APHANOMYCOSIS IN AFRICAN CATFISH "CLARIAS GARIEPINUS"

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ABSTRACT

Aphanomycosis was reported in about 8% of the examined *Clarias gariepinus.* The disease was recorded during spring and autumn. Aphanomyces fungus was found as sparsely branched-thin-long non septated hyphae in squash preparation from autolysed infected tissues. The organism was isolated on glucose peptone yeast broth using 5 steps culture technique with some modifications as addition of metalexyle, amphotricin B and fluconazol (antimycotics). Culture characters on glucose peptone yeast broth and agar were studied. On sporulating media, sporangia were formed at the hyphal tips where spores encysted in clusters from which secondary zoospores released. Biochemical identification of the isolated fungus was recorded. Clinical signs and lesions on naturally and experimentally infected fish were also described. Histopathological examinations revealed presence of severe inflammatory reaction with heavy inflammatory cellular infiltration and multiple granulomas formed from aggregation of inflammatory cells mainly epitheliod cells. Muscle degeneration and necrosis were observed. Numerous multinucleated gaint cells were found and some of them showing fragmented fungal parts within their cytoplasm. Moreover, PAS positive spores and fungal hyphae were noticed.

Abbreviations : PAS = Periodic Acid Schieff

First Scientific Conference of Fac. Vet. Med.;Moshtohor,Sept. 1-4, Benha-Ras sedr.

INTRODUCTION

Aphanomycosis is a seasonal epizootic condition of great importance in wild and farmed freshwater and estuarine fishes. It was reported for the first time among tropical fish showing swelling from which thin hyphae extend to the outside (31). Also, Vishniac and Nigrelli (38) isolated aphanomyces sp. from wounded platyfish. Aphanomycosis is recognized as epizootic ulcerative syndrome (EUS) in fresh water fish species from south eastern Asia (6); red spot disease in mullets from Philippines (25) and Australia (5) and mycotic granulomatosis in *Pleogossus altivelis* in Japan (13). Recently ulcerative mycosis in Atlantic menhaden from the north America (18, 20). In Egypt, aphanomyces sp. isolated from Nile tilapia (8) and from skin erosion and ulcers of cultured striped and thin lip grey mullets (30). The present work was planned to investigate the isolation and identification of causative fungus, prevalence of aphanomycosis among the examined *Clarias gariepinus*, as well as histopathological changes induced in experimentally infected fish.

MATERIALS AND METHODS

Examined fish: One hundred apparently healthy and diseased African catfish, *Clarias gariepinus* weighted 150 ± 10 g were randomly collected from their natural sources (El-Riah El-Tawfiki and their tributaries). The fish were transported alive in large tanks and sent to the wetlab, Faculty of Veterinary Medicine, Moshtohor. They were subjected to clinical examination according to Amlaker (2), then sacrificed and examined grossly for lesions and microscopically for the presence of suspected fungus (11).

Mycological examination: Using aseptic technique, small pieces from skin, fins, eyes and gills of the examined fish were taken for isolation of aphanomyces fungus on glucose peptone yeast broth and agar (GPY) using modified five steps culture technique (41). The isolated fungus was identified as previously described (1, 12, 21, 43)

Aquaria: A clean glass aquaria each measured 1 × 0.4 × 0.4 m were used for holding fish. They were supplied with dechlorinated tap water(16) and sufficient aeration by using electric air pump (Rina Italy).

Experimental fish: A total number of 75 apparently healthy *Cl. gariepinus* weighted 150 ± 10 g obtained from a private fish farm and transported in large tanks to the wet lab where they kept in well prepared aquaria and left 7 days for acclimation. A random sample of five fish were sacrificed and examined mycologically to ensure that the fish were aphanomyces free. The remaining 70 fish were subdivided into 7 group each of 10 fish per aquaria. The fish received pelleted

commercial ration (32% crude protein) and daily provided as 3% of their body weight (10). Uneaten food and excreta were aspirated regularly.

