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Effect of dietary selenium nanoparticles and chitosan oligosaccharide on biochemical parameters of Caspian roach (*Rutilus caspicus*) under malathion stress

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ABSTRACT

The present study was conducted to investigate the protective effects of selenium nanoparticles (nano-Se) and chitosan oligosaccharide (COS) against the malathion-induced blood hematological and biochemical alterations in Caspian roach (*Rutilus caspicus*). The fish treated with 0.5 mg L^{-1} malathion in water and supplemented with selenium nanoparticles (1 mg kg^{-1}) and /or COS (600 mg kg^{-1}) in their diet for 28 days. At the end of the experiment, the blood samples were collected and hematological indices were assayed including serum total protein, albumin, globulin, glucose, cholesterol and triglyceride as well as enzymes such as aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase (LDH), alkaline phosphatase and acetyl cholinesterase (AChE). Significant decreases were observed in LDH level, AChE activities and hematological indices such as the white and red blood cell count, hemoglobin and hematocrit levels of the fish exposed to malathion ($p < 0.05$). Dietary selenium nanoparticles and COS could significantly ($P < 0.05$) reduce hematological indices, lysozyme value and the blood antioxidant enzyme activities in malathion-exposed Caspian roach. Briefly, this suggests that supplementation of dietary Caspian roach with nano-selenium (1 mg kg^{-1}) and /or COS (600 mg kg^{-1}) may have positive effects on hematological and biochemical indices in fish exposed to malathion stress.

Key words: Biochemical markers, Nano selenium, Pesticides, Caspian roach, Stress enzymes.

INTRODUCTION

Malathion is one of the most effective organophosphate insecticides which can be intentionally or unintentionally introduced to surface waters through rainfall run-off, runoff from agricultural applications and air-drift (Aker *et al.* 2008; Moore *et al.* 2011; Yonar *et al.* 2014). Upon malathion entrance to aquatic ecosystems, aquatic organisms such as fish will be exposed to this organophosphate insecticide. They may uptake it by their gills, skin or digestion system and hence distribute it to other tissues (Yonar *et al.* 2017). Malathion has exhibited numerous adverse effects on cell membrane function, physiological and biochemical indexes, hematological profiles and growth parameters of some fish species (Venkataramana *et al.* 2006; Banaee *et al.* 2013). One of the well-known subjects in ecotoxicology is the possibility to enhance the efficiency of the organism detoxification system against environmental pollutants by reinforcing their antioxidant defense system (Yonar *et al.* 2011). This could accelerate and facilitate the elimination of toxic compounds from animals, which in turn can decline the severity of the biological damage of these compounds (Kaur *et al.* 2006; Sharifinasab *et al.* 2016). Moreover, the stress induced by organophosphate insecticides can threaten the health and growth of fish in aquatic environments (Yonar *et al.* 2011). Some studies exhibited that many antioxidant compounds can protect the fish cells against the destructive impacts of environmental pollutants (Aker *et al.* 2008; Kucukbay *et al.* 2009; Yonar *et al.* 2012).

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Alternations in blood biochemical parameters have been widely considered for evaluating the adverse effects of pollutants (Kucukbay *et al.* 2009). In addition, blood is easily accessible and is one of the major biological fluids of the body which may change in response to different physiological states (Yonar *et al.* 2014).
Essential elements such as selenium, iron, zinc, copper and manganese are required as metal co-factors for optimum catalytic activity and normal and adequate function of antioxidant enzymes. Dietary supplementation is the major method to provide the fish with these micronutrients. Deficiency of these elements may play a negative role in normal biochemical and physiological functions imposing adverse impacts on cells through oxidation damages (Shi *et al.* 2010). Selenium is an important trace mineral whose significance role to promote the fish growth rate is undeniable (Kucukbay *et al.* 2009; Wang *et al.* 2013). In the form of selenocysteine, selenium is an integral part of glutathione peroxidase (GPX) active site which plays an important role in protecting the cells against oxidative damage. This element is effective in the treatment of various diseases, especially those associated with oxidative stress (Shi *et al.* 2010). Selenoprotein, with a significant role in normal body function, mainly includes selenium (Wang *et al.* 2013). Recently, nano-Se has drawn considerable attention due to its higher effectiveness and far lower toxicity compared to its organic and inorganic forms (Ashouri *et al.* 2015). Some studies have demonstrated that nano-Se has more effective impacts on growth performance, blood indices and antioxidant defense system of *Cyprinus carpio* (Ashouri *et al.* 2015; Saffari *et al.* 2017).
Furthermore, the great efforts have been devoted to develop chitosan as a drug and hormone carrier (Santhosh *et al.* 2006; Muzzarelli 2010). After cellulose, chitosan is the second abundant natural polysaccharide obtained from N-deacetylation of chitin (Karimzadeh & Pormehr 2017). The degraded products of chitosan or chitin, is known as chitooligosaccharides (COS), which is oligomers of β-(1-4)-linked-d-glucosamine. Chitosan oligosaccharides (COS), hydrolyzed products of chitosan, is possessed various biological activities (Ciu *et al.* 2012). Owing to its remarkable biological properties COS can be supplemented to fish and shrimp diets to enhance growth, hematological profiles and nonspecific immunity (Wang *et al.* 2016). *Rutilus caspicus* is considered as a commercially valuable species in the Caspian Sea which is regarded as a suitable feed source for beluga sturgeon (Keyvanshokooh & Kalbassi 2006). Some studies have been conducted on the protective impact of nano-Se on fish species (Yonar *et al.* 2014; Sharifinasab *et al.* 2016). However, data on the effects of nano-Se and its combination with chitosan on hematological indices in *R. caspicus* are scarce. Regarding the pharmacological properties of chitosan along with antioxidant effects of nano-Se on bio-systems, this hypothesis can be introduced that application of nano-Se and COS, combined or alone, can be effective in declining the toxic effects of malathion on blood biochemical indices in Caspian roach. Therefore, the aim of this study was to investigate the effect of nano-Se and COS on blood biochemical and hematological indices in malathion-exposed Caspian roach.

