MYCOFLORA OF SMOKE-DRIED FISH FROM ROAD SIDE TABLE MARKETS IN TORU-ORUA, SAGBAMA, BAYELSA STATE, NIGERIA

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ABSTRACT
Occurrence of mycoflora on smoke-dried *Chrysichthys nigrodigitatus*, *Clarias gariepinus* and *Parachana obscura*, obtained from roadside table marketers in Toru-Orua was studied. The fungal infestation was examined prior to the standard treatment of each fish species tissue to sterile media preparation (Potato Dexirose agar) isolation, serial dilution plating, incubation, counting isolator and inventory/identification. All the fish species examined had signs of fungal infections which include *Mucor racemosus*, *Asperigillus fumigatus*, *A. niger*, *A. flavus* and *Penicillum marneffei*. The specimens, *P. obscura* and *C. gariepinus* had 3 fungal isolates, whereas the *C. nigroditatus* had 2 fungal isolates. And the rate of infestation ranged from $4x10^3$/gm (*A. niger*) to 10 $x10^3$/gm (*Mucos racemos*) in the Northern Station, while in the Southern Station the range was $2.0x10^3$/gm (*A. falavus*) to $5.0x10^3$/gm (*A. fumigatus*). Mean percentage infection was, *Asperigillus spp*. 87.5%, *Mucor spp*. 37.5% and *penicillum marneffei* 37.5%. Also the northern Toru-Orua roadside table market had 5 fungi isolated during the studied, while the southern Toru-Orua roadside table market had 4 fungi isolated. Though, the mean rates of infestation in the two locations were not significantly difference (P>0.05), *Parachanna obscura* in the North Station was found to be the most infested fish species with these three fungal isolates; *Asperigillus fumigatus, Mucor racemosus, and Penicillum marnieffi*. The study suggested mycoflora infestation of sold smoke-dried fish in Toru-Orua town and the need for improved handling, processing, and storage methods for the availability of safe smoke-dried fish for consumption.

Keywords: Fish spoilage, Mycoflora isolation, Mycoflora identification, Rates of infestation.

INTRODUCTION

Fish and fisheries products are frequently perishable, which necessitates adequate preparation, preservation, and processing to ensure consumers are receiving wholesome products. A popular traditional method of keeping fishery product in wholesome condition is smoke drying (Olokor *et al.*, 2007, and Foline *et al.*, 2011). However, the stored smoked dried fishes are still susceptible to the invasion of rodents, insects, and micro-organism such as fungi (Eyo, 2012).

The occurrence of mycoflora as micro-organism in fish spoilage has not only been recognized earlier, but its public health implications well emphasized (FAO, 2002, Okonta and Ekelemu, 2005, and Bankole and Kpodo, 2005). The study by Falioye *et.al*. (2002) had identified some fungi in the smoke-dried fishes in Ago-Iwoye, Ogun State, such as *Mucor spp*., *Asperigillus spp.*, *Rhizopus spp*., and *Fuscavim spp*. In fact, Bukola *et al*. (2008) investigated mycoflora of smoke-dried fishes sold in Akwa Ibom State and identified numerous mycoflora including *Asperigillus flavus, A. tereus, A. fumigatus, Absida spp*. and *Penicillum spp*. with *A. tereus* occurring the highest. According to Walter *et al*. (2020), fungi associated with smoke-dried fish belong to five Genera: *Aspergillus, Penicillum, Candida, Acremonium, and Rhizopus*. Also, Fasuan *et al.* (2022) stated that the handling process in the area of storage of smoke-dried fish, facilitate microbial deterioration under relatively high humidity condition. With the establishment that fish is a vector for the transmission of some mycoflora agents especially in humid environment, this study was designed. Its objective was to investigate the occurrence of mycoflora on smoke-dried fishes sold at roadside table marketing outlets in Toru-Orua, a fast-developing University and deltaic community in Bayelsa State.

MATERIALS AND METHODS

STUDY AREA

The study was carried out at Toru-Orua Town, a community located along the Forcados River. The town is 13.6Km from Sagbama Town, the Administrative Headquarters of Sagbama Local Government Area of Bayelsa State. Toru-Orua shares similar vegetation and climate conditions with other communities in Sagbama and Ekeremor LGAs which are under the West Senatorial District

of Bayelsa State (Daukere, *et al*., 2020). The area is characterized by a tropical rain forest with rainfall occurring generally every month of the year and ranged 2000m-2500m and with short dry season. The temperature is high all-round the year with relatively constant high humidity within the range of 75-85% (Google Weather Forecast in September, 2021). The vegetation in the area is freshwater swamps and lowland rainforest. Enamored by the location of the River Forcados as a result of their ancestral fishing occupation, communities settlement pattern are mostly linear, paralleled along the river/coastal banks (Mnom and Akpi, 2014). Toru-Orua town lies between Latitude 5° 06′ 07″ North and Longitude 6° 03′ 59″ East.

