Ann. Anim. Sci., Vol. 22, No. 4 (2022) 1323–1331 *DOI: 10.2478/aoas-2022-0036*

sciendo

DIETARY *SARGASSUM ANGUSTIFOLIUM* (MACRO-ALGAE, SARGASSACEAE) EXTRACT IMPROVED ANTIOXIDANT DEFENSE SYSTEM IN DIAZINON-EXPOSED COMMON CARP, *CYPRINUS CARPIO*

Widodo Brontowiyono[1], Saade Abdalkareem Jasim[2♦], Mustafa Z. Mahmoud[3,4♦], Lakshmi Thangavelu[5], Samar Emad Izzat[6], Ghulam Yasin[7], Hawraa Jabbar Mohammad[8], Yasser Fakri Mustafa[9], Mahboubeh Balvardi[10]

[1]Department of Environmental Engineering and Centre for Environmental Studies, Islamic University of Indonesia, Yogyakarta, Indonesia
[2]Al-maarif University College, Medical Laboratory Techniques Department, Al-anbar-Ramadi, Iraq
[3]Department of Radiology and Medical Imaging, College of Applied Medical Sciences, Prince Sattam bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia
[4]Faculty of Health, University of Canberra, Canberra, ACT, Australia
[5]Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai, India
[6]Al-Nisour University College, Baghdad, Iraq
[7]Department of Botany, Bahauddin Zakariya University, Multan, Pakistan
[8]Al-Manara College For Medical Sciences, Maysan, Iraq
[9]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Mosul 41001, Iraq
[10]Department of Biology, Payame Noor University, Tehran, Iran
♦Corresponding authors: Saade Abdalkareem Jasim: saade.a.j@uoa.edu.iq, Mustafa Z. Mahmoud: alfayrouz_clinic@yahoo.com

Abstract

The effects of different dietary levels of algae (*Sargassum angustifolium*) extract were investigated on the antioxidant system of common carp, *Cyprinus carpio*. Fish (30.2 ± 2.1 g) were fed 0 (control), 5, 10 and 15 g/kg basal diet of *Sargassum angustifolium* extract (SAE) for 60 days and then exposed to an environmentally relevant concentration of diazinon (2 mg/l) for 24 h. The biochemical assays were conducted two times including at the end of feeding period and after 24 h exposure to diazinon. According to the results, malondialdehyde (MDA) levels in the liver remained unchanged (P>0.01) during feeding period, while significantly increased in response to diazinon in control and fish fed 5 and 10 g/kg diet SAE (P<0.01). The hepatic metabolic enzymes (AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, CK: creatine kinase) showed no significant changes in all groups during feeding period, while these enzymes increased in non-SAE supplemented fish and those fed 5 and 10 g/kg SAE after exposure to diazinon (P<0.01). Although little elevations were observed in the activity of hepatic antioxidant enzymes (CAT: catalase, SOD: superoxide dismutase, GPx: glutathione peroxidase) in fish fed SAE, these elevations were not significant (P>0.01). After exposure to diazinon, antioxidant enzymes significantly decreased in control and fish fed 5 g/kg diet SAE, while the fish of 10 and 15 g/kg diet SAE treatments showed significant elevations (P<0.01). The antioxidant-related genes (*sod*, *cat*, *gpx*) significantly expressed more in response to dietary SAE compared to control (P<0.01). After exposure to diazinon, all groups showed significant elevations in antioxidant-related genes (P<0.01). In conclusion, the results of the present study revealed the antioxidant enhancing effects of SAE at dietary levels of 10 and 15 g/kg diet, and this effect may be attributed to some antioxidant components in the chemical composition of the macro-algae or to the direct effect of SAE on antioxidant defense system of the fish.

Development and industrialization of agriculture and horticulture have significantly increased the use of pesticides, fungicides and insecticides to control pests in the world (Murthy et al., 2013; Hedayati et al., 2015; Ullah and Zorriehzahra, 2015; Shahbazi Naserabad et al., 2015; Shahbazi Naserabad et al., 2017). However, this issue has raised environmental concerns about the use of these chemicals. Pesticides usually enter aquatic environments from the drainage of fields and gardens and have adverse impacts on aquatic life, especially on fishes (Brodeur et al., 2016; Poorbagher et al., 2018; Hajirezaee et al., 2019 a, b).

There are many studies reporting the adverse impacts of pesticides on vital aspects of fish such as immunity, osmoregulation, growth and reproduction (Kime, 1995; Moore and Waring, 2001; Köprücü et al., 2006; Katuli et al., 2014; Díaz-Resendiz et al., 2015).

According to literature, fish usually coped with pollutions at various levels including behavioral, histological, hematological and enzymatic, and molecular responses (Banaee, 2013; Blahova et al., 2014; Abarghoei et al., 2015; Khabbazi et al., 2015; Farsani et al., 2017; Ullah et al., 2018; Mirghaed et al., 2018; Hajirezaee et al., 2019 c; Hedayati et al., 2019, 2021). As the main organ

for detoxification, liver plays major role in reducing oxidative stress induced by pollutants, mainly by activating metabolic and antioxidant enzymes (Van der Oost et al., 2003; Hajirezaee et al., 2020; Vali et al., 2020; Ghafarifarsani et al., 2021 a). In fish, pollution-related fluctuations of hepatic enzymes have been widely reported in many studies (Slaninova et al., 2009; Banaee 2013; Naz et al., 2019); however, the change pattern of the enzymes depended mainly on species, pollutant concentration, and type of pollutant and duration of exposure. Today, the use of pesticides is inevitable due to the development and mechanization of agriculture. Therefore, providing solutions to moderate the effects of these chemicals on fish could be a helpful strategy. Due to the harmful effects of chemicals on fish health and the aquatic environment, the use of plants and plant-derived substances (PDSs) as alternatives have increased to treat diseases as well as to enhance the immune system of fish (Galina et al., 2009; Hajiahmadian et al., 2012; Bulfon et al., 2015; Al-Shawi et al., 2017; Durgawale et al., 2019; Datkhile et al., 2020; Elumalai et al., 2020; Khan et al., 2020; Ibrahem et al., 2021; Qahir et al., 2021; You et al., 2021; Lade et al., 2022).

Until now, many researchers have investigated the effects of PDSs on fish immunity, especially on the antioxidant defense system (Galina et al., 2009; Bulfon et al., 2015; Elumalai et al., 2020; Brown et al., 2021). However, few studies have investigated the ameliorating effects of these natural products on oxidative stress induced by pesticides in fish (Al-Shawi et al., 2021).