MATERIALS AND METHODS

Reagents

Chitosan oligosaccharide lactate ($C_{12}H_{24}N_2O_9$) was provided by Sigma-Aldrich Company, USA. Malathion (active ingredients about 57 % w/w) was obtained from GCE (Goodrich Chemical and Emory). Selenium nanoparticles were supplied from Pishgaman Nano Company, Mashhad, Iran. Other chemical materials were purchased from Merck Chemical Company (Germany).

Preparation of fish

A total of 630 samples of Caspian roach (1.72 ± 0.06 g in weight) was provided from a private fish farm in Rasht, Guilan Province and transferred to the laboratory of Mirza Kochak Khan Higher Education Center for Fisheries Sciences in Rasht. The samples were approached according to the National Ethical Framework for Animal Research in Iran. They were allowed to acclimate for two weeks in the aerated 1000-L fiberglass tanks prior to the experiment. During this period fish were fed twice a day at a rate of 5% of body weight, with commercial basal diet (Khorak-e Damo Abzian Co., Sari, Iran). Thereafter, the fish were randomly stocked to 21 tanks (100 L) (30 fish per tank) with triplicate in seven treatment groups. The diet contained with approximately 41% crude protein, 6 % lipid, 5 % fiber and 12% moisture were prepared to have 1 mg nano-Se and/ or 600 mg COS per 1 kg of diet according to the levels suggested in previous studies (Ashouri *et al.* 2015; Wang *et al.* 2016). Every day, each tank was cleaned and the water volume partially changed (about 40 %). A photoperiod of 14 h light and 10 h dark was applied. During the experiment, the mean water quality parameters were recorded as follows: temperature 24.2 ± 0.2 °C, dissolved oxygen 6.4 ± 0.7 mg L^{-1}, pH 7.6 ± 0.1. The experiment was assigned out in

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three replicates to seven groups including group I: control group fed with commercially formulated basal diet; group II: fed diet containing 1 mg nano-Se per kg of feed; group III: fed a diet containing 600 mg COS per 1 kg of feed; group IV: fish were exposed to 0.5 mg L^{-1} malathion (Yonar *et al.* 2014) and fed commercially with basal diet; group V: exposed to 0.5 mg L^{-1} malathion + simultaneous administration of nano-Se; group VI: exposed to 0.5 mg L^{-1} malathion with simultaneous administration of COS; group VII: exposed to 0.5 mg L^{-1} malathion + diet containing 1 mg nano-Se combined with 6000 mg COS per kg of feed. The water was exchanged daily and malathion was added to keep concentration near the nominal level. The experiment was performed for 28 days. Then, fish were anesthetized with benzocaine and blood was collected from the caudal vein of each individual fish. Blood was collected into heparinized glass vials (50 IU Sodium heparin/ mL of blood) and was used for hematological assays.

Hematological indices

The hematological profiles were measured according to the unified methods for hematological determination of fish (Svobodova *et al.* 1991). An aliquot of the whole blood sample was used to determine the erythrocyte count (RBC), leukocyte count (WBC), hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC).

Lysozyme activity

Serum lysozyme activity was determined according to Ellis (1990) method. A 10 µL of serum was mixed with 200 µL of a *Micrococcus lisodeichticus* suspension at 0.2 mg mL^{-1} in 0.05 M sodium phosphate buffer (pH 6.2). The optical density (OD) of the mixture was measured at 530 nm using an ELISA (enzyme-linked immunosorbent assay) plate reader. One unit of lysozyme activity was described as the amount of enzyme leading to a decreased absorbance of 0.001 min mL^{-1} of serum. Lysozyme concentrations were calculated from a standard curve using chicken egg white lysozyme (Sigma) as standard.

Blood biochemical indices

For serum separation, blood samples were centrifuged at 2000 rpm for 10 min at 4 °C. All biochemical indices were determined using the kits supplied by the Pars Azmun Company (Tehran, Iran) and a UV-VIS spectrophotometer (Shimadzu 1800, Japan). Total serum protein level was determined based on Biuret reaction in the wavelength of 540 nm (Reinhold 1953). Serum albumin was also measured based on the bromocresol green reaction in the wavelength of 630 nm (Doumas *et al.* 1971). Serum globulin was assessed based on the ratio of albumin versus total protein. Serum glucose and cholesterol levels were determined by Ashwell and Naito methods respectively (Ashwell 1957; Naito 1985).

Triglyceride was evaluated based on GPO-PAP enzymatic method at the wavelength of 510 nm (Rifai *et al.* 1991). Acetyl cholinesterase activity was also measured by acetylcholiniodide and dithiobis- nitrobenzoic acid as substrate at the wavelength of 405 nm as described by Knedel & Boetteger (1967). The aspartate aminotransferase and alanine aminotransferase enzyme activities of plasma were measured based on NADPH consumption and its conversion to NAD at a wavelength of 340 nm (Wootton 1964). For quantification of serum lactate dehydrogenase, the transformation of pyruvate to lactate was recorded at 340 nm (Wroblewski & Ladue 1955). Alkaline phosphatase was determined based on the transformation of nitrophenyl phosphate to nitrophenol and phosphate at a wavelength of 405 nm according to the method of Garen & Levinthal (1960).

