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Some Bacterial and Fungal affections Causing Disease Problems in Cultured Seabream *Sparus Aurata* in Damietta Governorate and Trials for Control

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ABSTRACT

Seabream (*Sparus aurata*) is a 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. So the present study was conducted to through alight spot on some diseases of seabream which interfering culturing of it specially bacterial and fungal diseases and some trials for control of these problems. Total number of one hundred and twenty seabream (*Sparus aurata*) weighted 150g ± 5 and 18 ± 0.2 cm in length were collected from earthen pond in Damietta and submitted to full clinical investigation, postmortem, bacteriological, mycological and histopathological examination. Also, water quality analysis of earthen ponds holding seabream was measured. Affected seabream clinically inactive, loss of balance and loss of escape reflex, sluggish swimming and swim near to the water surface, distended abdomen, focal or diffused hemorrhages on different parts of the body, fins and tail rot, uni or bilateral corneal opacity, loss of scales and pigmentation, erosion and ulceration in the lateral aspect of the body, appearance of cotton wool tufts like growth on the ulcerated parts, fins and eyes causing blindness. Internally the affected seabream showed congested gills. Liver showed reddish necrotic foci occur on its surface, distended gall bladder, congested spleen and accumulation of bloody tinged exudates in the abdominal cavity with offensive odour. The bacteriological examination of affected seabream revealed the isolation of *Vibrio alginolyticus* and *Aeromonas hydrophila*, according to morphological, biochemical and API. 20 E system. *V. algindyticus* was isolated in a total number one hundred and eighteen while *A. hydrophila* isolated in seventy one strains. From the macroscopic and microscopic characters of isolated fungi, it cleared that the isolated fungi was *Aphanomyces sp.* in a total ninety eight isolates. The infectivity tests and histopathological examination were done. The antibiogram sensitivity test of isolated strains were done and showed that, *V. algiolyticus* and *A. hydrophila* were sensitive to enrofloxacin, ciprofloxacin and florfinicol while they were resistant to ampicillin, lincomycin, colistin sulphate and neomycin and control trails were performed.

Keywords: Sea bream, bacteria and fungi, Beta polo.

INTRODUCTION

Aquaculture has an important role in the development of many national economics and plays a role in rural development and play a role in meeting demands for aquatic animal production, Haylor and Bland, (2001). Aquaculture industry gradually developed in the world as well as in Egypt especially marine aquaculture. Seabream (*Sparus aurata*) is marine Fish with economic value and wide spread all over the world, especially in the Mediterranean Sea. Seabream (*Sparus aurata*) Culture was known recently in Egypt and need for progressive development especially in

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feeding and health care. Farming of seabream (*Sparus aurata*) is still at an early stage development, it has started in late 1980 `s and there is still much to be learned with regard to health and disease diagnosis and prevention. As with all species of fish, crustacean and shell fish that are farmed intensively, seabream (*Sparus aurata*) has arrange of organisms that parasitize and cause diseases of it the success in Aquaculture industry depend up on the selection of reared species of fish, healthy aquatic environment and realizing the relationship between fish, their environment and pathogens. Fish in intensive Culture are continuously affected by environmental changes and bad management practice such as transportation, handling, crowding and deterioration of water chemistry and quality, all these factors will predisposing the fish to in factious diseases, specially bacterial, fungal, parasitic and viral diseases Noga, (1996). New diseases and condition are regularly being identified and reported as more effort is placed on investigation the health and wellbeing of the species seabream (*Sparus aurata*) under intensive farming conditions. Bacterial infections are the most significant diseases to be encountered in sea bass and seabream culture at present; several bacterial species are common pathogens in seabream Toranzo et al., (1999) and Balebona et al., (1998b). The most serious infection come form vibrionacea, pseudomonodacea, Pasteurella and genus micrococcus. Chabrillon et al., (2005), Rasado et al., (2007) and Marzouk et al., (2009). Vibrio alginolyticus is one of the pathogenic vidrio have been reported in seabream (*sparus aurata*) Bakhrouf et al., (1995), Balebona et al., _1998a), Zarrilla et al., (2003a) and Nakbi et al., (2006). Outbreaks of water born fungal infection of fish, amphibian and reptiles are common problem especially in fish under farm condition and hatcheries, also fungal infection considered one of the most significant diseases affect seabream (*sparus aurata*). *Aphanomyces sp.* is an infectious fungal disease that is wide spread in all stages of the life cycle of fish and considered as single largest cause of economic loss in aquaculture Meyer,(1991) and Hussein et al., (2001). *Aphanomyces* considered agent of secondary infection arising from conditions as bacterial infections, poor husbandry, including poor water quality, adverse water temperature, all these factors in creased occurrence of *Aphanomyces* infections Bailey, (1984). So this study was designed to through some light on diagnosis of most common infectious diseases of cultured seabream (*Sparus aurata*), specially bacterial and fungal diseases and trials for control of these infections.

MATERIALS AND METHODS

Water quality analysis

Water quality analysis was done in the field immediately, for measuring the water parameters in different bonds holding seabream (*sparus aurata*) in the farm at the occurrence disease. The water parameters are temperature, dissolved oxygen, saturation of the oxygen, pH, salinity, total solid, electric conductivity (E.C), nitrate, nitrite, Secchi disk (S.D) and also determination of green algae, blue green algae, diatom and Euglena. Some of these parameters were done by using oxygen meter ph meter Salinometer and Secchi disk but other parameters determined in the laboratory by taken water samples in glass bottles from different ponds.

