Isolation and pathological study of Branchiomycosis from the commercial pond of common carp (*Cyprinus carpio)* fish, in Governorate of Duhok / Iraq.

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Summary

Branchiomycosis is a fungal disease that infects fish gills. It was identified by isolation and histopathological changes of examined gills in common carp fish (*Cyprinus carpio*) which, were obtained from fish farm in Duhok Governorate, Iraq. The infected fish were suffering from respiratory disorders; gulping air at the water surface, rapid movement of operculum and massive mortality, which resulted in the loss of 95% of fish pond. The gills appear marbled appearance with necrotic areas on the localized damage gills. The causal pathogen was identified as *Branchiomyces sanguinis*, in which the diameter of spores and non-septated hyphae are 5-7 µm and 12 – 20 µm, respectively. In histopathological preparation, the spores and the non-septated hyphae have been shown to be embedded in the gill tissues contained undivided and sporulating stages.

Key Words: Branchiomycosis ; infected fish farm Duhok Government, Iraq ; fungal fish disease .

عزل ودراسة التغيرات النسجية للمرض برانكيومايكوسس في حقول الأسماك الكارب الأعتيادية نوع *(Cyprinus carpio)* في محافظة دهوك, العراق.

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الخلاصة

برانكيومايكوسس مرض فطري يصيب خياشيم الأسماك و تم تشخيصه بعد عزل الفطر المسبب وفحص التغيرات النسيجة للخياشيم في أسماك الكارب الأعتيادية (*Cyprinus carpio*) من حقل تربية الأسماك في محافظة دهوك, العراق.

و كانت اهم العلامات المرضية للاسماك المصابة صعوبة التنفس وأزدراد الهواء الموجودة على سطح الماء مع سرعة حركة طبقة الخياشيم مع نسبة هلاكات عالية وصلت الى 95% في حوض تربية الاسماك. اظهر الفحص المظهري لخ ياشيم الاسماك المصابة برخامية المظهر مع تنخر في المناطق المتضررة. تم التعرف على الفطر (*Branchiomyces sanguinis*) اعتمادا على قطر الأبواغ وخيوط الفطرية عديمة الحواجزوالتي كانت تتراوح ما بين 5-7 مايكرومتر و 12-20 مايكرومتر على التوالي. واظهر الفحص المجهري وجود الأبواغ والهايفي مغمورة في النسيج الخيشومي وأحتوت على أبواغ غير مقسمة وفي مراحل مختلفة.

Introduction

Branchiomycosis is a much feared fungal disease of fishes almost all over the world, especially on carp farms and other fish farm (1, 2 and 6). This fungal disease, sometimes called gill rot, which has caused acute, often high mortality in a number of fresh water fish (4, 5, 6, 11 and 19). The disease occurs most frequently in the warm climatic regions (1 and 2). The rise, and course of the disease depend on factors that underline them; water temperature is one of the factors that play the most important part with a high load of organic matter, ponds fertilized by organic manure, and high levels of unionized ammonia in the water increase the incidence of fungal gill rot due to gill epithelial cell hyperplasia (1 and 3).

Branchiomycosis is caused by two species; *Branchiomyces sanguinis* in carp and / or tench, crucian carp and sticklebacks and *Branchiomyces demigrans*, which infects large-mouth bass, northern pike, tench and striped bass in Europe, Taiwan, or the USA (2, 4, 10, 11, 12, 19). *Branchiomyces sanguinis* is generally located in the blood vessels of the gill arch and gill filaments. The diameter of the hyphae is 8–20 μm, the thickness of the hypha wall is 0.2 μm and the diameter of the spore is 5–9 μm while *Branchiomyces demigrans* produces hyphae which are able to penetrate into the gill filaments and spread on their surface. The diameter of the hyphae usually is 13–14 μm and may be up to 22–28 μm at the end of the hypha. The thickness of the wall is 0.5–0.7 μm and the diameter of the spore is 12–17 μm (1).

There is no treatment for branchiomycosis (5, 8, 9 and 10). Surviving fish are carriers of the infection and should not be transferred into *Branchiomyces*-free geographical areas. Dead affected fish should be burned and/or buried (9 and 10).

The aim of present study includes: Isolating and identifying of the causative agent of Brnachiomycosis by cultivation on culture media and histopathology study of the affected common carp (*Cyprinus carpio*) , fish pond in Mankishik district, Duhok government, Iraq.

