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RESEARCH PAPER

Moderating Effects of Dietary Oregano Extract (*Origanum vulgare*) on the Toxicity Induced by Organophosphate Pesticide, Diazinon in Rainbow Trout, *Oncorhynchus mykiss*: Metabolic Hormones, Histology and Growth Parameters

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Abstract

The present study was carried out to evaluate the moderating properties of oregano (Origanum vulgare) (ORG) extract on the toxic effects of organophosphate pesticide, diazinon on growth and some metabolism associated components of rainbow trout, Oncorhynchus mykiss. In non-diazinon-exposed fish, the triiodothyronine (T3) levels in serum and body weight index (BWI %) and specific growth rate (SGR) values were higher in fish supplemented with 2, 6 and 10 g ORG/kg diet compared to control (non-ORG-supplemented fish) and fish fed 14 g ORG/kg diet after 60 days feeding trial (P<0.05). Furthermore, the serum thyroxine (T4) levels increased in fish fed 6 and 10 g ORG/kg diet compared to control and those supplemented with 2 and 14 g ORG/kg diet (P<0.05). Fish supplemented with 14 g/kg ORG diet showed the lowest BWI %, SGR and serum thyroid hormones (P<0.05). The lowest FCR values were observed in fish supplemented with 6 and 10 g ORG/kg diet (P<0.05). In diazinon exposed fish, thyroid hormones, BWI % and SGR significantly declined during 60 days exposure to diazinon in control and ORG-supplemented fish (P<0.05). However, these parameters were found to be higher in fish supplemented with 6 and 10 g ORG/kg diet compared to other exposed fish (P<0.05). FCR values significantly increased in control and fish supplemented with 2, 6 and 14 g ORG/kg diet after exposure (P<0.05). The levels of cortisol significantly elevated during exposure to diazinon in control and fish supplemented with 2 and 14 g ORG/kg diet (P<0.05). The glucose levels significantly increased in fish fed 6 and 10 g ORG/kg diet (P<0.5). During exposure period, diazinon induced ranges of histological lesions in liver, which the severity of theses lesions was lower in ORG-supplemented fish (P<0.05). In conclusion, ORG at optimum dietary levels (6-10 g/kg diet) could moderate the growth suppressing effects, stress and tissue lesions induced by diazinon. In addition, ORG at high dietary levels had toxic effects on fish metabolism.

Introduction

The contamination of aquatic ecosystems by a wide ranges of pollutants is becoming a global concern (Schwarzenbach, Egli, Hofstetter, Von Gunten, & Wehrli, 2010). Annually, a lot of pollutants are entered water bodies as a consequence of industrial and agricultural activities, affecting all biological aspects of aquatic organisms including fish (Derraik, 2002; Islam, & Tanaka, 2004).

As one of the most pollutants, the agricultural pesticides (PCs), mainly reach surrounding water through runoff and drainage of farms (Islam, & Tanaka, 2004). There are several studies, confirming the adverse effects of PCs on fish biology. PCs reduce reproductive efficiency and disrupt also fish hemostasis in relation to

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surrounding environment (Bonga, & Lock, 1991; Kime, 1995; Barry, O'Halloran, Logan, Ahokas, & Holdway, 1995; Chang, Liu, Zhou, Wang, & Zhu, 2013; Katuli, Amiri, Massarsky, & Yelghi, 2014; Hajirezaee, Mirvaghefi, Farahmand, & Agh, 2016). Furthermore, many studies have shown the immune-suppressing effects of PCs in fish (Blakley, Brousseau, Fournier, & Voccia, 1999; Dunier, & Siwicki, 1993; Bols, Brubacher, Ganassin, & Lee, 2001).

However, little information is available regarding the adverse of PCs on components of fish metabolism, particularly metabolic hormones. Increases in cortisol levels (Ibrahim, & Harabawy, 2014) and the suppression of thyroid hormones have been reported as main consequences of PC-exposures (Ibrahim, & Harabawy, 2014; Katuli *et al.* 2014; Khatun, & Mahanta, 2014; Hajirezaee *et al.* 2016).

However, little information is available regarding the adverse of PCs on components of fish metabolism, particularly metabolic hormones. Khatun and Mahanta (2014) reported the suppression of thyroid function and significant decreases in serum levels of Triiodothyronine (T3), thyroxine(T4) and thyroid stimulating hormone(TSH) in Stinging Catfish, Heteropneustes fossilis after exposure to chlorpyrifos. In African catfish, Clarias gariepinus, long-term exposure to Carbofuran caused a significant increase in serum levels of cortisol and T3, although serum T4 levels showed significant decreases after exposure (Ibrahim and Harabawy, 2014). The serum levels of cortisol increased in Atlantic salmon, Salmo salar after exposure to atrazine (Waring and Moore, 2004). In Persian sturgeon, Acipenser persicus, the treatment of fish with diazinon significantly increased serum levels of cortisol, whereas, the T3 and T4levels showed significant decreases during exposure (Katuli *et al.*, 2014; Hajirezaee *et al.*, 2016).

Today, use of PCs shows an increasing trend due to the growing development of intensive agriculture. Nevertheless, finding the ways to moderate the toxic effects of PCs on fish is of essentials.

During recent two decades, medicinal herbal extracts (MHEs) have used as an alternative to the drugs, chemicals and antibiotics to prevent and treat diseases in human and animals, particularly fish (Galina, Yin, Ardo, L., & Jeney, 2009; Reverter, Bontemps, Lecchini, Banaigs, & Sasal, 2014; Van Hai, 2015; Bulfon, Bongiorno, Messina, Volpatti, Tibaldi, & Tulli, 2013). Immunostimulating, antioxidant, antimicrobial, anti-stress and growth promoting effects of MHEs have been well documented in fish (Prasad, & VariyurPadhyoy, 1993; Citarasu, VenketRamalingam, Raja JeyaSekar, MichealBabu, & Marian 2002; Sivaram, Jian, & Wu, 2004; Galina *et al.* 2009; Chakraborty, & Hancz, 2011; Reverter *et al.* 2014; Van Hai, 2015). However, there are too little information about the modifying effects of MHEs on oxidative stress induced by PCs in fish (Banaee, Sureda, Shahaf, & Fazilat, 2015; Rabie, & Ahmadi, 2016).

