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Full Length Research Paper

Fresh water fungi associated with Eggs and Broodstock of African Catfish (*Clarias Gariepinus,* Burchell 1822) in fish hatchery farms, Zaria, Kaduna State, Nigeria

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Abstract

A total of 150 randomly selected eggs and 15 broodstocks were investigated. A sterile swab was taken from outer surface of fish body (mouth, skin, gills, fins), as well as eggs. Sabouraud dextrose agar was used for this isolation. Identification of the fungi was based on their vegetative organs: hyphae, shape and size, asexual reproductive organs, shape of sporangium and spores, and generative organs, structure of oogonium, zoosporangium, and antheridium. In this study, six genus and species were identified; the most common were *Penicillium sp*., *Acreomonium sp., Fusarium solani, Aspergillus sp., Mucor sp., Saprolegnia sp*. Among the species observed, *Penicillium sp.* had 23% occurance, while *Saprolegnia sp*. had the least with 3% occurrence. Identification of *Saprolegnia sp*. which is an important pathogen in aquaculture needs further study in the future. The aim of this study was to investigate the aquatic fungal flora associated with eggs and brood stock of hatcheries from Zaria, Kaduna State, Nigeria.

Keywords: Isolation, African Catfish, Eggs, Fungal Infection, Hatchery.

INTRODUCTION

In seed production of African Catfish (*Clarias gariepinus*), loss during egg stage is one of the factors which decrease the number of production. The main reduction is caused by fungal infection. Fungal infection of eggs has been reported from many fish species *(*Czeczuga and Wornowicz, 1993; Czeczuga and Muszynska, 1997; Kitancharoen *et al*., 1998; Eli and Abowei, 2011). The growth of fish culture has also raised issues of fish health. (Bacterial hemorrhagic septicemia, lernaeasis, saprolegniasis, and anoxia are the most commonly occurring fish disease in pond fishes (Iqbal *et al*., 2012). Fungi are known to attack fish eggs, fries, fingerlings and adult fish. Water moulds infection cause great losses of freshwater fishes and their eggs in both natural and commercial fish farms (Bangyeekhun and Sylvie, 2001).

The mortality rate due to fungal infection may reach some time up to 80-100% in incubated eggs (Chukanhom and Hatai, 2004). Post-harvest landing of fishes may also result in infection with microorganisms such as bacteria and fungi (Akande and Tobor, 1992). Primary infections of fungi fishes and fish eggs by Oomycetes have been reported (Walser and Phelps, 1993). Fadaeifard *et al.,* (2001) isolated 8 species of fungi from eggs and brood stock of rainbow trout *Oncorhynchus mykiss,* these isolates were *penicillium* spp, *Acreomonium* spp, *Alternaria* spp, *Fusarium solani*, *Aspergillus* sp, *mucor* sp, *Saprolegnia* sp, and *Cladosporium* sp. Although, infection as a result of microbial contamination does not usually result in disease, but environmental stress may upset the balance between the potential pathogens and their hosts (Iqbal *et al*., 2012).Under such conditions the chances of infection increases. Hence this study was to survey the diversity of fungal species isolated

from eggs and brood stocks of *Clarias gariepinus* in Zaria, Kaduna state, Nigeria.

MATERIALS AND METHODS

Collection of fish and egg Samples

Collection of fish (brood stock) and egg samples were accomplished during spawning season (August-December, 2012). A total of 15 brooders and 150 eggs of African catfish (*Clarias gariepinus*) were collected alive from three hatcheries in Zaria Nigeria. In each farm, 50 eggs were collected with sterilized forceps from incubators and transferred to a screw cap tube which has been sterilized with distilled water. These randomly chosen samples were transported alive to the Microbiological Laboratory of Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria.

Isolation of Fungi

For the isolation of fungi associated with fish and egg; swap of mouth, gills, fins skin and eggs were cultured in sabouraud dextrose agar (SDA) and Saprolegniaceae specific medium, glucose-yeast extract (GY) agar. The GY agar consists of 10g of glucose, 2.0g yeast extract and 15g agar in 1,000ml distilled water (Hatai and Egusa, 1979). To inhibit the bacterial growth, 500ug/ml each of Septromycin and Penicillin was added to the medium. The isolates were incubated at 25^0c on GY agar and transferred to fresh GY agar. For purification, grown fungi were transferred to fresh medium. Samples cultured in sabouraud dextrose agar (SDA) were incubated at 28^0-30^0C and fungal growth was observed after 4-7 days. To inhibit the bacterial growth, 500µg/ml each of chlorophenicol was added to the medium, while another sample was cultured without inhibiting the bacterial growth (control experiment).