Preparation of inoculum: Sporulating media containing pure aphanomyces spores in a rate of 6000 spore per each 0.1 ml was prepared (12). A synthetic corticosteroid suspension dexamethazone (El-Amria company) was used as an immunosuppressant (3).

Experimental design: The fish were subdivided into seven groups (A-G) each of ten fish. Group A (with scrafied skin) and B (with intact skin) were exposed to aphanomyces spores (12000 spores/Liter) via bath challenge. Group C and D were infected via intramascular rout with 0.2 ml sporulating media each 0.1 ml contained 6000 spores, where fish of group D were previously treated with dexamethazone in a dose of 20 mg/kg fish. Group E were exposed to sterile sporulating media (0.2 ml) via bath challenge, while group F and G were injected intramuscularly with 0.2 ml of sterile sporulating media, where group G were previously treated with dexamethazone in a dose of 20 mg/kg fish. The last three groups used as controls. Both infected and control groups were kept under observation for 15 days.

Five fish from each group were scarified on days 7 and 15 post exposure. Clinical and postmortem examinations were carried out. Reisolation of aphanomyces fungus was done.

Histopathological examination: Specimens for histopathological examination were obtained from skin and underlying muscles of experimentally infected fish on day 7 post infection and fixed in 10% buffered formalin. The prepared sections were stained with hematoxylin and eosin stain (27). Periodic acid schief staining technique (PAS) was also used (9).

RESULTS AND DISCUSSIONS

In the present study, mycological examination of the collected *Cl. gariepinus* revealed presence of aphanomyces fungus in about 8% of the examined fish. Positive aphanomycosis fish showing clinical signs of excess mucous secretion covering the skin, dark gray coloration or even blackness of the skin, hemorrhagic inflamed destructed fin and small skin ulcers (Fig. 1A). These observations were nearly similar with those recorded in *M. cephalus* (4, 11, 30); *Cinhinus mirgala* (33) and menhaden (20).

The prevalence of infection among the examined fish was 8%. Such obtained results were lower than that detected in stripd and thin lip gray mullets (30). This may be due to the difference in the fish species or the virulence of the aphanomyces strain. The disease mainly recorded during spring and autumn. These findings

supported the results given by Virgona (37) who observed that environmental stress in mullet during autumn represented by fluctuating temperature from 24-30°C and sharp decrease in salinity prior to outbreak of red spot disease. Also, outbreaks of aphanomycosis were recorded in association with rapidly changing in temperatures (15, 24, 29). Squash preparations from autolized infected tissues revealed presence of thin long non septated sparsely branched hyphae of aphanomyces (Fig. 1B). These findings were inconsistent with other studies (7, 11).

Concerning the isolation, aphanomyces sp. was isolated on glucose peptone yeast broth and agar using 5 steps culture technique with some modifications by addition of antifungal drugs as metalexyl, amphotricin B and fluconazole in a way to overcome other fungal contamination. On glucose peptone agar, the suspected aphanomyces colonies appeared slightly opaque and had white velvety surface (Fig. 1C). With prolonged incubation, linear hyphal growths that fulfill the entire plate surface were appeared two week post inoculation (PI) (Fig. 1D). These observations were nearly similar to that obtained by some investigators (5,11, 30).

Microscopical examination of wet mount preparations from growing cultures on GPY agar and broth revealed the presence of sparsely branched non sepetated, long to very long hyphae with tapered end. The hyphae had cytoplasmic organelles (Fig. 1E). On GPY agar the hyphae obtained from the periphery of the growing colonies were thinner with smooth outline than those obtained from the center which appeared slightly wider, coarser with undulating wavy outline (Fig. 1F). These results were nearly similar with that observed by Callinan and Eroses (4) and Roberts *et al*. (28).