Today, macro-algae and their derivates are widely used in many industries such as pharmaceuticals, cosmetics and food industries (Tierney et al., 2010; Ariede et al., 2017; Wan et al., 2019; Lourenço-Lopes et al., 2020; Leandro et al., 2020; Teo et al., 2021). These algae include *Ecklonia maxima, Sargassum siliquosum*, *Sargassum swartzii* and *Gracilaria salicornia*.

The strong antioxidant properties of macro-algae have been reported by many studies (Cornish and Garbary, 2010; Tierney et al., 2010). Based on a literature review, there seems to be a lack of knowledge about the effect of these natural antioxidants on modulating the oxidative stress induced by pollutants, including pesticides.

Sargassum spp. are a group of marine macro-algae with strong antioxidant properties (Cho et al., 2007; Zahra et al., 2007; Ye et al., 2008; Namvar et al., 2013; Tian et al., 2020). Owing to this, several studies have used *Sargassum* spp. as food additive to enhance antioxidant and immune system of fish and other animals.

The present study was conducted to examine the potentials of the macro-algae, *Sargassum angustifolium* extract, as a dietary food additive in enhancing the antioxidant defense and ameliorating oxidative stress induced by diazinon in the most commercial fish, common carp. The results may be useful for carp aquaculture enhancement.

Material and methods

Preparation of algae extract

The *Sargassum angustifolium* were collected from intertidal regions of Chabahar Bay, Oman Sea, Iran. The algae were dried at 40°C for 48 h and then pulverized and homogenized by a mill. The extraction was done on the basis of the method suggested by Mora et al. (2005) by a rotary (Buchi, Switzerland) at 80°C. The concentrated extract was finally dried at 40°C to make powder. The powders were eventually kept at 0°C till the beginning of the feeding experiment. In addition, the chemical composition of *S. angustifolium* extract was assayed by gas chromatography (Table 1).

Table 1. The compounds of *Sargassum angustifolium* extract identified by GC-mass chromatography

No.	Compounds	R. time	Percentage
1	*Tritriacontane*	32.1	4.3
2	2-*Butyloctanol*	33.3	5.6
3	Pentacosane	34.2	3.2
4	Eicosane	35.5	12.8
5	Dotriacontane	36.1	8.5
6	Octatriacontyl	37.5	2.5
7	*Pentafluoropropionate*	38.1	14.6
8	Nonacosane	38.9	13.1
9	Hentriacontane	39.2	3.2
10	Tritriacontane	40.2	2.2
11	Phenols	41.3	36.8
12	Thiophene	43.2	1.2

Fish and feeding trial

A total number of 360 fingerling carp (TW: 30.2 ± 2.1 g; TL: 13.2 ± 1.5 cm) were provided from Karaj, Iran and distributed into 12 tanks (500 l) (30/tank) containing aerated and disinfected water. After 7-day acclimation period, fish were fed various dietary levels of *Sargassum angustifolium* extract (SAE) in three treatment and one control group including: control: non-SAE-supplemented fish, SAE1: 5 g SAE/kg diet, SAE2: 10 g SAE/kg diet, SAE3: 15 g SAE/kg diet. A commercial common carp basal diet (crude protein: 41%; lipid: 8%, fiber: 6%; ash: 11%, Faradaneh Co., Shahrekord, Iran) was used to make experimental diets. In this regard, the basal diet was powdered by a mill, the dried SAE added into the diet and mixed to make paste by adding 100 ml of distilled water. Then, an appropriate size of food (as granule; diameter: 2.2 ± 0.1 mm, length: 2.4 ± 0.2 mm) was made using a sieve. Then, the foods were respectively kept at room temperature (away from light) and in an oven (at 60°C) to dry. Finally, the foods were stored in a refrigerator (4°C). Feeding operation was performed daily and at a rate of 6% of total fish weight. The amount of needed food was

regulated with intervals of 10 days through estimating total fish weight. Throughout the feeding trial, the water quality properties were: pH: 6.8 ± 0.1 (APX15/C-WTW-330i), temperature: 15.7 ± 1.1°C, ammonia: 0.02 ± 0.002 mg/L (colorimetrically at 670 nm) and dissolved oxygen: 7.2 ± 0.15 mg/L (OxyGouard). Also, the numbers of dead fish were recorded during the experiment to estimate mortality percent.

Diazinon exposure challenge

After a 60 days feeding trial, fish were exposed to a mean environmentally relevant concentration of 2 mg/l diazinon (60% EC) (Shayeghi et al., 2006) for 24 h. Before exposure, no diazinon was detected in water based on outputs of gas chromatography–mass spectrometry (*GC-MS*) (Shimadzu QP2010 Ultra series instrument, 120 columns, each column: 30 m length, flow rate: 0.5 L/h). Throughout the experiment, water exchange (90%) was done every 24 h and oxygen level was kept at optimized range by continuous aeration. Also, the renewal of diazinon concentration was performed simultaneously with water renewal.

Liver sampling

The liver tissue of 10 fish/tank was sampled after 60 days feeding trial and after 24 h exposure challenge. Liver tissue samples were taken after dissecting out the fish and then kept in liquid nitrogen (–196°C) for further assays.

Liver biochemical assays

All assays were done by commercial assay kits (Sigma-Aldrich Co, USA) and according to manufacturer's instructions.

Lactate dehydrogenase (LDH) levels were measured at 450 nm on the basis of the oxidation rate of nicotinamide adenine dinucleotide. The activity of creatine kinase (CK) was assayed at 340 nm through measuring the amount of the phosphate transferred from phosphocreatine to ADP during phosphorylation of glucose. The aspartate aminotransferase (AST) levels were measured at 450 nm through calculating the deamination rate of aspartate to a-ketoglutarate and following generation of glutamate (at 37 C, pH: 8.0). The activity of alanine aminotransferase (ALT) was assayed at 570 nm based on the production rate of pyruvate. The ALP activity was measured at 405 nm through estimating the conversion rate of p-nitrophenol phosphate to nitrophenol. The activity of catalase (CAT) activity was assayed at 240 nm based on the production of water and oxygen from catalysis of hydrogen peroxide (H_2O_2) (Claiborne, 1985). The superoxide dismutases SOD activity was determined colorimetrically at 440 nm through oxidation of xanthine to superoxide radicals (SOR) and further reaction of SOR with WST-1 reagent to create a red formazan dye (Tozoglu et al., 2021; Salah Noori and Abdul-RedhaIsmaiel, 2022). The glutathione peroxidase (GPx) levels were determined at 340 nm based on the generation of oxidized glutathione from glutathione (GSH) upon GPx action.

