Iranian Journal of Fisheries Sciences 20(5) 1409-1425 2021
DOI: 10.22092/ijfs.2021.125041

Research Article

The adjuvant effect of Myrtle, *Myrtus communis*, extract on hematological, Immuno-physiological, antioxidant responses, and tissue histomorphology of gill and liver in juvenile Siberian sturgeon, *Acipenser baerii*

Barzegar R. [1]; Farokhrouz M.[1]*; Khara H. [1]; Shenavar Masouleh A.[2]; Ahmadnezhad M. [3]

Received: December 2020 Accepted: May 2021

Abstract

This study aimed to evaluate the effect of Myrtle, *Myrtus communis*, extract on hematological, immuno-physiological, antioxidant responses, bactericidal activity, and tissue histomorphology of gill and liver in juvenile Siberian sturgeon, *Acipenser baerii*. Siberian sturgeon were exposed to 4 doses of myrtle extract including 25% (67.4 mg/L; M_{25}), 50% (134.9 mg/L; M_{50}), 75% (202.0 mg/L; M_{75}), and 100% (269.8 mg/L; M_{100}) of the maximum allowable concentration and a control treatment (without exposure myrtle extract). Hemoglobin and red blood cell values were significantly increased in fish exposed to the myrtle extracts (p<0.05). The white blood cell was lower in M_{25} and M_{75}, while the highest value was found in M_{100} treatment (p<0.05). Myrtle extract did not affect the lymphocyte value in the course of exposure (p>0.05). The highest albumin and total protein levels were observed in M_{25} and M_{50} groups. The highest values of lysozyme and total immunoglobulin (Ig) activities were observed in M_{50}, M_{75} and M_{25}, M_{50}, respectively (p<0.05). Superoxide dismutase and catalase activities of those fish exposed to M_{50} and M_{75} were significantly higher than the control and M_{100} groups (p<0.05). The lowest glutathione peroxidase value was observed in the control group compared to the others (p<0.05). The severe changes such as adhesion and curling of gill lamella discern were observed in fish exposed to different levels of myrtle extracts. Moreover, in the control group, severe hepatocyte destruction was accompanied by nucleus pyknosis, but the severity of atrophy was observed in M_{75} and M_{100} treatments. Overall, the *r*esults suggested that myrtle in the range of 67.4-202 mg/L could be applied as a stimulant agent to Siberian sturgeon aquaculture.

Keywords: Exposure, Myrtle, Immuno-physiological, Siberian sturgeon

1-Department of Fisheries, Lahijan Branch, Islamic Azad University, Lahijan, Iran
2-International Sturgeon Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Rasht, Iran
3-Inland Waters Aquaculture Research Center. Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Bandar Anzali, Iran.
*Corresponding author's Email: dr.farokhrouz@gmail.com

[DOR: 20.1001.1.15622916.2021.20.5.16.3]

Introduction

Sturgeon farming is a fast-growing practice worldwide, and nowadays, noteworthy attention has been paid to this operation (Banavreh *et al*., 2019a). Siberian sturgeon, *Acipenser baerii*, is one of the most important sturgeon species with a fast growth rate, feeding adaptability, and stress resistance and has been used as a biological model to initiate investigations (Fontagné-Dicharry *et al*., 2018; Mirzakhani *et al*., 2020). The high density of fish in the rearing system provides a stressful environment for the aquatic animal and promote the sensitivity of the fish to suppressing the immune system and ultimately leading to infectious diseases in the animal (Long *et al*., 2017; Banavreh *et al*., 2019b). Traditionally, antibiotics and chemotherapeutics are regularly used to prevent aquatic diseases. Their indiscriminate use produces resistance pathogens during antibacterial therapy, bioaccumulation in the aquatic animals, and environmental deterioration (Banavreh *et al*., 2019b).

Nowadays, the application of butanoic extracts as an immunostimulant in practical modern aquaculture has been recommended as a promising approach for ameliorating health conditions and disease prevention (Mansouri Taee *et al*., 2017). The medicinal plant's derivative stimulants of the immune system, growth promotion, improved appetite, and antimicrobial activity due to their natural compounds such as phenolics, essential oils, alkaloids, flavonoids, steroids, which are affordable and bio-compatible, biodegradable and eco-friendly agents without any side-effects (Harikrishnan *et al*., 2011; Afzali and Wong, 2017; Soltani *et al*., 2018). Immunostimulants can be applied by three routes of bathing, injection, or oral administration. Traditionally, herbal extracts have been utilized as an appetizer, antibacterial, antiviral, antibacterial anticancer, as well as an antioxidant agent while attenuating the free radicals (Amensour *et al*., 2010; Mousavi *et al*., 2011; Safari *et al*., 2017).

Myrtle, *Myrtus communis* L, is a medicinal plant with a perennial shrub and dispersed in the Mediterranean regions, especially in Iran. The leaves of this plant contain essential oil, myrtenyl acetate, limonene, polyphenolic compounds like saponins, flavonoids, and tannins (Mansouri Taee *et al*., 2017; Safari *et al*., 2017).

However, in our knowledge, there is no information on the effects of myrtle on the Siberian sturgeon. Hence, the objective of the current study was to determine the effects of biological activities and phytochemical composition of myrtle on immuno-physiological and tissue histomorphological responses and antimicrobial properties of Siberian sturgeon.

Materials and methods

Preparation of myrtle

The myrtle leaves were obtained from the central region of Iran (Yazd province). The leaves were dried at 37°C in an incubator for 4 days and mailed by

[DOR: 20.1001.1.15622916.2021.20.5.16.3]

a grinder. After that, leaves were powdered (10 g) and blended with ethanol (100 mL, 70%) and then shaked by rotary shaker for 24 hour (h). The collected extract was preserved in a dark container at 4°C, pending to use (Amensour *et al*., 2010).

Total phenolic and flavonoid content

The content of total phenol was quantified according to the Folin-Ciocalteu method (Stocker *et al*., 2004). In brief, an aliquot (0.25 mL of extract) was added with Folin-Ciocalteu reagent (1.25 mL) and distilled water (0.5 mL). The compound was agitated and allowed to stand for 5 min before adding 1.25 mL of sodium carbonate solution (7%). The absorbance of the resulting dilution was measured at 760 nm. The total flavonoid content was determined based on the method summarized by Wannes and Marzouk (2016). Briefly, 250 mL of diluted extract was blended with 75 mL $NaNO_2$ (5%). After 6 minutes, 500 mL of NaOH, and 150 mL of $AlCl_3$ were added to this mixture. Then, the mixture remains stable at room temperature for 15 min; the absorbance was quantified at 510 nm. The condense tannin was detected with Ferric (2% ferric ammonium sulphate in 2 N HCl) and HCL-Butanol reagents (Porter *et al*., 1986). The total flavonoid values of extract were displayed as milligram catechin equivalents per gram (mg CE/g) through a calibration curve with catechin (3 replicates).

