Animal Health Research Journal Vol. 7, No. 4, November 2019 pp. 788-797

ISSN: 2356-7767

Mycological and Pathological Investigation of Freshwater Cultured Tilapia nilotica in El-Bohaira Governorate.

Amal, A. Al-Said* and Safaa, H. Aboollo **

Microbiology Unit*, Pathology Unit**, Damnhour Provincial Lab.,
Animal Health Research Institute, Egypt
Agriculture Research Center

Received in 23/09/2019
Accepted in 02/11/2019

Abstract

Oreochromis niloticus (tilapia nilotica) one of commonly animal protein source on Egyptian table and the most cheaper one and the fungal infection is considered one of the most serious causes of losses in aquaculture. Therefore, this study was aimed to screen the fungal status of tilapia nilotica in El-Bohaira Governorate, Egypt.

A total of 100 fish samples of Tilapia nilotica were collected from fish cages along Nile branch, at El-Bohaira Governorate.

Mycological examination of 100 apparently healthy fish (600 samples from fins, fish flesh, gills, liver, kidney and spleen) were taken.it revealed the isolation of 840 fungal isolates (350 mould and490 yeast isolates),. Isolated moulds belonged to the following genera: Saprolegnia (15.5%), Aspergillus niger (5.5%), Fusarium (1.2%), Mucor (4.8%), Penicillium (6.4%), Curvularia (1.2%). Cladosporium (6.2%), Alternaria (1.2 %). Yeasts isolated also from both fish species ad the following incidence: Candida albicans (39.3 %), other Candida species (8.9%)and Rhodotorula species (10%).

Histopathological investigations revealed various degrees of pathological lesions in different organs like gills, hepatopancreas, spleen and muscles From this study it was obvious that mycological infection caused harmful effects on tilapia fish.

Keywords: *Fungi, mycotoxin, Oreochromis niloticus, tilapia, histopathology*

Introduction

Fishes are the primary source of protein for human in many areas of the world and this is also in Egypt. Fungal contamination of fish is considered the main cause of signs of spoilage as off flavor and unpalatable taste and it may constitute a public health hazard as well as many of economic losses **(Hassan *et al*., 2007 and El Ahl, 2010)**. Outbreaks of water born fungal infections of fish, are common problems especially in fish farms and hatcheries. Of particular concern is saprolegniosis, which is an infectious fungal disease that is wide spread in all stages of the life cycle of fish. Saprolegniosis infection may contribute to heavy mortality among fishes. In Egypt, the mycotic disease constitute one of the most important disease causing troubles in fresh culture with several economical losses (**Easa, 1984, Shaheen 1986 and El Zayat, 1988**). Saprolegnia considered agent of secondary infection arising from conditions as bacterial infections, poor husbandry including poor water quality, adverse water temperature, all of these factors increased occurrence of saprolegnia infections (**Pickering and Willoughby, 1982**).

Many of the fungi that affect fishes are attacking the fishes when they are stressed or immunocompromised because of unfavorable environmental conditions, or secondary to bacterial or viral infections, or when they have lost their mucus protection because of trauma or

excessive handling (**Roberts 1989 and Quiniou *et al*., 1998**).

Mycotic infections of fishes by Oomycetes are wide spread in freshwater and represent the most important fungal group affecting wild and cultured fishes. The Saprolegniaceae, in particular members of the genus Saprolegnia, are responsible for significant infections involving both living, dead fishes and eggs. Oomycetes are classical saprophytic opportunities, multiplying on fishes that are physically injured, stressed or infected (**Pickering and Willoughby, 1982**).

Moreover, there are other fungi that have been implicated in fish diseases. Some of the genera involved include Aspergillus (**Salem *et al.,* 1989b**), Fusarium (**Bisht *et al.,* 2000**), Ichthyophonus (**Faisal et *al.,* 1985**), Branchiomyces (**Easa 1984**), Phoma (**Hatai *et al.,* 1994**), Paecilomyces **(shaheen1986)**, Exophialia (**Langdon and MacDonald 1987**), Phialophora (**Ellis *et al*., 1983**), Rhizomucor and Candida (**Neish and Hughes 1980**). The most common soil fungi such as penicillia and aspergilli are likely to be present in high numbers as water inhabitants in sediments and biofilms where asfusaria may be less common, since they are associated with plants **(*Gonçalves et al.* 2006).** These fungal species are mostly associated with mycotoxin production (**Göttlich *et al*. 2002)** Most of these are multiple case reports or single, and causing systemic disease with high mortality rates in fishes.

