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Epidemiological investigations of Mycotic infections of cultured Gilthead seabream, *Sparus aurata* at Marriott Lake, Egypt

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Abstract
Surveillance and descriptive studies of mycotic infections were investigated throughout a period of one year (2013 to 2014). A total number of one hundred (100) of cultured Gilthead seabream at Marriott Lake were surveyed for mycotic infections, whereas clinical and PM lesions were defined. Morphological and cultural characters of the isolated fungi and yeast were identified from fish tissues and organs. Moreover, their prevalence, incidence and relationship with physico-chemical properties and heavy metals content in water and tissues were evaluated. Infected fish have torned vertebral column, congested kidney with pale liver, fungal patches on the GIT and mottled appearance of the liver with severely congested heart. Results were confirmed with histopathological examination, which revealed the presence of fungal hyphae and spores in different organs. It was found that about eighty percentage (80%) of the examined fish were infected and total Aspergillus species were predominant in prevalence of mycotic isolates (32.12%) followed by Cladosporium (20.86%) and Fusarium species (14.45%). Moreover, the incidence of the mycotic isolates was higher in liver and kidney of the infected fish. The results of water quality parameters indicate that levels of nitrite, ammonia, organic matter as well as cadmium (Cd), lead (Pb) and copper (Cu) were higher than the permissible limits. We can conclude that the higher mycotic infections of cultured seabream were parallel together with unsuitable water quality and higher heavy metal levels.

Keywords: Mycotic infections - Seabream - Marriott Lake – Epidemiology.

1. Introduction
Seabream (*Sparus aurata*) is marine Fish with economic value and wide spread all over the world, especially in the Mediterranean Sea. Seabream culture was known recently in Egypt and need for progressive development especially in feeding and health care.
Fungi are members of Thalophyta, lacking of chlorophyll completely therefore are bound to live as a saprophytic or parasites in existence. Fungi were reported to be responsible for many fish diseases. These fungi are belonging to wide range of genera which were found associated with several mycotic diseases of fish [1]. The importance of fungal diseases in freshwater fish not stopped only for incidence of mortalities but also as economic importance such as decrease growth rate, hatchability in chronaic infection or by mycotoxins production by contaminated fungus in case of bad storage feed [2]. In spite of the fungal infections importance our knowledge about them is still poor for two basic reasons: difficult identification of pathogenic fungi and the prolific growth of saprophytic fungi once the fish is dead [3]. Many of the fungi that affect fishes are considered opportunists, 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 [4, 5].
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 [3].

Members of this group are generally considered agents of secondary infection arising from conditions such as bacterial infections, poor husbandry, and infestation by parasite and social interaction. However, there are several reports of Oomycetes as primary infectious agents of fishes [6] and their eggs [7].

Moreover, there are other fungi that have been implicated in fish diseases. Some of the genera involved include Aspergillus [8], Fusarium [9], Ichthyophonus [10], Branchiomycosis [11], Phoma [12], Paecilomyces [2, 13], Exophiala [14], Phialophora [15], Rhizomucor [16] and Candida [17]. Thus the present study was aimed to spot light on isolation and identification of mycotic infections of cultured seabream and their relationship with physico-chemical and heavy metals of water.

2. Material and Methods

2.1. Fish samples

In our investigation, a total number of one hundred cage-cultured marine fishes of Seabream (*Sparus aurata L.*), of different body weight ranged (50 gm ± 30 gm), fishes were from private fish farm at Wadi-Mariut region at west Alexandria governorate, Egypt. The sources of water in Wadi-Mariut are numerous; underground water, drainage of canal originated from El-Banger area as well as from rainfall water downstream from Borg-El-Arab city. Fishes were collected showing clinical signs in plastic bags containing about 1/3 volume of water at site of collection and filled with oxygen. They were transferred immediately to the laboratory in Animal Health Research Institute Damanhur branch. The freshly dead fish specimens were subjected to full clinical, postmortem (PM) lesions, and mycological examinations.

2.2. Gross clinical examination

Clinical examination of naturally infected fishes was performed to investigate any clinical abnormalities [18, 19].

2.3. Postmortem (PM) examination

Necropsy was performed on variable number of freshly dead and moribund fishes for detection of PM lesions [20, 21].

2.4. Mycological examination

The fish surface was disinfected with a swab of cotton moistened with 70% ethyl alcohol. Spleen, liver, heart and kidney of killed fish were collected under complete aseptic conditions and inoculated into Sabouraud's dextrose agar medium (SDA) with 0.05 mg/L chloramphenicol, Potato-dextrose agar and corn-meal agar plates **[22]**. The plates were incubated at 25 – 28 °c for 3-5 days. Negative plates were not discarded before 2 weeks **[23]**. All the positive moulds cultures were purified by sub culturing on Sabouraud's dextrose agar plates incubated at 25 - 28c for 3-5 days and examined for gross and micro morphological characteristics [24, 25].

2.5. Identification of mycotic isolates

2.5.1. Identification of moulds

All the purified mould cultures were examined for macro and micro-morphological characteristics. This was carried out according to the methods of [25, 26, 27, 28, 29 & 30].

2.5.1. a. Microscopical examination

The gross morphological examination included the rate of growth, texture, changes in color during growth, final color of the surface and reverse sides of the colonies.

2.5.1. b. Microscopical examination (Solutip method)

For micro morphological studies, a small portion of the periphery of a fresh colony was picked using the sticking surface of a piece of solutip and placed with its sticking surface down on a clean slide with a drop of lacto phenol cotton blue stain **[31]** and examined microscopically. Microscopically examination was carried out to detect septation of hyphae, roughness or smoothness of conidiophores, shape of vesicles, arrangement and number of the rows of the strigmata.

