



PROTECTIVE EFFECTS OF DIETARY LAVENDER (*LAVANDULA OFFICINALIS*) ESSENTIAL OIL AGAINST MALATHION-INDUCED TOXICITY IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Abstract

The present study was aimed to evaluate the moderating properties of lavender (*Lavandula officinalis*) essential oil (LEO) against immunotoxic effects of the organophosphate pesticide, malathion in rainbow trout, *Oncorhynchus mykiss*. For this purpose, fish were supplemented with LEO at dietary concentrations of 0.5, 1, 2 and 4 ml/kg diet LEO for 56 days. A non-LEO supplemented group was also considered as control. After 57 days feeding trial, biochemicals were assayed in the blood and kidney tissue and then fish exposed to a sub-lethal concentration of malathion [0.24 mg/l equal to 30% of LC₅₀ (0.8 mg/L)]. After 57 days feeding trial, the serum total immunoglobulin, respiratory burst activity, lysozyme activity and complement activity were significantly elevated in fish treated with 1 and 2 ml LEO/kg diet compared to non-LEO supplemented individuals ($P < 0.01$). Such changes were not observed in non-LEO supplemented fish ($P > 0.01$). Significant elevations were observed in the expression of the immune genes (iNOS and C₃ genes) in fish treated with 0.2–2 mg LEO/kg diet compared to non-supplemented ones ($P < 0.01$). The lysozyme and complement activity significantly decreased in fish fed 4 ml/kg diet ($P < 0.01$). After exposure to malathion, all immune components significantly declined in control and those treated with 0.5, 1 and 4 ml LEO/kg diet ($P < 0.01$). In contrast, the immunity components exhibited no significant changes in fish treated with 2 ml LEO/kg diet after exposure ($P > 0.01$). The expression of iNOS and C₃ genes were significantly reduced in control and fish fed 0.5, 1 and 4 ml LEO/kg diet in response to malathion ($P < 0.05$). Furthermore, the expression of these genes showed no significant changes in fish fed with 2 ml LEO/kg diet after exposure ($P > 0.01$). The findings of the present study suggested an immunoprotective role for dietary LEO at optimized dietary concentrations of 1 and 2 ml LEO/kg diet against oxidative stress and toxicity induced by malathion. Nevertheless, LEO at high dietary concentration (4 ml/kg diet) had reducing effects on the fish immunity.

Key words: *Lavandula officinalis*, immunity, malathion, rainbow trout

Aquatic ecosystems are always influenced by a large spectrum of land-based contaminants (Islam and Tanaka, 2004; Gashi et al., 2020; Mohamed et al., 2020). As main groups of contaminants, pesticides (PCs) usually enter water environment through direct runoff, drift, drainage and leaching (Hedayati et al., 2015; Vymazal and Březinová, 2015; Ghafari Farsani et al., 2016 a, b).

The toxicity of PCs on aquatic organisms, particularly fish has been well documented in many toxicological studies (Khan and Law, 2005; Slaninova et al., 2009; Dawood et al., 2020 a; Khafaga et al., 2020; Farag et al., 2021 a). The oxidative stress imposed by PCs has been observed to disturb fish osmoregulation, reproduction,

growth, and immunity. PCs reduce the reproductive potentials mainly through disrupting endocrine hormones and development of gonads (De Silva and Samayawardhena, 2005; Denslow and Sepúlveda, 2007; Agbohessi et al., 2015; Shahbazi Naserabad et al., 2015; Poorbagher et al., 2018). Also, PCs considerably reduced the osmoregulation capabilities of fish by disrupting the activity of gill Na⁺-K⁺-ATPase, plasma concentrations of thyroid hormones and damaging gill tissue (Rao et al., 2003; Velmurugan et al., 2009; Katuli et al., 2014; Ghasemzadeh et al., 2015; Hajirezaee et al., 2016). There are also some studies, reporting the disturbing effects of PCs on fish immune system (Khafaga et al., 2020; Naiel et al., 2020;