Fish samples

One hundred and twenty moribund and diseased fish Seabream (*Sparus aurata*) weighted 150g ± 5g and 18 ± 0.2cm in length were collected from earthen ponds in International rood of Damietta – Port said in December f 2012. The collected fish (Sea bream) immediately were subjected to full clinical, postmortem, bacteriological and mycological investigation.

Clinical investigation and postmortem examination

Moribund and diseased Fish, were properly examined for any external clinical abnormalities and clinical alterations on the skin, scales, eyes, abdomen, peduncle, fins and abnormal behaviors, the postmortem examination was done on freshly dead fish to examine, all internal organs including gills, liver, spleen, kidney and intestine, the clinical investigation and postmortem examination were done according Amlacher (1970) bacteriological examination. Samples for bacteriological examination collected under strictly aseptic precaution from gills skin ulcer fins liver, spleen, kidney, blood samples and inoculated onto typtic soy broth, tryptic soy agar. The suspected purified colonies picked up and streaked over specific medium, acrimonies selective agar base , and Thiosulphate Citrate Bile salt Sucrose agar (TCBS) (Biolife, Milan, Italy). The inoculated media were incubated at 25 ^{o}c for 48 – 96hrs, then isolated bacteria were subjected to taxonomical analysis according to Bergey's manual of Determinative Bacteriology (1994) also isolated bacteria were identified by using API_{20} E system (A analytical profile index) Biomerioux, France.

Mycological examination

The isolation Fungi were carried out from moribund and diseased Fish (sea bream). The sample were taken From eyes, skin ulcer, fins gills, mouth and inoculated onto Sabaroud's Dextrose Agar (SDA) medium plates and incubated at 20 ± 2^{o}c For 3 - 4 days and subculture on the Same medium (SDA) For purification, all positive culture were examined for colonial growth, morphological feature and microscopic characteristic. The microscopical examination was done from wet preparation from skin ulcer, eyes, gills, mouth and fins and also from growth on SAD to detect septation of hyphae according to **Dvorak and Atanoesk (1969).**

Histopathological examination

Specimens of gills, Liver, spleen, kidney and fins were fixed in 10% neutral buffer formalin gills and fins were decalcified in (EDTA) 12.5%. Paraffin section (5µm thick) was prepared and stained hematoxylene and eosin (HBE) and examined microscopically for detection of any pathological alteration Robert, (2001).

Pathogenicity test (Infectivity test)

A total number of forty apparently healthy Seabream (*Sparus aurata*) were collected with average 25g ± 0.5g kept in glass aquaria for tow weeks in the same environmental condition in the pond (salinity, pH and temperature) and fed with 5% body weight commercial a ration and divided into four groups, 10 fish in each group, 1st group injected with 4 ml x10^{7} Cfu of *V. alginolyticus* intrapretoneal (I / p), 2sd group injected with 1 ml x10^{6} Cfu of *A. hydrophila* I /p, 3rd group injected with bath *V. alginolyticus* and *A. hydrophila* I /p in some doses mentioned above and 4th group injected with 1 ml sterile saline as shown in Table (1). All groups observed daily for 10 days to record any clinical signs, behaviors and daily mortality and postmortem examination was done on freshly dead fish and resolution of injected strains.

Antibiogrom sensitivity

Were done according to the limits given by Schaperclaus et al. (1992)., using the disc diffusion method on Muller's Hinton agar medium and the interpretations zones of inhibition were done using the following discs Ampicillin, Enorfloxacin, ciprofloxacin, Nalidixic Acid, lincomycin sulphate, Neomycin and Florfenicol as recoded in Table (2).

Trials for control of the disease

1- Addition of lime and salt 1:1 at a concentration 0.5% for 3 successive days.

2- Improving the immune state of fish by addition Beta – polo (B' 1, 3 glucan, 30000 mg propylene glycol 1000 mg and purified water up to 1000ml).

Table (1): ***patogenicity and mortality rate of experimentally infected Seabream (Sparus aurata).***

Group	1st	2nd	3rd	4th
No of fish	10	10	10	10
Injected strain	*Vibrio alginolyticus*	*Aeromonas hydrophila*	*Vibrio Alginolyticus* + *Aeromonas ydrophila*	Sterile broth (Control)
Route of injection	I/P	I/P	I/P	I/P
Dose of injection	$1x10^7$	$2x10^6$	$1x10^7$+ $2x10^6$	1 ml
1st day	0	0	0	0
2nd day	2	1	10	0
3rd day	3	3	0	0
4th day	3	2	0	0
5th day	2	2	0	0
6th day	0	2	0	0
7th day	0	0	0	0
8th day	0	0	0	0
9th day	0	0	0	0
10th day	0	0	0	0
Total	10	10	10	0
Mortality %	100%	100%	100%	0%

I/P= Interaperitoneal ***No= number***

Table (2): ***Antibiogram sensitivity of isolated strains.***

Antibiotic disc	Code symbol	Concentration µg	Reaction	
			Vibrio Alginolyticus	***Aeromonas hydrophila***
Ampicillin	A	10	R	R
Enrofloxacin	E	10	S (++++)	S (+++)
Ciprofloxacin	Cip	5	S (+++)	S (+++)
Nalidixic acid	NA	30	S (++)	S (+++)
Lincomycin	L	2	R	R
Colistin	CL	10	R	R
Neomycin	N	30	R	R
Florfenicol	Ffc	30	S (+++)	S (+++)

RESULTS

The results of water quality analyses as shown in Table (3) revealed that decreasing in dissolved oxygen (D.O), temperature, salinity but slight increasing in total algae specially green algae specially green algae and blue green algae, and pH while other parameters in the normal limit.

