

Isolation and identification of fungi from apparently healthy and diseased *Clarias gariepinus* from freshwater in Zaria, Kaduna State, Nigeria

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ABSTRACT

Fish and fishery products have been documented as a major source of food-borne pathogens of which bacteria and fungi play major roles. The fungal organisms contaminating apparently healthy and diseased *Clarias gariepinus* in Zaria, Kaduna State was investigated. The standard mycological procedure revealed isolation of similar fungal organism been associated with apparently and diseased fish. The fungal organism isolated from the skin and gills of diseased fish were 26 (13.1%) *Aspergillus fumigatus*, 25 (12.6%) *A. flavus*, 36 (18.1%) *Aspergillus niger*, 37 (18.7%), *Penicillium* species, 14 (7.1%) *Mucor* species, 4 (2.0%) for *Rhizopus* species, 14 (7.1%) *Trichophyton* sp., and 42 (21.2%) yeast isolates. While the gills and the skin of apparently healthy *C. gariepinus* yielded 73 (18.6%) *Aspergillus fumigates*, 53 (13.5%) *A. flavus*, 57 (14.5%) *A. niger*, 1 (0.25%) *A. parasiticus*, 45 (11.55%) *Penicillium* sp., 38 (9.7%) *Mucor* species, 6 (1.5%) *Rhizopus* sp. and 119 (30.4%) yeast. There was no statically significant ($P > 0.05$) difference in isolates from skin and gills. All the fish were positive for multiple fungal isolates. The diseased *C. gariepinus* were characterised with skin ulcer, fin rot and tail rot. The study showed the majority of the isolates belonging to *Aspergillus* sp. and potential aflatoxin producers. Isolation of pathogenic fungi from skin and gills of *C. gariepinus* from cultures, meant for human consumption is alarming which can be a source of fish born infections. Attention should be paid to fish health in the culture so as to conserve public health and safeguard the farmer's investment.

Keywords: *Clarias gariepinus*, diseased, apparently healthy, fish, yeast and filamentous fungi.

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INTRODUCTION

Nigeria is blessed with over 14 million of hectares of reservoirs, lake, ponds and major rivers which account for about 12.4% of the total surface area of the nation (Olaosebikan and Raji, 1998). These water bodies are capable of producing over 980,000 metric tonne of fish annually (FDF, 2007). These bodies of water in addition to aquaculture and fish culture constitute sources of fish supply from inland fisheries of Nigeria (Adesulu et al., 2004).

The disease is one of the most important and universally recognized constraints of fish production in

Nigeria posing threats to the commercial success of aquaculture and fish farming (Nkemakolam et al., 2011). Diseases of fish may be of viral, bacterial, Chlamydia, fungal, rickettsial or parasitic origin.

Fungal diseases are the most serious cause of losses in aquaculture (Meyer, 1991) being the most recurrent type of disease problem in all types of aquaculture facilities resulting in considerable economic losses in the industries (Meyer, 1991). Many of the fungi that affect fishes are considered opportunists, attacking the fishes when they are stressed or immune-compromised, or they

may be secondary to bacterial or viral infections, or when they have lost their mucus protection because of trauma or excessive handling or due to inadequate nutrition (Roberts, 1989; Quiniou et al., 1998). Many fungal organisms infect fish causing spoilage leading to huge financial losses either in term of cost of medication to treat or outright loss of the fish to diseases; while some are of zoonotic potential, causing diseases in animal and human, and many may produce metabolites that can cause deleterious effect in man and animals (Douglas *et al.*, 2000).

Many fungal species can spoil food products or produce mycotoxins or both (Anderson and Thrane, 2006). Fungal mycotoxins produce a wide range of injurious effects in animals, in addition to causing food borne hazards to humans and these mycotoxins may result in decreased productivity, immune suppression and chronic damage to vital tissues and organs of animal and humans (CAST, 2003). Some of the fish pathogens are of public health importance, while others are part of the microbiota of both *Clarias* and *Tilapia* (Olojo et al., 2010, Efuntoye et al., 2012). Hence it is necessary to identify fungal organisms associated with diseased and apparently healthy fish in the target area.