Statistical analysis

All data were examined for normality with the Kolmogorov-Smirnov test and then data analysis was carried out by One-Way ANOVA at a confidence level of 95 % using SPSS (IBM 19). Mean values were also compared via a multiple Duncan's test. The results were reported as mean ± standard deviation.

RESULTS

Table 1 summarizes the effects of nano-Se, COS and their combined administration on hematological indices in fish exposed to malathion. Red and white blood cell counts, hematocrit, hemoglobin, MCH, MCV and MCHC significantly decreased in malathion-exposed fish as compared to the control group ($P < 0.05$). Administration of

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nano-Se and COS and their combination resulted in an increased hematological indices and those remained at the normal levels.

Table 1. Alterations in hematological indices assayed in Caspian roach fed with the control and experimental diets for 28-days rearing period.

	Hematological parameters							
Treatment	RBC (10^6)	WBC (10^3)	Hb (g dL^{-1})	Ht (%)	MCV (μ^3)	MCH (µg)	MCHC (%)	Lysozyme (U ml^{-1})
Control	1.68 ± 0.31^{c}	23.48 ± 1.45^{bc}	7.57 ± 0.70^{bc}	28.32 ± 2.66^{b}	187.91 ± 22.60^{c}	52.40 ± 6.30^{b}	28.14 ± 2.05^{c}	0.66 ± 0.19^{ab}
nano-Se	1.64 ± 0.23^{bc}	25.19 ± 1.24^{c}	7.92 ± 0.69^{c}	32.72 ± 1.09^{c}	218.11 ± 32.50^{abc}	50.39 ± 5.80^{b}	23.15 ± 1.45^{bc}	1.24 ± 0.29^{c}
COS	1.60 ± 0.15^{bc}	24.35 ± 1.50^{c}	7.43 ± 0.90^{bc}	32.31 ± 1.45^{c}	220.17 ± 20.35^{bc}	51.60 ± 6.30^{b}	21.91 ± 2.20^{ab}	1.33 ± 0.22^{c}
Malathion	1.20 ± 0.15^{a}	18.26 ± 1.93^{a}	4.83 ± 0.41^{a}	20.50 ± 2.62^{a}	170.44 ± 18.10^{d}	29.85 ± 5.35^{a}	17.40 ± 3.90^{a}	0.31 ± 0.18^{a}
Malation + nano - Se	1.31 ± 0.11^{ab}	19.44 ± 1.26^{a}	6.85 ± 0.41^{bc}	28.78 ± 1.67^{b}	220.84 ± 24.95^{ab}	54.69 ± 1.50^{b}	27.80 ± 2.00^{c}	0.99 ± 0.35^{bc}
Malathion + COS	1.53 ± 0.12^{abc}	20.13 ± 1.65^{a}	6.54 ± 0.24^{b}	27.44 ± 2.09^{b}	216.43 ± 18.65^{abc}	53.85 ± 2.55^{b}	26.24 ± 3.25^{bc}	1.10 ± 0.42^{bc}
Malathion + nano-Se + COS	1.45 ± 0.09^{abc}	21.13 ± 2.10^{ab}	6.82 ± 0.81^{bc}	27.78 ± 1.45^{b}	214.27 ± 18.65^{a}	52.90 ± 3.30^{b}	28.19 ± 4.30^{c}	1.15 ± 0.28^{bc}

RBC: Erythrocyte counts, WBC: Leucocyte counts, Ht: Hematocrit, Hb: Hemoglobin concentration, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin and MCHC: Mean corpuscular hemoglobin concentration. Values were expressed as means ± SE. Means with different superscripts are significantly different ($p < 0.05$), while means with the same superscripts indicate non-significant changes.

A significant decrease was observed in the blood Lysozyme content in fish exposed to malathion. Lysozyme significantly increased in the presence of nano-Se and COS, alone or in combination, in the diet of malathion-exposed fishes. The blood biochemical parameters in control and treated groups are presented in Table 1. Total plasma protein was significantly lower in malathion-exposed fish samples. Fish fed with the COS-supplemented diet did not exhibit alterations in their blood protein content. Administration of nano-Se alone or in combination with COS resulted in regulation of total plasma protein in malathion-exposed groups and its return to the level of the control group.

Albumin level was significantly lower in groups exposed to malathion ($P < 0.05$). Nano-Se (alone and in combination with COS) caused positive effects on albumin levels of fish exposed to malathion. The results also indicated a significant decrease in the plasma globulin level of the malathion-exposed fish (Table 2). Although addition of nano-Se significantly increased the globulin level in the blood plasma (in comparison with malathion-exposed groups), but co-administration of nano-Se and COS as well as COS alone displayed no impact on its level. A significant increase was also observed in the blood glucose level of malathion-exposed fish and those fed with COS-containing diet. Fish fed with diets supplemented with nano-Se alone or combined with COS regulated the blood glucose level and maintained it on the level of the control group (Table 2).

Table 2. The blood biochemical indices of Caspian roach in different dietary groups after rearing for 28 days.

