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RESEARCH ARTICLE

Use of Thyme and Thymol as Immunostimulant Agents to control Experimental *Aeromonas hydrophyla* Infection in Nile Tilapia (*Oreochromis niloticus*)

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Abstract

In the current investigation, a total of 110 *Oreochromis niloticus* were collected from aquaculture farm at Sahl Eltina area, North Sinai Governorate suffering from skin and fin ulcers with ascites, exophthalmia, and high mortalities. *Aeromonas hydrophyla* was isolated with a prevalence rate of 40% and identified biochemically. *A. hydrophyla* was used to induce experimental infection to Nile Tilapia in which thyme and thymol were used as feed additive. Evaluation of the protective effect of thyme and thymol as immunostimulant agents on fish tissue against experimental infection with *Aeromonas* infection was studied. A total of ninety apparently healthy fish tilapia were divided into three groups (GA, GB and GC of 30 fish per group) in triplicate. GA (infected non treated group) was fed on basal diet. GB and GC were fed on diet supplemented with *Thyme vulgaris* (10 g/ kg diet) and Thymol (3 g/ kg diet), respectively for 8 weeks and then the groups were subjected to experimental infection with *Aeromonas hydrophyla* isolate. The thyme and thymol groups had a nearly similar significant immunostimulant effect than infected non treated group by using lysozyme activity assay. Sever hyperemia of gill filaments and gill arch, epithelial desquamation of the gill filament, enteritis, necrosis and desquamation of epithelial cells of intestine, muscle necrosis, myocardiolysis of heart, hydropic degeneration of liver and hemosiderosis of spleen were observed in infected non treated group. In the treated groups, the prophylactic effect of thyme and thymol on fish tissues were clear against experimental infection with *A. hydrophyla*. These results indicate that the using thyme or thymol as feed additive could enhance the immunity and protect fish tissues from pathological deterioration induced by infection with *Aeromonas hydrophila* which is considered as one the main fish pathogens in Egypt.

Keywords: *Oreochromis niloticus*, *Aeromonas*, thyme, thymol, Immunostimulant, histopathology.

Introduction

Nowadays, Aquaculture has appeared as an essential source of food systems to meet global food demand and sustainability challenges [1]. In Egypt the fish production has decreased in 2020 than 2019 by 1.4% however Egypt has the largest aquaculture industry with Nile tilapia as the main farmed fish in Africa [2]. Different risks have negative impacts on fish production, one of them is linked to spreading of diseases especially zoonotic ones which have become more frequent and intense [3]. *Aeromonas* species are zoonotic facultative anaerobic bacteria, non-spore forming, Gram-negative rods, and ferment glucose, oxidase positive. It is widely distributed in marine environments, estuarine and fresh water [4, 5]. The organisms able to grow at different degrees of temperatures and its infection has elevated might be due to climatic changes [6]. *Aeromonas* species has got a wide spectrum of disease syndromes among cold- and warm-blooded animals, including, amphibians, mammals, fish, reptiles and humans [7- 9].

An opportunistic bacterium, *A. hydrophila* is common in Egypt and cause Motile

Aeromonas Septicemia [MAS] which consider the most serious infectious disease in warm water aquaculture, in per acute phase the disease characterized by high mortality rates without any symptoms but in acute phase, ascites in the abdomen skin and fin ulcers clearly appeared on the external surface of fish with and exophthalmia in addition to presence of drug resistant mutants which increase the severity of the problem and significant losses in Egyptian aquaculture industry [10- 13]. On the other hand, the use of antimicrobials in treatment has been proven leading to antimicrobial resistance, which is considered by the World Health Organization the one of the most dangerous risks to global health which has new expression as one health [14-16]. Nowadays the directions to herbal feed additive [phytotherapy] for enhancement of fish immune system against infectious diseases as Thyme and its extract [thymol] are used for medicinal purposes. Thyme includes thymol (44-60%) having good antiseptic properties, rich in, vitamins E, A and C and antioxidants [17].

Recent studies showed the effects of Phyto additives usage including immunostimulant, bio-productive, antioxidant, antimicrobial effects and keeping fish tissues healthy. The most advantage of these Phyto additives is that they are natural components that don't introduce any threat to fish and human health or to the environment. The therapeutic properties of thyme and its extracts in aquaculture also, include anesthetic, digestion stimulant and increase growth performance [18-21].

Fish histopathology has great diagnostic tool not only of the potential pathogens and their effects on fish tissues but also of the environmental factors as feed additives to infected non treated fish diseases in farmed production [22].

The goal of this work was to evaluate the effect of thyme and thymol on immuno-hematological parameters and protection the tissues of Oreochromis niloticus against damage induced by Aeromonas hydrophila infection.

Materials and Methods

Fish samples

One hundred and ten naturally infected Nile tilapia (*Oreochromis niloticus*) were freshly collected from intensive aquaculture farm at Sahl Eltina area, North Sinai Governorate. The collected fish was suffering from ulcers appear on the external surface of fish (skin and fin) with ascites in the abdomen, exophthalmia, and mortalities. After that the collected samples immediately transported in a cleaning bag in ice box with cooled ice bags to the laboratory and processed in less than 4hrs.

Bacteriological examination

Swabs were collected from the surfaces of ulcers, erosion, tail, fin, gills, muscles, liver, spleen, kidney, eye and ascetic fluids which were firstly sterilized with heated spatula. The sterile swab was inserted through the sterilized area and then inoculated into trypticase soya broth. The inoculated tubes were incubated at 24-25ºC for 24 h. A loopful from the broth was streaked onto tryptic soya agar (Oxoid, USA), Aeromonas selective agar (Oxoid, USA) with Ampicillin supplement media and Rimler's – Shotts medium (R-S) (Oxoid, USA)then incubated at 24-25ºC for 24-48 h. The pure colony was picked up to be subculture on blood agar medium and incubated at 25 C^{o} for 24 hours for detecting the hemolytic activity. A loopful of pure colony was inoculated on nutrient agar slant for further identification. Another loopful was inoculated on semisolid nutrient agar for testing the motility. Bacterial isolates were identified according to Schäperclaus *et al.* [23] using conventional methods as morphological characters and biochemical testing using analytical profile index of API 20 E system (BioMérieux, France).

Tested compound

Thyme vulgaris which was used as a whole plant that was collected from natural pasture at Matrouh Governorate. Thymol which was a Crystal (2- isopropyle-5- methyl-phenol, W224502, Sigma Aldrich, Munich, Germany).

Experimental design

Ninety apparently healthy fish tilapia (50 gm) were allocated into 3 equals groups (GA, GB and GC) with triplicate (30 fish / group; 10 fish/ replicate). Fish was acclimated for 2 weeks and fed on a basal commercial diet (Table 1) which contained 30.17 % crude protein twice daily at 3% of their body weight. The infected non treated group (GA) was fed on normal basal diet. The second (GB) and third groups were fed on diet supplemented with *Thyme vulgaris* (10 g/ kg diet) and Thymol (3 g/ kg diet), respectively [20,24, 25]. The aquaria were supplied with sufficient chlorine free tap water. Change of water was done every seven days to maintain good water quality. Aeration was done by electric aerator. The pH and temperature were adjusted at 7.4 and 25 ± 2 C respectively.

