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PREVALENCE OF BACTERIAL INFECTION ASSOCIATED WITH *CALIGUS* INFESTATION IN CULTURED *MUGIL CEPHALUS* WITH TRIAL TO CONTROL

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Abstract

One hundred and fifty clinically infected *Mugil cephalus* 40 ±5gm and 10 ±2cm in length were collected from earthen pond from Tri-angular of El Deeba, Damietta Governorate and subjected to clinical, postmortem investigation, bacteriological and parasitological examination. Also, the physicochemical analysis of water pond holding *Mugil cephalus* were recorded. The common clinical signs observed were lethargic, listless, cease to food, excessive mucus and nervous manifestation, infested fishes were rubbed themselves on solid substrate in attempt to dislodge the crustacean parasites which appeared attached to gills, skin & fins rays, head region and buccal cavity. Opaque of skin, hemorrhagic spots scattered on all parts of infested fishes specially perianal, caudal and pectoral region, mechanical damage of skin, erosion, sometimes reach ulceration of skin were observed. Infested fishes were showed gasping of air and accumulated around the water inlet (High current water). The postmortem finding showed presence of large number of parasitic crustaceans on the body surface, also occurred in oral cavity and under gills cover. In some cases, the gills were pale with brown focci look like marbling appearance, pale liver, congested spleen and kidney. Other cases showed congested liver and enlarged gall bladder and distended with bile. The result of bacteriological examination revealed the isolation of *Vibrio alginolyticus* and *Vibrio species*. *V. alginolyticus* was isolated with 40% from infested fishes with *Caligus*. *V. alginolyticus* was isolated with high percent from necrotic skin, gills, liver and kidney respectively while in low percent from spleen and blood. The pathogenicity of isolated *V. alginolyticus* was done and recorded. The result of antibiogram revealed that, florefenicol and ciproflox were

highly effective against *V. alginolyticus*. The parasitological examination revealed that the crustacean parasites were identified as copepods of *Caligus curtus*, the total prevalence of infestation was 100% and the mean number of individuals per host was thirty five. The histopathological finding of infested skin and gills of infested *Mugil cephalus* were studied. The trial of treatment by using Butox ® 50 in dose 0.2 ml /L (10 ppm/L) as bath for 5 minutes gave effective control of *Caligus* and should be repeated after 7th day of first application.

INTRODUCTION

Aquaculture has an important role in the development and meeting the increase demand for aquatic animal production Haylor and Bland (2001). Aquaculture industry gradually developed in the world as well as in Egypt especially marine aquaculture. The health keeping of fish depend upon the relationship between fish, environment and pathogens. *Mugil* fish species culture is one of an important aquaculture activity in Egypt and other countries. The major factor which would hamper its successful development and sustainability would be diseases which may bacterial, viral or parasitic diseases Austin and Austin (1993). Parasites have recently been highlight at serious pathogenic problems in cultured mullet fish in marine and brackish water Paperna (1986). In marine cultured fish, 54% of copepod infestations are Caligids, and their impacts range from mild skin damage to stress-induced mortality of the fish Costello (2006). The Caligidae is the largest family of parasitic Copepoda which include more than 450 species classified in 33 genera Boxshall and Halsey (2004). The genus *Caligus* Müller, 1785 is the broadest genus with more than 250 species Ho *et al*., (2000). Copepoda is commonly found on fishes cultured in marine, fresh and brackish water (Paperna, 1986, Menezes *et al*., 1990, Hoole *et al*., 2001 and Vinoth *et al*., 2010). Crustacean parasites belonging to Branchiura, Copepoda, Isopoda and Amphipoda are frequenly found on the body surface and/or gills of marine, fresh and brackish water fishes Venmathi *et al*., (2009). In Egypt, such parasites were isolated from mullet *Mugil cephalus*

Eissa (2004) and Noor El Deen (2012). Heavy infestation with *Caligus* in large numbers don't actually kill the fish but the growth rate and market value are reduced, also heavy infestation with *Caligus* cause emaciation, abrasions and in severe cases where the epidermis is breached death may be occurred due to loss of physiological homeostasis including osmotic stress, anemia and hypoprotein anemia Woo *et al*., (2002). The losses associated with *Caligus* infestation are the result of direct mortality due to immune state decreasing and suppressed and so secondary bacterial infection occurs (Lin *et al*., 1994 and Ho *et al*., 2000) specially vibriosis. *Vibrio alginolyticus* was isolated from marine, brackish and fresh water fishes as well as *Mugil cephalus* Wafeek *et al*., (2007). The present study was planned to the most common crustacean parasites specially copepod *Caligus* species and related bacterial infection affecting *Mugil cephalus* cultured in marine water and possible control and treatment using nature and chemotherapeutic agent.

MATERIALS AND METHODS

Naturally infected fish:

One hundred and fifty clinically moribund and diseased *Mugil cephalus* weighted 40 ±5gm and 10 ± 2 cm in length were collected from earthen pond from Tri-angular of El Deeba, Damietta Governorate and immediately were subjected to full clinical examination and postmortem finding also, bacteriological and parasitological investigation.

Water quality analysis:

The physico-chemical analysis of water holding *Mugil cephalus* were done in the field immediately for measuring the water parameters as (temperature, salinity, pH, dissolved oxygen, saturation, electric conductivity, alkalinity, nitrate, nitrite, phosphate and total solid). The physicochemical analysis of water samples were carried out in accordance to standard analytical methods APHA (2005).

Clinical investigation and Post-mortem examination of fish:

Moribund and clinically diseased fish were properly examined for any external clinical abnormalities and lesions and clinical alternations on the skin, scales eyes, abdomen, fins and any abnormal behaviors. The postmortem examination was done on moribund fish or freshly dead fish to examine all internal organs including gills, liver, kidney, spleen and intestine. The clinical investigation and postmortem examination were done according to Amlacher (1970).

Bacteriological examination:

Samples for bacteriological examination collected under aseptic precaution from gills, liver, spleen, skin ulcers, blood, kidney and fins and inoculated onto tryptic soy broth (TSB) and streaked tryptic soy agar (TSA) with 1% sodium chloride (NaCl), the suspected purified colonies were picked up and streaked over specific media as Thiosulphate Citrate Bile Salt sucrose Agar (TCBS) (Biolife, Milan, Italy). The inoculated media were incubated at 25°c for 24-48 hrs; the isolated bacteria were subjected to taxonomical analysis according to Bergey's Manual of Determinative Bacteriology (1994).

