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Studies on Some *Oreochromis niloticus* fungal Infections

Mohammad Abdel Razek[1]; Somayh, M. Mahmoud Awad[2]; Saleh, F. Sakr[2]; Amany, A. El-Aasar[1] and Ahmed M. M. Al-Ashram[3]

1- Botany and Microbiology department, Faculty of science, Suez Canal University.
2-Department of fish health and Management, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharkia, Egypt**.**
3- prophessor doctor from supreme council of university 2014 and Chief recearcher in aquaculture research center 2008

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ABSTRACT

Aquaculture remains one of the fastest-growing food-producing sectors and is set to play a key role in meeting the rising demand for fishery products that consider one of the cheapest sources of protein, Nile tilapia is the most economically important Freshwater Fish in Abbassa Farms and the most popular in Egypt. A total number of 100 randomly collected *O. niloticus* fish of different stages and different weights were obtained alive taken to lab. of fish health and management department in central lab for Aquaculture Research (El-Abbasssa), Sharkia, during the period from December 2018 to May 2019. The fish were subjected to clinical, post-mortem examination, Fungal isolation and identification revealed ***Saprolegnia* sp.**, ***Penicillium* sp., *Rhodotorulla*, *Aspergillus niger*., *Fusarium sp.*, *Aspergillus Ochraceus, Aspergillus flavus, Aspergillus tamari, Absidia sp*** and ***Aspergillus versicolor sp.***

And histopathological for pathogenic fungus (*Saprolegnia* sp.), tissue specimens were taken from suspected lesions for Histopathological examination and showed severe hyperplasia of epithelial cell covering of secondary lamella of gills, alternative area of hyperplasia of sloughing of epidermis, increase number of mucus secreting cell; severe congestion of hebatoportal vein and central vein in liver; increase numbers of MMCs in spleen; condensation of glomuler tubules with edema in Bowman's capsule in kidney.

Key words: Fish Fungal diseases, *Saprolegnia* sp., Histopathology.

Introduction

The world population will be 9.3 billion by 2050. Meeting basic human needs for animal protein is a challenge in an increasingly over populated world. Besides livestock, fish is the major source of cheap animal protein supply. The wild fish resource is limited for fisheries **(FAO. 2014).** Depleting wild stocks is an increasing concern for fishermen, environmental organizations and policy-makers. Sustainable aquaculture can play an important role in the transition to a more environmentally and economically viable fish production **(Cressey, 2009).** Land-based recirculating aquaculture **(Martins *et al.*, 2010)**, and cage and offshore aquaculture **(Ferreira, *et al*., 2009)**. Offer a unique combination of protection of wild stocks, socioeconomic benefits, and potential for sustainability and scalability.

Fungal infections (fungal infections are called mycoses) are among the most common diseases seen in temperate fish because fungal spores are found in all fish ponds and create problems in stressed fish. Poor water quality can also lead to an increase in fungal infections in an otherwise healthy fish population. Most fungal infections invaded on external tissues and only few fungal infections affect the internal organs of fish (Verma, 2008).

Histopathological changes were commonly observed in organs such as kidney, liver, gills, stomach and spleen infected with saprolegnia have also been studied recently and similar clinical signs and histological manifestation have been observed ((Rao. and Benarjee, 2015).

Materials and methods

Studying area:

Samples collected from two different fish farms, Abbassa fish farms that located in Abbassa District, Abou Hammad, Sharkia. The other is private fish farm in the way of Ismailia canal, Sharkiya Governorate.

Water Supply: Agricultural drainage water. The source of water from the Ismailia canal by way of the El Wadi El Quadim supply canal. The Ismailia canal originates from the Nile River.

Sampling

Water samples were taken from the two different farms irrigated with agricultural drainage water. Fish samples were collected from the same two studied areas. A total number of 100 *O. niloticus* fish was randomly collected with average 75 ± 5 gm body weight and length 13 ± 2 cm. were obtained alive in taken to lab. of fish health and management department in central lab for Aquaculture Research (El-Abbasssa), Sharkia, during the period from December 2018 to May 2019.

Clinical and post mortem examination of fish under investigation:

Fish were transported alive to the laboratory. The apparently healthy fish, diseased and freshly dead fish were subjected to clinical and post mortem examination **(lucky, 1977).**

Isolation of microbial pathogens from naturally infected and apparently healthy Nile tilapia (*Oreochromis niloticus*).

