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POTENTIAL PROTECTIVE EFFECTS OF THYME (*THYMUS VULGARIS*) ESSENTIAL OIL ON GROWTH, HEMATOLOGY, IMMUNE RESPONSES, AND ANTIOXIDANT STATUS OF *ONCORHYNCHUS MYKISS* EXPOSED TO MALATHION

Saade Abdalkareem Jasim[1♦], Reza Davoodi[2], Ghulam Yasin[3], Rustem Adamovich Shichiyakh[4], Ola Kamal A. Alkadir[5], Ahmed Taifi[6], Yasser Fakri Mustafa[7], Safoura Abarghouei[8]

[1]Medical Laboratory Techniques Department, Al-Maarif University College, Iraq
[2]Fisheries and Aquaculture Department, Agricultural and Natural Resources College, Persian Gulf University, Bushehr, Iran
[3]Assistant Professor, Department of Botany, Bahauddin Zakariya University, Multan, Pakistan
[4]Kuban State Agrarian University named after I.T. Trubilin, Krasnodar, Russia
[5]Al-Nisour University College, Baghdad, Iraq
[6]Al-Manara College for Medical Sciences, Iraq
[7]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Mosul-41001, Iraq
[8]Baharavaran Nastaran Agricultural Applied Scientific Training Center, Applied Scientific University, Qom, Iran
♦Corresponding author: saade.a.j@uoa.edu.iq

Abstract

As an abundant source of antioxidants and diet flavor enhancers, the plant essential oils can have positive effects on fish growth, and resistance against environmental stressors. In this study, garden thyme (*Thymus vulgaris*) essential oil (TEO) was used in the diet of rainbow trout, *Oncorhynchus mykiss*, to evaluate its protective effect against malathion pesticide exposure. Tested fish (19.99±0.01 g) were divided into six groups (three replicates), namely: T1: control diet; T2: control diet + 0.025 mg L^{-1} malathion; T3: control diet + 0.075 mg L^{-1} malathion; T4: control diet + 1% TEO; T5: control diet + 0.025 mg L^{-1} malathion + 1% TEO and T6: control diet + 0.075 mg L^{-1} malathion + 1% TEO. After 21 days, T4 fish had the highest final body weight (FW), weight gain (WG), specific growth rate (SGR), and the lowest feed conversion ratio (FCR) among experimental treatments (P<0.05). The blood parameters including the red blood cells (RBC), white blood cell count (WBC), hematocrit (Hct), and hemoglobin (Hb) values were the highest in T4 treatment, displaying a significant difference with T1 treatment (P<0.05). Fish in the T4 groups had the highest total protein (TP) and albumin (ALB), while fish of T3 showed the lowest levels of these parameters (P<0.05) and also had the highest level of triglycerides (TRG), cholesterol (CHOL), lactate dehydrogenase (LDH), and urea (Ur). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) enzymes recorded the lowest levels in T4 treatment, which showed a significant difference with T1 group. The catalase (CAT) and superoxide dismutase (SOD) showed the highest activities in T4 treatment, while the lowest SOD and the highest malondialdehyde (MDA) levels occurred in T3 group (P<0.05). Total immunoglobulin (total Ig) level, alternative complement (ACH_{50}) and lysozyme in the serum and skin mucus of T4 treatment of rainbow trout showed the highest activities with a significant difference from groups (P<0.05). From the results of the present study, it can be concluded that 1% of *T. vulgaris* as a supplement to the diet of rainbow trout can stimulate and improve the immune system of the fish. TEO can have a protective effect against unfavorable effects of malathion and improves the growth of the fish.

Key words: organophosphate, plant medicine, thyme, welfare, antioxidant, fish

The rapid growth of human population leads to more demands for food and consequently impels the human race towards the increase of agricultural production. To increase production and income and to overcome the vermin that cause damage to crops, farmers use pesticides which can be in the form of insecticides or herbicides. When used in excess, organophosphorus pesticides can enter the environment and harm non-target organisms including humans (Hedayati et al., 2015; Kadiru et al., 2022; Santana et al., 2022). Also, these types of residues may enter the aquatic ecosystems by surface runoff polluting the aquatic environment. Ali et al. (2021) indicated that the aquatic environment is threatened by the excess use of synthetic pesticides.

Among different classes of pesticides, organophosphorus insecticides account for a large share in developing countries (Kumar et al., 2010; Fu et al., 2022). Although organophosphorus pesticides have a low toxicity level for mammals and rapidly biodegrade in the environment, they can be highly toxic to fish and other aquatic organisms (Singh et al., 2009; Kumar et al., 2021). Malathion is an organophosphorus pesticide that is widely used for insect control and agricultural purposes (Shayeghi et al., 2001; Shahbazi Naserabad et al., 2015; de Souza et al., 2021; Vasseghian et al., 2022). Malathion enters the surface waters and accumulates in the aquatic animals (Gao et al., 2009). The bioconcentration factor values for some fish species have been reported (Howard, 1991; Deka

and Mahanta, 2016). Upon entering an organism's body, malathion binds to acetylcholinesterase enzyme (AChE) at the end of the nerve system, thus over-stimulating the nervous system (Bilal et al., 2022). Reduction of antioxidant defense capacity due to oxidative stress and increase in lipid peroxidation (Abdel-Salam et al., 2016) are other proposed mechanisms for malathion toxicity. Several studies have reported the adverse effects of malathion on fish (Yonar et al., 2014; Shahbazi Naserabad et al., 2015; Bharti and Rasool, 2021; de Souza et al., 2021). A decrease in antioxidant defense mechanism due to malathion toxicity has also been reported for some fish species (Patil and David, 2013; Topal et al., 2015; de Souza et al., 2020).

Plants contain phenolic compounds as secondary metabolites with antioxidant power which help in omitting the free radicals (Huang, 2010; Mohammadi et al., 2020; Rudiansyah et al., 2022; Yousefi et al., 2022). Accomplished investigations indicate the ability of plant supplementary and their derivatives to reduce the negative effects of contaminants on fish (Al-Shawi et al., 2022; Dawood et al., 2020). In fact, these investigations show the potential of plant essential oil and extracts to ameliorate the biochemical changes in the blood and liver as well as antioxidant and immune of the fish which are exposed to pollutants and contaminations (Hoseini et al., 2018; Mirghaed et al., 2019; Fazelan et al., 2020; Hoseini et al., 2020; Rudiansyah et al., 2022). Therefore, it is important to use the natural antioxidants as nutritional supplements to encounter the effect of stresses on fish (Dawood et al., 2020; Omidifar et al., 2021).

Thymus vulgaris is a species of the mint family with strong antioxidant properties (Grigore, 2010; Aldosary et al., 2021; Yousefi et al., 2021) and phenolic compounds including carvacrol and thymol (Bagamboula et al., 2004; Parsaei et al., 2016; Ghafarifarsani et al., 2021 a, 2022 a, b, c). Rehman et al. (2006) suggested that any interruption in oxidant/antioxidant balance negatively affects an animal's health status.