Fish and experimental conditions

A total number of 500 Siberian sturgeon (average weight: 15.1±1.03 g) were distributed into 15 circular fiberglass tanks (350 liters) at the International Sturgeon Research Institute (Rasht, Iran). Water quality indices including dissolved oxygen, water temperature, pH, nitrite, and NH_3 were measured as 7.19±0.5 mg/L, 19.1±1.52°C, 7.35±0.65, <0.1 mg/L and <0.05 mg/L, respectively.

Determination of trial dose and preparation of the experiment

The acute toxicity was tested for myrtle extract followed the guidelines for chemical tests approved by the OECD (1998). The experiment was carried out in a static system without water renewal. Sturgeon mortality at each myrtle extract concentration was recorded at 24, 48, 72, and 96 h. LC_{50}-96 h and maximum allowable concentration (MAC) were quantified in 269.8 mg/L by a computer program (CEAM, 1999) using Finney (1952) Probit Analysis. In the next step, 4 doses of myrtle extract were used including 25% (67.4 mg/L), 50% (134.9 mg/L), 75% (202.0 mg/L), and 100% (269.8 mg/L) of the MAC value and a control treatment (without exposure myrtle extract), hereafter named as M_{25}, M_{50}, M_{75}, M_{100}, and control, respectively. Each treatment contained 3 replications. During the test experiment, the water inlet was stopped. The tanks were continuously aerated with air pumps. Siberian sturgeon was exposed to the suspected doses for 96 h.

[DOR: 20.1001.1.15622916.2021.20.5.16.3]

Sampling and blood collection

At the end of the 4th day, blood sampling was performed. It should be noticed that tranquilizer was not utilized in the samplings because they can be an influence on the blood indices. In order to solve this bottleneck, fish were sacrificed by a sharp blow to the head. After that, the blood samples were randomly collected via the caudal vein of three fish per replicate. An aliquot of the blood sample in the heparinized vial was used for hematological assays, and the second aliquot in the non-heparinized vial was centrifuged at room temperature. The sera preserved at -20°C until further analysis.

Blood processing and analyses

The number of white blood cells (WBCs) and red blood cells (RBCs) were counted by hemocytometer after diluting blood samples by adding Turk solution and Hayem solution (isotonic solution), respectively (Blaxhall and Daisley, 1973). The estimation of the differential leukocyte counts, including lymphocyte, neutrophil, eosinophil, and monocyte were manually counted and determined using a light microscope. The standard microhematocrit method was utilized to measure hematocrit (Hct), and the values expressed as a percentage of erythrocytes. The hemoglobin (Hb) concentration was determined using spectrophotometry (540 nm) with the cyanomethahemoglobin method, blood mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were quantitated based on the method described by Blaxhall and Daisley (1973). Hemato-biochemical factors including albumin, glucose, globulin glucose, cortisol, and hepatic attributes of serum such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), as well as immunological indices such as lysozyme and total immunoglobulin, were quantified by an autoanalyzer instrument (Perstige 24i, Boeki, Japan) using commercial kits (Pars-Azmoon, Karaj, Iran) according to the manufacturer's protocol.

Antioxidant activities

The assay of catalase (CAT) in serum samples was accomplished, according to Aebi (1984), by assaying the reduction of H_2O_2 value at 240 nm. One unit of CAT activity was defined as the quantity of CAT needed to transform 1 μmol of H_2O_2/min. The superoxide dismutase (SOD) activity was defined using the method of Beauchamp and Fridovich (1971). SOD value was calculated using the sample that catalyzes the breakdown of μmol of O^{-2} to hydrogen peroxide and oxygen/min. Glutathione peroxidase (GPx) activity was detected using the technique illustrated by Adel *et al*. (2017).

Gill and liver histopathology

At the end of the experiment, three fish per treatment euthanized with 300 mg/L clove powder (Barij Essence, Iran;

[DOR: 20.1001.1.15622916.2021.20.5.16.3]

Banavreh *et al*., 2019a) to remove the gills (second-gill arches of the left side) and liver under aseptic conditions for histopathological analysis. The respective specimens were fixed in Bouin's solution for 18-24 h, dehydrated in soaring concentrations of ethanol, cleaning with xylene, and then paraffin embedding, and prepared for histological studies. The combined tissues were sectioned with a thickness of 4-6 µm using a microtome (Leitz-1512, Germany) (Rezakhani *et al*., 2020). Thereafter, these segments were stained with H&E. Histopathological modifications were appraised by a light microscope (Nikon, Ni-U, Japan).

Statistical analysis

After normality verification using a Shapiro-Wilk test, data were analyzed by a one-way ANOVA followed by Tukey's test. All comparisons were accomplished by SPSS software version no. 22.0 (Chicago, USA), and variations were considered significant at $p<0.05$. The quantitative analyses of the data were displayed as mean±SD.

Results

Phenolic contents

The phenolic compounds of the extract of myrtle are described in Table 1.

Table 1: Phenolic compounds of myrtle extract.

	leaves		
Sample	**Total phenols**	**Proanthocyanidins[1]**	**Flavonoids**
Myrtle extract	22.63±0.03	0.52±0.02	0.56±0.01

Total phenolic was expressed by mg gallic acid/g dry matter; flavonoids and proanthocyanidin values were expressed by mg catechin/g dry matter.
[1]As condense tannins equivalents

Hematological indices

As shown in Table 2, Hb and RBC values were significantly increased in fish exposed to the myrtle extracts (M_{25}, M_{50}, and M_{75}) compared with those of the control treatment ($p<0.05$; Tab 2). A similar tendency was obtained for MCV values, while most of the values were observed in M_{50} treatment. Hct values showed no variation in different groups ($p>0.05$). The WBCs was lower in M_{25} and M_{75}, while the highest value was recognized in M_{100} treatment ($p<0.05$).

Monocyte value was higher in more than 50% of the MAC value compare to the control and M_{25} groups. However, an increasing percentage of lymphocyte was observed in M_{25}, M_{50}, and M_{75} groups, but no significant differences were detected between any groups ($p>0.05$; Table 3). Unlike monocyte, the lowest eosinophils and neutrophils were found in M_{25}, M_{50} groups ($p<0.05$).

Fish in M_{25} and M_{50} groups had the highest albumin, which differs significantly from the other treatments (Table 4).

[DOR: 20.1001.1.15622916.2021.20.5.16.3]

Table 2: Hematological values of juvenile Siberian sturgeon exposed to different concentrations of myrtle.

