Special Conference Edition November 2017 http://dx.doi.org/10.4314/bajopas.v10i1.119S

Bayero Journal of Pure and Applied Sciences, 10(1):642 - 646
ISSN 2006 – 6996

FUNGI ASSOCIATED WITH AFRICAN MUDFISH (*Clarias gariepinus*, Burchell 1822) IN SELECTED FISH FARMS AND DAMS IN ZARIA AND ITS ENVIRONS, KADUNA STATE, NIGERIA.

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ABSTRACT
An investigation was conducted to determine the presence of fungi in C. gariepinus harvested from selected farms and dams in Zaria and its environs. A total of 360 randomly sampled Clarias gariepinus (African mudfish) and 144 fish holding water samples were collected from three dams (wild captured) and three fish farms (cultured). They were screened for fungi using Sabouraud Dextrose Agar as isolation medium. Identification of the fungi was done by macroscopic observation of the growth morphology followed by microscopy after staining with lactophenol cotton blue. Four fungal genera namely; Mucor (12.50%), Aspergillus (18.06%), Tricophyton (5.56%) and Penicillium spp. (4.17%) were isolated from water samples. Similar fungi were also isolated from the fish skin swabs in the following order; Mucor spp. (49.57%), Aspergillus sp.(31.30%), Trichophyton sp.(10.43%) and Penicillium sp.(8.0%). Finding these fungi in the fish holding water might have occurred through the use of contaminated and/or decomposed feed, from runoffs, waste water discharges from nearby human settlements into the aquatic environment. The infection of fish by the isolated fungi diminishes both the aesthetic and economic value of fish. Attention must therefore be paid to fish hygiene and fish health management practices must be upheld. We hereby recommend that fish meant for human consumption be properly exposed to temperature high enough to eliminate contaminating fungi.
Keywords: Fungi, African Mudfish, Clarias gariepinus, Dams (wild captured) Fish Farms (cultured).

INTRODUCTION

It has been estimated that fish provides only about 17% of the protein intake of the world's population and in sub-Saharan Africa, it provides 22% of required protein (Food and Agricultural Organization, 2003b). In Nigeria, the story is not much different because there is a deficit in meeting the FAO recommended minimum fish consumption rate of 12.5 Kilograms per head per year (FAO, 2009). Globally, aquacultural activities has been fastly growing (FAO, 2012). This increased aquaculture operations has provided new opportunities for the transmission of aquatic diseases(Woo and Bruno, 1999; Shagar El-Refaee, 2012).The occurrence of diseases may be a significant limiting factor not only for aquaculture production but also for the sustainability of biodiversity in the natural environment (Crane and Hyatt, 2011; Prabhu and Balasubramnian, 2012).

Among the many fungi that have been associated with diseases in fish, some are primarily pathogenic, while others would require such predisposing factors as environmental stress in the form of malnutrition, unstable water temperature, poor water quality, handling, or overcrowding in order to establish infection (Shagar and El-Refaee, 2012). Either of these factors is capable of upsetting the balance between the potential pathogens and their hosts to result in disease (Abolude *et al*., 2013).

Fungi are known to attack all the life stages of fish from egg to adult where they cause serious losses in aquaculture (Iqbal and Saleemi, 2013). Abolude *et al*. (2013), reported that water moulds infection cause great losses of freshwater fishes and their eggs in both natural and commercial fish farms. Even though every freshwater fish is exposed to at least one species of fungi during its lifetime, only stressed and poorly fed fishes are more susceptible to fungal infection. Water moulds infection cause great losses of freshwater fishes and their eggs in both natural and commercial fish farms (Bangyeekhun and Sylvie, 2001).

The quality of water in which fish are cultivated, largely, determines the microbial quality of such fish (Prabhu and Balasubramnian, 2012). Studies have revealed that the microbial flora of caught fish and other aquatic specimens is largely a reflection of the microbial quality of the water where they have been harvested. Therefore, the aim of this study was to survey the diversity of fungi associated with African mudfish (*Clarias gariepinus* , Burchell 1822) in selected fish farms and dams in Zaria and its environs, Kaduna State, Nigeria.

