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**SOME MICROBIOLOGICAL STUDY ON TWO SPECIES
OF NILE FISHES**
(With One Table)

By

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دراسة بكتريولوجية على نوعين من أسماك النيل
في صعيد مصر

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في هذا البحث تم عزل وتصنيف خمسة وعشرون صنفاً من الفطريات ونوع واحد من فطر الاسبيرجيلس نديبولانس التي تنتمي إلى ثلاثة عشر جنساً من الفطريات ، بالإضافة إلى ثلاثة أنواع من البكتريا الهوائية . تم عزل هذه العترات من خياشيم وجلد وزعانف وكبد وكلى أمعاء نوعين من الأسماك هي البلطي النيلي والبياض وكذلك من عينه من المياه المحيطة بهم في محافظة أسيوط .

SUMMARY

Twenty-five fungal species and one variety of Aspergillus nidulans which related to thirteen fungal genera in addition to three aerobic species of bacteria were isolated during this investigation. These organisms were isolated from gills, skins, fins, livers, kidneys and intestines of two species of fishes namely, Tilapia nilotica and Bagrus bayad as well as from water, from which, the two species fishes were caught.

INTRODUCTION

The association of water microorganisms with fishes could be either saprophytic, parasitic or pathogenic. The fungai reported as being responsible for or associated with diseases of aquatic animals belong to a wide range of taxa.

The most frequent in all types of host and environment are the so-called water moulds-the Oomycetes- but in recent years there has been an increasing recognition of the importance of various members of the higher fungi as parasites of aquatic animals. Members of the genus Saprolegnia have been known to infect the eel, Anquilla anguilla L. for a considerable length of time (SMITH, 1878). SHERESHEVSKAYA (1932) and TIFFNEY (1939 a,b) studied the water moulds population of fish hatchery, taxonomy and pathology of the fungi causing Saprolegniosis, COPLAND and WILLOUGHBY (1982)

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and NEISH and HUGHES (1980) studied the pathogenicity of *Saprolegnia* infections of *Anguilla anguilla* L. elvers. OLUFENI *et al.* (1983) recorded that *Aspergillus flavus* and *A. niger* were found to cause Aspergillomycosis in *Tilapia sp.* collected from water in Kenya.

In Egypt, a little works EL-ZAYAT (1988) reported the association between fungi and fishes. However, the present investigation aimed to isolate the microorganisms with special reference to fungi associated with two species of fishes.

MATERIAL and METHODS

A total of 28 living *Tilapia nilotica* and *Baqrus bayad* were caught by nets from small canal in Assiut province and as soon as possible were transferred to the laboratory in clean plastic bags. The two species of fishes were examined for clinical signs according to BAUER *et al.* (1973). In addition, a water sample, from which the fishes were caught, was brought to the laboratory in sterile conical flask (litre capacity).

Isolation and Identification of fungal genera and species :

Specimens of fish were obtained from the skins, fins, gills, kidneys, livers and intestines of the two fishes species as well as the water sample.

For isolation of terrestrial fungi, about 1/2 gram of each organ was placed in a sterile flask (250 ml.) which contained 100 ml. sterile distilled water. flasks were shaken by hand in a rotating motion for ten minutes. Aliquots (1 ml each) were introduced into Petri-dishes. To each of which about 20 ml. of appropriate agar medium were added when just in a molton condition. The petri-dishes were subsequently rotated in different directions, so that the suspension was thoroughly mixed up with the culture medium and inocula was uniformly distributed in the plates. In both media, Rose bengal was added as a bacteriostatic agent (SMITH and DAWSON, 1944). Ten plates were used for each organ; five of them were poured with modified Czapek's agar and the other five with cellulose Czapek's agar. Five samples of each two fish species were used.

According to RAPER and THOM (1949); De VRIES (1952); RAPER and FENNEL (1965); DOMSCH and GAMS (1972) and BOOTH (1977). The fungal genera and species were identified.