Fish fungal isolation

Dilution plate **(Garrett, 1981)** technique was applied in order to get as good diversity as possible. One gram of each fish part (skin, liver, spleen, fins) 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 pour media. To obtain as much species as possible, six plates isolation medium were prepared from every fish part. After inoculating, plates were incubated at 27 °C which is suitable for growth of most molds, for 5 to 7 days, thereafter; developing colonies were identified.

Fungal microscopic examination:

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

Direct microscopical examination of skin and gills was carried out according to **(Shaheen, 1986).**

Yeast morphological and microscopic examination:

The colonies were described considering its size, consistency and pigmentation. Also, yeast isolates were microscopically examined by placing part of the colony on a slide with one drop of distilled water then cover with coverslip and examined under high power magnification for the presence of yeast cells(,).

Histopathological examination:

Tissue specimens were taken from suspected lesions and fixed in 10% phosphate buffered formalin, embedded in paraffin, sectioned and stained with Haematoxylin and Eosin stains (H&E) **(Carleton *et al*., 1967).**

Results and Discussion

Water is anatural habitat for a wide variety of microorganisms including bacteria, fungi, protozoa and algae. Hence, fishes and other aquatic animals may accumulate these organisms from their environment **(Edun, *et al.*, 2007).** Fungi can become a problem if fish are stressed by disease, poor environmental conditions, receive poor nutrition or are injured. These factors weaken the fish or damage its tissue; fungus can infect the fish **(Klinger and Francis-Floyd, 2008).**

A wide variety of fungi can act as pathogenic agents for freshwater fishes and may serve as primary or secondary pathogens. There are also numerous fungi that occur as saprophytes on freshwater fishes. Dermatomycosis in fish may result in economic losses to farming projects due to increased mortality, poor weight gain, besides low market value **(Rezeaka, 1991).**

Clinical signs & P/M findings of naturally infected fishes: *O. niloticus* revealed eroded fins, redness on the skin, congested protruded anal opening and sometimes darkness on skin, ulcers with different sizes, and eye turbidity especially in fish naturally infected with *Aspergillus*, *Fusarium* and *Pencillium.*

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)**. *Saprolegnia* is one of the most important genera of oomycetes (water molds) that causes major economic losses in nature and at freshwater fish farms **(Diéguez-Uribeondo, *et al*., 2007; El-Ashram, *et al.*, 2007 and Woo, *et al.*, 2011).** Infected fish usually show cottony white to gray, brown, red, or greenish masses on skin and gills **(Yanong, 2003).**

The general signs of the clinically diseased *O. niloticus* appeared as eroded fins, redness on skin, congested protruded anal opening and sometimes darkness on skin, ulcers with different sizes, and eye turbidity. The ulcerative areas over the skin and fins erosion might be expected to the lytic action of primary bacterial infection as all fungal infections are considered as secondary invader pathogen; these results concurred with many authors **(Chauhan, 2014 and Bassiouny, *et al.*, 2019)**. Redness on the skin may be attributed to the extracellular products of molds resulting in severe symptomatic changes that appear on the fish in the form of hemorrhagic patches; the result agreed with **(Bassiouny, *et al*., 2019)** where hemorrhagic patches was observed in both examined diseased Nile tilapia and African catfish. Regarding the postmortem lesions, enlarged liver with moderate hemorrhagic spots were the most characteristic. This result may be ascribed to either the fact that most mycotic diseases are secondary invaders following fundamental systemic bacterial infection which is responsible mainly for this internal lesions and/or because of extracellular products produced by fungi that meddle with capacity of liver

leading to congestion in internal organs particularly liver **(Abou El-Atta, 2008; Mohamed, *et al.*, 2017; Bassiouny, *et al.*, 2019 and Abd El Tawab, *et al.*, 2020).**

Naturally infected fish were characterized by some clinical signs as slacken motion, swimming near the surface of water and loss their appetites when fed. Infected fishes with *A. flavus* and showed scales loss and ulceration of the skin. Postmortem lesion was yellow liver. Infected fishes with *A. niger* showed emaciation with congestion in the internal organs beside enlargement in the spleen and gall bladder, and showed severe erosions in the posterior part of the body leading to exposure of the muscles. Infected fishes with *Saprolegnia* spp. Showed cotton-wool like appearance on the head region in addition to severe ulceration on the head region. The different species of molds (*Saprolegnia* spp., *A.flavus* sp., and *A. niger* sp.) which isolated from different localities in Egypt caused skin lesions in Tilapia nilotica **(Nagib, 1994).** Saprolegniasis is generally external and usually starts as a cotton wool – like white to dark grayish or brownish growth on the head region in the form of focal patches **(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).**

Saprolegnia spp. was isolated from the skin of *O. niloticus* with incidence of (19.51%). between October and March, This result matches with Winter Saprolegniasis usually occurs when water temperatures are below 15 °C (59 °F). Mortality usually increases as temperatures rise in early spring. **(Durborow, *et al.*, 2003)** reported that, the disease has been reported as early as September and as late as April. Also it is supported by **(Wise, 2008)** who reported that, The occurrence of the disease appears to be dependent primarily on a rapid drop in water temperature which induces immunosuppression 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. **(Eid, *et al.*, 2017)** recorded that the highest infection rate was during winter season 66.6% of *Saprolegnia* spp.