MATERIALS AND METHODS

Collection and analysis of fish samples for fungal isolation

From each of three fish farms and three dams in Zaria, Kaduna State Nigeria, five live *Clarias gariepinus* were randomly sampled. The samples were collected between the months of July 2015 to June 2016. Fish samples were then placed in plastic buckets containing some holding water collected of the dams and fish farms from which the fish were harvested. The fish samples were then transported directly to the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria, Nigeria. The samples were coded as; ADM for A.B.U DAM, ZDM for Zango Dam, SDM for Shika Dam, ZGF for Zango Farm, SBF for Sabo Farm and BZF for BZ Farm.

Skin swabs of the *Clarias gariepinus* samples were cultured on Saboraud Dextrose Agar (SDA) and incubated at 28^{o}C for 5days. The isolated fungi were macroscopically examined for their morphological characteristics. They were then identified using microscopy with lactophenol cotton blue stain. This was aided by the help of available fungi identification keys (Abolude *et al,* 2013; Willoughby, 1994).

Collection and analysis of water samples for fungal isolation

Water samples were collected fortnightly into sterile bottles and immediately in insulated boxes to the laboratory for analysis. One hundred millilitres each of the water samples were dispensed into sterile test tubes and centrifuged. They were then filtered through a small disc of sterilized filter paper of 0.45*µm* pore size. The filter paper was placed on Saboraud Dextrose Agar (SDA) plates and labeled accordingly. Plates were then incubated in an incubator at 28^{o}C for 5 days (Cheesbrough, 2000). The resulting isolates were subjected to macroscopic examination for such morphological characteristics as size, shape and colour of the colonies on the plates.

Fungal Identification from water and fish samples isolates

Identification of fungi was done phenotypically based on such macroscopic and microscopic morphological features of cultivation as colony shape, size, colour, and growth pattern. The fungal species were identified using lactophenol cotton blue stain with the help of available fungi identification keys in literature (Abolude *et al,* 2013; Willoughby, 1994).

Results

The Relative positivity rate of fungi in water from the sampling sites is shown in Table 1. Four fungal genera namely; *Mucor (*12.50%), *Aspergillus* (18.06%), *Tricophyton* (5.56%) and *Penicillium* (4.17%). were isolated from water of the six sampling sites. Out of 24 water samples, seven (7) were positive for *Aspergillus* spp. representing the highest positivity rate (29.19%) in ZDM while the lowest positivity rate was found to be *Tricophyton* spp. 1(4.17%) and *Penicillium* spp. 1(4.17%) respectively which were both recorded in samples from ADM and ZDM respectively.

Table 2 is showing the distribution of fungi in *C. gariepinus* from selected farms and dams in Zaria and its environs. From the six sites, 360 fish samples were collected. Four fungal genera were isolated viz; *Mucor , Aspergillus, Trichophyton* and *Penicillium*. The most occurring was *Mucor* with 49.57% occurrence followed by *Aspergillus* spp. whose occurrence was 31.30%. The least in isolation having a positivity rate of 8.00% was *Penicillium* spp. In ter ms of sampling sites, ZDM had the highest fungal isolation rate from 27 fish samples out of 115 positive fish for fungal isolation, this translates to 23.48% . SDM and SBF each had 15(13.04%) fish sample respectively showing fungal growth representing the least positivity rate. From the dams, *Aspergillus* spp. had the most occurrence with 16(13.91%) fish samples showing growth from SDM and the least was *Penicillium* spp. having a positivity of 1(0.87%) each from ADM and ZDM respectively. From the Fish farms however, *Mucor* spp. had the highest isolation rate and also most prevalent among the studied farms, having a positivity of 9(7.83%) in samples from ZGF, SBF and BZF respectively. *Trichophyton* spp. was isolated from one (0.87%) fish sample each from SBF and BZF making it the least prevalent genus.

Figure 1.0 is the result of the comparism between dams and farms as sampled water bodies. It revealed that the relative positivity of fungi isolated from dams (both from water and fish) was higher than that of the fish farms.

Table 1: Relative positivity rate of fungi in water from selected Dams and Farms in Zaria and its environs .