The medicinal plant, oregano, Origanum vulgare is well known as a strong antioxidant, because of its capabilities in scavenging the ROS produced during oxidative stress (Lagouri, Blekas, Tsimidou, Kokkini, & Boskou, 1993; Yanishlieva, Marinova, Gordon, & Raneva, 1999; Kulisic, Radonic, Katalinic, & Milos, 2004). The chemical composition of oregano includes high content of polyphenols, thymol and carvacrol, which have been recognized to have antioxidant effects (Lagouri *et al.* 1993; Taimidou & Boskou, 1994). Several studies have shown the immunostimulating and antioxidant role of oregano in fish (Zheng, Tan, Liu, Zhou, Xiang, & Wang, 2009; Abdel-Latif & Khalil, 2014; Haghighi & Rohani, 2015; Diler, Gormez, Diler, & Metin, 2017; Beltrán, Espinosa, Guardiola, & Esteban, 2018), However, there are no information regarding the protective effects of oregano in relation to ROS resulting from pesticide-induced oxidative stress.

In the present study, we have studied for the first time the effects of ORG extract in moderating the diazinon-induced-oxidative stress in rainbow trout by measuring liver histology (as the main organ for energy metabolism), metabolic hormones (cortisol, T3 and T4), plasma glucose and growth parameters. The aims of the present study were: (a) examining the toxicity of diazinon on metabolic hormones and growth to obtain a comprehensive understanding of pesticide its effects on fish metabolism; (b) evaluating the moderating effects of ORG on possible toxicity of diazinon on metabolism. As we mentioned previously, although, a wide spectrum of studies have reported the impacts of PCs on various aspects of fish biology, too little data is available regarding the effects of these chemicals on fish metabolism. In addition, to our knowledge, no suggestion has yet been made to moderate the adverse PCs on fish metabolism. Therefore, theThe findings of the present study may help us to find a natural way to moderate the deleterious effects of PCs on fish metabolism.

Materials and Methods

Plant Extract

Dried leaves of oregano, Origanum vulgare L. were purchased from a local herbal market and then authenticated by a plant taxonomist. The hydro-alcoholic extract of oregano (ORG), Origanum vulgare L. was prepared using percolation method. For this purpose, the oven dried leaves (at 40ºC) were ground to powder by a manual mortar and then soaked in 80% (v/v) aqueous ethanol (1:5 w/v). After 72 h, the suspension was filtered and separated supernatant was evaporated to dryness in aan rotary evaporator (Buchi, Switzerland) at 80°C with reduced pressure. The concentrated extracts were then incubated at 40°C to complete dryness. Eventually, the dried extracts were grounded to powder and stored at 0°C under dark condition until feeding experiment.

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Fish and Experimental Design and Exposure

One thousand eight hundred Rainbow trout (n= 1800, total mean individual weight: 24.4±5.5 g; and mean total individual length: 12.2±1.8 cm) were considered for the experiment. At first, fishFish were distributed in fifteen 500l 500-L experimental tanks (at stocking rate: of 120 fish/ per tank) containing aerated and dechlorinated water for adaptation. After 3 days acclimation, fish were divided into two groups including diazinon and non-diazinon exposed fish. In each group, fish were sub-divided into five groups within 100l tanks (55 fish per tank) and fed experimental diets containing various levels of ORG including 0 (control), 2 g ORG/kg diet, 6 g ORG/kg diet, 10 g ORG/kg diet and 14 g ORG/kg diet in three replicates for 60 days. Totally 30 experimental tanks were considered for the experiment (non-diazinon exposed group: 15 tanks; diazinon exposed group: 15 tanks). The dietary levels of ORG extracts used in the present study were adjusted according to a previous study on rainbow trout (Pourmoghim, Haghighi, & Rohani, 2015).

Diet Preparation and Feeding

To prepare the experimental diets, dry pellets (Biomar, ORBIT INTRO pellets, size: 4.5 mm, crude protein: 45.7±1.02%, crude lipid: 25.6±0.96%, carbohydrates 14.5±1.29 %, crude fiber: 1.8±0.47%, Ash: 8.6±0.84% and digestible energy 19.7 MJ/kg) were grounded into a powder form in a mortar. Then, dried ORG extract was added to powdered diet at levels of 0, 2, 6, 10 and 14 g ORG/Kg diet. After homogenization by a mixer, the 50 ml distilled water was added to each mixture to form a paste. The paste was then sieved using a fine wire mesh household sifter to produce food particles (mostly as pellet and granule with 3±0.1 mm in diameter and 3.5±0.2 mm in length). The food particles were shade-dried at room temperature, then oven-dried at 60°C and finally storied at 2°C until usage (Nya, & Austin, 2009). Throughout 60 days feeding trial, fish were fed daily at 3% of body weight. The feeding rate was determined every 10 days based on total fish biomass per tank.

Exposure Trial

In diazinon-exposed groups, fish were exposed to a sublethal nominal concentration (25% of the LC50or 0.287 mg/l or 0.942 µM) of diazinon (60% purity, Arysta lifescience Company, France) for 60 days. The value of LC50 had been previously calculated during 96 h in a separate toxicity test. Before exposure, no diazinon was found in water according to the results of a Gas chromatography–mass spectrometry (GC-MS) (Shimadzu QP2010 Ultra series instrument, with a silica capillary 120 column with a length of 30 m, at a flow rate of 0.5 L/h). Throughout the experiment period, 50 % of water in each tank was exchanged every 24 h and the diazinon dosing dose was renewed accordingly. After water exchange, the diazinon concentration in water was checked by analyzing water by GC-MS. In this regard, a concentration of 0.288±0.00102 mg/l diazinon was measured for all assays during the experiment period.

Experiment Condition

Furthermore, water Water quality was checked daily for pH: 6.7±0.1 (by a pH metre: Model 6 APX15/C-WTW-330i), temperature: 15.6±1.2°C, ammonia: 0.02±0.001 mg/L (colorimetric method at 670 nm) and dissolved oxygen: 7.1±0.14 mg/L (by an oxygen the experiment.

Blood and Liver Sampling

Blood sampling was conducted by cutting of the caudal peduncle of 20 fish per tank at the end of the experiment. Serum samples were separated by centrifuging at 13,000 g for 10 min and then stored in liquid nitrogen (–196°C) until biochemical assays. Also, fish were dissected out to collect liver tissue samples. Liver tissues were then stored in Bouin's solution for histological investigations.

