Isolation of *Aphanomyces invadans* Associated with Skin Lesions in African Catfish" *Clarias gariepinus*"

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A B S T R A C T

The present study aimed to isolate and identify of *Aphanomyces invadans* from naturally infected African Catfish" *Clarias gariepinus*". A total number of 105 cultured *Clarias gariepinus* collected from private fish farms in Kafr Al-Sheikh & Behera Governorates. Result revealed that the prevalence of infection among the examined fish was 8.6 % and the disease mostly recorded during winter and spring. The infected fish developed characteristic ulcerative lesions and fin rot. Fungus-like oomycetes *Aphanomyces invadans* grow on glucose peptone yeast (GPY) agar as opaque colonies with uneven white transparent velvets surface at room temperature. Microscopically, lactophenol cotton blue stained fungal growth appeared as non-septated thin long branched hyphae with tapered end containing cytoplasmic organelles. On sporulating media, the rectangular shaped spores appeared inside the hyphae connected together by thin filament. In conclusions, EUS is an invasive disease of *Clarias gariepinus* and care should be taken with low temperature in managing fish pond.

Key words: *Aphanomyces invadans; Clarias gariepinus;* identification; prevalence; sporulation.

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1. INTRODUCTION

Recently, Epizootic ulcerative syndrome recorded as one of the most destructive diseases causing substantial losses to fresh water aquaculture system in Africa. Epizootic ulcerative syndrome (EUS) was first isolated from farmed freshwater ayu (*Plecoglossus altivelis*) in Japan in 1971 (Egusa and Masuda, 1971). In 1972, a EUS-like condition affected estuarine fish particularly grey mullet (*Mugil cephalus*) in Australia (McKenzie and Hall, 1976). The disease spread progressively across the Asia–Pacific region and North America. Papua New Guinea experienced the outbreak during 1975–1976 (Haines, 1983). EUS-like condition was recorded in East coast of the United States in 1980 (Hargis, 1985). In Egypt, *Aphanomyces invadans* was isolated from naturally infected Striped and thin lip grey mullet (Shaheen et al., 1999), and African catfish "*Clarias gariepinus*" (Amany et al., 2004). In late 2006 an unusual ulcerative condition in wild fish was reported for the first time in Africa from the Chobe and upper Zambezi Rivers in Botswana and Namibia. Concern increased with subsistence fishermen reporting large numbers of ulcerated fish in their catches. In April 2007 the condition was confirmed as an outbreak of epizootic ulcerative syndrome (EUS) (Huchzermeyer and van der Waal, 2012). Some fish, such as common carp (*Cyprinus carpio*), Nile tilapia (*Oreochromis niloticus*) are considered to be resistant to EUS as they were either not found to be infected naturally or experimentally (Pradhan et al., 2008) (Anon, 2010). EUS in freshwater fishes has been associated with low temperature, and has often occurred after periods of heavy rain in Bangladesh (Khan and Lilley, 2002).

The present work was planned to isolate and identify the *aphanomyces invadans* from *Clarias gariepinus*, and determine seasonal prevalence of the induced disease.

2. MATERIALS AND METHODS

2.1. Fish samples

A total number of 105 cultured *Clarias gariepinus* with an average body weight 250±10g. Fish were collected from private fish farms in Kafr Al-Sheikh & Behera Governorates. The samples were collected during the period from May 2015 to April 2016. The obtained fishes were transported alive in large tanks with oxygen pump. The live

fish was kept in well prepared glass aquaria supplied with dechlorinated tap water pumped with oxygen. The freshly dead fish were labeled, packed and transferred in ice boxes (Huet, 1986). All the samples were transported to the wet lab of fish disease and management, faculty of Veterinary Medicine, Benha University. The clinical examination of naturally infected fish was done according to (Amlacher, 1970).

2.2. Culture characters

Fish samples were prepared and the samples for mycological examination were taken from skin and underlying musculature under complete aseptic condition and culturing on glucose peptone yeast extract broth (GPY) broth. Using the modified 5 stages culture technique of (Willoughby and Roberts, 1994) for isolation of the suspected fungus.