Therefore, according to the stated information, the importance of rainbow trout all over the world on one hand, and as a source of energy and food, and extended uses of organophosphorus poisons, especially malathion, in the field of agriculture and its development, and the confluence of rivers – the place for nurturing rainbow trout in the vicinity of gardens and fields. Other results of previous studies showed the damage of toxins, especially malathion poison, to aquatic organisms of fishes and mortality, damage and economic losses caused by it. It was necessary to seek to reduce the stress caused by the presence and exposure of fish to this poison, and now, considering the characteristic features of garden thyme, this path was chosen for this study.

Therefore, we evaluated the influence of antioxidant efficiency of garden thyme (*Thymus vulgaris*) essential oil (TEO) as a potential protection measurement against malathion pesticide toxicity in rainbow trout (*Oncorhynchus mykiss*).

Material and methods

Experimental fish and design

Prior to the experiment, 450 rainbow trout juveniles were acclimatized to the experimental conditions for two weeks. During this period, water was constantly aerated and fish were fed twice a day with a basal feed from Faradaneh Co., Shahrekord, Iran (Table 1). The behavior and swimming pattern of the fish were checked daily. No mortality and disease were seen during the acclimatization period. After the acclimation period and initial biometry, 360 fish (average weight of 19.99±0.01 g; Mean ± SE) were distributed between five treatments and a control (all in triplicate) in a manner that there was no significant difference between the biomass of each tank. Twenty fish were put into each fiberglass tank (150 L) containing 120 L of freshwater.

Table 1. Biochemical composition of the basal feed

Ingredients	Percentage
Crude protein	41
Crude lipid	12
Crude fiber	3
Ash	9
Moisture	7
Phosphorus	1.25

Table 2. Chemical specifications of essential oil of *Thymus vulgaris* (Maleki Commercial Co., Fars, Iran)

Compound name	Percentage
Thymol	37–55
Carvacrol	0.5–5.5
p-Cymene	14–28
γ-Terpinene	4–12
Linalol	1.5–6.5
β-Myrcene	1–3
α-Terpinene	0.9–2.6

Treatments included: T1: control or basal diet, T2: control diet + 0.025 mg L^{-1} malathion, T3: control diet + 0.075 mg L^{-1} malathion, T4: control diet + 1% TEO, T5: control diet + 0.025 mg L^{-1} malathion + 1% TEO, T6: control diet + 0.075 mg L^{-1} malathion + 1% TEO.

TEO was purchased from Maleki Commercial Co., Fars, Iran (Table 2). To prepare the experimental diets, the basal feed was supplemented with 1% TEO sprayed over an appropriate weight of the feed. No essential oil was added to the control diet. All the feeds were then coated with 1% bovine gelatin solution, air-dried at room temperature, and stored at 4°C.

Malathion (57% EC) was purchased from an agricultural store. Two sub-lethal doses of malathion (0.025 and 0.075 mg L^{-1}), selected based on the previous studies on rainbow trout (Ghafari Farsani et al., 2016; Poorbagher et al., 2018), were added to the water of the intended treatments.

During the experiment, physico-chemical parameters of water including temperature (16±1.2°C) (by a thermometer, ZEAL, UK), dissolved oxygen (7.7 mg L^{-1}) (by portable oxygen meter: Oxyguard Polaris Dissolved Oxygen Meter, Dynamic Aqua Supply Ltd, Canada) and pH (7.8±0.5) (by portable pH meter, Model AE-PH501) were measured and kept constant. The photoperiod was 12:12 h (dark: light) during the experiment. Experimental fish were fed twice a day at a rate of 3% of the body weight (Akrami et al., 2012) for 21 days. To remove the feces and uneaten feed, 50% of the tank water was renewed every day and malathion concentrations were set according to the treatments (Kaya et al., 2015).

Growth parameters

Sampling was done after 21 days. All the fish from each tank were collected, anesthetized using 200 mg L^{-1} (2–3 minutes) clove powders) Hajirezaee et al., 2020; Hedayati et al., 2019) and weighed. Growth parameters were calculated as follows:

Weight gain (WG) (g) = mean initial weight – mean final weight

Specific growth rate (SGR) (% d^{-1}) = ({ln final wt(g) – ln initial wt(g)} / days of study) × 100

Feed conversion rate (FCR) = total feed given (g) / weight gain (g)

Survival rate (SR) (%) = (final fish count / initial fish count) × 100

Hematological parameters

From each tank, 3 fish were randomly selected and using 2 ml syringes, 1.5 cc of blood was taken from the caudal vein of each fish. To measure the hematological parameters, blood was centrifuged at 2000 rpm for 15 minutes. The percentage of hematocrit (Hct) was determined by the microhematocrit method. The measurement of hemoglobin (Hb) was accomplished by the manual method of cyan-meth hemoglobin which read at 540 nm wavelength. WBC and RBC were counted by means of a hemocytometer slide after diluting the blood. Other hematological parameters including mean corpuscular hemoglobin (MCH), mean concentration of corpuscular hemoglobin (MCHC), and mean corpuscular volume (MCV) were determined by the below-listed formula (Ciesla, 2018):

MCHC= Hb×10/Hct; MCV= Hct×10/RBC (million); MCH= Hb×10/RBC (million)

Biochemical parameters

To obtain sera, blood taken from the caudal vein was centrifuged for 15 minutes at 2000 rpm. The values of biochemical parameters including total protein (TP), albumin (ALB), cholesterol (CHOL), triglyceride (TRG), glucose (GLU), serum urea (Ur) and creatinine (CRT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were immediately obtained by using chemistry analyzer system (Roche Hitachi 911, Tokyo, Japan) and Pars Azmun kits (Pars Azmun Co, Tehran, Iran). The serum cortisol (CORT) level was measured by using a commercial ELISA kit (ZellBio, Germany), as well as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and malondialdehyde (MDA) levels were measured by using Zellbio kits (Zellbio®, Berlin, Germany) following manufacturer protocols. Serum globulin (GLO) was expressed as the difference between serum TP and ALB (Mohammadi et al., 2020; Rudiansyah et al., 2022).

Immunological parameters

From each tank, five fish were selected to collect their skin mucus (Ross et al., 2000). After cleaning the fish with 50 mM NaCl solution, they were kept in polyethylene bags containing 10 mL NaCl and by rubbing fish's body, their mucus was collected. Collected mucus was transferred to falcon tubes and diluted with 10 mmol phosphate buffered saline (PBS) (pH 7.5, containing 115 mmol sodium) before centrifugation. Supernatants were separated and stored at –20ºC for further assessment (Hoseinifar et al., 2014). In the collected mucus/serum, lysozyme activity was measured by lyophilized *Micrococcus luteus* (Ellis, 1990). Total immunoglobulin (total Ig) of the mucus/serum was quantified using polyethylene glycol (Siwicki, 1993). The alternative complement activity (ACH_{50}) was measured by calculating the amount of the mucus that induces 50% hemolysis of the rabbit red blood cells (RaRBC) (Yano, 1992: Ortuno et al, 2001). The activity of alkaline phosphatase (ALP) in the mucus samples was quantified using a commercial assay kit (Sigma-Aldrich Co., USA). Nitroblue tetrazolium (NBT) reduction test was to determine respiratory burst activity in blood samples. Briefly, 100 µl of heparinized blood and 100 µl of 0.2% NBT solution were mixed and incubated for 30 min. 100 µl of the mixture was mixed with 2 ml of N, N-dimethylformamide and centrifuged at 3000 × g for 6 min and the adsorption was read at 630 nm (Anderson and Siwicki, 1994).