Pathogenicity test:

Thirty *Mugil cephalus* were collected alive and apparently healthy with average body weight 40 ±5gm kept in glass aquaria for two week and fed with 5% body weight commercoid ration and divided into 3 groups each group contain 10 fish (10 fish/group); 1st group injected I/P with isolated strain in a dose 1×10^{7} cfu/ml. 2nd group injected I/M with same dose and the 3rd group injected with 0.5 ml of sterile saline, injected fish were observed daily for 10 days to record any clinical signs behaviors and daily mortalities. Postmortem examination was done on freshly dead fish to record gross signs and reisolation of injected strain.

Antibiogram sensitivity:

The antibiogram sensitivity test was done according to the limits givin by Schäperclaus *et al*., (1992) using disc diffusion method on Muller's Hinton agar medium and interpretations of zones of inhibitions were recorded. The antibiotic testes used were ciprofloxacin, colistin sulphate, rifampine, florefenicol, lincomycin, nalidixic acid.

Parasitological examination:

Macroscopic examination was done for detection of any abnormalities in different parts of fish body by naked eyes and hand lens. Skin, fins, gills, eyes and opercula were dissected and examined for presence of parasitic crustaceans. The attached crustaceans to the gills, skin, fins, head region and buccal cavity were carefully removed with the help of needle and soft brush, under a low power binocular microscope, washed with distilled water, They were fixed in 3% formol saline, preserved in equal amount of 70% alcohol-5% glycerin and permanent amounts were prepared by passage in descending grades of alcohol (70, 50 and 30%), cleared in glycerin and mounted in glyceringelatin according to Lucky (1977). The isolated Copepods species were identified according to Kabata (1988).

Histopathological examination:

Samples for histopathological examination were freshly taken from skin and gills of infested fish. Histopathological techniques were carried out according to Roberts (2001).

Treatment trials:

Forty infested *Mugil cephalus* collected from the same pond with average 40 ±5gm body weight and 10 ± 2 cm in length kept in glass aquaria 40×40×80 cm at the same environment specially salinity and divided into 4 groups each group contain 10 infested fish and treatment trials applied as the following:

- 1-Fresh water treatment, it was as a dip for 20 minutes
- 2- Butox ® 50 in a dose 0.1ml/L (5ppm) for 5 minutes
- 3- Butox ® 50 in a dose 0.2ml/L (10ppm) for 5 minutes
- 4- Butox ® 50 in a dose 0.3ml/L (15ppm) for 5 minutes

RESULTS

Results of physico-chemical analysis of water:

The physico-chemical analysis of water holding *Mugil cephalus* were recorded in Table (1) which considered in normal limit.

Results of clinical signs:

The results of clinical investigation of infested *Mugil cephalus* in cultured pond were lethargic, listless, cease feeding, nervous manifestation, infested fish with *Caligus* were rubbed themselves on solid substrate, excessive mucus. *Caligus* appeared attached to fish in different area of the body gills, skin and fins and buccal cavity. Opaque of skin Plate(1A), frayed fins, hemorrhagic spots scattered on all parts of the body specially perianal, caudal and pectoral region Plate(1B); in severe case fish were observed mechanical damage of skin, erosin and ulceration Plate(1C), also fish infested with *Caligus* showed gasping of air and accumulation around the water inlet (High current water).

Results of postmortem findings:

Infested fish showed presence of large number of *Caligus* on the body surface, also occur in oral cavity and under gill cover, the total number of *Caligus* different from fish to another almost reach 35 *Caligus* /fish. Pale gills with brown focci look like marbling appearance, pale liver and all internal organs; but most infected cases showing congested gill and liver while gall bladder distended with bile, congested spleen and kidney and accumulation of blood fluid in abdominal cavity, Plate (1D).

Results of bacteriological examination:

The result of bacteriological examination revealed the isolation of gram negative bacteria and according to cultural, morphological and biochemical characters recorded in Table (2) it was identified as *Vibrio alginolyticus* and *Vibrio species*. As showed in Table (3) the infection with *Vibro* bacteria reached 40% from total infested from total infested with *Caligus* which reached 100%. The distribution of isolated *V. alginolyticus* in different organs of infected *Mugil cephalus* as shown in Table (4). The *V. alginolyticus* isolated with high number from skin 47 strain reach 27.32% follwed by gills 40 strains with 23.25%, liver 35 strains with 20.34%, kidney 21 strains with 12.20%, spleen 17 strains with 9.88% finally blood 12 strains with 6.97%.

Results of pathogenicity:

As shown in Table (5) the clinical sings seen after 24h post I/P injection with *V. alginolyticus*, included inflammatory changes at the site of inoculation, hemorrhages allover the body surface in different parts of the body, congested gills, liver, spleen and kidney showed internally. Reisolation of *V. alginolyticus* was obtained on TCBS agar from freshly dead *Mugil cephalus*. Intraperitoneal injection I/P was high effect than I/m route which cause100% while I/M cause 70% mortality.

Results of antibiogram sensitivity:

As shown in Table (6), the antibiogram sensitivity of *V. alginolyticus* revealed that it was highly sensitive to florefenicol and ciprofloxacin and moderate sensitive to nalidixic acid and rifampine while it was resistant to colistin sulphate and lincomycin.

Results of parasitological examination:

The parasitological examination revealed severe infection by large numbers of crustacean species which was attached firmly to gills, skin and fins and buccal cavity of *Mugil cephalus* Plate (1A). Some

development stages were found, Plate (2). All isolated crustacea which were collected from *Mugil cephalus* according to the morphological characters were belonged to the Copepoda, family Calgidae and were identified as *Caligus curtus* Plate (3).