Rhodotorula sp. was isolated with percentage of (17.07%). 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).**

Aspergillus sp is the highest prevalence of mold infection in fish this result agrees with **(Pani, *et al.*, 2010)** who found that the highest prevalence of mold

infection in fish with *Aspergillus* sp. This may attributed to the faster growth rate of this fungus in addition to its better intrinsic prolific sporulation capacity to utilize the substrate.

Penicillium sp. was the most common mold which is isolated with percentage of (19.51%) with the highest incidence similar result was obtained by **(Shaheen, 1986)** who isolated *Penicillium* sp. with high incidence from skin, gills and with low incidence from kidney, it 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. **(El-Abbassy, 2007)** mentioned that, *Penicillium* is considered a thermo tolerant mold which grows at a temperature degree ranges from 8- 45°C.

Aspergillus niger was isolated with high percentage of (12.19%). this result are in coordenance with **(Younis, *et al*., 2020)**. results which showed the highest incidence of *Aspergillus niger* infection occur with percentage of (51.3%) as the most dominant species among 500 samples from *Oreochromis niloticus* and Clarias gariepinus. **(Samson, *et al*., 2007)** reported that all black aspergilla strains are not able to grow at 6°C and 9°C. These findings were confirmed by **(Hassan, *et al*., 2010)**, who found that, ochratoxin A was produced by *A. niger*. This result in harmony with **(El-Abbassy, 2007)** who mentioned that, *A. niger* is considered a thermo tolerant mold which grows at a temperature degree ranges from 8- 45°C, also **(Zhao, *et al*., 2009)** mentioned that, the maximum temperature that *Aspergillus niger* could tolerate was 40°C. Aspergillus niger is the most prevalent species in the genus Aspergillus. It is a common food contaminant that produces a sickness known as black mould on various fruits and vegetables such as grapes, anions, and peanus. It is found in soil and is frequently reported from indoor stituations, **(Samson *et al*., 2001)** contamination of food and bad storage of food lead to infection with aspergillus

Aspergillus flavus or *Aspergillus niger* could be isolated from both clinically diseased and apparently healthy *O. niloticus* mainly from internal organs supporting the fact that Aspergillosis is a systemic disease. Briefly, although most fungi are considered to be typical mycoflora, but they still can cause infections to fish. Therefore, it is suggested that proper health management practices should be adapted while rearing fish, so that the chances of fungal infection can be limited **(Abd El Tawab1, *et al*., 2020).**

(Ayansina and Owoseni, 2010) recorded that, mycotoxins, the secondary metabolites of toxigenic fungi exert injurious effects upon animal and human health and causing damage during metabolism and this induces vital changes in the chemical constituent of the associated substrate. The most significant

mycotoxinsare aflatoxins and ochratoxins. Aflatoxins that are primarily produced by the mold *Aspergillus flavus* and *Aspergillus parasiticus* are among the most toxic and carcinogenic compounds occurring naturally. Also **(Chang and Ehrlish, 2010)** mentioned that, all toxigenic *A. flavus* isolates produce B aflatoxins. In addition, **(Russo and Yanong, 2008)** reported that, aflatoxicosis is a disease that can affect many species of fish, and results when feed on contaminated with aflatoxins.

Aspergillus ochraceus was isolated with percentage of (7.31%). This result was consistent with **(Abd El-Ghany, 1998)** who isolated *Aspergillus ochraceus* from skin, liver and kidney of C. gariepinus. **(Hassan, *et al.*, 2010)** who mentioned that ochratoxin is a toxic secondary fungal metabolite produced by *Aspergillus ochraceus*. **(Chakraborty and Verma, 2010)** reported also that, ochratoxin exposure was found to exerts nephrotoxic, cytotoxic and carcinogenic effect on various laboratory animals and on humans. **(Spring and Fegan, 2004)** reported that, the key target organ of ochratoxin in aquaculture is the kidney, where it causes necrotic lesions in the proximal tubules. *Aspergillus flavus* and *Aspergillus niger* were the most predominant fungi isolated from either apparently healthy or even diseased one mainly from internal organs **(Abd El Tawab, *et al.*, 2020).**
Absidia sp. with percentage (2.43%) fungi were isolated and not detected in lesions. That may match with **(Shaheen, 1986)** who mentioned that zygomycetes species were not associated with any disease processes.

