

Isolation and Identification of Pathogenic Fungus from African Catfish (*Clarias gariepinus*) Eggs and Adults in National Fishery and Aquatic Life Research Center Hatchery, Ethiopia

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Abstract

Isolation and identification of pathogenic fungi from African Catfish (*Clarias gariepinus*) eggs and adults in Ethiopian National Fishery and Aquatic Life Research Center hatchery was conducted from October 2015 to May 2016. The aim of this study was to investigate the aquatic fungal flora associated with eggs and brood stock from African catfish in the center. A total of 16 egg samples, 77 swab samples from skin of adult fishes (brood stock) and water samples from 14 incubating containers were investigated. Samples were collected from water, outer body surface of fish as well as from artificially hatched eggs. Isolation and identification of the fungus was done on colonial and microscopic characteristics. In this study, 84.11% samples were positive for fungal growth and fungus isolates belonging to seven different genera were identified. *Tricophyton*, *Saprolegnia*, *Rhizopus*, *Penicillium*, *Mucor*, *Microsporium* and *Alternaria* were among the dominant isolated fungal genera. Among the isolated genera, *Tricophyton* was detected from 13.08% of the samples, while *Alternaria* had the least with 3.74%, unidentified fungi accounts for 14.02% and unidentified yeast were 20.56%. Based on the results, various pathogenic fungi species were identified from fish body, water and hatched eggs, hence are potential causes of mortality and decreased egg hatchability. In the light of this, appropriate egg and water disinfection methods are essential for hatchery management. Fishes in rearing facilities must be given proper health management monitoring to prevent outbreak of fungal disease.

Keywords African catfish; Fungus; Egg; Isolation; Identification

Introduction

Over 80% of the earth surface is covered by water. Fish are ubiquitous inhabitants of this ecosystem. With over 20,000 identified species and about twice that number of species which have not been discovered [1]. Fish are a paraphyletic group of animals that consists of all gill bearing aquatic vertebrate animals that lack limbs with digits [2]. There are over 200 fish species reported to occur in lakes, rivers and reservoirs in Ethiopia and the bulk of production is made of Nile Tilapia, Cat fish, Labeo barbuis, and common Carp species [3].

Catfishes are a monophyletic group belonging to the super order called the Ostariophysi. They are usually of dark gray or black coloration on the back, fading to a white belly, with a characteristic slender bodies, flat bony heads and broader in size and terminal mouths with four pairs of barbels. The African sharp tooth catfish is also gifted with large accessory breathing organs composed of modified gill arches sometimes referred to as rudimentary lungs and only the pectoral fins have spines. It is ideal for farming since it requires less space, time, money and has a higher feed conversion ratio. The African catfish is a nocturnal fish like many other species of catfish it feeds on living and dead animal matters. It is also able to swallow relatively large prey whole, because of its wide mouth [4].

In both natural and culture conditions, disease has a serious impact on total fish production. It is universally recognized as one of the most serious threats to the commercial success of aquaculture [5,6]. In seed production of African catfish (*Clarias gariepinus*), high losses are recorded during egg stage and this is responsible for reduction in total production. The main reduction is caused by fungal infection especially at the spawning stage. They also hinder proper functioning of organs especially in young fishes and can also cause skin irritation which may lead to reduction in commercial value of adult fishes. Almost every freshwater fish is exposed to at least one species of fungus during its life time [5].

Disease outbreaks have threatened profitable and viable aquaculture operations throughout the world. Parasitic and bacterial causes of fish diseases have been reported by many authors in Ethiopia [7-10]. However, studies on pathogenic or opportunistic fungi from fish farms and aquacultures are very few, quite limited and in many instances missing. To the best of author's knowledge, there are no published literatures concerning major fungal pathogens affecting African Catfish from Ethiopian aquacultures. Hence, this study was proposed to isolate and identify fungal flora associated with eggs, water and brood stock of African catfish (*Clarias gariepinus*) at the Ethiopian National Fish and Aquatic Life Research center.