Statistical analysis

The test plan was based on a completely random design. The data was analyzed by SPSS software (version 21.0, IBM Statistics, USA). After confirmation of normality (Shapiro–Wilk test), two-way ANOVA analysis of variance and Tukey's test were run to detect the significant differences among the treatments at a 95% confidential level. All the data was presented as Mean ± SE (Standard error). The P-value of <0.05 was considered statistically significant.

Results

Growth performance

Table 3 indicates growth parameters of the fish at the end of study. The two-way ANOVA results indicated that, except FCR ($P<0.05$), the interaction of dietary TEO and

malathion did not significantly affect the other growth parameters of the experimental groups. The highest WG, SGR, and survival, and the lowest FCR were observed in T4 which received TEO supplementary diet with significant differences to other groups (P<0.05). In contrast, the lowest growth parameters including the lowest SR (P>0.05) belonged to the T3 groups with malathion alone.

Hematological parameters

Hematological values of different groups are given in Table 4. The highest and lowest RBC, WBC, and Hct were noticed in the T4 treatment which received TEO alone. The lowest counts of RBC and WBC belonged to the T2 groups, while Hct percentage was lower in T3 group (Table 4). The Hb value of T4 was significantly higher than other groups (P<0.05), but no significant differences were observed between Hb value of other treatments. The levels of MCHC, MCH, and MCV were statistically similar among treatments (P>0.05). Interaction effects of TEO and malathion exposure were found on Hb and MCHC (P<0.05) but no significant effect was seen in the interaction of TEO and malathion on RBC, WBC, Hct and MCH (Table 4).

Table 3. Effects of dietary thyme essential oil administration and malathion exposure on growth parameters (mean ± SE) of *Oncorhynchus mykiss*

Malathion	TEO	IW (g)	FW (g)	WG (g)	SGR (% d^{-1})	FCR	SR (%)
0	0	19.91±0.01 a	30.67±0.27 b	10.76±0.27 ab	0.71±0.01 ab	1.38±0.00 c	96.33±0.33 b
0.025	0	20.03±0.04 a	27.03±0.59 cd	6.99±0.57 cd	0.49±0.03 cd	1.49±0.00 b	94.00±0.57 c
0.075	0	20.01±0.04 a	25.89±0.72 d	5.87±0.67 d	0.42±0.04 d	1.53±0.00 a	91.66±0.66 d
0	1	20.02±0.04 a	33.00±0.78 a	12.97±0.47 a	0.83±0.02 a	1.23±0.01 d	100.00±0.00 a
0.025	1	19.97±0.02 a	28.62±0.29 bc	8.64±0.30 bc	0.59±0.01 bc	1.40±0.00 c	96.00±0.57 bc
0.075	1	19.99±0.07 a	26.79±0.39 cd	6.80±0.43 cd	0.48±0.02 cd	1.46±0.00 b	94.33±0.33 bc
Two-way ANOVA (P-value)							
malathion		0.734	0.000	0.000	0.000	0.000	0.000
TEO		0.839	0.002	0.001	0.002	0.000	0.000
malathion × TEO		0.189	0.374	0.426	0.643	0.001	0.245

*IW: Initial weight; FW: Final weight; WG: Weight gain; SGR: Specific growth rate; FCR: Feed conversion ratio; SR: Survival rate. Different letters (a–d) in the same row indicate significant differences (P<0.05). T1: control diet; T2: control diet + 0.025 mg L^{-1} malathion; T3: control diet + 0.075 mg L^{-1} malathion; T4: control diet + 1% TEO; T5: control diet + 0.025 mg L^{-1} malathion + 1% TEO; T6: control diet + 0.075 mg L^{-1} malathion + 1% TEO.

Table 4. Effects of dietary thyme essential oil administration and malathion exposure on hematology parameters (mean ± SE) in the blood of *Oncorhynchus mykiss*

Malathion	TEO	RBC (× 10^6/µl)	WBC (× 10^3/µl)	Hct (%)	Hb (g/dl)	MCHC (g/dl)	MCH (pg/cell)	MCV (nm^3)
0	0	1.09±0.02 ab	3.27±0.04 b	31.66±0.79 ab	7.48±0.20 b	23.67±0.86 a	68.40±3.31 a	289.18±11.81 a
0.025	0	1.03±0.03 b	3.11±0.01 c	30.13±0.20 bcd	7.60±0.31 b	25.21±0.95 a	74.11±5.08 a	293.23±9.81 a
0.075	0	1.10±0.02 ab	3.24±0.02 bc	28.60±0.26 d	7.05±0.13 b	24.67±0.69 a	63.95±1.29 a	259.52±7.59 a
0	1	1.20±0.01 a	3.43±0.02 a	32.40±0.32 a	8.76±0.18 a	27.05±0.42 a	73.12±2.57 a	270.14±5.53 a
0.025	1	1.12±0.01 ab	3.19±0.01 bc	30.76±0.31 abc	7.38±0.20 b	24.02±0.91 a	66.00±2.49 a	274.78±4.08 a
0.075	1	1.15±0.01 a	3.29±0.02 b	29.73±0.20 cd	7.56±0.16 b	25.42±0.39 a	65.78±2.11 a	258.63±4.36 a
Two-way ANOVA (P-value)								
malathion		0.020	0.000	0.000	0.005	0.618	0.150	0.016
TEO		0.001	0.001	0.027	0.010	0.132	0.837	0.065
malathion × TEO		0.451	0.171	0.812	0.014	0.030	0.130	0.437

*RBC: Red blood cell; WBC: White blood count; Hct: Hematocrit; Hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MCH: Mean corpuscular hemoglobin; MCV: Mean corpuscular volume. Different letters (a–d) in the same row indicate significant differences (P<0.05). T1: control diet; T2: control diet + 0.025 mg L^{-1} malathion; T3: control diet + 0.075 mg L^{-1} malathion; T4: control diet + 1% TEO; T5: control diet + 0.025 mg L^{-1} malathion + 1% TEO; T6: control diet + 0.075 mg L^{-1} malathion + 1% TEO.

