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Mycobiota associated with some Red Sea fish, shellfish and their environment at Hurghada, Egypt

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Abstract: Red Sea fish and shellfish (120 samples) representing 10 species were collected and reared in a contaminated earthen pond at National Institute of Oceanography and Fisheries (NIOF), Hurghada to simulate contaminated aquaculture conditions. The mycobiota associated with fish samples and their environment were examined using two isolation media at 28 °C during the period from October 2014 to September 2015. Twenty-six species related to 11 fungal genera were isolated from the examined fish samples on both media. The most common fungal genera on fishes were; *Aspergillus, Penicillium* and *Purpureocillium*. From the above genera the most predominant species were: *A. flavus, A. fumigatus, A. niger, A. sydowii, A. terreus, A. versicolor, P. aurantiogriseum* and *Purpureocillium lilacinum*. *Cladosporium* and *Exophiala* were isolated from some fish and shellfish in the following descending order; *E. xenobiotica, C. sphaerospermum, C. antarcticum, C. velox, C. colombiae* and *E. salmonis*. Fish skin and gills yielded relatively higher fungal count than liver. Ten and 14 species belonging to 2 and 8 genera were isolated from earthen pond water and sediment samples, respectively. Fungal isolation showed a correlation between species isolated from water and sediments and those isolated from fish and shellfish especially for *Aspergillus* species. Otherwise *Penicillium donkii*, *Fusarium solani* and *Acremonium strictum* were recorded only from sediments. All of the 25 isolates tested for enzyme production were positive for lipase, whereas, 16 (64%) isolates exhibited different capabilities of proteolysis.

Keywords: fungi, Red Sea, fish, shellfish.

Introduction

The Red Sea is characterized by warm water with temperatures ranging from 21 to 30 °C (Hawkins and Roberts 1994). It is one of the most important repositories of marine biodiversity in the world. It has an extraordinary range of biological diversity and endemism (Lieske and Myers 2004). It is one of the most saline water masses with a mean surface salinity of 42.5 ppt (Sofianos *et al.* 2002). Fungi are universally distributed, occurring from arctic to tropical waters in temporary pools to the deep-sea where, they tolerate extreme conditions of temperature, hydrostatic pressure and nutrient availability (Wong *et al.* 1998). Common fungal species isolated from infected eggs and brood stock of rainbow trout were *Penicillium*, *Aspergillus*, *Alternaria*, *Acremonium*, *Fusarium*, *Cladosporium*, *Mucor* and *Saprolegnia* sp. (Shahbazain *et al.* 2010, Fadaeifard *et al.* 2011). Also, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillum* and *Rhizopus* were isolated from infected ornamental freshwater fishes (Refai *et al.* 2010, Zafar and Rabia 2013). Gold fish challenged with *Aspergillus niger* (Chauhan 2013) and *Labeo calbasu* fish with *A. flavus* and *A. terreus* (Chauhan *et al.* 2014). *Achlya*, *Aspergillus*, *Penicillium*, *Acremonium*, *Fusarium*, *Sepedonium*, *Alternaria*, *Rhizopus* and *Cladosporium* spp. were isolated from Salmon fish (Gholampour *et al.* 2014). *Aspergillus*, *Blastomyces* and *Pencillium* spp. were isolated from control and infected *Channa punctatus* and *C. striatus* (Koteshwar and Benarjee 2015) and *Aspergillus*, *Blastomyces*, *Penicillium* and *Rhizopus* sp. were also obtained from carp fish (Ali 2015).

In Egypt, numerous genera and species were isolated from different fish species. In this respect, *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus*, *Mucor*, *Cladosporium*, *Phoma*, *Trichoderma* and *Alternaria* were the common terrestrial fungi on River Nile Tilapia fish (Badran 1989). Also *Saprolegnia*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, *Scopulariopsis*, *Paeciliomyces* and *Curvularia* were isolated from *Oreochromis* sp. and *Clarias gariepinus* fish species (Salem *et al*. 1989). Fungal species such as *A. niger*, *A. fumigatus* and *Penicillium* sp. were associated with freshwater fish (Abd El-Mageed 2002), Recently, *Aspergillus*, *Cladosporium* and *Fusarium* were obtained from *Gilthead seabream* and *Sparus aurata* (Abdel-Latif *et al.* 2015).

A revision of all available data sources in Egypt reveals that the total number of marine and aquatic fungi known is 207 taxa (87 Ascomycota, 117 anamorphic taxa, and 3 Basidiomycota) (Abdel-Azeem 2010).

Extracellular fungal enzymes are highly important in providing soluble and readily absorbable nutrients from the environment, and in promoting the penetration of both plant and animal tissues (Cole and Hoch 1991, St Leger *et al.* 1986).

The aim of this work was to study the mycobiota associated with fish and shellfish. The

fungi inhabiting fish's environment (water and sediment) were also assessed during the period from October 2014 to September 2015. Also, the capabilities of commonly isolated fungi for lipase and protease production were determined.

Materials and Methods

Fish and shellfish sampling

A total of 120 samples of Red Sea fish (102) and shellfish (18) representing ten species were collected during the period from October 2014 to September 2015. Samples were taken from polluted earthen pond (10 m L, 4 m W and 1.5 m d) at the National Institute of Oceanography and Fisheries (NIOF), Hurghada (5 samples each 2 weeks). Water and sediment samples (one sample each 2 weeks) were also tested (Table1). All samples were mycologically analyzed during the period of the study.

Physico-Chemical analysis of the earthen pond water

Earthen pond temperature, pH, total dissolved solid (TDS) and conductivity were determined seasonally. Also total organic carbon (TOC) was measured directly in water using a total organic carbon analyzer (TOC-V CSN, Shimadzu Corporation, Japan), Nitrite (NO_2^- - N) was determined by colorimetric method, Nitrate (NO_3^- - N) with ultraviolet spectrophotometric method, Ammonia (NH_4^+– N) by Pant method and orthophosphate was determined spectrophotometrically according to APHA (2005).

Isolation of fungi from fish samples

Fish samples were surface sterilized by dipping in 70% alcohol for 3 min and washing with sterilized distilled water several times. Segments were taken from the skin, gills and liver (3 segments each) with a sterile needle and inoculated onto agar plates (3 plates each). Potato Dextrose agar (PDA) (potato extract, 4.0 g; Dextrose, 20.0 g; Agar, 20.0 g; seawater 1,000 ml) and Peptone Yeast Extract Sea Water agar (PYGS) (0.125% peptone, 0.125% yeast extract, 3% glucose and 1.2% agar in seawater) were used. To avoid bacterial contamination 500 mg/l chlormenphicol were incorporated in media. Cultures were incubated at 28°C and observed after 7-14 days for fungal development. The growing fungi were isolated, identified and the total count was calculated per 9 segments for each sample.

Isolation of fungi from water and sediments

One ml of sea water and approximately 0.1 g of sediments were diluted with sterile seawater and vortexed for 1 min. Aliquots of 0.1 ml were spread on the surface of the isolation media distributed in 9 cm plates. After incubation at 28°C for 7 days, the growing fungi were isolated and identified according to the keys of Raper and Fennell (1965), Pitt (1979), Moubasher (1993), Pitt and Hocking (2004), De Hoog *et al.* (2000) and Colin *et al.* (2012).