Based on a recognizance study, two major areas where table markets are prevalent were identified and designated as North (Station A) and South (Station B) locations of the town with distance of 225.01m apart. Fishes for the study were selected based on their prevalence and selected fish species were *Chrysichthys nigrodigitatus, Clarias gariepinus* and *Parachana obscura*.

SAMPLE COLLECTION

Smoke-dried *C. nigrodigitatus, P. obscura* and *C. gariepinus* samples were purchased from roadside table marketing outlets in Toru-Orua, Sagbama Local Government Area of Bayelsa State and kept in polythene bags to the laboratory for immediate study.

Between May and June, 2020, a total of twenty-four samples of smoke-dried fish comprising of eight *C. nigrodigitatus, C. gariepinus* and *P. obscura* each from both sampling stations, were purchased.

LABORATORY PREPARATION AND ANALYSIS

In the laboratory, each fish sample was examined with a hand lens to ascertain that there are no micro-organisms infestation such as whitish or web-lick cover on the external skin. Micro-organismal growth was promoted by aseptically cutting about ten grams portion of each fish tissue and macerated. Each macerated tissue was mixed with 90ml of peptone water and placed on a medium of Potato Dextrose Agar in which 250ml of Chloramphenicol as antibacterial agent was added. After thorough mixing, 1ml each of the sample solution was diluted and placed on petri dishes in duplicates. Thereafter, the petri dishes were incubated at 30°C for 7days and observed regularly for fungal infestation.

After the incubation period, the whole medium on the petri-dish was observed with hand-held lens to note the arrangement of the spores. Then, a piece of the Mycelium was removed with a needle and mounted on a slide, stained, following Gram staining Process, dried, and observed microscopically and fungi seen were identified to species level by using identification keys (Joseph, 1974, Samson *et al*., 1981, and Barley and Scott, 2003).

DATA ANALYSIS

Data collected were analyzed using descriptive statistics and subjected to student t-test, to compare means of microbial counts using SPSS statistics 23.

RESULTS

The identification of the isolated fungal organism was aided with the morphological characteristics shown in Table 1, especially, colour, size, and results of the Gram staining process. The colour observed were creamy white (*A. fumigatus*), white buffy (*M. racemosus*), brown black A. niger and grey fluffy (*Penicillium marneffei*). The sizes of the mycelium also varies ranging from 3mm in *A. Fumigatus*, *A. niger* to 1mm in *Penicillium marneffei*. Also the Gram staining process in this study gave Gram positive hypha for *A. fumigatus, Mucor racemosus* and Gram positive mycelium in *P. marneffei*, whereas *A. niger* showed Gram positive rough wallconidia conidiophore.

Table 1: Morphological Characteristics of Fungal Isolates, after Gram Staining & Identified Fungi.

SN	COLOUR OF SPORE	APPROXI. SIZE OF SPORE	PRESENCE OF MYCELIUM	GRAM STAINING POSITION (+VE & -VE)	FUNGI ISOLATED & IDENTIFIED
1	Creamy white	3mm	Yes	Positive hypha	*Asperigillus fumigatus*
2	White buffy	2mm	Yes	Positive hypha	*Mucor racemosus*
3	Brownish black	3mm	No conidiophore	Positive rough wallconidia	*Asprigillus niger*
3	Grey flubby	1mm	Yes	Positive mycellium	*Penicillum marneffei*

The fungal organisms isolated and identified from the three fish specimens and the number of organism per gram of fish tissue from the two sampling stations (North and South) are shown in Table 2. The result revealed that fungi infestation on the smoke-dried fishes studied were fish specific, for example, *P. obscura* and *C. gariepinus* had three fungi species isolated and identified as *Aspergilli fumigatus, Mucor racemosus* and *Penicillium marneffi*, whereas, in *C. gariepinus*, *A. niger, A. falvus* and *Penicillum marneffi* were isolated and identified. In fact, *Aspergillis spp*. were the most frequently occurring fungi in smoked-dried fishes in Toru-Orua. In terms of sampling stations, the Northern sampling station had more fungi infestation on smoke-dried fishes with seven species, whereas the Southern area had only four isolated and identified fungi organisms such as *Mucor racemosus, A. famigatus, Penicillum marneffi* and *A. falvus*. The rate of infestation (Table 2) further showed that there is more infestation $10x10^3$ organisms per gram of fish flesh in *P. obscura* followed by *Clarias gariepinus*, $9x10^3$ per gram of fish flesh. In the Southern Toru-Orua area, the rate of infestation was $5x10^3$ per gram of fish flesh in *P. obscura* followed by *C. nigroditatus* $4x10^3$ and *C. gariepinus* $1x10^3$ per gram.

Table 2: Summary of the Fungal Species Isolated, Identified, and Rate of Infestation on Smoked Dried Fishes in Toru-Orua, Bayelsa State.