Indices	Treatments				
	Control	**M_{25}**	**M_{50}**	**M_{75}**	**M_{100}**
Hb (g/dL)	5.17±0.15^{a}	6.13±0.21^{b}	6.37±0.15^{b}	6.17±0.25^{b}	5.13±0.25^{a}
RBC ($\times10^3$/mm^3)	510.01±0.04^{a}	595.00±0.01^{d}	598.1±0.01^{e}	584.99±0.01^{c}	531.01±0.02^{b}
WBCs ($\times10^3$/mm^3)	4.90±0.08^{d}	4.09±0.05^{a}	4.80±0.07^{c}	4.29±0.04^{b}	5.89±0.06^{e}
Hct (%)	24.67±2.52	30.33±0.57	30.01±3.61	30.06±1.02	25.67±1.53
MCV (fl)	489.67±10.07^{a}	506.01±4.58^{ab}	519.67±7.02^{c}	511.33±10.60^{bc}	491.67±3.79^{ab}
MCHC (g/dL)	20.83±0.06^{b}	20.57±0.25^{b}	20.30±0.10^{b}	20.20±0.50^{ab}	19.57±0.15^{a}

Notes. M_{25}: 25% of MAC; M_{50}: 0% of MAC; M_{75}: 75% of MAC; M_{100}: 100% of MAC. Data are presented as mean±SD. The presence of different superscript letters denotes significant variation between treatments (p<0.05). The absence of letters indicates no significant difference between treatments (p>0.05)

Table 3: Differential leukocyte counts of juvenile Siberian sturgeon exposed to different concentrations of myrtle.

Indices	Treatments				
	Control	**M_{25}**	**M_{50}**	**M_{75}**	**M_{100}**
Monocyte (%)	4.03±0.15^{a}	4.07±0.12^{a}	5.03±0.15^{b}	5.10±0.10^{c}	5.33±0.35^{c}
Lymphocyte (%)	79.67±1.53	84.33±2.8	84.67±2.52	85.01±3.04	81.67±1.53
Eosinophil (%)	0.97±0.25^{b}	0.97±0.15^{b}	0.00±0.00^{a}	0.00±0.00^{a}	1.97±0.15^{C}
Neutrophil (%)	15.13±0.35^{c}	10.17±0.29^{a}	11.97±0.55^{b}	11.13±38^{ab}	10.97±0.45^{ab}

Notes. M_{25}: 25% of MAC; M_{50}: 0% of MAC; M_{75}: 75% of MAC; M_{100}: 100% of MAC. Data are presented as mean ± SD. The presence of different superscript letters denotes significant variation between treatments (p<0.05). The absence of letters indicates no significant difference between treatments (p>0.05).

Table 4: Effect of various concentrations of myrtle extracts on hemato-biochemical parameters in juvenile Siberian sturgeon

Indices	Treatments				
	Control	**M_{25}**	**M_{50}**	**M_{75}**	**M_{100}**
Albumin (g/dL)	0.75±0.03^{a}	0.93±0.00^{d}	0.89±0.01^{cd}	0.82±0.02^{b}	0.84±0.01^{bc}
Total protein (g/dL)	1.76±0.01^{c}	1.95±0.01^{e}	1.93±0.00^{de}	1.81±0.01^{c}	1.79±0.00^{c}
Glucose (mg/dL)	49.01±2.65	47.33±2.58	52.33±1.53	52.46±2.65	56.67±3.53
Cortisol (µg/L)	104.33±5.13^{a}	110.21±5.01^{a}	108.11±7.02^{a}	117.52±4.08^{b}	122.32±7.37^{b}
ALP (U/L)	711.67±0.41^{c}	865.01±4.36^{d}	659.33±4.04^{b}	602.00±5.19^{a}	945.01±4.58^{e}
ALT (U/L)	18.00±1.73^{a}	34.67±2.08^{c}	28.03±3.61^{b}	22.33±2.52^{ab}	37.67±1.53^{c}
AST (U/L)	394.33±4.23^{b}	377.00±5.29^{c}	326.02±5.19^{a}	482.33±1.16^{c}	531.20±3.61^{d}
LDH (U/L)	984.70±1.53^{e}	661.03±9.54^{b}	826.72±5.69^{c}	887.7±14.01^{d}	984.71±1.53^{e}

Notes. M_{25}: 25% of MAC; M_{50}: 0% of MAC; M_{75}: 75% of MAC; M_{100}: 100% of MAC. Data are presented as mean±SD. The presence of different superscript letters denotes significant variation between treatments (p<0.05).

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.

Albumin and total protein levels wereparalleled each other. The glucose level was not significantly different in fish exposed to the tested concentrations, while cortisol value increased more than 202 mg/L in M_{75} group. The lowest ALP and ALT activity was recognized in the M_{75} group, while the inferior AST value was observed in the M_{50} group (p<0.05). LDH was decreased significantly in the M_{25} and then increased subsequently. The lysozyme activity of serum was elevated significantly in M_{50} and M_{75} groups compared to the control and M_{100}

[DOR: 20.1001.1.15622916.2021.20.5.16.3]

groups (p<0.05; Fig. 1). Serum total Ig content was higher in fish exposed to M_{25} and M_{50} than control and M_{100} groups (p<0.05; Fig. 2).

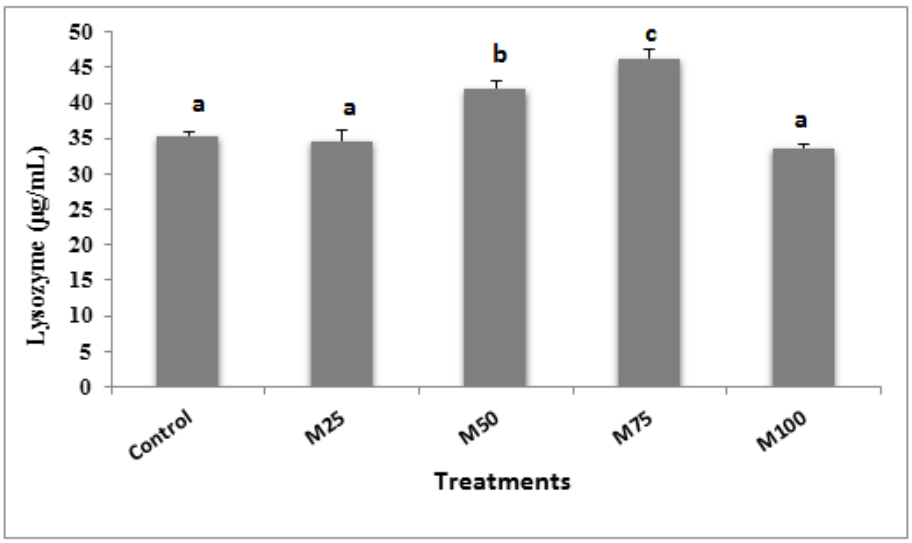

Figure 1: Lysozyme activity of juvenile Siberian sturgeon exposed to different concentrations of myrtle.