Table 1. Ingredient composition and calculated nutrient content values of the basal diet.

Feed ingredients	(%)
Yellow corn, ground [a]	28.00
Soya bean meal 46% CP [b]	32.00
Corn gluten meal 60 % CP	5.00
Fish meal 65% CP [d]	12.00
Wheat bran [e]	5.00
Rice bran [f]	16.20
Common salt [g]	0.20
DL-Methionine [h]	0.10
L-Lysine HCL [i]	0.10
Ground limestone [j]	1.00
Fish Premix [k]	0.30
Toxin binder [l]	0.10
Calculated composition (%)	
Crude Protein	30.17
Metabolizable Energy Kcal/Kg	2603
Ether Extract	5.02
Crude Fiber	4.46
Lysine	2.36
Methionine	0.81
Calcium	1.01
Available phosphorus	0.50

[a] Com (Argantina Co). [b] Soya bean meal (Argantina Co). [c] Corn gluten meal (US). [d] Fish meal 65% CP (Morocco). [e] Wheat bran (local Egypt). [f] Rice bran (Local Egypt). [g] Common salt (Local Egypt) [h] DL-Methionine (Evonik co). [i] L lysine (ADM Company, U.S). [j] Ground limestone (Egypt). [k] Fish premix (DSM Company). [l] Toxin binder (Kemin company, Bulgaria). Chemical analysis of fish diets according to Official Methods of Analysis of AOAC International [26].

Challenge test

At end of 8th weeks, all groups were injected with 0.2 mL (5x 10^5 Colony forming Unit/ml) of *Aeromonas hydrophyla* isolate intra peritoneal (I/P) according to Schaperclaus *et al.* [23].

Serum collection and lysozyme activity assay

At the end of feeding experiment (8 weeks) and at 96h after challenge, blood samples were collected without anti-coagulant for serum separation to be used in measuring serum lysozyme activity. Samples of serum were measured using lysozyme activity kit (Sigma, USA) through the turbid metric method as described by Esteban *et al.* [27].

Histopathological examination

Tissue samples of the gills, spleen, intestine, heart, liver and muscle of the different groups (GA, GB and GC). These samples collected after experimental infection and rapidly fixed at 10% neutral buffered formalin for histopathological examination, after that these samples were prepared and stained with Hematoxylin and Eosin according to Bancroft and Gamble [28].

Statistical Analysis

The data was analyzed by [SPSS] software. A value of $P < 0.05$ was considered significant.

Results

The clinical signs of naturally infected *Oreochromis niloticus* showed scale detachment, erosion on skin and with slight ascites; on the ventral abdominal wall and the base of the fins petechial hemorrhages were clearly appeared. While in naturally infected fish tilapia the postmortem lesions were congestion of kidney, spleen and liver with distended gall bladder, hemorrhage at intestine.

Bacteriological examination results were positive for Aeromonas infection in which these isolates were motile facultative anaerobic Gram-negative rods. They were catalase and oxidase positive. After using API

20E results indicated positivity for *Aeromonas hydrophila*. The bacterial isolation rate 40% (44 fish *Aeromonas* positive out of 110 fish).

Before and after experimental infection the fish groups treated with thyme and thymol revealed significant increase in lysozyme activity when compared with the infected non treated group. The fish groups treated with thyme and thymol have no significant differences (Table 2).

After Challenge test, the clinical signs of I/P experimental infected *Oreochromis niloticus* with *Aeromonas hydrophila* showed skin darkness, exophthalmia, external hemorrhages, scale detachment, gill congestion. Eye opacity, fin and tail rot. Ulcer at the late stage and some mortality in the fish. The results indicated a significant increase at the survivability percent of groups treated with thyme and thymol (GB and GC) in compare with the infected non treated group (GA) which revealed the low percent of survivability and the high percent of mortality (Table 3).

Histopathological examination

The liver tissue of GA showed severe and hydropic degeneration, infiltration of chronic inflammatory cells and slight hemorrhage. While GB showing slight hydropic degeneration, infiltration of chronic inflammatory cells. Fatty change was clearly detected in GC as shown in Figure 1. The spleen tissue showed clear appearance of hemosiderosis and lymphoid depletion and edema in case of group A&B in contrast in GC absence of hemosiderosis as shown in Figure 2. Heart tissue Showed sever hemorrhage and lymphocytic infiltration and clear appearance of sever myocardiolysis and myocardial necrosis in case of GA while GB have Appearance of slight to moderate myocardiolysis, and infiltration of chronic inflammatory cells (lymphocytic infiltration) but GC showed slight changes more or less to normal as shown in Figure 3.

Gills of GA showed sever hyperemia of gill filaments and gill arch, epithelial desquamation of the gill filament, epithelial hyperplasia at the base of the gill filament; sever hyperemia and epithelial lifting and lamellar fusion. While GB showed epithelial lifting of the gill filament and shortening of the gill filament and epithelial hyperplasia at the base of the gill filament. Epithelial lifting of the gill filament and shortening of the gill filament was clearly appeared in GC (Figure 4). Muscle of GA Showed muscle necrosis and myolysis the second group (GB) Showed myolysis and slight infiltration of inflammatory cells while in G C the severity of lesion begins to decrease (Figure 5). Intestine showed enteritis, sever infiltration of chronic inflammatory cells and edema of the lamina propria, necrosis and desquamation of epithelial cells in case of GA. But degenerated villi and desquamated epithelial cells at the tips of the villi slight infiltration of inflammatory cells and slight edema of the lamina propria was detected in GB while in GC beginning to return to normal as shown in Figure 6.

Table 2. Lysozyme activity in *Oreochromis niloticus* feed on Thyme and thymol before and after challenge infection with *Aeromonas hydrophila*.

	Before challenge			After challenge		
	GA	**GB**	**GC**	**GA**	**GB**	**GC**
Lysozyme	8.50± 0.73 [a]	14.93±1.15 [b]	13.88± 1.21 [b]	16.02± 0.49 [a]	26.98± 0.58 [b]	25.93± 0.57 [b]

Values with different superscripts [a, b] within the same row are significantly different (P<0.05). GA= served as infected non treated group, fish fed on normal basal diet. GB= fish were fed on diet supplemented with *Thyme vulgaris* (10 g/ kg diet). GC= fish were fed on diet supplemented with Thymol (3 g/ kg diet).

Table 3. Survivability and Mortality percent of *Oreochromis niloticus* challenged with *Aeromonas hydrophila*.

Fish group	Mortality % and survivability of *O. niloticus* challenged with *Aeromonas hydrophila* during the first ten days after challenge												
	No	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	Mortality %	Survivability
GA	30	2	1	9	6	4	2	0	0	0	0	80	20
GB	30	0	0	3	3	0	0	0	0	0	0	20	80
GC	30	0	0	3	3	3	0	0	0	0	0	30	70

GA= served as infected non treated group, fish fed on normal basal diet. GB= fish were fed on diet supplemented with *Thyme vulgaris* (10 g/ kg diet). GC= fish were fed on diet supplemented with Thymol (3 g/ kg diet).