2.5.2. Identification of yeasts

Suspected yeast like colonies were preliminary identified according to the scheme **[32]**. Suspected Candida species were scratched onto corn meal agar tween 80 for chlamydospore production **[33]** in very short 3 or 4 parallel lines using a mycological needle. The scratched lines were covered by sterile cover slides to provide an aerobic condition. Incubation was at 25 °C for 72 hours.

2.6. Collection of water samples

2.6.1. Assessment of physico-chemical water properties

The tools used for determination of Physico-chemical properties of water quality were namely; Dissolved Oxygen meter for measuring the level of Dissolved oxygen in the water, Salinometer for measuring of % of water salinity, PH meter for measuring the pH values and Kits for measuring the levels of unionized ammonia and Sulphate in the water (USA, Virginia Company, lot. No .201134).

2.6.2. Spectrophotometric method for detection the levels of heavy metals in water and fish tissues

The method for analysis of the heavy metals in water **[34]** and fish tissues **[35]** was carried out using Atomic Absorption Spectrophotometry. Atomic Absorption Spectrophotometer (Model Thermo Electron Corporation, S. Series AA Spectrometer with Gravities furnace, UK,) instrument was used to detect the heavy metals. The concentrations of heavy metals were expressed as mg/l for water and µg/g dry wt. for fish tissues.

Fish specimens were digested [36]. All frozen fish samples were allowed to thaw at room temperature, washed with distilled water and placed on filter paper to remove the excess liquid. Their gills and musculature tissues were dissected separately and minced using a domestic blender, then approximately 1.0 gm was placed in a 150 ml beaker and 10 ml concentrated nitric acid was added. After a short soaking period, 5 ml of 60% perchloric acid was added and the mixture was slowly heated on a hot plate until the conclusion of growth (approximately 2 hrs). The mixture was then heated until the appearance of dense white fumes that indicate the nitric acid had evaporated and perchloric acid had reached its boiling point. The mixture was cooled; 10 ml of 25% hydrochloric acid was added then, the solution was transferred to a 100 ml volumetric flask that was subsequently brought to volume with de ionized water. Blank solution was prepared for the background correction. Atomic absorption spectrophotometer instrument was used to determine As, Zn, Cu, Pb, Hg, and Cd concentrations which were expressed as µg / g dry weight in the Toxicology Unit of Central Laboratory, Faculty of Veterinary medicine, Alexandria University, Egypt.

Table 1: The important heavy metals and their recommended international permissible limits (PL) in water (mg / L.) and fish tissues (µg / g dry wt.).

Metals	PL in water	Reference	PL in fishes	Reference
Lead (Pb)	0.050	[37]	0.1	[39]
			0.5	[40]
Mercury (Hg)	0.001	[37]	0.5	[39]
			0.5	[40]
Cadmium (Cd)	0.005	[37]	0.10	[39]
			0.05	[40]
Copper (Cu)	0.2	[38]	10	[42]
Zinc (Zn)	2.0	[38]	60	[42]
Iron (Fe)	< 1.0	[37]	30	[41]

2.7. Histopathological examination

Specimens for histopathological techniques were freshly taken from infected organs and tissues of the infected Seabream. Samples were trimmed and fixed in 10% phosphate buffered formalin. Then washed in running tap water for 24 hours then dehydrated in different concentration gradients of alcohol and cleared in Xylol. Samples then embedded in paraffin wax and sectioned into thin sections of 5 microns thickness. Sections were stained with H & E stain and examined microscopically [1].

3. Results and Discussion

Mycotic affections have drastic economic significance in cultured fish, where fish become overcrowded and the diseases spread more easily among fish, these condition may contributed to high mortalities.

3.1. Results of clinical examination (clinical signs and PM lesions) of infected fish

Results of clinical signs and PM lesions of the infected Seabream were summarized in figures 1 and 2.

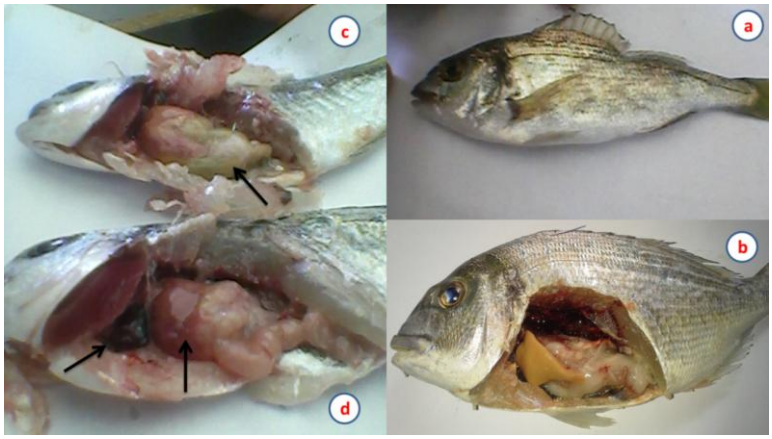

Fig 1: Naturally examined Seabream with torned vertebral column (Photo a), congested kidney with pale liver (Photo b), fungal patches on the GIT (Photo c) and mottled appearance of the liver (arrow) with severely congested heart (arrow) (Photo d).