Molecular identification

Sequencing analysis of the ITS region of rRNA gene was performed at Solgent Co., Ltd Bio industry development site, South Korea. Two universal primers (ITS1: CTTGGTCATTTAGAGGAAGTAA and ITS4: TCCTCCGCTTATTGATATGC) were used for sequencing. Sequences obtained were compared with the available sequences at the National Center for Biotechnology Information (NCBI). Neighbor-joining tree of the isolates and close taxa was constructed after alignment with the aid of MegAlign DNASTAR software version 5.01 (Altschul *et al.* 1997).

Screening for protease and lipase

Lipase production of fungal isolates was determined according to Hankin and Anagnostakis (1975) using Tween 80 in the test medium. Proteolytic activity was performed using casein-agar cup plate clearing zone assay (Badran1989, Cowan and Steel 1993).

Results and Discussion

1. Fungi isolated from fish and shellfish

Using PDA and PYGS media, 26 species related to 11 genera were isolated from the examined fish samples. The genera of high occurrence and their respective numbers (as percentages to gross total count) isolated from the tested samples were *Aspergillus* (89.7%), *Penicillium* (3.4%) followed by *Cladosporium* (3.1%) and *Exophiala* (1.45%). *Fusarium* (0.8%), *Pythium* (0.6%), *Nigrospora* (0.3%), *Stemphylium* (0.2%), *Rhizoctonia* (0.15%), *Alternaria* (0.12%) and *Pseudallescheria* (0.06%) were isolated in low frequency (Table 2). On PDA, the total counts fluctuated between 134-241 colonies/9 segments of skin samples; 117 – 219 of gills and 102 – 156 of liver samples. The highest total count was recorded during spring and winter. Also, total counts of fungi on PYGS varied from 49 - 194 colonies/9 segments of skin; 49 – 188 of gills and 69 – 107 of liver, showing their peaks during spring and autumn (Table 2 and Fig. 1).

In this respect, many conidial fungi were reported to be associated with fish diseases. Some of these fungi are *Aspergillus* (Salem *et al.* 1989) and *Fusarium* (Bisht *et al.* 2000). Refai *et al.* (2010) isolated *Aspergillus, Fusarium, Mucor* and *Penicillum* from infected freshwater ornamental fishes. Zafar and Rabia (2013) reported that *Aspergillus* spp. were the most prevalent fungi in Black moor fish followed by *Alternaria* sp., *Mucor* sp. and *Penicillium* sp. Zafar *et al.* (2014) isolated *Aspergillus* most commonly, and *Rhizopus*, *Penicillium* and *Mucor* less commonly from infected freshwater silver carp fish.

Aspergillus was the most common genus isolated from Red Sea fish and shellfish on PDA and PYGS. It was represented by 11 species of which *A. versicolor* (727 and 211 colonies/9 segments, each sample); *A. flavus* (472 and 408); *A. sydowii* (265 and 54); *A. niger* (196 and 105) and *A. terreus* (90 and 133) were the most prevalent species on the two isolation media respectively (Table 2). They occurred during most seasons. The remaining *Aspergillus* species were less common isolated from one or two seasons (Table 2).

In this instance, Olufemi *et al.* (1983) reported that *Aspergillus* spp. are capable of causing systemic disease in cultured Tilapia. Also, Olufemi (1986) postulated that *A. flavus* caused serious aspergillomycosis to cultured *Tilapia* in Scotland. The first record of *Aspergillus terreus* as fish pathogen was reported by Bhattacharya *et al.* (1988a). *A. niger* was also recorded to infect two species of Asian freshwater catfishes inducing hemorrhagic ulcer like patches on the gills and skin (Bhattacharya 1988b). *A. niger* was also identified from visceral lesions of tilapia cultured in Kenya and exhibiting Aspergillomycosis symptoms Paperna (1996). *A. flavus* and *A. terreus* were found in combination on *Labeo calbasu* fish (Chauhan *et al.* 2014). More recently *Aspergillus* was the most prevalent fungus infecting all the organs of *Channa punctatus* and *C. striatus* fish with severe infection during October, September, December and January (Koteshwar and Benarjee 2015). Gholampour *et al* (2014) isolated 11 fungal genera from Salmon fish and *Aspergillus* was the most prevalent. Ali (2015) isolated five fungal genera from different species of carps fish including *Aspergillus*. Abdel-Latif *et al.* (2015) recorded that 80% of the examined Gilthead seabream and *Sparus aurata* fish cultured at Marriott Lake showed mycotic infection and *Aspergillus* species were prevalent.

The second higher incidence rate was represented by *Penicillium aurantiogriseum* which shared with 1.9% of the total fungal population. It occurred in autumn and winter on PDA and PYGS with total counts of 28 colonies/9 segments, each sample and 35 colonies on the two media, respectively. *Purpureocillium lilacinum* was less common on the two types of isolation media (8 and 42 colonies, respectively) (Table 2).

Reichenbach (1956) described *Penicillium* species as a parasite of internal organs of several freshwater fish notably *Loricaria parva*. Blaylock *et al.* (2001) reported that wild-caught, tank-held red snapper fish was infected by *P. corylophilum* in Swim bladder. Jalilpoor *et al.* (2006) reported infection of *Aspencer percicus* eggs with *Penicillium* spp. However, Refai *et al.* (2010) has characterized *Penicillium* sp. as opportunistic pathogens as many of them possess virulence factors which enable them to cause disease, especially under favorable predisposing conditions. Shahbazain *et al.* (2010) isolated *Penicillium* sp. from infected eggs of rainbow trout. Fadaeifard *et al.* (2011) reported the occurrence *Penicillium* from the eggs and brood stock of rainbow trout. Abolude *et al.* (2013) reported that *Penicillium* sp. was associated with eggs and Broodstock African Catfish with 23% occurrence. *Penicillium* spp. were isolated from infected black moor fish (Zafar and Rabia 2013) and less commonly from silver carp fish (Zafar *et al.* 2014) and were represented by 20% of species from carps fish Ali (2015). Koteshwar and Benarjee (2015) found *Channa punctatus* and *C. striatus* fish infected with *P. chrysogenum* during the period from July up to February in case of C. *punctatus* and from July to January on *C. striatus*.

Cladosporium was represented by 103 colonies matching 3.1% of total of fungi. It occurred in three seasons on PDA showing the highest count in autumn of 2014. From the five species identified C. *antarcticum*, *C. sphaerospermum*, *C. velox* were the most common (Table 2). Some species of *Cladosporium* were reported to be associated with fish diseases (Bocklisch and Otto 2000). Infection with *Cladosporium* sp. caused a deep dermal ulcer that extended to bone in a cultured tomato clownfish, *Amphiprion frenatus* and tank-held red snapper, *Lutjanus campechanus* was infected with *C. sphaerospermum* (Blaylock *et al.* 2001). Silphaduang *et al.* (2000) reported *Cladosporium* sp. from infected Tomato clownfish and Fadaeifard *et al.* (2011) reported the occurrence of *Cladosporium* species from the eggs and brood stock of rainbow trout. Gholampour *et al.* (2014) isolated *Cladosporium* sp. from Salmon fish Abdel-Latif *et al.* (2015) recorded *Cladosporium* sp. from examined Gilthead seabream (*Sparus aurata*) fish cultured at Marriott Lake.