Hormonal Assays

Radioimmunoassay method (RIA) was used to measure the serum concentration of metabolic hormones including T3, T4 and cortisol. All assays were conducted using assay kit based on manufacturer's instructions (Cortisol [I-125] RIA KIT (Ref: RK-240CT); T4 [I-125] RIA KIT (REF: RK-11CT1); T3 [I-125] RIA KIT (REF: RK-609CT, izotop, Budapest, Hungary). The cross-reactivity of T3antiserum was little or negligible withT4. The cortisol antiserum had 8.5% cross-reactivity with corticosterone, 1.85% with 17-α-hydroxyprogesterone, <0.8 % with cortisone, <8% with 11-deoxycortisol, <1.7% with deoxy-corticosterone and <2.1% with dexamethasone. The cross-reactivity for the T4 antiserum was <12.6% with 3,5,3'-L-triiodothyronine (T3), <0.89% with 3',5',3, -triodo-L-thyronine (rT3) and <0.11% with 3,3'-diiodo-L-thyronine (3,3'-T2). Before assays, all reagents and samples were homogenized by gentle shaking to avoid foaming. Then, 10 µl of (25 µl for T4 assay and 50 µl for T3assay) of standard, control and samples were poured into assay tubes. Afterward, 500 µl of tracer (125I-labelled cortisol) [for T4assay: 100 µl tracer (125I-labelled T3); for T3 assay: 200 µl tracer (125I-labelled T3)] were respectively added into each tube and the tubes were fixed onto a shaker plate, sealed with a plastic foil and shacked gently. After shaking, tubes were kept at room temperature for 2 h and the supernatant was then decanted. Finally, tubes were placed upside down position on an absorbent paper for 2 min to dry and then subjected to a Gamma counter (LKB 182 Compugamma CS, LKB Wallac, Finland)

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for measurement of absorption spectrum and following calculation of hormone concentration using related standard curve.

Glucose Assay

A colorimetric method (at 540 nm) was used to assay serum glucose using Sigma Diagnostic (Product Code GAGO-20, St Louis, Missouri, USA) kit based on manufacturer's instructions. In this assay, gluconic acid and hydrogen peroxide (H_2O_2) were first generated from oxidation of glucose. The produced H_2O_2 reacted with o-dianisidine in the presence of peroxidase to form a colored product. Then, oxidized o-dianisidine reacted with sulfuric acid to produce a more stable colored product. For assay, 1 ml samples were added to tubes containing standard (0.05 ml Glucose standard + 0.95 ml distilled water) and 1 ml distilled water with mixing. After 30 min at 37°C, the reaction was stopped by adding 2 ml 12N H_2SO_4 and the wavelength was measured at 540 nm.

Histological Analyses

The preparation of liver tissues for histological examinations was conducted by an automatic tissue processor (DID SABZ, DS 2080/H). The samples fixed in Bouin's solution were first dehydrated serially by different grades of ethanol (70%, 80%, 90%, 95% and 100%) and then cleared in chloroform. Dehydrated and clarified tissues were then impregnated, embedded and blocked out in paraffin. The blocks were then sectioned at 5 μm thick, transferred onto glass slides, stained with hematoxylin and eosin (Figueiredo-Fernandes, Ferreira-Cardoso, Garcia-Santos, Monteiro, Carrola, Matos, & Fontaínhas-Fernandes, 2007) and finally examined under the light microscope to evaluate tissue lesions. Also, the histopathological lesions in liver were evaluated according to a histological grading system (Robbins, 2007; Van Dyk, Pieterse, & Van Vuren, 2007; Banaee, Antoni Sureda, Mirvaghefi, & Kamal Ahmadi, 2013). In this system, the numerical value of 1 was considered as no lesion in liver, the number 2 as a mild lesion (<25 %), 3 as moderate lesion (25–50 %), 4 as severe lesion (50–75 %) and 5 as very severe lesion (75 %<) (Robbins, 2007; van Dyk *et al.* 2007; Banaee *et al.* 2013).

Growth Parameters and Mortality Rate

Fish growth parameters including body weight indexgain (BWI WG %), specific growth rate (SGR %), feed conversion ratio (FCR) and mortality rate (%) were measured at the end of the experiment. All parameters were calculated by following equations:

$$BWI \% = (BWf − BWi)/BWi × 100 \text{ (Hung \& lutes Lutes, 1987):}$$

$$SGR = (LnBWf − LnBWi)/n × 100 \text{ (Zhou, Wu, Tan, Chi, \& Yang, 2006):}$$

$$FCR = F/(BWf − BWi) \text{ (Zhou } et al. \text{ 2006):}$$

$$\text{Mortality Rate (\%) = (initial fish number − final fish number) × 100.}$$

Where, BWf: the Final weight of fish before feeding trial (g); BWi: the Initial weight of fish after feeding trial (g); F: Total feed Feed intake during 60 days experiment (g)

Statistical Analysis

The data analysis was carried out by SPSS software (SPSS 19.0, IBM software, Inc., Chicago, IL, USA). Before analysis of variance (ANOVA), the normality of data was investigated using Kolmogorov–Smirnov (K-S) test. The analysis of parametric data was done by two-way analysis of variance and following calculating of significant F-ratios. The analysis was followed by Tukey test to determine which groups were different. Non-parametric data (histopathological score) were analyzed by the Kruskal–Wallis test. Then, the comparison of means was carried out using Mann–Whitney test. All data have been expressed as mean and standard deviation (SD).

Results

Non-Diazinon-Exposed Fish

There were no significant differences in mortality rate (%) between ORG-supplemented fish and control group during 60 days experiment (Figure 1, P>0.05).

The serum levels of T3 and (Figure 2A), BWI % (Figure 3A) and SGR % (Figure 3B) values were higher in fish supplemented with 2,6 and 10 g ORG/kg diet compared to control (non-ORG-supplemented fish) and fish supplemented with 14 g ORG/kg diet (P<0.05). Furthermore, the levels of T4 (Figure 2B) increased in fish supplemented with 6 and 10g ORG/kg diet compared to control and fish supplemented with 2 and 14 g ORG/kg diet (P<0.05). Fish fed 14 g/kg ORG diet showed lowest BWI (Figure 3A), SGR (Figure 3B) and thyroid hormones (Figure 2A, Figure 2B) compared to other groups (P<0.05). FCR values (Figure 4) significantly decreased in fish fed 6 and 10 g ORG/kg diet compared to control and other experimental groups (P<0.05). In addition, there were no significant differences in FCR values between control and fish supplemented with 2 g ORG/kg diet (P>0.05). Also, no significant alternations were observed in serum levels of cortisol (Figure 5) and glucose (Figure 6) and liver histology (Figure 7A) during 60 days feeding experiment (P>0.05).