	Blood biochemical indices					
Dietary treatment	**Total protein (g dL^{-1})**	**Albumin (mg dL^{-1})**	**Globulin (mg dL^{-1})**	**Glucose (mg dL^{-1})**	**Cholesterol (mg dL^{-1})**	**Triglyceride (mg dL^{-1})**
Control	4.6 ± 0.4^{bc}	2.4 ± 0.3^{abc}	2.11 ± 0.20^{c}	62 ± 6^{a}	135.4 ± 8.9^{c}	235 ± 23^{a}
Nano-Se	4.7 ± 0.2^{bc}	2.5 ± 0.3^{bc}	1.75 ± 0.30^{bc}	60 ± 7^{a}	130.6 ± 4.4^{abc}	221 ± 26^{a}
COS	4.3 ± 0.5^{b}	2.6 ± 0.3^{b}	1.81 ± 0.22^{bc}	59 ± 11^{a}	132.2 ± 4.0^{bc}	240 ± 16^{a}
Malathion	3.3 ± 0.1^{a}	2.3 ± 0.2^{ab}	1.25 ± 0.22^{a}	87 ± 8^{b}	170.6 ± 18.9^{d}	342 ± 36^{b}
Malation + Nano - Se	4.8 ± 0.3^{bc}	2.8 ± 0.2^{c}	2.08 ± 0.26^{c}	61 ± 6^{a}	116.1 ± 5.3^{ab}	323 ± 28^{b}
Malathion + COS	3.6 ± 0.3^{a}	2.0 ± 0.4^{a}	1.40 ± 0.18^{ab}	85 ± 4^{b}	120.3 ± 6.0^{abc}	327 ± 12^{b}
Malathion + Nano-Se + COS	4.9 ± 0.4^{c}	2.9 ± 0.1^{c}	1.30 ± 0.21^{a}	59 ± 4^{a}	114.1 ± 5.9^{a}	245 ± 19^{a}

Values were represented as means ± SE. Means with different superscripts are significantly different ($p < 0.05$), while means with the same superscripts indicate non-significant changes.

The cholesterol and triglyceride levels were higher in fish exposed to malathion compared to the other experimental and control groups (Table 2). Supplementation of diet with nano-Se and COS in malathion-exposed fish prevented upraising the cholesterol and triglyceride levels.

Caspian J. Environ. Sci. Vol. 18 No. 1 pp. 59~71 Received: July 06. 2019 Accepted: Nov. 25. 2019
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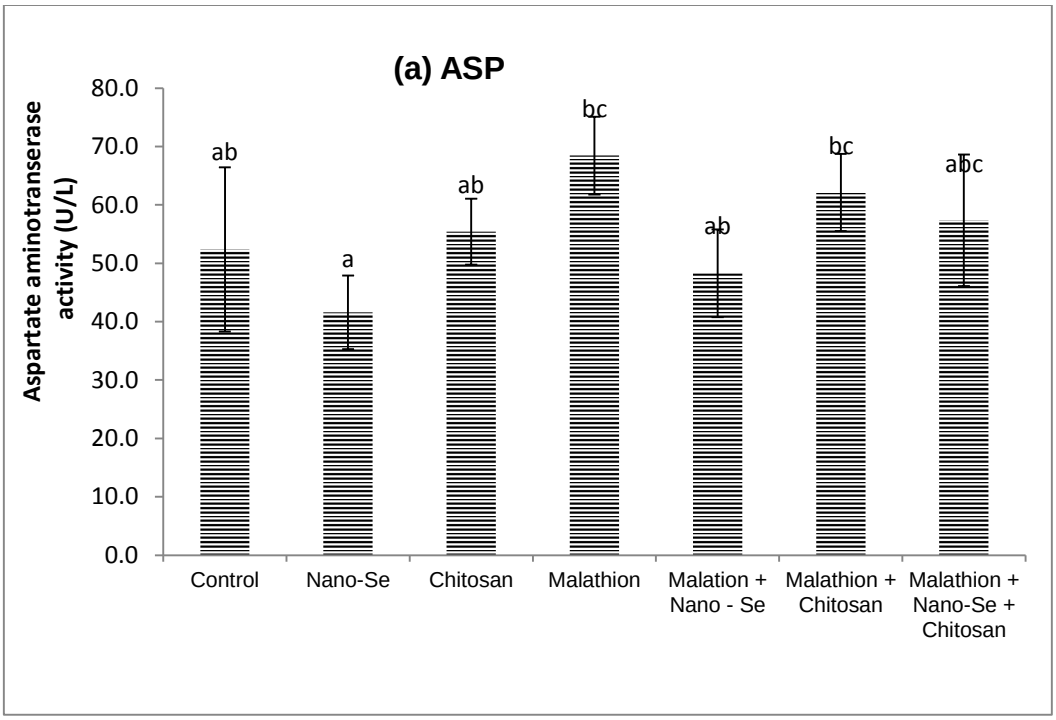
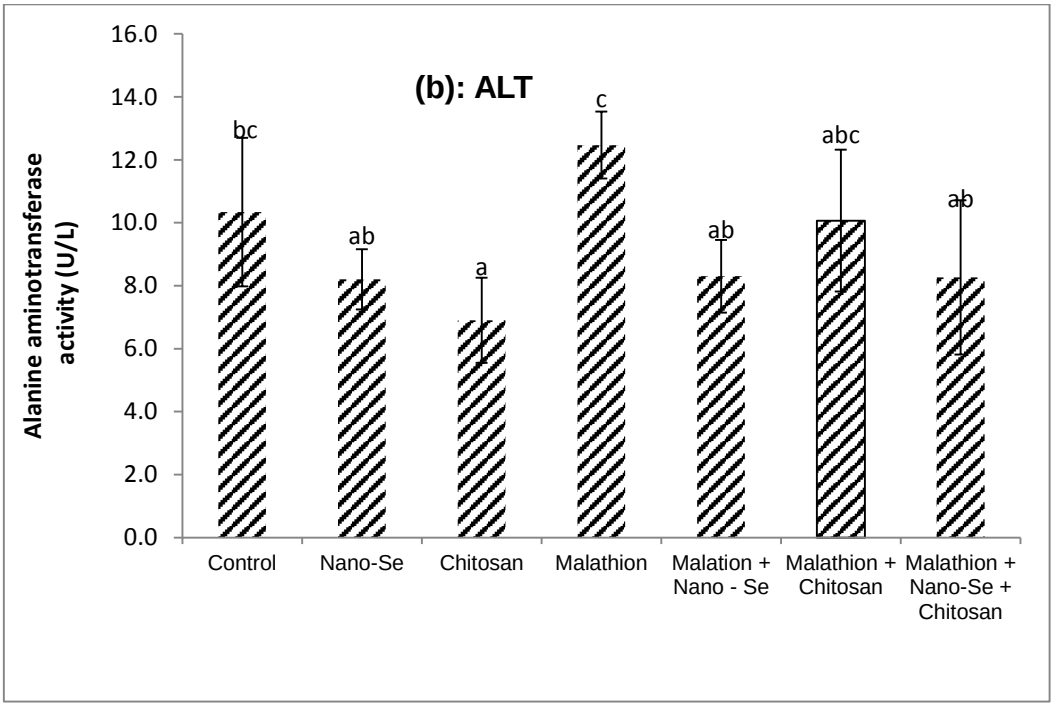
The effects of COS and/or nano-Se supplementation on blood biochemical enzymes in Caspian roach is illustrated in Fig. 1. Malathion significantly increased blood AST activity compared to the control group ($P < 0.05$). Administration of COS (alone) did not exhibit any effect on the blood AST activity as compared to malathion-exposed group. Administration of nano-Se alone or in combination with COS, however ameliorated malathion - induced alterations in AST activity (Fig. 1a).