MATERIALS AND METHODS

Samples

Seventy-eight diseased *Clarias gariepinus* of various sizes with the ulcer, fin rot, and tail rot and one hundred and seventy eight (178) apparently healthy *C. gariepinus* were sourced from integrated fish farms. The fish were raised in concrete ponds and fed on commercial feeds. Individual fish was collected using a sterile polyethylene bag and transported within an hour of the collection to the Department of Veterinary Microbiology Diagnostic Laboratory, Ahmadu Bello University. Skin scrapings, lesions scrapings and gills swabs were collected from diseased and apparently healthy *C. gariepinus* by first disinfecting individual fish with cotton wool soaked in 70% ethyl alcohol. Skin scrapings were collected by scraping the lesions and the surrounding skin of diseased *C. gariepinus* and the skin of apparently *C. gariepinus* using sterile scalpel blade into sterile Petri dishes. The operculum covering the gills was raised up then sterile swab rolled around the gills.

Fungal isolation and identification

Each sample was inoculated onto sabouraud dextrose agar (composition gm/ltr-mycological peptone-10, agar-16, dextrose-40, ph 5.6 ± 0.2) incorporated with chloramphenicol (to inhibit bacteria growth) and incubated at 25°C for 5 to 10 days and examined for fungal growth. Identification of fungal isolates was carried out by examining the morphology and microscopic features (Dugan, 2006) using the microscope model Boeco, Germany.

Morphological examination included growth rate, general topography, surface and reverse pigmentation if any. Microscopic identification of positive fungal cultures was carried out using the method described by Murray *et al.* (2005). Briefly, a drop of lactophenol cotton blue dye was placed on a clean glass slide; a portion of the fungal culture was transferred into the lactophenol cotton blue dye and teased with a 22 gauge nichrome needle to separate the hyphae. The coverslip was placed on the preparation

and examined under low and high power magnification using reduced light for identification. Microscopic characteristic of fungi such as hyphae, conidial heads and arrangements of conidia were observed. Slide culture was carried out on those isolates whose identification was inconclusive after staining with lactophenol cotton blue (Domsch et al., 2007).

Data analysis

Data generated from this work was presented in proportion and tables using Excel software (2007). Chi-square with contingency tables was used to analyze the data.

RESULTS

The sampled diseased *C. gariepinus* were characterized by skin ulcer, fin rot and tail rot (Figure 1" = A, B and C) while the apparently healthy *C. gariepinus* showed no clinical lesions. All the samples from the affected fish and apparently healthy fish were positive for multiple fungal growths after culture for 5 to 10 days and all isolates grew well on SDA incorporated with chloramphenicol except the dermatophytes that took up to ten days before any growths became visible. Occurrences of fungi in diseased and apparently healthy *C. gariepinus* were recorded in Tables 1 and 2. Morphologic and microscopic characteristics of various fungal species were shown in Figures 2 to 8.

Of the one hundred ninety eight (198) fungal isolates from 78 samples of skin and gills of diseased *C. gariepinus*, 26 (13.1%), *Aspergillus fumigatus*, 25(12.6%), *A. flavus*, 36 (18.1%), *Aspergillus niger*, 37 (18.7%), *Penicillium* species, 14 (7.1%) *Mucor* species, 4 (2.0%) for *Rhizopus* species, 14 (7.1%), *Trichophyton* sp., and 42 (21.2%) yeast isolates were isolated (Table 1).

A total of three hundred and ninety two (392) fungal isolates were recovered from the skin and gills of 178 apparently healthy *C. gariepinus* of which were 73 (18.6%) *Aspergillus fumigatus*, 53 (13.5%) *A. flavus*, 57 (14.5%) *A. niger*, 1 (0.25%) *A. parasiticus*, 45(11.55%) *Penicillium* sp., 38 (9.7%) *Mucor* species, 6(1.5%) *Rhizopus* sp. and 119 (30.4%) yeast (Table 2).

Aspergillus sp. was isolated from the two groups of fish as the predominant species. Aflatoxin-producing *A. parasiticus* and *A. flavus* were also isolated with *A. flavus* been the major sp. while *A. parasiticus* been the least. Similar isolates were recovered from skin and the gills of the two groups of fish except for *A. parasiticus* and *Trichophyton* sp. that were isolated from apparently healthy and diseased fish, respectively.

DISCUSSION

The fungal organisms isolated from diseased and apparently healthy fish in this research were somewhat similar. This was expected, as *Aspergillus* sp., *Mucor* sp.,



Figure 1. A = ulcer and fin rot, B = Caudal fin rot or tail rot, C = Skin ulcer.