Histopathological examination

Oreochromis niloticus suffer from *Saprolegnia* sp. Infection, in the gills revealed focal mild proliferative changes of the epithelial covering of the secondary lamellae or moderate hyperplasia. 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 microorganisms. The gills showing telagectiasis of the branchial capillaris, Other cases showed desquenation of epithelial covering of the primary lamellae together with complete slouphing of secondary lamellae. Most branchial blood vessels showed dilatation and congestion. That result also reported by **(Ferguson, 1989)** Telangiectasis 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 condensation or contraction of capillary tufts could be attributed to the pressure of the edematous fluid which accumulated in the Bowman´s Capsule.In the kidneys; showed condensation of capillary tufts with edema in

Bowman´s Capsule and separatation of epithelial cells of renal tubules from the basement membrane this matches with **(Erer, 1981 and Roberts, 2001)** who reported that subcapsular and interstitial haemorrhage, parenchyme degeneration and cytoplasmic vacuol formation.

The liver showing areas of necrosis beside congestion of both central and hepatoportal vein beside sidresis the results were previously reported by **(Miyazaki *et al*., 2000; Grizzle and Kirya, 1993 and Ciprians, 2001)** reported that the liver and kidneys are target organ of an acute septicemia.In liver severe congestion in hepatorial vein. In spleen revealed depletion of the hemopoietic elements in addition to activation of melanomacrophage centers. 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 microorganisms for destroying the host defense system in the spleen.

The fin revealed complete sloughing of the epidermis infiltration with melanin carrying cell. 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.

Table (1): Prevalence of infection among *O. niloticus* in relation to locality.

Locality	Fish species	No. of examined fish	No. of infected fish	% of infection in relation to species inside the locality
1st Fish farm	*O. niloticus*	50	38	76%
2nd Fishfarm	*O. niloticus*	50	46	92%

Table (2): Prevalence of fungal isolates isolated from naturally infected cultured *Oreochromis niloticus* (n=100):

Fungal isolates	1st fish farm	2nd fish farm	No	%
***Saprolegnia* sp.**	**5**	**3**	**8**	**19.51%**
***Penicillium* sp.**	**4**	**4**	**8**	**19.51%**
Rhodotorulla	**3**	**4**	**7**	**17.07%**
Aspergillus niger.	**5**	**-**	**5**	**12.19%**
Fusarium sp.	**4**	**-**	**4**	**9.75%**
Aspergillus Ochraceus	**3**	**-**	**3**	**7.31%**
Aspergillus flavus	**3**	**-**	**3**	**7.31%**
Aspergillus tamarii	**1**	**-**	**1**	**2.43%**
Absidia sp	**-**	**1**	**1**	**2.43%**
Aspergillus versicolor sp.	**-**	**1**	**1**	**2.43%**
total			**41**	**100%**

Table (3): Distribution of fungal species and their incidence in different tissues of (*O. niloticus*).

Fungi	Distribution of fungal species		skin		liver		spleen		kidney	
	No	%	No	%	No	%	No	%	No	%
			%							
Saprolegnia sp.	8	19.1	8	100	-	-	-	-	-	-
Penicillium sp.	8	19.1	3	37.5	3	37.5	2	25	-	-
Rhodotorulla	7	17.7	7	100	-	-	-	-	-	-
Aspergillus niger.	5	12.9	2	40	2	40	1	20	-	-
Fusarium sp.	4	9.75	2	50	1	25	-	-	1	25
Aspergillus Ochraceus	3	7.31	-	-	2	66.6	1	33.3	-	-
Aspergillus flavus	3	7.31	1	33.3	2	66.6	-	-	-	-
Aspergillus tamarii	**1**	**2.43**	**-**	**-**	**1**	**100**	**-**	**-**	**-**	**-**
Absidia sp	**1**	**2.43**	**-**	**-**	**1**	**100**	**-**	**-**	**-**	**-**
Aspergillus versicolor sp.	**1**	**2.43**	**1**	**100**	**-**	**-**	**-**	**-**	**-**	**-**