Wet preparations from fungal growth on a sterile tap water containing hemp seeds revealed abundant hyphal growth and the sporangia were formed from undifferentiated hyphae with septae at their bases. The sporangia were mostly formed at the hyphal tip. The primary zoospores were produced within the sporangium, which appeared as rectangular spores arranged in one row and linked together by thin cytoplasmic thread (Fig. 1G). The primary spores released and accumulated at the tip of the sporangia forming clusters of achyloid spores balls. The secondary zoospores were released from these balls leaving empty zoosporangium cyst in the terminal end (Fig. 1I). In some cases spores failed to release and encysted within the sporangium as refractile round encysted spores (Fig. 1H). These results were more or less similar with those reported by some investigators (13, 24 , 30 ,42). Regarding to sexual reproduction, the isolated Aphanomyces sp. failed to produce sexual structure on sterilized tap water containing hemp seed. These findings were similar with those

observed by Unestam and Weiss (36) who showed no sexual reproductive structures in Aphanomyces species.

With respect to the biochemical identification, all the obtained isolates of Aphanomyces species gave negative results toward carbohydrates utilization (glucose, manitol, sucrose, arabinose, starch and fructose) and urea utilization tests. The obtained results agree with that recorded by Yuasa and Hatai (43) who used the same biochemical tests as additional criteria for the identification of aphanomyces fungus.
Results of the experimental infection revealed that most fish of infected groups showed typical signs of the disease as haemorrhages allover the body surface as well as swellings, reddening and necrosis at the site of inoculation (Fig. 2A). Similar observations were previously recorded in Ayu *Plecoglossus altivetis* (26,39), snakehead *Chana striata* (35) and *menhaden.*(20).

The mortality rate among *C. gariepinus*, in groups infected by bath challenge was 40% and in fish infected intramuscularly was 60%, while in those infected intramuscularly and previously treated with dexamethazone was 80%. Similar findings were recorded in *O. niloticus* treated by cortizones and experimentally infected with *Saprolegnia parasitica* (17) and *Ichthyophonus hoferi* (14). Moreover, the inoculated aphanomyces spp. was reisolated from experimentally, infected fish and re-identified as previously described.

Histopathological examination of tissue sections from intramascular infected fish revealed heavy inflammatory cellular infiltration of the dermal and the underlying muscular layers mostly lymphocytes and macrophages was seen (Fig. 2B). Degeneration and necrosis of muscles represented by swelling, highly eosinophilic cytoplasm, loss of their striation and pyknosis of nuclei were noticed. Congestion of blood vessels and presence of multiple area of extravasation of erythrocytes were detected. Similar observations were also recorded in Ayu (39), snakhead (22) and menhaden fish (20). These pathological alterations could be attributed to the effect of fungi protease on the tissue (32). In addition numerous multinucleated giant cells with a circular or horse shoe-like arrangement of pyknotic nuclei and mononuclear inflammatory cells infiltrating the muscular layer were seen (Fig. 2C). Many fungal spores in between the muscles and small fragmented fungal parts within the cytoplasm of giant cells were noticed. These spores gave strongly positive PAS reaction (Fig. 2D). These findings were supported by those observed in dwarf goumi (*Colisa lata*) (39). Also, multiple fungal granulomas like formed from aggregation of inflammatory cells particularly epitheliod cells were noticed (Fig. 2E). Moreover large hyphae enclosed with inflammatory reaction mainly mononuclear cells and

reacted positively with PAS (Fig. 2F) were also recorded. These results came in accordance with that recorded in other studies (19, 20, 28, 34, 39, 40).