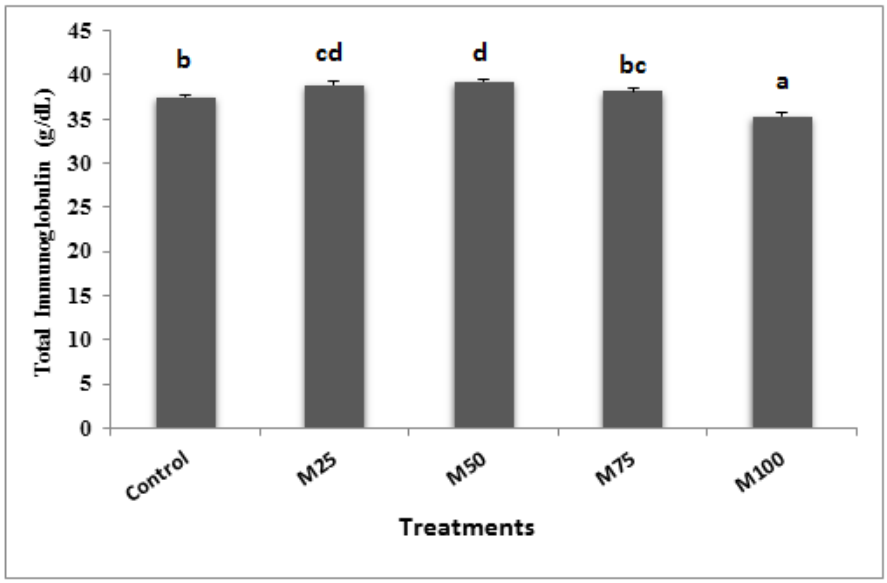

Figure 2: Total Ig of juvenile Siberian sturgeon exposed to different concentrations of myrtle.

Antioxidant enzymes

The antioxidant enzyme activities of serum are presented in Table 5. SOD and CAT activities of those fish exposed to M_{50} and M_{75} were significantly higher in comparison to the control and M_{100} groups (p<0.05). The lowest GPX activity was observed in the control group (p<0.05).

[DOR: 20.1001.1.15622916.2021.20.5.16.3]

Table 5: Antioxidant enzyme activities in serum of juvenile Siberian sturgeon exposed to different concentrations of myrtle.

Indices	Treatments				
	Control	M_{25}	M_{50}	M_{75}	M_{100}
SOD (U/mL)	55.67±0.58^{b}	52.13±1.73^{b}	63.02±3.12^{c}	66.33±2.89^{c}	40.67±1.53^{a}
GPX (U/mL)	324.67±1.53^{a}	551.67±5.69^{d}	404.33±5.13^{b}	478.33±5.69^{c}	621.33±3.51^{e}
CAT (U/mL)	63.33±1.53^{a}	71.00±2.00^{b}	80.33±0.58^{c}	85.00±1.00^{d}	74.33±1.16^{b}

Notes. M_{25}: 25% of MAC; M_{50}: 0% of MAC; M_{75}: 75% of MAC; M_{100}: 100% of MAC. Data are presented as mean±SD. The presence of different superscript letters denotes significant variation between treatments (p<0.05).
Abbreviations: SOD, Superoxide dismutase; GPX, glutathione peroxidase; CAT; Catalase.

Gill and liver histopathology

After 4th day of exposure, the histopathological variation in gill and liver are shown in Tables 6 and 7. The severity of changes in the histopathological attributes were specified as lack of tissue lesion (-), mild (+), moderate (++), and severe (+++).

Table 6: Semi-quantitative scouring of histopathology in the gill of Siberian sturgeon after 96 h exposure to different levels of myrtle extract.

Apoptosis	Control	M_{25}	M_{50}	M_{75}	M_{100}
Edema in the lamella epithelium	+++	++	+	+	++
Detachment of lamella epithelium	-	+	-	-	+
Adhesion of the lamellae	+	+++	+++	+++	++
Curling of lamella	+	+++	+++	++	+++
Dilation of filamentous capillaries	+++	-	-	-	++
Telangiectasia	-	-	-	-	+
Hemorrhages	-	-	-	++	+
Necrosis of the epithelial cell	-	-	-	++	-
Epithelial hypertrophy	-	+++	+++	+	+++
Hyperplasia	-	-	-	-	++

Score: Lack of alteration (-), Mild alteration (+), moderate alteration (++), severe alteration (+++).

Table 7: Semi-quantitative scouring of histopathology in the liver of Siberian sturgeon after 96 h exposure to different levels of myrtle extract

Apoptosis	Control	M_{25}	M_{50}	M_{75}	M_{100}
Atrophy	-	++	++	+++	+++
Nuclear pyknosis	+++	+	++	++	++
Necrosis	+++	-	++	++	++
Hyperemia in sinusoids	-	-	-	-	+
Accumulation of melanomacrophages	-	-	-	-	-

Score: Lack of alteration (-), Mild alteration (+), moderate alteration (++), severe alteration (+++).

The severity of lamella edema and filamentous dilation were observed in the control group compared to the other groups (Fig. 3). Juvenile fish exposed to the M_{25} and M_{50} treatments had severe adhesion and curling of lamella as well as epithelial hypertrophy. Overall, most of the number of gill changes was observed in fish exposed to the M_{100} group.

[DOR: 20.1001.1.15622916.2021.20.5.16.3]