El-Hameed et al., 2021; Ismael et al., 2021). The organophosphate pesticide, chlorpyrifos decreased the serum immunoglobulin (IgM) content and lysozyme activity in the serum, hepatopancreas, and spleen of common carp, *Cyprinus carpio* (Li et al., 2013). The serum IgM showed significant decreases in Nile tilapia, *Oreochromis niloticus* exposed to malathion, however, the lysozyme activity was elevated after exposure (Díaz-Resendiz and Girón-Pérez, 2014). In Nile tilapia, the serum bactericidal and lysozyme activity significantly decreased after exposure to chlorpyrifos (Zahran et al., 2018). Khoshbavar-Rostami et al. (2006) have reported decreased levels of white blood cells and liver and kidney lysozyme activity after long-term exposure to malathion in great sturgeon, *Huso huso*. Furthermore, serum complement activity, lysozyme activity, globulin and total protein decreased in rainbow trout, *Oncorhynchus mykiss* exposed to malathion (Ahmadi et al., 2014). The organophosphate pesticide, glyphosate significantly reduced the phagocytosis, lysozyme, and peroxidase activities in the silver catfish, *Rhamdia quelen* (Kreutz et al., 2011). Also, simultaneous use of diuron atrazine, isoproturon and simazine decreased the serum lysozyme activity in goldfish, *Carassius auratus* (Fatima et al., 2007).

Today, the rapid growth of agricultural activities has inevitably led to increasing use of PCs around the world. However, finding solutions to reduce or eliminate the adverse effects of PCs on aquatic life is essential. Pesticides enter water bodies mainly through runoff, drainage of agricultural fields, erosion of soil, spray drift during application, transportation by dust particles and volatilisation (Kookana et al., 2005). Use of medicinal herbs has a long history from ethnobotanical point of view (Othman et al., 2019; Khan et al., 2020 a, b; Olutayo and Friday, 2020; Pinmongkhonkul et al., 2021). Medicinal herbs (MHs) and their derivatives have been recognized to enhance the fish immune system against a wide spectrum of diseases (Düğenci et al., 2003; Reverter et al., 2014; Halim et al., 2019; Ridzuan et al., 2019; Abdel-Latif et al., 2021 a, b; Ibrahim et al., 2021; Raza et al., 2021, 2022; Ghafarifarsani et al., 2022). The antioxidants, antistress and antimicrobial properties of MHs have been well known in fish (Prasad and Variyur Padhyoy, 1993; Citarasu et al., 2002; Sivaram and Wu, 2004; Chakraborty and Hancz, 2011; Al-Shawi et al., 2017; Roslan and Shariff, 2019; Wulandari et al., 2021).

Despite the confirmed role of MHs in enhancing immunity and treating the diseases in fish, we have too little knowledge about the role of MHEs in moderating the toxicity of PCs; (Banaee et al., 2015; Rabie et al., 2016). Hajirezaee et al. (2019 a, b) reported the ameliorating effects of *Ginkgo biloba* extract on toxicity induced by diazinon in rainbow trout. In same species, oregano extract (*Origanum vulgare*) ameliorated the toxic effects of diazinon (Rafieepour et al., 2019 a, b). In Nile tilapia, use of thyme (*Thymus vulgaris*) powder in diet reduced the impaired immunity induced by insecticide, lambda-cyhalothrin (Khalil et al., 2020).

Apart from fish, the protective properties of MHs have been demonstrated in other vertebrates, where reactive oxygen species (ROS) resulting from oxidative stress were scavenged by MHs (Abid-Essefi et al., 2012; El-Boshy et al., 2017; Dawood et al., 2020 b; Farag et al., 2021 b).

As a medicinal herb, lavender (*Lavandula officinalis*) acts as a strong antioxidant (Gülçin et al., 2004), protects liver against oxidative stress (Selmi et al., 2015) and has anti-inflammatory properties (Cardia et al., 2018). The cineole and linalool in the biochemical composition of lavender extract are responsible for antioxidant, anti-inflammatory, and anti-stress effects (Peana et al., 2002; Ciftci et al., 2011; Juergens, 2014; Mirghaed et al., 2018; Yousefi et al., 2018). There are very few studies about the effects of lavender on the immune system of fish (Fazio et al., 2017). In addition, based on literature, we found no study on the protective effect of lavender on the toxicity induced by pesticides. The present study was aimed to evaluate the effects of dietary hydro-alcoholic extract of lavender in moderating the malathion-induced-oxidative stress in rainbow trout by assaying immune indices in the blood. The findings of this paper suggest a natural and efficient way to moderate the toxic effects of PCs.

Material and methods

Essential oil and food preparation

The lavender essential oil (LEO) was provided from Barij Essence Pharmaceutical CO. with 100% purity for food preparation. Essential oil at dietary levels of 0.5, 1, 2 and 4 ml/kg diet was sprayed evenly on the surface of a rainbow trout basal diet (total protein: 40%, crude lipid: 12%, Ash: 7%, Faradaneh Co., Iran). To prevent leaching of LEO in water, food prepared was covered by a layer of bovine gelatin (Ramsden et al., 2009). Finally, the prepared diets were stored in refrigerator (4°C) until usage.

