Histopathological Studies of *Oreochromis niloticus* affected by some pathogenic fungi from Abbassa Fish Farms

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Abstract

This study was conducted to investigate the fungal biota of cultured *O. niloticus* from Abbassa Fish Farms. Fishes were subjected to clinical, postmortem, mycological and histopathological examinations. The obtained results showed that, *(Saprolegnia* spp. *Cladosporium* sp., *Chrysosporium* sp., *Scopulariopsis brevicaulis, Aspergillus versicolor* sp., *Aspergillus ochraceus, Aspergillus niger, Aspergillus glaucus, Aspergillus wentii, Rhodotorulla* sp., *Penicillium* sp., *Syncephalastrum* sp., *Aspergillus tamarii., Aspergillus flavus, Trichoderma* sp., *Acremonium* sp., *Rhizopus* sp., *Fusarium* sp., *Phoma* sp., *Absidia* sp., *Aspergillus candidus, Alternaria* sp., *Aspergillus terreus, Aspergillus fumigatus and Paecilomyces* sp.) were isolated from the examined fish. *Penicillium* sp. is the most fungus distributed all over the year. Clinical signs and postmortem lesions of examined fish were slow swimming, loss of appetite, scale loss, ulceration, severe erosion and hemorrhagic skin, with septicemic picture in internal organs. Most histopathological alterations of infected fishes were recorded in skin, gills, liver and kidney and discussed.

Introduction

Increasing aquaculture has been accompanied by outbreaks due to diseases. Fungal infections are mainly secondary to environmental stressors exist in water environment and may result in economic losses to farming projects due to increased mortality, poor weight gain, besides low market value (**Rajinikanth *et al.*, 2010**). There are over 1.5 million fungal species distributed widely throughout the globe. Many of these fungi which are the chief agents of cellulose decomposition; have great potential in the daily life of human beings besides their utilization in industry, agriculture, medicine, biotechnology, in recycling nutrients and decomposing the dead organic matter in soil and litter (**Pani *et al.*, 2010**).

Fungi are present everywhere in salt water or fresh water, in cool or warm temperatures (**Elias and Abd El Ghany, 2008**).

The different species of molds (*Saprolegnia* spp., *Aspergillus* spp., *Penicillium* sp., *Mucor* sp., *Rhizopus* sp., *and Cladosporium* sp.) which isolated from different localities in Egypt caused tail and fin rot syndrome and skin lesions in Tilapia species and Nile catfish (**Marzouk *et al.*, 1990**). Saprolegniasis is a worldwide serious mycotic winter fresh water disease often affects wild and cultured fishes (**Osman *et al.*, 2010**). Hyphae of *Saprolegnia* sp. may invade deep tissues of fish and penetrate the vital organs even the central nervous system (**Zaki *et al*., 2003**). *Saprolegnia delica* and *Dictyuchus carpophorus* (the greatest fungal populations) were the most dominant isolated zoosporic fungal species where they were highly occurred especially at the hyper-polluted waters with the heavy metals (**Ali, 2007**).

Refai *et al*. (2010) isolated *Saprolegnia, Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus, Scopulariopsis, Paeciliomyces and Curvularia* from *Oreochromis* species.

Histopathologically different kinds of retrogression changes were observed in skin, gills and liver of the infected fish (**Rao *et al.*, 2015**).

This study was occurred to study clinical signs, postmortem findings and histopathological changes due to fungal infection in cultured *O. niloticus* with identification of the isolated fungi.

Material and methods

A total number (430) Nile tilapia (*Oreochromis niloticus*) at different stages was obtained alive from Abbassa Fish Farms in Sharkia Governorate. The fish were transported into Fish Diseases Department, Central Lab. for Aquaculture Research (El- Abbassa), Sharkia. Clinical and postmortem findings were recorded. Fungal examination using wet preparation, isolation and identification of fungi and histopathological examination of infected fishes were applied according to (**Carleton *et al.*, 1967**).

Isolation and identification of fungai

Under aseptic condition one gram of fish samples (skin & muscle, gills, liver, kidneys and intestine) was homogenized in 9 ml sterile water then diluted to 1/1000. One milliliter of the diluted sample (10^{-3}) was aseptically transferred into a sterile Petri dish then pours the medium (Czapek' s yeast extract agar (CYA) and potato dextrose agar (PDA)) using Dilution plate technique (**Garrett, 1981**) and supplemented with Rose Bengal (1/15,000) and chloramphenicol (50 ppm) for suppression of bacterial growth (**Smith**

and Dawson, 1944). After inoculation, plates were incubated at 27 °C for 5 to 7 days, thereafter; developing colonies were identified and counted.