Results of clinical investigation

Affected seabream (*Sparus aurata*) showed inactive, refused food intake, loss of escape reflex, loss of balance, sluggish swimming and swim near to the water surface, diseased fish in chronic state suffered form sever edema, focal or diffused hemorrhage on the different parts of the body of diseased fish at the abdominal region, tail and fins and tail rot, corneal opacity of the eye may be unilateral or bilateral, loss of scales and pigmentation, erosion may be extended to reach epidermal layers causing ulceration behind the head and trunk region, appearance of cotton wool tufts growth on the lateral aspect of the body when removed showed irregular margin ulceration, also, cotton wool tufts growth on mouth, eyes, causing blindness uni or bilateral, emaciation ended by death, all clinical signs shown in plate (A : Photos 1, 2, 3, 4, 5).

Postmortem examination:-

Internally the diseased fish showed congested gills and in some cases were pale, enlarged, congested with hemorrhagic foci on liver surface and in other cases showed whitish necrotic foci, distended gall bladder with bile secretion. Congestion in kidney and spleen, intestine was hemorrhaged and free from any food particles and accumulate ion of blood tinged exudates in the abdominal cavity with offensive odor as shown in plate (A: photo 6).

Table (3): ***Results of water analysis in different ponds holding Sea bream*.**

	Water parameters											
Pond No	**Green algae**	**Blue green algae**	**Diatom**	**D.O**	**S.O**	**T.S**	**T**	**S.D**	**S**	**pH**	**NO3**	**NO2**
	Cell/ml	Cell/ml	Cell/ml	Mg/L	%	Mg/L	^{O}C	Cm	ppt		Mg/L	Mg/L
1	1870	3100	600	3.83	45.00	68.1	19.2	19	5	7.8	0.16	0.024
2	1930	3170	780	4.40	54,00	85.2	18.7	21	4	7.9	0.21	0.021
3	1900	3110	690	4.60	56.00	67.0	19.5	18	6	8.1	0.15	0.019
4	1870	2716	720	4.00	49.00	59.7	19.6	22	3	8.7	0.14	0.016
5	1850	2890	750	4.30	53.00	66.3	19.8	25	5	8.6	0.25	0.024
6	1930	3190	760	3.60	44.00	90.2	18.9	23	4	9.2	0.15	0.018
7	1925	3150	780	3.50	44.00	87.3	20.0	18	4	9.4	0.23	0.022
8	1910	3120	654	3.70	45.00	86.4	18.8	19	5	7.9	0.24	0.021
9	1890	3105	683	4.20	54.00	67.3	19.3	21	6	8.8	0.22	0.025
10	1920	3001	710	3.40	43.00	88.2	19.9	24	3	9.2	0.13	0.018

D.O: dissolved oxygen ***S.O: saturation of oxygen*** ***T.S: total solids***
T.: temperature ***S.D: seccki disk*** ***S.: salinity***
NO3: nitrate ***NO2: nitrite***