Experimental design and exposure

Rainbow trout (*Oncorhynchus mykiss*) juveniles (n=720, total weight: 18.5 ± 2.1 g; and mean total length: 10.7 ± 0.9 cm) were purchased and transferred into twenty-four 300 L experimental tanks (30 fish per tank) containing aerated and disinfected freshwater. After 17-day acclimation period, fish were fed with different dietary concentrations of HEL including: 0.5, 1.0, 2.0 and 4.0 ml LEO/kg diet. One non-LEO supplemented group was considered as control. After 57 days feeding trial, blood was sampled and kidney specimens were taken for further analysis and remainders exposed to a sub-lethal concentration (0.24 mg/L equal to 30% of LC_{50}) of malathion (60% purity, Nambar Co., Iran) for 96 h. The 96 h LC_{50} (0.80 mg/L) was measured over an acute toxicity test. In acute toxicity test, fish were exposed to concentrations of 0, 0.2, 0.4, 0.6, 0.8, 1, 1.2 mg/l malathion over 96 h and the mortality percent was recorded. Finally, the LC_{50} value was estimated by probit analysis (Raymond,

1985). All stages of sampling and manipulation of fish have been performed in accordance with ethical standards approved by the Committee of Ethics of the Faculty of Sciences of the University of Tehran (357; 8 November 2000).

Throughout the experimental period, feeding was done at a rate of 3% of body weight. Furthermore, a sample of fish was taken every 10 days to adjust feeding rate based on the fish total biomass data. Over the course of the experiment, the water was daily replaced, and malathion dosing was renewed accordingly. Furthermore, the water quality parameters were at optimum ranges throughout the experiment, as follows: pH was: 6.7 ± 0.1 , temperature: $15.3 \pm 0.8^\circ\text{C}$, ammonia: 0.017 ± 0.001 mg/L and dissolved oxygen: 7.25 ± 0.11 mg/L. Also, the mortality percentage was calculated with counting dead fish.

Blood and liver sampling

The samples of blood (by cutting the caudal peduncle) and kidney tissues (by dissecting out the fish) were immediately collected after anesthetization of fish with clove extract (20 mg/l) (Wagner et al., 2002) in two times including at the end of feeding trial and after 96 h treatment with malathion. Totally, 10 fish were allocated for sampling operation. After 8 min centrifuging at 13,000 g (Hajirezaee et al., 2021), the separated plasma was kept in the refrigerator till biochemical assays. Also, kidney tissues were sampled after dissecting out the fish. Furthermore, the kidney tissues were also stored in liquid nitrogen tank till evaluation of gene expression.

Immunological and gene expression assays

All immune components were determined using commercial assay kits provided from Sigma-Aldrich Co. (USA) according to the assay procedures described. The assay procedures have been previously described by Hajirezaee et al. (2019 b) for ACH₅₀ (by haemolysis of rabbit red blood cells), IgM (by precipitating the immunoglobulin content by 12% polyethylene glycol solution), lysozyme (incubation of *Micrococcus lysodeikticus* suspension with a lysozyme substrate and estimating the decreases in optical density), peroxidase (by reacting tetramethylbenzidine hydrochloride and H₂O₂ and colorimetrically estimating the absorbance).

Before gene expression assay, the method of Awad et al. (2011) was used for the extraction of the RNA content from the kidney tissues (50 mg). After evaluating the quantity (spectrophotometrically at 260/280 nm) and quality (by electrophoresis on a 1.5% agarose gel) of the extracted RNA, 1 µg of RNA was allocated for the synthesis of cDNA [by previously designed qPCR primers (Table 1)] and then the expressions estimated by an iCycler (BioRad), as described in detail by Hajirezaee and Khanjani (2021).

Statistical analysis

Data (means + SD) of this paper was analyzed by SPSS software. An analysis of variance (ANOVA) was done after normality evaluation by Kolmogorov–Smirnov (K–S). One-way ANOVA was used to compare the groups after feeding trial. In addition, to compare the means after exposure to malathion, two-way ANOVA was applied, followed by Tukey's multi comparison test to identify the differences ($P < 0.05$) between the experimental groups. In addition, independent-sample-t-test was used to compare the means of each group before and after exposure to malathion.