As shown the Table (2), *Exophiala* was represented by *Exophiala xenobiotica* (12 isolates) on PDA medium and *E. xenobiotica* (28 isolates) *E. salmonis* (8) on PYGS medium. Fungal infections caused by the genus *Exophiala* has been reported in several species of fish. The first report was *Exophiala salmonis* from cutthroat trout, *Salmo clarki* and lake salmon, *Salvelinus namaycush* Carmichael (1966). *E. pisciphilus* was reported from channel catfish, *Ictalurus punctatus* (Fijan 1969) and from smooth dogfish (Gaskins and Cheung 1986). *E. salmonis* infection was also reported from Atlantic salmon, *Salmo salar* (Richard *et al.* 1978; Otis and Wolke, 1985). *E. psychrophila* was isolated from Atlantic salmon Pedersen and Langvad, (1989). A novel species of *Exophiala* was reported from leafy seadragons *Phycodurus eques* and weedy seadragons, *Phyllopteryx, taeniolatus* during the period from January 2002 to March 2007, namely *Exophiala angulospora* (Bonar *et al.* 2013). *Exophiala* infection was also observed in Japanese flounder, *Paralichthys olivaceus* in Japan (Kurata *et al.* 2008). *Exophiala* infection occurred in cultured striped jack, *Pseudocaranx dentex* (Munchan *et al.* 2009). *Exophiala angulospora* caused severe disease

and mortality in Atlantic halibut, *Hippoglossus hippoglossus* Overy *et al.* (2015). In the present study the fungus was classified into the genus *Exophiala* based on its morphology and as *Exophiala xenobiotica* based on sequences of the ITS1 5.8S-ITS2 regions of rDNA.

The remaining genera (represented each by one species) were of rare frequency of occurrence. They collectively accounted for 2.11% of total fungi (Table 2). Bian and Egusa (1981) recorded that *Fusarium solani* caused black gill disease of crustaceans, a problem in marine shrimp ponds. *Fusarium* infection was also found in cultured red sea bream, *Pagrus major*, in Japan (Hatai *et al*. 1986). Black tiger shrimp, *Penaeus monodon* infected with *Fusarium incarnatum* showed typical signs of black gill disease and mortalities about a month prior to harvest (Khoa *et al.* 2004). Zafar and Rabia (2013) reported *Alternaria* sp. from Black moor fish. Abdel-Latif *et al.* (2015) recorded *Fusarium* species from infected gilthead seabream (*Sparus aurata*) fish cultured at Marriott Lake. Gholampour *et al.* (2014) isolated *Alternaria* from Salmon fish.

Most of *Cladosporium* and *Exopiala* species were identified by molecular sequence analysis of the internal transcript spacer (ITS) region of the ribosomal RNA gene and compared with morphology (Table 3 and Fig. 2).

Table1: Names and numbers of fish and shellfish samples tested during the period from October 2014 to September 2015.

Type	English name	Scientific name	Number
Fish	Broomtail wrasse	*Cheilinus lunulatus* (Forsskål, 1775)	31
	Rabbit fish	*Siganus rivulatus* (Forsskål, 1775)	20
	Doublebar bream	*Acanthopagrus bifasciatus* (Forsskål, 1775)	17
	Blacktip mojarra	*Gerres oyena* (Forsskål, 1775)	11
	Picnic sea bream	*Acanthopagrus berala* (Forsskål, 1775)	8
	Blacktip grouper	*Epinephelus fasciatus* (Forsskål, 1775)	7
	Klunzinger's wrasse	*Thalassoma rueppellii* (Klunzinger, 1871)	3
	Sergeant major	*Abudefduf saxatilis* (Linnaeus, 1758)	3
	Crocodile fish	*Cymbacephalus beauforti* (L. W. Knapp, 1973)	2
Shellfish	Red Sea shrimp	*Litopenaeus vannamei* (Boone, 1931)	18
Total			120

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Table 2: Seasonal fluctuation of total count (calculated per 9 segments, each sample) of fungi isolated from Red Sea fish and shellfish samples during the period from October 2014 to September 2015 on PDA and PYGS media at 28°C.

Media	PDA													PYGS										
Season	Autumn			Winter			Spring			Summer			Total	Autumn			Winter			Spring			Summer			Total
Genus & Species	Skin	Gills	Liver	Skin	Gills	Liver	Skin	Gills	Liver	Skin	Gills	Liver	count	Skin	Gills	Liver	Skin	Gills	Liver	Skin	Gills	Liver	Skin	Gills	Liver	count
Alternaria alternata	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	2	0	0	0	0	2
Aspergillus	189	147	110	235	214	155	112	115	91	181	198	123	1870	181	183	98	94	67	49	47	37	51	74	134	86	1101
A. flavus	40	34	46	30	63	23	10	5	4	83	96	38	472	29	26	28	61	41	19	22	4	3	54	87	34	408
A. fumigatus	0	0	0	0	0	8	0	0	0	0	15	0	23	0	0	7	0	0	0	0	0	0	0	0	0	7
A. niger	11	0	5	20	53	10	0	8	2	4	41	42	196	6	0	4	15	10	6	0	14	7	10	15	18	105
A. ochraceus	0	7	0	0	0	0	0	0	2	0	0	0	9	7	0	0	0	0	0	0	0	7	0	0	0	14
A. oryzae	0	4	0	7	0	0	0	0	2	0	0	0	13	0	10	0	0	0	7	0	0	0	0	0	0	17
A. sulphureus	5	4	5	3	11	0	0	0	2	0	0	0	30	23	31	9	8	4	9	0	0	0	0	0	0	84
A. sydowii	62	49	17	42	14	0	23	21	9	10	12	6	265	12	32	10	0	0	0	0	0	0	0	0	0	54
A. tamarii	0	0	0	0	0	0	5	0	0	4	19	9	37	0	0	0	0	0	0	10	6	0	10	22	20	68
A. terreus	24	8		7	3	2	4	10	14	5	6	7	90	11	6	0	10	12	8	15	13	34	0	10	14	133
A. versicolor	47	41	37	126	70	112	70	63	56	75	9	21	727	93	78	40	0	0	0	0	0	0	0	0	0	211
A. wentii	0	0	0	0	0	0	0	8	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladosporium	0	41	16	0	0	0	9	2	4	6	7	7	92	0	0	0	0	0	0	0	0	0	4	0	7	11
C. antarcticum	0	12	0	0	0	0	6	2	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
C. colombiae	0	8	7	0	0	0	0	0	0	0	0	7	22	0	0	0	0	0	0	0	0	0	4	0	0	4
C. halotolerans	0	4	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0
C. sphaerospermum	0	14	0	0	0	0	0	0	0	0	7	0	21	0	0	0	0	0	0	0	0	0	0	0	7	7
C. velox	0	3	9	0	0	0	0	0	0	6	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladosporium sp.	0	0	0	0	0	0	3	0	4	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2: Continued.