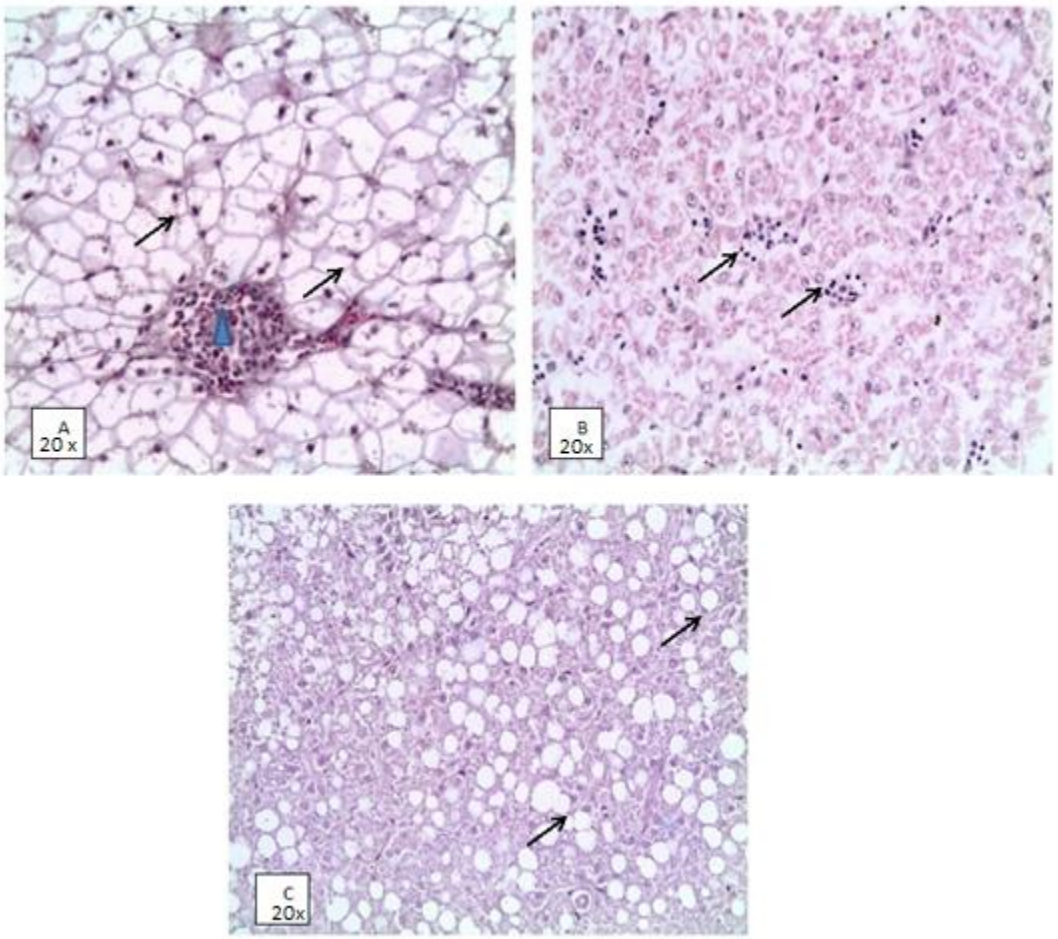

Figure 1: Representative photomicrograph of Hematoxylin and eosin (H&E) stained fish liver, magnification X20 showing: A) Liver of infected non treatedgroup (GA) showing severe and hydropic degeneration (black arrow), infiltration of chronic inflammatory cells and slight hemorrhage (triangle). B) Liver of GB group, which fish were fed on diet supplemented with *Thyme vulgaris* (10 g/ kg diet) showing slight hydropic degeneration, infiltration of chronic inflammatory cells (black arrow). C) Liver of GC group, which fish were fed on diet supplemented with Thymol (3 g/ kg diet) showing fatty change (black arrow).