The isolated fungi was identified using phenotypic (macroscopic and microscopic) approach down to the species level on standard morphology characteristics of fungi on standard media will mainly base on the following identification keys: (**Pitt, 1980**) *Penicillium*; (**Raper, and Fennell, 1965**) *Aspergillus*; **(Ellis, 1971& 1976)** dematiaceous hyphomycetes, and more dematiaceous hyphomycetes; **(Booth, 1971**) *Fusarium*; (**Domsch *et al.*, 2007**) for miscellaneous fungi; and (**Guarro *et al.*, 2012**) ascomycetes. Prevalence of isolated fungi to the infected fish and organs were recorded.

Histopathological examination

Tissue specimens were taken from suspected lesions and healthy tissues and fixed in 10% phosphate buffered formalin, washed and ethanol was used for removal of excess water then embedded in paraffin, sectioned and stained with Haematoxylin and Eosin stains (H&E) (**Carleton *et al.*, 1967**).

Results and Discussion

Clinical signs

Naturally infected fish showed loss of appetite, scale loss, increase of mucus secretion, fins and tail rot, ulcer and emaciation as show in figures (1, 2, 3 & 4) which agree with that recorded by (**Nagib, 1994**). Cotton wool like white to dark grey growth on the head region and dorsal fin then spread all over the body in the form of focal patches as recorded by (**Osman *et al.*, 2010 and Khoo, 2000**).

Postmortem findings

Postmortem lesions were yellow liver, congestion in the internal organs, enlargement in the spleen and gall bladder as show in figure (5). The severe emaciation and congestion in all internal organs may be due to production of some mycotoxins as ochratoxins, this finding was confirmed by (**Hassan *et al.*, 2010**), who found that, ochratoxin A was produced by *A. niger*.

Identification of isolated fungi

In this study the isolated fungi were suspected identified as *Saprolegnia* sp. (Fig. 6), *Cladosporium* sp. *Chrysosporium* sp., *Aspergillus ochraceus*, *Aspergillus candidus*, *Aspergillus niger* (Fig. 7), *Aspergillus tamarii.*, *Aspergillus flavus* (Fig. 8), *Aspergillus terreus* (Fig. 9&10), *Aspergillus fumigatus* (Fig. 11), *Aspergillus glaucus* (Fig. 12), *Aspergillus*

wentii (Fig. 13),, *Aspergillus versicolor* (Fig. 14), *Rhodotorulla* sp., *Acremonium* sp., *Rhizopus* sp., *Fusarium* sp., *Phoma* sp., *Absidia* sp. (Fig. 15), *Penicillium* sp. (Fig. 16), *Trichoderma* sp. (Fig. 17), *Syncephalastrum* sp. (Fig. 18), *Scopulariopsis brevicaulis* (Fig. 19), *Alternaria* sp. and *Paecilomyces farinosus*.

Prevalence of mycological infection in the examined *O. niloticus*

Our study revealed that, out of 430 examined fish of *O. niloticus* only 131 were infected with various fungi table (1). Distribution of different fungi isolated from different organs and tissues of infected *O. niloticus* revealed that, the highest organ infected by fungi was Gills (83.96%), followed by skin, fin and lesion (54.93%). The high prevalence of fungal infection in skin may be due to saprophytic nature of fungus on fish mucous, this finding was supported by (**Udomkusonsri *et al*., 2007**), who recorded that, fungal pathogens are generally found on fish skin, gill, water and environment surrounding fish. The lowest prevalence of fungal infection was occurred in the internal organs was (48.85%).

Aspergillus sp. was the highest prevalence of fungus infection in the examined fish, this may attributed to the faster growth rate of this fungus in addition to its better intrinsic prolific sporulating capacity to utilize the substrate, this result agrees with **Pani *et al*.** (**2010).**

Penicillium sp. was the most common fungi which was isolated (25.95%) from gills, skin and fins, internal organs and other lesions of the examined *O. niloticus*. The highest prevalence of *Penicillium* sp infection from gills (12.2%), this result agreed with (**Shaheen, 1986**) who isolated *Penicillium* sp. with high incidence from skin, gills and with low incidence from kidney, also this result was in consistence with (**Abd El-Ghany, 1998**) who recorded that, genus *Penicillium* is characterized by saprophytic existence and it was recorded as a rare disease producing agent. (**Randhawa *et al*., 2009**) reported also that *Penicillium* species are ubiquitous and their spores are spread by wind and insects and are usually regarded as unimportant in terms of causing disease.