Caspian J. Environ. Sci. Vol. 18 No. 1 pp. 59~71
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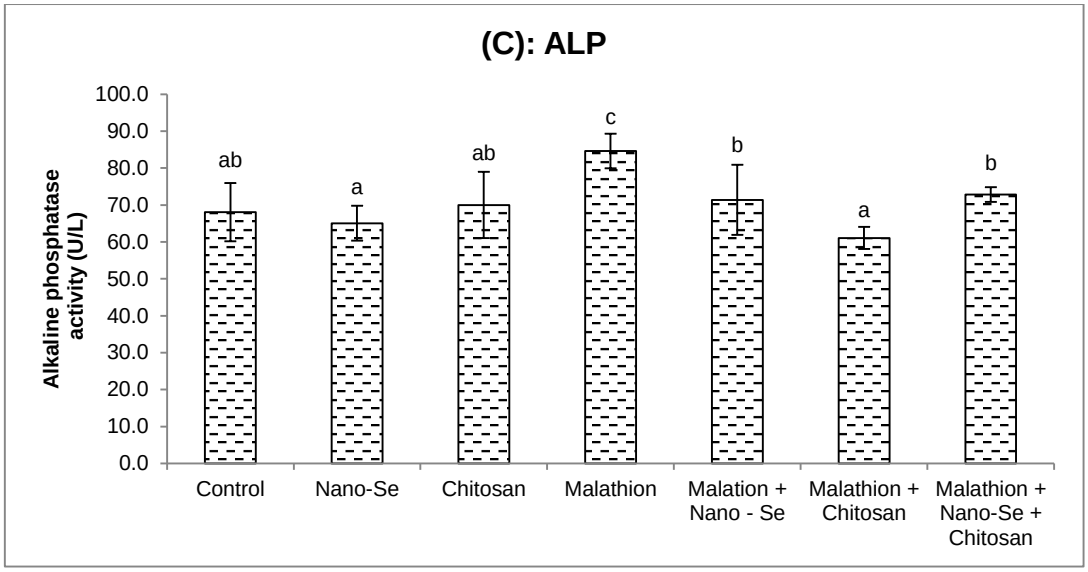