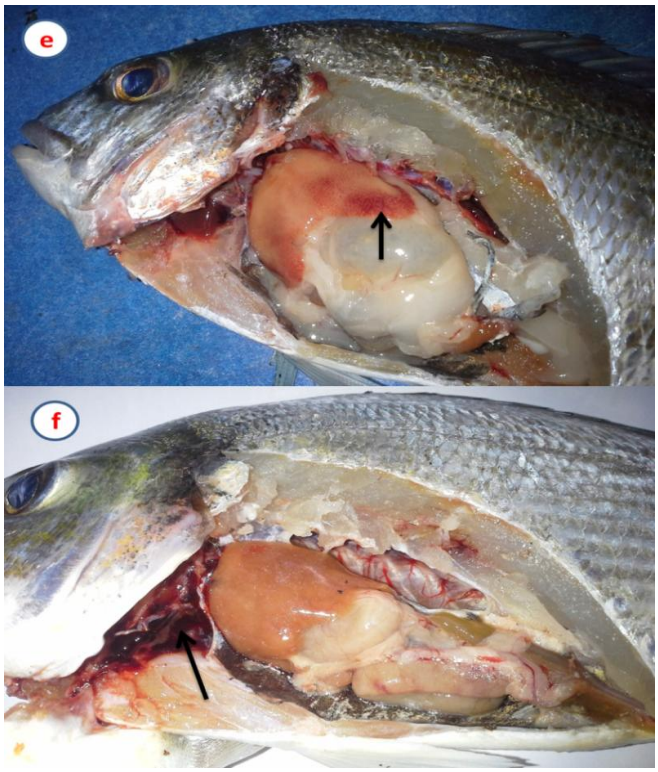

Fig 2: PM lesions of naturally examined Seabream characterized with pale liver with focal hemorrhages on its surface and have mottled appearance (arrow) (Photo d), congested heart and gills (arrow) (Photo e).

Results of clinical findings of Seabream were parallel to that obtained by [43], and similarly the postmortem findings were in agreement with those of [44].

3.2. Cultural and Morphological identification of mycotic isolates

3.2.1. Moulds

3.2.1.1. Aspergillus species

3.2.1.1. a. *Aspergillus flavus* (*A. flavus*)

Macroscopically, the growth appeared velvety with numerous aerial growths, at first the color was yellow and became yellowish green by aging. While, microscopically, the conidiophores were long and rough. The vesicles were large and rounded. The strigmata were biseriate, loose, and radiate and gave rise to ovoid rough conidia.

3.2.1.1. b. *Aspergillus fumigatus* (*A. fumigatus*)

Macroscopically, colonies have distinct margin with some shades of green, blue-green, surface has a powedrey appearance. A white apron was seen at the edge in the zone of active growth. While, microscopically, characterized by hyaline and distinctly septated hyphae, conidiophores were long with club -shaped vesicle, spherical conidia were born from single row of sterigmata.

3.2.1.1. c. *Aspergillus niger* (*A. niger*)

Macroscopically, the colonies were wooly in texture and spread rapidly .They were black in color with radiated rouge. While, microscopically, the conidiophores were very long, smooth and yellowish color. The vesicles were very large and globes while the strigmata were biseriate, compact and radiate. The conidia were globes and smooth.

3.2.1.1. d. *Aspergillus parasiticus* (*A. Parasiticus*)

Its characters are those of A. flavus group but colony color is predominantly greener, short stalks with usually a single series of sterigmata. No sclerotia have been seen. The mycelium is uncolored.

3.2.1.1. e. *Aspergillus terreus* (*A. terreus*)

Macroscopically, the colonies were buff to dark brown velvety folded. While, microscopically, small hemispherical vesicle with phialides born on prophialide.

3.2.1.2. Aphanomyces species

Macroscopically, characterized by flat, slight opaque colonies with an uneven white velvets surface. The hyphal growth increase with prolonged incubation to occupy the entire surface of the plate and appear as linear growth within 14 days of incubation. While, microscopically, characterized by branched non septated hyphae with tapered end contain cytoplasmic organelles.

3.2.1.3. Alternaria species

Macroscopically, colonies were dark greenish-black to grey-brown with a light border. Reverse is black. Furthermore, microscopically, the hyphae dark and septated. Conidia are large, brown, muriform, club-shaped and occur singly or in chains.

3.2.1.4. Cladosporium species

Macroscopically, colonies were dark, velvety and olive-green, with dark reverse. Moreover, microscopically, the conidiophores with varying lengths that produce long branching chains of brown, smooth-walled, oval, pointed conidia .The conidia are easily dispersed.

3.2.1.5. Fusarium species

Macroscopically, colonies were cottony or wooly in texture, snow white, pink-violet or rosy-red in color, with specific diffusion of colored pigments into the reverse surface of the medium. While, microscopically, they were long, branched and septated hyphae from which short conidiophores rose singly or in groups, and sometimes branched. Two types of conidia were observed, a large banana shaped, septated macroconidia and a small, round, non septated microconidia.

3.2.1.6. Geotrichum species

Macroscopically, colonies were whitish, flat, and moist and yeast like with a granular surface. Some strains produce short, white, cottony aerial hyphae. Moreover, microscopically, the septated mycelium fragments into arthrospores (arthroconidia), which are formed consecutively and become round. No blastoconidia, are produced.

3.2.1.7. Helminthosporium species

Macroscopically, colonies were cottony and dark grey to black. Reverse is black. Furthermore, microscopically, the unbranched conidiophores those are brown slightly curved, with conidia forming along the sides. The later are large, dark, multi-celled and club-shaped.