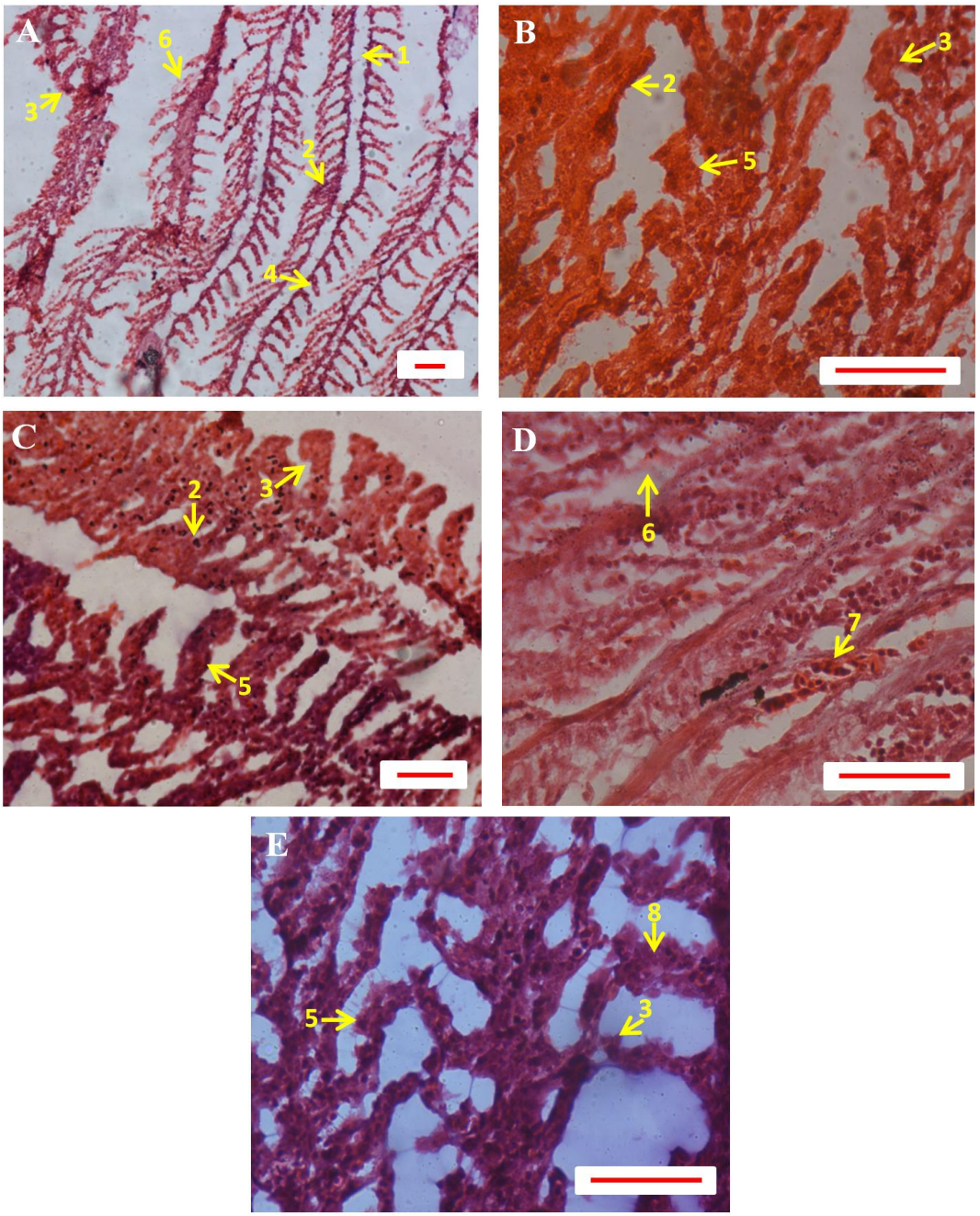

Figure 3: Longitudinal pathological section of the gill in juvenile Siberian sturgeon using H&E staining. A (control), B (M_{25}), C (M_{50}), D (M_{75}), E (M_{100}).1, dilation of filamentous capillaries; 2, adhesion of the lamellae; 3; curling of lamella; 4, edema in the lamella epithelium; 5, epithelial hypertrophy; 6, necrosis of the epithelial cell; 7, hemorrhages; 8, hyperplasia. Scale bar= 50 µm.

In the control group, severe hepatocyte destruction was accompanied by nucleus pyknosis (Table 7). The severity of atrophy was observed in M_{75} and M_{100} treatments (Fig. 4). Nevertheless, hyperemia in sinusoids was not an apparent discrepancy between treatments. Accumulation of melanomacrophages was not found in any treatments.

[DOR: 20.1001.1.15622916.2021.20.5.16.3]

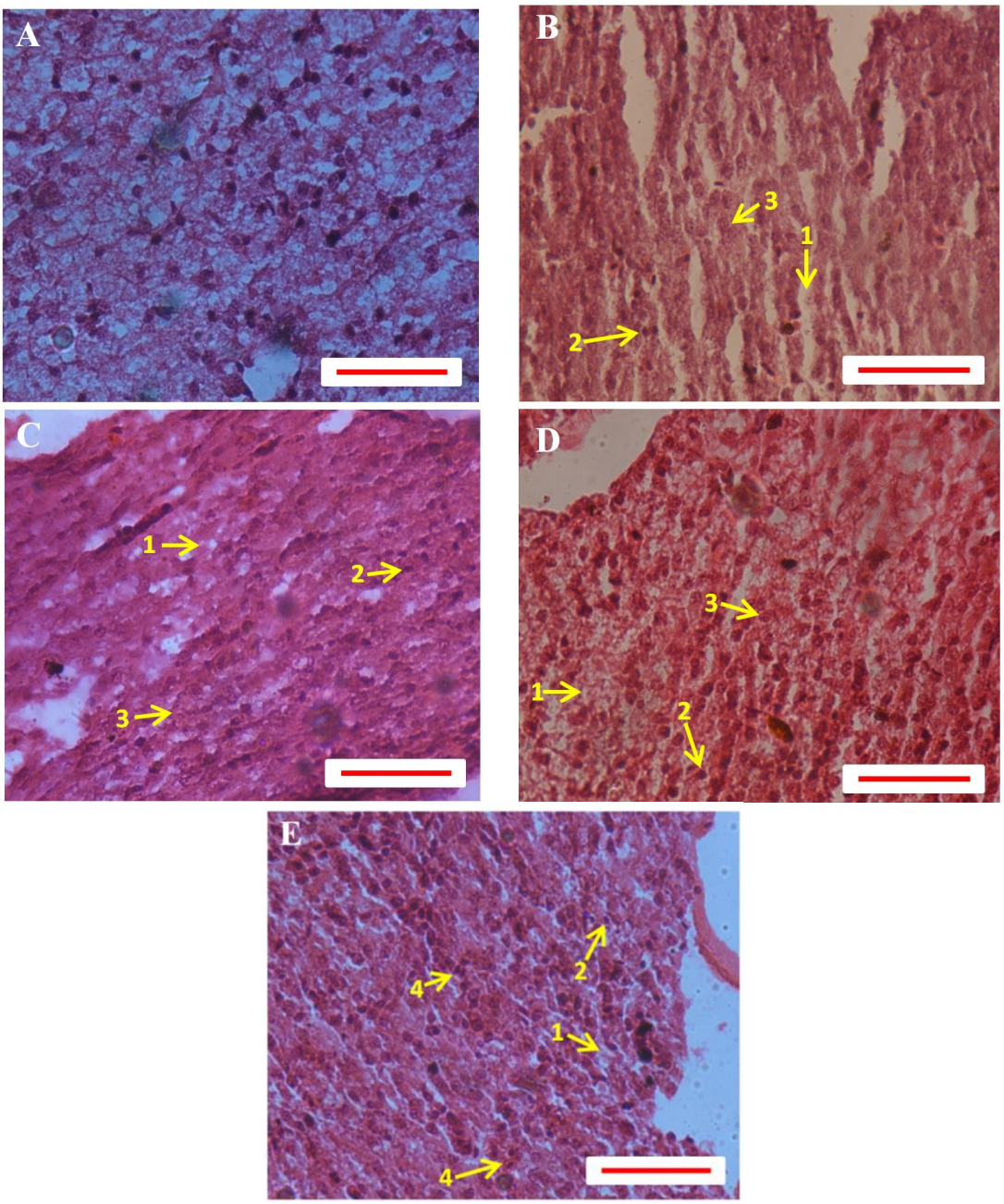

Figure 4: Longitudinal pathological section of the liver of juvenile Siberian sturgeon using H&E staining. A (control), B (M_{25}), C (M_{50}), D (M_{75}), E (M_{100}).1, necrosis; 2, nuclear pyknosis; 3, atrophy; 4, hyperemia in sinusoids. Scale bar= 50 µm.