Sampling Sites	*Mucor* sp. No.(%)	*Aspergillus* sp. No. (%)	*Trichophyton* sp. No. (%)	*Penicillium* sp. No. (%)
ADM	3(12.50)	4(16.67)	4(16.67)	1(4.17)
ZDM	4(16.67)	7 (29.19)	1(4.17)	2(8.33)
SDM	2(8.33)	3 (12.50)	0(0.00)	0(0.00)
ZGF	3(12.50)	6(10.00)	2(8.33)	2(8.33)
SBF	4(16.67)	2(8.33)	1(4.17)	1(4.17)
BZF	2(8.33)	4(16.67)	0 (0.00)	0(0.00)
Total	**18(12.50)**	**26(18.06)**	**8(5.56)**	**6(4.17)**

ADM=A.B.U DAM, ZDM=Zango Dam, SDM=Shika Dam, ZGF=Zango Farm, SBF= Sabo Farm, BZF=BZ Farm.

Table 2: Distribution of fungi in *C. gariepinus* from selected farms and dams in Zaria and its environs.

Sampling sites	*Mucor spp. No. (%)*	*Aspergillus spp. No. (%)*	*Trichophyton spp. No. (%)*	*Penicillium spp No. (%)*	*Total No. (%)*
ADM	8(6.96)	10(8.70)	2(1.74)	1(0.87)	21(18.26)
ZDM	16(13.91)	7(6.09)	3(2.61)	1(0.87)	27(23.48)
SDM	6(5.22)	4(3.48)	3(2.61)	2(1.74)	15(13.04)
ZGF	9(7.83)	6(5.22)	2(1.74)	3(2.61)	20(17.40)
SBF	9(7.83)	4(3.48)	1(0.87)	1(0.87)	15(13.04)
BZF	9(7.83)	5(4.35)	1(0.87)	2(1.74)	17(14.78)
Total	**57(49.57)**	**36(31.30)**	**12(10.43)**	**10(8.00)**	**115(100.00)**

ADM=A.B.U DAM, ZDM=Zango Dam, SDM=Shika Dam, ZGF=Zango Farm, SBF= Sabo Farm, BZF=BZ Farm. n= 24.

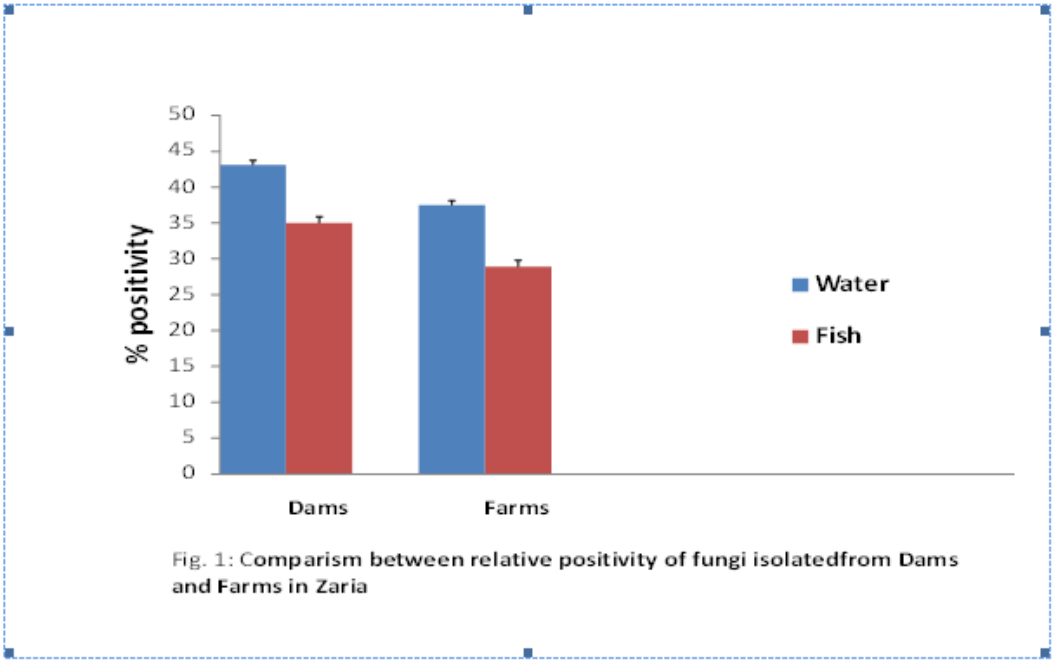

Fig. 1: Comparism between relative positivity of fungi isolatedfrom Dams and Farms in Zaria