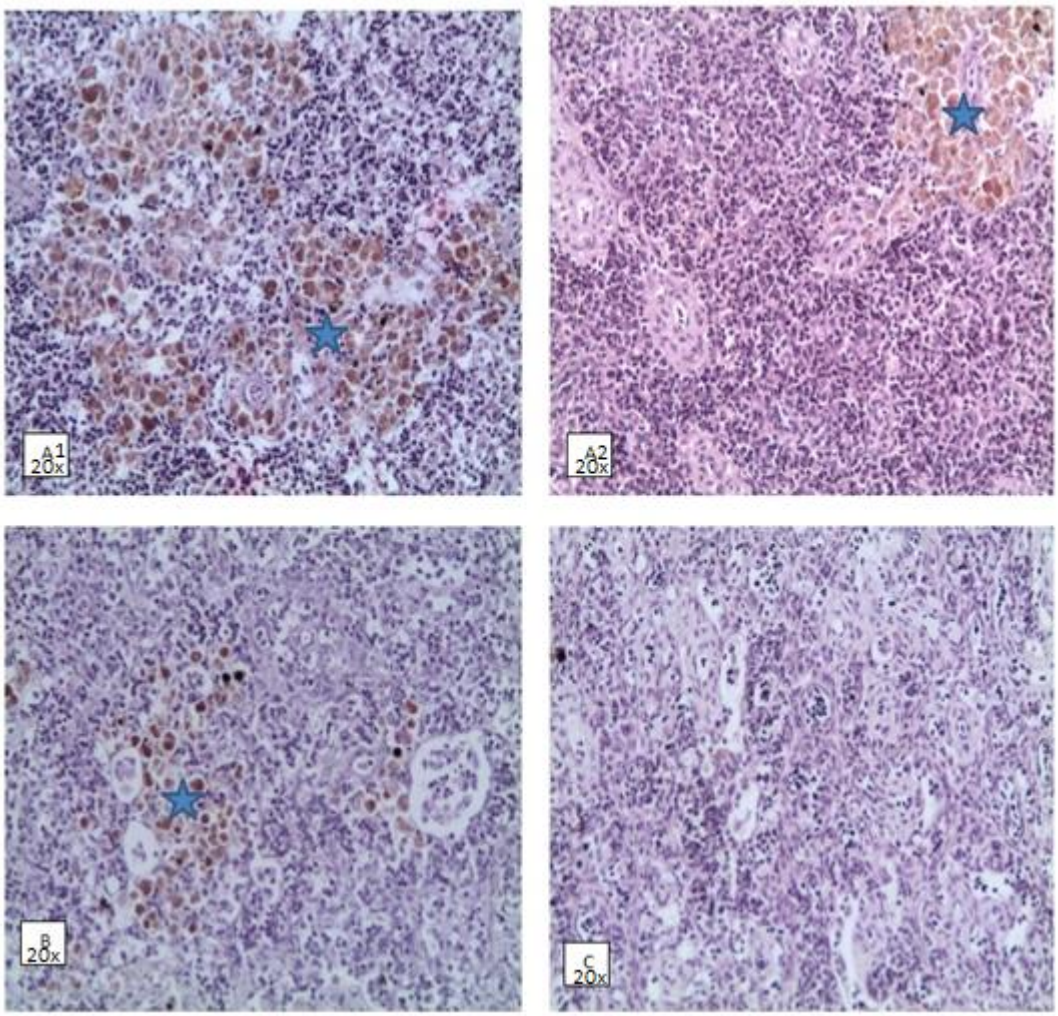

Figure 2: Representative photomicrograph of Hematoxylin and eosin (H&E) stained fish spleen, magnification X20 showing: A1) Spleen of infected non treated group (GA) showing wide area of hemosiderosis. A2) Spleen of infected non treated group (GA) showing lymphoid depletion (star). B) Spleen of GB group, which fish were fed on diet supplemented with *Thyme vulgaris* (10 g/ kg diet) showing small area of hemosiderosis (red star), edema and slight lymphoid depletion. C) Spleen of GC group, which fish were fed on diet supplemented with Thymol (3 g/ kg diet) showing clear appearance with absence of hemosiderosis and slight lymphoid depletion.

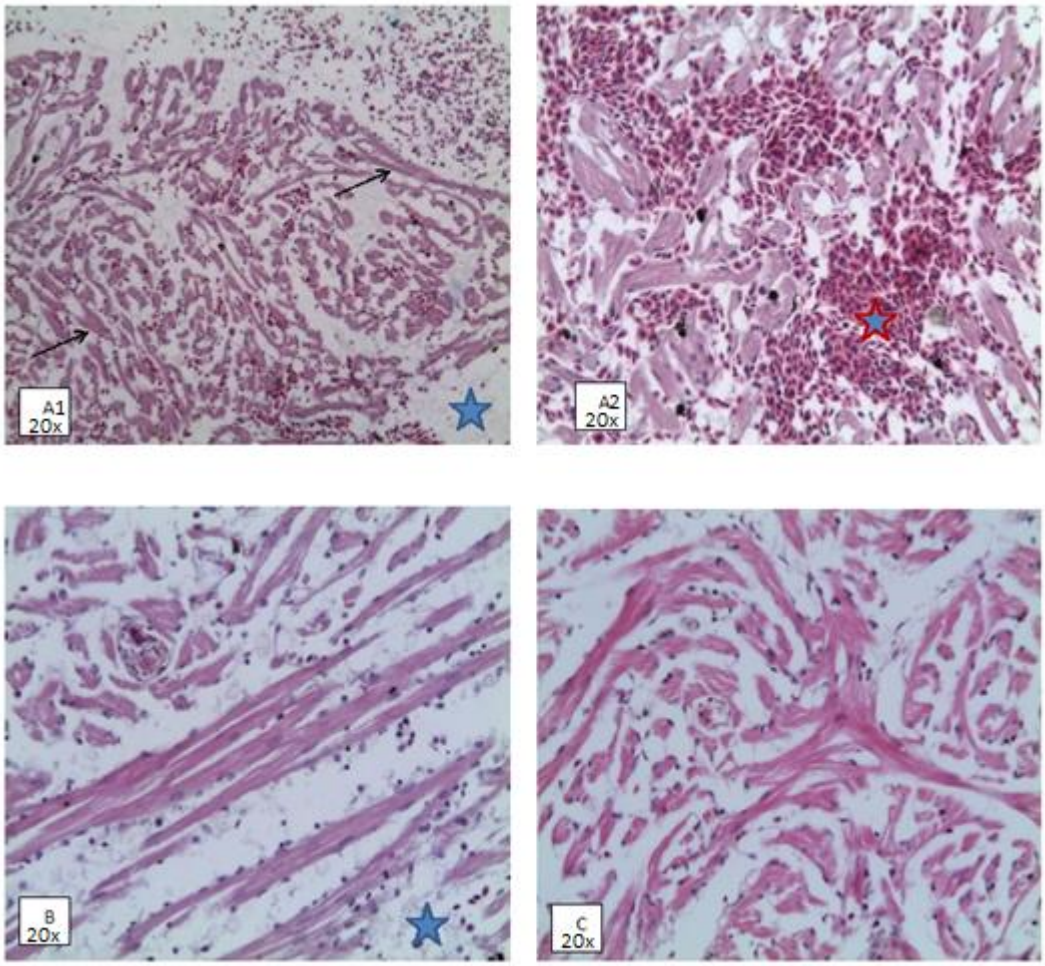

Figure 3: Representative photomicrograph of Hematoxylin and eosin (H&E) stained fish heart, magnification X20 showing: A1) Heart of infected non treated group (GA) showing clear appearance of sever myocardiolysis (blue star) and myocardial necrosis (arrow) A2) Heart of infected non treated group (GA) showing severe hemorrhage and lymphocytic infiltration (red star) . B) Heart of GB group, which fish were fed on diet supplemented with *Thyme vulgaris* (10 g/ kg diet) showing appearance of slight to moderate myocardiolysis, and infiltration of chronic inflammatory cells (lymphocytic infiltration) (Blue star). C) Spleen of GC group, which fish were fed on diet supplemented with Thymol (3 g/ kg diet) showing slight changes more or less to normal.