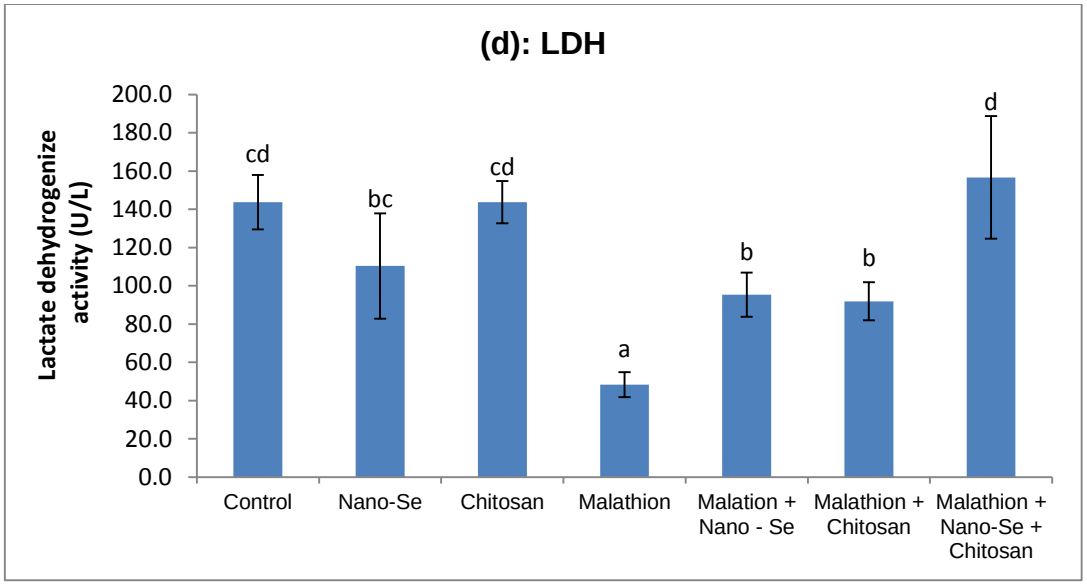

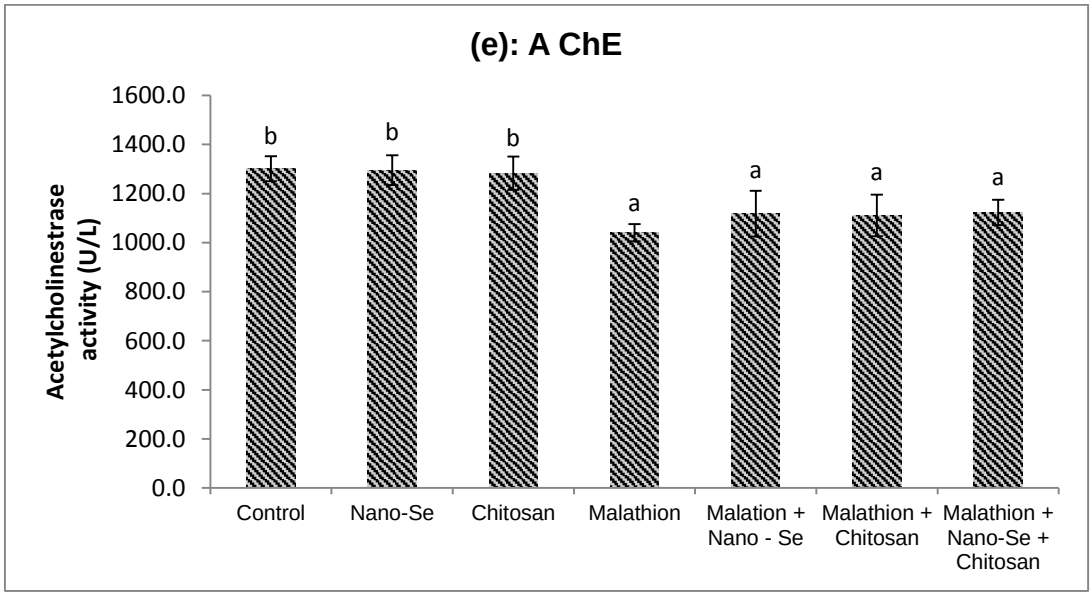

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Fig. 1. Ameliorative effects of nano-Se and COS on the serum enzyme activities of Caspian roach in different dietary treatments including the control and experimental groups exposed to malathion. The serum enzyme activities of: AST (a), ALT (b), ALP(c), LDH (d) and AChE (e) are illustrated. Significant differences between values when compared to control groups were represented by different letters ($p < 0.05$). Similar letters indicate no significant difference between experimental groups. Duncan post-hoc test was performed to show specific differences.

The blood ALT activity significantly upraised in malathion-exposed groups ($P < 0.05$), but co-administration of nano-Se with COS and nano-Se alone resulted in the regulation of ALT enzyme activity and its restoration to normal level (Fig. 1b).

The alkaline phosphatase (12.0 ± 0.15 UL^{-1}) activity in the bloodstream of malathion-exposed samples were significantly higher than in the control group. However feeding these fish with a nutritional supplement containing nano-Se and COS, alone or in combination, led to regulation of ALP activity and its restoration to near normal levels (Fig. 1c).

Our results revealed that malathion exposure led to a significant decrease ($P < 0.05$) in lactate dehydrogenize activity (LDH). Fishes fed with diets containing nano-Se or COS exhibited an increase in their LDH enzyme activity, but its activity was significantly lower than in the control group (Fig. 1d). Co-administration of nano-Se and COS in the blood of malathion-exposed fish regulated the activity of this enzyme.

Acetylcholinesterase activity level significantly declined ($P < 0.05$) in the blood of malathion-exposed samples in comparison with that of the control group. Although administration of nano-Se or COS alone and in combination had ameliorative effects on blood AChE enzyme activity in malathion-exposed fish, the AChE levels were still significantly lower than the normal level (Fig. 1e).

DISCUSSION

Hematological indices have been widely considered as valuable biomarkers for quantification of health alternations in fish species following exposure to chemical stressors (Modesto & Martinez 2010). In this study, a significant decrease was observed in the RBC count, hematocrit and hemoglobin (Hb) of the fish treated with malathion. According to Yonar *et al.* (2014) and Hamed (2015), pesticide exposure-induced acute stress in fishes is usually followed by inhibiting erythropoiesis, hemosynthesis or osmo-regulatory disorder. The observed decrease in the RBC counts, Ht and Hb levels can also be due to the increased rate of erythrocyte destruction in the hematopoietic organ and may indicate anemia (Vani *et al.* 2011; Prusty *et al.* 2011). This finding coincides with Yonar *et al.* (2014) and Al-Ghanim (2012) who recorded a decrease in levels of RBCs, Ht and Hb in *Cyprinus carpio* and *Oreochromis niloticus* respectively after their exposure to the sub-lethal concentration of malathion. Furthermore, decreased levels of the MCV, MCH, and MCHC can be attributed to the microcytic hypochromic type of anemia (Prusty *et al.* 2011). The number of WBC is one of the most important indicators of health and the status of the immune system (Yonar *et al.* 2014). It has a considerable role in phagocytic activity and immune response to bacterial, viral and parasitic infections (Hamed 2015).