Macroscopic Examination

Isolates were studied macroscopically by colony shape, size, colour, and growth pattern.

Microscopic Examination

Slides were prepared from each colony and stained with 0.05% Trypan blue in lacto phenol. The slide was observed under microscope and photographed. The existing septate wall, sexual organ structure, size, and arrangement of spores were also examined and documented.

Fungal Identification

The fungi species were identified with the help of available fungi identification keys and literature (Willoughby, 1994).

RESULTS

The result of this study showed that six fungal species, *Penecillium* sp., *Acreomonium* sp., *Fusarium solani*, *Aspergillus* sp., *Mucor* sp., and *Saprolegnia* sp. were isolated from *Clarias gariepinus* eggs and brood stocks respectively (Plates 1 to 7 and Table 1). Identification of isolates was accomplished on the basis of their vegetative, asexual reproduction and generative organs. *Saprolegnia* spp. with thick hypha, cylindrical zoosporangium, spheroid cysts and fungal colonies were seen as a white and cotton shape on the glucose-yeast agar which filled the entire medium after about a week. The highest infection with 45% was found in farm A and the lowest with 20% in farm C where only *Mucor*, *Yeast* and *Penicillum* spp. were observed. At Farm B however, *Yeast* and *Saprolegnia* spp. were not identified. The most occurrences of fungal isolates belong to *Aspergillus* spp. and the least was found in *Saprolegnia* spp. (Table 1). Diversity of fungal isolates according to selected farms is as shown in Table 1. Relative frequencies of identified fungi in different farms show that farm A had an isolation of 6 strains of fungi with 46% relative frequency and the highest fungal infection at the period of sampling and lowest in farm C with 10% relative frequency ($P>0.5$).

The observed physico-chemical parameters in the water (Table 2) showed that the pH observed at Farm A was the least while the highest was observed at Farm B. However, there was no significant difference in the values of pH observed at Farms A and C but the pH observed at Farm B was significantly higher than the observed trends at Farms A and C. Water temperature also ranged from 16.80 ^{0}C in December at Farm B to 25.00 ^{0}C in August also recorded at Farm B. There was no significant difference in the water temperature of Farms A and C but the water temperature observed at Farm B was significantly higher. The total organic matter was highest (225.00mg/l) at Farm C and the lowest (103mg/l) at Farm A. Also there was no significant difference between the total organic matter obtained from Farms A and B but the observed organic matter at Farm C was significantly higher than the observed values at Farms A and B (Table 2). The observed increase or decrease in the infection rate of hatcheries studied depends on management, circumstances, and the conditions of broodstock under investigation.

Table 1. Fungi isolated from eggs and brood stock of *Clarias gariepinus* from hatchery fish farms in Zaria.

Farm Fungi	Farm A	Farm B	Farm C
Mucor sp.	+	+	+
Apergillus flavor	-	+	-
Aspergillus niger	+	+	-
Yeast sp	+	-	+
Penicillum sp.	+	+	+
Saprolegnia sp.	+	-	-
Trichophyton sp.	+	+	-
Total	6	5	3

Table 2. Some Physicochemical parameters of the sampling sites between August and December 2012

Months	Physicochemical parameters								
	FARM A			FARM B			FARM C		
	pH	Water Temp. (^{0}C)	Total Org. Matter(mg/l)	pH	Water Temp. (^{0}C)	Total Org. Matter(mg/l)	pH	Water Temp. (^{0}C)	Total Org. Matter(mg/l)
AUGUST	7.97	24.00	103.00	8.59	25.00	113.50	8.36	23.0	190.00
SEPTEMBER	8.76	22.50	175.00	8.66	21.00	191.00	8.60	22.6	205.00
OCTOBER	7.50	21.00	153.00	7.56	19.00	155.00	7.83	21.6	225.00
NOVEMBER	8.10	22.00	160.00	8.05	23.00	139.50	7.68	20.6	198.00
DECEMBER	7.25	17.00	180.00	7.80	16.80	170.00	7.34	18.8	185.00
MEAN±SE	7.92±0.05	21.30±0.05	154.20±3.25	8.13±0.05	20.96±1.02	153.80±2.33	7.96±0.05	21.32±0.05	200.60±2.02

DISCUSSION

The most common strain in this study is *Penicillium* which in most cases counts as a ubiquitous fungi in nature but did not isolate from fishes as a pathogenic agent, although some species of *Penicillium* are able to make pathological signs in fish. Mycotic infections associated with Saprolegnia are widely reported in freshwater fish (Hussein and Hatai, 2002). They are rarely found in brackish water (Kwanprasert *et al*., 2007). Ogbonna and Alabi, (1991) carried out a survey on the species of fungi associated with mycotic infections of fish in a Nigerian freshwater fish pond. A total of 24 fungal species belonging to 6 genera of aquatic phycomycete were isolated from the infected fishes. From their observation, *Achlya racemosa, Aphanomyces laevis, Dictyuchus sterile, Saprolegnia ferax, S. litoralis and S. parasitica* had 100% frequency of occurrence amongst the infected fishes. There were similarities in the species of fungi isolated from the infected fishes in the fish pond and those isolated from the hatchery.