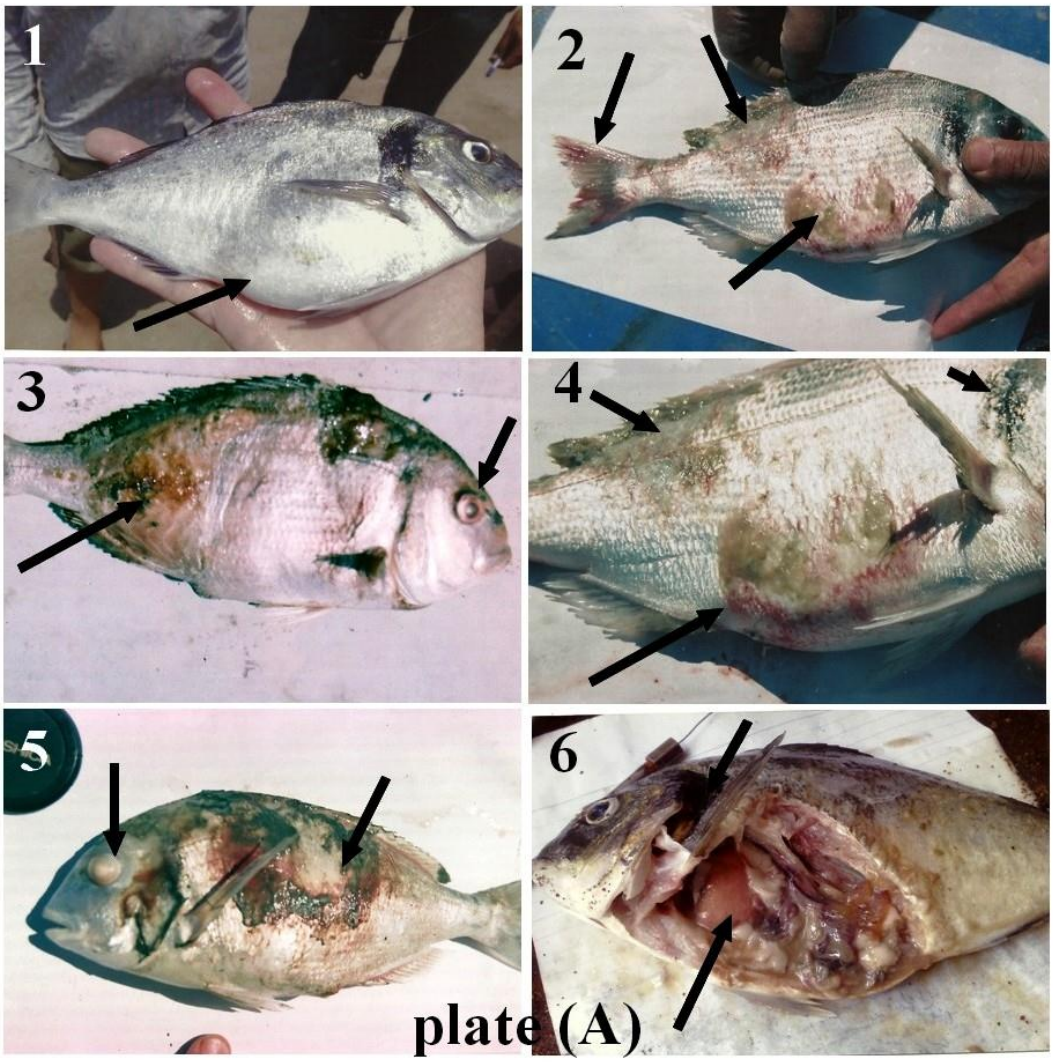

Photo (1) affected Seabreamshowed edema in the abdomen.
Photo (2) showed diffuse hemorrhages, fins and tail rot.
Photo (3) erosions, ulceration and opacity of the eyes.
Photo(4) cotton wool like tufts growth on the fin and abdomen.
Photo(5) cotton wool like tufts growth on the fin and abdomen with blindness of the eyes.
Photo (6) congestion of the liver, kidney, spleen with bloody tinged exudates in the abdominal cavity

The results of bacteriological examination of diseased seabream revealed the isolation of one hundred and eighty nine bacterial isolate of gram negative bacteria which identified according to morphological, biochemical and API_{20} into *Vibrio alginolyticus* and *Aeromonas hydrophila* as shown in plate (B: photos 1, 2) and Table (4).

The distribution of *V. alginolyticus* and *A.hydrophila* in different organs and tissues of diseased seabream (*Sparus aurata*) as shown in Table (6) *V. alginolyticus* was isolated in one hundred and eighteen strain fro skin, fins, gills, spleen, liver and blood but not isolated from eyes while *V. alginolyticus* was isolated in seventy one strain from skin, fins, gills, spleen and liver. *V. alginolyticus* was isolated with higher percentage from skin 27 (22.88%) and lowest percentage from blood and gills 12 (10.16%) while *A. hydrophila* was isolated in higher percentage from the skin 17 (23.94%) and lowest percentage from the spleen 8 (11.20%).

Table (4): ***The biochemical and morphological characters of isolated bacteria from seabream (Sparus urata).***

Characters	*Vibrio alginolyticus*	*Aeromonas hydrophila*
Gram stain	-ve	-ve
Motility	+ve motile (swarming)	+ve motile
Growth on TCBS	+ve yellow colonies	-ve
Growth on aeromonas base agar	+ve	+ve green colonies
Growth on Nacl %		
0 %	-ve	+ve
3 %	+ve	+ve
5 %	+ve	+ve
7 %	+ve	-ve
10 %	+ve	-ve
Growth on Novobiocin	-ve	+ve
Indole	+ve	+ve
Voges Proskaur	+ve	+ve
Methyl red	+ve	+ve
Arginine	-ve	+ve
Sensitivity to Cephalothin 30µg disc	Sensitive	Resistant
Sugar fermentation		
Glucose	-ve	+ve
Lactose	-ve	+ve
Sucrose	+ve	+ve
Arabinose	-ve	+ve

Table (5): ***The collective data about infection.***

Fish species	Diseased fish	Infected with bacteria	Infected with fungus	Total samples	Total isolates	Bacterial isolates	Fungal isolates
Seabream (S.aurata)	120	120	85	840	287	189	98
%	100	100	70.83		34.16	22.50	11.66

Table (6): ***Distribution of isolated strains in different organs and tissues.***

Organ →		Skin	Fin	Gill	Liver	Spleen	Kidney	Blood	Eye	Total
No of isolates →		84	69	45	35	17	19	12	16	287
Strain ↓										
V.alginolyticus	No	27	25	12	21	10	11	12	---	118
	%	22.88	21.18	10.16	17.79	8.47	9.32	10.16	---	
A.hydrophila	No	17	16	9	14	7	8	---	---	71
	%	23.94	22.53	12.67	19.71	9.85	11.26	---	---	
Aphanomyces sp	No	30	28	24	---	---	---	---	16	98
	%	30.61	28.57	24.48					16.32	

The results of mycological examination showed that, isolation of *Aphanomyces* according to wet mount preparation, microscopic characters and feature of growth on Sabroud's dextrose agar , showed long , branched , non septated hyphae Carrying cysts as shown in plate (B: photo 3). The *Aphanomyces* isolated from diseased Seabream(*S. aurata*) in a total ninety eight strain of *Aphanomyces sp.*. The distribution of *Aphanomyces* in different organs and tissues as shown in Table (6), it was isolated from infected skin, fins, gills, and eyes. Skin 30 (30.61%), fins 28 (28.57%), gills 24 (24.48) and eyes 16 (16.32%).