Table 5. Effects of dietary thyme essential oil administration and malathion exposure on biochemical parameters (mean ± SE) in the blood serum of *Oncorhynchus mykiss*

Malathion	TEO	TP (g/dL)	ALB (g/dL)	GLO (g/dL)	TRG (mg/dL)	CHOL (mg/dL)	GLU (mg/dL)	CORT (nmol/L)	CRT (mg/dL)	Ur (mg/dL)	LDH (U/L)
0	0	1.93±0.02 b	0.94±0.01 b	0.98±0.02 b	110.87±2.63 bc	94.09±1.48 b	65.87±1.16 b	81.06±1.78 c	0.62±0.008 b	0.41±0.006 d	185.68±4.24 c
0.025	0	1.96±0.01 b	0.90±0.01 bc	1.06±0.02 ab	121.04±2.62 ab	104.16±2.09 a	75.75±1.34 a	92.59±0.80 ab	0.67±0.012 a	0.47±0.008 b	208.45±3.60 ab
0.075	0	1.74±0.01 d	0.76±0.01 d	0.98±0.00 b	132.21±2.74 a	105.52±2.75 a	74.81±1.33 a	94.17±1.20 a	0.68±0.008 a	0.51±0.008 a	217.52±3.56 a
0	1	2.11±0.01 a	1.02±0.02 a	1.09±0.02 a	97.80±2.28 c	76.35±1.60 c	53.03±0.84 c	67.25±1.46 d	0.52±0.008 c	0.36±0.008 e	164.90±3.68 d
0.025	1	1.97±0.01 b	0.94±0.01 b	1.02±0.02 ab	114.06±2.20 b	98.63±1.85 ab	66.76±0.69 b	86.86±0.96 bc	0.64±0.008 ab	0.44±0.005 cd	193.28±3.50 bc
0.075	1	1.82±0.01 c	0.83±0.01 cd	0.99±0.00 b	121.45±4.27 ab	101.20±2.02 ab	66.02±1.05 b	88.79±0.89 ab	0.65±0.008 ab	0.46±0.003 bc	202.92±3.77 abc
Two-way ANOVA (P-value)											
malathion		0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TEO		0.000	0.000	0.112	0.001	0.000	0.000	0.000	0.000	0.000	0.000
malathion × TEO		0.000	0.357	0.008	0.580	0.011	0.158	0.008	0.004	0.590	0.668

*TP: Total protein; ALB: Albumin; GLO: Globulin; TRG: Triglyceride; CHOL: Cholesterol; GLU: Glucose; CORT: Cortisol; CRT: Creatinine; Ur: Urea; LDH: Lactate dehydrogenase. Different letters (a–e) in the same row indicate significant differences ($P<0.05$). T1: control diet; T2: control diet + 0.025 mg L^{-1} malathion; T3: control diet + 0.075 mg L^{-1} malathion; T4: control diet + 1% TEO; T5: control diet + 0.025 mg L^{-1} malathion + 1% TEO; T6: control diet + 0.075 mg L^{-1} malathion + 1% TEO.

Table 6. Effects of dietary thyme essential oil administration and malathion exposure on antioxidant indices (mean ± SE) in the blood serum of *Oncorhynchus mykiss*

Malathion	TEO	CAT (U/ml)	SOD (U/ml)	GPx (U/ml)	MDA (nmol/ml)
0	0	45.46±0.85 b	35.94±0.81 c	69.22±1.17 b	0.63±0.005 c
0.025	0	40.71±0.97 cd	38.55±0.80 bc	63.68±0.73 cd	0.70±0.014 b
0.075	0	35.87±0.81 e	31.63±0.83 d	60.47±1.26 d	0.83±0.012 a
0	1	51.94±0.80 a	46.14±0.82 a	74.68±1.27 a	0.39±0.007 e
0.025	1	41.96±0.84 bc	40.31±0.60 b	66.27±0.90 bc	0.56±0.009 d
0.075	1	37.01±0.64 de	35.35±0.76 c	64.24±0.85 bcd	0.69±0.008 b
Two-way ANOVA (P-value)					
malathion		0.000	0.000	0.000	0.000
TEO		0.001	0.000	0.001	0.000
malathion × TEO		0.11	0.000	0.418	0.001

*SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; MDA: Malondialdehyde. Different letters (a–d) in the same row indicate significant differences (P<0.05). T1: control diet; T2: control diet + 0.025 mg L^{-1} malathion; T3: control diet + 0.075 mg L^{-1} malathion; T4: control diet + 1% TEO; T5: control diet + 0.025 mg L^{-1} malathion + 1% TEO; T6: control diet + 0.075 mg L^{-1} malathion + 1% TEO.

Table 7. Effects of dietary thyme essential oil administration and malathion exposure on liver enzymes (mean ± SE) in the blood serum of *Oncorhynchus mykiss*

Malathion	TEO	ALT (U/ml)	AST (U/ml)	ALP (U/ml)
0	0	11.81±0.52 bc	16.04±0.31 c	27.04±0.83 bc
0.025	0	13.16±0.26 ab	18.17±0.34 b	25.50±0.50 c
0.075	0	14.36±0.58 a	20.14±0.31 a	29.80±0.57 ab
0	1	10.63±0.3 1c	15.75±0.39 c	25.51±0.65 c
0.025	1	12.80±0.37 ab	16.21±0.41 c	27.31±0.25 bc
0.075	1	13.55±0.28 ab	18.05±0.25b	30.74±0.72 a
Two-way ANOVA (P-value)				
malathion		0.000	0.000	0.000
TEO		0.036	0.000	0.436
malathion × TEO		0.619	0.042	0.50

*ALT: Alanine aminotransferase; AST: Aspartate transaminase; ALP: Alkaline phosphatase. Different letters (a–d) in the same row indicate significant differences (P<0.05). T1: control diet; T2: control diet + 0.025 mg L^{-1} malathion; T3: control diet + 0.075 mg L^{-1} malathion; T4: control diet + 1% TEO; T5: control diet + 0.025 mg L^{-1} malathion + 1% TEO; T6: control diet + 0.075 mg L^{-1} malathion + 1% TEO.

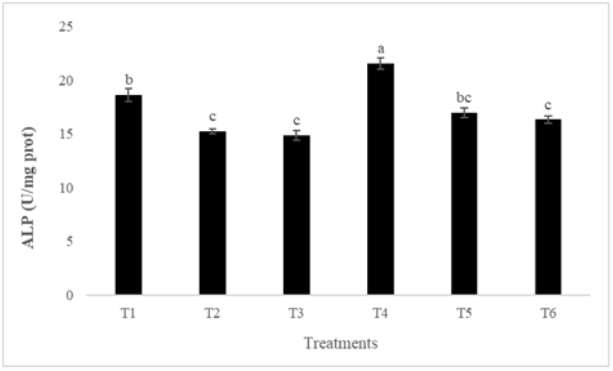
Biochemical parameters

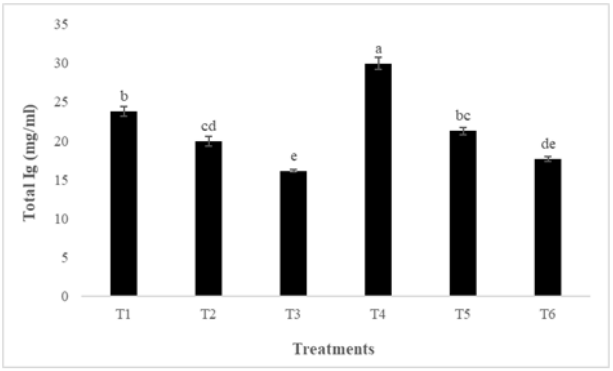
The serum biochemical parameters of the fish were presented in Table 5. A significant interaction between TEO dietary and malathion exposure was seen in TP, GLO, CHOL, CORT and CRT (P<0.05). No significant difference was seen in the interaction for ALB, TRG, GLU, UR and LDH (Table 5). With a higher amount of TP, GLO, ALB, and lower amount of TRG, CHOL, GLU, CORT, CRT, Ur, and LDH, T4 treatments showed the best results among the treatment groups. The worst biochemical values were significantly noted in the serum of the fish in T3 treatment. Antioxidant biomarkers study also revealed significantly high levels of CAT, SOD, and GPx and low level of MDA in the serum of the fish kept in T4 treatment (Table 6). The interaction of TEO and malathion showed a significant effect on antioxidant indices including SOD and MDA.