The morphological description of *Caligus curtus*:

The body length of the male measures 5.97 mm and the greatest width measures 2.91 mm. The cephalothorax is nearly as long as wide. Genital complex roughly circular, with protruding posterolateral corners, more than 1/2 length of thoracic zone of dorsal shield. Abdomen one-segmented, more than 1/2 length of genital complex. The caudal rami are longer than wide. Second antenna with bifid terminal claw. The body length of the female measures 5.53 mm and the greatest width measures 2.66 mm. The cephalothorax is nearly as long as wide. Genital complex of equal length with thoracic zone of dorsal shield. Abdomen one-segmented, less than 1/2 length of genital complex. The caudal rami are longer than wide. Female characterized by long bar-shaped egg pouches or strings.

The prevalence of *Caligus curtus* infestation in *Mugil cephalus* fish:

Table (3) showed the total prevalence of *Caligus curtus* in examined *Mugil cephalus* fish which was 100%.

Results of histopathological examination:

The results of histopathological examination of skin of affected *Mugil cephalus* infested with *Caligus curtus* and infected with bacteria showed hyperplasia of club cells of the epidermis, congested and hemorrhagic dermis with excessive aggregation of round cells, melano macrophage cell and edema of the dermis as shown in Plate (4A).

The results of histopathological examination of gills of affected *Mugil cephalus* infested with *Caligus curtus* showed that the parasite induced sever congestion of branchial blood vessels, telangectiasis, also

causing sloughing of secondary lamellae and desquamation of secondary lamellar epithelium as shown in Plate 4 (Fig B to Fig H).

Results of treatment trials:

Treatment trials of naturally infested *Mugil cephalus* with *Caligus curtus* at field area using freshwater for 10 hrs and Butox ® 50 in different concentration as bath for 5 minutes exposure as shown in Table (7). It was noted that, the freshwater treatment gave a good result in control of *Caligus curtus* attached to *Mugil cephalus* as a bath for 10 hrs. While the using of Butox ® 50 at different concentration 0.1 ml/L (5ppm), 0.2ml/L (10ppm) and 0.3ml/L (15ppm) revealed that application of 0.2ml/L (10ppm) as a bath for 5 minutes considered the best chemical and effective to eliminate *Caligus curtus* which infested and affect *Mugil cephalus* in marine aquaculture and has no effect on healthy state of treated fish.

Table (1): Physico-chemical analysis of water holding *Mugil cephalus*.

Pond Number	Water parameters										
	Temperature	Salinity	pH	DO	Saturation	EC	Alkalinity	NO2	NO3	PO4	TS
1	28°c	25%	7.8	5mg/L	57%	11000	100 mg/L	0.002 mg/L	0.1 mg/L	0.2 mg/L	140 mg/L
2	29°c	26%	7.8	4mg/L	54%	10000	100 mg/L	0.002 mg/L	0.3 mg/L	0.5 mg/L	110 mg/L

DO = Dissolved oxygen, NO2 = Nitrite, NO3 = Nitrate, PO4 = Phosphate, EC = Electric conductivity, TS = Total solid.

Table (2): Morphological and biochemical characters of isolated *vibrio.*

Test	Reaction
Gram stain	- ve
Mortality	swarming mortality
TCBS agar	yellow colonies
Oxidase	+ ve
OIF	+ Fermentive
Growth at	
29°c	+ ve
37°c	+ ve
43°c	+ ve
Growth on NaCl%	- ve
0%	+ ve
3%	+ ve
5%	+ ve
7%	+ ve
10%	+ ve
Indole	+ ve
V.P	+ ve
M.R	+ ve
H2s production	- ve
Citrate utilization	+ ve
Arginis hydrolysis	- ve
Catalase	- ve
Gelatin liquefaction	+ ve
Fermentation of sugar	
glucose	- ve
arabinose	- ve
sucarose	- ve
lactose	- ve

Table (3): The obtained data about infestation and infection.

Total No. of examined fish	No. of infested fish with *Caligus curtus*	No. of infected fish with bacteria	No. of samples for bacteriology	Total isolated
150	150	60	360	172
%	100	40	60	47.77

Table (4): Prevalence and distribution of isolated *V. alginolyticus* in different organs and tissues of infected *Mugil cephalus*.

Causative agent	No. of isolates	Gills		skin		liver		Kidney		Spleen		Blood	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
V. alginolyticus	172	40	23.25	47	27.32	35	20.34	21	12.20	17	9.88	12	6.97

Table (5): Pathogenicity and mortality rate of experimentally infected *Mugil cephalus* with *V. alginolyticus*.

Group	Dose of injection	Rate of injection	Fish No.	Dead fish during 10days										No. of dead	Mortality rate %
				1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1st	1×10^7 cfu	I/P	10	1	2	3	3	1	0	0	0	0	0	10	100%
2nd	1×10^7 cfu	I/M	10	0	1	1	2	1	1	1	0	0	0	7	70%
3rd	Sterile saline	I/P	10	0	0	0	0	0	0	0	0	0	0	0	0

Table (6): Antibiogram of sensitivity of *V. alginolyticus*.

Antbiotic disc	Code symbol	Concentration (µg)	Reaction
Ciprofloxacin	Cip5	5	(S)+++
Colistin Sulphate	CL10	10	R
Rifampine	RD30	30	(R)
Florefenicol	Ffc30	30	(S)+++
Lincomycin	L2	2	R
Nalidixic Acid	NA30	30	(S)++

Table (7): Condition of *Mugil cephalus* and copepods after different treatment and time of exposure to remove *Caligus curtus* from *Mugil cephalus*.

Group	No. of fish	Treatment	Time of exposure	Concentrate	Fish condition	Copepod condition	No. of attached copepods before treatment	No. of attached copepods after treatment
1st	10	Freshwater	12hrs		Good	Dead	30-40/ Fish	0
2nd	10	Butox ® 50	5 minutes	0.1ml/L (5ppm)	Good	Inactive	30-40/ Fish	4-5/Fish
3rd	10	Butox ® 50	5 minutes	0.2ml/L (10ppm)	Good	Dead	30-40/ Fish	0
4th	10	Butox ® 50	5 minutes	0.3ml/L (15ppm)	Stressed	Dead	30-40/ Fish	0