The morphological characters of the hyphal growth on (GPY) agar including gross appearance of the cultures, rates of growth, texture and color of the surface were noticed and recorded in the text. Wet mount preparation from hyphal growth examined microscopically for identify the suspected fungus (Dovorak and Atecenasek, 1969). Staining of hyphae of *Aphanomyces invadans* by lactophenol cotton blue and examined microscopically.

2.3. Detection of asexual characters of Aphanomyces invadans according to (Hatai and Egusa, 1979)

Briefly, a pure culture of Aphanomyces species isolated from *C. gariepinus* was incubated at room temperature for 10 days on glucose yeast (GY) agar and then the hyphae were cut into pieces in 1000 ml Erlenmyer flask containing 500 ml of GY broth. Mycelia were collected and washed twice with sterilized tap water. Sporulating media of sterilized tap water containing sterilized pierced hemp seed was inoculated with washed mycelia and incubated for 2 days at room temperature. Examination of sporulating media and the growing hyphae microscopically to detect the developed zoospores.

Along the period of 6 weeks post sporulation, samples were taken for microscopically examination every 48 h to investigate the developing reproductive organs.

3. RESULTS

In the present study, general signs of the clinically diseased fish appeared as redness on the body surface, erosions, small and extensive ulcers, excess mucous secretion, eroded fins, fin rot, congested protruded anal opening and darkness on the body surface. Positive Aphanomycosis fish showing clinical signs of destructed fin, superficial ulcers and sometimes extensive ulcers and hemorrhage on fins and body surface (Fig1. A, B).

Out of 105 *C. gariepinus,* the total prevalence of infection among the examined fish was 8.6%. With respect to the health status of the examined fish, the disease found in about 11.25% from the investigated clinically diseased 80 fish while the apparently healthy fish showed –ve fungi isolation table (1).

With respect to the seasonal prevalence, the disease only recorded in winter (20%), and spring (12%) (Fig 2). The culture characters of *Aphanomyces invadans* isolated on glucose peptone yeast agar showed opaque mycelia with uneven white transparent velvets surface (Fig1. C). The hyphae begin to grow on the agar plate at 3rd day from culturing of it and the size of growth increase gradually till fill the plate at the 7th day. Wet mount preparations from growing cultures on GPY agar and broth revealed the presence of branched non- septated long hyphae with tapered end. The hyphae contained a cytoplasmic organelle (Fig1. D, E).

Hyphae of *A. invadans* stained with lacto phenol cotton blue and some of them give -y- shape appearance (Fig1. F). With prolonged incubation, the hyphae obtained from the center of the growing colonies were thicker, undulating than those obtained from the periphery which were thinner (Fig1. G). In the wet mount preparations from the sporulating media 48 hrs. post incubation, the culture showed that the sporangia were mostly formed at the hyphae tip but the hyphae remain unchanged in their diameters. Rectangular shaped primary spores were produced within the sporangium arranged in chain linked together by thin filament (Fig1. H).

The primary spores released from the tip of the sporangia then encysted to form clusters at the mouth of the sporangium and quickly transform into the secondary form. In some cases, some spores failed to release and encysted within the sporangium as a refractile round encysted spores (retained spores). At weekly interval, wet mount preparation from sporulating media was examined and the hyphae showed no sexual organs along the whole incubation period (6 weeks).