Gene expression assays

RNA content was extracted from liver tissues using acid guanidinium thiocyanate-phenol chloroform. The quantity of extracted RNA was spectrophotometrically measured at 260/280 nm. Furthermore, the quality of extraction was evaluated by electrophoresis on a 1.5% agarose gel according to Miandare et al. (2013). 1 µg of extracted RNA was used to synthesize first-strand cDNAs using a Fermentas cDNA synthesis Kit for reverse transcription polymerase chain reaction (RT-PCR) based on manufacturer's instructions and a set of oligo-dT as primer. The qPCR primers were designed on the basis of the DNA sequences present in GenBank (accession numbers listed in Table 2) and using Gene Runner (version 6.) software. The expression levels were standardized using β-actine gene as reference gene. qPCR analysis was carried out by an iCycler (BioRad) with Fermentas Maxima SYBR Green qPCR Master Mix (2×) (Fermentas) and all primers at concentration of 100 nM after protocol described by Miandare et al. (2013). The fold change in *sod*, *cat* and *gpx* relative mRNA expression was calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The data were then analyzed by iQ5 optical system software version 2.0 (Bio-Rad).

Table 2. Sequences of oligonucleotide primers used for real-time PCR

Gene name	Sequences of primers	Accession number
gpx	Forward: CTCAACAGGAGAATGC-CAAGAATG	XM_019096258.1
	Reverse: CCTTGAGGAACACGAACA-GAGG	
sod	Forward: TGAGCTGTCGGAAGCCAT-CAAG	XM_019111527.1
	Reverse: TTGGTTCCCACATGCAG-CAATCC	
cat	Forward: AGACGACACCCATCGCT-GTTCG	XM_019068442.1
	Reverse: AAGGTCCCAGTTGCCCT-CATCG	

Statistical analysis

All statistical analyzes were performed using SPSS software. After the normality of data (mean ± standard deviation) was verified and confirmed by Kolmogorov–Smirnov test, one-way analysis of variance was used to detect statistical differences. Finally, the difference between the means was determined using the Tukey test. The comparison of the means before and after exposure to diazinon was carried out by independent-sample t-test for each group.

Results

Lipid peroxidation index

The levels of malondialdehyde (MDA) did not show significant changes in the experimental groups during 60 days feeding with dietary SAE (Figure 1, P>0.05). However, this index significantly increased in control (non-SAE supplemented fish) and fish supplemented with 5 and 10 g SAE/kg diet after diazinon exposure (Figure 1, P<0.05). Fish supplemented with 10 SAE/kg diet showed no significant changes in MDA levels after exposure to diazinon.

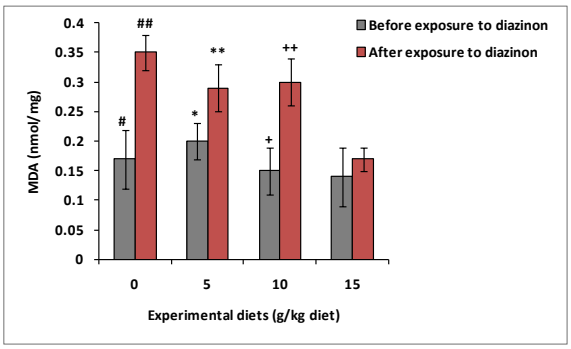


Figure 1. Effects of different dietary levels of SAE (*Sargassum angustifolium*) extract on the liver MDA levels in common carp, *Cyprinus carpio*. Different superscripted letters indicate significant differences between the dietary groups (P<0.01). Different superscripted symbols indicate significant differences in each group before and after exposure to diazinon (P<0.01). Data are presented as mean ± SD

Hepatic metabolic and antioxidant enzymes

No significant changes were found in concentrations of hepatic metabolic enzymes [AST (Figure 2 a), ALT (Figure 2 b), ALP (Figure 2 c), LHD (Figure 2 d), CK (Figure 2 e)] during feeding trial with SAE (Table 1, P>0.05). After exposure to diazinon, these enzymes were significantly elevated in control fish and those fed 5 and 10 SAE/kg diet (Figure 2, P<0.05). The activity of antioxidant enzymes [CAT (Figure 3 a), SOD (Figure 3 b), GPx (Figure 3 c)] showed some elevations in SAE supplemented fish compared to control, however these changes were not significant (Figure 3, P>0.05). After exposure to diazinon, antioxidant enzymes significantly decreased in control and fish fed 5 g SAE/kg diet (Figure 3, P<0.05), while the fish of 10 and 15 g SAE/kg diet treatments showed significant elevations (Figure 3, P<0.05).

Gene expressions

During 60 days feeding trial, the expression of antioxidant-related genes [*sod* (Figure 4 a), *cat* (Figure 4 b), *gpx* (Figure 4 c)] was significantly elevated in response to dietary SAE compared to control (P<0.01). After exposure to diazinon, considerable elevations were observed for the gene expressions in all groups (Figure 4, P<0.01).