3.2.1.8. Ichthyophonus species

Macroscopically, characterized by growing culture appeared as white hyphal growth with different levels both on the surface and into the substrate of the S.D.A. media the hyphal growth increased to full fill the plate within 10-14 days post inoculation. Furthermore, microscopically, characterized by branched non septated hyphae with spherical hyphal tips and various forms of resting spores.

3.2.1.9. Nigrospora species

Macroscopically, colonies were compact and wooly, white at first but black areas appear due to the production of black globose conidia. Reverse is black. While, microscopically, the short conidiophores that swell and then taper to the point of conidia formation. Conidia are large, black, round but slightly flattened.

3.2.1.10. Paecilomyces species

Macroscopically, colonies were flat surface, powdery or velvety, yellowish-brown or light pastel shades of pink, violet or gray green. Microscopically, resembles Penicillium (the conidiophores formed brush -like branches resembling the fingers, with long chains of small spherical conidia forming the flask-shaped sterigmata (metula) but the phialides are more elongated and taper into along slender tube. The conidia are elliptical or oblong and occur in chains.

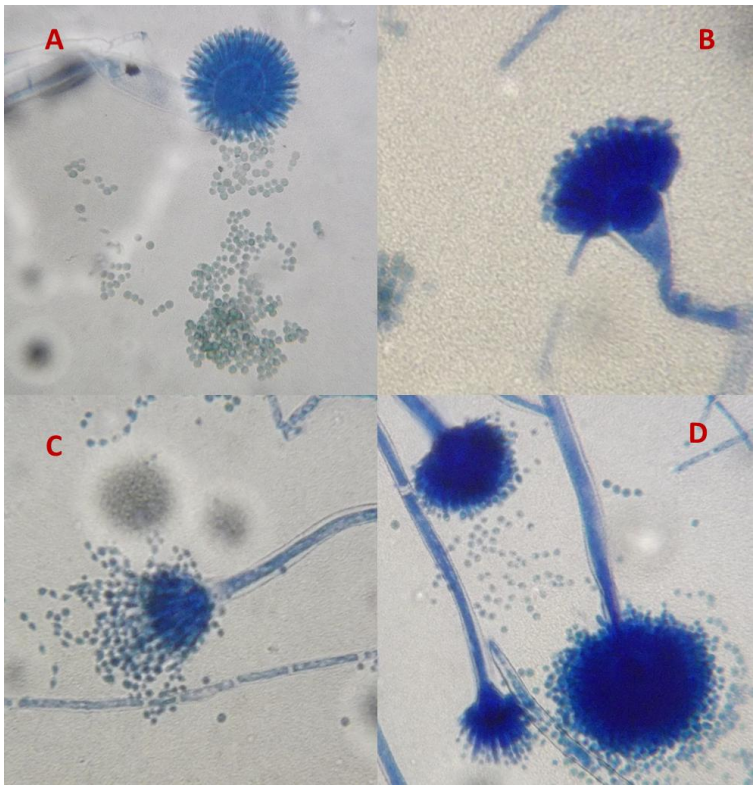

Fig 3: Light photograph of Lacto phenol cotton blue X 400 have A. flavus, 5-7 days old (**Photo A**), showing the large rounded vesicles were bearing the biseriate, loose and radiate strigmata which gave rise to ovoid rough conidia, A. niger (**Photo B**), showing conidial heads are short columnar in and biseriate. Conidiophore stipes is usually short, brownish and smooth walled Conidia are globose and rough-walled, A. fumigatus (**Photo C**), showing conidial heads are typically columnar but often much shorter and smaller) and uniseriate. Conidiophore stipeses are short, smooth-walled and have conical-shaped terminal vesicles which support a single row of phialides on the upper two thirds of the vesicle. Conidia are produced in basipetal succession forming long chains and are globose to subglobose and A. terreus (**Photo D**) showing conidiophore stipes are hyaline and smooth-walled Conidia are globes to ellipsoidal, hyaline to slightly yellow and smooth-walled.