Discussion

In the current study, increase in Hb and RBC were observed in fish exposed to the myrtle extract M_{75} (202 mg/L). In line with this, Goda (2008) stated that dietary immunostimulant (ginseng herb) levels in a dose-dependent manner lead to increase hematological indices such as RBC, Hb, and Hct in Nile tilapia (*Oreochromis niloticus*) fingerlings. The author believed that ginseng derivative enhances the hematological function in

[DOR: 20.1001.1.15622916.2021.20.5.16.3]

Nile tilapia. Moreover, Moghaddam *et al*. (2017) demonstrated that Siberian sturgeon fed supplemental *Aloe vera* extract had a significantly higher amount of hemoglobin than the control group. In contrast, the WBCs value in the mentioned above studies was higher compare with the control group, while in the present study, this trend in the eosinophils and neutrophils were lower up to 50% of the MAC value. Inconsistency results may be related to the routes of administration of extracts, species-specific, and fish age (Van Hai, 2015). The higher monocyte in the bloodstream is a crucial indicator of fish health (de Moraes *et al*., 2018). In the present study, monocyte was elevated in higher myrtle extract groups. This can be ascribed to an increase in fish immunocompetence in the exposed groups. In our finding, although the results revealed that lymphocytes did not manifest any variations, however, decreased with rising exposure to 202 mg/L (M_{75}) of myrtle extract in juvenile Siberian sturgeon. Dadras *et al*. (2016) found that dietary administration of rosehip and safflower ended in strengthening the nonspecific defense system to pathogens and bacteria in beluga (*Huso huso*).

It is demonstrated that, an increase in the biomolecules, such as total protein and albumin value, triggers the fortified immune response of fishes, stress level, and physiology welfare of fish (Damusaru *et al*., 2019; Mukherjee *et al*., 2019). Our finding revealed that the levels of albumin and total protein were fortified in M_{25} and M_{50} groups. In accordance with our result, Roychowdhury et al (2020) postulated that failures in liver function might lead to lower serum protein and albumin. Further, Harikrishnan *et al*. (2009) advocated that dip treatment with triherbal solvent extract from *Azadirachta indica*, *Ocimum sanctum*, and *Curcuma longa* aqueous leaf extract displayed a notable boost in serum total protein in goldfish, *Carassius auratus*.

Glucose and cortisol in blood serum are indices that are widely used as stress responses (Naderi, *et al*., 2019). Cortisol level was increased in fish exposed to more than 134.8 mg/L (M_{50}) of myrtle extract, but these differences had disappeared to this level of myrtle extract. This finding demonstrated a higher inclusion of extract or more prolonged exposure lead to higher levels of cortisol in the bloodstream, thereby low immunity and inflammatory responses and indicating that the fish were under stress.

Serum hepatic enzymes are very sensitive indices applied in diagnosing hepatic impairment because they are cytoplasmic enzymes and are delivered into the bloodstream after cellular damage (Sadeghi *et al*., 2013). Sadeghi *et al*. (2013) stated that myrtle oil extract added to diets containing aflatoxin of broiler chicks could decrease ALT, AST, and ALP activities. Likewise, Sen *et al*. (2016) declared that hepatic damage in the rats was markedly ameliorated by feeding with dietary myrtle extracts. In corroborated with our

[DOR: 20.1001.1.15622916.2021.20.5.16.3]

result, except for AST, the lowest amount of liver enzymes was observed in M_{50} treatment. Similar results were reported that the lower levels of hepatic enzymes might be due to the medicinal herbs' hepatoprotective influence (Dadras *et al*., 2016). Our finding revealed that LDH has a decreasing trend in M25, M50, and M75 groups following the enzymes mentioned above.

It has been illustrated that sturgeons have superior levels of lysozyme activities (Banavreh *et al*., 2019b). Lysozyme is a main first-line host fish defense agent that is responsible for destroying pathogens and is commonly measured as an important sign of innate immune function in fish (Verlhac *et al*., 1998; Dadras *et al*., 2016). Lysozyme hydrolysis of β (1-4) glycosidic bonds in the peptidoglycan of bacterial cell walls (Mirghaed *et al*., 2019). It is well documented that herbs bioactive compounds, like polyphenols and flavonoids, could be elevated lysozyme and immunity reinforcement in fish (Hwang *et al*., 2013; Van Hai, 2015; Soltani *et al*., 2018; Banavreh *et al*., 2019b). By far, little information is available regarding exposure of fish to herbal extracts. The present result revealed that juvenile sturgeons exposed to myrtle extract significantly affected lysozyme activity in M_{50} and M_{75} groups, while Mansouri Taee *et al*. (2017) suggested that rainbow trout fed with myrtle supplemented diet failed influenced on skin mouse lysozyme activity. The conflicting result may be related to the mode of administration, species, and fish exposure duration. In addition, many authors announced that phytoimmunostimulant could improved Ig (Dadras *et al*., 2016; Hoseinifar *et al*., 2016). In this vein, serum Ig of juvenile Siberian sturgeon increased in M_{25} and M_{50} groups; this can be attributed to the slight increase, but not significant changes of lymphocyte percentage.

CAT, SOD, and GPX activities usually imply an improved antioxidative defense system in fish and the initial step of the enzymatic antioxidative defense system that removes the reactive oxygen species to preserve tissues from oxidative damage (Liu *et al*., 2019). In rabbits, Sepici-Dincel *et al*. (2007) found an ameliorating effect of myrtle on the SOD and CAT activities in the liver. Similarly, Safari *et al*. (2017) reported that Zebrafish (*Danio rerio*) fed with myrtle powder led to positive effects of antioxidant enzymes gene expression. In our study, antioxidants activities were higher in fish exposed to the M_{50} and M_{75} groups, implying the occurrence of a compensatory response to protect against stress-induced impairment.

In piscine, the gill is a multifunctional organ that performs a crucial role in physiological functions likes ammonium excretion, acid-base regulation, and ion transportation. Accordingly, the modifications recognized in gill histomorphology may trigger disorder of the gill's physiological function; thereby, fish is threatened (Chupani *et al*., 2016). Unfortunately, no data have been

[DOR: 20.1001.1.15622916.2021.20.5.16.3]

reported on the effects of myrtle on histomorphological parameters of animals. Our findings showed that Siberian sturgeon exposed to myrtle in <202 mg/L does did not appear disruption of the physiochemical function of the gill, but more than this range could lead to disorder function. However, the paucity of data makes it hard to have definitive conclusions, and more investigation is required.

It is demonstrated that liver impairment can influence health status. Our research findings pointed out that there was no significant alteration in the liver in M_{25} and M_{50} groups in the course of exposure.

The results of current study indicated that the concentration of <202 mg/L could be proposed as an adjuvant immunostimulant in juvenile Siberian sturgeon. Thus, it is possible to increase the resistance of fish to diseases and reduce the use of drugs in aquaculture. More researches are needed to assess the potentials effects of physiological and osmoregulation indices in this spices.