The objective of this study was to determine the types of fungal pathogens affecting freshwater fishes specially those causing high mortality rates and their effect on fish health Therefore, the present study was carried out to study the mycological quality of fresh fish (Tilapia nilotica).

Materials and Methods

Fish samples: A total of 100 fresh fish (Tilapia nilotica), in different size about (150-250 gm) were randomly collected from fish cages along the Nile branch in El-Bohaira Governorate. The collected samples were directly identified and transferred to the laboratory in ice box, without delay. Fish were subjected for thorough inspection for the assessment of the general appearance, the odour, the texture and the conditions of the eyes and the gill. According to **(FAO 1985).**

Sampling for histopathology

Following necropsy of 100 fish samples, Tissue specimens from positively fungal isolation tilipia nilotica fishes including samples from gills, muscles, spleen and hepatopancreas, were rapidly fixed in 10% neutral buffered formalin. The fixed specimens were processed through the conventional paraffin embedding technique. Paraffin blocks were prepared, from which 5 microns thick sections were obtained. These sections were stained by Hematoxyline and Eosin (H&E) according to the method described by (**Culling 2013**). No specimens from kidney and fins were collected.

Mycological examination:

Mycological examination was done according to **Noga (1996**) and Identification of moulds was carried out according to **Refai (1987).**

Isolation of fungi: was carried out from fish, samples were taken from fins, fish flesh, gills, spleen, liver and kidney were collected and inoculated onto SDA medium plates and incubated at 25ºC for 3-5 days ,subculture on the same media was done for purification. {Preparation of samples according to **ISO 6887-3 (2003)**.

Isolation was carried out by cutting portions of fish flesh and other organs samples and were transferred to asterilized homogenizer containing 0.1% sterile peptone water. The homogenate was allowed to stand for 5 minutes at room temperature, then the homogenate was placed on the prepared isolation media seaboard's dextrose agar **(Cruickshank *et al.,* 1975),** with chloramphenicol and chlortetracycline (100mg of each) as described by **Koburger (1970).**

The inoculated plates were incubated at 25°C for up to 5days. The microbes that grow on the plates were sub-cultured on fresh agar plates using the same medium to obtain pure microbial isolates. The fungal isolates were mounted in lactophenol cotton blue stain solution on slides with cover slips and microscopically ex-

amined for spores and vegetative bodies according to the method described by **(Barnet and Hunter,1972).**

Gram stain used for yeast identification. The isolated fungi were identified individually by macro and microscopic characteristics according to **(Bailey and Scott 1998), (Pitt and Hoching 2009),** while yeast isolates according to **(Kriger Van Rij 1984)** and **(Tibor and Larry, 1996).**

Germ tube test (Martin, 1979):
Used for differentiation between Candida spp in which a very light suspension of yeast like organisms in 0.5-1.0 ml of sterile rabbit serum can be used . Incubation was occurred at 37°C for no longer than 3 hrs. then one drop of yeast - serum mixture
was placed on a slide slip and was examined microscopically for germ tube production.

Results

Table (1). Incidence of +ve mould and yeast samples from different organs and tissues of *T. niloticus*.

Fish species	Organs	Fins	Fish flesh	Gills	Liver	Kidney	Spleen	Total
100 apparently healthy *O. niloticus*	No. of samples	100	100	100	100	100	100	600
	No. of +ve mould samples	70	50	80	20	20	10	250
	%	28	20	32	8	8	4	100%
	No. of +ve yeast samples	80	80	70	60	60	30	380
	%	21.1	21.1	18.5	15.8	15.8	7.8	100%

Table (2). Incidence of mycological examination of fish samples and its organs (600 samples)

Total mould	Total yeast	Total
`350	490	840

Table (3). Incidence of *mould isolated* from different organs and tissues of *T.niloticus.*

Isolated mold spp.	Fins	Fish flesh	Gills	Liver	Kidney	Spleen	Total
Saprolegnia	40	33	45	5	7	0	130 (15.5%)
Asp. niger	12	10	16	3	3	2	46 (5.5%)
Pencillium	15	4	15	10	5	5	54 (6.4%)
Cladosporium	15	**12**	20	1	2	2	52 (6.2%)
Mucor	12	10	6	5	2	5	40 (4.8%)
Fusarium	2	3	5	0	0	0	10(1.2%)
Curivlaria	2	1	2	2	1	0	8(.9%)
Alternaria	2	4	4	0	0	0	10(1.2%)

*The percentage are calculated in relation to total mould and yeast isolated (840).