Materials and Methods

Description of study area

The study was conducted in Ethiopian National Fishery and Aquatic Life Research Center (NFALRC) which is located in Sebeta town, Oromia regional state from October 2015 to May 2016. Sebeta town, is at 08° 54'N and 38° 38'E° with an altitude of about 2225 m above sea level with maximum temperature of 27°C and minimum temperature of 12°C and an average annual rainfall of 1350 mm.

The study population: The study was conducted on African catfish from eggs, adult fishes and water from all growing and incubating ponds and containers managed artificially. Artificial reproduction of catfish was carried out at the NFALRC hatchery. Suitable brood stock was selected from ponds in the center, males and females were placed in separate tanks inside the hatchery. Water temperature of the incubating basin was maintained at 25°C using thermostat controlled heater. The brood-stock was fed suitable diet for 1-2 weeks inside the hatchery. Two days prior to injection with hormone all feeding was stopped. Pituitary hormone from carp, 3mg per kilogram was injected and dissolved using saline 2.5 ml above the lateral line. Stripping then carried out the next morning and fertilization of eggs with sperm from male donor was done immediately after receiving the eggs on a bowl. Fertilized eggs then placed in incubating basins which were equipped with continuous flow of water. Catfish started hatching after 24 hours at 25°C of incubation. The egg yolk sac was totally absorbed by the larvae after the third day of hatching before live feed feeding commenced. *Artemia naupli* (live feed) was fed four times a day until the tenth day after hatching. At this stage the fish had already become fry [11], the infected samples were taken from artificially hatched eggs, adults (brood stock) and water from the ponds. Adult male and female fishes were sampled from 2 ponds in the center.

Study design

Case study was conducted to isolate and identify the fungal agents from the cases suspected of having fungal infections; from artificially hatched eggs, water samples and skin swabs from the adult fishes.

Sample size and sampling method

The number of samples was dependent on the availability of fungal infected or suspected cases from ponds under study. Depending on this situation 107 suspected samples were processed during the study period. The sampling technique was purposive sampling, those fishes and eggs suspected of having fungal infections were considered.

Sample collection

Different types of samples were collected (fish skin swab, egg and water). Collections of skin swab samples were accomplished from a

total of 77 (42 males and 35 females) brood stock using sterilized swab stick both from eye region and all fins. Moreover, an average of 2 g of egg samples was collected from 8 female catfishes both before and after incubation. Eggs were randomly sampled with sterilized forceps from receiving bowls and incubators and stored in sterile criovials. Unfertilized and un-hatched eggs were collected from the base of the incubating basins after 24 h of incubation and immediately transported and cultured at NAHDIC. In addition, 2 ml of Water samples were collected from 7 incubating containers (basins), using aseptically sterilized plastic containers which have holding capacity of 10 ml both prior to the start of the incubation process and also after incubation.

Fungal identification

For the isolation and identification of fungi, swab samples from suspected fish, egg and water samples were collected and immediately transported and cultures on sabouraud dextrose agar (SDA) at NAHDIC. The inoculated samples were incubated at 26°C for up to 4 weeks (if no visible fungal growth was observed within this period, no growth was recorded). Isolates were examined macroscopically by colony shape, size, colour and growing pattern, Slides were prepared from each colony using scotch tape method where transparent scotch tape was lightly pressed to colony and then the tape was fixed to slide that had a drop of lacto phenol cotton blue stain. The slides were observed under microscope in X10 and X40 magnification power and were identified with the help of fungal identification key [12]. Pure fungal culture was then cultivated by picking a small portion of colony with the help of sterilized loop and culture again on SDA. The pure culture plates were incubated and observed for further identification. The presence of uniform colony character throughout the plate indicated the formation of pure fungal culture.

Data management and analysis

The data collected in the study was stored in the Excel Microsoft (MS excel) and descriptive statistics was employed by SPSS version 20 data analysis software.

Results

Initially, water chemical analysis was carried out and was found To=25°C, conductivity=175 µc/cm, PH=7.6, dissolved oxygen=5.4 mg/l, total ammonium nitrogen (TAN)=86%. According to CCAC [13], all parameters were within the normal range for African catfish aquaculture. The infected fertilized eggs in hatching containers appeared as tuft hairy like surrounded with a white cottony envelop. It didn't hatch and capitulated within 24-36 h. The infected eggs showed highly branched hyphae under microscope.