Materials and Methods

In this, study ten of common carp (*Cyprinus carpio*) , fish weight 700-1500 gm. were obtained from the commercial common carp (*Cyprinus carpio*) , fish pond of "Talal project in Mankishik –Dohuk," with neither no quarantine expectations for transferred new fish from farm fish in Aqraa or nor prior treatment . They were submitted to laboratory of clinical pathology laboratory of the College of Veterinary Medicine, Dohuk University, in container of freshwater for further investigation which include microbiological and histopathological examination . The affected fish were that referred from commercial fish from located in Mankishik region/ Duhok provide to College of

Veterinary Medicine/ University of Duhok . Fish were euthanized by 40% ethyl alcohol then examined for detection any abnormality of postmortem, of external and internal body fish. Gills examination was done by lifting the operculum and examined with naked eye. Gills from both side are kept in a Petri dish with little amount of normal saline 90 % then this gill were examined under a dissecting microscope. A wet mount of gill filaments were prepared and examine under a light microscope for detection of spores and hyphae as mentioned by (15 and 18).

All positive samples by direct microscopically examination were cultured on Sabouraud's dextrose agar 65 g with cychlohexamide 0.5 g, chloramphenicol 1x250 mg capsule, 0.65 ml of 40 mg/ml gentamycin, yeast extract 5 g, and distilled water 1000 ml and then incubated at 22-25 C^{o} for at least one weak. By both lactophenol cotton blue and iodine 10% stains were applied, for examination of fungal growth on microscopic slide. The shape and diameter of hayphae and spores isolated fungus were measured.

After the swab was taken from gills, the gills (especially the areas of gill section that appear pale or necrotic) were fixed in 10% neutral buffered formalin. The histopathological section from gills of fish were prepared by paraffin blocks which had been sectioned at 4-6 µm in thickness according to (13) and stained with Haematoxylin (Harris) and Eosin according to (14) which was carried out on histological laboratory at institute of Zakho.

Results

The affected fish manifested by appearance of respiratory disorders which include lethargy with gulping air at the water surface and rapid movement of operculum, with high mortality rate 95% . At postmortem examination there was no any abnormality on the surface of the morbid fish, while in the examined gills, the affected areas of gills were appeared that striated or marbled appearance with the pale areas representing the infection and the dying tissues and then become necrotizing as in (Fig. 1 A and B). The direct examination of the gills which was mounted under light microscope revealed the characteristic spores (Fig. 2).

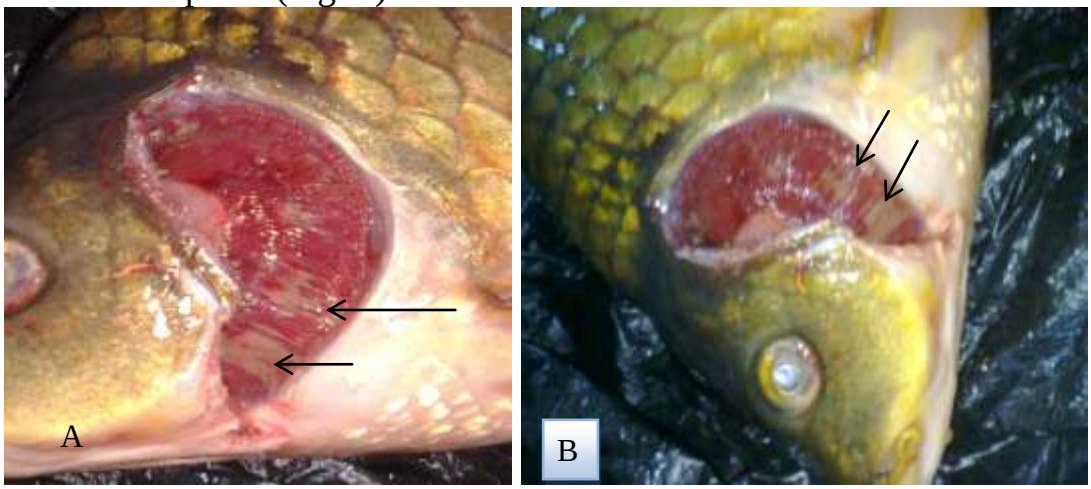

Figure 1 (A, B): *Cyprinus carpio* infected with *Branchiomyces* species showing marbled appearance with the pale and necrotizing areas (arrows).