Acknowledgement

Authors thank the staff of International Sturgeon Research Institute of the Caspian Sea for their assistance.

References

Adel, M., Gholaghaie, M., Khanjany, P. and Citarasu, T., 2017. Effect of dietary soybean lecithin on growth parameters, digestive enzyme activity, antioxidative status and mucosal immune responses of common carp (*Cyprinus carpio*). *Aquaculture Nutrition*, 23(**5**), 1145–1152. DOI: 10.1111/anu.12483.

Aebi, H., 1984. Catalase. *Methods Enzymol*, 105(**1947**), 121–126.

Afzali, S.F. and Wong, W.L., 2017. In vitro screening of Sonneratia alba extract against the oomycete fish pathogen, Aphanomyces invadans. *Iranian Journal of Fisheries Sciences*, 16(**4**), 1333–1340.

Amensour, M., Bouhdid, S., Fernández-López, J., Idaomar, M., Senhaji, N.S. and Abrini, J., 2010. Antibacterial activity of extracts of myrtus communis against food-borne pathogenic and spoilage bacteria. *International Journal of Food Properties*, 13(**6**), 1215–1224. DOI 10.1080/10942910903013399.

Banavreh, A., Soltani, M., Kamali, A., Yazdani-Sadati, M.A. and Shamsaie, M., 2019a. Effects of olive pomace on growth performance, digestibility, body composition and fatty acid profile in yearling Siberian sturgeon, *Acipenser baerii* (Brandt 1896). *Aquaculture Nutrition*, 25(**2**), 333–342. DOI:10.1111/anu.12858.

Banavreh, A., Soltani, M., Kamali, A., Yazdani-Sadati, M.A. and Shamsaie, M., 2019b. Immuno-physiological and antioxidant responses of Siberian sturgeon (*Acipenser baerii*) fed with different levels of olive pomace. *Fish Physiology and Biochemistry*, 45, 1419–1429. DOI: 10.1007/s10695-019-00649-y.

[DOR: 20.1001.1.15622916.2021.20.5.16.3]

Beauchamp, C. and Fridovich, I., 1971. Superoxide Dismutase. *Analytical Biochemistry*, 44, 276–287.

Blaxhall, P.C. and Daisley, K.W., 1973. Routine hematological methods for use with fish blood. *Journal of Fish Biology*, 5, 771–781.

CEAM (Center for Exposure Assessment Modeling)., 1999. U.S. E.P.A., "LC50 software program, version 1.00", CEAM Distribution Center.

Chupani, L., Zuskova, E., Stara, A., Velisek, J. and Kouba, A., 2016. Histological changes and antioxidant enzyme activity in signal crayfish (*Pacifastacus leniusculus*) associated with sub-acute peracetic acid exposure. *Fish & Shellfish Immunology*, 48, 190–195. DOI: 10.1016/j.fsi.2015.11.010.

Dadras, H., Hayatbakhsh, M.R., Shelton, W.L. and Golpour, A., 2016. Effects of dietary administration of Rose hip and Safflower on growth performance, haematological, biochemical parameters and innate immune response of Beluga, *Huso huso* (Linnaeus, 1758). *Fish and Shellfish Immunology*, 59, 109–114. DOI: 10.1016/j.fsi.2016.10.033.

Damusaru, J.H., Moniruzzaman, M., Park, Y., Seong, M., Jung, J.Y., Kim, D.J. and Bai, S.C., 2019. Evaluation of fish meal analogue as partial fish meal replacement in the diet of growing Japanese eel Anguilla japonica. *Animal Feed Science and Technology*, 247, 41-52. DOI: 10.1016/j.anifeedsci.2018.10.018.

de Moraes, A.V., Pereira, M. de O., Moraes, K.N., Rodrigues-Soares, J.P., Jesus, G.F.A. and Jatobá, A., 2018. Autochthonous probiotic as growth promoter and immunomodulator for *Astyanax bimaculatus* cultured in water recirculation system. *Aquaculture Research*, 49(**8**), 2808–2814. DOI: https://doi.org/10.1111/are.13743.

Finney, D.J., 1952. Probit analysis (2nd Ed). Journal of the Institute of Actuaries, 78(3), 388– 390.

Fontagné-Dicharry, S., Larroquet, L., Dias, K., Cluzeaud, M., Heraud, C. and Corlay, D., 2018. Effects of dietary oxidized fish oil supplementation on oxidative stress and antioxidant defense system in juvenile rainbow trout (*Oncorhynchus mykiss*). *Fish and Shellfish Immunology*, 74, 43–51. DOI:10.1016/j.fsi.2017.12.039.

Goda, A.M.A.S., 2008. Effect of dietary ginseng herb (Ginsana ® G115) supplementation on growth, feed utilization, and hematological indices of Nile Tilapia, *Oreochromis niloticus* (L.), fingerlings. *Journal of the World Aquaculture Society*, 39(**2**), 205–214. DOI: 10.1111/j.1749-7345.2008.00153.x.

Harikrishnan, R., Balasundaram, C., Kim, M.C., Kim, J.S., Han, Y.J. and Heo, M.S., 2009. Innate immune response and disease resistance in *Carassius auratus* by triherbal solvent extracts. *Fish and Shellfish*

[DOR: 20.1001.1.15622916.2021.20.5.16.3]

Immunology, 27(**3**), 508–515. DOI: 10.1016/j.fsi.2009.07.004.

Harikrishnan, R., Balasundaram, C., Heo, M.S., 2011. Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. *Aquaculture*, 317, 1–15.DOI:10.1016/j.aquaculture.2011.03.039.

Hoseinifar, S.H., Zoheiri, F. and Lazado, C.C., 2016. Dietary phytoimmunostimulant Persian hogweed (*Heracleum persicum*) has more remarkable impacts on skin mucus than on serum in common carp (*Cyprinus carpio*). *Fish and Shellfish Immunology*, 59, 77–82. DOI: 10.1016/j.fsi.2016.10.025.

Hwang, J.H., Lee, S.W., Rha, S.J., Yoon, H.S., Park, E.S., Han, K.H. and Kim, S.J., 2013. Dietary green tea extract improves growth performance, body composition, and stress recovery in the juvenile black rockfish, *Sebastes schlegeli*. *Aquaculture International*, 21(**3**), 525–538. DOI:10.1007/s10499-012-586-5.

Liu, G., Ma, S., Chen, F., Gao, W. and Zhang, W., 2019. Effects of dietary lysolecithin on growth performance, feed utilization, intestinal morphology and metabolic responses of channel catfish (*Ictalurus punctatus*). *Aquaculture Nutrition*, 26(**2**),456-465.
DOI: 10.1111/anu.13008.

Long, M., Lin, W., Hou, J., Guo, H., Li, L., Li, D. and Yang, F., 2017. Dietary supplementation with selenium yeast and tea polyphenols improve growth performance and nitrite tolerance of Wuchang bream (*Megalobrama amblycephala*). *Fish and Shellfish Immunology*, 68(**1**), 74–83. DOI: 10.1016/j.fsi.2017.07.017.