DISCUSSION

The success in aquaculture industry depend upon the selection of rearing species of fish, healthy aquatic environment and realizing the relationship between fish and pathogens or causative agent, regarding the physico-chemical analysis of water holding *Mugil cephalus*, they were in

normal range with exception slight decreasing in dissolved oxygen. The clinical investigation of affected *Mugil cephalus* showed that, lethargic, listless, cease feeding, nervous manifestation these sings were due to attachment of *Caligus curtus* with fish, excessive mucus secretion due to irritation. Infested *Mugil cephalus* with *Caligus curtus* rubbed themselves on solid substrate to try dislodge of parasite from their body, infested fish with *Caligus curtus* accumulate around high water current at water inlet and gasping of air at the water surface, these sings due to contact of parasites on gill filaments causing gill damage, destruction of gill tissue and secondary bacterial infection were occur resulted in death of infected fish, these result were nearly similar to that recorded by (Eissa, 2004, Lester and Hayward, 2006 and Noor El Deen *et al*., 2012). Opaque of the skin, frayed fins, hemorrhagic spots scattered on all parts of the body, erosion and ulceration occur in severe infested cases and mortality was occur, these signs as a result of penetration of skin with *Caligus curtus* for feeding while erosion and ulceration occur due to infested fish with rubbed themselves against solid substrate leads to physiological hemeostasis including osmotic stress anemia, hypoproteinanemia, immune state decreasing and suppressed, facilitate the invasion with secondary bacterial infection specially *Vibrio* species which it occur ready in water environment, these result agree with (Lin *et al*.,1994, Yambot and Lopez 1997, Ho *et al*., 2000, Johnonson *et al*., 2004, Lester and Hayward, 2006 and Noor El Deen *et al*., 2012). The postmortem examination of the affected *Mugil cephalus* showed presence of large number of *Caligus curtus* in gills, skin and fins and buccal cavity, the gills were pale with brown focci look like marbling appearance, these result agree with El-Lamie (2007) and Noor El Deen *et al*., (2012) but in most infected cases showing congested gill, liver, kidney and spleen and accumulation of blood fluid in abdominal cavity, these result were due to septicemia which caused by secondary bacterial infection specially *Vibrio* species which secret it's toxins and protedytic enzymes; the

accumulation of bile secretion in gall bladder was due to hepatomegaly which pressed on bile duct and ductules these result agree with Wafeek *et al*. (2007) and Enany *et al*. (2011). The results of bacteriological examination revealed isolation of *Vibrio* bacteria according to culture characters on solid media (TCBS) agar, morphological and biochemical characters identified as *Vibrio alginolyticus* which inhibit marine and brackish water and highly distributed between wild and cultured marine fishes. *V. alginolyticus* isolated from infested *Mugil cephalus* with high percentage reach 40%, these results agree with Samaha *et al*., (2004), Wafeek *et al.* (2007) and Enany *et al*. (2011). *V. alginolyticus* act as primary and secondary infected pathogen, it isolated with high percentage from skin, gills, liver, kidney and spleen and isolated with low percentage from blood system as shown in Table (4), these due to infestation with *Caligus curtus* which leads to erosion and ulceration of skin and excessive mucus secretion and decrease immune state and suppressed immune system these accelerate and enhance the bacterial infection, these result nearly accepted with those mentioned with Wafeek *et al*. (2007) and Enany *et al*. (2011). The result of pathogenicity of *V. alginolyticus*, showed that the *V. alginolyticus* was highly virulence which cause 100% mortality after injection I/P route while it cause 70% mortality after injection I/M route. So, *V. alginolyticus* considered as primary and secondary bacterial infection for fish and out break, these results agreed with Wafeek *et al*. (2007), El-Adawy (2010) and Enany *et al*. (2011) who recorded that I/P injection of *Mugil capito* with *V. alginolyticus* cause 100% mortality and gave the same clinical and postmortem lesions of naturally infection with *V. alginolyticus*. About the result of antibiogram sensitivity of isolated strain *V. alginolyticus* it was highly sensitive to florefenicol and ciprofloxacinand moderate sensitive to nalidixic acid and rifampine while it was resistant to colistin sulphate and lincomycin, these results agree with Wafeek *et al*. (2007) who recorded that *V. alginolyticus* was

sensitive to ciprofloxacin and agree also with Enany *et al*. (2011) who recorded that *V. alginolyticus* isolated from *Mugil capito* was sensitive to ciprofloxacin and moderate sensitive to nalidixic acid and rifampine while it was resistant to colistin sulphate and lincomycin. From obtained data, florefenicol was newly introduced in fish field for control of bacterial infection especially *V. alginolyticus*. The parasite under discussion isolated from *Mugil cephalus* this agrees with Eysa, and Abu-El-Wafa (1995) and Marzouk *et al*. (2001) who obtained the same species from the same host. Also, Eissa (2004) and Noor El Deen *et al*. (2012) recorded the same genus from the same host, while disagree with Hogans and Trudeau (1989) and Hamre *et al*. (2011) who obtained the same species from different host. The morphological characters and measurements of *Caligus curtus* were similar to that obtained by Kabata (1988). Regarding crustacean infestation rate, the total prevalence of infestation was 100% and these results were much higher than that obtained by Marzouk *et al*. (2001) who recorded the same parasite from the same host as the rate was 11%. This difference may be attributed to the locality from which fish samples obtained. In the present study majority of the *Caligus curtus* were adult, but also some developmental stages were found such as copepodid stage chalimus stage and preadult stage this agree with Lin *et al*. (1997) and Pike and Wadsworth (1999) who recorded that Caligid copepods have direct life cycles consisting of 2 free-living planktonic nauplius stages, 1 free-swimming infectious copepodid stage, 4 attached chalimus stages, 2 preadult stages, and 1 adult stage. The results of histopathological examination of skin of affected *Mugil cephalus* infested with *Caligus curtus* and infected with bacteria revealed hyperplasia of club cells of the epidermis, congested and hemorrhagic dermis with excessive aggregation of round cells, melano macrophage cell and edema of the dermis. These results may be due to the parasite and bacteria which a brad the skin surface and feed on cutaneous and subcutaneous tissue causing very extensive destruction.