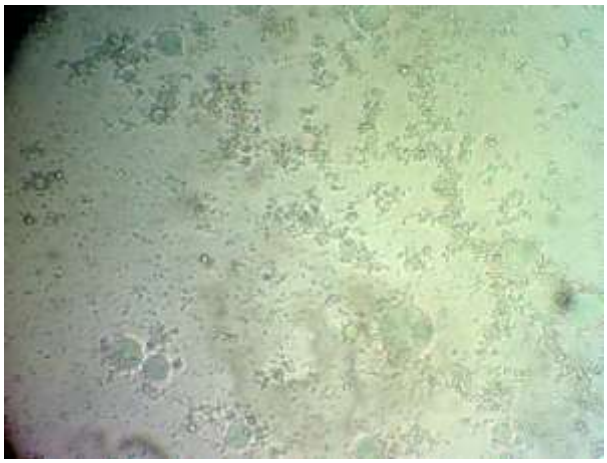

Figure 2: Direct examination of the gills spores of *Branchiomyces* spp.

Figure 3: Culturing of *Branchiomyces* spp. on the Sabouraud's dextrose agar after three days from *Cyprinus carpio.*

The positive samples of gill fish by direct microscopic examination were cultured on the specific media (Sabouraud's dextrose agar with cychlohexamide, chloramphencol, gentamycin, yeast extract) were daily examined for fungal growth. The primary growth was appeared after 3 days of culturing (Fig. 3) and the typical full growth of colonies were observed 7 days of culturing. The typical colonies appear as folded heaped, glabrous and velvety, white in color and with white –yellowish in revers site (Fig. 4). The diameter of the hyphae is 12 – 20 µm with spores ranging 5-7 µm diameters (Fig. 5 , 6 and 7) .

Figure 4: *Branchiomyces spp.* culture after 7 days of cultivation, the colonies shows as folded heaped, glabrous and velvety, white in color and with white –yellowish in reveries

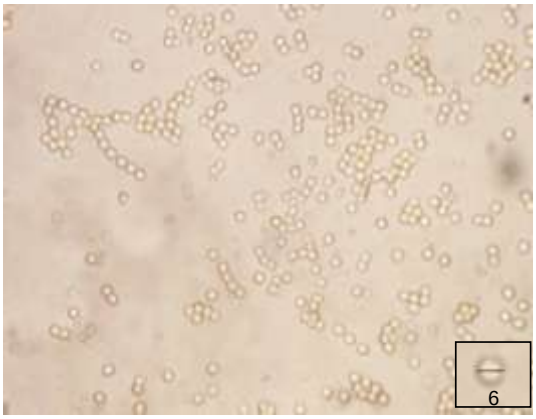

Figure 5: Spores of *Branchiomyces spp.* stained with Iodine from culture. Bar= 6 µm

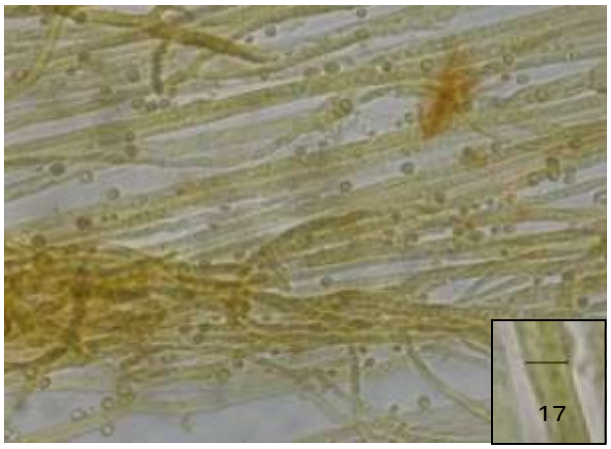

Figure 6: Spores and hyphae of *Branchiomyces spp.* stained with iodine from culture. Bar= 17 µm