Aspergillus niger was isolated with high percentage (14.5%) from gills, skin, fins and internal organs of examined *O. niloticus*. The prevalence of *A. niger* isolated from lesions of infected fishes was 1.52%. The severe emaciation and congestion in all internal organs may be due to production of some mycotoxins as ochratoxins, this finding was confirmed by (**Hassan *et al*., 2010**), who found that, ochratoxin A was produced by *A. niger*. Pathogenicity of *A. flavus* also may be due to its mycotoxins

production (**Ayansina and Owoseni, 2010**) and (**Chang and Ehrlish, 2010**).

A. ochraceus was isolated from gills, skin& fins and internal organs with percentage of (2.4%) of examined *O. niloticus*. The prevalence was 3.1, 3 and 1.8% with internal organs, skin& fins and gills respectively. These results were consistent with (**Abd El-Ghany, 1998**) who isolated *A. ochraceus* from skin, liver and kidney of *C. gariepinus.* (**Hassan *et al*., 2010**) mentioned that ochratoxin is a toxic secondary fungal metabolite produced by *Aspergillus ochraceus*.

Aspergillus fumigatus was isolated from gills only of examined *O. niloticus* and its prevalence was (0.9%) without lesion detected.

Alternaria sp. was isolated only from internal organs of examined *O. niloticus* with percentage (3.1%) with no lesion detected.

Cladosporium sp. was isolated from gills, skin& fin and internal organs of examined *O. niloticus* with percentage (4.8%).

Different species of zygomycetes *(Rhizopus* sp. *and Absidia* sp.*)* fungi were isolated from skin, gills and internal organs of examined O. niloticus, while did not isolate from lesion. That may match with **Shaheen** (**1986**) who mentioned that zygomycetes species were not associated with any disease processes.

Saprolegnia spp. was isolated from the skin and fins of examined *O. niloticus* with prevalence 15.15%. Mortality usually increases as temperatures rise in early spring. This is supported by (**Wise, 2008**) who reported that, occurrence of the disease appears to be dependent primarily on two factors: a rapid drop in water temperature which induces immunosuppressant in the fish and maintenance of low water temperature which favors the proliferation of *Saprolegnia* spp. and production of high number of motile zoospores in the water. *Saprolegnia* spp. occurred as a relatively superficial, cotton wool like white to dark grey growth on the head region and dorsal fin then spread all over the body in the form of focal patches. Similar findings were obtained by (**Osman *et al*., 2010**). Typically, *Saprolegnia* spp. presented as cottony white growth on the skin of fish when in water, while out of the water the cottony appearance is difficult to appreciate because the mycelium collapses into a slimy mass this was similar to the findings of (**Khoo, 2000**).

Yeasts are ubiquitous microorganisms, which disseminate with animals, air and water currents, and can grow in various environments where organs and substrate are available. The natural proliferation of yeasts in fish mucus may be generally considered as commensalism, in

spite of a few cases of pathological infections mainly due to opportunistic strains, that was reported by (**Gatesoupe, 2007**). *Rhodotorula* sp. was isolated from gills, skin& fins and internal organs of examined *O. niloticus* with 5.2%, but it isolated with high percentage from skin& fins (10.6%).

Seasonal prevalence of isolated fungi:

From table (2) the results revealed that, the highest percentage of infection was obtained during Summer season (46.6%), this result was in harmony with that obtained by (**El-Abbassy, 2007**) who reported that, thermo tolerant molds of *A. niger, A. flavus, A. ochraceus* and *Penicillium* sp. grow at temperature degree ranged from 8 - 45°C and agreed with (**Skupieka, 2010**) and (**Hennessey, 2010**) who mentioned that, molds prefer warm and humid environment. (**Gubbins, 2006**) also recorded that, once the thermal optimum is exceeded, the function of the immune system will decline and physiological stress and oxygen depletion (warmer water holds less oxygen in solution than cold water) may well lead to disease and welfare issues in fish and under higher temperature regimen, most fungal infections would be predicted to progress faster once the host was infected. In addition, **De Canals *et al*., (2001)** reported that, fungal infections often start when an immune-suppression is produced by a sudden variation in temperature. The infection prevalence of *Rhodotorula* sp. was very high in spring season (47.3%).