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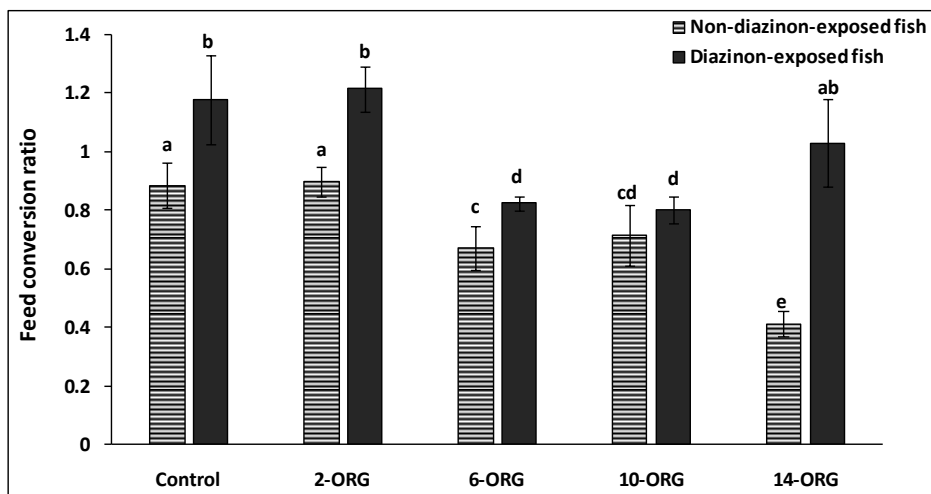*Turk. J. Fish.& Aquat. Sci. 20(3), 207-219*
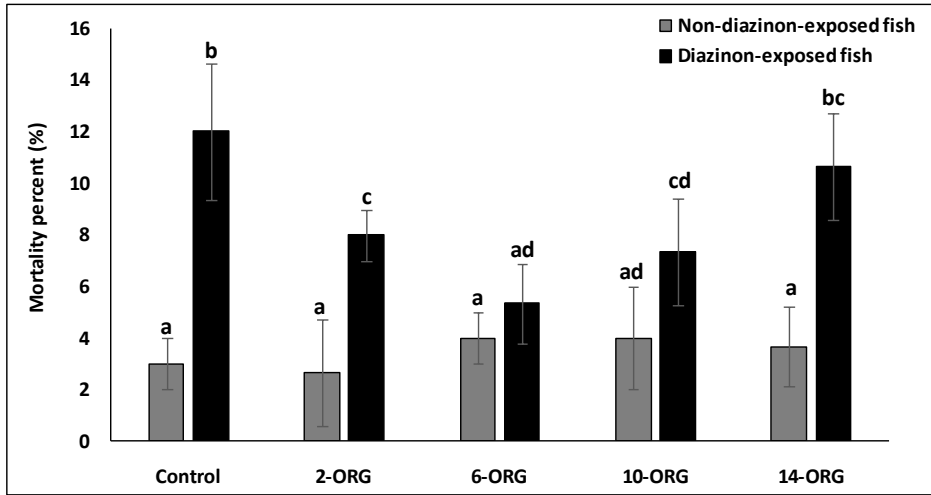
Figure 1. Effects of different dietary levels of *Origanum vulgare* on fish mortality in non-diazinon and diazinon-exposed rainbow trout. Control: non-ORG-supplemented fish, 2-ORG: fish supplemented with 2 g ORG/kg diet, 6-ORG: fish supplemented with 6 g ORG/kg diet, 10-ORG: fish supplemented with 10 g ORG/kg diet, 14-ORG: fish supplemented with 14 g ORG/kg diet. Means with the different superscript letters show significant differences between groups (P<0.05). Data are presented as mean ± S.D.
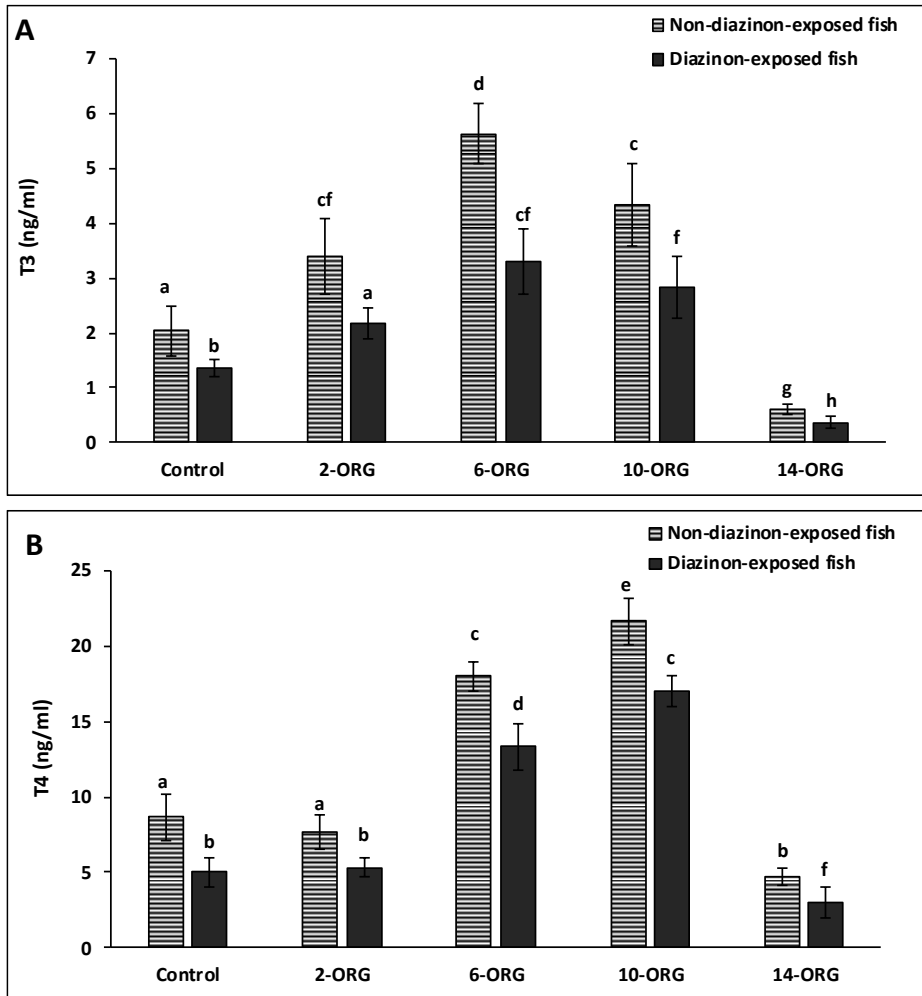
Figure 2. Effects of different dietary levels of *Origanum vulgare* on plasma levels of T_3 (plot A) and T_4 (plot B) in non-diazinon and diazinon-exposed rainbow trout. Control: non-ORG-supplemented fish, 2-ORG: fish supplemented with 2 g ORG/kg diet, 6-ORG: fish supplemented with 6 g ORG/kg diet, 10-ORG: fish supplemented with 10 g ORG/kg diet, 14-ORG: fish supplemented with 14 g ORG/kg diet. Means with the different superscript letters show significant differences between groups (P<0.05). Data are presented as mean ± S.D.