Media	PDA													PYGS										
Season	Autumn			Winter			Spring			Summer			Total	Autumn			Winter			Spring			Summer			Total
Genus & Species	Skin	Gills	Liver	Skin	Gills	Liver	Skin	Gills	Liver	Skin	Gills	Liver	count	Skin	Gills	Liver	Skin	Gills	Liver	Skin	Gills	Liver	Skin	Gills	Liver	count
Exophiala	0	0	0	0	0	0	0	0	0	12	0	0	12	0	0	6	0	1	21	0	1	6	0	0	1	36
E. salmonis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	1	0	0	1	0	0	0	8
E. xenobiotica	0	0	0	0	0	0	0	0	0	12	0	0	12	0	0	0	0	1	20	0	1	5	0	0	1	28
Fusarium oxysporum	6	0	0	0	0	0	0	0	0	0	0	0	6	4	0	0	0	17	0	0	0	0	0	0	0	21
Nigrospora sphaerica	0	0	0	0	0	0	10	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium aurantiogriseum	10	7	0	6	5	0	0	0	0	0	0	0	28	9	5	3	12	6	0	0	0	0	0	0	0	35
Purpureocillium lilacinum	0	0	0	0	0	0	0	0	7	1	0	0	8	0	0	0	27	12	0	0	0	0	3	0	0	42
***Pseudallescheria* sp.**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2
***Pythium* sp.**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	12	0	0	0	21
Rhizoctonia solani	0	0	0	0	0	0	3	0	0	2	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0
Stemphylium botryosum	5	0	0	0	0	0	0	0	0	2	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	210	195	126	241	219	156	134	117	102	204	205	130	2039	194	188	107	133	103	70	49	49	69	81	134	94	1271
No. of genera = 11	5			2			5			7			9	4			4			5			4			8
No. of species = 26	16			9			15			15			25	7			10			10			8			20

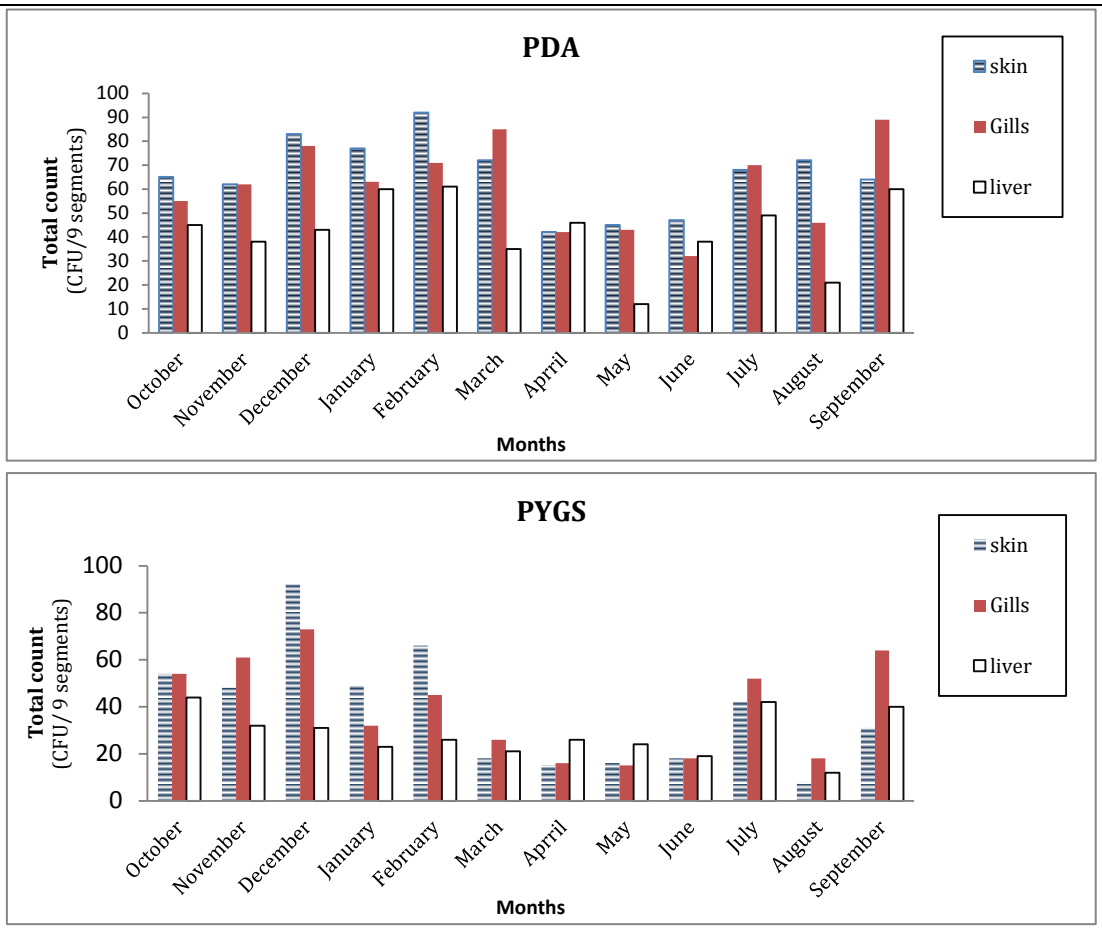

Figure 1: Monthly total count of fungi (CFU/9 segments, each sample) isolated from some Red Sea fish and shellfish during the period from October 2014 to September 2015 on PDA and PYGS media at 28°C.

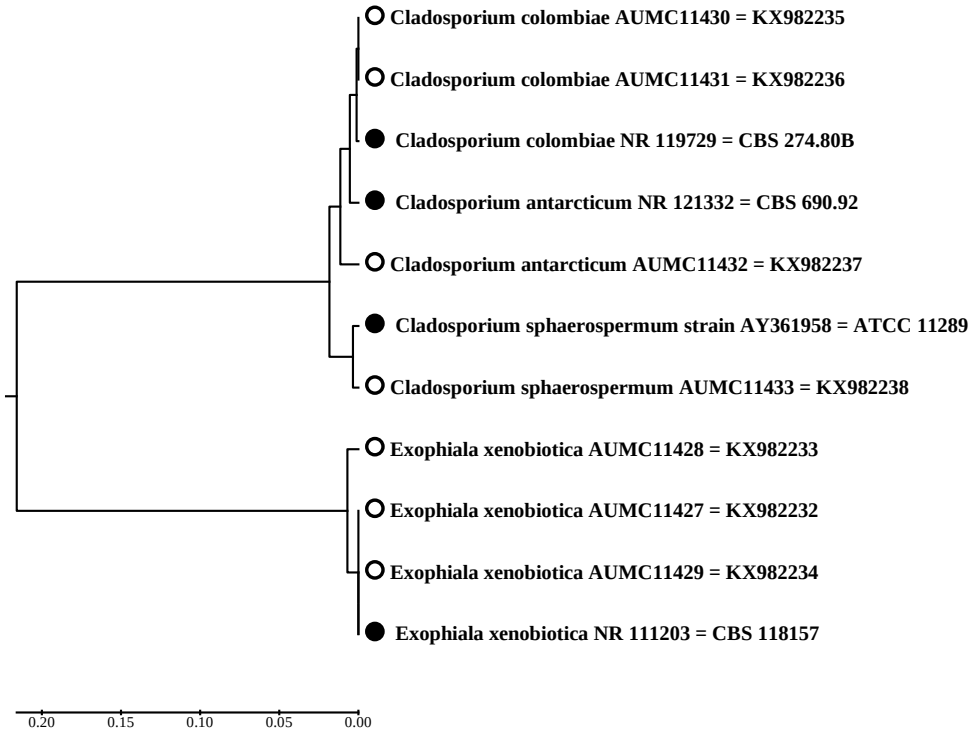

Figure 2: Phylogenetic tree based on ITS sequences of the isolated strains with the nearest species of the genera *Exophiala* and *Cladosporium*. Bar 0.05 substitutions per nucleotide position.