The seasonal prevalence of fungal infection of examined *O. niloticus* was high in Autumn (40.1%) after summer. This findings is supported by (**Rezeaka, 1991**) who mentioned that, the majority of infections occurred during Autumn season when sudden changes in temperature were detected. The prevalence of isolated fungi from examined *O. niloticus* was (18.2%) in Winter and the lowest percentage of infection was 17.1% in Spring season. This result agreed with (**Skupeika, 2010**) who reported that, rate of spore generation of molds is reduced when the temperature is low. However molds do not die in cold weather. They stay dormant and wait for the right conditions for life to come. While, this result disagreed with (**Moeller, 2010**) who mentioned that, most cases of fungal infections were detected in Winter months.

Histopathological study

The infected gills with fungi revealed focal mild proliferative changes of the epithelial covering of the secondary lamellae or moderate hyperplasia (fig 20). This matches with (**Ferguson, 1989**) who reported that hyperplasia was a simple response to cellular necrosis, desquamation

of epithelial covering of primary lamella and complete sloughing of secondary lamellae are commonly induced by the chemical pollutants or attributed to the toxins excreted by the pathogenic fungi. The infected gills with fungi showed telagectiasis of the branchial copillanis fig (21). Other cases showed desquamation of epithelial covering of the primary lamellae together with complete sloughing of secondary lamellae fig (22). Most branchial blood vessels showed dilatation and congestion fig (23) and the healthy fin fig (24). That result also reported by (**Ferguson, 1989**) telangiectasia of secondary lamellae was produced as a response to the branchial injury in which there is breakdown of vascular integrity due to rupture of pillar cells and pooling of blood.

The infected fins with fungi revealed complete sloughing of the epidermis infiltration with melanin covering cell fig. (25). That also recorded by (**Ferguson, 1989)** depletion and destruction of the host defense system may favor the bacteria to have direct action on the epithelial covering of the epidermis of fins resulting in degenerative changes of the upper covering epithelium. The fungal infection may cause a secondary invader leading to discharge of the goblet cells and hyperplasia of alarm substance cell, beside spongiosis and ballooning degeneration, when spongiosis was severe, cell1-cell contact might totally breakdown with the consequent formation of vesicles or bullae. They may become confluent with lifting and loss of epidermis.

The infected liver by fungi showed some hepatocytes suffer from vascular degeneration fig (26). Other cases showed some nucleated red blood cells in the vicenia of the central vein fig (27). This result was recorded as In case of acute and chronic exposure of *O. niloticus* to OTA. This change usually associated with acute stage of haemolytic anemia (**Chang *et al.*, 1979**) and destructive effects of OTA on spleen, kidney and liver (**Smith and Hamilton, 1970**). Moreover, (**Easa, 1997**) confirmed these results by recording depletion of hematopiotic elements due to the effects of OTA.

The spleen revealed depletion of the hemopoietic elements in addition to activation of melanomacrophage centers (Fig 28). This result goes in parallel with **(Ferguson, 1989**) who recorded that the depletion of the hemopoietic elements, in the spleen substantiates the cytolytic and fibrolytic capacities of pathogenic fungi for destroying the host defense system in the spleen.

The infected kidney with fungi revealed condensation of some gromeruli and edema was encountered in Bowman´s capsule beside glomerular lobulation fig (29). Some renal tubules showed hyaline droplet degeneration and necrosis fig (30). Some renal tubules showed detachment

of epithelial cell lining from basement membrane fig (31). Hyaline droplet degeneration suggests the existence of a glomerular diseases or breakdown the cement substance which binds the endothelial cell lining of glomerular tuft which lead to protein leakage into the filtrate and decreased osmotic pressure with its consequences which leads to edema in Bowman´s capsule and condensation of glomerular tuft.

Figure (1) Examined *O. niloticus* suffered from ulcer and hemorrhage.

Figure (2) scale loss and darkening of examined fish.

Figure (3) Fin and tail rote of examined fish.

Figure (4) Cotton-wool like on fish head, fins and tail of examined fish and infected with *Saprolegnia* spp.