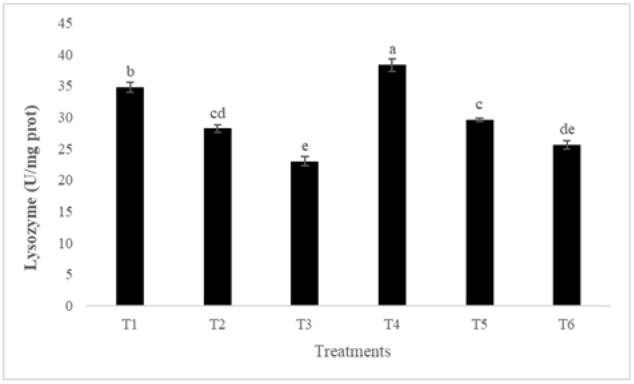
The serum ALT, AST, and ALP levels varied among study treatments (Table 7). T3 treatment showed the highest levels of ALT, AST with a significant difference with other treatments (P<0.05). In terms of ALP activity, the highest level occurred in the T6 treatment (P<0.05), although no significant difference was observed between T6 and T3 treatments (Table 7).

Immunological parameters

Table 8 and Figure 1 demonstrate the results of the immunological parameters in the serum and mucus of the fish under different treatments. The two-way ANOVA results show that the interaction of dietary TEO and malathion did not significantly affect lysozyme, ACH_{50} and NBT. But their effect on total Ig was evident. The highest level of lysozyme, ACH_{50}, total Ig and NBT of the serum was in the T4 treatment. The lowest level of lysozyme, ACH_{50} and NBT belonged to the T3 treatment. The lowest and the highest level of total Ig were recorded in T3 and T4 groups, respectively (P<0.05). Further, T3, T2, T5, and T6 treatments showed similar levels of serum total Ig (P>0.05).







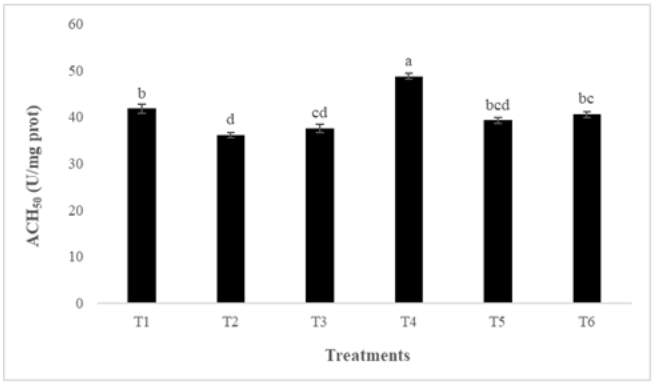


Figure 1. Immunological indices of fish mucus under different treatments. ALP: Alkaline phosphatase; Total Ig: Total immunoglobulin; $ACH_{50:}$ Alternative complement activity. Values are presented as the mean ± SE. Different letters (a–e) indicate the significant differences ($P<0.05$). T1: control diet; T2: control diet + 0.025 mg L^{-1} malathion (Low dose); T3: control diet + 0.075 mg L^{-1} malathion (High dose); T4: control diet + 1% TEO (TEO); T5: control diet + 0.025 mg L^{-1} malathion + 1% TEO (Low-TEO); T6: control diet + 0.075 mg L^{-1} malathion + 1% TEO (High-TEO)

Table 8. Effects of dietary thyme essential oil administration and malathion exposure on immunological indices (mean ± SE) in the blood serum of *Oncorhynchus mykiss*

Malathion	TEO	Lysozyme (U/ml)	ACH_{50} (U/ml)	Total Ig (mg/ml)	NBT (OD at 540 nm)
0	0	61.92±1.15 b	33.05±0.90 cd	22.40±0.53 b	0.36±0.014 b
0.025	0	56.20±1.13 c	34.61±0.98 bc	18.58±0.38 c	0.26±0.014 cd
0.075	0	50.48±1.11 d	29.69±0.88 d	17.20±0.39 c	0.23±0.008 d
0	1	70.17±0.83 a	39.69±0.81 a	28.85±0.85 a	0.43±0.014 a
0.025	1	59.07±0.73 bc	37.24±0.68 ab	19.67±0.41 c	0.31±0.008 bc
0.075	1	56.72±0.97 c	31.74±0.75 cd	19.20±0.36 c	0.25±0.008 d
Two-way ANOVA (P-value)					
malathion		0.000	0.000	0.000	0.73
TEO		0.000	0.000	0.000	0.001
malathion × TEO		0.057	0.038	0.001	0.102

*ACH_{50}: alternative complement activity; Total Ig: Total immunoglobulin; NBT: Nitro blue tetrazolium. Different letters (a–d) in the same row indicate significant differences (P<0.05). T1: control diet; T2: control diet + 0.025 mg L^{-1} malathion; T3: control diet + 0.075 mg L^{-1} malathion; T4: control diet + 1% TEO; T5: control diet + 0.025 mg L^{-1} malathion + 1% TEO; T6: control diet + 0.075 mg L^{-1} malathion + 1% TEO.

The immune indices of skin mucus varied among experimental groups (Figure 1). There was a significant difference between the amount of lysozyme, total Ig, and ACH_{50} of different treatments with the highest and lowest level belonging to T4 and T3, respectively (P<0.05).

Discussion

As shown, thyme oil contains a number of antimicrobial components including thymol, linalool, terpinene, *p*-cymene, carvacrol, and β-caryphyllene which inhibit pathogens. These phenolic components also act as strong antioxidants, improve the growth performance and defense mechanisms of the animal. Despite its advantages over other essential oils (Zarzuelo and Crespo, 2002), less attention has been given to the use of thyme oil in aquaculture (Zargar et al., 2019).

In this study, the impacts of TEO on growth and biochemical parameters of *O. mykiss* exposed to malathion were examined. According to the results, nutritional supplementation of TEO improved the SR and some growth indices, including FW and FCR of the fish. Although growth parameters in the treatments containing malathion were significantly reduced. Numerous studies described the perturbation in the growth parameters of fish due to poisoning and effect of contaminations (Mekkawy and Lashein, 2003; Huculeci et al., 2009; Al-Shawi et al., 2022). Also, previous studies showed that plant extracts increase the digestibility and nutritional availability and synthesize more proteins (Samadi et al., 2016). For instance, it has been demonstrated that Mooseer extract (Ebrahimi Dorche et al., 2013) and combination of oak acorn, coriander, and common mallow extracts (Raissy et al., 2022) affect protease and digestion enzymes and improve the digestion and ingestion of proteins. Pancreatic enzymes are important factors in feed digestion. Medicinal herbs can stimulate the pancreas gland to secrete digestive enzymes (Frankic et al., 2009). Similar to our results were observed in the study of Sönmez et al. (2015), where the higher WG was obtained in *O. mykiss* supplemented with thyme essential oil. Therefore in this experiment, the higher weight gain in T4 can be ascribed to thyme oil supplementation and subsequently increased secretion of digestive enzymes.