Table 3: ITS sequencing data of *Cladosporium* and *Exophiala* species isolated from some Red sea fish samples during the period from October 2014 to September 2015 on PDA and PYGS media at 28°C.

AUMC number	Isolation source	Isolation date (medium)	Accession GenBank number	Length (bp)	Closest Genbank match # ITS	Sequencing similarity (%)	Species identity
11427	*Cymbacephalus beauforti*	21-Oct-2015 (PYGS)	KX982232	568	NR_111203 =CBS118115^T	510/510 (100%)	*Exophiala xenobiotica*
11428	*Cymbacephalus beauforti*	21-Oct-2015 (PYGS)	KX982233	566	NR_111203 =CBS118115^T	503/513(98%)	*E. xenobiotica*
11429	*Cymbacephalus beauforti*	21-Oct-2015 (PYGS)	KX982234	566	NR_111203 =CBS118115^T	510/511(99%)	*E. xenobiotica*
11430	*Gerres oyena*	13-Jan-2015 (PDA)	KX982235	514	NR_119729 = CBS 274.80B^T	502/507(99%)	*Cladosporium colombiae*
11431	*Siganus rivulatus*	13-Jan-2015 (PDA)	KX982236	513	NR_119729 = CBS 274.80B^T	501/504(99%)	*C. colombiae*
11432	*Acanthopagrus bifasciatus*	17-Dec-2014 (PDA)	KX982237	515	NR_121332= CBS 690.92^T	485/487(99%)	*C. antarcticum*
11433	*Cheilinus lunulatus*	22-Feb-2014 (PDA)	KX982238	515	ATCC 11289= AY361958^T	491/492(99%)	*C. sphaerospermum*

2. Fungi isolated from water and sediment

The physico-chemical analysis of the earthen pond showet high values for total organic carbon (TOC), nitrate (NO_3-N), ammonia (NH_3-N), orthophosphate (PO_4-P) and low temperature than the surrounding Red Sea water during the period from October 2014 to September 2015. The highest temperatures were recorded from the earthen pond 27.8°C and from the Red Sea 33.12°C in summer while the lowest for the earthen pond 11.6°C and Red Sea 17.4°C was recorded during winter. The maximum values of electrical conductivity for the earthen pond 61.7 µmhos/cm^3 and Red Sea 62.2 µmhos/cm^3 were observed during spring, while the minimum for the earthen pond 45.8 µmhos/cm3 was determined during autumn and for the Red Sea 60.48 µmhos/cm^3 during summer. Values of pH always on the alkaline side, the maximum value for the earthen pond 9.1 was recorded during summer and the Red Sea 8.9 during autumn and the minimum for the earthen pond 7.8 and the Red Sea 8.1 were recorded during winter. Total organic carbon showed seasonal variations were the highest value for the earthen pond 75.8 ppm was observed during summer and for the Red Sea 57 ppm during winter and the lowest value for the earthen pond 42.4 ppm and Red Sea 33.5 ppm during autumn. Nitrate recorded high value for the earthen pond 11.05 ppm during autumn while the normal value observed on Red Sea 1.9 ppm during summer. The highest value of Ammonia NH_3-N 3.45 ppm was noticed during winter on the earthen pond whereas the normal concentration 0.9 ppm in the Red Sea. The highest O-phosphate 4 ppm was found during winter on the earthen pond while the normal value on the sea was 1 ppm (Table 5). Beltagi (1984) reported that the range of Red Sea water temperature was 19 -32°C and salinity was 39.38–39.97 ‰. Fahmy (2003) noticed that the temperature varied from 16.84 during January to 33.24 °C in July, and the salinity fluctuated from 38.99 to 40.22 ‰ with an annual mean of 39.80 ‰. Abdelmongy and El-Moselhy (2015) studied seasonal variations of the physical and chemical properties of the northern Red Sea water at the National Institute of Oceanography and Fisheries (NIOF) site the temperature ranging from 17.96 °C in winter to 31.34 °C in summer and salinity was 41.28 ppt in winter to 41.84 ppt in summer.

The pH is one of the vital environmental characteristics for the survival, metabolism, physiology; growth of aquatic organisms and chemical processes (Ramanathan *et al.* 2005). pH value of water is controlled by the dissolved oxygen, water temperature, sewage discharge, decomposition of organic matter and photosynthetic activities (Nassar and Hamed 2003). Fahmey (2003) found pH values of the Red Sea coastal water varied from 8.13 to 8.34 in September and January, respectively with an annual mean of 8.19. Abdelmongy and El-Moselhy (2015) recorded that the pH ranged from 7.93 in winter to 7.89 in summer at NIOF site.

Beltagi (1984) observed that phosphate concentrations in the Red Sea water ranged from 0.12 to 0.14 mM PO_4-P. Fahmey (2003) noticed that the annual average value of NH_4-N was 0.59 mM, Nitrite was 0.04 mM and always less than 0.1 mM and nitrate annual average was 0.39 mM. Abdelmongy and El-Moselhy (2015) observed that the mean value of ammonia was 2.38 mMl-1 and nitrite was 0.11 mMl-1 at NIOF site.

A total of 10 fungal species related to 2 genera were isolated from the earthen pond water on PDA and PYGs at 28 °C. Seasonally the total count fluctuated between 48 – 73 CFU/ml in winter and spring on PDA and from 10 – 63 CFU/ml in autumn and winter on PYGS. Fungal genera isolated from water were *Aspergillus* (98.7%) and *Stachybotrys* (1.3%). Of the 9 species of *Aspergillus*, *A. flavus*, *A. fumigatus*, *A. sydowii*, *A. terreus* and *A. versicolor* were the most prevalent species. The remaining species were less common. *Stachybotrys chartarum* was isolated only in winter on PYGS medium (Table5). Jaber *et al.* (2012) isolated *Aspergillus sydowii*, *A. wentii*, *A. flocculosus*, *Penicillium expansum* and *Eupenicillium javanicum* from sediments and waters of the Gulf of Aqaba, Red Sea.

From earthen pond sediment samples, 15 species appertaining to 8 genera were isolated on PDA and PYGS at 28 °C (Table 6). Seasonally, the total count on PDA fluctuated between 91 CFU/g in summer to 78 CFU/g in winter and on PYGS from 54 CFU/g in summer and winter to 34 CFU/g in autumn. Fungal genera isolated from sediment were *Aspergillus* (320 CFU/g matching 67.1%), *Acremonium* (22, 4.6%), *Alternaria* (27, 5.6%), *Exophiala* (31, 6.5%), *Fusarium* (25, 5.2%), *Geotrichum* (44, 7.3%), *Penicillium* (34, 7.1%) and *Stachybotrys* (17, 3.5%). A clear correlation was observed between species isolated from water (9 species) and sediment (8 species) and those isolated from fish and shellfish (11 species) especially for *Aspergillus*. *Alternaria* and *Exophiala* species. *Stachybotrys Chartarum* was isolated from water and sediment only and *Penicillium donkii*, *Fusarium solani* and *Acremonium strictum* were recorded on sediment only. Our results were generally similar to those reported by Khallil *et al.* (1991). They isolated terrestrial fungi from Red Sea mud samples, namely *Aspergillus*, *Penicillium*, *Cladosporium*, *Mucor* and *Scopulariopsis.* From the Red Sea coast, Abd-Elaah (1998) identified fungal genera such as *Aspergillus*, *Penicillium*, *Fusarium*, *Neurospora* and *Rhizopus* and noticed that *Aspergillus* was the most frequent genus, represented by seven species of which *A. niger*, *A. flavus* and *A. ustus* were the most common. Kutty *et al*. (2013) reported that *Penicillium* was occurred less frequently and was represented by two species, while *Fusarium* was infrequent and contributed four species. Occurrence of black yeasts in the slope sediments of the Bay of Bengal was very scanty and identified as *Hortaea werneckii* by Internal Transcribed Spacer (ITS) sequencing.