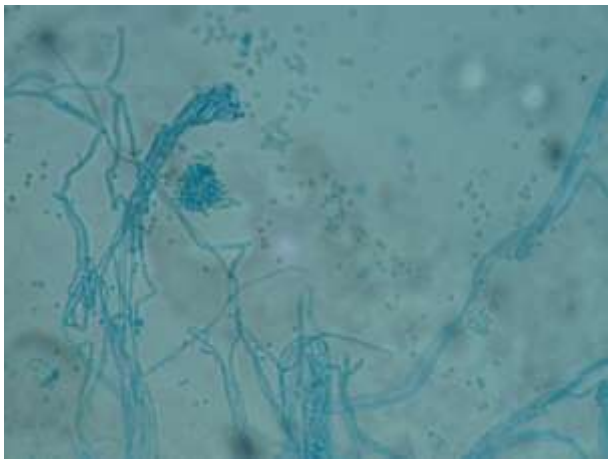

Figure 7: Spores and hyphae of *Branchiomyces spp.* stained with Lactophenol cotton blue stain from cultivation.

The Histopathological examination of the infected site of gill was appeared as fusing of primarily lamellae and heavy proliferation of gill filament with the appearance of heavy numbers of spores and unseptated hyphae (Fig. 8). The spores and hyphae in the blood vessels of the gill were cause blockage, haemostasis and thromboses, which consequently caused extensive necrosis of the gill filaments and areas of the gill filaments turned brown . The process was fast and was accompanied by proliferation of the gill epithelium with resulting adhesions of the filaments. Single, hard-walled sporangium-like bodies (Fig. 9). Other sections of hyphae contained numerous uninucleate or binucleate bodies at various stages of differentiation (sporonts), with a thin and hard wall, and with either many vacuoles or a dense cytoplasm (Figs. 10, 11 and 12). When spread into the surrounding tissue, they also became aggregated within large macro- phage-like cells with a marginal, flattened nucleus. The proliferated epithelial cells were organized into concentric layers around the fungal hyphae. The hyphal wall was embedded in a narrow layer of homogeneous matrix, apparently an end product of local cellular necrosis (Fig. 12). Signs of localized cellular damage (including spongiosis or cellular degeneration, karyorrhexis and pyknosis) were also evident throughout the epithelial layer (Figs. 11, 12). The proliferated epithelium was infiltrated by erythrocytes and macrophages. Such gill filaments contained necrotic residues with residues of congestion and some remains of fungal sporonts (Fig. 13)

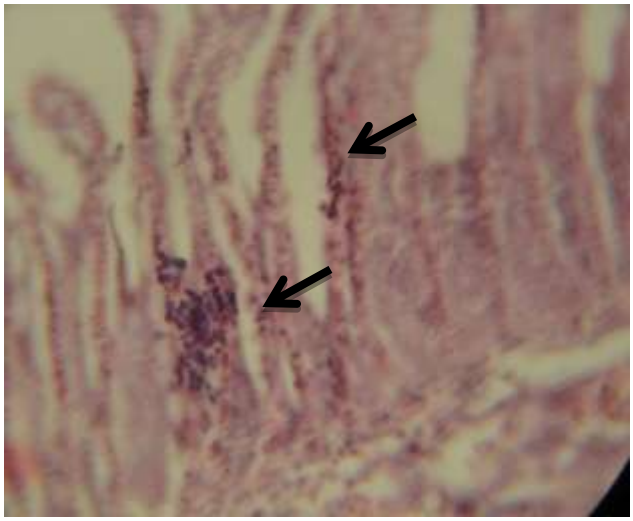

Figure 8: Histological section of gill *Cyprinus carpio* infected with *Branchiomyces spp.*, revealing heavily proliferated gill filaments (arrows): H & E 400 X.

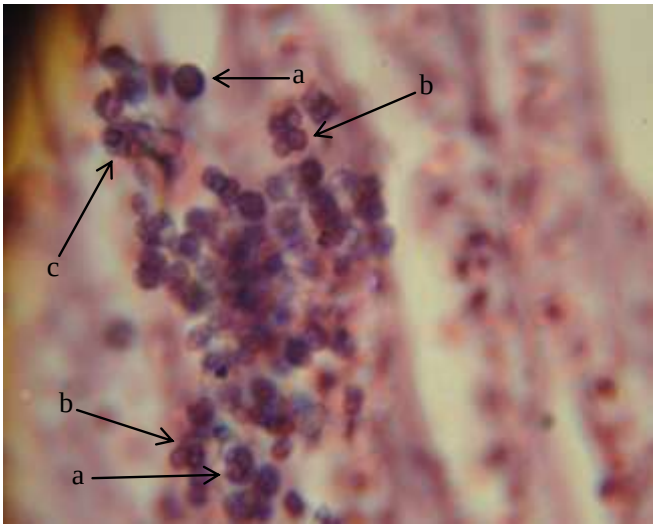

Figure 9: Histological section of gill *Cyprinus carpio* infected with *Branchiomyces spp.*, revealing (a) Multinucleate sporangium-like body; (b) divided sporonts ; (c) uninuclear progemy of plasmodium.). H & E 1000 X.