Table 1. Rate of fungal occurrence on the skin and gills of diseased *Clarias gariepinus* from Zaria, Kaduna State (P > 0.05).

Isolates	Skin no.	Gills no.	Total (No.)	Percentages (%)
<i>A. fumigatus</i>	13	13	26	13.1
<i>A. flavus</i>	12	13	25	12.6
<i>A. niger</i>	19	17	36	18.2
<i>Penicillium</i> sp.	15	22	37	18.7
<i>Mucor</i> sp.	9	6	14	7.1
<i>Rhizopus</i> sp.	1	3	4	2.0
<i>Trichophyton</i> sp.	5	9	14	7.1
Yeast	23	19	42	21.2
Total	97	102	198	100

$$\chi^2 = 4.48, df = 7, P = 0.72.$$

Table 2. Rate of fungal occurrence on the skin and gills of apparently healthy *Clarias gariepinus* from Zaria, Kaduna State (P > 0.05).

Isolates	Skin no.	Gills no.	Total (No.)	Percentages (%)
<i>A. fumigatus</i>	35	38	73	18.6
<i>A. flavus</i>	22	31	53	13.5
<i>A. niger</i>	29	28	57	14.5
<i>A. parasiticus</i>	0	1	1	0.3
<i>Penicillium</i> sp.	23	22	45	11.5
<i>Mucor</i> sp.	19	19	38	9.7
<i>Rhizopus</i> sp.	4	2	6	1.5
Yeast	62	57	119	30.4
Total	195	197	392	100

$$\chi^2 = 4.13, df = 7, P = 0.76.$$

Penicillium sp. and *Rhizopus* sp. were categorized by Refai et al. (2010) as normal mycobiota of freshwater fish. Even though these isolates are normal mycobiota of both *Clarias* and *Tilapia*, they can, however, produce disease because they are opportunistic fungi and many of them possess virulence factors, which enable them to cause diseases (Refai et al., 2004) especially under favourable predisposing condition. This finding agrees

with the reports of other investigators that fungal infections are among the most serious types of disease problems in all types of aquaculture facility causing economic losses in fish farming in tropical Africa, and in other developing countries (Meyer, 1991). Several studies have documented several fungal agents associated with freshwater fish in Nigeria and all over the world. Christianah and Fagade (2010) while evaluating

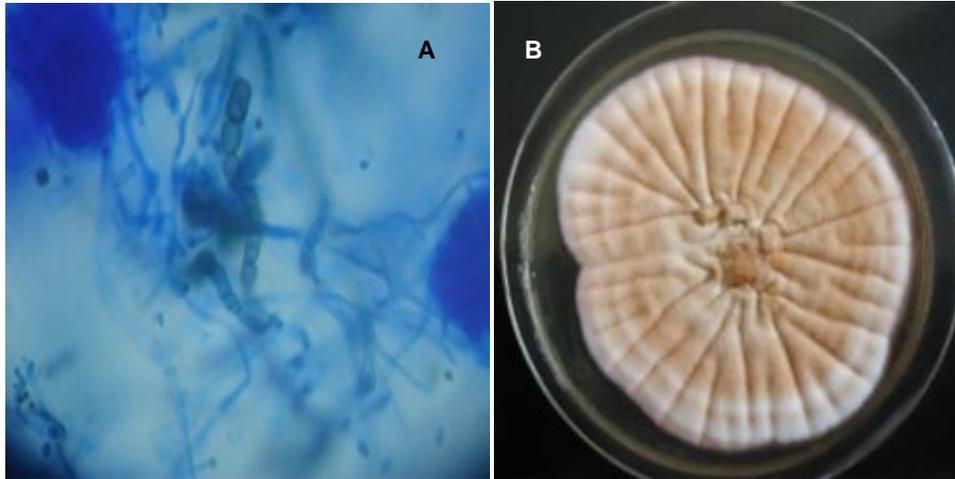


Figure 2. A = Microscopic view (x40 magnification) of *Trichophyton* sp. B = Culture of *Trichophyton* sp on SDA.