SN	SCIENTIFIC NAME OF STUDIED FISH	COMMON NAME OF FISH	FUNGAL SPECIES ISOLATED AND IDENTIFIED	SAMPLE STATION IN TORU-ORUA ROADSIDE TABLE OUTLET NORTHERN (A) AREA RATE OF INFESTATION	SOUTHERN (B) AREA RATE OF INFESTATION
1	*Chrysichthys nigrodigitatus*	Grey Catfish	⋆*Asperigillus fumigatus* ⋆*Mucor racemosus*	$7.0x10^3$/g 5.0 $x10^3$/g	------- 4.0 $x10^3$/g
2	*Parachanna obscura*	Snakehead	⋆*Asperigillus fumigatus* ⋆*Mucor racemosus* ⋆*Penicillum marnieffi*	8.0 $x10^3$/g 10.0 $x10^3$/g 4.0 $x10^3$/g	5.0 $x10^3$/g ----- 2.0 $x10^3$/g
3	*Clarias gariepinus*	Africa walking catfish	⋆*Asperigillus niger* ⋆*A. falvus* ⋆*Penicillum marnieffi*	4.0 $x10^3$/g ----- 9.0 $x10^3$/g	----- 1.0 $x10^3$/g -----

DISCUSSION

The five fungal isolates encountered in the study were; *Mucor racemosus*, *Asperigillus fumigatus*, *Asperigillus niger*, *Asperigillus flavus* and *Penicillum marneffei* which is similar to the report of Walter *et al*. (2020), all being found during storage of the fish. The result on morphological characteristics were like the colours and sizes of

fungi isolated in the study by Okonta and Ekelemu (2005) and Oku and Amakoromo (2013). The same characteristics were also observed during the study of traditional smoke-dried freshwater fish in Ago-Iwoye, Ogun State, by Faloye, *et al*., (2002). The identified fungi which are also storage fungi are often known to be associated with area of high relative humidity which are above the critical value of 80-86% (Eyo, 2001, and Akani and Nwankwo, 2019, and Fasuan *et al.*, 2022). Since Toru-Orua is located within the Niger Delta with a high relative humidity of 80-90% and within the critical relative humidity, suggests that one primary cause of these fungi identified is high relative humidity which promotes the growth of fungi on smoke-dried fishes.

Asperigillus spp. were the most prevalent in this study. Similar observation was also reported by Faloye *et.al*. (2002), Bukola *et al*., (2008), Wogu and Iyayi (2011), Akani and Nwankwo (2019). The study of Chukwuemeka *et al*., (2020) reported that three of the five different markets sampled smoke-dried fishes indicated highest mean fungal counts of *Asperigillus spp*. In fact, they observed the prevalence of *Asperigillus spp.*, *Mucor spp*., and *Penicillum spp*. on most of the smoke-dried fish sold in their study area. In this study, fungi infestation were more in the North sampled station than South sampled station where only four fungi organisms were identified. This observation may be as a result of higher relative humidity in the Northern part and inadequate processing and handling techniques giving rise to harmful micro-organisms as reported by Hagos (2021) and Fasuan *et al.* (2022). This micro-organisms thrive in acid content medium of different fish flesh, i.e. various micro-organisms react differently in an acid medium. In fact, it is reported that moulds develop readily in an acidic medium and can thrive for long time (Eyo, 2001, De Meridol, 1969, Faloye *et al*., 2002).

On the rate of infestation, the Northern station had the highest rate per gram of fish flesh than Southern station. This could be due to higher relative humidity in the Northern part and inadequate processing and handling techniques. With *Parachana obscura* showing the highest ($10x10^3$), followed by *Clarias gariepinus*, $9x10^3$ per gram of fish flesh.

The implications of the results are that fungal and moulds infestation of smoke-dried fishes in Toru-Orua, deltaic positioned community with high relative humidity that promotes the growth of the micro-organisms, could be due to inadequate processing and handling techniques. Also, the practice of the mode of displaying fish for sale once removed from the smoking kiln may expose the fish that are not well dried into the environment that is highly humid. This in addition to the mode of storage and the handling methods may have led to the observations made in this study which agrees with the report of Bukola *et al*., (2008). The use of polythene bags/sheets encourages warmth and prevent proper air circulation leading to the accumulation of moisture, then infestation of moulds, fungi and insects (Eyo, 2001 and Faloye *et al*., 2002).

CONCLUSION

The findings of this study showed that fungal infestation is a common occurrence in smoke-dried fish in Toru-Orua, Bayelsa State especially for the species sampled. The fungi isolated and identified in this study gave an indication of the species that are most likely to cause spoilage of smoke-dried fish during storage in Toru-Orua, Bayelsa State. However, more studies on processing and handling techniques are needed to obtain a comprehensive checklist of all possible fungal on available fish species. This shall help provide ways of preventing their infestation so as to provide high quality smoke-dried fish for the ever-increasing number of consumers in Toru-Orua a rapidly expanding University community in Bayelsa State.

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