Mansouri Taee, H., Hajimoradloo, A., Hoseinifar, S.H. and Ahmadvand, H., 2017. Dietary Myrtle (*Myrtus communis* L.) improved non-specific immune parameters and bactericidal activity of skin mucus in rainbow trout (*Oncorhynchus mykiss*) fingerlings. *Fish and Shellfish Immunology*, 64, 320–324. DOI: 10.1016/j.fsi.2017.03.034.

Mirghaed, A.T., Yarahmadi, P., Soltani, M., Paknejad, H., Hoseini, S.M., 2019. Dietary sodium butyrate (Butirex® C4) supplementation modulates intestinal transcriptomic responses and augments disease resistance of rainbow trout (*Oncorhynchus mykiss*). *Fish & Shellfish Immunology*, 92, 621–628. DOI: 10.1016/j.fsi.2019.06.046.

Mirzakhani, M.K., Abedian Kenari, A., Motamedzadegan, A. and Banavreh, A., 2020. Apparent digestibility coefficients of crude protein, amino acids, crude lipid, dry matter and gross energy of ten feedstuffs for yearling Siberian sturgeon (*Acipenser baerii)*. *Iranian Journal of Fisheries Sciences*, 1–17. DOI: 10.22092/ijfs.2020.120754.

Moghaddam, B.S., Haghighi, M., Rohani, S.M., Hamidi, M. and Ghasemi, M., 2017. The effects of different levels of *Aloe vera* extract on some of the hematological and

non-specific immune parameters in Siberian sturgeon (*Acipenser baerii*). *Iranian Journal of Fisheries Sciences*, 16(**4**), 1234–1247. DOI: 10.22092/IJFS.2018.114733.

Mousavi, S.M., Wilson, G., Raftos, D., Mirzargar, S.S. and Omidbaigi, R., 2011. Antibacterial activities of a new combination of essential oils against marine bacteria. *Aquaculture International*, 19(**1**), 205–214. DOI: 10.1007/s10499-010-9354-3.

Mukherjee, A., Chandra, G. and Ghosh, K., 2019. Single or conjoint application of autochthonous Bacillus strains as potential probiotics: Effects on growth, feed utilization, immunity and disease resistance in Rohu, *Labeo rohita* (Hamilton). *Aquaculture*, 512, 734302. DOI:10.1016/j.aquaculture.2019.734302.

Naderi, M., Keyvanshokooh, S., Ghaedi, A. and Salati, A.P., 2019. Interactive effects of dietary Nano selenium and vitamin E on growth, haematology, innate immune responses, antioxidant status and muscle composition of rainbow trout under high rearing density. *Aquaculture Nutrition*, 25(**5**), 1156–1168. DOI: 10.1111/anu.12931.

OECD, 1998. Test No. 212: Fish, Short-Term Toxicity Test on Embryo and Sac-Fry Stages. OECD Publishing.

Porter, L.J., Hrstich, L.N. and Chan, B.G., 1986. The conversion of proanthocyanidins and prodelphinidins to cyanidin and delpinidin. *Phytochemistry*, 25, 223–230.

Rezakhani, S., Mohammadizadeh, F., Khara, H., Bahri, A.H. and Ahmadnezhad, M., 2020. Immunological, hematological, and histological responses in blood, spleen and gill of *Salmo caspius* juveniles exposed in different water temperatures, Iranian Journal of Fisheries Sciences, 19(**6**), 2925-2942. DOI: 10.22092/IJFS.2020.122842.

Roychowdhury, P., Aftabuddin, M., Pati, M.K., 2020. Thermal stress altered growth performance and metabolism and induced anaemia and liver disorder in Labeo rohita. *Aquaculture Research*, 51(**4**), 1406-1414. DOI: 10.1111/are.14486.

Sadeghi, A.A., Saei, M.M., Nikkhah, A. and Ahmadvand, H., 2013. The effect of *Myrtus communis* oil extract on growth performance, serum biochemistry and humoral immune responses in broiler chicks fed diet containing aflatoxin B1. *Archives Animal Breeding*, 56(**1**), 842–850. DOI:10.7482/0003-9438-56-084.

Safari, R., Hoseinifar, S.H., Van Doan, H. and Dadar, M., 2017. The effects of dietary Myrtle (*Myrtus communis*) on skin mucus immune parameters and mRNA levels of growth, antioxidant and immune related genes in zebrafish (*Danio rerio*). *Fish and Shellfish Immunology*, 66, 264–269. DOI: 10.1016/j.fsi.2017.05.007.

Sen, A., Ozkan, S., Recebova, K., Cevik, O., Ercan, F., Kervancıoglu Demirci, E. and Sener, G., 2016.

[DOR: 20.1001.1.15622916.2021.20.5.16.3]

Effects of Myrtus communis extract treatment in bile duct ligated rats. *Journal of Surgical Research*, 205(**2**), 359–367.DOI: 10.1016/j.jss.2016.06.094.

Sepici-Dincel, A., Açikgöz, Ş., Çevik, C., Sengelen, M. and Yeşilada, E., 2007. Effects of in vivo antioxidant enzyme activities of myrtle oil in normoglycaemic and alloxan diabetic rabbits. *Journal of Ethnopharmacology*, 110(**3**), 498–503. DOI: 10.1016/j.jep.2006.10.015.

Soltani, M., Lymbery, A., Song, S.K. and Hosseini Shekarabi, P., 2018. Adjuvant effects of medicinal herbs and probiotics for fish vaccines. *Reviews in Aquaculture*,11(**4**), 1325-1341. DOI: 10.1111/raq.12295.

Stocker, P., Yousfi, M., Djerridane, O., Perrier, J., Amziani, R., El Boustani, S. and Moulin, A., 2004. Effect of flavonoids from various Mediterranean plants on enzymatic activity of intestinal carboxylesterase. *Biochimie*, 86(**12**), 919-925. DOI:10.1016/j.biochi.2004.09.005.

Van Hai, N., 2015. The use of medicinal plants as immunostimulants in aquaculture: A review. *Aquaculture*, 446, 88–96. DOI: 10.1016/j.aquaculture.2015.03.014.

Verlhac, V., Obach, A., Gabaudan, J., Schuep, W. and Hole, R., 1998. Immunomodulation by dietary vitamin C and glucan in rainbow trout (*Oncorhynchus mykiss*). *Fish & Shellfish Immunology*, 8(**6**), 409–424. DOI:10.1016/0044-8486(95)01238-9.

Wannes, W.A. and Marzouk, B., 2016. Characterization of myrtle seed (*Myrtus communis* var. baetica) as a source of lipids, phenolics, and antioxidant activities. *Journal of Food and Drug Analysis*, 24(**2**), 316–323. DOI: 10.1016/j.jfda.2015.11.001.

[DOR: 20.1001.1.15622916.2021.20.5.16.3]