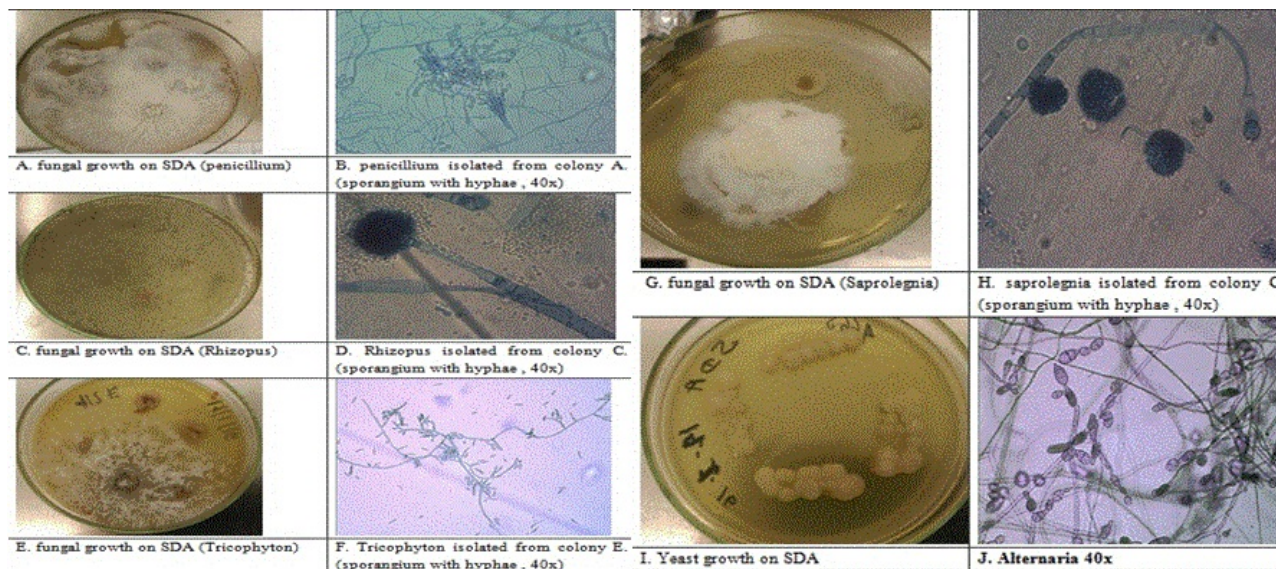


Figure 1: Growth colony morphology and microscopic characteristics of some of the isolated fungal genera. Description; whitish, rugose, slightly raised colony (A) Dense broom like phialides (B), Whitish, long sporangiophores with dark round sporangium (C) smooth walled, non-septate sporangiophores with dark globose sporangia (D) whitish slightly raised colony (E), numerous numbers of pyriform microconidia near the hyphae (F) White cottony colony (G) Masses of sporangia filled with large number of sporangiospores (H), white to creamy colored, smooth, glabrous colony (I) Macroconidia are drumstick-shaped with elongated beak of one conidium butting against the rounded blunt end of the next (J).

In this study, 84.11% samples were positive for fungal growth (Table 1). It was possible to isolate and identify seven major fungal genera; these were *penicillium*, *Rhizopus*, *Mucor*, *Tricophyton*, *Saprolegnia*, *Alternaria* and *Microsporium*. These fungi were identified from *Clarias gariepinus* eggs, brood stock and water samples as shown in Table 2.

The most frequently occurred fungal isolate was *Tricophyton* and the least was from *Alternaria* genus. Moreover, yellowish mucoïd and pink colonies of yeasts were also isolated and categorized as unidentified yeasts (Table 2 and Figure 1).

Source of sample	Status of fungal growth			Positive (fungal growth) percentage
	No growth of fungus	Growth of fungus	Total sample	
Skin sample from male fish	3	39	42	92.90%
Skin sample from female fish	10	25	35	71.40%
Egg before incubation	0	8	8	100%
Egg after incubation	1	7	8	87.50%
Water before set on incubation process	1	6	7	85.70%
Water after set on incubation process	2	5	7	71.40%
Total	17	90	107	84.11%

Table 1: Growth status of fungi from skin, egg and water samples.