Table (4). Incidence of *yeast isolated* from different organs and tissues of T. niloticus.

Isolated yeast spp.	Fins	Fish flesh	Gills	Liver	Kidney	Spleen	Total
Candida albicans	80	65	60	50	45	30	330 (39.3%)
Other Candida spp.	20	10	15	3	20	5	75 (8.9%)
Rhodotorula	15	10	20	10	20	5	85 (10%)

*the percentage are calculated in relation to total mould and yeast isolated (840).

Macroscopic examination:
Examination of fishes revealed the liver was enlarged, pale, and friable. The spleen was congested. The gills were dark red in colour. (Fig. 1).

Histopathological findings of positively fungal isolation samples:

Hepatopancreas:
of positively fungal isolation samples showed congestion of hepatic sinusoids and degeneration of the hepatocytes with infilteration of mononuclear inflammatory cell. (Fig. 2).

Gills:
the gills revealed severe hyperplasia in the epithelial lining of the secondary lamellae and diffuse filamentous clubbing due to fusion of the secondary lamellae(fig.4) associated with degenerative changes and necrosis and Congestion of lamellar and branchial blood vessels. (Fig. 3).

Muscles:
Showed degenerative changes and necrosis of muscle fibers. (Fig. 5).

Spleen:
Showed severe congestion and hyperactivation of melanomacrophage center along with lymphocytic depletion. (Fig. 6).

Figure (1). Tilipia nilotica fish showing congested gills (blue arrow) congested spleen (yellow arrow) and pale enlarged friable liver (red arrow).

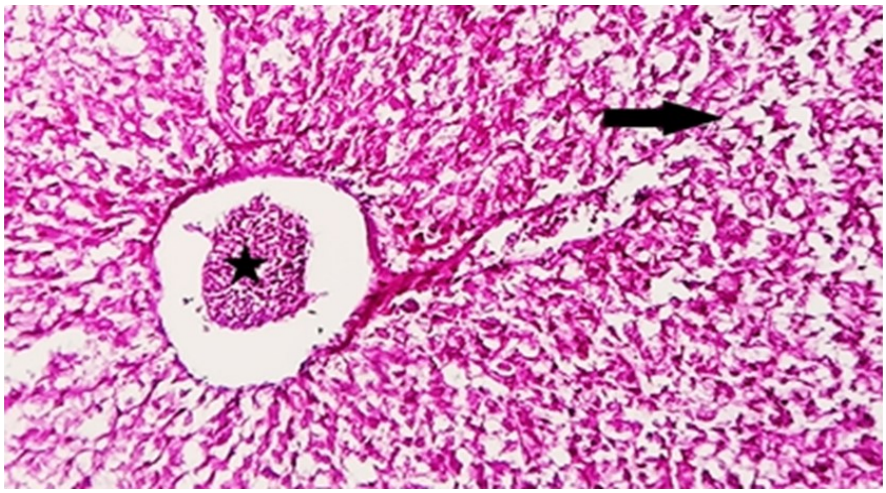

Figure (2). Hepatopancreas of tilapia nilotica showing congestion of hepatic blood vessel (star) and degeneration of hepatocytes (arrow). H&E.(X250).

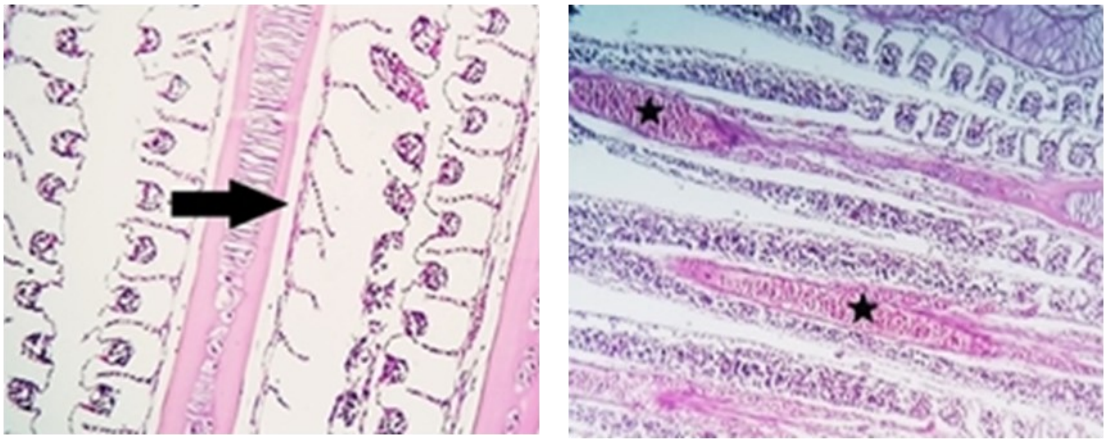

Figure (3). Gills of tilapia nilotica showing degeneration of lamellae (arrow) and Congestion of lamellar and bronachial blood vessels (stars) H&E.(X400), (X200) respectively