Screening for Protease and lipase production by fungi

Twenty-five fungal isolates were tested for their capabilities to produce lipase and protease enzymes. All isolates were capable for producing lipase but with different degrees. Only 6 isolates were high producers and these were *Aspergillus sulphureus*, *A. vesicular*, *A. ochraceus*, *A. oryzae*, *Fusarium oxysporum* and *F. subglutinans*. On the other hand 16 of 25 isolates were protease-producing also with different capabilities. Ten strains were high-producers related to *Aspergillus sydowii*, *A. versicolor A. terreus*, *A. oryzae*, *A. tamarii*, *Fusarium oxysporum*, *F. subglutinans*, *Geotrichum candidum*, *Pseudallescheria* sp. and *Nigrospora sphaerica* (Table 7).

According to several studies, penetration of fish and shellfish by mycopathogens is likely achieved by mechanical pressure Nyhlen and Unestam (1975), and, more importantly, the extracellular enzymatic activity of infection structures. However, because fungal invasion of host tissues is often achieved by the germinating spores, the extent to which these enzymes from these mycelial stages are involved in fish and shellfish pathogenesis is unclear and deserves further attention (Boucias and Pendland 1991).

In an investigation of Kudoa thyrsites infections in Pacific hake, *Merluccius productus*, the flesh softening was apparently caused by a proteolytic enzyme produced by the parasite (Tsuyuki *et al.* 1982). Parasite proteases are presumed to facilitate the invasion of host tissues, allow parasites to digest host proteins, help parasites evade the host immune response, and prevent blood coagulation (McKerrow 1989). Fungi which have ability to produce proteinase and phospholipase-like enzyme facilitate the adhesion and invasion in host tissue, because they can easily degrade cell membranes which are composed mainly of proteins and lipids (Cruz *et al.* 2011).

Table 4: Seasonal Physico-chemical analysis of the earthen pond and the Red Sea water during the period from October 2014 to September 2015

Parameter	Unit	Autumn (October2014)		Winter (December2014)		Spring (march 2015)		Summer (June 2015)	
		Pond	Sea	Pond	Sea	Pond	Sea	Pond	Sea
pH		6.87	8.9	7.8	8.1	8.1	8.5	9.1	8.63
Temp.	°C	25.9	28.9	11.57	17.4	21.6	32.6	27.8	33.12
TDS	ppm	28.33	30.5	29	30	31	32	30.4	30.25
Cond.	mS/cm	45.8	61.07	59.6	61	61.7	62.2	60.8	60.48
Sal.	%	28.3	40.9	39.9	41.2	14.3	41.6	40.9	40.58
TOC	ppm	42.4	30.55	146	57	48.7	46	75.8	52.3
NO_3	ppm	11.05	ND	3.55	ND	0.8	0.3	0.1	1.9
NH_4	ppm	3.45	2.4	0.05	0.95	0.15	0.21	2	1.25
O-phosphate	ppm	2.85	1	4.3	0.6	3.5	1.5	3.2	2.2

Table 5: Seasonal fluctuations of total count of fungi isolated from earthen pond water (CFU/ml) during the period from October 2014 to September 2015 on PDA and PYGs media at 28°C.

Media	PDA					PYGs				
Genus & species	Winter	Spring	Summer	Autumn	Total count	Winter	Spring	Summer	Autumn	Total count
Aspergillus	73	48	65	45	231	58	29	53	10	150
A. flavus	19	5	12	3	39	10	3	6	3	22
A. fumigatus	9	11	5	6	31	5	4	0	3	12
A. niger	4	2	2	4	12	12	1	0	0	13
A. ochraceus	7	5	3	6	21	5	2	0	2	9
A. oryzae	0	2	3	3	8	4	4	7	0	15
A. sulphureus	0-	0	4	2	6	5	3	10	0	18
A. sydowii	10	6	15	5	36	6	3	8	0	17
A. terreus	19	7	16	9	51	6	2	7	2	17
A. versicolor	5	10	5	7	27	5	7	15	0	27
Stachybotrys chartarum	0	0	0	0	0	5	0	0	0	5
Total	73	48	65	55	231	63	29	53	10	155
No. of genera =2	1	1	1	1	1	2	1	1	1	2
No. of species = 10	7	8	9	9	9	10	9	6	4	10

Table 6: Seasonal fluctuation of total count of fungi isolated from earthen pond sediment (CFU/g) during the period from October 2014 to September 2015 on PDA and PYGs media at 28°C.

Media	PDA					PYGS				
Genus & species	Winter	Spring	Summer	Autumn	Total count	Winter	Spring	Summer	Autumn	Total count
Acremonium strictum	0	4	5	3	12	5	0	5	0	10
Alternaria alternata	0	0	2	5	7	3	2	6	9	20
Aspergillus	56	72	63	50	241	23	20	20	16	79
A. flavus	10	18	26	16	37	10	5	15	5	35
A. fumigatus	4	9	3	5	21	0	4	0	0	4
A. niger	9	3	5	6	23	3	0	0	2	5
A. ochraceus	3	7	5	6	21	0	0	0	4	4
A. oryzae	2	4	3	4	13	0	0	0	5	5
A. sydawii	7	9	6	2	24	0	5	3	0	8
A. terreus	11	6	9	3	29	5	0	2	0	7
A. versicolor	10	16	6	8	40	5	6	0	0	11
Exophiala salmonis	0	0	2	9	11	2	6	12	0	20
Fusarium solani	6	6	2	5	19	0	6	0	0	6
Geotrichum candidum	5	0	0	9	14	12	3	6	9	30
Penicillium donkii	8	2	15	4	29	0	0	5	0	5
Stachybotrys chartarum	3	2	2	1	8	9	0	0	0	9
Total	78	86	91	86	341	54	37	54	34	179
No. of genera = 8	5	5	7	8	8	6	5	6	3	8
No. of species = 15	12	12	14	15	15	9	8	8	6	15

Table 7: Protease and lipase production (mean diameters of clear zones in cm) by fungi isolated from the Red sea fish and shellfish samples during the period from October 2014 to September 2015 on PDA and PYGS media at 28°C.

Fungi species	Lipase	Index	Protease	Index
Aspergillus flavus	0.43	L	0	0
A. fumigatus	0. 3	L	0.47	L
A. niger	0.3	L	0	0
A. ochracceus	0.67	H	0	0
A. oryzae	0.67	H	1.37	H
A. sulphureus	0.97	H	0.4	L
A. sydowii	0.43	L	0.7	H
A. tamarii	0.4	L	0.9	H
A. terreus	0.22	L	0.85	H
A. versicolor	0.67	H	0.6	H
A. wentii	0.5	M	0.3	L
Cladosporium colombiae	0.17	L	0.3	0
C. halotolerans	0.17	L	0	0
C. sphaerospermum	0.07	L	0	0
C. velox	0.1	L	0	0
Exophiala xenobiotica	0.18	L	0	0
E. xenobiotica	0.1	L	0	0
Fusarium oxysporum	0.7	H	0.9	H
F. subglutinans	0.67	H	1.07	H
Geotrichum candidum	0.4	L	0.6	H
Nigrospora sphaerica	0.37	L	1.2	H
Stachybotrys chartarum	0.2	L	0. 3	L
Penicillium aurantiogriseum	0.37	L	0	0
P. donkii	0.3	L	0.4	L
Pseudallescheria sp.	0.43	L	0.63	H
No. of isolates tested	25		25	
No. of isolates positive	25		16	

0= zero indicates negative result, H >0.5, L<0.5.