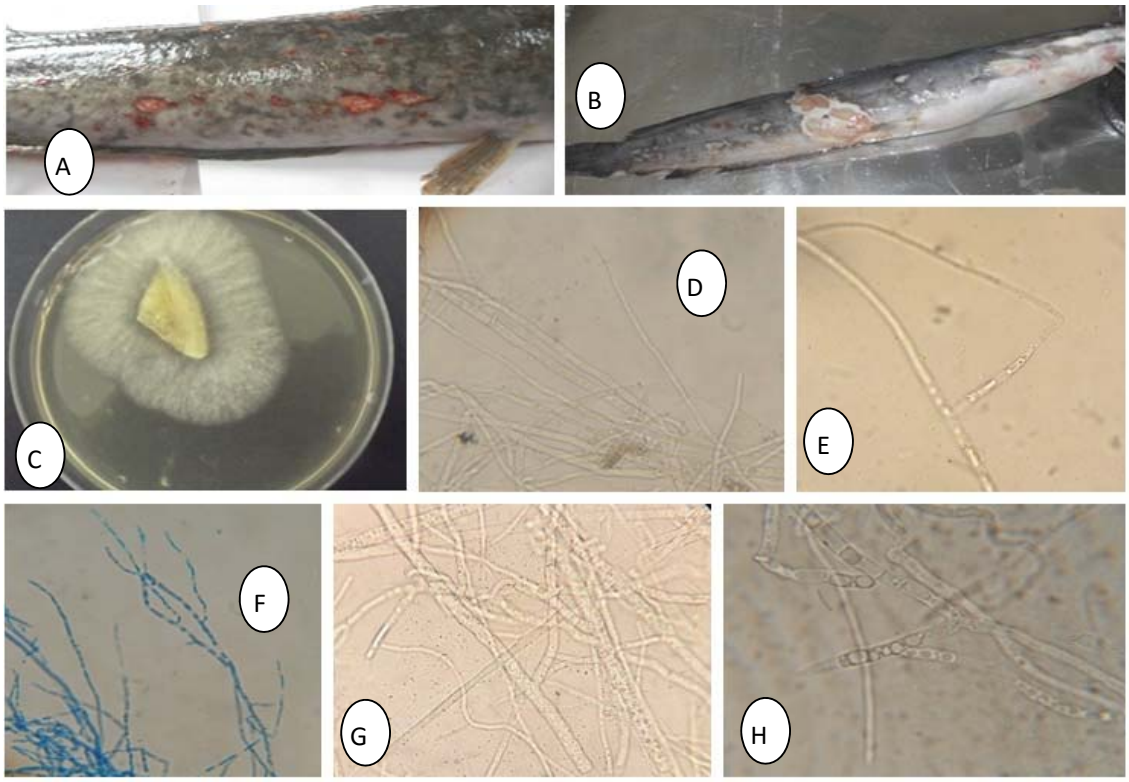


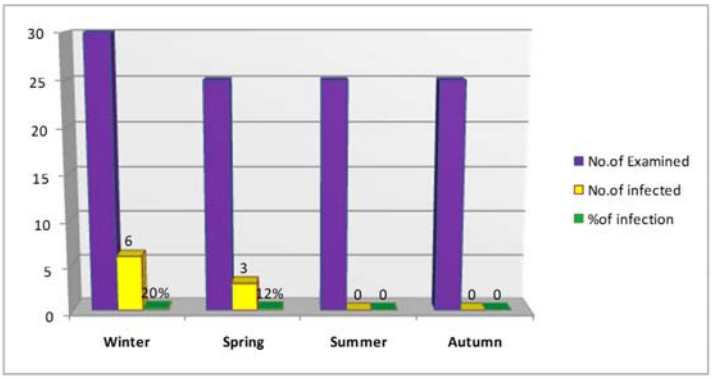
Figure (1): (A): Naturally infected *C.gariepinus* with *A. invadans* showing multiple small ulcers with hemorrhagic border all over the body. (B): Naturally infected *C.gariepinus* with *A. invadans* showing diffuse large ulcer with fin erosion. (C): growth of *A. invadans* on GPY agar revealing opaque mycelia with uneven white transparent velvets surface. (D): Mass of mycelia of branched non septated hyphae obtained from periphery of the culture. (E): Hyphae appeared as branched non septated with tapered end contain cytoplasmic organelles. (F): Hyphae of *A. invadans* stained with lactophenol cotton blue and give –y- shape appearance. (G): Thick and undulating hyphae obtained from the center of old culture. (H): *A. invadans* hyphae from tap water contain hemp seed showing primary zoospores as chain like connecting each other by thread.

Table (1): Prevalance of *Aphanomyces invadans* among the examined *C. gariepinus*.

	No. of Examined	Of % Examination	No. of Infected	of % Infection
Clinically diseased fish	80	76.2	9	11.25
Apparently healthy fish	25	23.8	0	0
Total	105	100	9	8.6

(% calculated according to the number of the examined fish)

Fig (2): Seasonal prevalence of *Aphanomyces invadans* among the examined *C. gariepinus*.