In the present study, the WBC counts were significantly decreased in fish treated with malathion. A similar pattern was reported after exposing *Clarias gariepinus*, *Oreochromis niloticus* and *Cyprinus carpio* to malathion (Al-Ghanim *et al.* 2012; Ahmad 2012; Yonar *et al.* 2014). Decrease in the leukocyte counts following exposure to malathion can be attributed to the formation of leukocytosis with heterophilia and lymphopenia (Hamed 2015).

Although there are few conflicting reports about the role of COS in the growth and hematological parameters (Aathi *et al.* 2013; Sharifinasab *et al.* 2016), however, its significant role was documented in preventing from anemia through a positive effect on hematopoietic function and hematological parameters in some fishes (Saurabh & Sahoo 2008; Harikrishnan *et al.* 2012). In the present study, the hematological indices of fish fed with diets supplemented with nano-Se, COS and their combination improved and retained to levels near the normal values.

Lysozyme is the main component of the fish innate immune system. It has been documented that fish lysozyme possesses lytic activity against bacterial infections and stressful conditions (Harikrishnan *et al.* 2012).

In the present study, a significant decrease was observed in the lysozyme content of malathion-exposed fish in comparison with the control group. Administration of nano-Se and COS, alone or in combination, resulted in enhancement of lysozyme level. The impact of COS on the increase of immune system response of *Oncorhynchus mykiss* and *Cyprinus carpio* and the role of nano-Se in enhancement of lysozyme activity of *Cyprinus carpio* were also corroborated (Wang *et al.* 2016; Majumder & Kaviraj 2018). The results of total protein, globulin and albumin

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of plasma in this study came in accordance with the previous works (Al-Ghanim *et al.* 2012; Amin & Hashem 2012). Such reduction in total protein may be ascribed by hypo proteinemia which can be related to cellular degradation, imperfect protein synthesis and protein loss due to pathological changes in the kidney (Hamed 2015). Administration of nano-Se, alone or in combination with COS, may maintain the total plasma protein in normal level in malathion-exposed fish due to prevention from cell damage and the antioxidant role of nano-Se and COS in regeneration of the liver damages.

In the present study, diet supplementation with nano-Se alone or co-administrated with COS led to regulation of blood glucose. The significant role of selenium in the decrement of stress was also documented in cadmium-treated fishes (*Oreochromis niloticus*) (Hamed 2015). Selenium is capable of reducing lipid peroxidation in liver tissues and enhancing the hepatic antioxidant enzymes and subsequently, resulting in decreased glucose level and the biochemical parameters (Ashouri *et al.* 2015).

The significant increase in blood glucose of treated fishes may be due to enhanced energy demand by releasing more glucose via glycogenolysis (Al-Ghanim *et al.* 2012). Similar increased glucose was also reported in plasma of *Labeo rohita* exposed to Imidacloprid (Prusty *et al*. 2011; Qadir *et al.* 2014) and *Oreochromis niloticus* treated with chlorpyrifos (Majumder & Kaviraj 2018).

The hyperglycemic condition observed in the present study is in agreement with the observations of several authors which suggested the significant role of glucocorticoids and catecholamines in glucose mobilization from the adrenal tissue of the stressed fish (Yousef *et al.* 2003; Abalaka *et al.* 2011).

The required lipids for cells membrane are provided by cholesterol. Cholesterol has a significant role as a precursor information of biliary salts and corticosteroid compounds. An increased level of cholesterol in malathion-exposed groups may be due to lipoprotein metabolism disorder, liver and kidney pathologic damages and disturbed endocrine system (Yonar *et al.* 2017). Similar effects were also reported in *Oreochromis niloticus* exposed to chlorpyrifos (Majumder & Kaviraj 2018). Triglycerides are the source of energy for various metabolic processes and its extra amounts will be stored in form of fat in adipose tissue. So the observed elevation in serum triglyceride in malathion-treated fish could be attributed to degradation of the stored fats for producing demand energy to overcome the toxic effects of malathion (Yousef *et al.* 2003; Ahmad 2012). Besides, free radical-induced oxidative stress may be attributed to hepatopathy and cirrhosis of the liver which was observed in *L. rohita* exposed to Imidacloprid (Qadir *et al.* 2014) and in *O. niloticus* exposed to chlorpyrifos (Majumder & Kaviraj 2018). Oxidative stress induced by organophosphate pesticides like malathion can result in formation of reactive oxygen species (ROS) which can react with the cellular macromolecules and enhance lipid peroxidation. Therefore, the cell membrane damage can lead to mobilization of cellular enzymes such as ALT, ASP, and ALP into the blood (Yousef *et al.* 2003; Majumder & Kaviraj 2018). AST, ALT are presented in the liver. Under stressful condition, these enzymes released into bloodstream and gluconeogenic mechanisms are stimulated. Also, ALP has a serious role in the transport of phosphorylated intermediates through cell and carbohydrate metabolism (Yousef *et al*. 2003).