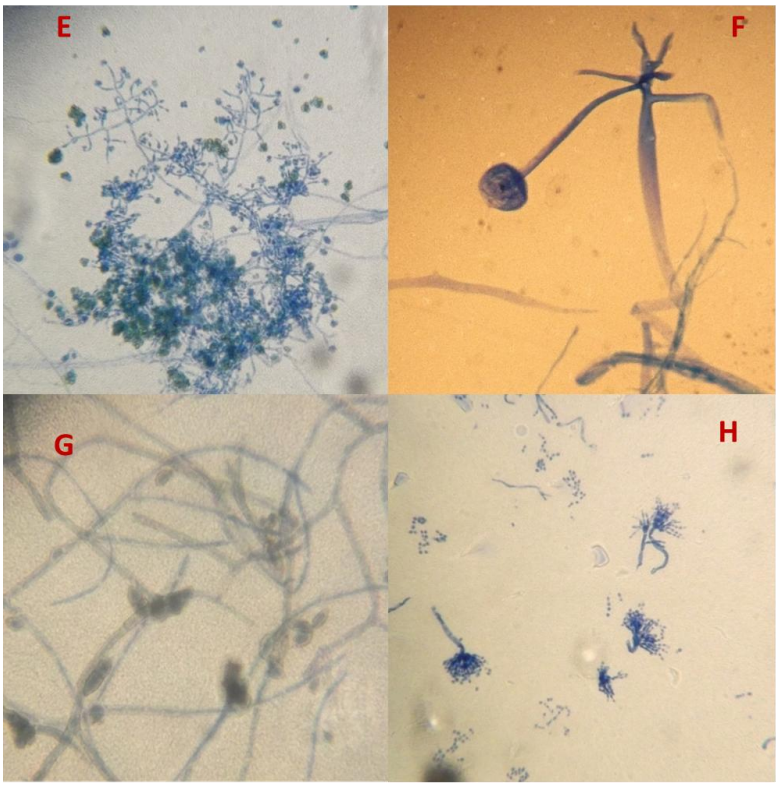

Fig 4: Light photograph of Lacto phenol cotton blue X 400 have Paecilomyces spp. showing phialides are long, slender and graceful and broad, non-septated hyphae (**Photo E**) and Conidiophores bearing dense, vertically arranged branches bearing phialides. Phialides are cylindrical or ellipsoidal, tapering abruptly into a rather long and cylindrical neck (**Photo H**), Rhizopus spp. (**Photo F**) showing rhizoids of the colony formation and Cladosporium carrionii (**Photo G**) showing ascending to erect, apically branched, elongate conidiophores producing branched acropetal chains of smooth-walled conidia. Conidia are pale olivaceous, smooth-walled or slightly verrucose, limoniform to fusiform.

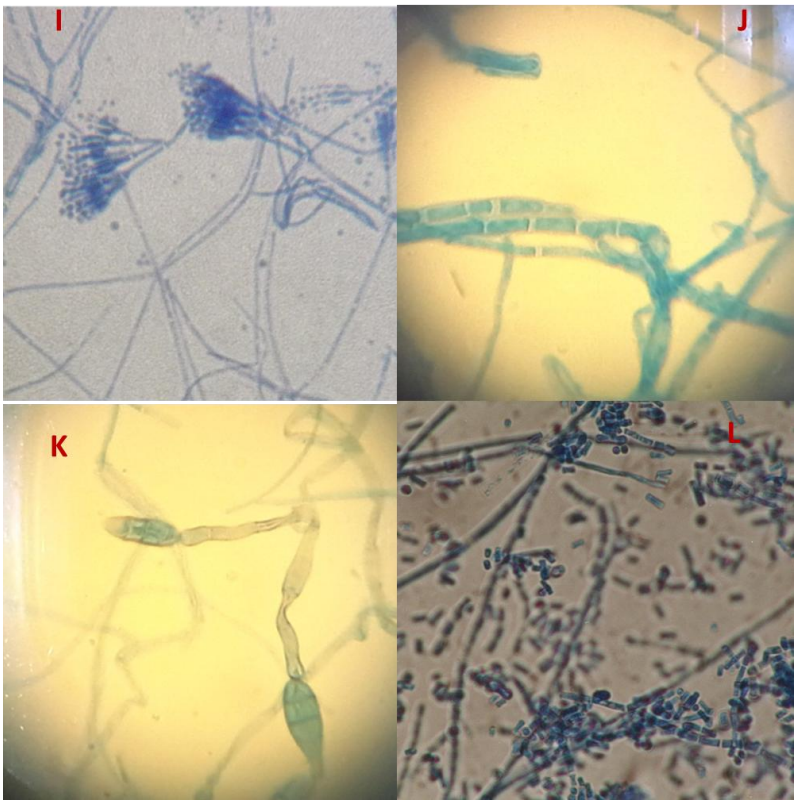

Fig 5: Light photograph of Lacto phenol cotton blue X 400 have Penicillium spp. (**Photo I**) showing conidiophores are hyaline, smooth walled and bear terminal verticals of 3-5 metulae, each bearing 3-7 phialides. Conidia are globose to subglobose, smooth-walled and are produced in basipetal succession from the phialides, *Aphanomyces* spp. (**Photo J**) Showing arrangement of zoospores in one row, Exophiala spp. (**Photo K**) showing aggregations of cylindrical spores at the end of hyphae and Alternaria spp. (**Photo L**) showing macroconidia divided by alteration of spores.

3.2.2. Yeasts

3.2.2.1. Torulopsis species

Characterized by white to cream colored, later become grayish-white or brown on S.D.A. at 25oc to 37oc after 3-4 days. The colonies are moist, smooth and shiny initially. Older cultures may become wrinkled. Microscopically, no pseudo hyphae were formed on rice agar and growth at 37oc on S.D.A.

3.2.2.2. Cryptococcus species

Could be identified by positive urease test, no pseudohyphae on rice agar medium.

3.2.2.3. Rhodotorula species

Characterized by budding of round, oval cells, absence of pseudohyphae on rice agar medium and colony on Sabouraud's Dextrose Agar was characterized by formation of carotenoid pigments; that vary from orange to red.