4. DISCUSSION

EUS is currently defined as a seasonal epizootic condition of freshwater and estuarine warm water fishes of complex infectious etiology characterized by the presence of invasive Aphanomyces spp. ((Vijayakumar et al., 2013). A number of etiologies have been proposed for the outbreak of EUS in which the primary agent is thought to be viruses, bacteria, parasites and fungi (Kamilya and Baruah, 2014). An unusual ulcerative condition in wild fish was reported for the first time in Africa (Huchzermeyer and van der Waal, 2012). Concerning the total prevalence, the infection rate was 8.6% among the examined *C. gariepinus*. These finding were nearly similar to Amany et al. (2004) who found that the prevalance of infection was 8%in *C.gariepinus*. Meanwhile, the disease was recorded in naini and rohu (Indian major carps) with prevalence rate of (97.1%) (Baidya and Prasad, 2013). Among the examined clinically diseased fish, 11.25% of them were positive for *Aphanomyces invadan*s. This means that *Aphanomyces invadans is* infectious to *C.gariepinus* but it spread mostly among the clinically diseased fish rather than the apparently healthy fish. So *Ahanomyces invadans* need predisposing factors that affect skin integrity to initiate the infection. Seasonal prevalence in *C. gariepinus* revealed that the rate of infection of Aphanomyces sp. was 20, 12 % in winter, spring respectively. No infection recorded in autumn and summer. These findings supported by Baldock et al. (2005) who found that the disease (EUS) often occurred when water temperatures drop. Which inversely induced a retarded immune response in the fish (Huchzermeyer and van der Waal, 2012). In the same manner, EUS in freshwater fishes in Bangladesh has been associated with low temperature, and has often occurred after periods of heavy rain (Khan and Lilley, 2002). The differences in prevalence may be attributed to fish species, water temperature, water salinity and virulence of aphanomyces spp.

C. gariepinus infected by Aphanomycois showing clinical signs of excess mucous secretion covering the skin, dark gray coloration of the skin, hemorrhagic inflamed destructed fin, superficial ulcers. Similar findings were recorded in *C. gariepinus* (Abd el-Latif, 2003; Amany et al., 2004). Meanwhile, loss of appetite, erratic swimming and red spots on the body surface observed in snakehead *Channa marulius* (Saylor et al., 2010) *Labeo* and *Catla* (Baidya and Prasad, 2013) infected with EUS.

Regarding the morphological characters of *Aphanomyces invadans* isolated on glucose peptone yeast agar showed opaque colonies with uneven white transparent velvets surface. Similar results were reported by several investigators (Abd el-Latif, 2003; Amany et al., 2004; Shaheen et al., 1999). Microscopically examination of wet mount preparations from growing cultures on GPY agar and broth revealed the presence of non septated thin branched long hyphae with tapered end. The hyphae contained a cytoplasmic organelle. These observations supported by the results of Afzali et al. (2013) who observed that that Aphanomyces spp. isolates exhibited vegetative mycelium about 5–10 lm in diameter, aseptate, smooth, slightly wavy, moderately branched.

In the wet mount preparations from the sporulating media, the sporangia were mostly formed at the hyphae tip but the hyphae remain unchanged in their diameters. The spores were rectangular like arranged inside the hyphae as chain connecting each other by thin filament. Unlike Saprolegnia., the present study revealed that the examined *Aphanomyces invadans* did not produce sexual structures in the autoclaved incubated tap water containing hemp seed cultures. In the same manner, a study carried out by Callinan et al. (1995) showed that the culture of Aphanomyces spp. had filamentous sporangia indistinguishable from hyphae, an 'achlyoid' manner of primary spore discharge and encystment and no of oogonia and antheridia were observed. The results are supported by Afzali et al. (2013) who found that Aphanomyces produced sporangia with a single row of primary spores and the primary spores were eventually released and encysted at the hyphal tip forming spore-balls, characteristic for the genus Aphanomyces, Zoosporangia were slender with the same diameter as hyphae and the strains appeared to be sterile and lacked sexual reproduction as (No oogonia or antheridia were observed).

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