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References

Abdel-Azeem AM (2010): The history, fungal biodiversity, conservation, and future perspectives for mycology in Egypt. IMA Fungus 2(1): 123-142.

Abd-Elaah GA (1998): The occurrence of fungi along the Red Sea coast and variability among isolates of *Fusarium* as revealed by isozyme analysis. Journal of basic Microbiology 5(6): 303–311.

Abdel-Latif HMR, Khalil RH, El-Hofi HR, Saad TT and Zaied SMA (2015): Epidemiological investigations of mycotic infections of cultured gilthead seabream, *Sparus aurata* at Marriott Lake, Egypt. International Journal of Fisheries and Aquatic Studies 2(3): 05-13.

Abd El-Mageed AE (2002): Studies of some bacteria and fungi isolated from fish. M. Sc., Zagazig University (Benha branch), Egypt.

Abdelmongy AS and El-Moselhy KM (2015): Seasonal variations of the physical and chemical properties of seawater at the northern Red Sea, Egypt. Open journal of ocean and coastal sciences 2 (1): 1-17.

Abolude DS, Opabunmi OO and Davies OA (2013): Fresh water fungi associated with Eggs and Broodstock of African Catfish (*Clarias Gariepinus*, Burchell 1822) in fish hatchery farms, Zaria, Kaduna State, Nigeria. Journal of Research in Environmental Science and Toxicology 2(7): 131-135.

Ali HH (2015): Isolation and identification of pathogenic fungi from carp fish in Suliamania province, Global Journal of Bio Sciences and Biotechnology 4 (4) 356-363

Altschul SF, Madden TL and Schaffer AA (1997): Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25: 3389-3402.

APHA (2005): Standard methods for the examination of water and wastewater. 21st Ed. American Public Health Association, Washington, D.C

Badran RAM (1989): Studies on fungi associated with tilapia fish in river Nile water, Ph.D. Botany Department, Faculty of Science, Qena, Assiut University.

Beltagi AI (1984): Oceanographic and fisheries investigations in the Egyptian Red Sea. Special publication, Academy of Scientific Research and Technology, NIOF Egypt, p. 98.

Bhattacharya U, Prasad J and Dubey NK (1988a): *Aspergillus terreus* (Thom) A new record as fish pathogen. Current Science, 57 (11): 622-623.

Bhattacharya U (1988b): *Aspergillus niger*: a new record as a fish pathogen. Environmental Ecology 6: 231-233.

Bian BZ and Egusa S (1981): Histopathology of black gill disease caused by *Fusarium solani* (Martius) infection in the Kuruma prawn, *Penaeus japonicus* Bate. Journal of Fish Diseases 4(3): 195-201.

Bisht D, Bisht GS and Khulbe RD (2000): *Fusarium* a new threat to fish population in reservoirs of Kumaun India. Current Science 78(10): 1241-1245.

Blaylock RB, Overstreet RM and Klich MA (2001): Mycoses in red snapper (*Lutjanus campechanus*) caused by two deuteromycete fungi (*Penicillium corylophilum* and *Cladosporium sphaerospermum*). Hydrobiologia 460: 221–228.

Bocklisch H and Otto B (2000): Mycotic diseases in fish. Mycoses 43: 76-78.

Bonar CJ, Garner MM, Weber ES and Keller CJ (2013): Pathologic findings in weedy (*Phyllopteryx taeniolatus*) and leafy (*Phycodurus eques*) seadragons. Veterinary Pathology 50(3): 368-376.

Boucias DG and Pendland JC (1991): Attachment of mycopathogens to cuticle: the initial event of mycoses in arthropod hosts. In: Cole GT, Hoch HC (Eds.), The Fungal Spore and Disease Initiation in Plants and Animals. Plenum Press, New York, pp. 101–127.

Carmichael JW (1966): Cerebral mycetoma of trout due to a *Phialophora*-like fungus. Sabouraudia 6: 120–121.

Chauhan R, Nisar Z, Baig AH (2014): Studies on Aspergillomycosis in *Labeo calbasu* found infected with *Aspergillus flavus* and *A. terreus*. World journal of pharmacy and pharmaceutical sciences 3(7): 1842-1848.

Chauhan R (2013): Studies on conidial fungi isolated from some fresh water fishes, International journal of advanced life sciences 6 (4) 131-135.

Cole T and Hoch CE (ed.) (1991): The Fungal Spore and Disease Initiation in Plants and Animals. New York: Plenum Press 101-127.

Colin KC, Elizabeth MJ and David WW (2012): Identification of pathogenic fungi. John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK.

Cowan ST and Steel AR (1993): Cowan and steel's Manual for the Identification of Medical Bacteria, 3nd ed. Cambridge University Press, Cambridge.

Cruz da Silva LR, Camilo de Souza O, Dos Santos Fernandes MJ, Massa Lima DM, Rodrigues Coelho RR and Souza-Motta CM (2011): Culturable fungal diversity of shrimp *Litopenaeus vannamei* boone from breeding farms in Brazil. Brazilian Journal of Microbiology 42(1): 49-56.

De Hoog GS, Guarro J, Gene´ J and Figueras MJ (2000): Atlas of Clinical Fungi. The Netherlands: Centraalbureau voor Schimmelcultures.

Fadaeifard F, Raissay M, Bahrami H, Rahimi E and Najafipoor A (2011): Freshwater fungi isolated from eggs and broodstocks with an emphasis on *Saprolegnia* in Rainbow Trout farms in west Iran. African journal of Microbiology 4: 3647–3651.

Fahmy MA (2003): Water quality in the Red Sea coastal waters, Egypt: Analysis of spatial and temporal variability. Chemistry and Ecology 19: 67-77.

Fijan N (1969): Systemic mycosis in channel catfish. Bulletin of the Wildlife Disease Association 5: 109-110.

Gaskins JE and Cheung PJ (1986): *Exophiala pisciphila*, a study of its development. Mycopathologia 93: 173–184.

Gholampour A, Hosseinfard SM, Rouhi S and Hamid MH (2014): isolation and recognition of infection fungus of Salmo trutta of caspius skin in fish farming of Mazandaran province, Northern Iran. Journal of Animal Biology 6 (4): 51-59.

Hankin L and Anagnostakis SL (1975): the use of solid media for detection of enzyme production by fungi. Mycologia 67: 597-607.

Hatai K, Kubota SS, Kida N and Udagawa S (1986): *Fusarium oxysporum* in Red Sea bream (*Pagrus* sp.). Journal of Wildlife Diseases 22: 570–571.

Hawkins, JP and Roberts, CM (1994): The growth of coastal tourism in the Red Sea: present and future effects on coral reefs. Biological Conservation 76(2): 216-216.