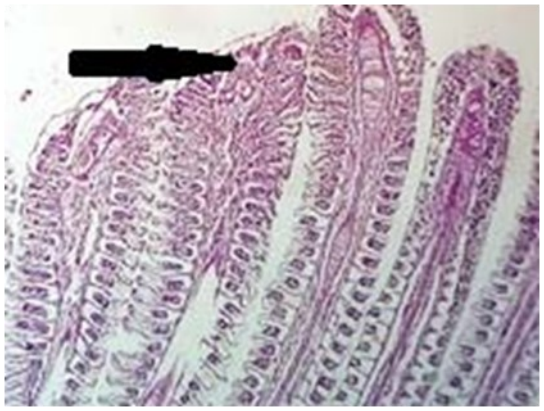

Figure (4). Gills of tilipia nilotica showing severe epithelial hyperplasia at the tips of gill filaments and diffuse filamentous clubbing due to fusion of the secondary lamellae (arrow). H&E, (X100)

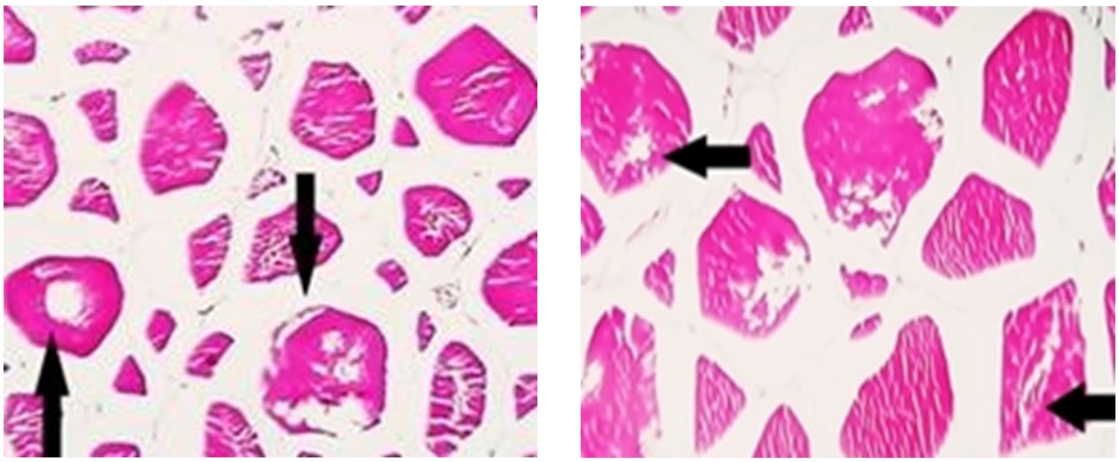

Figure (5). Muscles of tilapia nilotica showing degenrative changes and focal areas of necrosis of muscle fiber (arrows). H&E. (X400)

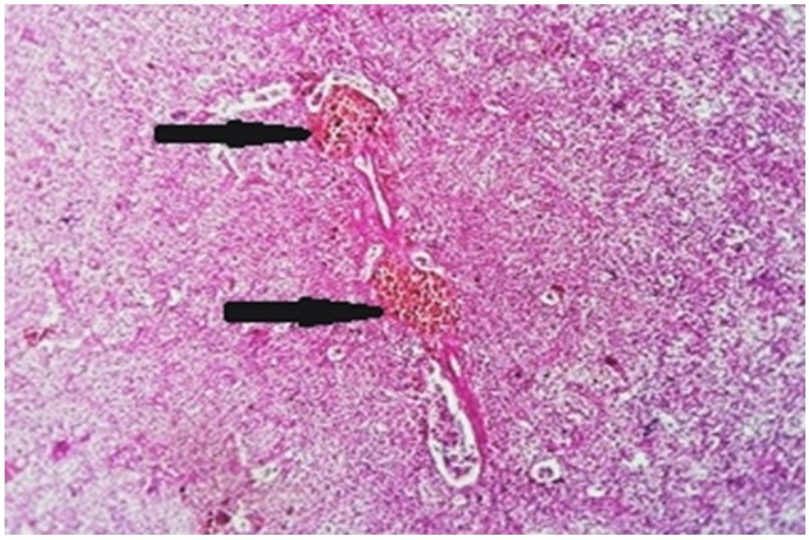

Figure (6). Spleen of tilapia nilotica showing marked hyperactivation of melanomacrophage center and slight lymphocytic cell depletion (arrows), H&E, (x250)