The present findings nearly agree with results reported by Udomkusonsri *et al*. (2004) and Claudia and Jeffrey (2009). While the histopathological examination of infested gills revealed that the parasite induced sever congestion of branchial blood vessels, telangectiasis and causing sloughing of secondary lamellae and desquamation of secondary lamellar epithelium. These results may be attributed to the mechanical injury induced by the parasite that ingest infiltrated cells and epithelial cells that proliferate due to stimulation of attachment leads to slow hemorrhage, rapid blood clot, thrombus, ischemia and finally necrosis which manifests the picture of marbling appearance. These results nearly agree with that recorded by Eissa *et al*. (1996) and Noor El Deen *et al*. (2012). About treatment trials of naturally infested *Mugil cephalus* with *Caligus curtus* were applied using both of freshwater and Butox ® 50 in different concentration as shown in Table (7). It was noted that, the freshwater treatment gave effective treatment against attached *Caligus curtus* after exposure for 10 hrs, the free *Caligus curtus* were completely dead, these attributed to the parasite *Caligus curtus* cannot tolerate osmotic change and concentration. So, freshwater considered as natural control of *Caligus* which infest *Mugil cephalus* in marine water, these results agree with Osmanov and Yusupov (1985), Hogans and Trudeau (1989) and Noor El Deen *et al*. (2012) who recorded that, the freshwater treatment of *Caligus* attached to *Mugil cephalus* gave effective treatment within 12 hrs. The using of Butox ® 50 at different concentration 0.1 ml/L (5ppm) as a bath not effective for control because the *Caligus* still attached with *Mugil cephalus*, but the using of Butox ® 50 at concentration 0.2 ml/L (10ppm) as a bath gave effective treatment and control of all attached *Caligus curtus* and free *Caligus curtus* in glass aquaria were completely dead with no effect on health state of fish, while the using of Butox ® 50 at concentration 0.3 ml/L (15ppm) as a bath for five minutes gave effective treatment and control on attached *Caligus curtus* but has effect on health state and causing stress on treated fish. In the present study, from

concluded results florefenicol can used for control of bacterial infectionin concentration 30µg/kg body weight fish and using freshwater to control *Caligus curtus* affect *Mugil cephalus* in brackishwater and using Butox ® 50 at concentration 0.2ml/L (10ppm) as a bath for 5 minutes for control *Caligus curtus* attached with *Mugil cephalus* in marine water environment.

CONCLUSION

To control *Caligus* infestation in cultured *Mugil* farms must be apply the management strategies such as the farm should be located in which the water current is strong to flash away copepod stage; using wooden slats for trapping eggs of parasite; also, filtering of incoming water to remove larval stages of parasite and finally stocking clean fish and quarantining incoming fish with suitable treatment before stocking. For treatment of *Caligus* using freshwater for treating *Caligus* in brackishwater *Mugil* farm and Butox ® 50 for treating *Caligus* in marine *Mugil* farm as a bath for 5 minutes exposure at concentration 0.2ml/L (10ppm).

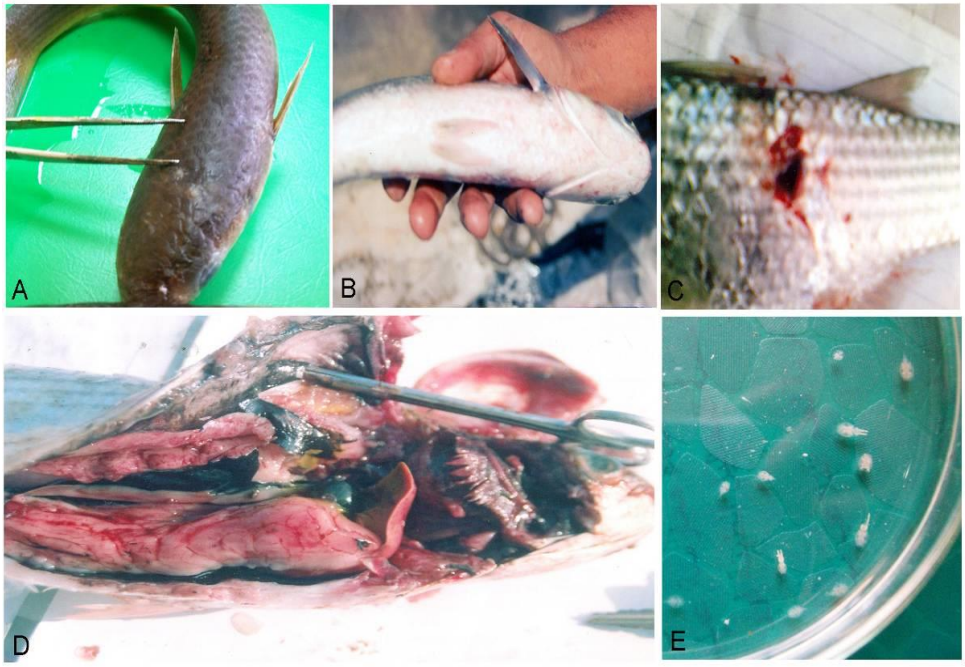

Plate (1): A- Affected *Mugil cephalus* showing opaque of skin and brownish *Caligus* attached to skin. B- Affected *Mugil cephalus* showing hemorrhagic spots scattered on different parts of the body from ventral region. C- Infested *Mugil cephalus* showing different hemorrhage, erosion and irregular ulceration. D- Infested *Mugil cephalus* internally showing excessive mucous on gills and brown focci like marbling appearance, congested and hemorrhaged liver, kidney, spleen and accumulated bloody fluid in abdominal cavity. E- *Caligus curtus* recovered from infested fishes.

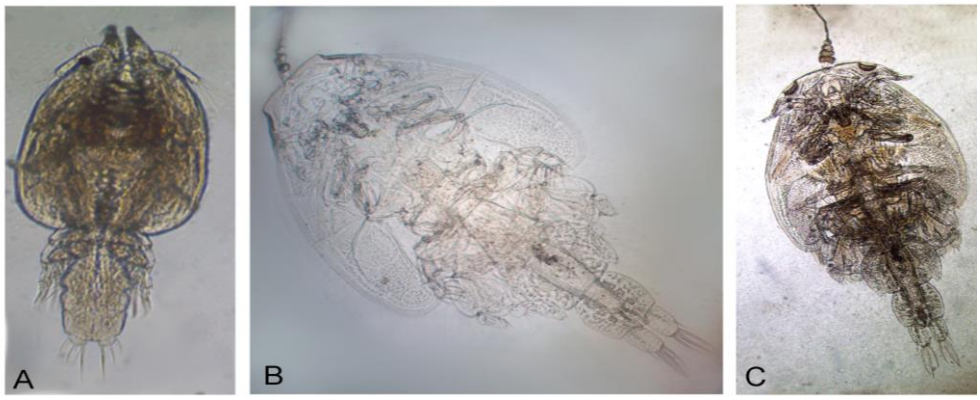

Plate (2): Light photomicrograph of some developmental stages of *Caligus curtus* showing A- Copepodid stage (X 40); B-Fourth chalimus stage (X 40); C- Pre-adult stage (X 40).