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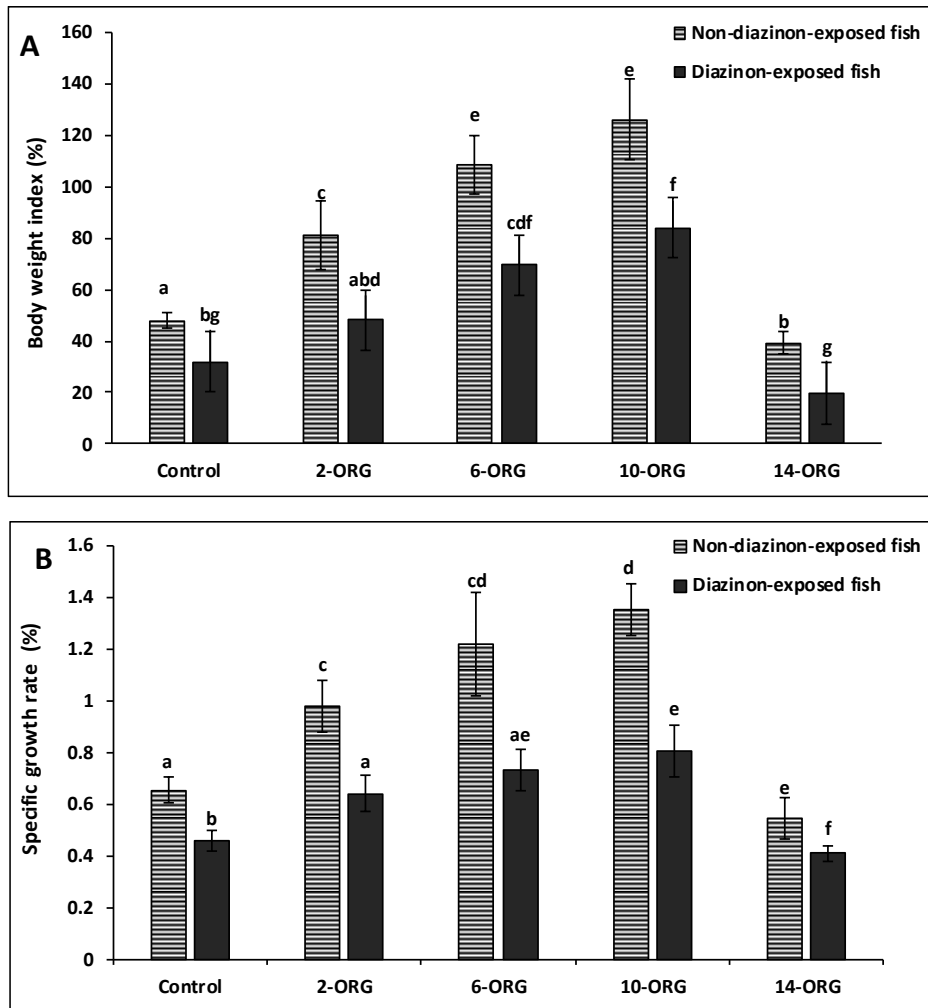
Figure 3. Effects of different dietary levels of *Origanum vulgare* on body weight index (plot A)and specific growth rate (plot B) in non-diazinon and diazinon-exposed rainbow trout. Control: non-ORG-supplemented fish, 2-ORG: fish supplemented with 2 g ORG/kg diet, 6-ORG: fish supplemented with 6 g ORG/kg diet, 10-ORG: fish supplemented with 10 g ORG/kg diet, 14-ORG: fish supplemented with 14 g ORG/kg diet. Means with the different superscript letters show significant differences between groups (P<0.05). Data are presented as mean ± S.D.
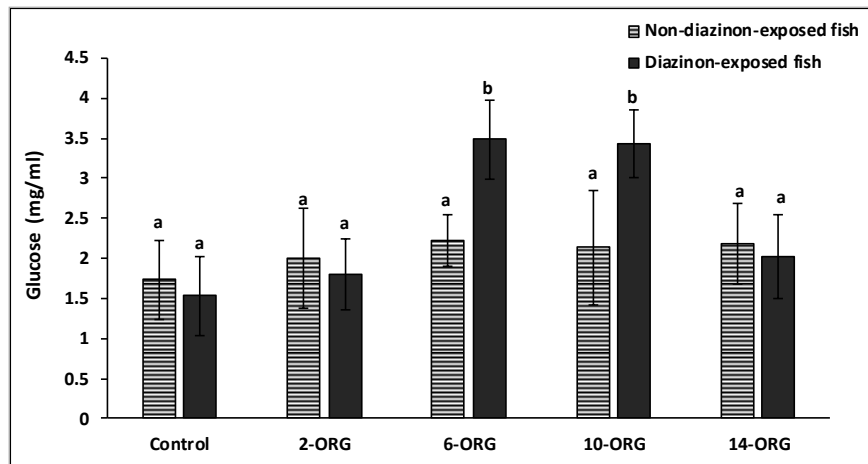

Figure 4. Effects of different dietary levels of *Origanum vulgare* on feed conversion ratio in non-diazinon and diazinon-exposed rainbow trout. Control: non-ORG-supplemented fish, 2-ORG: fish supplemented with 2 g ORG/kg diet, 6-ORG: fish supplemented with 6 g ORG/kg diet, 10-ORG: fish supplemented with 10 g ORG/kg diet, 14-ORG: fish supplemented with 14 g ORG/kg diet. Means with the different superscript letters show significant differences between groups (P<0.05). Data are presented as mean ± S.D.