4. Histopathological findings of the infected Seabream

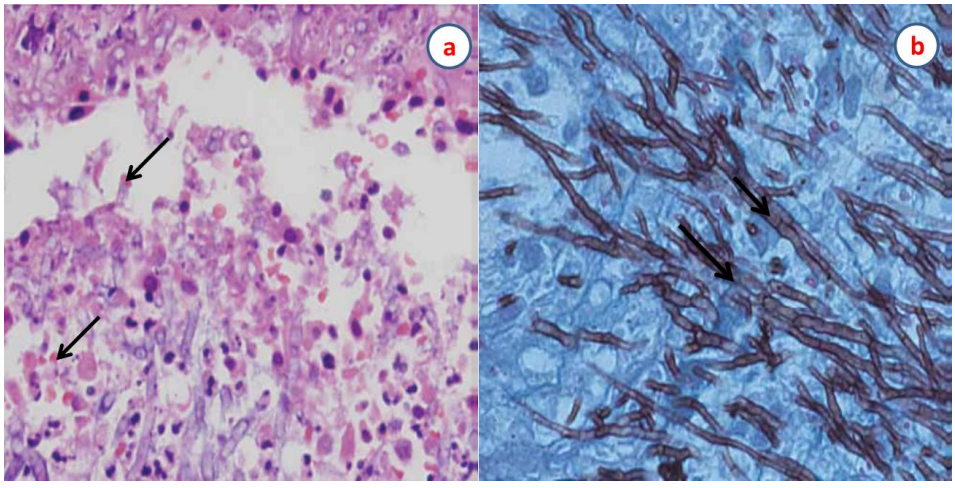

Fig 6: Histopathological section of liver of seabream (**Photo a**) showing hydropic degeneration and distributed fungal elements (arrows) in the most disarrangement hepatic cells, while the musculature (**Photo b**) showing myelitis of the muscle fibers associated with embedded hyphal elements along the course of muscle fibers (Arrows) (Periodic Acid Schiff stain 100X).

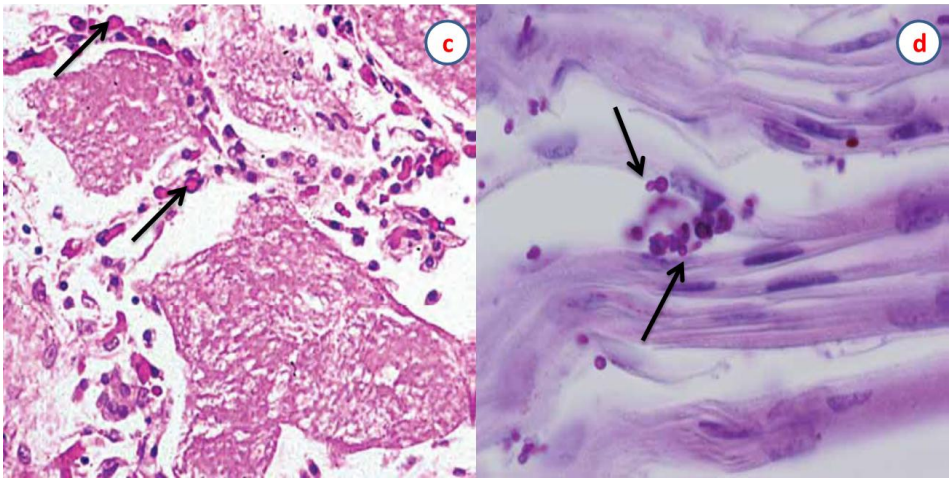

Fig 7: Histopathological section of the liver of Seabream (**Photo c**) showing severe congestion and hyaline cost masses as well as distributed fungal elements (arrows) in the most disarrangement hepatic cells while the musculature of Seabream (**Photo d**) showing myelitis of the muscle fibers associated with aggregation of budding spores of fungus in the center of muscle cells (Arrows) (Periodic Acid Schiff stain 100X).

5. Prevalence of mycotic infections in cultured Seabream

A total of 100 cultured Seabream were mycologically examined. Specimens from different organs (600) of different species were mycologically examined only 480 samples were positive with affection percentage of 80%.

In this study 13 genera of mould and four genera of identified yeast beside to the unidentified yeasts were isolated. This was expected, as almost all these fungi were categorized [45] as normal mycoflora. This does not mean that they cannot produce disease. They can better be considered as opportunistic fungi [30] as many of them possess virulence factors, which enable them to cause diseases [46], particularly under favorable predisposing condition.

Table 2: Prevalence of fungal isolates from cultured Seabream.

Fungal isolates	Prevalence	
	No.	%
A. niger	128	9.43
A. flavus	150	13.27
A. terreus	60	4.42
A. fumigatus	68	5.01
A. parasiticus	30	2.21
Total Aspergillus species	436	32.15
Paecilomyces species	104	7.66
Fusarium species	196	14.45
Ichthyophonus species	68	5.01
Aphanomyces invadans	12	0.88
Alternaria species	112	8.25
Cladosporium species	280	20.64
Helminthosporium species	8	0.58
Nigrospora species	32	2.35
Achlya species	12	0.88
Phomaherbarum	4	0.29
Legnadium	36	2.65
Exophiala	16	1.17
Geotrichum species	40	2.94
Total number of fungal isolates	1356	

Table 3: Prevalence of yeast isolates from cultured Seabream.

Yeast isolates	Prevalence	
	No.	%
Unidentified yeast	280	66.03
Rhodotorula species	36	8.49
Candida species	94	22.16
Cryptococcus species	4	0.94
Torulopsis species	10	2.36
Total number of yeast isolates	424	

6. Incidence of mycotic infections in different organs and tissue of cultured Seabream

Incidence of fungal infections from different organs and tissues of cultured Seabream were summarized in table 3.

Table 4: Incidence of fungal isolates in different organs and tissue of cultured Seabream.