Figure (5): examined *O. niloticus* infected with fungi had enlarged yellow liver and congested kidney

Figure (6) *Saprolegnia* sp

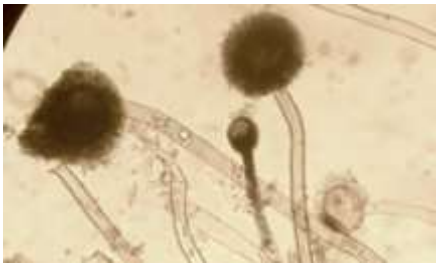

Figure (7) *Aspergillus niger*

Figure (8) *Aspergillus flavus*

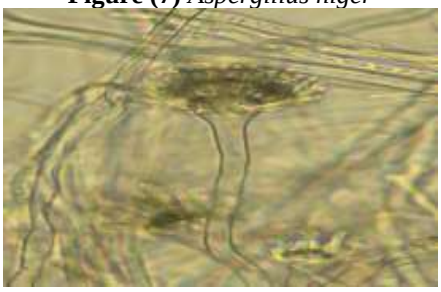

Figure (9) *Aspergillus terreus* Micromorphology conidiophore & head

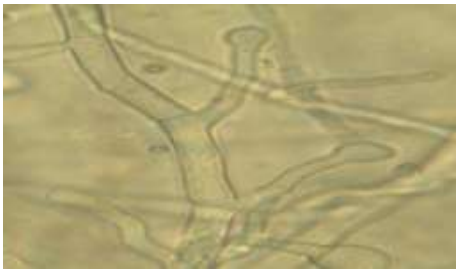

Figure (10) *Aspergillus terreus* Blastoconidia.

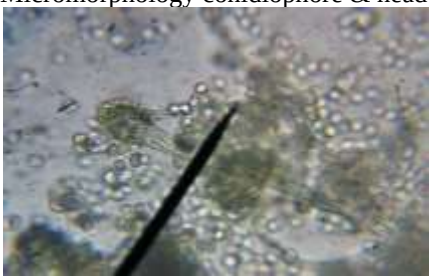

Figure (11) *Aspergillus fumigates*

Figure (12) *Aspergillus glaucus*

Figure (13) *Aspergillus wentii*

Figure (14)*Aspergillus versicolor* sp.

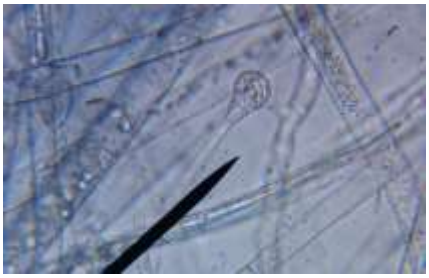

Figure (15) *Absidia* sp.

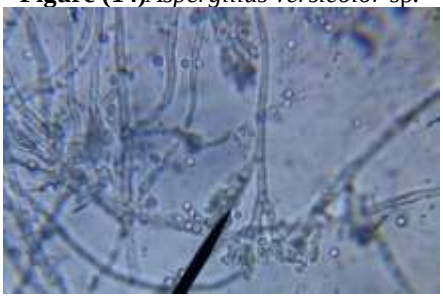

Figure (16) *Penicillium* sp.

Figure (17) *Trichoderma* sp.

Figure (18) *Syncephalastrum* sp.

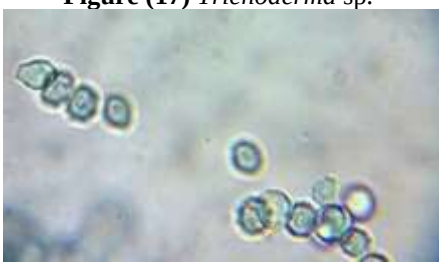

Figure (19) *Scopulariopsis* sp.

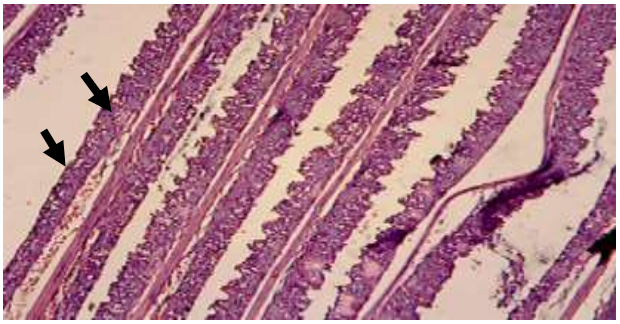

Figure (20) gills of *O. niloticus* naturally infected by some pathogenic molds as (*Penicillium* sp. and *A. niger*), and other nonpathogenic as (*A. tamarii*,) showing focal mild proliferative changes of the epithelial covering of the secondary lamellae or moderate hyperplasia. H&E. (X10).