Isolation and Identification of aerobic bacteria :

Samples from skin, gills, fins, kidneys, livers and intestine were aseptically taken and cultivated on nutrient agar and blood agar. The sediment obtained from centrifugation of 100 ml. of the water sample was inoculated into the same enriched media and incubated at 22 C for 48 hours. The presumptive identification of the isolated bacteria were performed according to KATHRYN *et al.* (1973); CRUICKSHAK *et al.* (1975) and SHOTTS & BULLOCK (1975).

Tilapia nilotica, Bagrus boyad**RESULTS**

A total of twenty-five fungal species and one variety of Aspergillus nidulans were recovered from the 28 examined fish. Total counts of terrestrial fungi isolated from two species of fishes and water sample were illustrated in (Table 1).

Three species of aerobic bacteria were isolated from the organs of two investigated species of fishes as well as from water sample.

Table (1): Total counts (per ml of washing water) of terrestrial fungi isolated from two species of fishes and water sample on glucose and cellulose Czapek's agar medium at 28°C.

	Water	Gill		Skin		Fin		Liver		Kidney		Intes- tine	
	sample	T	B	T	B	T	B	T	B	T	B	T	B
Total count	9	5	5	4	2	2	7	5	8	5	5	2	12
<i>Aspergillus</i>	4	0	1	2	0	1	3	1	6	4	2	1	7
<i>A. fumigatus</i>	3	0	1	2	0	1	2	1	1	3	1	1	5
<i>A. sydowi</i>	0	0	0	0	0	0	1	0	0	0	0	0	1
<i>A. parasiticus</i>	0	0	0	0	0	0	0	0	5	0	0	0	0
<i>A. flavus</i>	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>A. nidulans</i> var. <i>latus</i>	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. versicolor</i>	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>A. niger</i>	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Penicillium</i>	1	1	0	2	0	0	1	1	1	1	1	0	2
<i>P. oxalicum</i>	1	1	0	1	0	0	1	0	1	1	0	0	0
<i>P. verruculosum</i>	0	0	0	1	0	0	0	0	0	0	0	0	1
<i>P. mertensii</i>	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>P. cyclopium</i>	0	0	0	0	0	0	1	0	0	0	0	0	1
<i>Fusarium</i>	2	1	1	0	1	0	0	1	0	0	0	0	0
<i>F. moniliforme</i>	2	0	1	0	1	0	0	1	0	0	0	0	0
<i>F. poae</i>	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Cladosporium</i>	2	1	1	0	1	1	1	0	1	1	1	0	1
<i>C. cladosporioides</i>	0	0	1	0	1	1	0	0	0	1	0	0	1
<i>C. herbarum</i>	1	0	0	0	0	0	1	0	1	0	1	0	0
<i>C. sphaerospermum</i>	1	1	0	0	0	0	0	0	0	0	0	0	0
<i>Alternaria alternata</i>	0	0	0	0	0	0	1	1	0	0	0	0	0
<i>Stachybotrys atra</i>	0	1	1	0	0	0	0	0	0	0	0	0	0
<i>Scopulariopsis</i>	0	0	0	0	0	0	0	1	0	0	0	1	1
<i>S. brevicaulis</i>	0	0	0	0	0	0	1	0	0	0	0	1	0
<i>S. koningii</i>	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Machrophomonia phaseolina</i>	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Paecilomyces inflatus</i>	0	0	1	0	0	0	0	0	0	0	0	0	0
<i>Chaetomium globosum</i>	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Curvularia lunata</i>	0	0	0	0	0	0	1	0	0	0	0	0	0
<i>Botryotricum atrovirens</i>	0	0	0	0	0	0	0	0	0	0	0	0	1
* <i>Saprolegnia</i> sp.	+	+	+	+	+	+	+	-	-	-	-	-	-
** <i>Aeromonas</i> sp.	+	+	+	+	+	-	+	+	-	+	-	+	-
** <i>Staphylococcus</i> sp.	+	+	-	+	-	-	-	-	-	-	-	+	-
** <i>Pseudomonas</i> sp.	+	-	+	-	+	-	-	-	+	-	+	-	-

T = *Tilapia nilotica*B = *Bagrus boyad*

* Member of Zoosporic fungi.