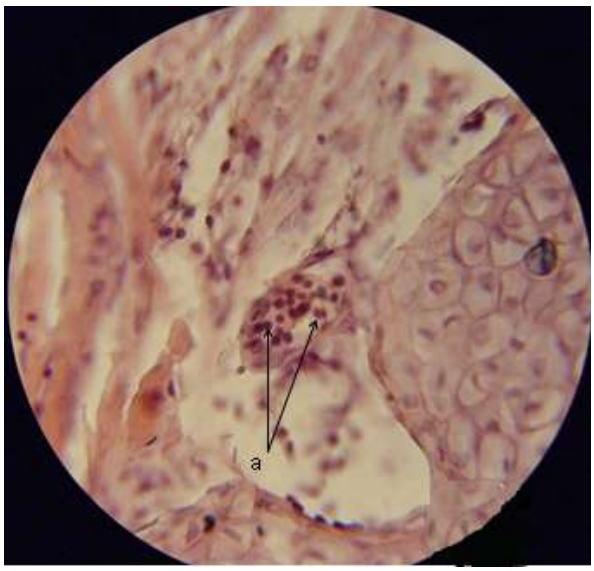

Figure 10: Histological section of gill *Cyprinus carpio* infected with *Branchiomyces spp.*, revealing, hyphae containing (a) uninucleate and binucleate progeny of plasmodia. H & E 400 X.

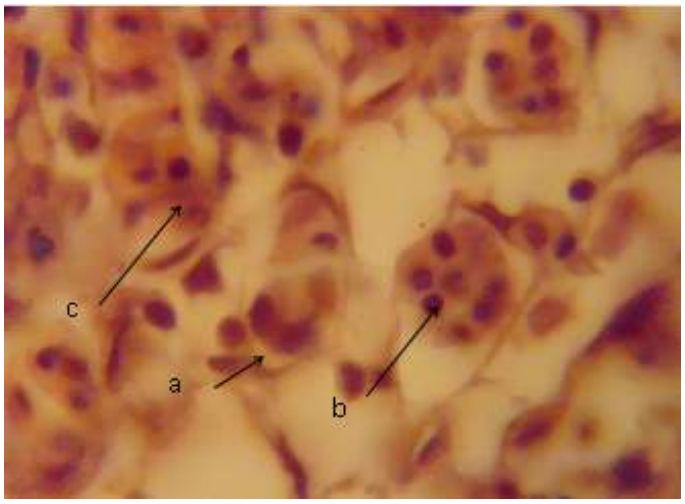

Figure 11: Histological section of gill *Cyprinus carpio* infected with *Branchiomyces spp.*, revealing hypha containing (a) plasmidium (b) uninuclear progemy of plasmodium (c) adjoining area of epithelial degeneration pyknosis, keryorrhexis. H & E 1000X

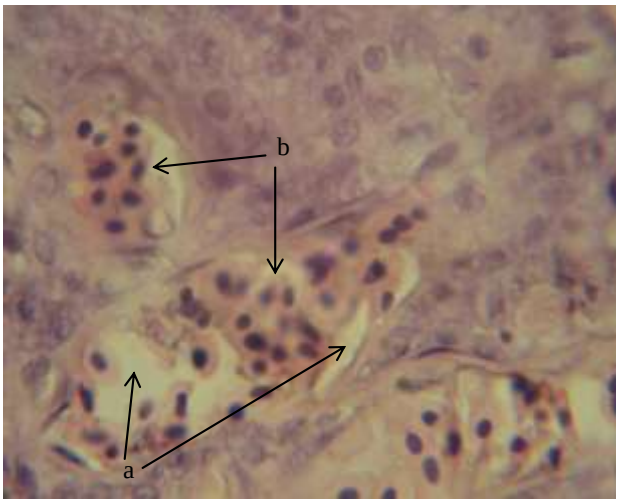

Figure 12: Histological section of gill *Cyprinus carpio* infected with *Branchiomyces spp.*, revealing (a) hyphae; (b) hyphae loaded with progeny of plasmodia (sporonts) at various stages of differentiation (arrow: thin-walled sporonts, or 'daughter' plasmodia). H & E 1000 X.