DISCUSSION

The diversity of fungi isolated from the fish samples is similar to those isolated from the water samples (Table 1). The trend in terms of positivity rate is also similar to what was observed in the water samples with Mucor spp. having the highest occurrence of 49.57% followed by *Aspergillus* spp. (31.30%). *Penicillium* spp. was the least occurring (8.0%). The fungi isolated in this study are comparable to the observation of Abolude *et al.* (2012), in his study of fresh water fungi in eggs and broodstock of *C. gariepinus* in Zaria. The result of the present study also compares with the

work of Njoku *et al* (2015) who evaluated the microbial profile of some fish ponds in the Niger Delta region of Nigeria and also Iqbal and Saleemi (2013) who assessed the presence of pathogenic fungi on *C. catla* in Pakistan and found out that *Aspergillus* spp. was mostly isolated from fish the samples.

The isolation of these fungi in the present study might be of public health significance because of the possible acute deleterious effects they can cause which could also lead to mass mortality of fish and morbidity in humans who consume them (Olufemi, 1985). Improper pond management, excessive amounts of decomposing organic matter, sick or injured fish and other stressful conditions are all possible sources of fungi in the study sites (Iqbal and Saleemi, 2013). The mixing of fungal spores through surface water runoff of catchment and nearby forest areas along with rain water flowing into the river, might also have been responsible for higher isolation of water fungi than fish fungi.

For both water and fish samples, it was revealed in this study that dam samples recorded higher fungal positivity than the farm samples (Figure 1). This finding is in parity with the study of Atawodi and Bichi (2013) in which they reported that dams were more contaminated than fish ponds or farms. This might have been as a result of some anthropogenic activities. The work of Nnaji *et al.,* (2011) revealed that domestic and industrial wastes and effluents are directly or indirectly discharged into the river Galma in zaria, two of the sampled dams in this study namely; Zaria dam (ZDM) and Shika dam (SDM) are located on the river Galma.

Run off and soil leaching from the surrounding communities as well as from proximal agricultural fields into the dams could also have possibly contaminated the dams.

Isolating fungi from the dam and fish farm water samples has given an indication of water contamination. This makes it possible for the fish from such contaminated water to get infected, and infection of fish by pathogenic fungi diminishes the value of its flesh and may cause fish diseases when predisposing environmental conditions become favourable (Iqbal and Saleemi, 2013). In a study by Kelley *et al*. (2003), it was concluded that mycotoxins and other metabolites can be produced by fungi in water which will usually be extremely diluted, but consumption of mycotoxin in small amounts over a long period of time may be hazardous to human health (Paterson & Lima, 2005). For instance, *Aspergillus* sp. found in water was reported to have been the causative agents of kidney and liver disorders, allergic sinusitis burns, otitis media and increase the risk of other invasive infections De-hoog *et al*. (2000).

CONCLUSION

Four fungal genera (*Mucor, Aspergillus, Tricophyton and Penicilolium* spp.) were isolated from both water and fish from all the sampled dams and fish farms in Zaria and its vicinity.

The isolation of these fungi in the present study might be of public health significance because of the possible deleterious effects they can cause which could also lead to mass mortality of fish and morbidity in humans who consume them. Furthermore, several species of Penicillium and *Aspergillus* are known to produce mycotoxins in food and beverages.

Recommendation

Based on the findings in this study, we hereby make the following recommendations;

1) Awareness programs should be created to educate the public on the potential health implications associated with consumption of fungi contaminated fish and fish products.
2) Fish hygiene and fish health management practices should be improved so as to minimize those factors that could encourage fungal introduction and establishment on fish and in the aquatic system.
3) We also recommend that fish meant for human consumption be properly exposed to temperatures high enough to eliminate contaminating fungi before they are consumed.

Author's contribution

Atawodi, J.C conceived / designed the research, conducted the field and laboratory work and performed the initial data analysis as well as manuscript preparation.

Yola, I.A, Kawo, A.H and Abdullahi, B.A supervised the design and oversaw the conduct of field and laboratory work as well as data analysis and manuscript preparation.

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