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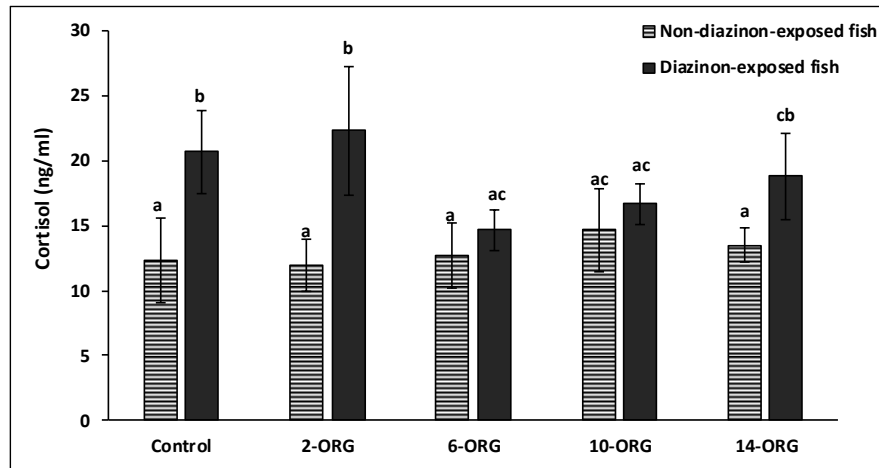
Figure 5. Effects of different dietary levels of *Origanum vulgare* on plasma levels of cortisol in non-diazinon and diazinon-exposed rainbow trout. Control: non-ORG-supplemented fish, 2-ORG: fish supplemented with 2 g ORG/kg diet, 6-ORG: fish supplemented with 6 g ORG/kg diet, 10-ORG: fish supplemented with 10 g ORG/kg diet, 14-ORG: fish supplemented with 14 g ORG/kg diet. Means with the different superscript letters show significant differences between groups (P<0.05). Data are presented as mean ± S.D.

Figure 6. Effects of different dietary levels of *Origanum vulgare* on plasma levels of glucose in non-diazinon and diazinon-exposed rainbow trout. Control: non-ORG-supplemented fish, 2-ORG: fish supplemented with 2 g ORG/kg diet, 6-ORG: fish supplemented with 6 g ORG/kg diet, 10-ORG: fish supplemented with 10 g ORG/kg diet, 14-ORG: fish supplemented with 14 g ORG/kg diet. Means with the different superscript letters show significant differences between groups (P<0.05). Data are presented as mean ± S.D.

Diazinon-Exposed Fish

The mortality rate increased in control and fish supplemented with 2 and 14 g ORG/kg diet after exposure to diazinon (Figure 1, P<0.05). Fish supplemented with 6 and 14 g ORG/kg diet showed lower mortality compared to other groups (Figure 1, P<0.05).

The thyroid hormones (Figure 2A, Figure 2B) and the values of BWI (Figure 3A) and SGR (Figure 3B) significantly reduced during exposure to diazinon in control and ORG-supplemented fish (P<0.05). However, these parameters were higher in fish supplemented with 2, 6 and 10 g ORG/kg diet compared to other exposed fish (P<0.05). In this regard, the highest values of thyroid hormones (Figure 2A, Figure 2B), BWI (Figure 3A) and SGR (Figure 3B) were observed in fish

supplemented with 6 and 10 g ORG/kg diet (P<0.05). There were no significant differences in BWI (Figure 3A) and SGR (Figure 3B) between fish supplemented with 2 and 6 g ORG/kg diet (P>0.05). FCR values (Figure 4) significantly increased in control and fish supplemented with 2, 6 and 14 g ORG/kg diet after exposure (P<0.05). In fish fed 10 g ORG/kg diet, FCR (Figure 4) showed no significant changes after exposure (P>0.05). Furthermore, no significant differences were observed between FCR (Figure 4) values of 2, 6 and 10 g ORG/kg diet treatments and also between control and fish of 14 g ORG/kg diet treatment (P>0.05). Also, the lowest values of thyroid hormones (Figure 2A, Figure 2B), BWI (Figure 3A), and SGR (Figure 3B) were observed in groups supplemented with14 g/kg ORG diet (P<0.05).

The levels of cortisol (Figure 5, P<0.5) significantly increased during exposure to diazinon in control and

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groups supplemented with 2and 14 g ORG/kg diet, while the levels of this hormone remained unchanged in fish supplemented with 6 and 10 g ORG/kg diet (Figure 5, $P>0.5$). The glucose levels showed no significant changes in control and groups supplemented with 2 and 14 g ORG/kg diet (Figure 6, $P>0.5$), while the glucose significantly elevated in fish supplemented with 6 and 10 g ORG/kg diet (Figure 6, $P<0.5$).

During exposure period, diazinon induced ranges of histological lesions in liver including: hypertrophy of the hepatocytes (HT), cytoplasm vacuolization (VG), nucleus atrophy (NA) and hepatocyte cloudy swelling (CS) (Figure 7B). The severity of theses lesions was lower in ORG-supplemented fish compared to control group (Figure 8, $P<0.05$). In this regard, the lowest liver lesions were found in groupsfed6 and 10 g ORG/kg diet (Figure 8, $P<0.05$).

Discussion

Today, iIt was is well recognized that PCs reduce the fish growth in aquatic environments (Woltering,

1984; Murthy, Kiran, & Venkateshwarlu, 2013). However, no practical solutions have been suggested to eliminate counteract this problem so far. During last decades, the medicinal herbs (MHs) have been used in aquaculture as a natural substitution for chemicals to improve the immune system, stress-resistance capabilities, growth and reproduction in fish (Galina *et al.* 2009; Reverter *et al.* 2014; Van Hai, 2015; Bulfon *et al.* 2017). Nevertheless, too little attentions have has been paid to possible modifying effects of MHs on toxicity induced by toxicants, particularly PCs.

In the present study, the possible moderating effects of a medicinal plant, oregano, Origanum vulgare were investigated for the first time on toxicity induced by organophosphate pesticide, diazinon in rainbow trout by measuring some components of fish metabolism i.e. serum metabolic hormones, growth parameters and histological alternations alterations in liver (as the main organ for energy metabolism).