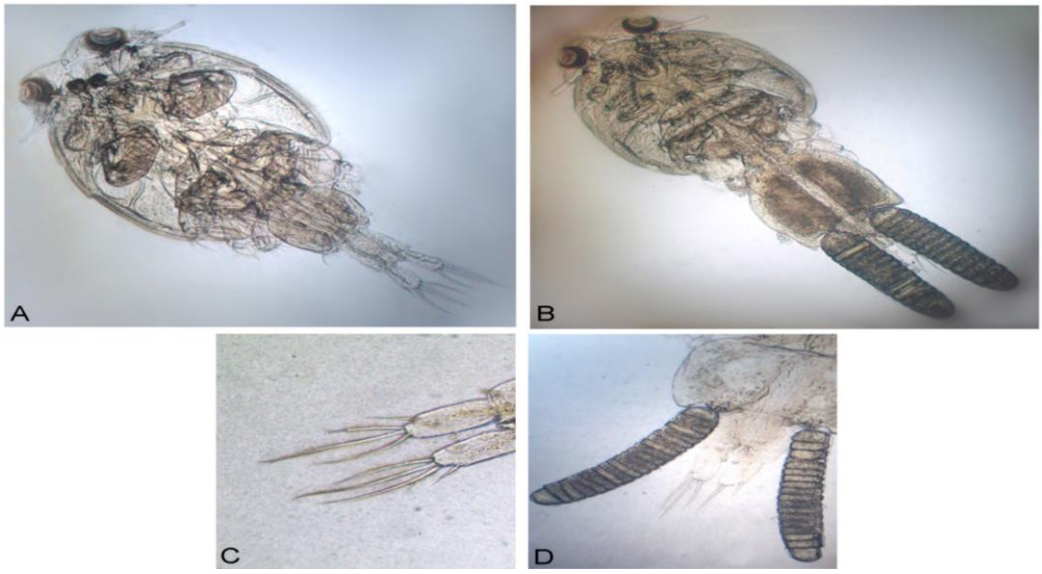

Plate (3): Light photomicrograph of *Caligus curtus* showing A-Adult male (X 40); B-Adult female (X 40); C-Caudal ramai (X 100); D-Egg strings (X 100).

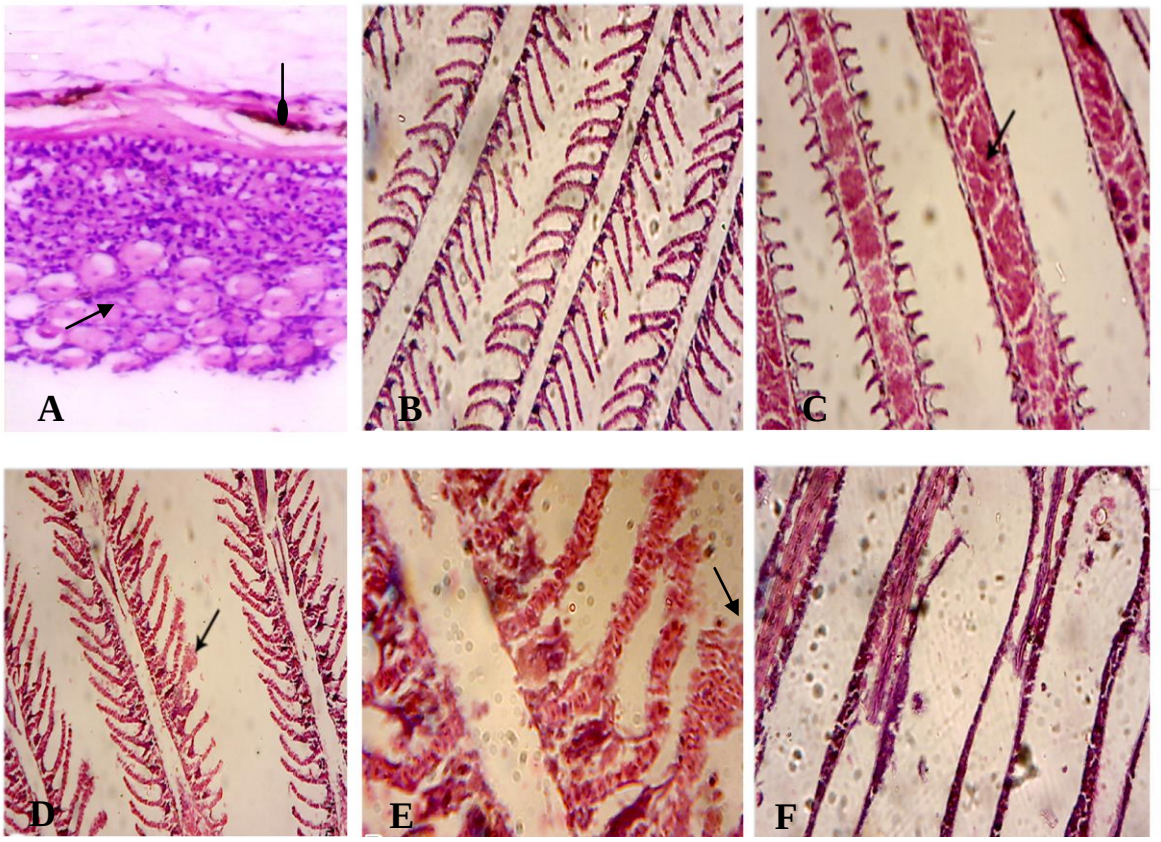

Plate (4): A- Photomicrograph of the skin section of *Mugil cephalus* showing hyperplasia of club cells of the epidermis "arrow", congested and hemorrhagic dermis with excessive aggregation of round cells, melano macrophage cell and edema of the dermis "oval arrow" (H&E X 300). B- Photomicrograph of the gill section of *Mugil cephalus* in control showing normal architecture of gills (H&E X 300).C- Photomicrograph of the gill section of *Mugil cephalus* infested with *Caligus curtus* showing sever congestion of branchial blood vessels "arrow" (H&E X 300). D- Photomicrograph of the gill section of *Mugil cephalus* infested with *Caligus curtus* showing telangectiasis "arrow" (H&E X 300).