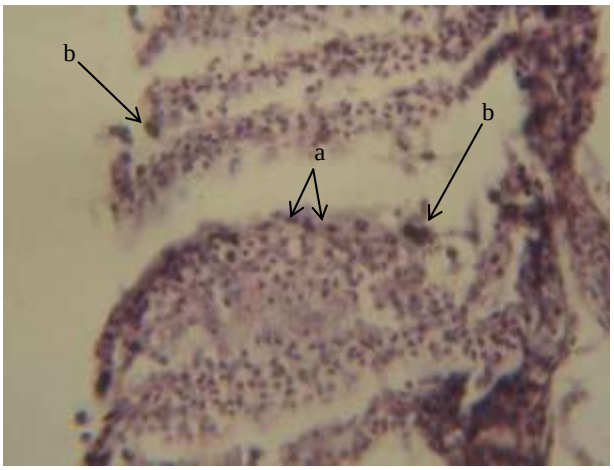

Figure 13: Histological section of gill *Cyprinus carpio* infected with *Branchiomyces spp.*, revealing, Branchiomycosis-induced necrosis of the gill filament with (a) residues of congestion and (b) some remains of fungal sporonts. H & E 400 X.

Discussion

This is the first report for the occurrence of Branchiomycosis on fish in Duhok Governorate, Kurdistan region of Iraq. Branchiomycosis affects a wide variety of cultured fish throughout the world but particularly carp family. Generally, the diagnosis of the disease depend on the field observation which includes case history, clinical and gross signs of the diseases, while laboratory diagnosis of the disease depends mainly on the demonstration of spores and hyphae of the fungi by isolation of fungi in the culture technique and histopathological examination.

The present study shows that the infected fish which are clinically characterizes by respiratory disorder including rapid movement of operculum and gasping of the fish on the surface of the water and these signs are similar to those reported by (4, 5, 6 and 11), and gills appear striated or marbled with the pale areas which is a pathognomic lesions and this result is in agreement with those reported by (4 and 8).

This study also shows that Sabouraud's dextrose agar containing cychlohexamide, chloramphencol, gentamycin, yeast extract, and agar is selective media for isolation of the *Branchiomyces* spp. from infected fish samples and this result agrees with (19).

Hyphal diameters of our isolates *Branchiomyces* spp. are ranging from 12 to 20 µm and spores diameters are ranging from 5 to 7 µm. These measurements

of fungi are in agreement with those given by (1 and 18) which is assigned to *Branchiomyces sanguinis*.

These fungi affect only the gills, leading to localized gill damage, therefore called gill rot which causes acute signs of respiratory disorder and high mortality, due to anoxia and these result are similar to those reported (4, 5, 6, 11 and 16). This study shows that the spores and hyphae of fungi infect; blood vessels of gill, gill arches and base of the primary lamellae cause an infarctive necrosis of gill that cause blockage and thrombosis, therefore termed "gangrenous branchitis," for which there is no treatment and these in agreement with (2, 3, 4, 7 and 18). These results are in agreement with that of (6) who reported that *Branchiomyces* infections in carp fish in Europe, which is very pathogenic to fish, and difficult to control.

The histopathology of the effected fish's gills shows the fusion of primary lamellae and the heavy proliferation of gill filaments with numerous numbers of spores and non-septated hyphae. These hyphae contain; multinucleated sporangium-like body, divided sporonts which could be uninucleated or binucleate bodies in various stage (sporonts). The localized cellular damage of the epithelial layer and the necrotic residues of congestion remain of some fungal sporonts and these results are in agreement with (16).

The outbreak of the disease occur due to environmental factor like high-temperature of the summer, and the bad-management such as transported and bad handling of entering new fish from other pond, which contributed into acceleration a favorable environment for the proliferation of this fungus and this in agreement with (17 and 19).

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