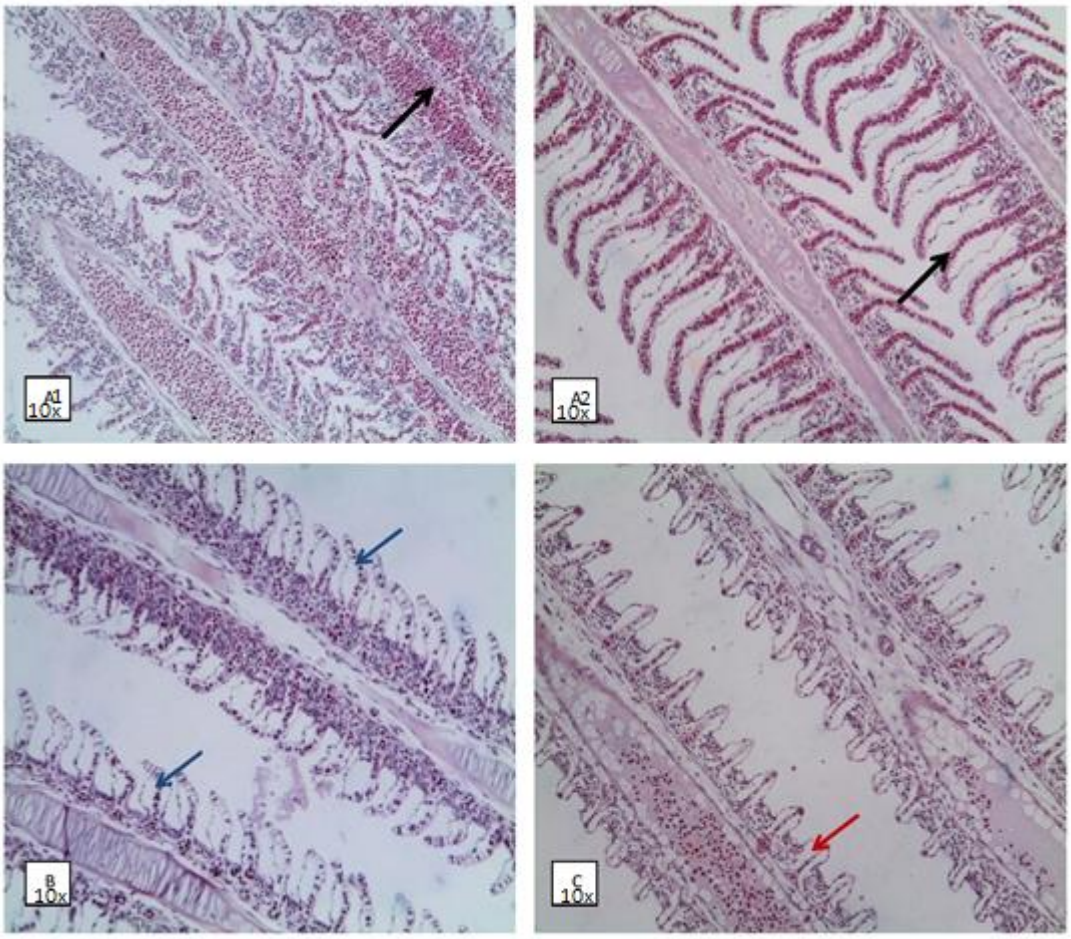

Figure 4: Representative photomicrograph of Hematoxylin and eosin (H&E) stained fish gills, magnification X10 showing: A1) Gills of infected non treated group (GA) showing sever hyperemia of gill filaments. A2) Gill arch, epithelial desquamation of the gill filament, epithelial hyperplasia at the base of the gill filament; sever hyperemia and epithelial lifting and lamellar fusion (arrow). B) Gills of GB group, which fish were fed on diet supplemented with *Thyme vulgaris* (10 g/ kg diet) showing epithelial lifting of the gill filament and shortening of the gill filament and epithelial hyperplasia at the base of the gill filament (blue arrow). C) Gills of GC group, which fish were fed on diet supplemented with Thymol (3 g/ kg diet) showing epithelial lifting of the gill filament and shortening of the gill filament (red arrow).

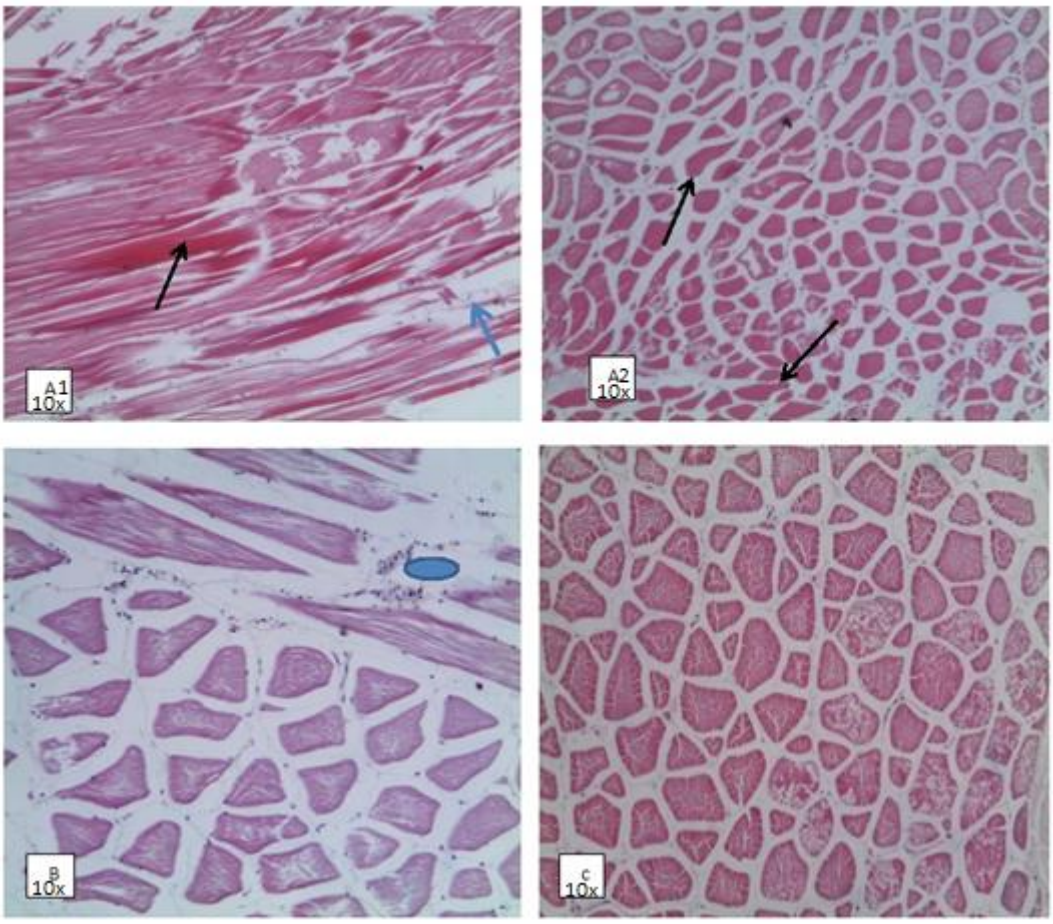

Figure 5: Representative photomicrograph of Hematoxylin and eosin (H&E) stained fish muscles, magnification X20 showing: A) Muscles of control group (GA) showing muscle necrosis and myolysis (arrow). B) Muscles of GB group, which fish were fed on diet supplemented with Thyme vulgaris (10 g/ kg diet) showing myolysis and slight infiltration of inflammatory cells (circle). C) Muscles of GC group, which fish were fed on diet supplemented with Thymol (3 g/ kg diet) showing more or less to normal.