E-High power of Fig. (c) showing telangectiasis "arrow" (H&E X 1200) F- Photomicrograph of the gill section of *Mugil cephalus* infested with *Caligus curtus* showing sloughing of secondary lamellae and desquamation of secondary lamellar epithelium (H&E X 300).

REFERENCES

Amlacher, E. 1970. Text book of fish diseases. TFH Publication, New Jarsey, U.S.A., 117-135.

APHA. 2005. Standard methods for the examination of water and waste water, 21th edition, Washington, American Public Health Association.

Austin, B. and D.A. Austin. 1993. Bacterial fish pathogens disease in farmed and wild Fish. (2nd Ed.), Ellis Horwood Ltd. Chichester, New York, London, England. 384 pp.

Bergey, D.H. 1994. Bergey's manual of determinative bacteriology, ed. Buchanan, R.E. and Gibbons, N.E. 9th ed. Baltimore, Williams and Wilkins.

Boxshall, G.A. and S.H. Halsey. 2004. An introduction to copepod diversity. The Ray Society, London. 966 pp.

Claudia, H. and C. Jeffrey. 2009. Wolf morphologic effects of the stress response in fish ILAR Journal, 50 (4): 387-396.

Costello, M.J. 2006. Ecology of sea lice parasitic on farmed and wild fish. Trends Parasitol. 22: 475-483.

Eissa, I.A.M. 2004. Parasitic fish diseases in Egypt. Dar El- Nahda El-Arabia publishing, 2nd Edit. 23 Abd El- Khalak Tharwat St. Cairo, Egypt.

Eissa, I.A.M.; A.S. Diab and A.F. Badran. 1996. Studies on some internal parasitic diseases among wild and cultured *Oreochromis*

niloticus. 7th Sci. Cong., 17-19. Fac. Vet. Med., Assiut, Egypt, 274-289.

El Adawy, M.M. 2010. Studies on bacterial pathogens affecting in cultured *Mugil Capito*. M. V. Sc. Thesis, Fac. Vet. Med., Suez Canal University.

El-Lamie, M.M. 2007. Studies on the parasitic diseases in some marine fish. Ph. D. Thesis, Fac. of Vet. Med., Dept. of Fish Diseases and Management, Suez Canal University.

Enany, M.E.; H.M. Ibrahim; M.E. Abou El Atta; and M.M. El Adawy. 2011. Bacteriological and Histopathological studies on some bacterial pathogens diseases in cultured *Mugil Capito* in Ismailia Governorate. SCVMJ., (1): 1-10.

Eysa, M. and Abu-El-Wafa, S.A. 1995. Pathological studies on an epidemic of *Caligus curtus* among captive *Mugil* and *Sparus* in Egypt with reference to Malathion control. J. Applied. Aquaculture, 5 No. 2: 25–30.

Hamre, L.A.; B.T. Lunestad; R. Hannisdal and O.B. Samuelsen. 2011. An evalulation of the duration of efficacy of emamectin benzoate in the control of *Caligus curtus* Muller infestations in Atlantic cod, Gadus morhua L. Journal of Fish Diseases, 34 (6): 453-457.

Haylor, G. and S.A. Bland. 2001. Integrating aquaculture into rural development in coastal and inland areas. In Aquaculture in the third millennium technical., 73-81.

Ho, J.S.; C.L. Lio and S.N. Chen. 2000. Species of *Caligus* Müller, 1785 (Copepoda: Caligidae) parasitic on marine fishes of Taiwan, 46 (3): 159-179.

Hogans, W.E. and D.J. Trudeau. 1989. *Caligus elongatus* (Copepoda: Caligoida) from Atlantic salmon (*Salmo salar*) cultured in in

marine waters of the lower Bay of Fundy. Canadian Journal of Zoology, 67: 1080–1082.

Hoole, D.; O. Bucke; P. Burgess and I. Wellby. 2001. Diseases of carp and other cyprinid fishes, Fishing News Books, A division of Blackwell Science, Ltd., Oxford, UK, 264pp.

Johonson, S.C.; J.W. Treasurer; S. Bravo; K. Nagasawa and Z. Kabata. 2004. A review of the impact of parasitic copepods on marine aquaculture. Zoological Studies, 43: 229-243.

Kabata, Z. 1988. Copepoda and Branchiura. p. 3-127. *In* L. Margolis and Z. Kabata (eds.) Guide to parasites of fishes of Canada. Part II. Crustacea.. Can. Spec. Publ. Fish. Aquat. Sci. 101. 184pp.

Lester, R.J.G. and C.J. Hayward. 2006. Phylum Arthropoda, pp 466-565. In P.T.K., Woo. (ed.). Fish diseases and disorders Vol 1: Protozoan and Metazoan Infections. 2nd edition. CAB international, London.

Lin, C.C.; J.S. Ho and S.N. Chen. 1994. Two species of *Caligus* (Copepoda, Caligidae) parasitic on black sea bream (*Acanthopagrus schlegeli*) cultured in Taiwan. Fish Pathology, 29: 253–264.

Lin, C.L.; J.S. Ho and S.N. Chen. 1997. Development of *Caligus multispinosus* Shen, a caligid copepod parasitic on the black sea bream (*Acanthopagrus schlegeli*) cultured in Taiwan. Journal of Natural History, 31: 1483–1500.

Lucky, Z. 1977. Methods for the diagnosis of fish diseases American Publishing Co., Pvt. Ltd., New Delhi, Bombay Calcutta and New York., 1977.

Marzouk. M.S.M.; N. Ezz El Din; M.E. Mohamed; A.M. Mahmoud and S.M. Rawia. 2001. Epizological and pathological investigations

on fishes of Lake Manzala infested with some gill and intestinal parasites. Egypt. J. Aquat. Biol. and fish, (2): 901-913.