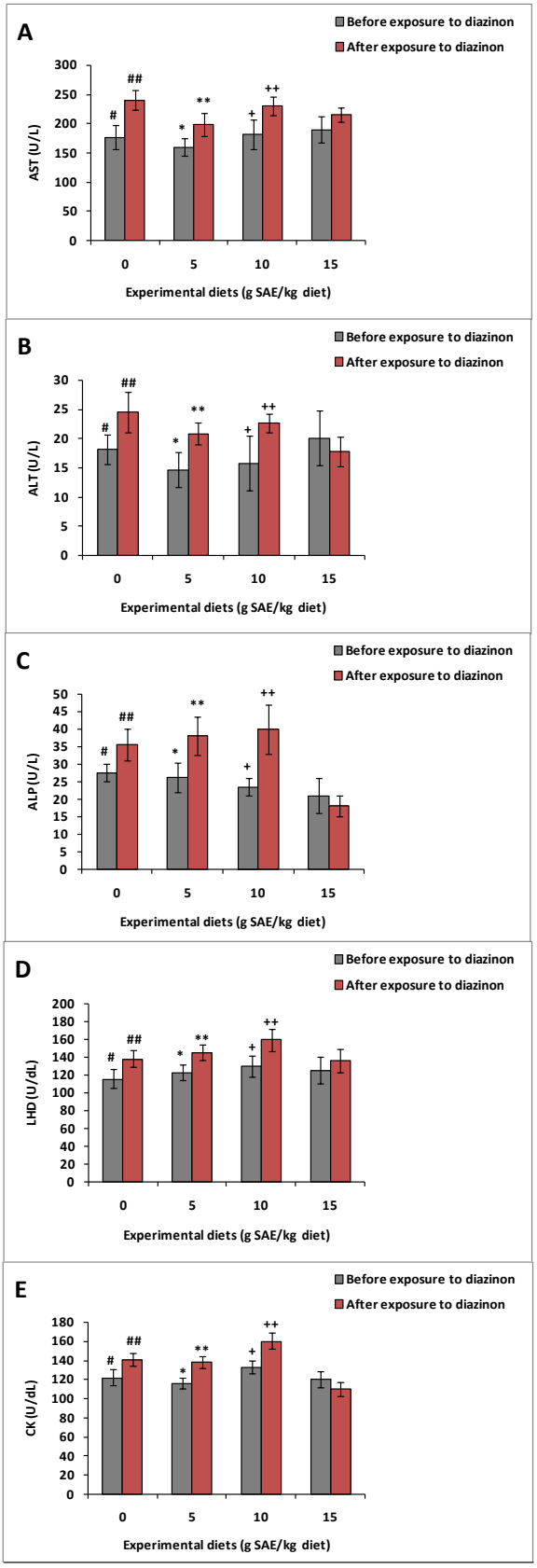


Figure 2. Effects of different dietary levels of SAE (*Sargassum angustifolium*) on the hepatic metabolic enzymes in common carp, *Cyprinus carpio*. AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, CK: creatine kinase. Different superscripted letters indicate significant differences between the dietary groups (P<0.01). Different superscripted symbols indicate significant differences in each group before and after exposure to diazinon (P<0.01). Data are presented as mean ± SD

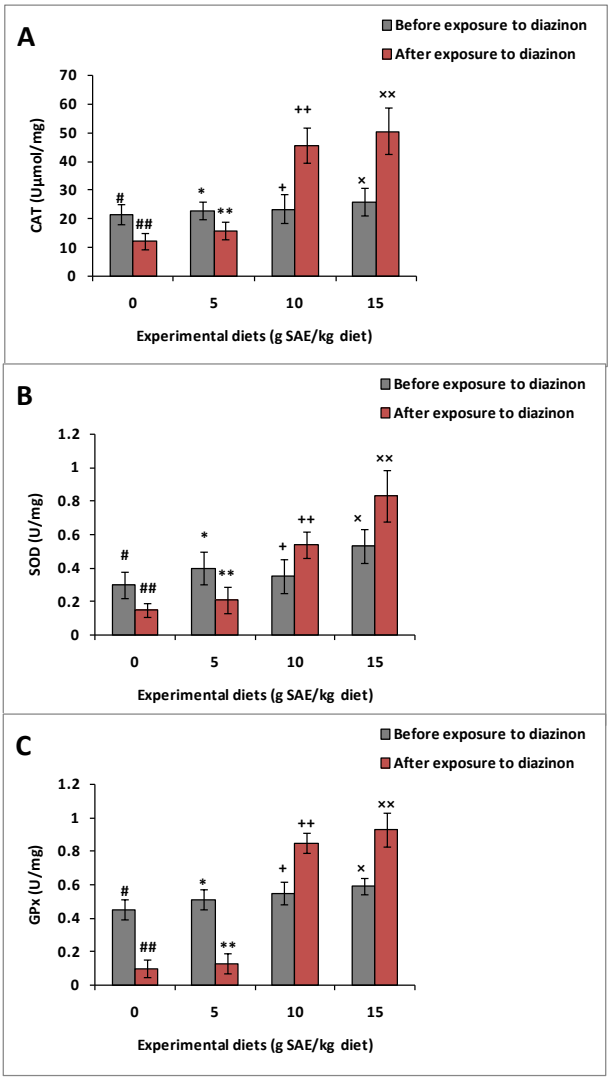


Figure 3. Effects of different dietary levels of SAE (*Sargassum angustifolium*) on the liver antioxidant enzymes in common carp, *Cyprinus carpio*. Different superscripted letters indicate significant differences between the dietary groups (P<0.01). CAT: catalase, SOD: superoxide dismutase, GPx: glutathione peroxidase. Different superscripted symbols indicate significant differences in each group before and after exposure to diazinon (P<0.01). Data are presented as mean ± SD

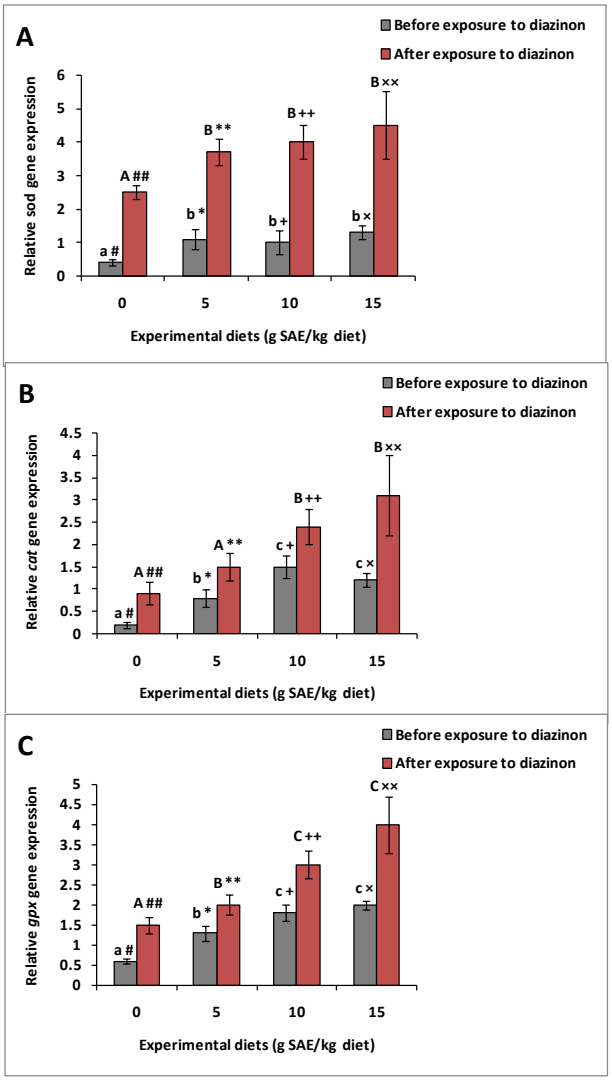


Figure 4. Effects of different dietary levels of SAE (*Sargassum angustifolium*) on antioxidant-related gene expressions in common carp, *Cyprinus carpio*. Different superscripted letters indicate significant differences between the dietary groups (P<0.01). Different superscripted symbols indicate significant differences in each group before and after exposure to diazinon (P<0.01). Data are presented as mean ± SD

Survival rate

No significant changes were found in survival rate of the fish between experimental groups during feeding trial with SAE (Figure 5, P>0.05). After exposure to diazinon, considerable decreases were observed in survival of control and fish fed 5 SAE/kg diet (Figure 4, P<0.01).