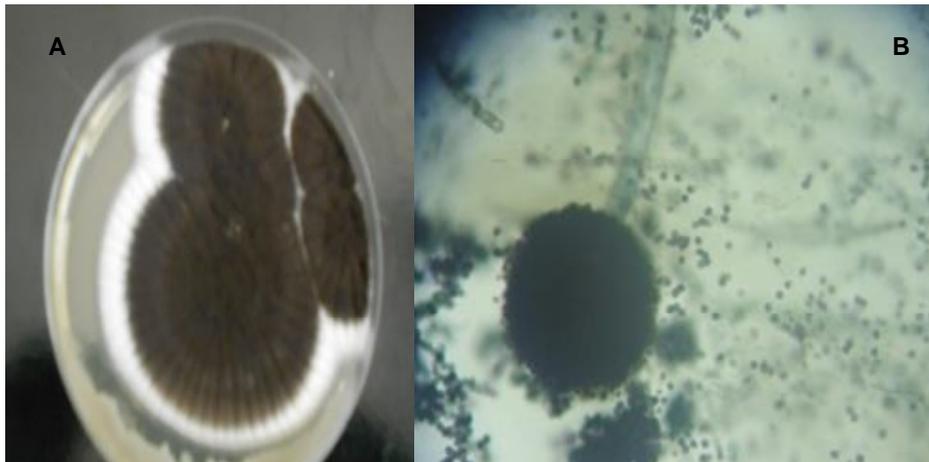


Figure 3. A = Culture of *Aspergillus niger* on SDA. B = microscopic view (x40 magnification) of *Aspergillus niger*.

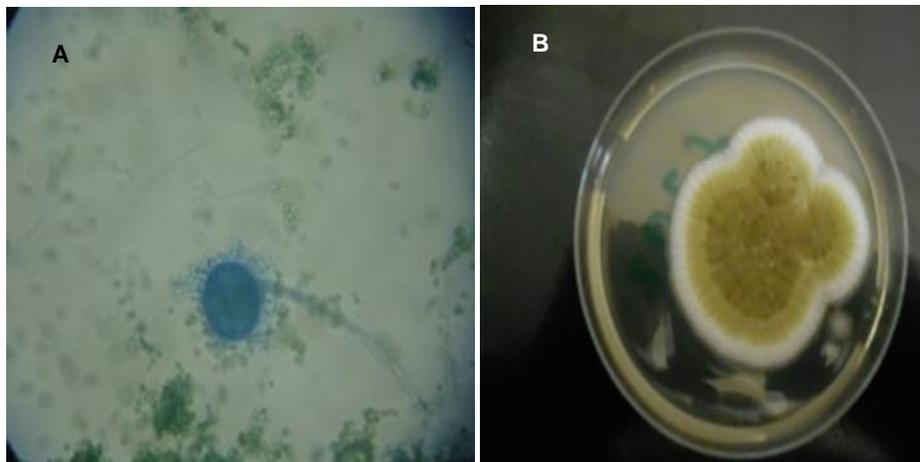


Figure 4. A = Microscopic view (x40 magnification) of *Aspergillus flavus*. B = culture of *A. flavus* on SDA.

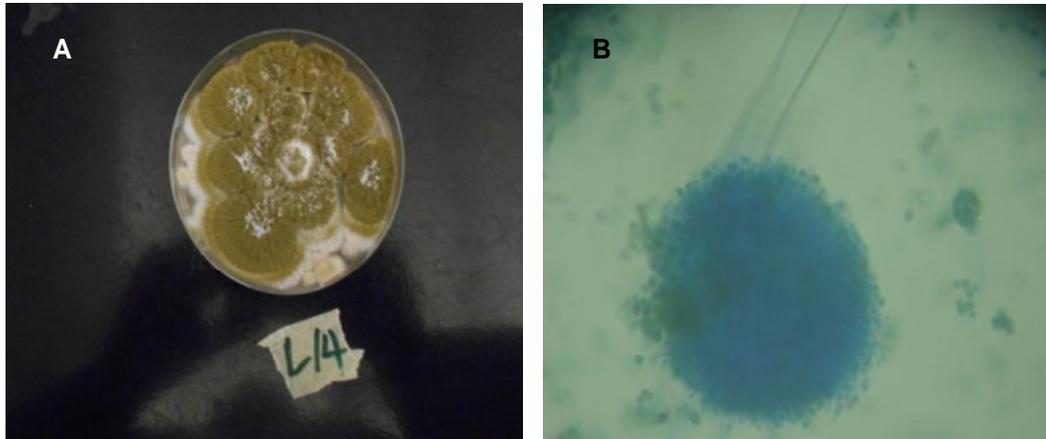


Figure 5. A = Culture of *Aspergillus parasiticus* on SDA. B = Microscopic view (x40 magnification) of *A. parasiticus*.