Results of histopathological examination

Skin showed hyperplasia of the club cells of the epidermis, Congested and hemorrhagic dermis with excessive aggregation of round cells and melanomacrophage. Other lesions showed epithelial desquamation in the epidermis, the other epidermal cells suffered vacuolar degeneration and focal necrosis, the underling dermis was edematous with focal aggregation of melanomacrophage cells. The necrotic muscle infiltrated with mononuclear leukocytes and some melanamacrophage. Plate (C: photo 1).

Gill, showed hyperplasia of the epithelial covering, beside congestion of branchial blood vessels and fusion of secondary lamellae. Plate (C: photos 2, 3).

The liver showed lipid infiltration of the hepatocytes, congestion of the hepatoportal vein, central vein and congestion of the sinusoid and hyperplasia of the epithelial lining bile duct, Plate (C: photos 4, 5)

Kidney showed congestion of some renal blood vessel and depletion of hemopiotic elements in the interstitial tissues. Collapse of glomerulli and edema of Bowman's capsule with degenerative change, Plate (C: photos 6, 7)

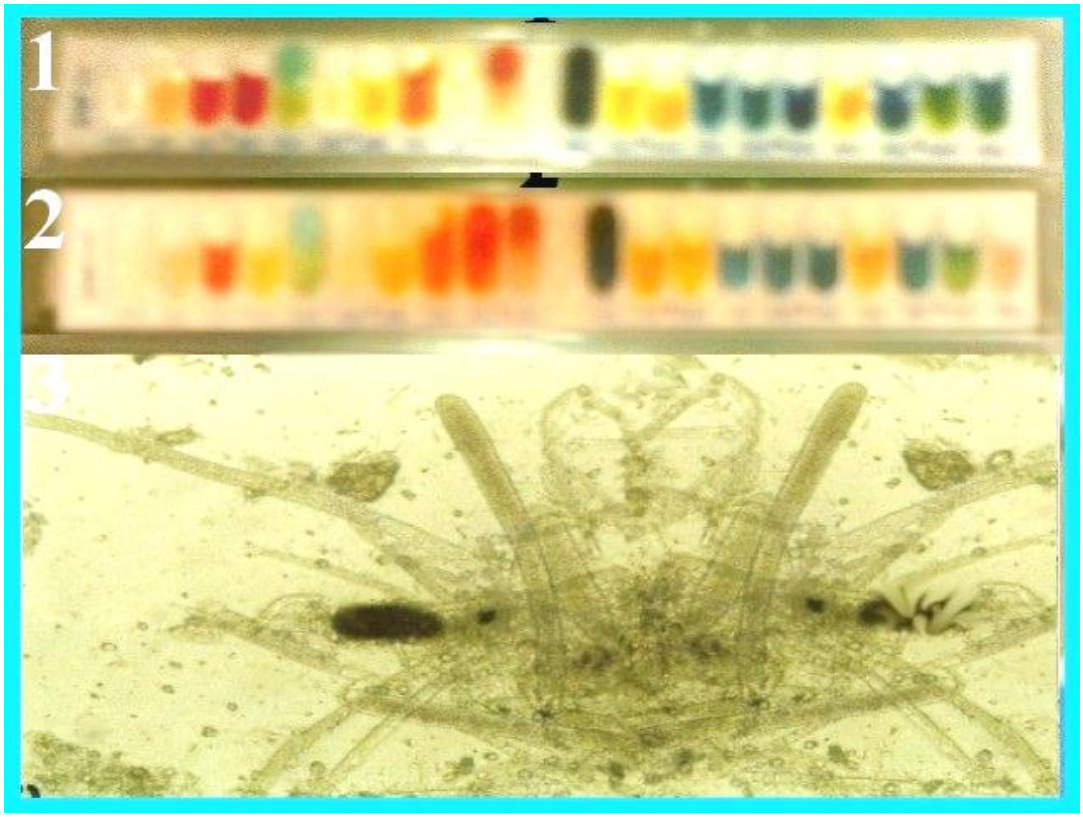

Plate (B)
Photo (1) API20$_E$ analysis of Vibrio alginolyticus.
Photo (2) API20$_E$ analysis of Aeromonas hydrophila.
Photo (3) microscobic examination of the fungal growth showed branched non septated hyphae of Saprolegnia parasitica isolated from skin lesions of sea bream.

Spleen showed separation of the spleenic capsule with multiple melanomacrophage cells and alternative area of depletion and proliferation of hemopiotic elements, Plate (C: photos 8)**.**

The results of infectivity tests (pathogencity test) as shown in Table (1).

The clinical signs of the disease were seen after 24 hrs post injection with *V. alginolyticus*, *A. hydrophila* and in both strains (*V. alginolyticus* + *A. hydrophila*). It showed that I/P route of ignition was highly effective in *V. alginolyticus* and *A. hydrophila* and cause 100% mortality wither 5th day in *V. alginolyticus*, and within 6th day post injection in *A. hydrophila* while cause 100% mortality within 2nd day post injection in both (*V. alginolyticus* + *A. hydrophila*).

Results of antibiogrom sensitivity test, showed that *V. alginolyticus and A. hydrophila* were sensitive to, they were resistant to ampicillin, lincomycin, colistin sulphate and neomycin as clear in Table (2).