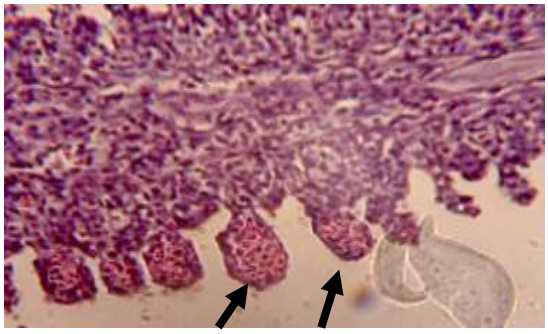

Figure (21) gills of *O. niloticus* naturally infected by some pathogenic molds as *Penicillium* sp. and *A. niger*), and other nonpathogenic as (*A. tamarii*,) showing telangiectasia of the brachial capillaries H&E. (X40).

Figure (22) gills of *O. niloticus* naturally infected by some pathogenic molds as (*Penicillium* sp), and other nonpathogenic as (*A. niger*) showing desquamation of epithelial covering of the primary lamellae together with complete sloughing of secondary lamellae. H&E. (X4).

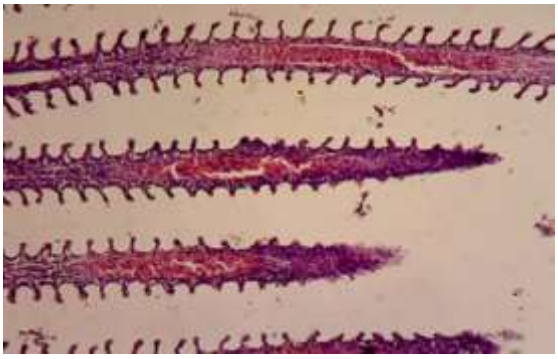

Figure (23) gills of *O.* niloticus naturally infected by some pathogenic molds as *Penicillium* sp., *A. niger*), and other nonpathogenic as (, A. tamarii) showing dilatation and congestion of brachial blood vessels. H&E. (X10).

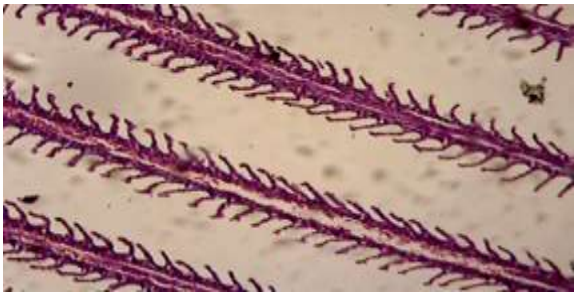

Figure (24) gills of *O. niloticus* not infected by any pathogenic molds or nonpathogenic showing normal architecture of gills. H&E. (X10).

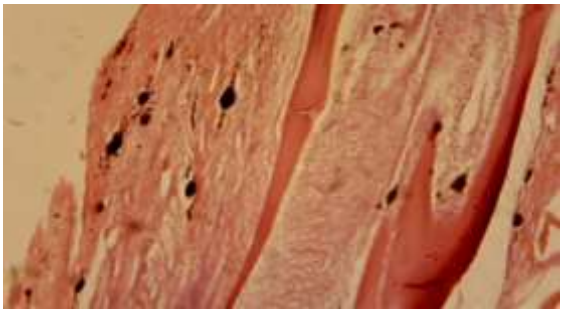

Figure (25) fins of naturally infected *O. niloticus* by fungi showing- complete sloughing of the epidermis together with infiltration with melanin carrying cells. H&E. (X10).

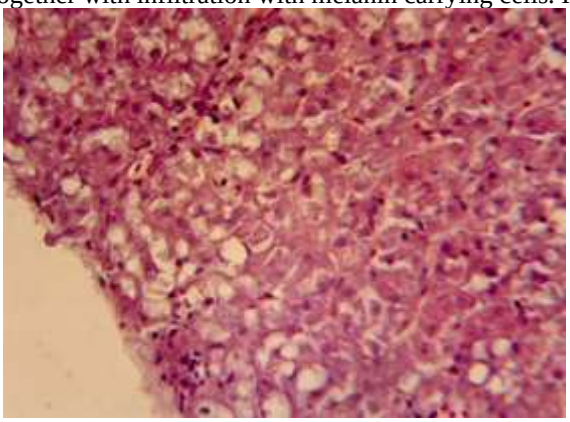

Figure (26) liver of *O. niloticus* naturally infected by some pathogenic molds as (*A. flavus*), showing hepatocytes suffer from vacuolar degeneration. H&E. (x40).