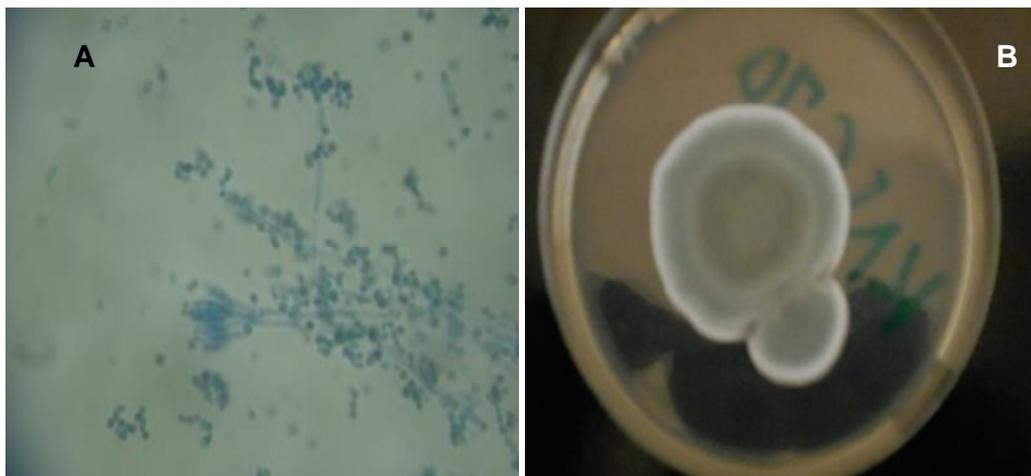


Figure 6. A = Microscopic view of *Penicillium* sp. (x40). B = Culture of *Penicillium* sp. on SDA.

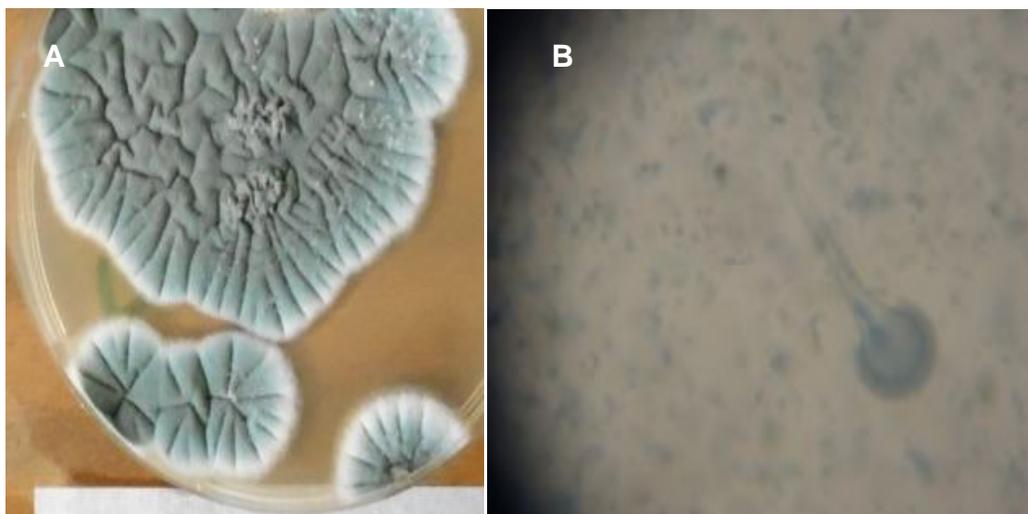


Figure 7. A = Culture of *Aspergillus fumigatus* on SDA. B = Microscopic view of *Aspergillus fumigatus* (x40), Microscope model, Boeco Germany.