Discussion

Mycological examination of (100) fresh water fish (*Tilapia nilotica*) in table (1) show the Incidence of +ve. mould and yeast samples from different organs and tissues in which mould isolated from (250) samples of different organs and tissue and yeast isolated from (380) samples.

Identification of fungi into yeasts and moulds revealed that the percentage of yeast isolates per fish was slightly higher in which from (840) fungal isolates (350 mould) and (490 yeast). (table 2) .

In table (3) Isolated moulds belonged to the following genera: Saprolegnia (15.5%), Aspergillus niger (5.5%), Fusarium (1.2%), Mucor (4.8%), Penicillium (6.4%), Curvularia (0.9%). Cladosporium (6.2%), lternaria (1.2%).

Nearly similar results were recorded by (**Ammar, 2001**; and **El- Ahl ,2010**).

But these results disagree with **(Refai *et al.*, 2010)** in which their results were Saprolegnia (4.2%), Aspergillus (43.0%), Fusarium (14.1%), Mucor (14), Penicillium (17.2),

The fungal contamination of fish could be attributed to improper sanitation during catching, handling, transportation and marketing of fish. These findings were supported by the view reported by (**Ward and Baji, 1988 and Hassan, 2003**). The contaminated feeds, water supply and worker hands used fish breeding play the essential role on the health status of fish (**Hassan and Abdel Dayem, 2004**). The current results disagree with (**Ibrahim, 2000 and Nasser, 2002**)

And also with (**Atef *et al.,* 2011)** who isolated A.flavus from most of all samples of fish examined in their studies. In our study we cant isolate Aspergillus flavus which considered the most toxogenic mould.

In table (4) isolated yeast belonged to the following genera: Candida albicans (39.3 %), other Candida species (8.9%) and Rhodotorula species (10%) the obtained results are in agreement with **(Ammar, 2001; and El- Ahl, 2010)** and with **(Ibrahim, 2000 and Nasser, 2002) and also with (Refai *et al.,* 2010).**

Fish are more liable to contamination with moulds and yeasts from animal and human reservoirs which may contaminate the water in the fishing area. Furthermore, contamination during handling. The contamination was increased in cases of fish caught from polluted areas **(Hassan and Abdel Dayem, 2004).**

The incidences of isolated moulds from different organs of fish were detected. This was expected, most of these fungi were categorized as normal mycoflora. This does not mean that they cannot produce disease. They can better be considered as opportunistic fungi **(Refai, 1987)** as many of them possess virulence factors, which enable them to cause diseases **(Refai *et al.,* 2004)**, particulary under favourable predisposing condition. Saprolegnia species were isolated with high incidence.

Oreochromis niloticus (Tilapia nilotica) are one of the most sensitive aquatics which easily gets damaged when exposed to pollutants, even at minimal concentration levels **(Karlsson, 1983)** or change in environmental conditions.

The results are in line with previous studies findings which conclude that the different pollutants had effect on fishes as will as the physical status of aquatic environment **(Redner BD, Stickney RR., 1979) (Mallatt J., 1985)**.

Histopathologic changes detected in samples were carried out of positively mycological isolation fishes, gills pathological changes are in line with many studies that had been reported to react to pollutants, leading to changes in anatomical structure and physiology of gills tissues and therefore be regarded as evidence of response to stress in general. **(Filfilan WM, Aljahdali MO., 2019) (Speare DJ, Ferguson HW., 2006)**. Gills showed lamellar edema which is following exposure to chemical pollutants. Complete separation of the respiratory epithelium of primary and secondary lamellae with necrosis of lamellar epithelial cells result in respiratory and osmoregulatory distress **(Yang and Albright, 1992).** Also epithelial proliferation of secondary gill lamellae, which resulted as a response of the malpighian cells to chemical irritation, as they migrate distally, often in the early stages, resulting in an accumulation of cells at the edge of the secondary lamella, progression of this migration leads to lamellar fusion and terminal lamellar clubbing **(Robert, 2001).**