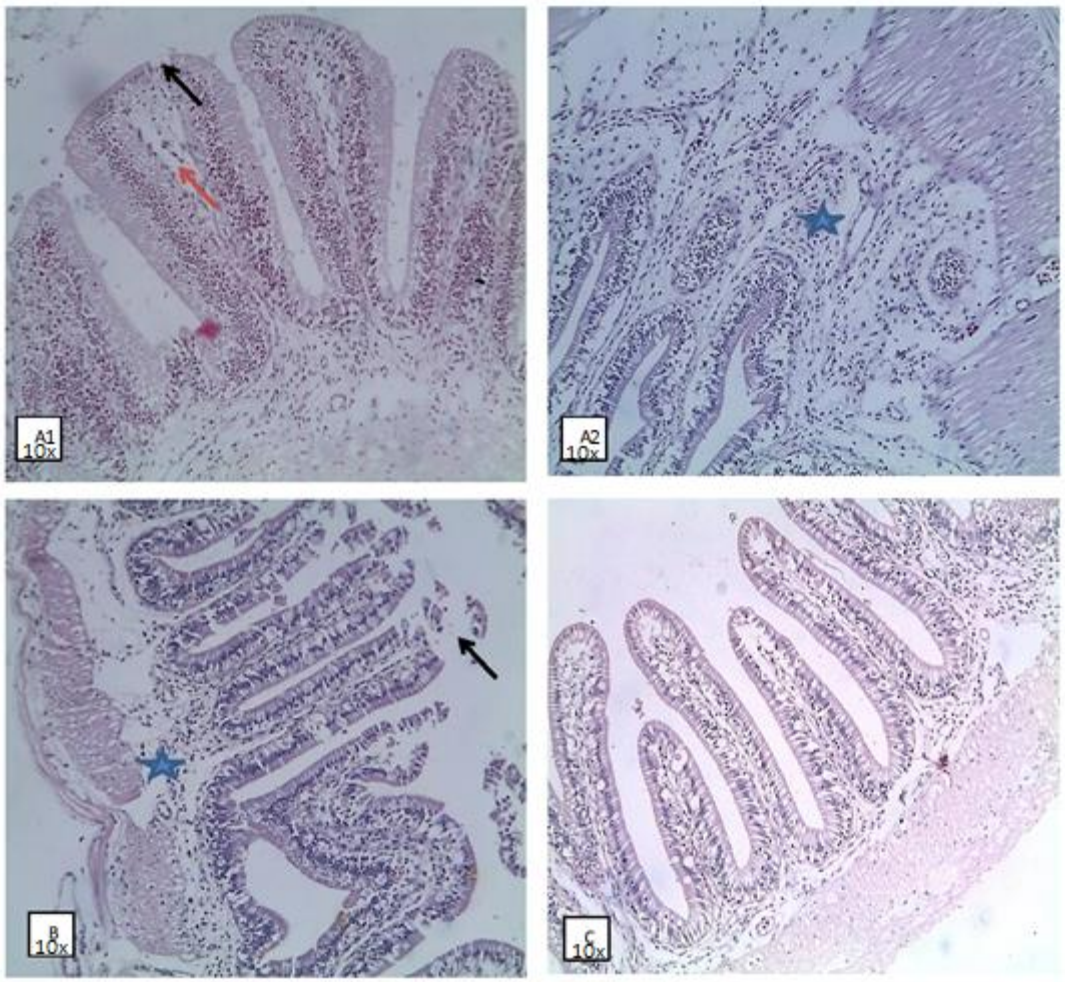

Figure 6: Representative photomicrograph of Hematoxylin and eosin (H&E) stained fish intestine, magnification X10 showing: A1) Intestine of infected non treated group (GA) showing enteritis, sever infiltration of chronic inflammatory cells (star) and edema of the lamina propria (red arrow). A2) Intestine of infected non treated group (GA) showingnecrosis and desquamation of epithelial cells (black arrow). B) Intestine of GB group, which fish were fed on diet supplemented with Thyme vulgaris (10 g/ kg diet) showing degenerated villi and desquamated epithelial cells at the tips of the villi (black arrow), slight infiltration of inflammatory cells and slight edema of the lamina propria. C) Intestine of GC group, which fish were fed on diet supplemented with Thymol (3 g/ kg diet) showing more or less normal appearance of intestinal villi.

Discussion

MAS is a serious disease facing Egyptian aquacultures, causing severe economic losses. Protection fish farming industry in Egypt from this serious problem is mandatory. *A. hydrophila*, has a global spreading and infecting fishes, and birds as well as human. Moreover, the high prevalence of *A. hydrophila* may be due to its presence as a part of intestinal flora of healthy marine and freshwater fish [12, 29]. The bacteriological results were positive for *Aeromonas* infection. Also, hemorrhage at base of fins or on the skin, protruded eyes, distended abdomens, which are the same lesions of septicemia disease caused by *Aeromonas* spp. The isolation rate per fish was 40% (44 fish *Aeromonas* positive out of 110 fish samples) in accordance with Beaz-Hidalgo and Figueras [30] and Hassan *et al.* [31]. In this study the

PM examination of naturally infected *O. niloticus* showed inflammatory reaction and congestion especially in haemopoietic organs as spleen, liver, kidney and gill. Skin and muscles could be attributed to bacterial pathogenesis and virulence factors. These factors have effect on structural features (pilli, S layer, lipopolysaccharide), pathogenicity are extracellular toxins (enterotoxin, hemolysin and protease), adhesion and invasion. This also recorded by Eissa *et al.* [32]. Hemorrhagic mucous desquamative catarrh clearly appeared and this may be due to the bacterial multiplication inside the intestine. Toxemia was induced due to absorption of toxic metabolites of *Aeromonas* species by the intestine. Capillary hemorrhage occurs in the trunk, dermis of fins and in the sub mucosa of stomach. The clinical signs of experimental infected fish in our study are similar to those described during the naturel infection these results supported by Eissa *et al.* [32]. The main lesions of infected fish were hemorrhagic septicemia as hemorrhage at base of fins. Or on the skin, protruded eyes and distended abdomens. There are significant losses in aquaculture industry because of reduced growth due to infection by *Aeromona*s. The results agree with Pachanawan *et al.* [11]. On the other hand, the immunostimulant effect of thyme and thymol in prophylaxis of *Aeromonas* infection was clear through the results of lysozyme activity assay and challenge test after feeding on thyme and thymol. The measured humoral innate parameter in this work was the Lysozyme activity which considered an indispensable tool of fish to fight against infectious agents and one of the important bactericidal enzymes. Lysozyme activity in this study was significantly increased before and after experimental infection the fish groups treated with thyme and thymol. These results were nearly similar to Zheng *et al.* [33]. Lysozyme is constitutively expressed, synthesized and secreted by monocytes, neutrophils and macrophages; there is directly proportional with the leukocytic count and the greatest concentration of lysozyme [34]. About the challenge test it was no doubt of the protective effect of thyme and thymol. The comparative challenge tests results which carried out with *Aeromonas* Spp showed that there was a significant increase in survivability percent of treated groups with thyme and thymol in compare with the infected non treated groups which revealed the low percent of survivability and the high percent of mortality, which goes hand in hand with Yassen *et al.* [35].

The PM examination in our study revealed that the naturally infected *O. niloticus* showed inflammatory reaction, congestion and degenerative changes, especially in hematopoietic organs like spleen, kidney, gill and liver. The histopathological examination of *Aeromonas* spp infected groups showed severe damage in intestine, spleen, and kidney. while the infected groups fed on thyme and thymol extract showed significant alleviation of damage of intestine, spleen and kidney. The histopathological examination *Aeromonas* spp. infected groups revealed sever changes in intestine as enteritis, sever infiltration of chronic inflammatory cells, and edema of the lamina propria, necrosis and desquamation of epithelial cells. gills showed sever hyperemia of gill filaments and gill arch, epithelial desquamation of the gill filament, epithelial hyperplasia at the base of the gill filament, sever hyperemia and epithelial lifting and lamellar fusion. Liver showing hydropic degeneration, hemosiderosis of pancreas, muscle necrosis and myocardiolysis and hemorrhage of heart. These result in accordance with AlYahya *et al.* [36] and Mostafa *et al.* [37]. Many studies showed that the chronic infections of *A. hydrophila* have led to dermal ulceration. the hepatocyte of liver showing vacuolation and severe necrosis. In agree with Afifi *et al*. [38]. Also, in this study the histopathological examination of treated group with thyme revealed decrease the severity of lesion while the treated group with thymol the lesion decreased and beginning to return to normal. Also confirm the prophylactic effect of thymol is slight better than thyme on fish tissues against experimental infection with *Aeromonas hydrophila*.