The interactions of physic-chemical factors generally have influence on the diversity of water molds (Paliwal and Sati, 2009). It is proven that ecological differences in different geographical locations also play an important role in the species diversity of the fungi that developed on both fish and eggs (Wood and Willoughby, 1986). Although environmental variables were not investigated in this study, they are known to influence the growth, reproduction, and intensity of aquatic fungal infections. In addition, the occurrence of fungal infections may be related to environmental changes or seasonal variations, water quality, temperature as well as physiological changes and the immune response of fish. According to Willoughby (1994), fungi which belong to the genus of *Saprolegnia* could cause disease in freshwater fishes and their eggs. The results of the present study from the point of relative frequency confirm this. Kitancharoen and Hatai, (1997) found that signs of *Saprolegniosis* subside when water temperature reaches above 18°C and this indicated that *Saprolegnia* species in lower temperature are able to become severe and epidemic. Thus, depending on environmental differences and management conditions of farms, fungi have wide range of infection (Willoughby, 1994) and thereby plays an important role in causing fungal infections on eggs. It has been proved that *Saprolegnia* when in the same culture with *Fusarium* has less growth in comparison to when it is alone in the environment where it grows and penetrates into the cell wall and brings about reduction in water flow

Plate 1: *Aspergillus niger*

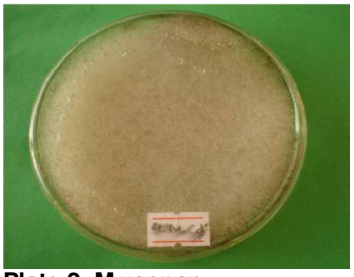

Plate 2: M*ucor sp*

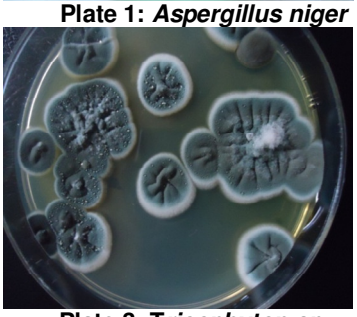

Plate 3: T*ricophyton sp*

Plate 4: *Aspergillus sp*

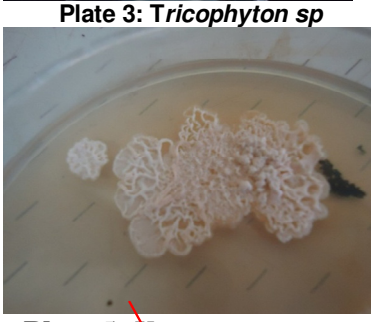

Plate 5: ***Yeast sp***

Plate 6. Macroscopic view of some fungi isolated from brooders of *Clarias gariepinus* (Arrows indicating white slough on fungi infected Broodstock of *Clarias gariepinus)*

Plate 7. Macroscopic view of some fungi isolated from eggs of *Clarias gariepinus*

Figure 1. Provide Legend

and enzyme secretion which eventually lead to death of fish eggs.

It is evident from the results presented in Table 2 that the frequency of water molds and concentration of different nutrients varied considerably at different seasons in all the three sampled hatcheries. Cultured water was found to be alkaline throughout the study period (Table 2). It was observed that cultured water varied from site to site due to environmental factors, such as vegetation cover, human activities, etc. Water mold is of ephemeral nature and consequently exhibits seasonality in aquatic system. During the present study, Farm A showed highest diversity of water molds (6 spp.). This might be due to wide range of pH (7.25-8.76) with moderate water temperature (17.0-24.0 ^{0}C) and the relatively low organic matter observed at Farms A and B compared to the high organic matter observed at Farm C. The mixing of fungal inoculums or spores through surface water runoff of catchment and nearby forest area along with rain water flowing into the river, might be responsible for higher diversity of water molds during the rainy season. Thus, in the process of handling fishes seed production of African Catfish (*Clarias gariepinus*), the following should be paid attention to: using healthy brood stocks without any external injuries, prevention from stress during the incubation period, disinfection of troughs and hatchery water using suitable disinfectants, observing the egg ripening process and keeping the fertile eggs in suitable condition.

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