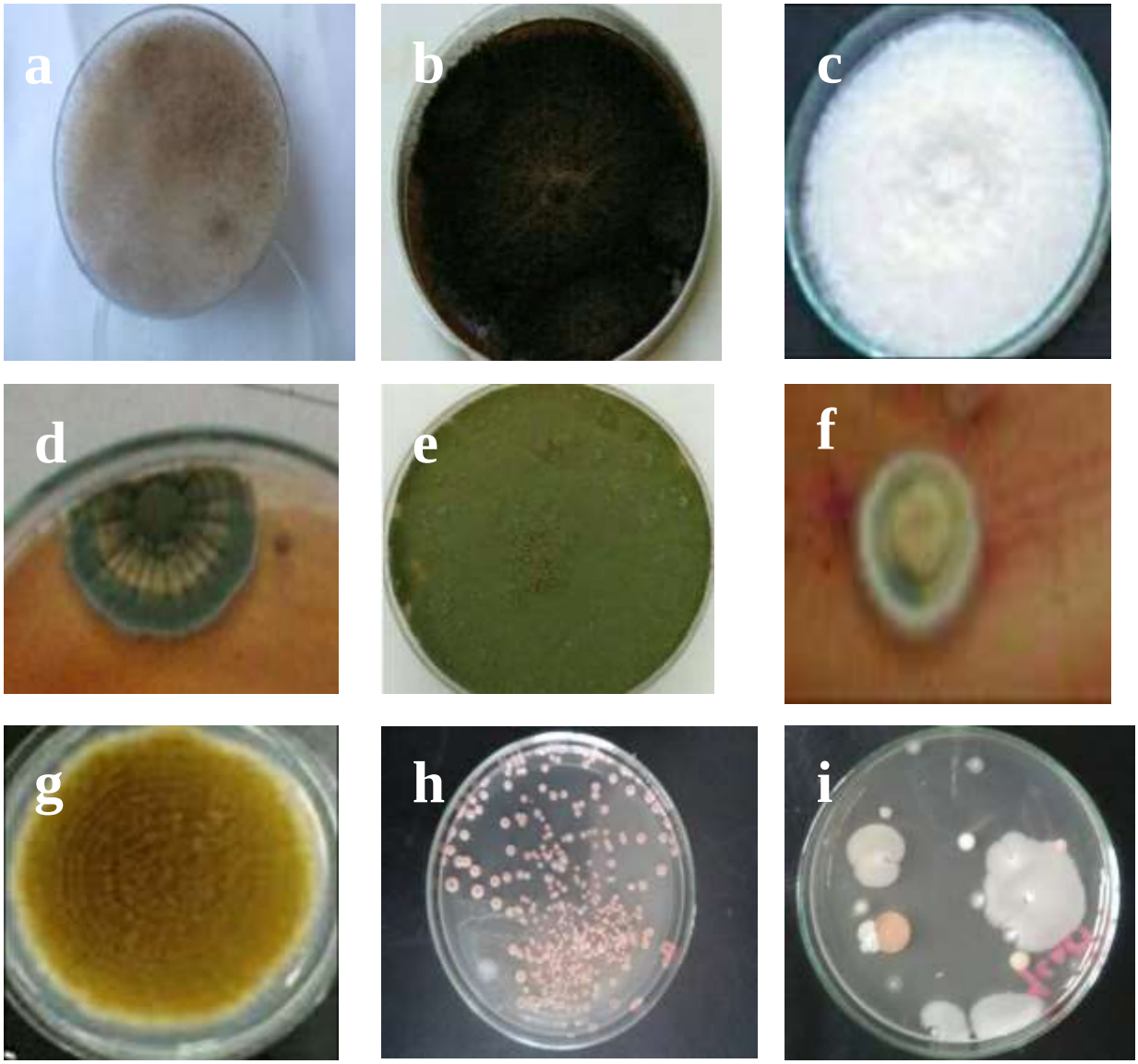


Fig . (1): Macroscopic identification of various mold and yeast isolates: **a:***Saprolegnia* sp**. b**: *Aspergillus niger*, **c**: *fusarium*, **d**: *Penicillium*, **e**:*Aspergillus flavus*, **f:***Aspergillus versicolor*, **g:** *Aspergillus tamerrii*, **h**: *Rhodotorulla* sp., **i.** *saccharomyces cerevisiae*. **j.** *absidia sp***.** And **k:** *aspergillus ochraceus***.**