Conclusion

Aeromonas spp. which is considered as fish pathogen, it could be isolated from *Oreochromis niloticus* (Nile tilapia) that was the mean public health hazards of the human handling diseased fish. Feed addition with thyme and thymol could enhance the immunity in fish experimentally challenged with *Aeromonas hydrophila* and increase resistance of *O. niloticus* against such disease.

Conflict of interest

The Authors declare that they don't have any conflict of interest.

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References

[1] Bohnes, F.A.; Michael Zwicky; Hauschild, M.Z.; Schlundt, J.; Nielsen, M. and Laurent, A. (2022): Environmental sustainability of future aquaculture production: Analysis of Singaporean and Norwegian policies. Aquaculture, 459, 737717.

[2] CAPMAS (2022): Egypt in figures report 2022. Central Agency for Public Mobilization and Statistics. https://www.capmas.gov.eg/Pages/StaticPages.aspx?page_id=5035

[3] Stel, M.; Eggers, J. and Nagelmann, S. (2022): Accuracy of Risk Perception of Zoonoses Due to Intensive Animal Farming and People's Willingness to Change Their Animal Product Consumption. Sustainability, 14(2): 589.

[4] Holmes, P.H; Niccolls, L.M.; Sartory, D.P. (1996): The ecology of mesophilic Aeromonas in the aquatic environment. In: The Genus: Aeromonas, First Edition, Austin B, Altwegg M, Gosling PJ, Joseph SW (Eds), John Wiley & Sons, Ltd, Chicester. p.127.

[5] Martin-Carnahan, A. and Joseph, S.W. (2005): Aeromonas. In: Bergey's Manual of Systematic Bacteriology, Second Edition, Brenner, Krieg, Staley, Garrity (Eds), Williams and Wilkins, New York. Vol 2.

[6] Gehad R. Donia; Amani A. Hafez and Islam M. Wassif (2017): Studies on Some Heavy Metals and Bacterial Pollutants in Tilapia Fish of El Salam Canal, Northern Sinai, Egypt. Egypt. J. Aquat. Biol. Fish.21(4): 67 – 84.

[7] Roberts, R. (1993): Motile aeromonad septicaemia. In Inglis, V.; Roberts, R.J. and Bromage, N.R. editors. Bacterial diseases of fish. Institiute of Aqua Culture, Blackwell Scientific Publications, London. 143-155.

[8] Gosling, P.J. (1996): Aeromonas species in diseases of animals. In: The Genus: Aeromonas, First Edition, Austin B, Altwegg M, Gosling PJ, Joseph SW (Eds), John Wiley & Sons, Ltd, Chicester. p.175.

[9] Janda, J.M. and Abbott, S.L. (2010): The genus Aeromonas: taxonomy, pathogenicity, and infection. Clin. Microbiol. Rev., 23(1): 35–73.

[10] Bondad-Reantaso, M. G.; Subasinghe, R. P.; Arthur, J. R.; Ogawa, K..; Chinabut, S.; Adlard, R.; Tan, Z.and Shariff, M. (2005): Disease and health management in Asian aquaculture. Vet. Parasitol, 132(3-4): 249–272.

[11] Pachanawan, A.; Phumkhachorn, P. and Rattanachaikunsopon, P. (2008): Potential of Psidium guajava supplemented fish diets in controlling *Aeromonas hydrophila* infection in tilapia (*Oreochromis niloticus*). J. Biosci. Bioeng, 106 (5):419-424.

[12] Austin, B. and Austin, D.A. (2012): Aeromonadaceae representatives (motile aeromonads). In: Austin, B., Austin, D.A. (Eds.), Bacterial Fish Pathogens, Diseases of Farmed and Wild Fish. 5th ed. Springer, New York, London. 119-146.

[13] El-Sherbeny, E.M.; Khoris, E.A. and Kassem, S. (2022): Assessment the efficacy of some various treatment methods, in vitro and in vivo, against *Aeromonas hydrophila* infection in fish with regard to side effects and residues. Comparative Biochem. and

Physiol. C: Toxicol Pharmacol, 253:109246.

[14] Santos, L. and Ramos, F. (2018): Antimicrobial resistance in aquaculture: Current knowledge and alternatives to tackle the problem. Int. J. Antimicrob. Agents., 52(2):135-143.

[15] World Health Organization (WHO) (2018): Global Action Plan on Physical Activity 2018-2030: More Active People for a Healthier World (p. 104). Geneva: World Health Organization. http://apps.who.int/iris/bitstream/handle/10665/272722/9789241514187-eng.pdf

[16] Wu, H.; Shinoda, R.; Murata, M.; Matsumoto, H.; Ohnuki, H. and Endo, H. (2019): Real-time fish stress visualization came true : A novel multi-stage color-switching wireless biosensor system. Biosens. Bioelectron. 130: 360-366.

[17] Alçiçek, Z. (2011): The effects of thyme (*Thymus vulgaris* L) oil concentration on liquid-smoked vacuum-packed rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) fillets during chilled storage. Food Chem., 128 (3): 683- 688.

[18] Rota, M.C.; Herrera, A.; Martínez, R.M.; Sotomayor, J.A. and Jordán, M.J. (2008): Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Food Control, 19 (7): 681-687.

[19] Erol-Florian, G.; Şara, A.; Molnar, F. and Benţea, M. (2011): The Influence of Some Phytoadditives on Growth Performances and Meat Quality in Rainbow Trout (*Oncorhynchus Mykiss*). J. Anim. Sci. Biotechnol.44 (2): 13-18.

[20] Ahmadifar, E.; falahaktar, B. and Akrami, R. (2011): Effect of dietary thymol-carvacol on growth performance, hematological parameters and tissue compsition of juvenile rainbow trout, Oncorhynchus mykiss. J. Appl. Ichthyol., 27(4):1057-1060.

[21] Azad, I.S., Al-Yaqout, A. and Al-Roumi, M. (2014): Antibacterial and immunity enhancement properties of anaesthetic doses of thyme (*Thymus vulgaris*) oil and three other anesthetics in *Sparidentax hasta* and *Acanthopagrus latus*. J. King Saud Univ. Sci., 26 (2): 101- 106.

[22] Roberts, R. J. (Eds) (2012): *In* Fish Pathology, Fourth Edition. Published by Blackwell Publishing Ltd. Preface p.vii

[23] Schäperclaus, W.; Kulow, H. and Schreckenbach, K. (1992): Bacteriological techniques. In: Schäperclaus, W., Kulow, H., Schreckenback, K. (Eds.), Fish diseases, Vol. 1, 5th ed. AA Balkema / Rotterdam Publisher. 117-141.

