | Un F      | Un F      |
| F16  | PDA   | 3 F        | 1 F        | 0         | 0         | 0         | 0         | 0         |
| F17  | PCA   | 4          | 0          | 0         | 0         | 0         | 0         | 0         |
|      | PCA+  | Un B       | Un B       | 0         | 0         | 1         | 0         | 0         |
|      | MSA   | 3          | 0          | 0         | 0         | 1         | 0         | 0         |
| F18  | YEA   | Un F       | 0          | 0         | 0         | 0         | 0         | 0         |
|      | PDA   | Un F       | Un F       | Un F      | Un F      | Un F      | 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 |
|------|----------------|--------|--------|---------------|
| F1   | $R_1$ and $R_2$ | YEA    | Black  | Aspergillus niger |
|      | $R_3$          | PDA    | Green  | Aspergillus flavus |
|      | $R_4$          | PDA    | Black  | A. niger       |
| F2   | $R_5$          | YEA    | Black  | A. niger       |
|      | $R_6$          | YEA    | Grey   | Rhizopus stolonifera |
|      | $R_7$          | YEA    | Grey   | R. stolonifera |
|      | $R_8$          | PDA    | White  | R. stolonifera |
| F3   | $R_9$          | PDA    | Black  | A. niger       |
The highest fungal count in mature wet salted fish was in *Hydrocyamus* sp. with 49% moisture and 27% organic content, followed by *Alestes* sp. with 48% moisture and 32% organic content. The least fungal count was in dry salted *O. niloticus* with 90% organic content. The least fungal count was in dry salted *O. niloticus* with 90% organic content. In the rest of the samples single or bi-blum fungi were encountered. In the rest of the samples single or bi-blum fungi were encountered. In the rest of the samples single or bi-blum fungi were encountered. The fungal species occurrence in a descending order was *A. niger* (60%), *R. stonloifara* (26%) *A. flavus* (6%), *Alternaria* sp. (4%) and *Penicillium* 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 Yousuf 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.

Fasiyo et al. [23] reported that fungi of samples of traditionally smoke-dried fishlike Clarisas 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 Yemen. 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 *Polypaecium pisce*, 3 *Eurotium* spp., 5 *Aspergillus* spp. and a variety of *Penicillium* spp. Atapattu and Samarajewa [26] in their study of fungi of dried fish in Sri Lanka, reported *Aspergillus* flavus, *A. fumigatus*, *A. glaucus*, *A. restrictus*, *Aureobasidium* spp. *Bispetospora 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 Kejek samples (probably *Clarias* spp.). Occurrence of *Penicillium* spp. in some samples during this investigation is in agreement with Yousuf et al. [14] work on Malooha and Shamsan and Al-Jobory [11] on sun-dried fish. In the present study the least isolated fungi species was *Alternaria* sp. It was found in 8 and 25-old wet salted *Alestes* spp. as well as in mature wet salted *Hydrocyamus* 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 al. [22] found that the samples that had moisture content above 15 % recorded the highest fungal count.

| R | PDA | Black | Alternaria sp. |
|---|-----|-------|----------------|
| F4 | R11 | PDA | Black | *A. niger* sp. |
| F5 | R12 | YE A | Black | *R. stonloifara* |
| F6 | R13 | YE A | Black | *Pencillim* sp. |
| F7 | R14 | YE A | Black | *Alternaria* sp. |
| F8 | R15 | YE A | Green | *A. Flavus* |
| F9 | R16 | PDA | Black | *A. niger* |
| F10 | R17 | PDA | Grey | *R. stonloifara* |
| F11 | R18 | PDA | Black | *A. niger* |
| F12 | R19 | PDA | Grey | *R. stonloifara* |
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 Hydrocyamus 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 attacks are encouraged by unhygienic methods by fishers, mongers, processors and sellers as stated by Eyo [30]. Mycotic 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.

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