Figure 5. Effects of different dietary levels of SAE (*Sargassum angustifolium*) on survival rate of common carp, *Cyprinus carpio*

Discussion

The immunostimulating effects of macro-algae have been reported in many studies with fish and shellfish. However, there is a very significant lack of knowledge about the effects of these algae on the potentials of fish immune system, especially in fish exposed to contaminants. In this study, the extracts of the marine macro-algae, *Sargassum angustifolium* were used as food additive to examine their possible enhancing effects on the antioxidant defense system of the common carp against oxidative stress induced by the organophosphate pesticide, diazinon. In the present study, exposure to diazinon triggered oxidative stress in liver, because we observed significant elevations in MDA levels in control and fish fed diet containing 5 and 10 g SAE/kg diet. In organisms, MDA is known as an indicator of oxidative stress

and lipid peroxidation (Janero, 1990; Ayala et al., 2014; Ghafarifarsani et al., 2021 b). In fish fed 10 g SAE/kg diet, MDA showed no changes after the exposure, which may be due to the ameliorating effects of SAE (at dietary level of 10 g/kg diet) on oxidative stress. Similar to our results, a few studies have shown the ameliorating effects of dietary *Sargassum* spp. on lipid peroxidation in fish exposed to various environmental stressors. In black sea bream, *Acanthopagrus schlegelii*, dietary *Sargassum horneri* reduced MDA levels, which was attributed to the ameliorating impacts of the algae on oxidative stress induced by hypoxia and ammonia (Shi et al., 2019, 2020). In addition, many studies have reported the antioxidant properties of the macro-algae, in particular *Sargassum* spp. (Anggadiredja and Andyani, 1997; Choi et al., 2006; Cho et al., 2007; Cornish and Garbary, 2010; Tierney et al., 2010; Costa et al., 2011; Budhiyanti et al., 2012; Lim et al., 2019). In the present study, hepatic metabolic enzymes (HMEs) (AST, ALT, ALP, LDH, CK) remained unchanged during feeding with SAE, while these enzymes were elevated in control fish and those fed 5 and 10 g SAE/kg diet. These elevations could be a physiologic reaction to detoxify the pollution. It seems that SAE at 15 g SAE/kg diet concentration ameliorates the diazinon-induced increases in HMEs, because these enzymes remained unchanged in response to diazinon challenge. The HMEs play important role in decreasing the oxidative stress-induced disruptions in liver. To this end, the HMEs are considered biomarkers of the pollutants including pesticides.

In the present study, supplementation of fish with SAE increased the activity of liver antioxidant enzymes (SOD, CAT, GPx), however the increases were not significant. The liver antioxidant enzymes constitute the first line of defense against oxidative stress. SOD is responsible for removal of superoxide radicals (Kappus, 1985; Kohen and Nyska, 2002; Mohammadi et al., 2020). CAT exerts its antioxidant function through removing hydrogen peroxide radicals (Van der Oost et al., 2003). Furthermore, GSH-Px catalyzes hydrogen peroxide radicals to water and also eliminates lipid peroxide radicals through converting them to lipid alcohols. Among diazinon challenged fish, the antioxidant enzymes decreased in control and fish fed 5 g SAE/kg diet, while the activity of these enzymes was elevated in fish fed 10 and 15 g SAE/kg diet. These results clearly reveal the enhancing effects of dietary SAE on antioxidant capacity of the liver. The stimulating effects of dietary *Sargassum* spp. on liver antioxidant enzymes have been reported in fish and shellfish. In the study of Seraj and Ghorbani Ranjbari (2016), dietary administration of common carp, *Cyprinus carpio* with *Sargassum angustifolium* extract increased the activity of SOD in liver. Use of *S. horneri* in diet of black sea bream increased total antioxidant capacity, SOD and CAT activities in the liver (Shi et al., 2019). Wang et al. (2019) revealed that the catalase activity was elevated as the dietary *S. horneri* increased. In the study of Baleta et al. (2013), the activity of SOD and GPx in white shrimps, *Litopenaeus vannamei* supplemented with *Sargassum oligocystum* extract was investigated. In this study, the analysis of the chemical composition of the SAE was conducted by GC-mass chromatography. Phenols formed the main compounds of the SAE, which may have antioxidant properties (Maqsood et al., 2014).

In this study, similar to liver antioxidant enzymes, antioxidant-related genes (*sod, cat, gpx*) were elevated in response to dietary SAE. This result may be a reason for the increased levels of antioxidant enzymes in SAE-supplemented fish. Interestingly, the gene expressions considerably increased after exposure to diazinon, while the activity of antioxidant enzymes showed decreases in control and fish fed 5 g SAE/kg diet. This result may be due to the disrupting effect of diazinon on the production of the enzymes at levels other than the level of the gene expression. It seems that this adverse effect does not include fish fed 10 and 15 g SAE/kg diet, because in these treatments, a simultaneous increase of antioxidant enzyme-related gene expressions and activity of antioxidant enzymes were observed.

Conclusion

In conclusion, the results of the present study indicated the antioxidant enhancing effects of SAE at dietary levels of 10 and 15 g SAE/kg diet, and this effect may be attributed to some antioxidant components in the chemical composition of the macro-algae or to the direct effects of SAE on liver antioxidant enzymes.

Ethical Approval

In this study, all stages of sampling and manipulation of animals have been performed in accordance with ethical standards.

Data availability statements

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

The authors declare that they have no competing interests.

Authors' contributions

All the authors of this article have made important contributions to testing, collecting data, analyzing results, and writing the article.

Conflict of interest

The authors have no conflict of interest

Acknowledgments

The authors would like to thank all those who helped us to do the research.

References

Abarghoei S., Hedayati S.A., Ghafari Farsani H., Gerami M.H. (2015). Hematological responses of Goldfish (*Carassius auratus*) to different acute concentrations of silver sulfate as a toxicant. Pollution, 1: 247–256.

Al-Shawi S.G., Al-Younis Z.K., Abd Al-Kareem N.F. (2017). Study of cumin antibacterial and antioxidant activity of alcoholic and aqueous extracts. Pak. J. Biotechnol., 14: 227–231.