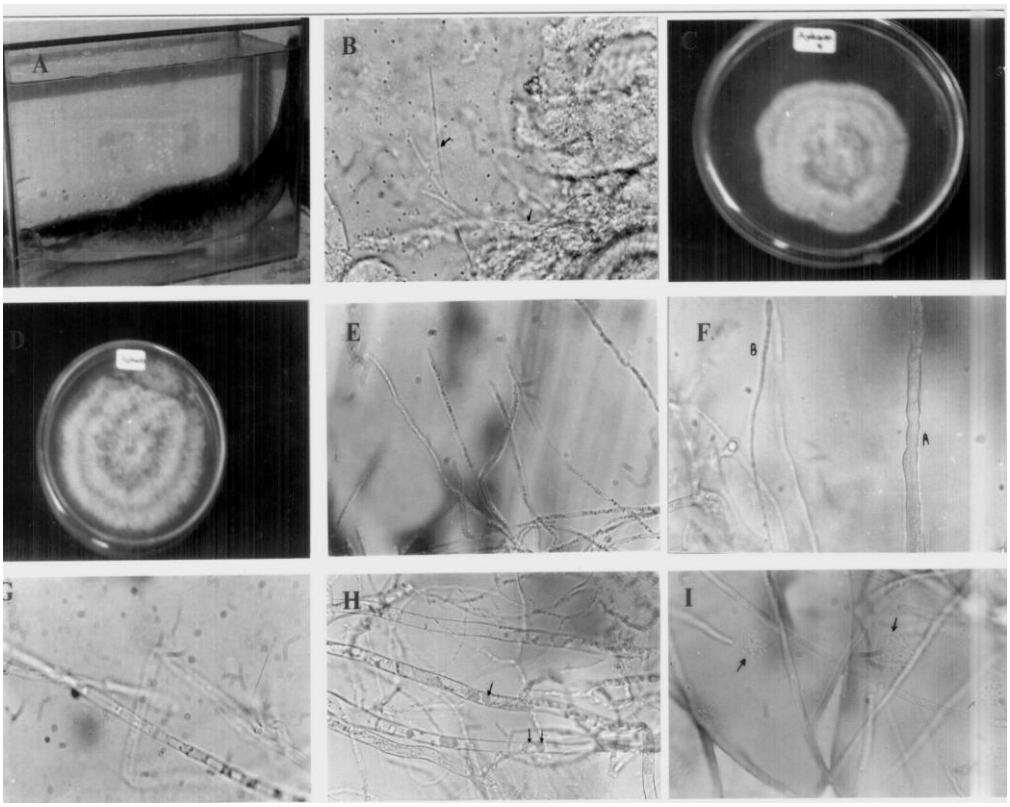

Fig. 1. showing naturally infected *C. gariepinus* with reddening of the entire surface focal ulcer and erosions (A); squash preparation of autolysed infected tissue showing hyphae of ahanomyces x 40 (B); fungal growth on GPY agar appeared slightly opaque and had white velvety surface (C), and at 2 weeks PI linear growth fulfill the entire plate (D);wet preparation from GPY agar culture showing sparsely branched non septated hyphae, with tapered end and contain cytoplasmic organelles (E), the hyphae at the center appeared wide, coarse and with undulating outline while those at the periphery were thin and with smooth outline (F); wet preparation from culture on tap water contained hempseed showing primary zoospore as one row linked together by a thin cytoplasmic thread (G); retained refractile encysted mature spores within the sporongia (H) and clusters ball of encysted zoospores at the hyphal tip (I).