The histopathological finding of The hepatic tissue showed congestion of blood vessels with degenerative changes which may due to mycotic infection and environmental pollutant on hepatocytes since the hepatopancreas is the site of detoxification of all chemicals and toxins **(Robert, 2001).**

The activation of melanomacrophage centers either in spleen or hepatopancreas is a result to infection or irritation in fish leading to fish immune response **(Robert, 2001).**

As will as splenic changes represnted in increased melanomacrophages **(Montero *et al.*, 1999), (Gogal *et al.*, 1999; Garcia-Abiado *et al.*, 2004)**. The findings obtained in the present investigation are in line with studies explain the effect of environmental pollutants on normal fish physiology **(David M. and Kartheek R.M., 2015)**. the pathologic finding of gills ,

liver, muscels and spleen are in line with studies that reported to react to chemical and toxins in tilapia nilotica (**Amany, M. Kenawy *et al.*, 2009), (Gaafar, A.Y. *et al.*, 2010)**.

Conclusion and Recommendation

It can be concluded from the results obtained in the present work that, though most fungi isolated from fishes are considered by several authors as normal mycoflora, yet we could prove in the present study that many fungi can cause natural infections. This was confirmed by histopathological reactions characteristic of fungal infection fishes. This conclusion should give attention to the possible role of fungi in affecting fishes industry .The risks of fungal infection in *Tilapia nilotica* fishes increase due to poor aquarium management. Also, the basic health management practices could be simply unnoticed due to lack of expert personals.

In order to decrease the chance of spreading fungal infection in the native fish species,we need good water supply, control diseased fish and expert personals, avoiding damage of skin during transportation of fish, right kind of food with sufficient amount must be provided to fish. over crowding of fish must be prevented. preventing the introduction of new fish to the fish farm until known that fish are free from disease. disinfection of the equipments and utensils to prevent spread of the infection.

References

Ammar, M.A.M. (2001). "Sanitary assessment of some common fresh water fish in Assiut." M.V.Sc. Thesis, Fac. Vet. Med., Assuit Univ., Egypt

Amany, M. Kenawy; Hala, M. El-Genaidy; Mohammad, M.N. Authman and Abdel-Wahab, M.A. (2009). Pathological studies on effects of aflatoxin on Oreochromis niloticus with application of different trials of control .Egypt. J. Comp. Path. & Clinic. Path. Vol. 22 No. 1 (January); 175 - 193 .

Atef, A. Hassan; Manal, A. Hassan; Howayda, M. El Shafei; Rasha, M.H. S. El Ahl and R.H. Abd El-Dayem (2011). Detection of Aflatoxigenic Moulds Isolated From Fish and their Products and its Public Health Significance]. Natureand Science 2011; 9(9):106-114] (ISSN:1545-0740

Barnett, H.L. and Hunter, B.B. (1972). Illustrated genera of imperfect fungi. 2nd Ed., Burgess Put. Co..

Bailey, W.R. and Scott, E.G. (1998). Diagnostic Microbiology. A Textbook for the isolation and identification of pathogenic microorganisms. The C.V. Mosby Company Saint Louis, US.

Bisht, D.; G.S. Bisht and R.D. Khulbe (2000). Fusarium A new threat to fish population in reservoirs of Kumaun India .Curr. Sci., 78 (10): 1241-1245

Cruickshank, K.R.; Duguid, J.P.; Marmion, B.D. and Swain, R.H.A. (1975). MedicalMicrobiology.12thEd.,Vol.2,Churchill Livi ngstone Limited, Edinburgh, London and New York.

Culling, C.F.A. (2013). Handbook of histopathological and histochemical techniques: including museum techniques, (Butterworth-Heinemann).

David M. and Kartheek R.M. (2015). Histopathological alterations in spleen of freshwater fish *Cyprinus carpio* exposed to sublethal concentration of sodium cyanide .Open Vet J.; 5(1): 1–5.

Easa, M. EI-S; M.E. Hatem; E.E. Sark and M. Refai (1984). *Phoma herbarum* as a mycotic fish pathogen in *Clarias lazera*, Armout catfish. *Vet. Med. J.*, 32, 257- 267

Ellis, A.E.; I.F. Waddell, and D.W. Minter (1983). A systematic fungal disease in Atlantic *salmon parr*, *Salmo salar L.*, caused by a species of Phialophora . *J. Fish. Dis*., 6 :511-523.

El-Ahl, M.H.S. Rasha (2010). Studies on fungi in fish and fish products and their control. Ph. D. Thesis, Dept. of Microb, Fac. of Vet. Med., Cairo Univ.