Both in non-diazinon and diazinon-exposed groups, the serum thyroid hormones, BWI and SGR were found to be higher in fish supplemented with 2, 6 and 10
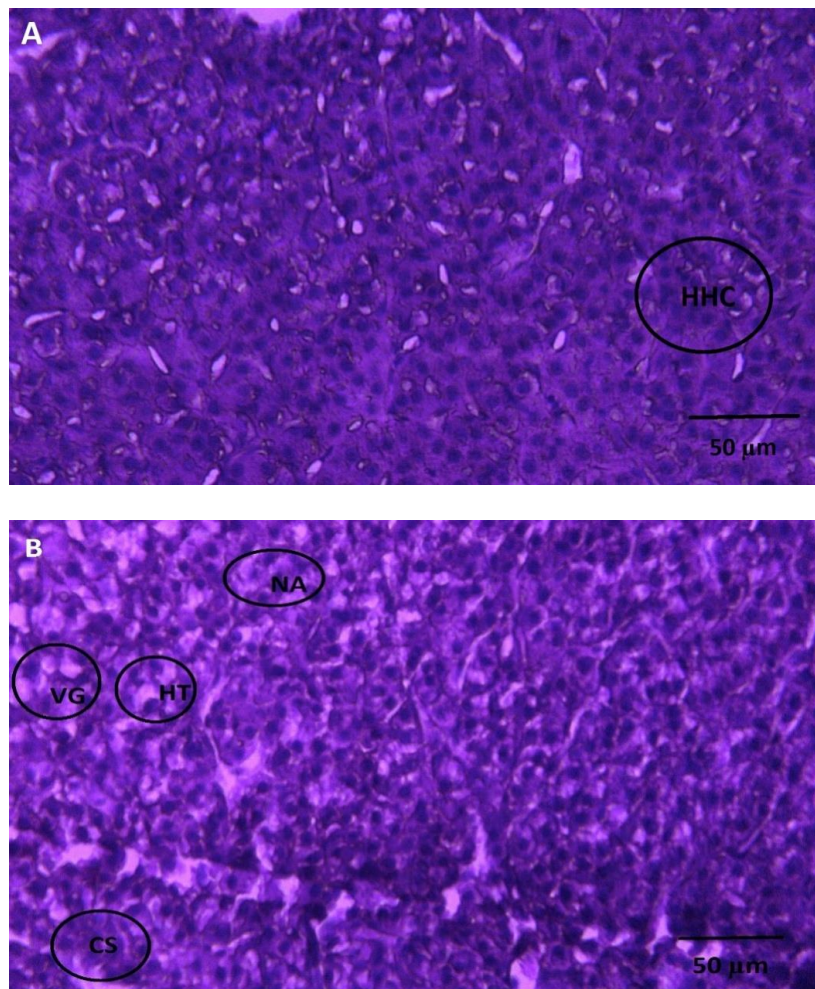
Figure 7. Histopathology of the rainbow trout (*Oncorhynchus mykiss*) liver tissue after 60 days experiment. A) Liver of control fish (non-ORG-supplemented and non-diazinon exposed fish): HHC: hexagonal hepatic cells surrounded with the sinusoidal portal blood (SI); B) Liver of control group exposed to 0.287 mg/L diazinon: hypertrophy of hepatocytes (HT), vacuolization of cell cytoplasm (VG), hepatocyte cloudy swelling (CS), nucleus atrophy (NA). Figures are representatives of pictures taken from all experimental groups (magnification of the sections 400×).

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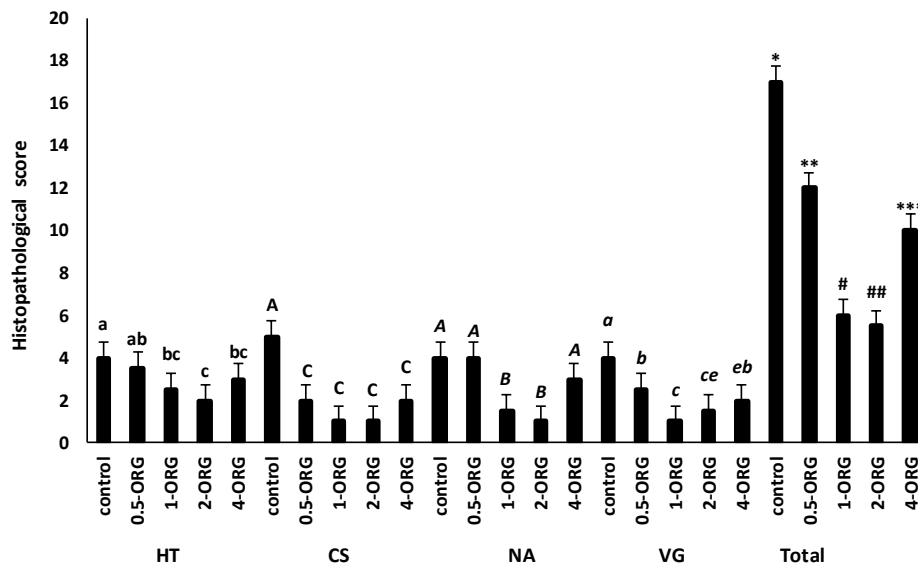
Figure 8. Total and categorical (HT: hypertrophy of the hepatocytes, VG: cytoplasm vacuolization, NA: nucleus atrophy, CS: hepatocyte cloudy swelling) liver histopathological lesions (mean ± S.D.) for rainbow trout exposed to 0.287 mg/L diazinon. Control: non-ORG-supplemented fish, 2-ORG: fish supplemented with 2 g ORG/kg diet, 6-ORG: fish supplemented with 6 g ORG/kg diet, 10-ORG: fish supplemented with 10 g ORG/kg diet, 14-ORG: fish supplemented with 14 g ORG/kg diet, Different alphabet and symbols represents significant differences between experimental groups (nonparametric analysis, using Kruskal–Wallis test and Mann–Whitney U test, $P<0.05$). Columns sharing the same superscript letter or symbol show no significant differences between groups ($P>0.05$). Data are expressed as mean ± S.D.

g ORG/kg diet compared to non-supplemented fish, indicating the growth promoting properties of ORG at optimum dietary levels. In coincidence parallel with our results, the supplementation of Nile tilapia, Oreochromis niloticus and channel catfish, Ictalurus punctatus with oregano improved the growth performance, muscle protein content and feed utilization (Seden, Abbass, & Ahmed, 2009; Zheng *et al.* 2009). AlsoMoreover, the use of oregano essential oil in diet of for rainbow trout enhanced the growth performance (Diler *et al.* 2017). The growth promoting effects of ORG could be related to its aromatic flavour, which makes it a strong appetizer. The feed intake induced by flavour of ORG could eventually improve fish growth (Abdel-Latif & Khalil, 2014). In addition, the growth promoting effects of ORG may also be attributed to some bioactive compounds in the biochemical composition of ORG, which stimulate the secretion of digestive enzymes and following increases in food consumption and absorption (Radhakrishnan, Saravana Bhavan, Seenivasan, Muralisankar, & Shanthi, 2015). In our study, the improved growth observed in ORG-supplemented fish may also be in relationrelated to the levels of thyroid hormones. In fish, thyroid hormones play crucial role in control of growth, development, metamorphosis, reproduction and behavior. The thyroid hormones have been found to enhance growth in salmonids (Fontaine & Baraduc, 1955; Refstie, 1982; Boeuf & Gaignon, 1989; Saunders, Henderson, & Harmon, 1985), probably via affecting appetite or digestion (Fagerlund *et al.* 1980; Refstie, 1982).