In this experiment malathion and thyme oil affected the blood indicators of rainbow trout in such a manner that the highest level of Hct, RBC and WBC were seen in the fish fed with supplemented thyme oil. Moreover, toxic and stress conditions change the physiological indices including hematological parameters of the animals (Ates et al., 2008; Abarghoei et al., 2015; Mirghaed et al., 2018). This experiment showed that thyme oil in the diets can increase the count of RBC, Hct percentage, and Hb values. This agrees with previous studies where gibel carp (*Carassius auratus gibelio*) (Zadmajid and Mohammadi, 2017) and Nile tilapia (*Oreochromis niloticus*) (Antache et al., 2014) were fed diets containing thyme as compared to control. A significant increase in the number of RBC and Hb content was also recorded in *O. mykiss* (Ahmadifar et al., 2011) and *O. mossambicus* (Gultepe et al., 2014) fed thymol-carvacrol and 1% of thyme supplemented diets, respectively, which were similar to our results. Increase in the WBS of the fish in our experiment indicates that the presence of thymol, eugenol, and carvacrol as phenolic compounds in the thyme oil could stimulate the immune system of animals, thereby raising the WBC levels. In contrast, amount of Hct and RBC in the fish exposed to both the doses of malathion were reduced as compared to control group. Since Hct is the volume percentage of RBC, declined number of RBC or Hb reduces the amount of Hct (Ghafari Farsani et al., 2016 a, b; Mohammadi et al., 2020). Number of RBC can be reduced due to the exposure to contaminants or their

accumulation in the gill tissues due to the stresses arising from the pollutants (Adeyemo, 2007; Ghafari Farsani et al., 2016 a, b).

Our data indicated an improvement in the level of biochemical components of the blood of the fish fed TEO-contained diet. The serum total protein is an important indicator of fish's nutrition and health status (Yousefian et al., 2011). Albumin is synthesized in the liver and acts as an antioxidant agent against oxidative stress induced by free radicals (Adler and Edwards, 2000). Globulin is another essential component of the innate immune system of fish (Yousefi et al., 2021). The same result was obtained by Gulec et al. (2013) who found a higher level of total protein, albumin, and globulin when feeding fish on diets supplemented with a mixture of thyme and fennel. Exposing fish to higher doses of malathion significantly reduced the total protein which could be due to the disturbance in the protein synthesis in the liver and absorbance of amino acids from the intestine. Also, a lower level of ALB in the fish exposed to malathion alone and the combination of TEO and higher dose of malathion might be due to the lower biosynthesis of protein in the fish liver. A higher level of TRG was recorded in the fish exposed to malathion which can be a consequence of breaking down of fats from the body tissues (Banaee et al., 2019). In contrast, a lower level of TRG was recorded in the fish fed with thyme oil. Reductions in TRG levels were recorded by Hoseini and Yousefi (2019) and Mousavi et al. (2016) when rainbow trout and common carp (*Cyprinus carpio*) were fed with *T. vulgaris*, respectively. The levels of CHOL had slight changes during this experiment but significantly high levels of it were recorded in T2 and T3 treatments as compared to the control group. Malathion can disrupt the excretion of bile cholesterol and reduces lipoprotein synthesis which therefore raises the level of cholesterol (Abdel-Daim et al., 2020). Also, at the end of the experiment, CORT values showed significant differences between the experimental groups. In this investigation, the amount of cortisol and Ur were significantly elevated in the fish exposed to malathion. Cortisol secretion and increasing levels of glucose are signs of stress in the exposed fish to overcome the energy needed for these conditions (Banaee et al., 2020). On the other hand, fish fed diet supplemented with thyme oil presented lower contents of CORT and Ur, indicating an improvement in these parameters. Antache et al. (2014) showed dietary *Thymus vulgaris* reduced the serum concentration of cortisol in *O. niloticus*.

Based on the literature, an elevated level of hepatic metabolic enzymes is redolent of hepatic injury and malfunction (Yousefi et al., 2018). SOD and CAT act as body defense mechanisms against oxidative stress induced by superoxide radicals and H_2O_2 (Sönmez et al., 2015). Their inhibitory function on oxygen free radicals formation reduces oxidative stress (Pandey et al., 2003). In the present study, significantly high levels of CAT, SOD, and GPx but lower levels of MDA were observed in the serum of fish in T4 treatment. Elevated levels of SOD and CAT beneficially neutralize adverse effects of reactive oxygen species (ROS) on body cells, which is confirmed by a lower level of MDA (John et al., 2001). Therefore, it can be said that different contaminants in aquatic environments produce ROS and damage the cells of aquatic organisms (Xiong et al., 2011). The defense mechanism of cells reduces the injuries of ROS radicals (Monteiro et al., 2006). By maintaining the redox homeostasis, cell defense mechanism normalizes the cellular functions by metallothionein (MTs), glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) (Le Bras et al., 2005). Results of Al-Shawi et al. (2022) on the antioxidant enzymes of *C. carpio* exposed to cadmium and fed with *Silybum marianum*, confirm our results. Other studies also show the improvement of antioxidant enzymes due to the supplemented feeding of plants (Mohammadi et al., 2020; Veisi et al., 2021; Ghafarifarsani et al., 2021 b and 2022 a; Raissy et al., 2022; Rudiansyah et al., 2022).

Measuring the level of ALT, ALP, and AST enzymes is advantageous in the evaluation of diet metabolism and the negative effect of a toxicant on fish liver (Zadmajid and Mohammadi, 2017). A significant decrease in the level of ALP, AST, and ALT was observed in the fish fed with TEO. Low levels of these enzymes in the serum of fish fed TEO as compared to fish exposed to malathion suggest a healthier liver that successfully controls the amino acid metabolism. These beneficial effects may be attributed to the phenolic compounds found in thyme oil, which stimulate the antioxidant status and immune system of fish. Significant reduction in the level of ALP, AST, and ALT of gibel carp was recorded when the amount of thyme oil in their feed increased to 800 mg kg^{-1} (Zadmajid and Mohammadi, 2017). Valladão et al. (2019) also reported no significant difference between ALT and AST levels of tilapia fed with 0.1–1% of thyme oils. A two weeks dietary experiment demonstrated that thyme extract at 5–20 g kg^{-1} of feed suppressed AST and ALT levels in *O. mykiss* (Hoseini and Yousefi, 2019).