El-Zayat, S.A.M. (1988). studies on freshwater fungi of Aswan high Dame lake. Ph.D. Thesis, Botany Dept. Faculty of Science (Aswan), Assiut University, Egypt

Faisal, M.; H. Torky and H.H. Reichenbach -Klinke (1985). A note on swimming disease among the Labyrinth catfish (*Clarias lazera*). *J. Egypt. Assiut Vet. Med*., 45: 53-60

F.A.O., Fisheries Technical paper (1985). Avoidness of losses in preserved fishes. Food and Agriculture Organization of United Nations, Rome. No. 219: 16-100.

Filfilan, W.M. and Aljahdali, M.O. (2019). Histological Changes in the Gills of Marine Cultured Tilapia (*Oreochromis spilurus*) at Larvae Stage Treated by Phenanthrene. J Aquat Poll

Gaafar, A.Y.; El-Manakhly, E.M.; Soliman, M.K.; Soufy, H.; Mona, S. Zaki; Safinaz, G. Mohamed and Shahenaz, M. Hassan (2010). Some pathological, biochemical and hematological investigations on Nile tilapia (*Oreochromis niloticus*) following chronic exposure to edifenphos pesticide. Journal of American Science; 6(10).

Garcia-Abiado, M.A.; Mbahinzireki, G.; Rinchard, J.; Lee, K.J. and Dabrowski, K. (2004). Effect of diets structure in tilapia, Oreochromis sp., reared in a recirculating system. J. Fish Dis.; 27: 359–368.

Gogal, R.M.; Smith, B.J.; Robertson, J.L.; Smith, S.A. and Holladay, S.D. (1999). Tilapia (*Oreochromis niloticus*) dosed with azathrioprine display immune effects similar to those seen in mammals, including apoptosis. Vet. Immunol. Immunopathol.; 68: 209–227.

Göttlich, E.; van der Lubbe, W.; Lange, B.; Fiedler, S. and others (2002). Fungal flora in groundwater-derived public drinkingwater. Int J Hyg Environ Health 205: 269–279

Gonçalves, A.B.; Russell, R.; Paterson, M. and Lima, N. (2006). Survey and significance of filamentous fungi from tap water. Int JHyg Environ Health 209: 257–264

Hassan, A.A. (2003). Detection of some mycotoxinsand mycotoxins producing fungi in both macro- and microenvironment of diseased animals. 7th Sci. Cong. Egyptian Society for Cattle Diseases, pp. 112 – 119,Assiut, Egypt.

Hassan, A.A. and Abdel- Dayem, R.H. (2004). "Prevalence of fungi and mycotoxins in fresh and salted fish. J. Egypt. Vet. Med. Assoc. 64 (1): 1-11 and 59- 68.

Hassan, A.A.; Hammad, A.M; El Barawy, A.M. and Manal, A.H. (2007). Incidence of aflatoxigenic fungi in frozen and canned fishes and trials to inhibit aflatoxin production by use of some minor elements and *lupinustermis* seeds. Egypt. J. Appl. Sciences, 22 (10B) 351-360.

Hatai, K. and G.I. Hoshiai (1994). Pathogenicity of *Saprolegnia parasitica* coker. *In* Salmon Saprolegniasis. *Edited by* G. J. Mueller. U.S. Department of Energy, Bonneville Power Administration, Portland, Oregon. pp. 87-98.

Ibrahim, H.A.M. (2000). Incidence of fungal contaminants in fish and fish products. M.V.Sc., Thesis, Zag. Univ. Benha Branch, Egyp.

International Orgaanization For Standardization (ISO) 6887-3 (2003). Microbiology of food and animal feeding stuffs-Preparation of test sampl, initial suspension and decinal dilutions for microbiological examination-Part 3:specific rules for the preparation of fish and fishery products.

Karlsson, L. (1983). Gills morphology in the zebra fish, *Brachydanio rerio* (Hamilton-Buchanan). J Fish Bio 23: 511-524.

Koburger, J.A. (1970). Fungi in Foods. 1-Effect of inhibitor and incubation temperature on enumeration. J. Milk and Food Technol., 33(10): 433-434.

Kriger Van Rij, N.J.W. (1984). The yeasts: A taxonomic study. 3rd Ed. Amsterdam, Elsevier

Langdon, J.S. and W.L. MacDonald (1987). Cranial *Exophilia piscphilia* infection in *Salmo salar* in Australia. *Bull. Eur. Ass. Fish Pathol.* 7:35-37.