Results

LEO-supplemented fish

After 57 days feeding trial, the serum total immunoglobulin (Figure 1), respiratory burst activity (RBA) (Figure 2), lysozyme activity (Figure 3) and complement activity (Figure 4) significantly increased in fish fed 1 and 2 ml LEO/kg diet compared to non-LEO supplemented ones ($P < 0.05$). The activity of lysozyme (Figure 3) and complement significantly (Figure 4) decreased in fish fed diet containing 4 ml LEO/kg diet compared to control ($P < 0.05$). The peroxidase activity (Figure 5) did not show significant changes in all experimental groups ($P > 0.05$). Significant elevations were observed in the expression of the immune genes, iNOS (Figure 6) and C₃ (Figure 7) in fish fed with 0.5–2 ml LEO/kg diet compared to non-supplemented ones and those treated with 4 ml LEO/kg diet ($P < 0.05$). There were no significant differences in mortality rate between the groups during feeding trial (Table 2, $P > 0.05$).

Table 1. Sequences of oligonucleotide primers used for real-time PCR

Genes	Primers	Primer sequences	Amplification size (bp)	ID number
Nitric oxide synthase (iNOS)	iNOS Forward	CGAATGGAGCTATCGTCAGACC	602	100136347
	iNOS Reverse	CGGGAACGTTGTGGTCATAATACC		
Complement	C3 Forward	AGCTTGCTGACTGGCTTTGT	152	100136951
	C3 Reverse	TCATAACGGTGACCCCAAC		

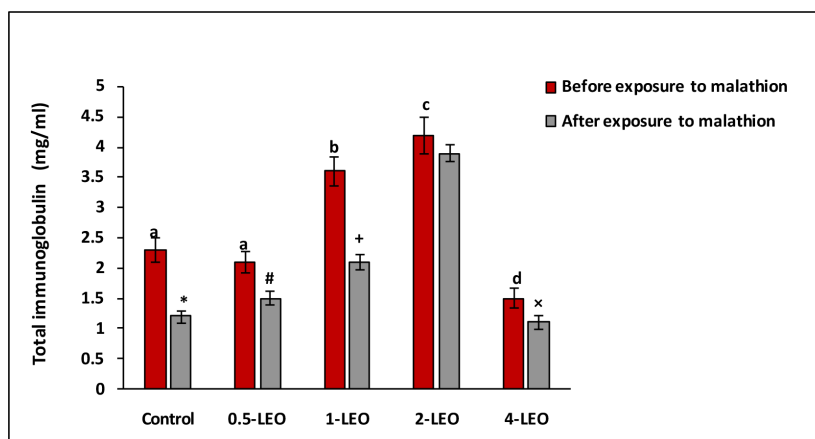


Figure 1. Effects of different dietary levels of lavender (*Lavandula officinalis*) essential oil (LEO) on plasma total immunoglobulin. Control: non-LEO-supplemented fish, 0.5-LEO: fish supplemented with 0.5 ml LEO/kg diet, 1-LEO: fish supplemented with 1 ml LEO/kg diet, 2-LEO: fish supplemented with 2 ml LEO/kg diet, 4-LEO: fish supplemented with 4 ml LEO/kg diet. Data (mean $\pm$ S.D) with the different superscript letters indicate significant differences between groups during feeding trial ($P < 0.05$). The symbols indicate the significant differences for each group before and after exposure to malathion ($P < 0.05$)

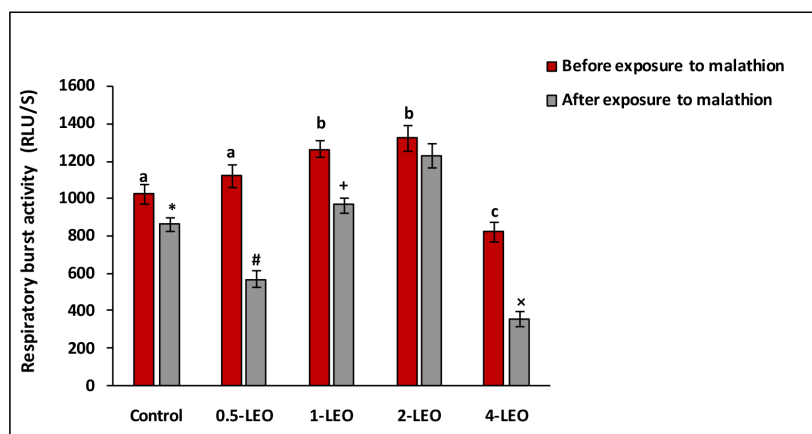


Figure 2. Effects of different dietary levels of lavender (*Lavandula officinalis*) essential oil (LEO) on plasma respiratory burst activity. Control: non-LEO-supplemented fish, 0.5-LEO: fish supplemented with 0.5 ml LEO/kg diet, 1-LEO: fish supplemented with 1 ml LEO/kg diet, 2-LEO: fish supplemented with 2 ml LEO/kg diet, 4-LEO: fish supplemented with 4 ml LEO/kg diet. Data (mean $\pm$ S.D) with the different superscript letters indicate significant differences between groups during feeding trial ($P < 0.05$). The symbols indicate the significant differences for each group before and after exposure to malathion ($P < 0.05$)