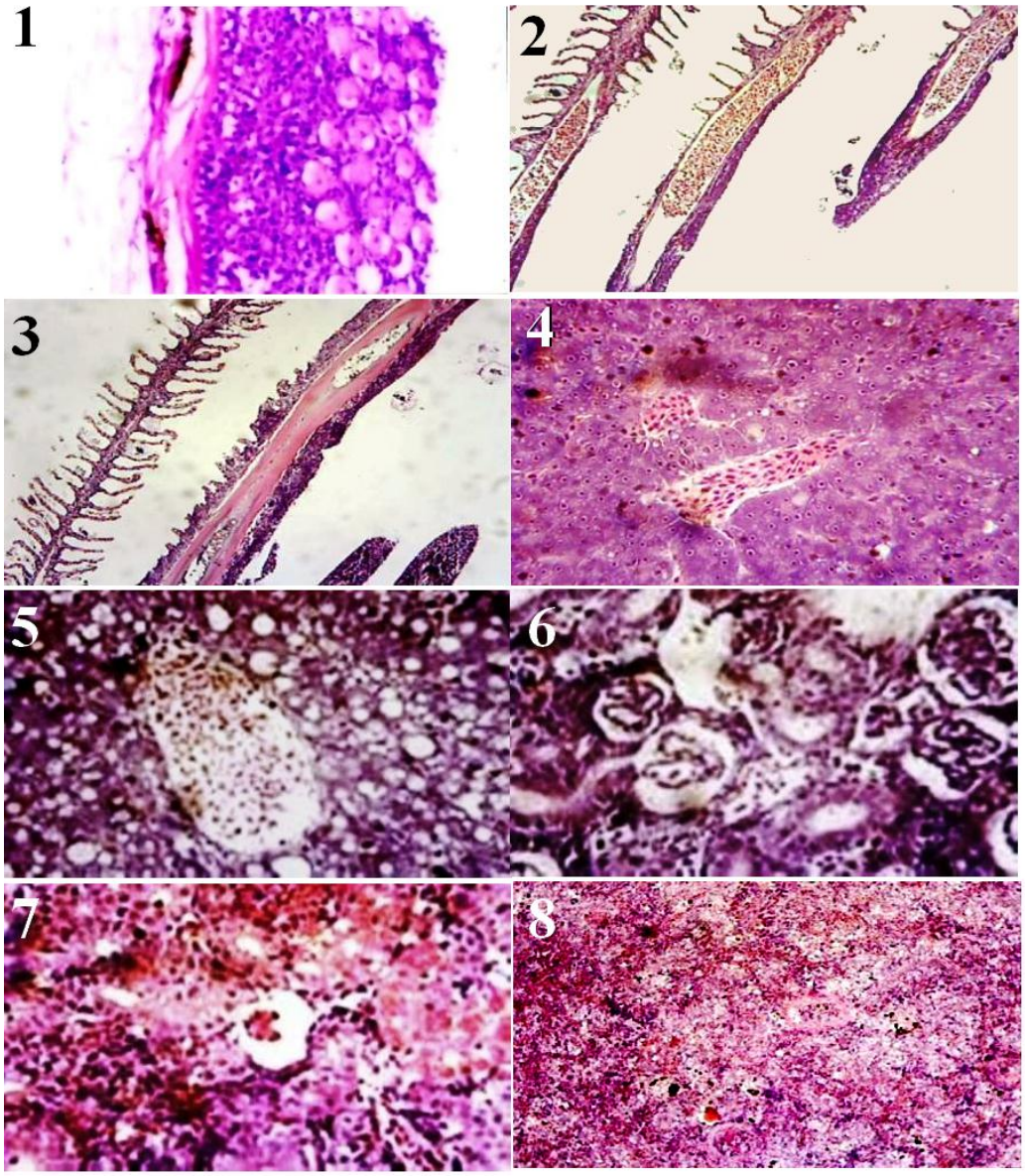

Plate (C)

Photo (1) skin showed hyperplasia of the club cells of the epidermis, Congested and hemorrhagic dermis with excessive aggregation of round cells with increased melanomacrophage cells.

Photo (2, 3) gills showed focal hyperplasia, cellular necrosis and congestion of the bronchial vessels.

Photo (4, 5) liver showed congestion of the central vein and lipid droplet degeneration.

Photo (6,7) kidney showed collapse of the capillary tuft (Glomeruli) with accumulation of edematous fluids in the Bowman's capsule, with hyaline droplet degeneration.

Photo (8) spleen showed depletion of the hemopiotic elements.

The results of control trials, first of all remove the causes and correct the environmental condition specially salinity by using lime and salt for 3 successive at a ratio 1:1 in a concentration of 0.5 gave a good results in controlling fungal infection, and using sea water supply after partial removal of pond water improved of immune state of fish by addition of Beta polo in a dose 1m /hg. Feed for 7 days gave good results in improving health state of fish and addition of florfenicol in a dose 30 mg 1 kg fish for 7-10 days.