Menezes, J.; M.A. Ramos; T.G. Pereira and A.M. da Silva. 1990. Rainbow trout culture failure in small lake as a result of massive parasitosis related to careless fish introductions. Aquaculture, 89: 123-126.

Noor El- Deen, A. E; O.K. Abdel Hady; S.I. Shalaby and S. Z. Mona. 2012. Field studies on *Caligus* disease among cultured *Mugil Cephalus* in brackish water fish farms. Life Science Journal, 9 (3): 733-737.

Osmanov, S.O. and O. Yusupov. 1985. Influence of the increasing salinity of the Aral Area on the *Mugil cephalus* parasite. Fauna. Parazitologicheskii- Sbornik, (33): 14- 43.

Paperna, I. 1986. Parasitic infestation and diseases of fish in Africa. FAO, CIFA Technical paper, 51- 62.

Pike A.W. and S.L. Wadsworth 1999. Sea lice on salmonids: their biology and control. Advances in Parasitology, 44: 234-337.

Roberts, R.J. 2001. Fish pathology 3rd ed . WB. Saunders, Philadiphia, PA. U.S.A., 472 pp.

Samaha, H.A.; Y.N. Hagga and M. Nadia. 2004. Brackish and marine water fish as a source of certain bacterial pathogens to human beings. In the Proceedings of the 4th Sci. Conference for Vet. Med. Researches, Fac. Vet. Med., Alex. Univ., 2-4 October.

Schäperclaus, W.; H. Kulow and K. Schreckenbach. 1992. Fish diseases, Vol. I. A.A. Balkema /Rotterdam, septicemia of fish, Fish disease leaflet 68(1- 24).

Udomkusonsri, P; E.J. Noga and N.A. Monteiro-Riviere. 2004. Pathogenesis of acute ulceration response (AUR) in hybrid striped bass. Dis Aquat Organ, 61: 199-213.

Venmathi, B.A.; L.T. Seng; S. Ohtsuka and K. Nagasawa. 2009. Record of *Caligus* (Crustacea: Copepoda: calgidae) from Marine Fish Cultured in floating Ponds in Malaysia with a Redescription of the Male of *Caligus longipedis* Bassett-Smith, 1898. Zoological studies, 48 (6): 797-807.

Vinoth, R.; T.T. Ajith Kumar; S. Ravichandran; M. Gopi and G. Rameshkumar. 2010. Infestation of copepode parasites in food fishes of Vellar Estuary, southeast coast of India. Acta Parasitologica Globalis, 1 (1): 01-05.

Wafeek, M; M. Abo El Atta and G. Ebrahim. 2007.Heavy metals and bacterial distribution in different rgans of Gray mullet (*Mugil Cephalus*)cultured in different environmental conditions. Egypt. J. Aquat. Biol. and fish, 11 (3): 1125-1144.

Woo, P.T.K; D.W. Bruno and L.H.S. Lim. 2002 Infectious diseases of warm water Fish in marine and brackish waters. CAB international 2002. Diseases or disorders of finfish in cage, 217-219.

Yambot, A.V. and E.A. Lopez. 1997. Gill parasite *Lambroglena monody*, Capart, infecting the Nile tilapia, *Oreochromis niloticus* L., cultured in philipines. Proceedins of the 3rd Symposium on Diseases in Asian Aquaculture, Bangkok,Thailand, 175-177.

معدل انتشار العدوى البكتيرية المرتبطة بالاصابة بقمل السمك فى أسماك البورى المستزرعة و محاولة السيطرة عليها

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الملخص العربى

بفحص عدد ١٥٠ سمكة مريضة ظاهريا من اسماك البورى تم تجميعها من منطقة مثلث الديبة– محافظة دمياط حيث تم فحصها ظاهريا و بكتريولوجيا و طفيليا. وكذلك تم قياس بعض الصفات الفيزيائية والكيميائية للمياة. أسفر الفحص الاكلينكي للأسماك المصابة إنها كانت تعانى من رفض الغذاء، التوتر، زيادة كمية المخاط على الجسم و اعراض عصبية حيث كانت الأسماك المصابة بقمل السمك تحك جسمها بالمواد الصلبة داخل الحوض فى محاولة للتخلص من قمل السمك مع وجود عتامة الجسم ، انتشار البقع النزيفية والتقرحات الجلدية على معظم اجزاء الجسم كما أظهرت الأسماك المصابة التراكم حول مدخل المياة. وأظهرت الصفة التشريحية وجود اعداد كبيرة من قمل السمك على سطح الجسم و فى تجويف الفم وعلى الخياشيم وكانت الخياشيم فى معظم الأسماك باهتة اللون و كذلك الكبد و البعض الاخر وجد إن الأعضاء الداخلية مختنقة. من الفحص البكتريولوجى تم عزل ميكروب الفيبرو الجينولتكس بنسبة ٤٠% من الأسماك المصابة بقمل السمك بنسبة عالية من التقرحات الجلدية، الخياشيم، الكبد و الكلى على التوالى و بنسبة اقل من الطحال و الدم. وقد وجد ان هذا الميكروب حساس للسيبروفلوكساسين و الفلوروفنيكولز و ان ميكروب الفيبرو الجينولتكس يحدث نفوق بنسبة ١٠٠% عند الحقن البريتونى. طفيليا تم عزل نوع من القشريات الطفيلية (كاليجس كارتس) كما تمت مناقشة ووصف التركيب المورفولوجى للطفيل وكانت النسبة الكلية للاصابة ١٠٠% حيث كان متوسط الأصابة ٣٥ طفيل للسمكة. و قد تم دراسة التغيرات الهستوباثولوجية التى حدثت فى الجلد و الخياشيم للأسماك المصابة. و لقد تبين من محاولات العلاج ان استخدام الجرعة بيوتكس ٥٠® ٢ ملى لكل ١٠ لتر ماء كحمام مائى لمدة ٥ دقائق فعالة فى التخلص من قمل السمك فى المياة المالحة دون التأثير على الحالة الصحية للأسماك المعالجة مع مراعاة تكرارها بعد اليوم السابع من التطبيق الأول و لذلك يوصى باستخدام بيوتكس ٥٠® فى علاج الطفيليات القشرية و خاصة قمل السمك.