Al-Shawi S.G., Yousif A.Y., Al-Younis Z.K., Shichiyakh R.A., Zekiy A.O., Naserabad S.S. (2021). Dietary silymarin, *Silybum marianum* extract ameliorates cadmium chloride toxicity in common carp. Ann. Anim. Sci., 22: 741–750.

Anggadiredja J., Andyani R. (1997). Antioxidant activity of *Sargassum polycystum* (Phaeophyta) and *Laurencia obtusa* (Rhodophyta) from Seribu islands. J. Appl. Phycol., 9: 477.

Ariede M.B., Candido T.M., Jacome A.L.M., Velasco M.V.R., de Carvalho J.C.M., Baby A.R. (2017). Cosmetic attributes of algae – A review. Algal Res., 25: 483–487.

Ayala A., Muñoz M.F., Argüelles S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid. Med. Cell. Longev., 2014: 360438.

Baleta F.N., Lin Y., Chen Y., Chen J.C., Yeh S.T., Putra D.F., Huang C.L. (2013). Efficacy of *Sargassum oligocystum* extract on the innate immunity of white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. J. Fish. Soc. Taiwan., 40: 241–56.

Banaee M. (2013). Physiological dysfunction in fish after insecticides exposure. In: Insecticides – development of safer and more effective technologies, Trdan S. (ed.). InTech, DOI: 10.5772/54742.

Blahova J., Modra H., Sevcikova M., Marsalek P., Zelnickova L., Skoric M., Svobodova Z. (2014). Evaluation of biochemical, haematological, and histopathological responses and recovery ability of common carp (*Cyprinus carpio* L.) after acute exposure to atrazine herbicide. Bio. Med. Res. Int., 2014: 980948.

Brodeur J.C., Malpel S., Anglesio A.B., Cristos D., D'andrea M.F., Poliserpi M.B. (2016). Toxicities of glyphosate- and cypermethrin-based pesticides are antagonic in the tenspotted livebearer fish (*Cnesterodon decemmaculatus*). Chemosphere, 155: 429–435.

Brown R., Moore L., Mani A., Patel S., Salinas I. (2021). Effects of ploidy and salmonid alphavirus infection on the skin and gill microbiome of Atlantic salmon (*Salmo salar*). PLOS One, 16(2): e0243684.

Budhiyanti S.A., Raharjo S., Marseno D.W., Lelana I.Y. (2012). Antioxidant activity of brown algae Sargassum species extracts from the coastline of Java island. Am. J. Agric. Biol. Sci., 7: 337–346.

Bulfon C., Volpatti D., Galeotti M. (2015). Current research on the use of plant-derived products in farmed fish. Aquacult. Res., 46: 513–551.

Cho S.H., Kang S.E., Cho J.Y., Kim A.R., Park S.M., Hong Y.K., Ahn D.H. (2007). The antioxidant properties of brown seaweed (*Sargassum siliquastrum*) extracts. J. Med. Food., 10: 479–485.

Choi S.Y., Kim S.Y., Hur J.M., Choi H.G., Sung N.J. (2006). Antioxidant activity of solvent extracts from *Sargassum thunbergii*. J. Korean Soc. Food Sci. Nutr., 35: 139–144.

Claiborne A. (1985). Catalase activity. In: CRC handbook of methods in oxygen radical research, Greenwald R.A. (ed.). Boca Raton: CRC Press, pp. 283–284.

Cornish M.L., Garbary D.J. (2010). Antioxidants from macroalgae: potential applications in human health and nutrition. Algae, 25: 155–171.

Costa L.S., Fidelis, G.P., Telles C.B.S., Dantas-Santos N., Camara R.B.G., Cordeiro S. L., Costa M.S.S.P., Almeida-Lima J., Melo-Silveira R.F., Oliveira R.M., Albuquerque I.R.L. (2011). Antioxidant and antiproliferative activities of heterofucans from the seaweed *Sargassum filipendula*. Mar. Drugs., 9: 952–966.

Datkhile K.D., Patil S.R., Durgawale P.P., Patil M.N., Jagdale N.J., Deshmukh V.N. (2020). Studies on phytoconstituents, *in vitro* antioxidant, antibacterial and cytotoxicity potential of *Argemone mexicana* Linn. (family: Papaveraceae). J. Nat. Sci. Biol. Med., 11: 198–205.

Díaz-Resendiz K.J.G., Toledo-Ibarra G.A., Girón-Pérez M.I. (2015). Modulation of immune response by organophosphorus pesticides: fishes as a potential model in immunotoxicology. J. Immunol. Res., https://doi.org/10.1155/2015/213836

Durgawale P., Patil M., Joshi S., Korabu K., Datkhile K. (2019). Studies on phytoconstituents, *in vitro* antioxidant, antibacterial, antiparasitic, antimicrobial, and anticancer potential of medicinal plant *Lasiosiphon eriocephalus* decne (Family: Thymelaeaceae). J. Nat. Sci. Biol. Med., 10: 38–47.

Elumalai P., Kurian A., Lakshmi S., Faggio C., Esteban M.A., Ringø E. (2020). Herbal immunomodulators in aquaculture. Rev. Fish. Sci. Aquacult., 29: 33–57.

Farsani H.G., Doria H.B., Jamali H., Hasanpour S., Mehdipour N., Rashidiyan G. (2017). The protective role of vitamin E on *Oreochromis niloticus* exposed to ZnONP. Ecotoxicol. Environ. Saf., 145: 1–7.

Galina J., Yin G., Ardo L., Jeney Z. (2009). The use of immunostimulating herbs in fish. An overview of research. Fish Physiol. Biochem., 35: 669–676.

Ghafarifarsani H., Imani A., Niewold T.A., Pietsch-Schmied C., Moghanlou K.S. (2021 a). Synergistic toxicity of dietary aflatoxin B_1 (AFB_1) and zearalenone (ZEN) in rainbow trout (*Oncorhynchus mykiss*) is attenuated by anabolic effects. Aquaculture, 541: 736793.

Ghafarifarsani H., Kachuei R., Imani A. (2021 b). Dietary supplementation of garden thyme essential oil ameliorated the deteriorative effects of aflatoxin B_1 on growth performance and intestinal inflammatory status of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 531: 735928.

Hajiahmadian M., Vajargah M.F., Farsani H.G., Chorchi M.M. (2012). Effect of *Spirulina platensis* meal as feed additive on growth performance and survival rate in golden barb fish, *Punius gelius* (Hamilton, 1822). Int. J. Fish., 7: 61–64.

Hajirezaee S., Rafieepour A., Shafiei S. (2019 a). A NMR-based metabonomic study on the ameliorating effects of *Ginkgo biloba* extract in rainbow trout, *Oncorhynchus mykiss* exposed to organophosphate pesticide, diazinon. Aquaculture, 513: 734450.