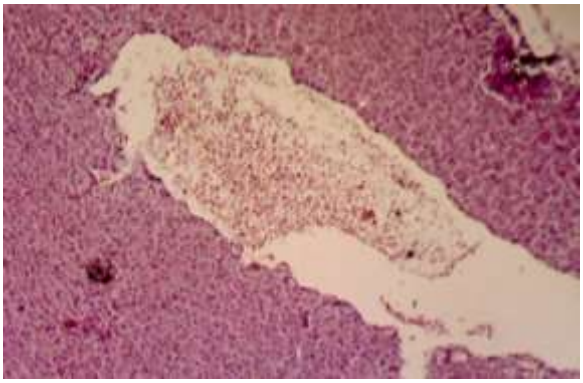

Figure (27) liver of *O. niloticus* naturally infected by some pathogenic molds *A. flavus* showing some nucleated red blood cells in the vicenia of the central vein. H&E. (X10).

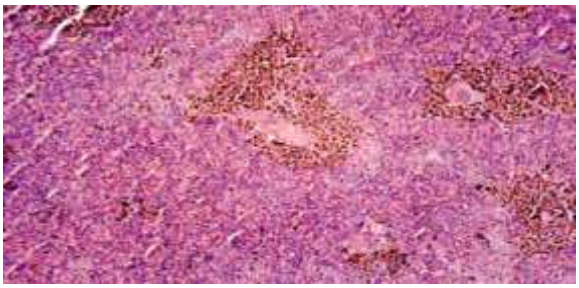

Figure (28) Spleen of *O. niloticus* naturally infected by some pathogenic molds as (*Penicillium* sp.*) and* nonpathogenic *(A. tamarii)* showing depletion of the hemopoietic elements in addition to activation of melanomacrophage (MMC). H&E. (X10).

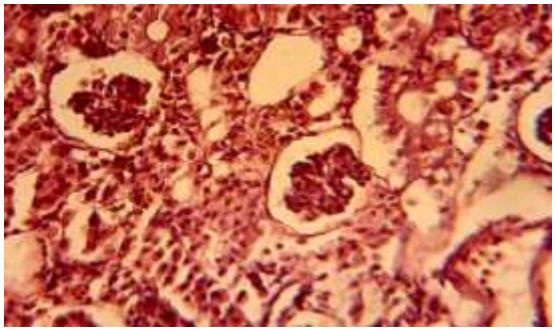

Figure (29) kidney of *O. niloticus* naturally infected by fungi showing condensation of some gromeruli and edema was encountered in *Bowman´s* capsule beside glomerular lobulation. H&E. (X40).

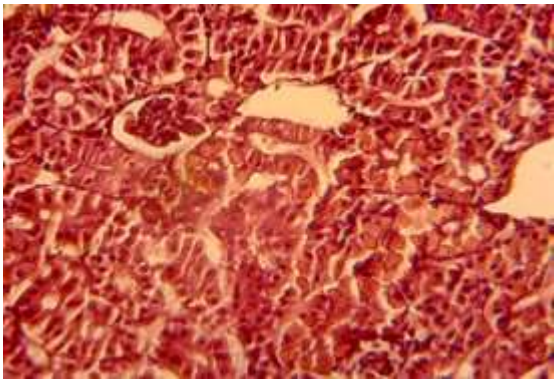

Figure (30) kidney of *O. niloticus* naturally infected by fungi showing hyaline droplet degeneration and necrosis. H&E. (X40).

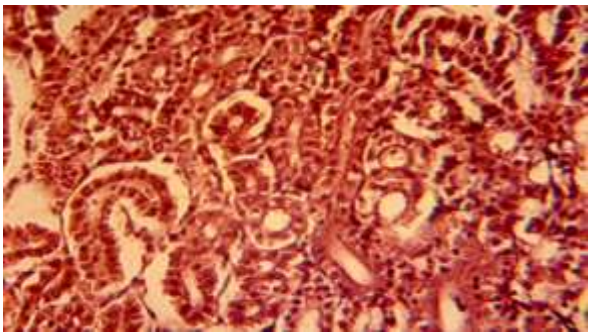

Figure (31) kidney of *O. niloticus* naturally infected by fungi showing detachment of epithelial cell lining from the basement membrane of some renal tubules .H&E. (X40).