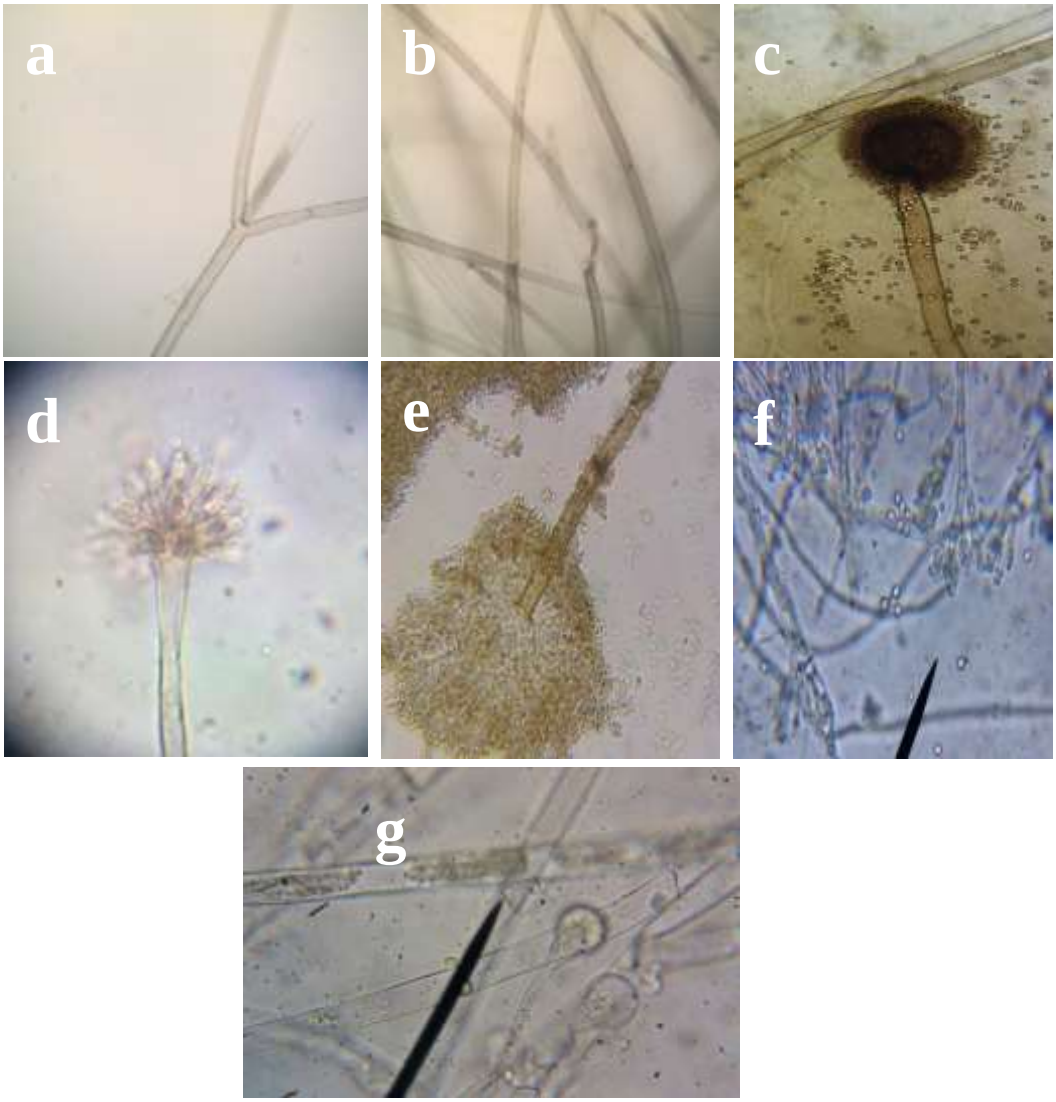


Fig. (2) : Microscopic identification of molds from naturally infected fishh a, b: *Saprolegnia* sp**., c:***Aspergillus niger***, d:***Aspergillus versicolor group***, e:***Aspergillu ochraceus***, f:***Penicillium* sp**., g:***Absidia*