The current study indicated the role of TEO as a growth enhancer and an immunostimulant. The immunostimulatory effects of thyme oil components have been reported previously (Rota et al., 2008). Lysozyme activity and WBC were improved in *O. mykiss* treated with *Thymus vulgaris* essential oils (Zargar et al., 2019). Elevation of ACH_{50} was observed in fish fed with TEO diets. It seems that a higher level of TP in the blood serum has led to higher levels of ACH_{50} and a better immune system in the TEO treatments which help the phagocytosis of harmful agents. The amount of total Ig was also increased in TEO treatment groups. Similar results were reported by Hoseini and Yousefi (2019), where thyme (*Thymus vulgaris*) extract increased total Ig, ACH_{50}, total protein, and lysozyme values.

Conclusion

Overall, it can be inferred that thyme oils can enhance the growth, blood, and biochemical parameters immune

system and the defense mechanism of *O. mykiss* against malathion toxicity. These inverse effects are due to the phenolic compounds including carvacrol and thymol in the oil. The best result was obtained in 1% thyme oil supplementation. Therefore, *T. vulgaris* essential oil is a suitable supplement for stimulating the growth and immune system of rainbow trout under stresses of exposure to pollutants and contaminants. Additional research on the effect of thyme oil against aquaculture toxicants is encouraged.

Compliance with ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Author contributions

Conceptualization: Saade Abdalkareem Jasim; Methodology: Reza Davoodi, Safoura Abarghouei; Software: Ghulam Yasin; Validation: Reza Davoodi; Data curation: Saade Abdalkareem Jasim; Writing original draft preparation: Reza Davoodi, Ahmed Taifi, Yasser Fakri Mustafa; Writing – review and editing: Rustem Adamovich Shichiyakh, Ola Kamal A. Alkadir, Safoura Abarghouei; Supervision: Saade Abdalkareem Jasim. All authors have read and agreed to the published version of the manuscript.

Data availability

The datasets in this study are available from the corresponding author on reasonable request. All data and materials are available for publication.

Consent to publish

All authors give consent for publication.

Conflict of interest

The authors declare that they have no conflict of interest.

References

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

Abdel-Daim M.M., Abushouk A.I., Bungău S.G., Bin-Jumah M., El-Kott A.F., Shati A.A., Aleya L., Alkahtani S. (2020). Protective effects of thymoquinone and diallyl sulphide against malathion-induced toxicity in rats. Environ. Sci. Pollut. Res., 27: 10228–10235.

Abdel-Salam O.M., Youness E.R., Mohammed N.A., Yassen N.N., Khadrawy Y.A., El-Toukhy S.E., Sleem A.A. (2016). Novel neuroprotective and hepatoprotective effects of citric acid in acute malathion intoxication. Asian Pac. J. Trop. Med., 9: 1181–1194.

Adeyemo O.K. (2007). Haematological profile of *Clarias gariepinus* (Burchell, 1822) exposed to lead. Turkish J. Fish. Aquat. Sci., 7: 163–169.

Adler A.J., Edwards R.B. (2000). Human interphotoreceptor matrix contains serum albumin and retinol-binding protein. Exp. Eye Res., 70: 227–234.

Ahmadifar E., Falahatkar B., Akrami R. (2011). Effects of dietary thymol-carvacrol on growth performance, hematological parameters and tissue composition of juvenile rainbow trout, *Oncorhynchus mykiss*. J. Appl. Ichthyol., 27: 1057–1060.

Akrami R., Chitsaz H., Hezarjaribi A., Ziaei R. (2012). Effect of dietary mannan oligosaccharide (MOS) on growth performance and immune response of Gibel carp juveniles (*Carassius auratus gibelio*). J. Vet. Adv., 2: 507–513.

Aldosary S.K., El-Rahman S.N.A., Al-Jameel S.S., Alromihi N.M. (2021). Antioxidant and antimicrobial activities of *Thymus vulgaris* essential oil contained and synthesis thymus (*Vulgaris*) silver nanoparticles. Braz. J. Biol., 83.

Ali S., Ullah M.I., Sajjad A., Shakeel Q., Hussain A. (2021). Environmental and health effects of pesticide residues. Sustain. Agric. Res., 48: 311–336.

Al-Shawi S.G., Al-Younis Z.K., Yousif A.Y., Shichiyakh R.A., Zekiy A.O., Naserabad S.S. (2022). Dietary silymarin, *Silybum marianum* extract ameliorates cadmium chloride toxicity in common carp, *Cyprinus carpio*. Ann. Anim. Sci., 22: 741–750.

Anderson D.P., Siwicki A.K. (1994). Duration of protection against *Aeromonas salmonicida* in brook trout immunostimulated with glucan or chitosan by injection or immersion. Prog. Fish Cult., 56: 258–261.

Antache A., Cristea V., Grecu I., Dediu L., Cretu M., Bocioc E., Petrea S.M. (2014). Effects of dietary supplementation at Nile tilapia with *Thymus vulgaris*, *Trigonela foenum graecum* and *Azadirachta indica* on welfare status. Bulletin of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Anim. Sci. Biotechnol., 71: 115–122.

Ates B., Orun I., Talas Z.S., Durmaz G., Yilmaz I. (2008). Effects of sodium selenite on some biochemical and hematological parameters of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) exposed to Pb^{2+} and Cu^{2+}. Fish Physiol. Biochem., 34: 53–59.

Bagamboula C.F., Uyttendaele M., Debevere J. (2004). Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. Food Microbiol., 21: 33–42.

Banaee M., Tahery S., Nematdoost Haghi B., Shahafve S., Vaziriyan M. (2019). Blood biochemical changes in common carp (*Cyprinus carpio*) upon co-exposure to titanium dioxide nanoparticles and paraquat. Iran. J. Fish. Sci., 18: 242–255.

Banaee M., Akhlaghi M., Soltanian S., Sureda A., Gholamhosseini A., Rakhshaninejad M. (2020). Combined effects of exposure to sub-lethal concentration of the insecticide chlorpyrifos and the herbicide glyphosate on the biochemical changes in the freshwater crayfish *Pontastacus leptodactylus*. Ecotoxicology, 29: 1500–1515.

Bharti S., Rasool F. (2021). Analysis of the biochemical and histopathological impact of a mild dose of commercial malathion on *Channa punctatus* (Bloch) fish. Toxicol. Rep., 8: 443–455.

Bilal S., Nasir M., Hassan M.M., Rehman M.F., Sami A.J., Hayat A. (2022). A novel construct of an electrochemical acetylcholinesterase biosensor for the investigation of malathion sensitivity to three different insect species using a $NiCr_2O_4$/g-C_3N_4 composite integrated pencil graphite electrode. RSC Adv., 12: 16860–16874.

Ciesla B. (2018). Hematology in practice. F A Davis, 432 pp.

Dawood M.A., Abdel-Tawwab M., Abdel-Latif H.M. (2020). Lycopene reduces the impacts of aquatic environmental pollutants and physical stressors in fish. Rev. Aquac., 12: 2511–2526.

De Souza S.S., Machado R.N., da Costa J.C., Campos D.F., da Silva G.S., de Almeida-Val V.M.F. (2020). Severe damages caused by malathion exposure in *Colossoma macropomum*. Ecotoxicol. Environ. Saf., 205: 111340.

De Souza S.S., da Silva Castro J., Campos D.F., Pereira R.S., Bataglion G.A., da Silva G.S., de Almeida-Val V.M.F. (2021). Temporal exposure to malathion: Biochemical changes in the Amazonian fish tambaqui, *Colossoma macropomum*. Aquat. Toxicol., 241: 105997.