DISCUSSION

The high morbidity and mortality rates of cultured seabream *(sparus aurata)* may be attributed to ad verse of same parameter of water quality specially decreasing of water temperature, decreasing of water salinity , slight in creasing in pH , ammonia , slight decreasing in dissolved oxygen and in creasing in total algae in the ponds holding seabream (blue green, green and diatom), all these factors considered to be predisposing factors to appear the disease due to low activity of cultured seabream and their resistant to in factious was lowered and resulted in significant losses , so , the fish in intensive culture are affected by environmental changes and bad management these results agree with that mentioned by Roberts (1989) and Noga, (1996). It suggest that seasonal suppression of immune state due to decrease of water temperature surrounding fish also decrease of the salinity, leads to suppression of antibody formation so, the antibody production of the fish was seasonality dependants , these was Mentioned by yamaguchi et al., (1980) the results of clinical examination of diseased fish (Sea bream) showed that, affected seabream in active, are fused of food intake, loss of escape reflex, loss of balance, sluggish movement and summing near to the water surface, these results were due to frayed tail and fin rot l also diseased seabream gasping of the air near to the water surface as a result of fungal growth on gills. Diseased fish suffered from sever odema, focal or diffused hemorrhage on the different parts of the body as results of releasing of powerful bacterial proteolytic enzymes, catatonic and neuron toxic effects on the diseased fish., these results were similar to that mentioned and with Morita (1975), Zorrilla et al., (2003_a) Toranzo et al ., (2005) and kahla-Nakbi et al., (2006), unilateral or bilateral corneal opacity pf the eyes maybe attributed to inflammatory local odema due to in cease permeability of the capillary endothelium leading to escape of plasma protein under the effect of exotoxin or cytotoxin produced by infected microorganisms. The cataract maybe developed as results of degenerative changes these results of postmortem were identical to those obtained by Balebona et al., (1998b) Kala-Nakbi et al., (2006) and Taghrid (2011). According to morphological, physiological, biochemical characters and $API_{20}E$ revealed that isolation of gram negative bacteria identified as *V. algindyticus* and *A. hydeophila*. One hundred and eighty nine bacterial strains of *V. algindyticus* and *A. hydeophila* from seabream (*S. aurata*). The *V. algindyticus* was obtained with high prevalence from skin 27 strains with percentage (22.88 %) these due to increase of mucous on the skis of diseased seabream and slight increase of pH can be conductive to the intensity of the number of vibrio bactria in the water interacting with fish skin mucous, these result agree Bolebona et al., (1995). The higher isolation of the *V. algindyticus* was recorded in winter due to decrease of the temperature and decrease of salinity these results were similar to those mentioned by Zorrilla et al., (2003a) also agree with the results mentioned with Balebone et al., (1998a,b) and Le Breton (1999). They recorded that *V. algindyticus* was isolated from cultured diseased seabream and considered as one of the main bacterial pathogens described in seabream and seabass cultured in Mediterranean Sea coastal areas. While *A. hydrabhila* was isolated in a total number seventy one isolates with higher percentage from skin 17 strains (23.94%), *A. hydrophila* can persist in the salinity environment reach 6-7% so it cause Aeromonas septicemia in fresh, brackish and

marine environment, these results agree with Abou El Atta and Saleh (2010) whom isolated *A. hydrophila* from Meager fish reared in brackish water. According to morphological , microscopic features of isolated fungi it cleared that, isolation of *Aphanomyces* which it was identified as *Aphanomyces sp.*, the higher prevalence of *Aphanomyces sp.* obtained from skin 30 isolates (30-61%) followed by fins , gills and eyes as shown in Table (6). The high percentage of *Aphanomyces* was due to low temperature, low salinity and increasing of bacterial infection as vibrio and Aeromonas, these factors act as a good media for infection, so sapralegnia considered as secondary invader to bacterial or ectoparasitic infestation. Isolation of *Aphanomyces sp.* considered as the first record in seabream.

Saprolegnia parasitica isolated from Meager fish reared in brackish water as mention by Abou El-Atta and Saleh (2010), also, isolated from seabass reared in brackish water (Cook and Unwin., (1985), press communication). From the results of infectivity test which applied on apparent healthy seabream (*S. aurata*) , it cleared that *V. alginolyticus* was virulence in I/p route under same condition (salinity 4% and temperature 18-5^{o}c) it cause 100% mortality within 4th day post injection, these result agree with those recorded with Kala-Nakbi et al., (2006), while *A. hydrophila* cause 100% mortality with in 6 day post injection I/P, these result accepted with Abou El Atta and Saleh (2010), Enany et al., (2011) and Abou El-Atta and El Ekiaby (2012) But *V. alginolyticus* and *A. hydrophila* cause 100% mortality with in 2nd day post injection I/P due to *V. alginolyticus* can produce two toxins Catatonic and neurotoxin effects on fish and cell lines, these recorded with Balebona et al., (1998a,c) and Kala-Nakbi et al., (2oo6) while *A. hydrophila* produce proteolytic enzyme and hemolysin enzyme, these recorded with Morita (1975) both strains *V. alginolyticus* and *A. hydrophila* enhance the disease and cause mortality within 2nd day post injection.

From the results of hitopathological findings the congestion and hemorrhages of the skin attributed to the extra cellular enzymes which had hemolytic and photolytic activity, such enzymes have toxic effect on the epithelial cells leading to irritation and subsequent hyperplasia of the alarm substance and mucus cells as a defense mechanism against the hazarders of toxins and affect the endothelial lining of subcutaneous blood vessels leding to escape of RBCs, leukocytes to the surrounding tissues as well as plasma protein causing edema, congestion and hemorrhages at the site of infection, these finding agree with Sakr and Aabou El-Atta (2006), Avci et al., (2013). The gills showed focal hyperplasia was a simple response to the cellular necrosis, the congestion of the branchial vessels may be attributed to the reaction of the inter leukins which cause vasodilatation of blood vessels. The process of acute inflammation was initiated by the action of the action of vasoconstrective amines on the microcirculation. As mentioned by Avci et al., (2013). The liver showed congestion of the central vein and lipid droplet degeneration in the hepatocytes, such hepatic lesions are indicative of septicemia as the liver was damaged by blood born pathogenic bacteria and it's metabolites. Similar lesions were described by Avci et al., (2013). The kidney showed collapse of the capillary tufts (Glomeruli) and could attributed to the presence of edematous fluids which accumulated in the Bowmn;s capsules. The presence of hyaline droplet degeneration suggested the existence of glomerular disease which can present in the protein leakage in it, so, the filtrates decreased and make pressure on the cells. The edema of the Bowmn;s capsules resulted in hypoproteinamia, decrease the colloidal substances, breakdown the cement substance and the endothelial cells leads to the passage of fluids to the surrounding media. These histopathological changes are in agreement with those obtained by Marzouk et. al., (2009) and Avci et al.,(2013). The spleen showed depletion of the hemopiotic elements due to cytolytic and fibrolytic capacities of *V.*

alginolyticus and *A. hydrophila* activity which destroy the host defense system in the spleen. This results recorded by Avci et al., (2013).