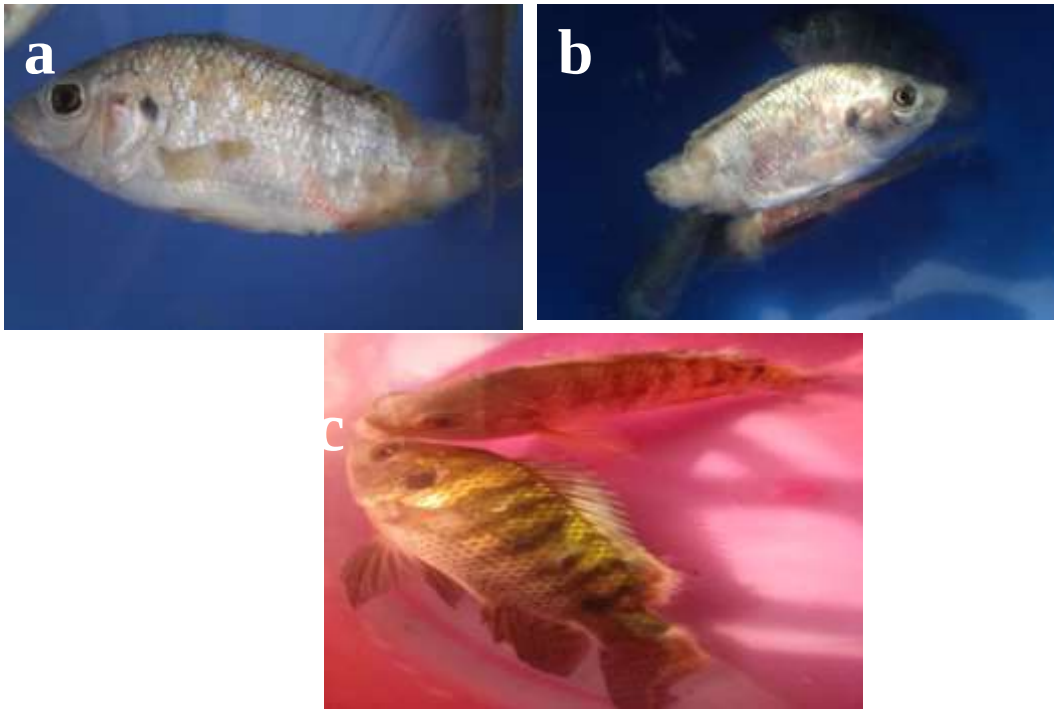
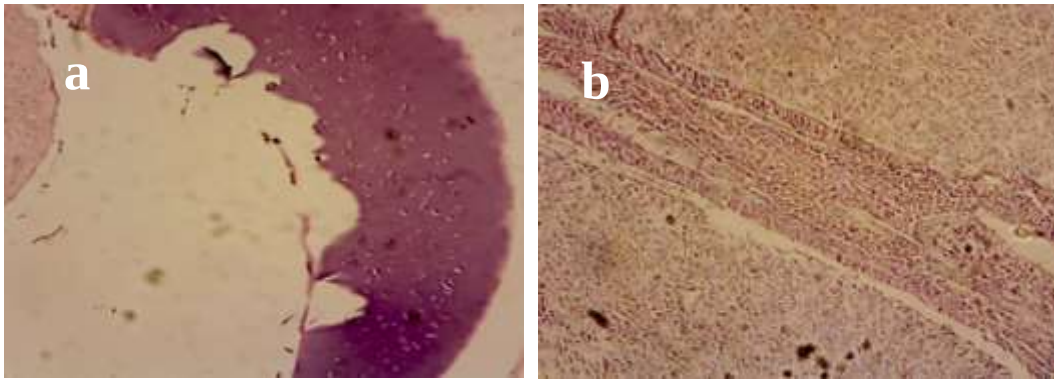


Fig. (3): a, **b** and **c** *o. niloticus* naturally infected by *saprolegnia* sp. shown cotton-wool like appearance on different parts of the body.

Histopathological examination: *Oreochromis niloticus* suffer from mold infection (*Saprolegnia* sp.) showed in different tissues of fishes (gills, liver, spleen, kidney and fins).