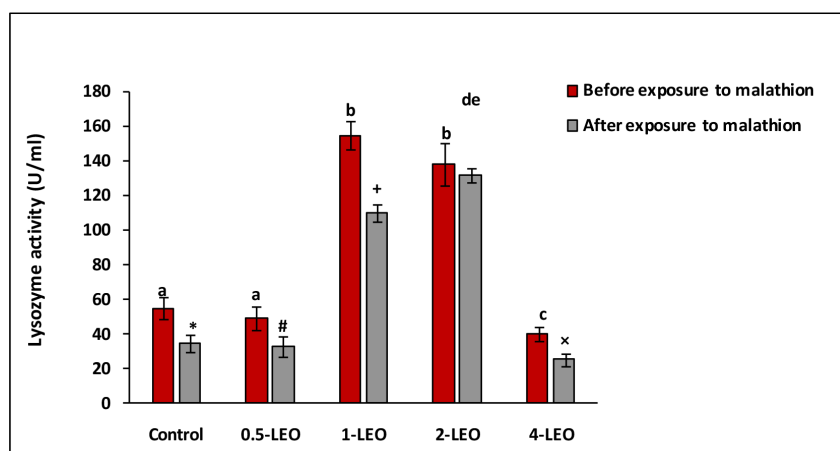


Figure 3. Effects of different dietary levels of lavender (*Lavandula officinalis*) essential oil (LEO) on plasma lysozyme activity. Control: non-LEO-supplemented fish, 0.5-LEO: fish supplemented with 0.5 ml LEO/kg diet, 1-LEO: fish supplemented with 1 ml LEO/kg diet, 2-LEO: fish supplemented with 2 ml LEO/kg diet, 4-LEO: fish supplemented with 4 ml LEO/kg diet. Data (mean $\pm$ S.D) with the different superscript letters indicate significant differences between groups during feeding trial ($P < 0.05$). The symbols indicate the significant differences for each group before and after exposure to malathion ($P < 0.05$)

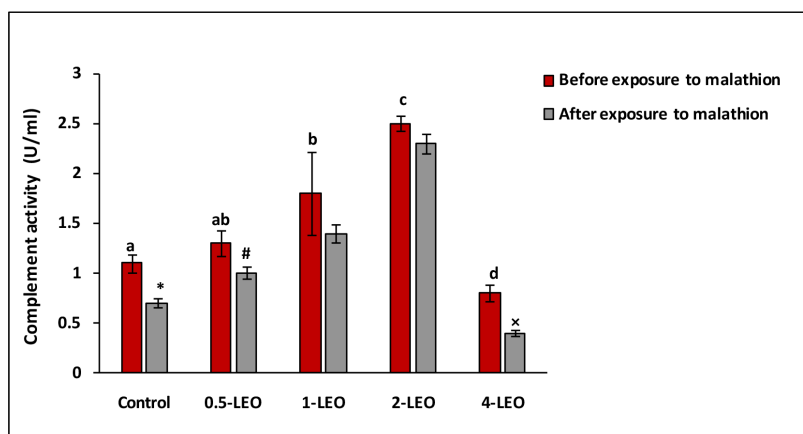


Figure 4. Effects of different dietary levels of lavender (*Lavandula officinalis*) essential oil (LEO) on plasma complement activity. Control: non-LEO-supplemented fish, 0.5-LEO: fish supplemented with 0.5 ml LEO/kg diet, 1-LEO: fish supplemented with 1 ml LEO/kg diet, 2-LEO: fish supplemented with 2 ml LEO/kg diet, 4-LEO: fish supplemented with 4 ml LEO/kg diet. Data (mean $\pm$ S.D) with the different superscript letters indicate significant differences between groups during feeding trial ($P < 0.05$). The symbols indicate the significant differences for each group before and after exposure to malathion ($P < 0.05$)