** Aerobic bacteria.

DISCUSSION

It was observed that cotton like structure over the skins and fins with area of erythema at the margin of these growth. Gills were pale in colour. fins were rot specially on tail fins. All internal organs appeared clinically normal but a slight distention was observed in gall bladder specially in Tilapia nilotica. WOLKE (1975) and MEYER and ROBINSON (1973) mentioned that some fish diseases caused by aquatic fungi. These diseases are Saprolegniasis, Achliasis, Aphanomycosis and Branchiomyces (gillrot) which are incited by certain species of Saprolegnia, Achlya, Aphanomyces and Branchiomyces respectively.

Mycological examination :

In this investigation twenty-four fungal species in addition to one variety of Aspergillus nidulan which related to thirteen terrestrial fungal genera were isolated (Table 1). The richest organs with fungal population were intestine of Bagrus bayad (8 species constituting 16.67% of total isolated fungal colonies) and fins of Bagrus bayad (6 species constituting 9.72% of total isolated colonies). The poorest organs were intestine and fins of Tilapia nilotica and skin of Bagrus bayad (each yielded 2 species constituting 0.3% of total isolated fungal colonies). The water sample yielded seven species and nine colonies (0.13% of total isolated colonies).

Aspergillus, Cladosporium and Penicillium were the most common genera and were represented in 11, 10 and 9 samples out of 13 samples respectively. Aspergillus fumigatus was the dominant species and was isolated from 11 samples yielding 21 colonies constituting 65.62% of total Aspergillus and 29.17% of total fungal colonies respectively. Two species namely Fusarium poae and Machrophomonia phaseolina emerged from the gills of Tilapia nilotica only. Paecilomyces inflatus was isolated from the gills of Bagrus bayad only. Curvularia lunata appeared in the fins of Bagrus bayad only. Botryotrichum atrogriseum was isolated from the intestine of Bagrus bayad only. Chaetomium globosum was isolated only from the kidney of the Bagrus bayad. One variety (A. nidulans var. latus) emerged from water sample and disappeared completely in all organs of two examined species. On the other side, there are variations concerning the fungal diversity and populations between two investigated species as well as the organs examined (Table 1).

These terrestrial fungi can be either saprophytic, parasitic or pathogenic to fishes. Some works were conducted concerning this respect, EL-ZAYAT (1988) isolated 52 species which belong to genera of terrestrial fungi from the scales of Tilapia species collected from Aswan high dam lake (Egypt). She reported that the most prevalent genera were Mucor (4 species), Aspergillus (12 species) and Fusarium (4 species). Penicillium was described as a parasite of the internal organs of several freshwater fish, notably Loricaria parva (REICHENBACK-KLINK, 1956). Phoma herbarum, fungal plant saprophyte have several times been reported from salmonid fry and fingerlings (WOOD, 1974; ROSS *et al.*, 1973). This is in contrast, with Van HYNING and SCARBOROUGH (1973) who described Phoma herbarum, as a cause of the black mat syndrome of the tanner crab Chionoecetes bairdi. NEISH and HUGHES (1980) reported

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that, Phoma herbarum infections have been found in three species of Salmonids (Oncorhynchus kisutch, O. tshawytscha and Salmo gairdneri).

Fusarium culmorum has been reported as being responsible for mortality of 200 carp in a new earthen ponds (HORTER, 1960), the infection possibly being derived from a large inoculum of sores present because of layers of beech leaves on the pond bottom. EGUSA and UEDA (1972) isolated Fusarium solani from the Kuruma prawn, Pennaeus japonicus. BURNS et al. (1979) isolated Fusarium from cuticular lesions on the Malaysian freshwater prawn Macrobrachium rosenbergii.