From the results of antibiogram sensitivity test, it cleared that *V. alginolyticus* war sensitive to enrofloxacin, ciprofloxacin, as nalidixic acid and florefenicol, these were similar with those recorded with Enany et al., (2012) and Abou El-Atta and El-Ekiaby (2012), while *A. hydrophila* was sensitive to enrofloxacin, ciprofloxacin, nalidixic acid and florefenicol, these results were similar to results recorded by Sakr and Abou El-Atta (2006), Enany et al., (2011) and Enany et al., (2012).

CONCLUSION

To overcome the bacterial and fungal infection in cultured seabream (*sparus aurata*) in earthen pond, removal of the causes of the diseases and remove the predisposing factors, improve the salinity of pond water since the minimal salinity keeping seabream was 7% by addition of salt and lime suspension at are a ratio 1:1 in concentration of 0.5% of the mixture gave a good results in controlling the fungal infection and addition of florfenicol 30mg/10kg. B.w. to control the bacterial infection, to improve the immune state of cultured fish Beta polo must be added in a concentration of 1ml/ kg food.

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الاصابات البكتيرية و الفطرية المسببة للمشاكل المرضية فى اسماك الدنيس المستزرعه فى محافظة دمياط و محاولة السيطرة عليها

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اسماك الدنيس من اسماك المياه المالحة ذات القيمة العالية وواسعة الانتشار فى جميع انحاء العالم وخاصة منطقة البحر المتوسط . استزراع اسماك الدنيس يعتبر حديثا فى مصر و يحتاج الى عمل متقدم وخاصة من ناحية التغذية و الرعاية الصحية. هذا العمل سيلقى الضوء على بعض الامراض التى تعوق استزراع مثل هذه الانواع ذات القيمة العالية حيث تم تجميع ١٢٠ سمكة مصابة من اسماك الدنيس تزن ١٥٠ جرام ± ٥ جرام و طوله ١٨ سم ± ٠,٢ سم من الاحواض الترابية الخاصة بتربية الدنيس فى محافظة دمياط و تم عمل الفحص الظاهرى و الداخلى و البكتريولوجى و الفطرى و الهستو باثولوجى للاسماك المصابة و اختبار الضراوة و الحساسية الدوائية للميكروبات المعزولة و تم تحليل جودة المياه المستخدمة فى الاستزراع.

ظاهريا كانت الاسماك المصابة غير حيوية و غير نشطة وكانت تعانى من فقدان التوازن و الاستجابة للمؤثرات الخارجية مثل الهروب و تعوم وقريبا من السطح و التنفس بصعوبة واستسقاء البطن و انزفه من مناطق مختلفة من الجسم و تعفن الذيل و الزعانف و عتامة العينين - فقدان القشور و تغير لون الجلد الخارجى- تهتكات و تقرحات للجلد على جانبى الجسم- ظهور نموات قطنية الشكل على مناطق التقرحات الجلدية و كذلك على الزعانف و الذيل و على العينين مسببة العمى- داخليا وجد احتقان للخياشيم و الكبد و وجود بقع حمراء على سطح الكبد و امتلاء الحويصلة المرارية بسوائلها و احتقان الطحال و الكلى و تجمعات دموية داخل التجويف البطنى ذو رائحة كريهة. من الفحص البكتريولوجى تم عزل ميكروب الفيبريو الجينوليتكس و الايروموناس هيدروفيلا و ذلك حسب الخواص المورفولوجية و البيوكيميائية و كذلك ال API (20) وتم عزل ١١٨ عترة من الفيبريو و ٧١ عترة من الايروموناس و تم عزل ٩٨ عترة من الفطر الافانومايسيس وذلك حسب الفحص الظاهرى و الميكروسكوبى للعينات الخاصة بالفطر و تم عمل التحليل الهستوباثولوجى للعينات وتم عمل اختبار الضراوة و الحساسية الدوائية للميكروبات المعزولة حيث وجد ان ميكروب الفيبرو و الايرو موناس كانتا حساسة للانروفلوكساسين و السيبروفلوكساسين و الفلورفينيكول وكانت مقاومة للامبسيللين و اللينكومايسين و الكولستين سلفات و النيومايسين- للتغلب على الاصابات البكتيرية و الفطرية فى اسماك الدنيس فى الاحواض الترابية يجب المحافظة على الملوحة عند درجة ٧% كحد ادنى و ذلك باستخدام مياه البحر فى الرى و اضافة الجير و الملح بنسبة ١:١ تركيز ٠,٥ % . للتغلب على الاصابات الفطرية- و اضافة ٣٠ مجم من فلورفنيكول لكل كجم وزن حى من الاسماك وتحسين الحالة المناعية و ذلك باضافة بيتابولو ١ مللى لكل كجم علف.