Isolates	Fish organs and tissues					
	Gills	Musculature	Liver	Heart	Spleen	Kidney
A. niger	8	4	36	8	32	40
A. flavus	56	16	56	8	16	28
A. terreus	4	36	8	-	4	8
A. fumigatus	12	4	12	12	12	16
A. parasiticus	-	-	-	-	-	-
Paecilomyces species	40	4	4	4	12	40
Fusarium species	32	16	24	52	32	40
Ichthyophonus species	20	24	12	-	4	8
Aphanomyces invadans	8	-	-	-	-	4
Alternaria species	20	24	4	20	16	28
Cladosporium species	64	40	60	24	60	32
Helminthosporium species	-	4	4	-	-	-
Nigrospora species	-	-	4	8	8	12
Achlya species	8	-	-	-	-	4
Phomaherbarum	-	-	-	-	-	4
Legnadium species	4	-	4	20	4	4
Exophiala species	4	12	-	-	-	-
Geotrichum species	4	-	-	32	4	-
Total of moulds	284	184	228	188	204	268

Table 5: Incidence of yeast isolates in different organs and tissue of cultured Seabream.

Isolates	Fish organs and tissues					
	Gills	Mus culature	Liver	Heart	Spleen	Kidney
Candida species	36	20	-	20	16	12
Cryptococcus species	-	-	4	-	-	-
Rhodotorula species	4	-	-	-	-	32
Torulopsis species						
Unidentified yeast	4	32	96	40	100	8
Total of yeasts	44	52	120	40	116	52

7. Results of water quality parameters

The role of water quality on spreading of systemic mycotic affections in some cultured freshwater fish was studied in the present work.

Table 6: Results of water quality parameters.

Parameters	water	P. L
D.O.	4.4 mg / L	4-5
Ammonia (NH3)	0.31 mg / L	0.01
Nitrite (NO2)	0.023 mg / L	0.01
PH	8 – 8.3	7.8-8.3
Salinity	17 PPT	----------
Hardness	151 mg / L	160
Organic matter	5.55 mg / L	2-3

8. Levels of heavy metals in both fish tissues and water samples

8.1. Determination of levels of heavy metals in water samples

Table 7: Heavy metal concentrations (mg/l) in marine water of cultured seabream.

Parameters	water
Arsenic(As)	1.45
Mercury (Hg)	0.0249
Cadmium (Cd)	0.0969
Lead (Pb)	0.054
Zinc	0.195
Copper	0.76

8.2 Determination of levels of heavy metals in fish tissues

Table 8: Heavy metal concentrations (µg/g dry wt.) in musculature and liver of cultured seabream.

Heavy metals	Liver	Musculature
Arsenic(As)	1.75	1.258
Mercury (Hg)	1.025	0.90
Cadmium (Cd)	1.59	0.26
Lead (Pb)	2.364	0.82
Zinc (Zn)	123.16	53.72
Copper(Cu)	118.93	28.18

9. Conclusions

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 in naturally infected fishes, and the presence of fungal elements in the lesions. This should direct our attention to the possible role of fungi in affecting fishes industry.

10. References

1. Roberts RJ. Fish Pathology, Edn 3, W. B. Saunders, Philadelphia, PA, 2001.
2. Lightner D, Redman RM, Mohney L, Sinski J, Priest D. A renal mycosis of an adult hybrid red tilapia, *Oreochromis mossambicus* and *O. hornorum*, caused by the imperfect fungus, Paecilomyces marquandii. J Fish Dis 1988; 1(1):437-440.
3. Pickering AD, Willoughby LG. Saprolegnia infections of salmonid fish. In Microbial Diseases of Fish, Edit Roberts, RJ Academic Press, London, 1982, 271–298.
4. Roberts RJ. The mycology of teleosts, in Roberts RJ (ed). Fish Pathology, Edn 2, London, England, Baillere Tindall, 1989, 320-336
5. Quiniou SMA, Bigler S, Clem LW. Effects of water temperature on mucous cell distribution in channel catfish epidermis: a factor in winter saprolegniasis. Fish Shellf Immunol 1998; 8:1-11.
6. Pickering AD, Christie P. Sexual differences in the incidence and severity of ectoparasitic infestation of the brown trout, Salmo trutta L. J Fish Biol 1980; 16:669–683.
7. Walser CA, Phelps RP. The use of formalin and iodine to control Saprolegnia infections on channel catfish, Ictalurus punctatus, eggs. J Appl Aquacult 1993; 3:269–278.
8. Salem AA, Refai MK, Eissa IAM, Marzouk M, Bakir A, Moustafa M, Manal Adel. Some studies on aspergillomycosis in Tilapia nilotica. Zagazig Vet J 1989; 17(3):315-328.
9. Bisht D, Bisht GS, Khulbe RD. Fusarium: A new threat to fish population in reservoirs of Kumaun India. Curr Sci 2000; 78(10):1241-1245.
10. Faisal M, Torky H, Reichenbach-Klinke HH. A note on swimming disease among the Labyrinth catfish (Clarias lazera). J Egypt Assiut Vet Med 1985; 45:53-60.
11. Easa M. EI-S, Hatem ME, Sark EE, Refai M. Phomaherbarum as a mycotic fish pathogen in Clarias lazera, Armout catfish. Vet Med J 1984; 32:257- 267.
12. Hatai K, Fujimaki Y, Egusa S. A visceral mycosis in ayu fry, Pleoglossus altivelis Temminck & Schlegel, caused by a species of Phoma. J Fish Dis 1986; 9:111-116.
13. Khalil RH, Safinaz G, Mohmad N, Mahfouz El-Banna S, Soliman MK. A New Fungal Disease in Cultured Monosex Oreochromis Niloticus Caused By Paecilomyces Marquandii. Issn 110-2047. Alex J Vet Science 2004; 21(1).
14. Langdon JS, MacDonald WL. Cranial Exophiala piscphilia infection in Salmo salar in Australia. Bull Eur Ass Fish Pathol 1987; 7:35-37.
15. Ellis AE, Waddell IF, Minter DW. A systematic fungal disease in Atlantic salmon parr, Salmo salar L., caused by a species of Phialophora. J Fish Dis 1983; 6:511-523.
16. Wolf JC, Smith SA. Systemic zygomycosis in farmed tilapia fish. J Comp Pathol 1999; 121(3):301-306.
17. Neish GA, Hughes GC. Diseases of fishes, Book 6. Fungal diseases of fish. TFH Publications, Neptune, Ng, 1980; 1159.
18. Amlacher E. Textbook of fish diseases. TES Publication New Jersey USA P, 1970, 117-135.
19. Austin B, Austin DA. Bacterial Fish pathogens: Diseases in Farmed and Wild fish. Ellis Harwood limited England, 1987, 250-262.
20. Conroy DA, Herman LR. Text book of fish diseases. TFH