Martin, M.V. (1979). Germ tube formation by oral strains of *Candida albicans. J.Med. Microbiol*., 12:187-193.

Mallatt, J. (1985). Fish gills structural changes induced by toxicants and other irritants: A statistical review. Can J Fish Aquat Sci 42: 630-648.

Montero, D.; Blazer, V.S.; Socorro, J.; Izquierdo, M.S. and Tort, L. (1999). Dietary and culture influences on macrophage aggregate parameters in gilthead.

Nasser, L.A. (2002). Mycological status of importedcanned fish consumed in Saudia Arabia with special reference to proteolytic activity. Assuit Vet. Med. J., 47: 125.Nowakowska, D.; Gaj, Z.; Sobala, W. and Wilczynski.

Neish, G.A. and G.C. Hughes (1980). Diseases of fishes, Book 6. Fungal diseases of fish. TFH Publications, Neptune, Ng.1159

Noga, E.J. (1996). Fish Disease Diagnosis and Treatment. Mosby-Year Book, Inc. St. Louis, MO. 367 p.

Pickering, A.D. and L.G. Willoughby (1982). *In* Microbial Diseases of Fish. *Edited by* R.J. Roberts. Academic Press, London, England. pp. 271-297.

Pitt, J.I. and Hocking, A.D. (2009). Fungi and Food spoilage. 3rd Ed. Published by Springer Dordrecht Heidelberg, London, New York.

Quiniou, S.M.A.; S. Bigler and L.W. Clem (1998). Effects of water temperature on mucous cell distribution in channel catfish epidermis: a factor in winter saprolegniasis. *Fish Shellf Immunol.* 8:1-11

Redner, B.D. and Stickney, R.R. (1979). Acclimation to ammonia by *Tilapia* aurea. Trans Am Fish Soc 108: 383-388.

Refai, M. (1987). Isolation and identification of fungi. Fac. Vet. Mid. Cairo University.

Refai, M.; M.M. Abdel halim; M.M.H Afify; H. Youssef and K.M. Marzou (1987). Studies on aspergillomycosis in catfish (*Clarias Lasera*). Allgemeine Pathologic and pathologische Anatomic. Tagung der Deutachen Veterinar - Medizinischen Gesellschaft. der Europeischen Gesellschaft fur Vet. Pathol. 63, 1-12.

Refai, M.; S. Attia; R.M. Salem and E.M. El -Dahshan (2004). Studies on the pathogenicity of *Aspergillus* fumigatus, *A. flavus* and *A. niger* isolated from chickens and their enviroment. *Egypt. J. Comp. Path., Clinic. Path.,* 17 (2): 193-205.

Refai, M.K.; Laila, A. Mohamed; Amany, M. Kenawy and Shimaa, El-S.M.A. (2010). The Assessment Of Mycotic Settlement Of Freshwater Fishes In Egypt. Journal of American Science; 6(11): 595-602]. (ISSN: 1545-1003).

Roberts, R.J (1989). The mycology of teleosts, in Roberts RJ (ed). Fish Pathology, 2nd edition. London, England, Baillere Tindall, pp 320-336.

Robert, R.J. (2001). Fish Pathology. 3rd Ed., Bailliere Tindall, London, Philadelphia, Sydney, Tokyo, Tornonto.

Shaheen, A.A.M. (1986). Mycoflora of some freshwater fish. M.V.Sc. Thesis, Zagazig University.

Speare, D.J. and Ferguson, H.W. (2006). Systemic pathology of fish: A text and atlas of normal tissues in teleosts and their **responses** in diseases. In: Ferguson HW editor. Chapter 2 Gills and Pseudobranchs. Scotian Press, London. Pp: 25-63.

Salem, A.A.; M.K. Refai; I.A.M. Eissa; M. Marzouk; A. Bakir; M. Moustafa and Manal Adel. (1989b). Some studies on aspergillomycosis in Tilapia nilotica. *Zagazig Vet. J.,* 17(3): 315-328

Tibor, D. and R.B. Larry (1996). Handbook of food spoilage yeasts. 1st Edition (Contemporary Food **Science**) by CRC Press, Boca Raton, New York, London and Tokyo.

Ward, D.R. and Baaji, N.J. (1988). Factors affecting microbiological **quality** of sea foods. Food Technol., 42: 85.

Yang, C.Z. and Albright, L.J. (1992). Effects of the harmful diatom *Cheatoceros concavicornis* on respiration of rainbow trout *Onchorynchus mykiss*. Disease of Aquatic Organisms. 19: 51-55.