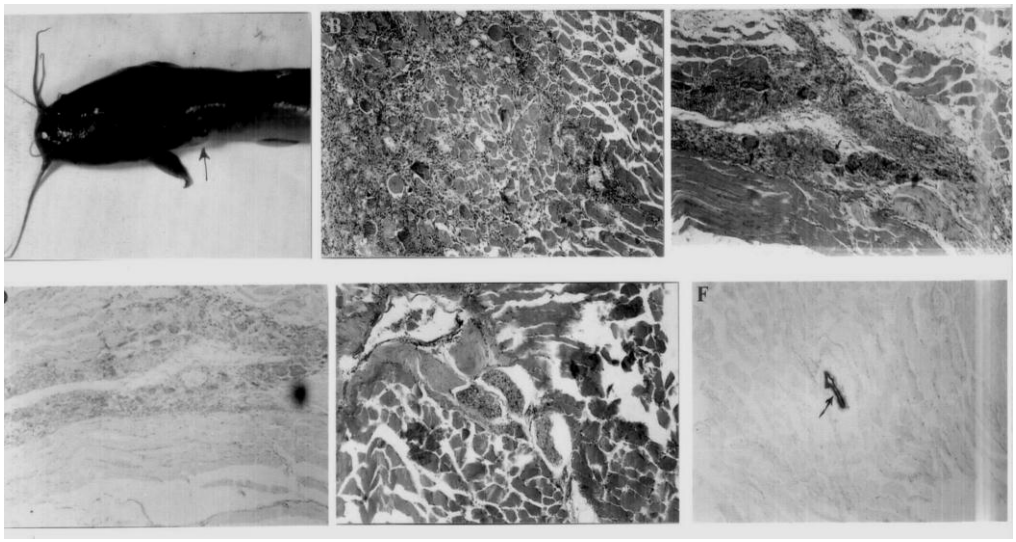

Fig. 2 . showing experimentally infected *C. gariepinus* with aphanomyces sp. with reddening swelling and necrosis at the site of inoculation 6 day post infection (A); Skin of catfish (*C. graiepinus*) inoculated with spores of aphanomyces sp. showing inflammatory cellular infiltration mostly lymphcytes and macrophages together with necrosis of the muscles. H & E stain × 100 (B); mononuclear inflammatory cells and numerous multinucleated giant cells contained fungal element. H & E stain × 200 (C) and PAS stain × 100 (D); multiple fungal granulomas formed from aggregation of inflammatory cells particularly epitheliod cells. H & E stain × 200 (E) and positive PAS stained fungal hyphae – PAS stain × 100 (F).

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مرض الأفانوميسيز فى أسماك القرموط الأفريقى (النيلى)

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** المستشفى البيطرى – كلية الطب البيطرى بمشتهر جامعة الزقازيق / فرع بنها*

أظهرت الدراسة وجود الأفانوميسيز بنسبة 8% فى أسماك القرموط النيلى التى تم فحصها وقد سجل المرض فى فصلى الربيع والخريف وبالفحص المجهرى للأنسجة المتحللة من الأسماك المصابة ظهر فطر الأفانوميسيز على هيئة غزل فطرى غير مقسم طويل رفيع قليل التفرع. تم عزل الفطر بالزرع على بيئة جلوكوز ببتون وخلاصة الخميرة مضافاً إليها مضادات للفطريات مثل ميتالبكسيل وأمفوترسين ب فلوكونازول وقد تم دراسة الصفات المورفولوجية للفطر على هذه البيئة وبالزرع على البيئة الخاصة بالتجرثم كون الفطر الحافظة الجرثومية التى تتحور على شكل كريات عند نهاية الغزل ويخرج منها الجراثيم الثانوية. تم تسجيل التصنيف البيوكيميائى للفطر المعزول وكذلك العلامات المرضية والصفة التشريحية للأسماك المصابة طبيعياً والمعداة صناعياً. أوضح الفحص الميكروسكوبى لأنسجة سمكة القرامبط المعدى بفطر الأفانوميسيز وجود غزل فطرى بداخل أنسجة العضلات وأيضا داخل الخلايا العملاقة عديدة الأنوية. وكذلك وجود عقيدات تتكون من تجمعات من الخلايا الالتهابية خاصة الخلايا الظهرانية Epitheliod cell كما شوهد احتقان ومناطق أنزفة وتجمعات من الخلايا العملاقة عديدة الأنوية بين العضلات بالإضافة إلى وجود بعض الغزل الفطرى موجبة الصبغة لـ PAS محاطة بخلايا التهابية.