Hajirezaee S., Rafieepour A., Rahimi R., Shafiei S. (2019 b). Effects of gingko, *Ginkgo biloba* extract on metabolic hormones, liver histology, and growth parameters of rainbow trout, *Oncorhynchus mykiss* exposed to diazinon. Toxin Rev., 40: 632–644.

Hajirezaee S., Rafieepour A., Shafiei S., Rahimi R. (2019 c). Immunostimulating effects of *Ginkgo biloba* extract against toxicity induced by organophosphate pesticide, diazinon in rainbow trout, *Oncorhynchus mykiss*: innate immunity components and immune-related genes. Environ. Sci. Pollut. Res., 26: 8798–8807.

Hajirezaee S., Mohammadi G., Naserabad S.S. (2020). The protective effects of vitamin C on common carp (*Cyprinus carpio*) exposed to titanium oxide nanoparticles (TiO_2-NPs). Aquaculture, 518: 734734.

Hedayati S.A.A., Ghafari Farsani H., Shahbazi Naserabad S., Gerami M.H. (2015). Acute toxicity and behavioral changes associated with diazinon in *Rutilus rutilus caspicus* and *Hypophthalmicthys molitrix*. Iran. J. Toxicol., 9: 1354–1359.

Hedayati S.A., Farsani H.G., Naserabad S.S., Hoseinifar S.H., Van Doan H. (2019). Protective effect of dietary vitamin E on immunological and biochemical induction through silver nanoparticles (AgNPs) inclusion in diet and silver salt ($AgNO_3$) exposure on Zebrafish (*Danio rerio*). Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol., 222: 100–107.

Hedayati S.A., Sheikh Veisi R., Hosseini Shekarabi S.P., Shahbazi Naserabad S., Bagheri D., Ghafarifarsani H. (2021). Effect of dietary *Lactobacillus casei* on physiometabolic responses and liver histopathology in common carp (*Cyprinus carpio*) after exposure to iron oxide nanoparticles. Biol. Trace Elem. Res., 200: 3346–3354.

Ibrahem A.A., AL-Temimi W.K.A., Al-Shawi S.G. (2021). The optimum conditions for fructooligosaccharide production by using crude fructosyltransferase of plant origin. Plant Cell Biotechnol. Mol. Biol., 22: 252–265.

Janero D.R. (1990). Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic. Biol. Med., 9: 515–540.

Kappus H. (1985). Lipid peroxidation: mechanisms, analysis, enzymology and biological relevance. Oxidative Stress, 273: 273–310.

Katuli K.K., Amiri B.M., Massarsky A., Yelghi S. (2014). Impact of a short-term diazinon exposure on the osmoregulation potentiality of Caspian roach (*Rutilus rutilus*) fingerlings. Chemosphere, 108: 396–404.

Khabbazi M., Harsij M., Hedayati S.A.A., Gerami M.H., Ghafari-Farsani H. (2015). Histopathology of rainbow trout gills after exposure to copper. Iran. J. Ichthyol., 1: 191–196.

Khan J., Leenoos L.M., Ruhi S., Al-Dhalli S., Kaleemullah M., Saad R., Ali H.S., Sahu R., Florence M., Rasny Yusuf E. (2020). Development and evaluation of polyherbal halal ointment using honey and papaya. Int. J. Med. Toxicol. Legal Med., 23: 232–238.

Kime D.E. (1995). The effects of pollution on reproduction in fish. Rev. Fish Biol. Fisheries, 5: 52–95.

Kohen R., Nyska A. (2002). Invited review: Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol. Pathol., 30: 620–650.

Köprücü S.Ş., Köprücü K., Ural M.Ş., İspir U., Pala M. (2006). Acute toxicity of organophosphorous pesticide diazinon and its effects on behavior and some hematological parameters of fingerling European catfish (*Silurus glanis* L.). Pestic. Biochem. Physiol., 86: 99–105.

Lade S., Burle S., Kosalge S.B., Bansode M.N. (2022) Antimicrobial and antioxidant activity of *Hibiscus sabdariffa* Linn (Roselle). IJPRT, 12: 22–27.

Leandro A., Pereira L., Gonçalves A.M. (2020). Diverse applications of marine macroalgae. Mar. Drugs., 18: 17.

Lim S., Choi A.H., Kwon M., Joung E.J., Shin T., Lee S.G., Kim N.G., Kim H.R. (2019). Evaluation of antioxidant activities of various solvent extract from *Sargassum serratifolium* and its major antioxidant components. Food Chem., 278: 178–184.

Livak K.J., Schmittgen T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods, 25: 402–408.

Lourenço-Lopes C., Fraga-Corral M., Jimenez-Lopez C., Pereira A.G., Garcia-Oliveira P., Carpena M., Simal-Gandara J. (2020). Metabolites from macroalgae and its applications in the cosmetic industry: A circular economy approach. Resources, 9: 101.

Maqsood S., Benjakul S., Abushelaibi A., Alam A. (2014). Phenolic compounds and plant phenolic extracts as natural antioxidants in prevention of lipid oxidation in seafood: A detailed review. Compr. Rev. Food Sci. Food Saf., 13: 1125–1140.

Miandare H.K., Farahmand H., Akbarzadeh A., Ramezanpour S., Kaiya H., Miyazato M., Nikinmaa M. (2013). Developmental transcription of genes putatively associated with growth in two sturgeon species of different growth rate. Gen. Comp. Endocrinol., 182: 41–47.

Mirghaed A.T., Yarahmadi P., Craig P.M., Farsani H.G., Ghysvandi N., Eagderi S. (2018). Hemato-immunological, serum metabolite and enzymatic stress response alterations in exposed rainbow trout (*Oncorhynchus mykiss*) to nanosilver. Int. J. Aquat. Biol., 6: 221–234.

Mohammadi G., Rashidian G., Hoseinifar S.H., Naserabad S.S., Van Doan H. (2020). Ginger (*Zingiber officinale*) extract affects growth performance, body composition, haematology, serum and mucosal immune parameters in common carp (*Cyprinus carpio*). Fish Shellfish Immunol., 99: 267–273.

Moore A., Waring C.P. (2001). The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (*Salmo salar* L.). Aquat. Toxicol., 52: 1–12.

Mora S., Diaz-Veliz G., Lungenstrass H., García-González M., Coto-Morales T., Poletti C., Tortoriello J. (2005). Central nervous system activity of the hydroalcoholic extract of *Casimiroa edulis* in rats and mice. J. Ethnopharmacol., 97: 191–197.