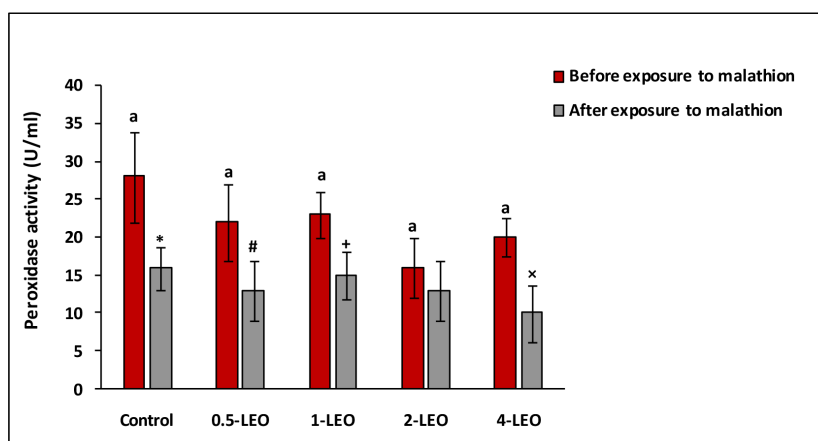


Figure 5. Effects of different dietary levels of lavender (*Lavandula officinalis*) essential oil (LEO) on plasma peroxidase activity. Control: non-LEO-supplemented fish, 0.5-LEO: fish supplemented with 0.5 ml LEO/kg diet, 1-LEO: fish supplemented with 1 ml LEO/kg diet, 2-LEO: fish supplemented with 2 ml LEO/kg diet, 4-LEO: fish supplemented with 4 ml LEO/kg diet. Data (mean $\pm$ S.D) with the different superscript letters indicate significant differences between groups during feeding trial ($P < 0.05$). The symbols indicate the significant differences for each group before and after exposure to malathion ($P < 0.05$)

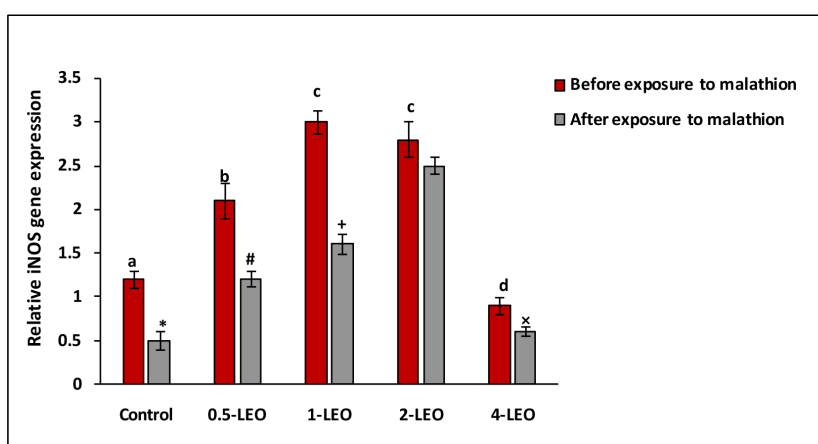


Figure 6. Effects of different dietary levels of lavender (*Lavandula officinalis*) essential oil (LEO) on expression of iNOS (Nitric oxide synthase) gene in kidney. Control: non-LEO-supplemented fish, 0.5-LEO: fish supplemented with 0.5 ml LEO/kg diet, 1-LEO: fish supplemented with 1 ml LEO/kg diet, 2-LEO: fish supplemented with 2 ml LEO/kg diet, 4-LEO: fish supplemented with 4 ml LEO/kg diet. Data (mean $\pm$ S.D) with the different superscript letters indicate significant differences between groups during feeding trial ($P < 0.05$). The symbols indicate the significant differences for each group before and after exposure to malathion ($P < 0.05$)