Jaber B, Al-Silawi R and Al-Najjar T (2012): Isolation and molecular identification of Ascomycetes in sediments and waters of the Gulf of Aqaba, Red Sea Natural Science 4(8): 555-561.

Jalilpoor J, Mosouleh S.A. and Masoumzadeh M. (2006): Fungal flora in *Acipenser persicus* eggs with particular emphasis on *Saprolegnia* sp. (Oomycetes) and mortality during mass incubation at the Shahid Behesti hatchery. Journal of Applied Ichthyology 22: 265-268.

Khallil RMA, EL-Hissy FT and Bagy MMK (1991): Mycoflora of Mangroves of Red Sea in Egypt. Folia Microbiologica 36 (5): 456-464.

Khoa L, Hatai K and Aoki T (2004): *Fusarium incarnatum* isolated from black tiger shrimp, *Penaeus monodon* Fabricius, with black gill disease cultured in Vietnam. Journal Fish Diseases 27: 507–515.

Koteshwar RP and Benarjee G (2015): Studies on haematological and histological *Channa punctatus* (Bloch) found infected with *Aspergillus fumigatus* and *Aspergillus niger* spp. exhibited EUS Charecterstics. World Journal of Pharmaceutical Research 4(7) 1233-1246.

Kurata O, Munchan C, Wada S, Hatai K, Miyoshi Y and Fukuda Y (2008): Novel *Exophila* infection involving ulcerative skin lesions in Japanese flounder *Paralichthys olivaceus*. Fish Pathology 43: 35–44.

Kutty SN, Lawman D, Singh ISB and Philip R (2013): Black yeasts from the slope sediments of Bay of Bengal: phylogenetic and functional characterization. Mycosphere 4 (3) 346-361.

Lieske E and Myers RF (2004): Coral Reef Guide Red Sea: The Definitive Guide to Over 1200 Species of Underwater Life. London, Harper Collins.

McKerrow JH (1989): Minireview: parasite proteases. Experimental Parasitology 68:11-115

Moubasher AH (1993): Soil Fungi in Qatar and Other Arab Countries, the Scientific and Applied Research Centre. University of Qatar, Doha, Qatar.

Munchan C, Kurata O, Wada S, Hatai K, Sano A, Kamei K. and Nakaoka N (2009): *Exophiala xenobiotica* infection in cultured striped jack, *Pseudocaranx dentex* (Bloch & Schneider), in Japan. Journal of Fish Diseases 32:893–900.

Nassar MZ and Hamed MA (2003): Phytoplankton standing crop and species diversity in relation to some water characteristics of Suez Bay (Red Sea), Egypt. Egyptian Journal of Aquatic Biology & Fisheries, 7(3):25–48.

Nyhlen L and Unestam T (1975): Ultrastructure of the penetration of the crayfish integument by the fungal parasite Aphanomyces astacii oomycete. Journal of Invertebrate Pathology 26: 353-366.

Olufemi BE (1986): Indication of clinical Aspergillomycosis caused by feeding contamination diet to Tilapia, Oreochromis niloticus (L.). Journal of fish Diseases 9:123-128.

Olufemi BE, Agius C and Roberts RJ (1983): Aspergillomycosis in intensively cultured Tilapia from Kenya. Veterinary Record 112(9):203-204.

Otis E. J. and Wolke R. E. (1985): Infection of *Exophiala salmonis* in Atlantic salmon (*Salmo salar* L.).Journal of Wildlife Diseases 21:61–64.

Overy DP, Groman D, Giles J, Duffy S, Rommens M and Johnson G (2015): *Exophiala angulospora* causes systemic mycosis in Atlantic halibut (*Hippoglossus hippoglossus*) - A Case Report. Journal of Aquatic Animal Health 27(1):12-9.

Paperna I (1996): Parasites, infections and diseases of fishes in Africa. An update CIFA Tech. Pap. No. 31. 220 p. FAO, Rome.

Pedersen OA and Langvad F (1989): *Exophiala psychrophila* sp. nov., a pathogenic species of the black yeast isolated from farmed Atlantic salmon. Mycological Research 92: 153–156.

Pitt JI and Hocking AD (2004): Fungi and food spoilage, Springer- science and business media.

Pitt JI (1979): The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press Inc, London.

Ramanathan N, Padmavathy P, Francis T, Athithian S and Selvaranjitham N (2005): Manual on polyculture of tiger shrimp and carps in freshwater, Tamil Nadu Veterinary and Animal Sciences University, Fisheries College and Research Institute, Thothukudi, 1–161.

Raper KB and Fennell DI (1965): The genus *Aspergillus*, Williams & Wilkins, Baltimore.

Reichenbach - Klinke H. H. (1956): Uber einige bisher ubekannte Hyphomyce Hyphomyceten bei Verscheidenen Suswasser und Meeresfi schen. Mycopathologia et Mycologia Applicata 7: 333–368.

Refai MK, Laila A Mohamed, Amany M Kenawy and Shimaa El-SMA (2010): The assessment of mycotic settlement of freshwater fishes in Egypt. Journal of American science 6(11): 595- 602.

Richard R. H., Holliman A. and Helgason S. (1978): *Exophiala salmonis* infection in Atlantic salmon *Salmo salar* L.. Journal of Fish Diseases 1: 357–368.

Salem AA, Refai MK, Eissa IAM, Marzouk M, Bakir A., Moustafa M., and Manal Adel. (1989): Some studies on aspergillomycosis in Tilapia nilotica. Zagazig Veterinary Journal 17(3): 315-328.

Shahbazain N, Ebrahimzadeh M, Soltani M, Khosravi AR, Mirzagai S and Sharifpour I (2010): Fungal contamination in rainbow trout eggs in Kermanshah Province Propagation with emphasis on Saprolegniaceae. Iranian Journal of Fisheries Sciences 9(1): 151 - 160.

Silphaduang U, Hatai K, Wada S and Noga E. (2000): *Cladosporium* infection in tomato clownfish. Journal of Zoo and Wildlife Medicine 31: 259–261.

Sofianos SS, Johns WE, and Murray SP (2002): Heat and freshwater budgets in the Red Sea from direct observations at Bab el Mandeb. Deep Sea Research part II 49(7-8): 1323-1340.

St Leger RJ, Charnley AK and Cooper RM (1986): Cuticle-degrading enzymes of entomopathogenic fungi: Synthesis in culture on cuticle. Journal of Invertebrate Pathology 47: 85-95.

Tsuyuki H, Williscroft SN, Kabata Z and Whitaker DJ (1982): The relationship between acid and neutral protease activities and the incidence of soft cooked texture in the muscle tissue of Pacific hake Merluccius productus infected with *Kudoa paniformis* and/or *K. thyrsites*, and held for varying times under different pre-freeze chilled storage conditions. Canadian Technical Report of Fisheries and Aquatic Sciences 1130, 39 pp.

Wong G., Kaattari S. L. and Christensen J. M. (1998): Effectiveness of an oral enteric coated vibrio vaccine for use in salmonid fish. Immunological Investigations 21: 353–364.

Zafar I and Rabia M (2013): Some fungal pathogens of an ornamental fish, Black moor (*Carassius auratus* L.). European Journal of Veterinary Medicine, 2 (1): 1-10

Zafar I, Uzma N and Saira S (2014): Fungal infection in silver carp, *Hypophthalmichthys moltrix* (Valenceinnes) reared in earthen pond. Science International (Lahore) 26(1):261-266.