Our findings indicated that the observed increase in AST, ASP and ALT serum activity of malathion-exposed Caspian roach could be ascribed to cellular cytotoxicity and the liver damage which were observed before in *Cyprinus carpio* exposed to paraquat (Qadir *et al.* 2014). Similar findings were observed in *L. rohita*, *C. carpio* and *Alburnus mossulensis* after exposure to fenvalerate, paraquat and fenpropathrin, respectively (Sharifinasab *et al.* 2016). However, administration of nano-Se alone or combined with COS to fish exposed to malathion reversed the activity of AST and ALT to their normal levels. Similar results were reported by Yonar *et al.* (2011) and Sharifinasab *et al.* (2016) who observed that propolis, combination of vitamin C and chitosan could reduce malathion and paraquat toxicity in *C. carpio*, respectively.

The significant drop of the serum LDH in malathion-treated fish compared with the control group suggests the liver dysfunction and a lower rate of the glycolytic process due to decreased metabolic rate (Sharifinasab *et al.* 2016).

This result is in agreement with data on *C. carpio* and *O. niloticus* exposed to diazinon and cypermethrin, respectively (Hamed 2015; Sharifinasb *et al.* 2016). Feeding fish with diets containing nano-Se and COS regulated the activity of LDH and restored it to the normal level. Cholinesterase enzyme inhibition is one of the most important mechanisms for dealing with toxic organophosphate compounds. This is necessary to maintain the normal function of the nervous system (Hamed 2015).

Caspian J. Environ. Sci. Vol. 18 No. 1 pp. 59~71
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The decreased acetylcholinesterase activity in the bloodstream of malathion-exposed fish may indicate the adverse effect of malathion on inhibiting the serum AChE activity. The results are consistent with several observations reporting decreased plasma AChE activity in different fishes such as *Clarias gariepinus* (Bakhshwan *et al.* 2009; Harabawy & Ibrahim 2014), *Oreochromis mossambicus* (Jordan *et al.* 2013) and *C. carpio* (Yonar *et al.* 2014; Sharifinasab *et al.* 2016) exposed to various organophosphorus pesticides. This reduction can be attributed to accumulation of free acetylcholine at the end of the nerve resulting in the stimulation of continuous electrical activity and degeneration in the functioning of the nervous system (Yonar *et al.* 2017).

Free radical mobilization during oxidation stress as the result of pesticides exposure may lead to a decrease in the total cellular antioxidant level (Yonar *et al.* 2014). Our findings suggested that nano-Se alone and in combination with COS could modulate and decrease the adverse effects of malathion. The inclusion of COS in the diet as an immune-stimulant, antioxidant and transporting minerals for improving fish health was documented (Cui *et al.* 2012; Wang *et al.* 2016). The significant protective role for selenium was considered in fish and rat exposed to pesticide (Nazıroglu *et al.* 2004; Khan *et al.* 2017). So, this might exhibit that nano- Se and COS can enhance the fish antioxidant enzyme system and restore oxidative effect induced by malathion.

CONCLUSION

The imbalance between ROS and total cellular antioxidant capacity of the malathion-exposed fish is one of the major causes of adverse alterations in their blood biochemical parameters. An increase in level of hematological and blood biochemical indices in diets containing nano-Se and COS, can be due to the improvement of the fish antioxidant system. Our findings elucidated that nano-Se, COS and their complex may be effective in reduction of malathion undesirable effects by restoration of blood biochemical indices in Caspian roach. Further studies are essential to define the exact protective mechanism of nano-Se and COS against pesticide toxicity.

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تاثیر نانوذره سلنیوم و کیتوزان الیگوساکارید جیره بر پارامترهای بیوشیمیایی ماهی کلمه تحت استرس با مالاتیون

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چکیده

مطالعه حاضر با هدف بررسی اثرات محافظتی نانوذرات سلنیوم (nano-Se) و الیگوساکارید کیتوزان (COS) در برابر تغییرات خون‌شناختی و بیوشیمیایی سرم ماهی کلمه (*Rutilus caspicus*) تحت استرس با مالاتیون انجام شد. ماهیان در معرض مالاتیون با غلظت ۰/۵ میلی‌گرم در لیتر آب قرار گرفته و با جیره‌های حاوی نانوذرات سلنیوم، الیگوساکارید کیتوزان یا مخلوط توأمان آنها به مدت ۲۸ روز تغذیه شدند. در پایان آزمایش، نمونه‌های خون جمع‌آوری شده و وضعیت خون شناختی، شاخص‌های بیوشیمیایی سرم (پروتئین کل، آلبومین، گلوبولین، گلوکز، کلسترول و تری‌گلیسرید) آنزیم‌هایی مانند آسپارتات آمینوترانسفراز، آلانین آمینوترانسفراز، لاکتات دهیدروژناز(LDH)، آلکالین فسفاتاز و استیل کولین استراز (AChE) سنجش شدند. کاهش قابل توجهی در فعالیت LDH، AChE و شاخص‌های خون‌شناختی مانند شمارش گلبول‌های سفید و قرمز، غلظت هموگلوبین، سطح هماتوکریت در ماهیان مواجه شده با مالاتیون مشاهده شد. نانوذرات سلنیوم و COS به طور معنی داری ($P <$ ۰/۰۵) توانستند کاهش مشاهده شده در شاخص‌های خون‌شناختی، میزان لایزوزیم و فعالیت آنزیم ضد اکسایش خون در ماهی کلمه تحت القای مالاتیون را به حالت اولیه بازگردانند. در کل، نتایج نشان دادند که غنی‌سازی جیره با نانوسلنیوم (به میزان ۱ میلی‌گرم در کیلوگرم) و کیتوزان الیگوساکارید (به مقدار ۶۰۰ میلی‌گرم در کیلوگرم) می‌تواند تأثیر مثبت بر شاخص‌های خون‌شناختی و بیوشیمیایی ماهی کلمه در معرض استرس مالاتیون داشته باشد.

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