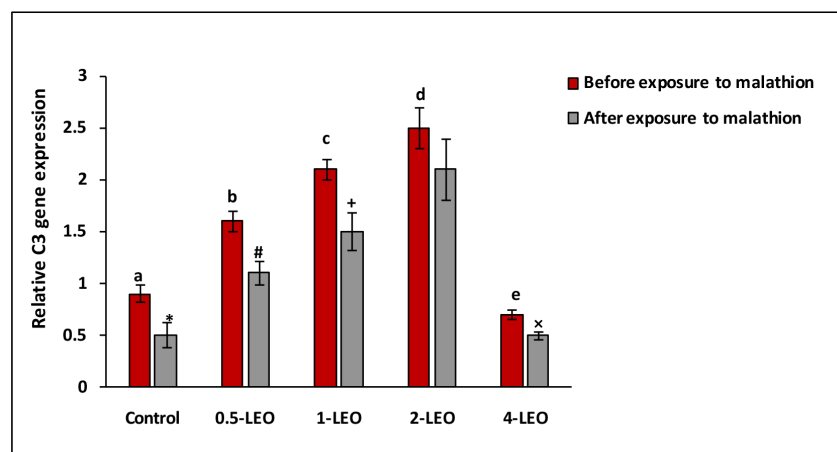


Figure 7. Effects of different dietary levels of lavender (*Lavandula officinalis*) essential oil (LEO) on expression of C_3 (complement gene) gene in kidney. Control: non-LEO-supplemented fish, 0.5-LEO: fish supplemented with 0.5 ml LEO/kg diet, 1-LEO: fish supplemented with 1 ml LEO/kg diet, 2-LEO: fish supplemented with 2 ml LEO/kg diet, 4-LEO: fish supplemented with 4 ml LEO/kg diet. Data (mean $\pm$ S.D) with the different superscript letters indicate significant differences between groups during feeding trial ($P < 0.05$). The symbols indicate the significant differences for each group before and after exposure to malathion ($P < 0.05$).

Table 2. Effects of different dietary levels of lavender (*Lavandula officinalis*) essential oil (LEO) and exposure to malathion on fish mortality. Data (mean $\pm$ S.D) with the lower-case different letters indicate significant differences between groups in LEO supplemented fish after exposure to malathion ($P < 0.05$). For each group, the differences in mortality rate before and after exposure are presented as different symbols ($P < 0.05$).

	Mortality rate (%)	
	before exposure to malathion	after exposure to malathion
Control (non-supplemented fish)	1 $\pm$ 0.5*	16 $\pm$ 3.1 a **
0.5 ml LEO/kg diet	1.5 $\pm$ 0.7 #	9 $\pm$ 2.2 a ##
1 ml LEO/kg diet	2.1 $\pm$ 1	1.6 $\pm$ 0.7 b
2 ml LEO/kg diet	2.2 $\pm$ 1.2	2.5 $\pm$ 1.4 ab
4 ml LEO/kg diet	1.9 $\pm$ 1 †	11.5 $\pm$ 1.8 a ††

Malathion-exposed fish

After exposure to malathion, all immune parameters significantly declined in control and fish treated with 0.5, 1 and 4 ml LEO/kg diet (Figure 1–5, $P < 0.05$). In contrast, the immunity components remained unchanged in fish treated with 2 ml LEO/kg diet after exposure (Figure 1–5, $P > 0.05$). The expression of iNOS (Figure 6) and C_3 (Figure 7) genes was significantly reduced in control and fish fed 0.5, 1 and 4 ml LEO/kg diet in response to malathion ($P < 0.05$). Furthermore, the expression of these genes showed no significant changes in fish fed with 2 ml LEO/kg diet after exposure (Figure 6–7, $P > 0.05$). Fish mortality (%) significantly increased in control and those treated with 0.5 and 4 ml LEO/kg diet after exposure to malathion (Table 2, $P < 0.05$).

Discussion

Today, PCs have been well known to adversely affect all biological properties of life in aquatic environments. In this regard, it was clearly recognized that PCs

reduce fish immunity, which makes fish susceptible to diseases and various environmental stressors (Mondon et al., 2000). As an alternative to chemicals, use of medicinal herbs and their derivatives has increased to improve fish immunity against a wide spectrum of diseases and stressors in fish farms. Although there are several studies reporting the immunostimulating role of plant-based supplements in fish, too little data is available about their immunogenic effects against oxidative stress induced by toxicants, particularly PCs. The present study evaluated the protective role of the medicinal plant, lavender (*Lavandula officinalis*) essential oil (LEO) against oxidative stress induced by malathion in rainbow trout through assessing some immunological components and the expression of the immune genes.

In LEO-supplemented fish, the serum total immunoglobulin, respiratory burst activity, lysozyme activity and complement activity were significantly raised in fish fed 1 and 2 ml LEO/kg diet and expression of the immune genes (iNOS and C_3 genes) in fish of 0.2–2 ml LEO/kg diet treatments compared to non-supplemented individuals, indicating the immunostimulatory properties of LEO