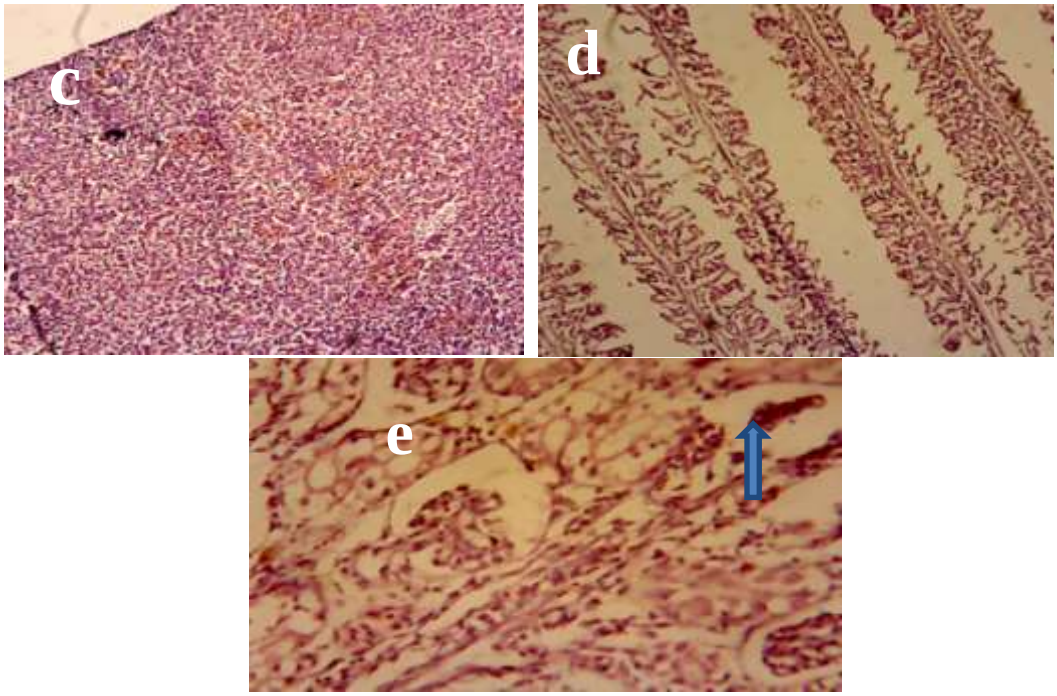


Fig. (4): a: showing thickening of deforming of sclera H&E x10. **b**: severe congestion of hebatoportal vein beside hemosidrosis. H&E; x10. **C** : spleen; increase numbers of MMCs. H&E; x10. **d** : showing slight to moderate hyperplasia of epithelial cell coverind of Secondary lamella. H&E; x10. **e** :kidney; condensation of capillary tufts with edema in Bowman´s capsule. H&E x100.

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دراسة عن بعض أنواع العدوى الفطرية فى أسماك البلطى النيلى

محمد عبد الرازق محمد[١]،سمية محمد محمود عوض[٢] صالح صقر[٢]، أمانى عبدالمنعم الاعصر [١] احمد محمد الاشرم[٣]

١- قسم النبات والميكروبيولوجى كلية العلوم- جامعة قناة السويس

٢- قسم صحة وأمراض الأسماك مركز البحوث الزراعية المعمل المركزى لبحوث الثروة السمكيه العباسة- شرقية

٣- استاذ من المجلس الاعلى للجامعات عام ٢٠١٤ و رئيس بحوث قسم صحه و امراض الاسماك مركز البحوث الزراعيه ٢٠٠٨

الملخص العربى

تربية الأحياء المائية لاتزال واحده من أسرع قطاعات إنتاج الأغذية نموا، ومن المقرر أن تلعب دورا رئيسيا فى تلبية الطلب المتزايد على المنتجات السمكية التى تعتبر من أرخص مصادرالبروتين فى مزارع العباسه التى هى واحده من أهم مزارع الأسماك فى مصر.

تم فحص ١٠٠ سمكة من أسماك البلطى النيلى بأوزان مختلفة تم تجميعها من مزرعتين مختلفتين احداهما مزرعة من مزارع العباسة أبو حماد شرقية والأخرى مزرعة خاصة على طريق ترعة الإسماعيليه محافظة الشرقية وتم نقلهم إلى معمل صحة الأسماك ورعايتها بالمعمل المركزى لبحوث الثروة السمكية بالعباسه محافظة الشرقية فى الفترة مابين ديسمبر٢٠١٨ إلى مايو٢٠١٩. وقد خضعت تلك الأسماك للفحص الأكلينيكى والتشريحى الفطرى والهستوباثولوجى .

الأسماك المريضه تمثلت العلامات المرضية على تثاقل فى الحركة والسباحه بالقرب من سطح المياه وفقدالشهيه, يتم عزل وتعريف الفطريات المسببة للأمراض مثل (***Saprolegnia* sp.** ***Penicillium* sp., *Rhodotorulla*, *Aspergillus niger.*, *Fusarium sp.*, *Aspergillus Ochraceus*, *Aspergillus flavus*, *Aspergillus tamari*, *Absidia sp*** and ***Aspergillus versicolor sp.***)

تم بالفحص الهستوباثولوجى لأنسجة الأسماك المصابة ولوحظ وجود تغيرفى أنسجة الأسماك وحدوث تشوهات فى التركيب الطبيعى للخياشيم, سقوط كامل أو متباين فى طبقة البشرة فى الجلد, زيادة فى عدد الخلايا المفرزة للمخاط؛ إحتقان فى الأوعية الدموية للكبد؛ مناطق متباينه بين نشاط وإضمحلال مكونات الدم مع تفعيل مراكز الماكروفاج فى الطحال؛ إنكماش فى الجروميولاى فى كبسولة بومانز فى الكليه.