Genus of identified fungus	Source of sample			Total (%)
	Adult fish skin samples	Egg samples	Water samples	
No fungal growth	13	1	3	17 (15.89)
<i>Penicillium</i>	4	2	0	6 (5.61)

<i>Mucor</i>	4	0	2	6 (5.61)
<i>Rhizopus</i>	1	0	8	9 (8.41)
<i>Trichophyton</i>	9	5	0	14 (13.08)
<i>Saprolegnia</i>	7	2	0	9 (8.41)
<i>Microsporium</i>	5	0	0	5 (4.67)
<i>Alternaria</i>	4	0	0	4 (3.74)
un identified fungus	11	4	0	15 (14.02)
un identified yeast	19	2	1	22 (20.56)
Total number of samples	77	16	14	107

Table 2: Identified fungal genera from different sample sources.

Discussion

Seven fungal genera were isolated from this study; *Trichophyton*, *Rhizopus*, *Saprolegnia*, *Mucor*, *Penicillium*, *Microsporium* and *Alternaria*. It was possible to isolate all the seven fungal genera from adult fish skin swab sample with *Trichophyton*, *Saprolegnia* and *Microsporium* genera found to be highly prevalent. *Trichophyton*, *Saprolegnia* and *Penicillium* were also isolated from egg samples, whereas *Mucor* and *Rhizopus* were mainly identified from water samples collected from the incubating containers.

There are several reports of true fungi as primary infection agents of adult fish species of *Clarias* genus from the African continent. The current result have similarities with the study of [14] that reported six fungal genera namely; *Penicillium*, *Acreomonium*, *Fusarium*, *Aspergillus*, *Mucor*, and *Saprolegnia* isolated from *Clarias gariepinus* eggs and brood stocks. Moreover, [15] isolated about 6 genuses of fungus from ornamental fish Fantail they were *Penicillium*, *Fusarium*, *Rhizopus*, *Mucor*, and *Alternaria*. Refai et al. [16] also reported fungal isolates from diseased and apparently healthy fish samples. Isolated filamentous fungi belonged to the following genera; *Saprolegnia*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, *Scopulariopsis*, *Paecilomyces* and *Curvularia* from *Oreochromis* species and *Clarias gariepinus*. Shabazian et al. [17] also isolated 17 species of fungi from the rainbow trout eggs. In another study by Fadaefard [18] isolated eight genera from eggs and brood stock of rainbow trout and the most common were *Penicillium*, *Acreomonium*, *Alternaria*, *Aspergillus*, *Mucor*, *Saprolegnia* and *cladosporium*. But this report stated that, *Aspergillus* *Penicillium* and *Rhizopus* are normal mycoflora but are able to cause infections. On the other way Al-Niaeem et al. [19] has also reported that *Aspergillus* and *Mucor* species were the most common fungi species infecting fish. Though most fungi regarded as opportunistic pathogen but few of them are known to cause diseases such as *Saprolegniasis*, *Aspergillois*, *Scopulariopsis*, *Paecilomycosis* and *Penicillium* infection [16].

With the current study it was possible to identify genus *Saprolegnia* from adult fish and eggs and this finding was similar to many other reports. *Saprolegniasis* is a common and highly prevalent fungal disease that affects all species and ages of fresh water and estuarine fish [20]. Thoen et al. [21] noticed that the live egg contact with dead infected salmon egg had developed foci of white discoloration then death with total whitening. *Saprolegnia* species were reported to grow

on a surface of egg shell which affected it by direct adhesion mechanism. Similarly, Czczuga et al. [22] also reported that *Saprolegnia parasitica* was among the most commonly encountered species on the *C. gariepinus* eggs. Moreover, Eissa et al. [23-25] also reported that *Saprolegnia* species was the major cause for the mass mortalities of angelfish eggs accompanied with very low hatchability in a private ornamental fish farm in Egypt. From the current study it was concluded that fungi infestation can possibly challenge good quality fish production. So appropriate egg and water disinfection methods are necessary and attention must be paid to fish health management practices in rearing facilities.

Conflict of Interests

The authors have not declared any conflict of interests.

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