Both in non-diazinon and diazinon-exposed groups, the growth parameters and serum levels of thyroid hormones decreased in fish supplemented with the highest dietary ORG inclusion level (14 g/kg) ORG. These results probably suggest a growth-suppressing effect for ORG at high dietary levels. In sea bass (Dicentrarchus labrax), no growth-retarding effects were observed in fish supplemented with carvacrol, a main component of ORG (Volpatti *et al.* 2013).

Although the toxic effects of ORG has not been reported in fish so far, some studies on other vertebrates have suggested growth retarding effects for ORG at high dietary levels (Giannenas, Florou-Paneri, Papazahariadou, Christaki, Botsoglou, & Spais, 2003; Giannenas, Florou-Paneri, Botsoglou, Christaki, & Spais, 2005). In broiler, the growth retarding effects of dietary ORG was attributed to disruption of upper layer of mature enterocytes of the intestinal mucosa by carvacrol and thymol, as the major components of oregano essential oil (Sikkema, de Bont, & Poolman, 1994; Weber & de Bont, 1996; Giannenas *et al.* 2003).

In our study, the exposure to diazinon significantly decreased the fish growth and levels of circulating thyroid hormones, indicating the growth-retarding effects of diazinon. Similar to our results, many studies have reported the suppressing effects of PCs on fish growth (Cook, Paradise, & Lom, 2005; Sweilum, 2006; Hanson, Dodoo, Essumang, Blay, & Yankson, 2007; Baldwin, Spromberg, Collier, & Scholz, 2009) and also the levels of thyroid hormones in blood (Sinha & Singh, 1992; Singh & Canario, 2004; Hajirezaee *et al.* 2016).

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Nevertheless, some studies have also indicated an increases in serum thyroid hormones in response to pesticide toxicity. In malathion-exposed catfish, Heteropneustes fossilis, the serum T3 levels increased due to possible conversion of T4 to T3 or reduction in T3 excretion. The pesticide, Butachlor increased the serum T3 and T4 probably through activating the hypothalamus–pituitary–thyroid (HPT) axis (Chang *et al.* 2013). Considering these results, it seems that pesticide-induced changes of thyroid hormones could be different depending on fish species, pesticide type and concentration and or exposure duration. Among diazinon exposed groups, fish supplemented with 2, 6 and 10 g ORG/kg diet exhibited higher growth and serum levels of thyroid hormones, indicating moderating effects of ORG on toxicity induced by diazinon. The toxicant-moderating effects of ORG on diazinon induced toxicity may be related to antioxidative properties of this plant. The antioxidant effects of ORG are usually attributed to the its high content of phenolic compounds and flavonoids in the chemical composition of the plant (Koldaş, Demirtas, Ozen, Demirci, & Behçet, 2015; Han, Ma, Yang, Yan, Xiong, Shu, Zhao, & Xu, 2017; Hassanzadeh-Kiabi & Negahdari, 2018).

The changes of cortisol and glucose levels in blood are known as main indicators of stress in fish (Mazeaud, Mazeaud, & Donaldson, 1977; Wendelaar Bonga, 1997). In stressful condition, the elevated levels of cortisol activate glycogenolysis in liver to produce free glucose for meeting the energy demands of stress (Mazeaud *et al.* 1977; Wendelaar Bonga, 1997). In diazinon-exposed groups, the levels of cortisol elevated in control and fish supplemented with 2 and 14 g ORG/kg diet, which may be a result of resulting from the oxidative stress induced by diazinon. The elevations increase in serum cortisol of fish have has been well documented in response to oxidative stress induced by PCs (Katuli *et al.* 2014; Ghasemzadeh, Sinaei, & Bolouki, 2015). In fish Fish supplemented withfed 6 and 10 g ORG/kg diet do not exhibited variation in, cortisol levels had no changes after exposure, which may be due to the moderatingmoderating moderate effects of ORG (at optimum dietary levels) on diazinon-induced oxidative stress. In the present study, we observed no changes in serum glucose levels of control and fish supplemented with 2 and 14g ORG/kg diet after 60 days exposure to diazinon, which this result could be attributed toas a result of depletion of liver glycogen stories storage in liver during long-term exposure (De Aguiar, Moraes, Avilez, Altran, & Corrêa, 2004; Gagnon, Jumarie, & Hontela, 2006; Nieves-Puigdoller, Björnsson, & McCormick, 2007; Fırat, Cogun, Yüzereroğlu, Gök, Fırat, Kargin, & Kötemen, 2011). In fish supplemented withfed 6 and 10 g ORG/kg diet, the glucose increased during exposure, indicating the role of ORG (at optimum dietary levels) in moderating the stress and in keeping the liver glycogen stories during long-term exposure.

In the present study, long term exposure to diazinon caused liver tissue damages in non-ORG supplemented fish, as reported in other teleosts exposed to diazinon (Banaee *et al.* 2013; Hajirezaee *et al.* 2016). However, we observed no liver tissue damages in groups supplemented with ORG, suggesting a protective role for ORG against tissue lesions induced by diazinon. This protective role may be related to antioxidative nature of ORG and its abilities in scavenging the ROS produced during oxidative stress induced by diazinon. In fish, wide ranges of synthetic and natural antioxidant have been successfully used to reduce pesticide-induced tissue lesions (Korkmaz, Cengiz, Unlu, Uysal, & Yanar, 2009; Jia, Cao, Xu, Jeney, & Yin, 2012; Shivashri, Rajarajeshwari, & Rajasekar, 2013; Harabawy & Mosleh, 2014).

Conclusion

The findings of the present work suggest a growth promoting effect for ORG at optimum dietary levels (6-12 g/kg diet). In addition, ORG (6-12 g/kg diet) could moderate the growth suppressing effects, stress and liver tissue lesions induced by diazinon. Nevertheless, ORG at high dietary levels (14 g/kg diet) caused toxic effects on fish metabolism.

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