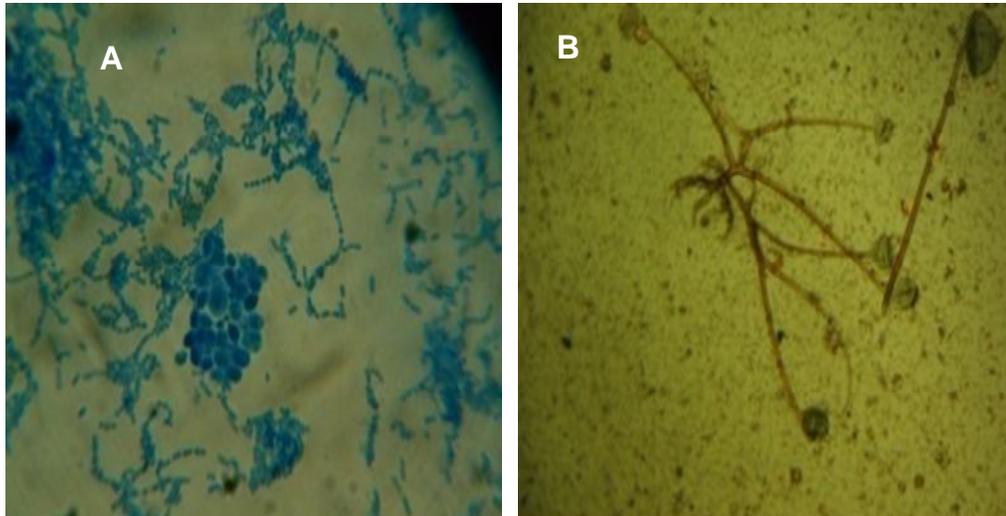


Figure 8. A = Microscopic view of yeast, B = Microscopic view of *Rhizopus* sp. both at x40 magnification.

smoked fish (*Ethmalosa fimbriata*) from retail outlets in Ago-Iwoye, Ogun State, Nigeria for fungal contamination isolated the genera *Aspergillus*, *Rhizopus* and *Penicillium* as the predominant isolates. All isolates were said to degrade the proteins of the fish with *A. ochraceous* having the highest rate of degradation followed by *A. niger* and *A. flavus*. Tsadu et al. (2006), in his survey of fungi contaminating some species of fish from Tagwai dam Minna Niger state, isolated *Aspergillus niger*, *A. flavus*, *A. parasiticus*, *A. fumigatus*, *Rhizopus* sp., *Mucor* sp., *Microsporum canis*, *Penicillium viridicalium* and *Fusarium* sp from the skin, gills and fins of fish with *A. niger* contaminating all species of the fish sampled. Fafioye et al. (2008) isolated *Aspergillus* sp., *Rhizopus* sp., *Fusarium* sp. and *Mucor* sp., from smoked-dried four species of freshwater fish obtained from Oja-Oba market, Ago-Iwoye in Ogun state with *Aspergillus* sp. as the most prevalent species isolated. Rashmi and Chandan (2015) isolated *Mucor* sp., *Rhizopus* sp., *Chaetomium globosum*, *Alternaria tenuis*, *Verticillium* sp., *Aspergillus fumigates*, *Penicillium funiculosum* from some economically important freshwater fishes in Gandak River near Muzaffarpur region of Bihar. Mohamed (2011) isolated mycotoxin producing fungi: *Aspergillus flavus*, *A. clavatus*, *A. ochraceous*, *A. parasiticus*, *A. terreus*, *A. vesicolor*, *Penicillium chrysogenum* and *Trichoderma viride* from five fish species including tilapia and catfish growing in aquaculture.

The fungal organisms isolated in this study have been documented to be associated with *C. gariepinus* and *Oreochromis niloticus*. Abolude et al. (2013) isolated *Penicillium* sp., *Acremonium* sp., *Fusarium solani*, *Aspergillus* sp., *Mucor* sp., and *Saprolegnia* sp. from eggs and brood stocks of *Clarias gariepinus* from hatchery farms in Zaria in Kaduna State, while Refai et al. (2010) isolated *A. fumigatus*, *A. niger*, *A. terreus*, *A.*

flavus, *Penicillium* sp., *Mucor* sp., *Rhizopus* sp., *Fusarium* sp. and *Saprolegnia* sp. from diseased and apparently healthy *C. gariepinus* and *O. niloticus* in a study to assess mycotic settlement of freshwater fish in Egypt. Most of the fungal isolates gotten from this research are of veterinary and medical importance (Beck-Sague and Jarvis, 1993; Denning, 1996).

The results of this study showed that all diseased and apparently healthy fish sampled were positive for multiple fungal organisms; this may be due to the opportunistic nature of most of the fungi. Isolation of the fungal agents from diseased and apparently healthy fish meant for human consumption in this study suggests that mycosis may be a major disease of *C. gariepinus* in Zaria areas and that the fungi isolated from this work might have been responsible for the lesions/disease conditions noticed on the fish or they might have occurred secondary to bacterial or viral infection.