in rainbow trout. Complement system includes a complex of proteins capable to destroy bacteria through opsonization of pathogens for phagocytosis and disruption of bacterial cell wall (Rooijackers and van Strijp, 2007). Lysozymes include a group of antibacterial enzymes that are produced by various types of leukocytes including neutrophils and macrophages. Lysozymes destruct the cell wall of a wide range of bacteria including Gram-positive and Gram-negative ones (Chipman and Sharon, 1969; Saurabh and Sahoo, 2008). Immunoglobulins (IGs) are also heterodimeric glycoproteins that have been found to have the main role in humoral innate and specific immunity in fish (Mashoof and Criscitiello, 2016). The immunogenic functions of IGs are mainly exerted through opsonization of pathogens for neutralization of toxins and pathogens, phagocytosis, and activation of the complement reactions (Mashoof and Criscitiello, 2016). RBA is known as an indicator of phagocytic activity of phagocytes and thus composes one of the crucial parts of non-specific immunity in fish (Secombes and Fletcher, 1992; Bols et al., 2001; Uribe et al., 2011).

Similar results were observed in the study of Yousefi et al. (2020), where the administration of common carp with lavender significantly increased total immunoglobulin, blood leukocytes, and plasma complement and lysozyme activity in the fish. The ACH₅₀, lysozyme activity, IgM and total Ig were significantly increased in rainbow trout fed with 200 ml/kg lavender essential oil nanoemulsion compared to non-supplemented individuals (Hassanalizadeh et al., 2020). Based on our results, it seems that LEO at high dietary level have suppressive effects on immunity components of rainbow trout, since lysozyme and complement activity were reduced in fish fed 4 ml/kg diet. This finding could be resulting from the existence of some immunotoxic compounds in the biochemical composition of lavender (El-Mehiry and Shalbaya, 2019). After exposure to malathion, the expression of iNOS and C₃ genes and all immune components decreased in non-LEO-supplemented fish and those fed diet containing 0.5, 1 and 4 ml LEO/kg diet, indicating the immunotoxic effects of malathion on the immune system of the fish. In agreement with our findings, there are many studies reporting the immunotoxic effects of PCs in fish. In common carp, *Cyprinus carpio*, the decreased levels of immunoglobulins were related to the toxic effects of bifenthrin and malathion on the hepatocytes, inhibiting the protein and immunoglobulin synthesis (Banaee et al., 2008; Velisek et al., 2009). The malathion-induced decreases in serum immunoglobulin have been also observed in other fish species (Khoshbavar-Rostami et al., 2006; Girón-Pérez et al., 2007; Banaee et al., 2011). In a study by Ahmadi et al. (2014), malathion caused considerable declines in lysozyme activity, ACH₅₀ and total immunoglobulin in rainbow trout. In zebra fish, the serum levels of lysozyme were reduced in response to permethrin (Nayak et al., 2007). Similar results were also found in rainbow trout exposed to other pesticides (Shelley et al., 2009; Siwicki et al., 2010; Tukmechi et

al., 2014). In silver catfish (*Rhamdia quelen*) and tilapia, the decreases in serum lysozyme activity were related to the disrupting effects of glyphosate on protein synthesis and on cells responsible for producing lysozyme (El-Gendy et al., 1998; Kreutz et al., 2011). In common carp, the pesticide glyphosate depressed the immunity by disrupting immune-related gene expression and decreasing lysozyme, ACH₅₀ and IgM levels in liver and spleen (Ma and Li, 2015). In silver catfish, the immunotoxic effects of glyphosate on ACH₅₀ were also attributed to the inhibitory role of this pesticide on production of complement proteins in liver or tissues responsible for synthesis of the complement (Kreutz et al., 2011).

In this study, the expression of the immune genes and all immune components remained unchanged in fish treated with 2 ml LEO/kg diet after exposure, suggesting an immunoprotective function for LEO at optimized dietary concentrations against malathion-induced oxidative stress. As a natural antioxidant, LEO probably is involved in scavenging free radicals produced during malathion-induced oxidative stress. The antioxidative properties of LEO may be attributed to high levels of cineole and linalool in the biochemical composition of lavender (Peana et al., 2002; Ciftci et al., 2011; Juergens, 2014; Mirghaed et al., 2018; Yousefi et al., 2018). In conclusion, the findings of this study suggested an ameliorating effect for LEO at optimum dietary levels (1 and 2 ml LEO/kg diet) against immunotoxicity induced by malathion. Nevertheless, LEO at high dietary concentration (4 ml LEO/kg diet) reduced the fish immunity.

Ethical approval

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

Compliance with ethical standards

The authors declare that they have no competing interests.

Author's contributions

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

Conflict of interest

All authors have no conflict of interest

Acknowledgments

The authors would like to thank all those who contributed to this research.

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Received: 12 X 2021

Accepted: 30 XII 2021