[24] Moustafa, M.M; Abdel Aziz, M.A; Wassif, I.M. and Mai, S. Yaseen (2017): Use of thyme and thymol as immune modulator for *Oreochromis niloticus* challenged with *Strept.* Sp. J. Egypt. Vet. Med. Assoc., 77(1):7-17.

[25] Antache, A.; Cristea, V.; Dediu, L.; Grecu, I.; Docan, A.; Vasilean, I.; Mocanu, M.C. and Petrea, Ş.M. (2012): The influence of some phytobiotics on growth performance at *Oreochromis niloticus* reared in an intensive recirculating aquaculture system, University of Agricultural Sciences and Veterinary Medicine Iasil University "Dunarea de Jos" Galati Romania., 180-199.

[26] AOAC (2006): Official Methods of Analysis of AOAC International, 18th Edition current through revision. Dr. William Horwitz, Editor Dr. George W. Latimer, Publisher: AOAC International, Gaithersburg, Maryland, USA.

[27] Esteban, M.A.; Mulero, V.; Cuesta, A.; Ortuno, J. and Meseguer, J. (2000): Effects of injecting chitin particles on the innate immune of sea bream (*Spareus aurata* L). Fish Shellfish immunol, 10 (6):543-54.

[28] Bancroft, J. D. and Gamble, M. (2008): "Theory and Practice of Histological Techniques", In: Swisher, B. (ed.), Microorganisms, Churchill Livingstone, Philadelphia.

[29] Wassif, I.M. (2018): Biochemical and Molecular Characterization of Aeromonas species Isolated from Fish. AJVS, 57 (1): 32-39 34.

[30] Beaz-Hidalgo, R. and Figueras, M. J. (2012): Molecular detection and characterization of furunculosis and other Aeromonas fish infections. *In*: E. Carvalho, (Ed.), Health and Environment in Aquaculture. InTech, Europe, China. 97-132.

[31] Hassan, M.A.; Noureldin, E.A.; Mahmoud, M.A. and Fita, N.A. (2017): Molecular identification and epizootiology of *Aeromonas hydrophyla* infection among farmed *Oreochromis niloticus* in Eastern Province, KSA. Egypt. J. Aquat. Res., 43: 161–167.

[32] Eissa, I.A.M.; El-Lamei, M.; Sherif, M.; Desuky, E.; Zaki, M. and Bakry, M. (2015): *Aeromonas hydrophyla* biovar *sobria* a Causative Agent of Mass Mortalities in Cultured Nile Tilapia in El-Sharkia governorate, Egypt. Life Science Journal, 12(5): 90-97.

[33] Zheng, Z.L.; Tan, J. Y., Liu, H.Y.; Zhou, X.H. and Wang, K.Y. (2009): Evaluation of oregano essential oil (*Origanum heracleoticum* L.) on growth, antioxidant effect and resistance against Aeromonas hydrophila in channel catfish (*Ictalurus punctatus*). Aquaculture 292(3-4): 214-218.

[34] Yano, T. (1996): The non-specific immune system Humoral defence. *In* Fish physiology, Vol. 15, Iwama,G.K.and Nakanishi T.(Eds) ,pp.105-157.

[35] Yassen, D.A.; El-Gawad, A. and Abbass, A.A. (2017): Effect of Herbal Plants *Thymus Vulgaries* and *Rosmarinus Officinale*" on Growth Performance and Protection of *Oreochromis Niloticus* against *Aeromonas hydrophila* Infection. Benha Journal of Applied Sciences (BJAS), 2(2): 9-17.

[36] AlYahya, S.A.; Ameen, F.; Al-Niaeem, K.S.; Al-Sa'adi, B.A.; Hadi, S. and Mostafa, A.A. (2018): Histopathological studies of experimental *Aeromonas hydrophila* infection in blue tilapia, *Oreochromis aureus*. Saudi J Biol Sci., 25(1): 182–185.

[37] Moustafa, E.M.; Dawood, M.A.; Assar, D.H.; Omar, A.A.; Elbialy, Z.I. Farrag, F.A.; Shukry, M. and Zayed, M.M. (2020): Modulatory effects of fenugreek seeds powder on the histopathology, oxidative status, and immune related gene expression in Nile tilapia (*Oreochromis niloticus*) infected with *Aeromonas hydrophila*. Aquaculture journal, 515:734589.

[38] Afifi, S.H.; Al-Thobiati, S. and Hazaa, M.S. (2000): Bacteriological and histopathological studies on *Aeromonas hydrophila* infection of Nile tilapia (*Oreochromis niloticus*) from fish farms in Saudi Arabia. Assiut. Vet. Med. J., 42 (84):195–205.

الملخص العربي

إستخدام الزعتر والثيمول كمحفز مناعي ضد العدوى التجريبية بميكروب الإيروموناس في أسماك البلطي النيلي

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فى الدراسة الحالية تم تجميع 110 من أسماك البلطي النيلي بمنطقة سهل الطينة بمحافظة شمال سيناء و التي كانت تعاني من تقرحات الجلد والزعانف مع الإستسقاء وجحوظ العين ثم النفوق. وقد تم عزل ميكروب الإيروموناس هيدروفيلا *Aeromonas hydrophyla* بنسبة 40٪ وتم االتصنيف على أساس الإختبارات البيوكيميائيةً وقد تم إحداث عدوى تجريبية للبلطي النيلي بهذه المعزولة حيث أستخدم الزعتر والثيمول كإضافات علفية. وتم دراسة تقييم التأثير الوقائي للزعتر والثيمول كمحفزات مناعية على أنسجة الأسماك ضد العدوى التجريبية. وكانت النتائج لمجموعة الزعتر والثيمول تأثير منشط مناعي ذات تاثير معنوي على عكس المجموعة الضابطة التي لم يضاف لها على العلف اي من الزعتر والثيمول وذلك بإستخدام إختبار نشاط الليزوزيم. وقد لوحظ إحتقان شديد بأنسجة الخياشيم و إلتهاب الأمعاء و نخر وتقرح للخلايا الطلائية للأمعاء ونخر العضلات وعضلة القلب بجانب تكيس مائي للكبد وتهتك في أنسجة الطحال وذلك في المجموعة الضابطة بينما لم يلاحظ هذه التغيرات النسيجية في المجموعتين المعالجتين مع عدم وجود فرق معنوي بين مجموعتي الزعتر و الثيمول ضد العدوى التجريبية. ومن خلال هذه النتائج يتضح ان إضافة الزعتر أو مستخلصه الثيمول إلي عليقة الأسماك يمكن أن يحفز الجهاز المناعي ويحمي أنسجة الأسماك من التغيرات الباثولوجية المصاحبة للعدوي بميكروب الإيروموناس هيدروفيلا والذي يعتبر واحدا من أهم المسببات المرضية قى الأسماك بمصر.