Scolecobasidium humicola was described from a case of systemic mycotic infection in coho salmon held for experimental purposes (ROSS and YASUTAKE, 1973). KENDRICK and CARMICHAEL (1973) reported that Verticillium piscis to be the cause of such granuloma in a gold fish.

Saprolegnia sp. which is known as strictly aquatic zoosporic fungus was recovered from water sample, gills, skins and fins of two investigated species. WILLOUGHBY (1968, 1977, 1978) has clearly shown that there is specific type of saprolegnia associated with salmonid fish lesion. DAVIS and LAZAR (1941) described Saprolegnia ferax as the cause of an alimentary tract infection of rainbow trout (Salmo gairdneri) fingerlings. Moreover, AGERSBERG (1933) reported that of "intestinal fungitions" of brook trout (Salvelinus fontinalis) fingerlings were the only ones describing the initial site of a saprolegian as the gut instead of a site somewhere on the surface of the fish (e.g. skin, gills, nares) until recently, when this phenomenon was confirmed by MIYAZAKI et al. (1977) and by HATAI and EGUSA (1977). These workers found that Saprolegnia diclina infected the guts of rainbow trout and amago salmon (Oncorhynchus rhodurus f. macrostoms). Saprolegnian fungi are not restricted to living as parasites of the skin, gills and musculature (BOOTSMA, 1973; NOLARED-TINTIGNER, 1973, 1974). RUCKER (1944) concluded that Saprolegnia parasitica Coker was a secondary invader which could not normally initiate an infection and that the primary cause of the infections was the Myxobacterium. Moreover, BÖHM and FUHRMANN (1984) isolated Saprolegnia species infected rainbow trout, Eel and other fish species. This species of Saprolegnia was found mostly in skin lesions and fins and in 18 cases, the fungus was isolated from pathologically changed gills. LENNON (1954) found that Leptomitius lacteus was associated with wounds of fish caused by sea lampreys (Petromyzon marianus).

Bacteriological examination :

Three species of aerobic bacteria isolated from the organs of investigated species as well as from water sample (Table 1). Aeromonas sp. was isolated from all investigated samples except the fins of Tilapia, liver, kidney and intestine of Bagrus bayad. Staphylococcus sp. was isolated from water sample, gills, skin and intestine of tilapia. It disappeared completely in all organs of Bagrus bayad.

Pseudomonas sp. was isolated from water sample as well as from gills, skin, liver and kidney of Bagrus bayad and was not isolated from neither all organs of

Tilapia nor fins and intestine of Bagrus bayad. This result is in agreement with EVELYN *et al.* (1961) and TRUST *et al.* (1974).

In this respect, HUME (1903) was convinced that the "Salmon disease" was due to bacterium. COLLINS (1970) found that Aeromonas liquefaciens and Pseudomonas fluorescens are common in fresh-water and can be isolated from healthy fish.

HATAI and WILLOUGHBY (1988) stated that the fungus Saprolegnia parasitica and the bacterium Pseudomonas fluorescens were present together in several lesions on rainbow trout.

FUHRMANN (1983) isolated Aeromonas sp., Pseudomonas sp., Flavobacteriu sp., Enterobacteriaceae, Anthobacter sp., Gram positive cocci and Saprolegnia species from infected rainbow trout, Crap teuch and eel. RICHARDS and PICKERING (1978) isolated Saprolegnia species, Aeromonas hydrophila, Actinobacter spp., Morascella spp. and Alcaligenes spp. from infected Brown trout and Char.

It is worth mentioning that, this investigation represents a preliminary study concerned with the isolation and identification of microorganisms (specially belonging to fungi) associated with two species of fishes. The nature of the relationship between these organisms and fishes needs further experimentation and hopefully will be forthcoming.

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