The result of this study demonstrated that infection of *C. gariepinus* with any of these fungal agents may result in the ulcer, fin rot and tail rot. The identification of the fungal organisms in this study was by culture, slide culture, Gram's staining and staining using lactophenol cotton blue. Isolation of fungal organism from the gills of fish may predispose the fish to respiratory disease (Srivastava, 2009).

Most of the fungal isolates in this research were from the genus *Aspergillus*; *A. fumigatus*, *A. flavus*, *A. niger* and *A. parasiticus*. Isolation of *Aspergillus* spp. as the major isolates from this study is significant because *Aspergillus* sp. have been documented to be associated with the disease outbreak in fish culture. *A. niger* is a human pathogen and environmental contaminant and *A. fumigatus* has been identified as important agents of nosocomial fungal infections and implicated as been responsible for life-threatening pulmonary disease of

animal and immuno-compromised human in the world (Denning, 1996). Isolation of *A. flavus* and *A. parasiticus* in this study may predispose fish to aflatoxin contamination and consumers to aflatoxicosis if the fish are consumed by man because they are responsible for aflatoxins production (Ghadeer and Al-Delamiy, 2012).

Many researchers categorised many of the isolates as normal mycobiota, however isolation of these agents from diseased as well as from apparently healthy fish signify they might have been responsible for the infections noticed on the fish or they might have colonized the fish secondary to bacterial organisms, because ulcer, tail rot and fin rot are found to be associated with infections caused by bacteria *Aeromonas* sp. and *Pseudomonas* sp. (Jawahar et al., 2004). *C. gariepinus* from Zaria area may serve as the reservoir for the fungal agents isolated and subsequently become the route through which they reach the human population that consume or handle the fish. Fungal organisms isolated from diseased and apparently healthy fish might not be pathogenic to apparently healthy fish but may cause disease in stressed and immune compromised fish. These isolates may, however, induce diseases in man when the fish are consumed.

Fungi organisms isolated from apparently healthy and diseased *C. gariepinus* in this study are similar and may be part of normal mycobiota of this fish in Zaria areas, but at the same time might have been responsible for the diseased condition noticed on the fish and subsequently may cause morbidity and mortality in catfish culture. These isolates may have arisen secondary to bacterial, parasitic or viral infection. The fungi organism isolated might have been introduced from the environment or through contaminated fish feeds because these fungal species are reported to be infectious through contamination of fish feeds (Saleem et al., 2012). Also these agents might have been introduced to the ponds from the poultry units by the attendants because all the farms practiced mixed farming and so it is easy for these isolates to be spread either by the attendants or by the wind dispersing the spores. Isolation of some of these isolates from fish in this study might make their consumption hazardous to human health.

Conclusion

This study to investigate fungal organisms associated with apparently healthy and diseased *C. gariepinus* from fish farm in Zaria, Kaduna State, Nigeria isolated and identified eight different fungi species and several yeast isolates from skin, gills and gut of the fish. Identification of the fungi was based on morphologies and with the help of authentic manuals of fungi. The most common species of fungi isolated from diseased and apparently healthy *C. gariepinus* were *Aspergillus* sp., *Trichophyton* sp., *Penicillium* sp. and *Mucor* sp. and yeast isolates. Similar fungal organisms were isolated from diseased and

apparently healthy fish. Potential aflatoxin producing species of fungi were isolated from diseased and apparently healthy fish meant for human consumption. Infection of *C. gariepinus* with any of these fungal organisms may result in ulcer, fin rot and tail rot thereby result in economic loss to the farmer as they rendered the fish unmarketable. Fungal organisms isolated may be part of mycobiota of *C. gariepinus* or they may take part in disease of the fish in Zaria, they may also be source of fungal infection and aflatoxicosis when fish are consumed. It is recommended that further study is carried out to determine the role play by individual specie of fungi in *Clarias* diseases, morbidity and mortality. There is the need to study individual fungus and their role in fish diseases in sampled area and in the country as a whole with a view to instituting preventive and control measures. It is recommended that periodic examination of fishes grown in aquacultures in Zaria is carried out to ensure that they are devoid of fungal contamination to conserve public health. Aflatoxin level in the fish raised in the culture in Zaria should be quantified to ensure they are not above the limit allowable for the human. Duties should be assigned on integrated farm to prevent spread of pathogenic organisms from one production unit to another.

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