publ West Sylvania, 1981.
21. Plumb JA, Bowser PR. A laboratory Manual of Microbial fish Diseases. Auburn Univ. Auburn Alabama 1982, 77.
22. Whitman KA. Finfish and Shellfish Bacteriology Manual; Techniques and Procedures. ISBN 0-8138-1952-0 – Iowa State Press, 2004.
23. Feingold SM, Baron EJ. Bailey and Scoll's Diagnostic Microbiology. The Cv. Mosby co., St. Louis, 1986.
24. Collins CH, Lyne PM. Microbiological Methods. Edn 5, Butterworth's & Co. Publishers, Ltd, 1984.
25. Ellis MB. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute: Kew Surrey, UK, 1971.
26. Raper KB, Fennell DI. The Genus Aspergillus. Williams and Wilkins, Baltimore, 1965.
27. Moss ES, McQuown AL. Atlas of medical mycology. Edn 3, Baltimore: The Williams and Wikins Company, 1969, 366.
28. Larone DH. Medically Important Fungi. A Guide to Identification, 3rd ed. Harper and Row Publishers, New York, 1976, 68, 73 and 102.
29. Frey D, Old-Field RJ, Bridjer RC. A colour atlas of pathogenic fungi. Wolfe Medical Public Ltd Holland, 1979.
30. Refai M. Isolation and identification of fungi. Fac Vet Mid Cairo University, 1987.
31. Cruickshank R, Duguid JP, Marimion BP, Swain RH. Medical microbiology. Edn 12, Vol. 11: The practice of medical microbiology. Churchill Livingstone, Edinburgh, London, 1982.
32. Terrence CD. A practical approach to identification of yeast like organisms. Amer. Jour Microbiol 1971; 35(5):580-590.
33. Larone, DH. Medically Important Fungi. A Guide to Identification, Edn 2, Washington, DC, 1987.
34. APHA (American Public Health Association). Standard Methods for the Examination of Water and Wastewater. 19th Edn., Washington DC, 1995.
35. Clesceri LS. Standard methods for the examination of water and waste water. In Collection and Preservation of Samples and Metals (Eds Arnold E, Greenbergy and Eaton AD), APHA, AWWA, WEF, Washington, DC, 1998.
36. AOAC (Association of Official Analytical Chemists). "Official Methods for Minerals in Plants. Method 975.03. B.b." In: Official Methods of Analysis of AOAC International, Edn 16, Vol 1, Arlington (Va.): AOAC International Section 1996; 3:3-4.
37. WHO, World Health Organization. Guidelines for drinking water quality. Geneva 1984, 111.
38. FAO. Water Quality for Agriculture. Irrigation and Drainage Paper No. 29, Rev. 1. Food and Agriculture Organization of the United Nations, Rome, 1985.
39. EOSQC. Egyptian organization for standardization and Quality control. 2760- 2005. Physical and chemical methods for testing fish and fishery products. Part 5: Crustacea and Mollusca, Egyptian Organization for Standardization and Quality Control. U.D.C.: 637/ 664-2005 Arab Republic, 2005.
40. FAO/WHO. Codex alimentarius commission, standard programme codex committee on food additives and contaminates. 24th Session, Hague, 23- 28 March, 1992.
41. Food Stuff. Cosmetics and Disinfectants–Act No. 54-Regulation No. R2064 Marin Food Government Gazett Governorate Particular, Pretoria, 1972.
42. FAO/WHO. List of maximum levels recommended for contaminants by the Joint FAO/ WHO Codex Alimentarius Commission. Second Series. CAC/FAL, Rome, 1984; 3:1–8.
43. Marzouk MS, Samira SR, El-Gamal MH. Mycological investigations on cultured Tilapia in Kafer El- Sheikh Governorate. Kafer El-Sheikh Vet Med J 2003; 1(2):97-114.
44. Refai M, Abdel halim MM, Afify MMH, Youssef H, Marzouk KM. Studies on aspergillomycosis in catfish (*Clarias Lazera*). Allgemeine Pathologic and pathologische Anatomic. Tagung der Deutachen Veterinar - Medizinischen Gesellschaft. der Europeischen Gesellschaft fur. Vet Pathol 1987; 63:1-12.
45. Shaheen AA. Mycoflora of some freshwater fish. MV Sc. Thesis, Fac. Vet. Med., Zagazic Univ, 1986.
46. Refai MS Attia R, Salem M, El-Dahshan EM. Studies on the pathogenicity of *Aspergillus fumigatus*, *A. flavus* and *A. niger* isolated from chickens and their environment. *Egypt.* J Comp Path Clinic Path 2004; 17(2):193-205.