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الدراسات الهستوباثولوجية لأسماك البلطى النيلى بفعل بعض الفطريات المسببة للأمراض فى مزارع أسماك العباسة

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الملخص العربي

تربية الأحياء المائيه لا تزال واحدة من أسرع قطاعات إنتاج الأغذية نموا, ومن المقرر أن تلعب دورا رئيسيا فى تلبية الطلب المتزايد على المنتجات السمكية التى تعتبر مصدر آخر للبروتين, فالبلطى النيلى من أسماك المياه العذبة الأكثرإقتصادا فى مزارع العباسة, واحده من أهم مزارع الأسماك فى مصر.

١) تم فحص" ٤٣٠ سمكة" من أسماك البلطي النيلى بأوزان مختلفة تم تجميعها من ثلاث مزارع مختلفة لأسماك البلطى بالعباسة أثناء الفترة من أكتوبر ٢٠١٤ الى يوليو ٢٠١٥. وقد خضعت هذه الأسماك للفحص الأكلينيكى والفطرى والهستوباثولوجى.

٢) الفطريات المعزولة من الأسماك المختلفة أشتملت على الأنواع التالية :

سبروليجنيا والكلادوسبوريم والكريسوسبوريم والاسكوبيولاريوبسس بريفيكالس والأسبراجلس فيرسى كلرجروب والأسبراجلس اوكراشيس والاسبراجلس نيجروالأسبراجلس جلاكس والأسبراجلس ونتياى والرودوتورولا والبنيسيليم والسينسيفالسترم والأسبراجلس تاميراى والألسبراجلس فلافس والتريكوديرما والأكريمونيوم والريزوبس والفيوزاريم والفوما والأبسيديا والأسبراجلس كانديدس والألترناريا والأسبرجلس تيريس والأسبرجلس فيوميجاتس والباسيلوميسزفارينوسيز.

٣) بالنسبة لمعدل الأصابة فى الأعضاء المختلفة كانت أعلى نسبة (٤٤٪) و(٢٦,٤٪) و(٢٥,٦٪) و(٤٪) من الخياشيم والجلد والزعانف والأعضاء الداخليه وكذلك منطقه الأصابه على التوالى. ولكن مع الأخذ فى الإعتبار بأن منطقة الإصابه هى تابعه للجلد فى الأساس , فالنسبة هى (٤٤٪) و(٣٠,٤٪) و(٢٥,٦٪) من الخياشيم ,الجلد والزعانف والأعضاء الداخلية على التوالى.

٤) بالنسبة للأعراض الظاهرة على الأسماك المصابة فى حالة الأصابة بالاسبرجلس فلافس هى سقوط للقشور وتقرحات للجلد وأهم الصفات التشريحية كانت إصفرار الكبد, بينما كانت الأعراض الظاهرة فى حالة الإصابة بالاسبراجلس نيجر ضعف شديد فى الجسم وأهم الصفات التشريحية كانت إحتقان شديد فى الأعضاء الداخليه بالاضافة الى زياده فى حجم الحويصله المراريه والطحال. وفى حاله الاصابه بالاسبرجلس أوكراشيس كانت احتقان شديد فى الكلى, وفى حاله الاصابة بالكلادوسبوريم كانت إسمرار الكبد بالاضافة الى إحتقان وزيادة فى حجم الحويصلة المراريه. الأسماك المصابة بالسبروليجنيا أظهرتقشر للبشرة وتساقط وتآكل خصوصا حول الذيلية والزعانف البطنية نادرا ما تم الكشف عن نزيف وفى بعض الحالات يعانون الهزال.

٥) وجد أن معظم التغيرات الباثولوجية فى الأسماك المصابة كانت عبارة عن خلل فى التركيب الطبيعى للخياشيم واحتقان فى الاوعية الدموية الخيشومية. واحتقان فى الاوعية الدموية للكبد. ونقص فى العناصر المكونة للدم مع تفعيل مراكز الماكروفاج فى الطحال. وضمورفى الجروميريولاى فى كبسوله بومانزفى الكلية. سقوط كامل لطبقة البشرة فى الجلد مع زيادة فى خلايا الميلانين فى منطقة الزعانف.