Murthy K.S., Kiran B.R., Venkateshwarlu M. (2013). A review on toxicity of pesticides in fish. Int. J. Sci. Res., 1: 15–36.

Namvar F., Mohamad R., Baharara J., Zafar-Balanejad S., Fargahi F., Rahman H.S. (2013). Antioxidant, antiproliferative, and antiangiogenesis effects of polyphenol-rich seaweed *(Sargassum muticum*). Bio. Med. Res. Int., Article ID 604787, 9 pp.

Naz H., Abdullah S., Abbas K., Hassan W., Batool M., Perveen S., Mushtaq S. (2019). Toxic effect of insecticides mixtures on antioxidant enzymes in different organs of fish, *Labeo rohita*. Pak. J. Zool., 51: 1355–1361.

Poorbagher H., Ghaffari Farsani H., Farahmand H. (2018). A method to quantify genotoxicity of malathion in rainbow trout using the weighted averaging. Toxicol. Mech. Methods, 28: 607–614.

Qahir A., Khan N., Hakeem A., Kamal R. (2021). The antioxidant, antimicrobial, and clinical effects with elemental contents of pomegranate (*Punica granatum*) peel extracts: A review. Baghdad J. Biochem. Appl. Biol., 2: 21–28.

Salah Noori R., Abdul-RedhaIsmaiel M. (2022). Relationship between oxidative stress and the blood iron concentration and antioxidant status in major ß-thalassemia in Iraq. Arch. Razi Inst., 77: 169–180.

Seraj B., Ghorbani Ranjbari N. (2006). Antioxidant and immune-stimulating effects of *Sargassum angustifolium* extract on the common carp (*Cyprinus carpio*) juvenile (in Persian). Physiol. Aquat. Biotechnol., 3: 15–32.

Shahbazi Naserabad S., Mirvaghefi A., Gerami M.H., Ghafari Farsani H. (2015). Acute toxicity and behavioral changes of the gold fish (*Carassius auratus*) exposed to malathion and hinosan. Iran. J. Toxicol., 8: 1203–1208.

Shahbazi Naserabad S., Mirvaghefi A., Rashidiyan G., Rostamian N., Ghafari Farsani H. (2017). Investigating the agent of temperature into acute toxicity (LC_{50} 96h) of edifenphos in *Rutilus frisii kutum* (Kamensky, 1901). Iran. J. Toxicol., 11: 39–44.

Shayeghi M., Hosseini M., Abtahi M. (2006). The determination of dimethoate insecticide residues upon the cucumber product (Fars Province). J. Environ. Sci. Technol., 27: 30–35.

Shi Q., Wen X., Zhu D., Aweya J.J., Li S. (2019). Protective effects of *Sargassum horneri* against ammonia stress in juvenile black sea bream, *Acanthopagrus schlegelii*. J. Appl. Phycol., 31: 1445–1453.

Shi Q., Yu C., Zhu D., Li S., Wen X. (2020). Effects of dietary *Sargassum horneri* on resisting hypoxia stress, which changes blood biochemistry, antioxidant status, and hepatic HSP mRNA expressions of juvenile black sea bream *Acanthopagrus schlegelii*. J. Appl. Phycol., 32: 3457–3466.

Slaninova A., Smutna M., Modra H., Svobodova Z. (2009). A review: Oxidative stress in fish induced by pesticides. Neuroendocrinol. Lett., 30: 2.

Teo B.S.X., Gan R.Y., Abdul Aziz S., Sirirak T., Mohd Asmani M.F., Yusuf E. (2021). *In vitro* evaluation of antioxidant and antibacterial activities of *Eucheuma cottonii* extract and its *in vivo* evaluation of the wound-healing activity in mice. J. Cosmet. Dermatol., 20: 993–1001.

Tian H., Liu H., Song W., Zhu L., Zhang T., Li R., Yin X. (2020). Structure, antioxidant and immunostimulatory activities of the polysaccharides from *Sargassum carpophyllum*. Algal Res., 49: 101853.

Tierney M.S., Croft A.K., Hayes M. (2010). A review of antihypertensive and antioxidant activities in macroalgae. Bot. Mar., 53: 387–408.

Tozoglu E.O., Ustundag M.F., Gokceimam P.S. (2021). The relationship between cognition and asymmetric dimethyl arginine, symmetric dimethyl arginine, nitric oxide levels and total antioxidant capacity in euthymic bipolar disorder patients. Archiv. Clin. Psych. (São Paulo), 48: 195–202.

Ullah S., Zorriehzahra M.J. (2015). Ecotoxicology: a review of pesticides induced toxicity in fish. Adv. Anim. Vet. Sci., 3: 40–57.

Ullah S., Li Z., Hasan Z., Khan S.U., Fahad S. (2018). Malathion induced oxidative stress leads to histopathological and biochemical toxicity in the liver of rohu (*Labeorohita*, *Hamilton*) at acute concentration. Ecotoxicol. Environ. Saf., 161: 270–280.

Vali S., Mohammadi G., Tavabe K.R., Moghadas F., Naserabad S.S. (2020). The effects of silver nanoparticles (Ag-NPs) sublethal concentrations on common carp (Cyprinus carpio): Bioaccumulation, hematology, serum biochemistry and immunology, antioxi-

dant enzymes, and skin mucosal responses. Ecotoxicol. Environ. Saf., 194: 110353.
Van der Oost R., Beyer J., Vermeulen N.P. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol., 13: 57–149.
Wan A.H., Davies S.J., Soler Vila A., Fitzgerald R., Johnson M.P. (2019). Macroalgae as a sustainable aquafeed ingredient. Rev. Aquac., 11: 458–492.
Wang C., Hu W., Wang L., Qiao H., Wu H., Xu Z. (2019). Effects of dietary supplementation with *Sargassum horneri* meal on growth performance, body composition, and immune response of juvenile turbot. J. Appl. Phycol., 31: 771–778.
Ye H., Wang K., Zhou C., Liu J., Zeng X. (2008). Purification, antitumor and antioxidant activities in vitro of polysaccharides from the brown seaweed *Sargassum pallidum*. Food Chem., 111: 428–432.
You S.H., Yoon M.Y., Moon J.S. (2021). Antioxidant and anti-inflammatory activity study of fulvic acid. J. Nat. Sci. Biol. Med., 12.
Zahra R., Mehrnaz M., Farzaneh V., Kohzad S. (2007). Antioxidant activity of extract from a brown alga, *Sargassum boveanum*. Afr. J. Biotechnol., 6.

Received: 27 I 2022
Accepted: 6 IV 2022