Deka S., Mahanta R. (2016). Malathion toxicity on fish – a review. Int. J. Curr. Res., 8: 44120–44128.

Ebrahim Dorche I., Tangestani R., Alizadeh Dvghyklayy E., Zare P. (2013). Effect of different levels of garlic essential oil on growth,

feed and carcass composition of beluga (*Huso huso*) rearing young. J. Mar. Sci. Technol., 11: 1–12.

Ellis A.I. (1990). Lysozyme assays. Techniq. Fish Immunol., 1: 101–103.

Fazelan Z., Hoseini S.M., Yousefi M., Khalili M., Hoseinifar S.H., Van Doan H. (2020). Effects of dietary eucalyptol administration on antioxidant and inflammatory genes in common carp (*Cyprinus carpio*) exposed to ambient copper. Aquaculture, 520: 734988.

Frankic T., Voljč M., Salobir J., Rezar V. (2009). Use of herbs and spices and their extracts in animal nutrition. Acta Agric. Slov., 94: 95–102.

Fu H., Tan P., Wang R., Li S., Liu H., Yang Y., Wu Z. (2022). Advances in organophosphorus pesticides pollution: Current status and challenges in ecotoxicological, sustainable agriculture, and degradation strategies. J. Hazard. Mater., 424: 127494.

Gao J., Liu L., Liu X., Zhou H., Lu J., Huang S., Wang Z. (2009). The occurrence and spatial distribution of organophosphorous pesticides in Chinese surface water. Arch. Environ. Contam. Toxicol., 82: 223–229.

Ghafari Farsani H., Hedayati S.A., Zare Nadimi Bin N., Azizpour S., Shahbazi Naserabad S. (2016 a). Effects of sub lethal concentrations of pesticide malathion on hematology parameters of rainbow trout (*Oncorhynchus mukiss*). J. Oceanogr., 7: 1–9.

Ghafari Farsani H., Poorbagher H., Farahmand H. (2016 b). Effects of malathion on DNA breakage in the liver and gill of rainbow trout (*Oncorhynchus mykiss*) using weighted averaging. J. Fish., 69: 89–99.

Ghafarifarsani H., Hoseinifar S.H., Adorian T.J., Ferrigolo F.R.G., Raissy M., Van Doan H. (2021 a). The effects of combined inclusion of *Malvae sylvestris*, *Origanum vulgare*, and *Allium hirtifolium* boiss for common carp (*Cyprinus carpio*) diet: Growth performance, antioxidant defense, and immunological parameters. Fish Shellfish Immunol., 119: 670–677.

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

Ghafarifarsani H., Hoseinifar S.H., Aftabgard M., Van Doan H. (2022 a). The improving role of savory (*Satureja hortensis*) essential oil for Caspian roach (*Rutilus caspicus*) fry: Growth, haematological, immunological, and antioxidant parameters and resistance to salinity stress. Aquaculture, 548: 737653.

Ghafarifarsani H., Hoseinifar S.H., Javahery S., Yazici M., Van Doan H. (2022 b). Growth performance, biochemical parameters, and digestive enzymes in common carp (*Cyprinus carpio*) fed experimental diets supplemented with vitamin C, thyme essential oil, and quercetin. Ital. J. Anim. Sci., 21: 291–302.

Ghafarifarsani H., Hoseinifar S.H., Javahery S., Van Doan H. (2022 c). Effects of dietary vitamin C, thyme essential oil, and quercetin on the immunological and antioxidant status of common carp (*Cyprinus carpio*). Aquaculture, 553: 738053.

Grigore A., Paraschiv I.N.A., Colceru-Mihul S., Bubueanu C., Draghici E., Ichim M. (2010). Chemical composition and antioxidant activity of *Thymus vulgaris* L. volatile oil obtained by two different methods. Rom. Biotechnol. Lett., 15: 5436–5443.

Gulec A.K., Danabas D., Ural M., Seker E., Arslan A., Serdar O. (2013). Effect of mixed use of thyme and fennel oils on biochemical properties and electrolytes in rainbow trout as a response to *Yersinia ruckeri* infection. Acta Vet. Brno, 82: 297–302.

Gultepe N., Bilen S., Yılmaz S., Güroy D., Aydın S. (2014). Effects of herbs and spice on health status of tilapia (*Oreochromis mossambicus*) challenged with *Streptococcus iniae*. Acta Vet. Brno, 83: 125–131.

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

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

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

Hoseini S.M., Yousefi M. (2019). Beneficial effects of thyme (*Thymus vulgaris*) extract on oxytetracycline-induced stress response, immunosuppression, oxidative stress and enzymatic changes in rainbow trout (*Oncorhynchus mykiss*). Aquac. Nutr., 25: 298–309.

Hoseini S.M., Hoseinifar S.H., Doan H.V. (2018). Effect of dietary eucalyptol on stress markers, enzyme activities and immune indicators in serum and haematological characteristics of common carp (*Cyprinus carpio*) exposed to toxic concentration of ambient copper. Aqua. Res., 49: 3045–3054.

Hoseini S.M., Khalili M., Rajabiesterabadi H., Hoseinifar S.H., Van Doan H. (2020). Effects of dietary monoterpene, myrcene, administration on immune-and health-related genes expression in common carp gill following exposure to copper sulfate. Fish Shellfish Immunol., 98: 438–445.

Hoseinifar S.H., Sharifian M., Vesaghi M.J., Khalili M., Esteban M.Á. (2014). The effects of dietary xylooligosaccharide on mucosal parameters, intestinal microbiota and morphology and growth performance of Caspian white fish (*Rutilus frisii kutum*) fry. Fish Shellfish Immunol., 39: 231–236.

Howard P.H. (1991). Handbook of environmental degradation rates. CRC Press.

Huang B., Ban X., He J., Zeng H., Zhang P., Wang Y. (2010). Hepatoprotective and antioxidant effects of the methanolic extract from *Halenia elliptica*. J. Ethnopharmacol., 131: 276–281.

Huculeci R., Dinu D., Staicu A.C., Munteanu M.C., Costache M., Dinischiotu A. (2009). Malathion-induced alteration of the antioxidant defence system in kidney, gill, and intestine of *Carassius auratus gibelio*. Environ. Toxicol., 24: 523–530.

John S., Kale M., Rathore N., Bhatnagar D. (2001). Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. J. Nutr. Biochem., 12: 500–504.

Kadiru S., Patil S., D'Souza R. (2022). Effect of pesticide toxicity in aquatic environments: A recent review. Int. J. Fish Aquat. Stud., 10: 113–118

Kaya H., Çelik E.Ş., Yılmaz S., Tulgar A., Akbulut M., Demir N. (2015). Hematological, serum biochemical, and immunological responses in common carp (*Cyprinus carpio*) exposed to phosalone. Comp. Clin. Path., 24: 497–507.

Kumar R., Nagpure N.S., Kushwaha B., Srivastava S.K., Lakra W.S. (2010). Investigation of the genotoxicity of malathion to freshwater teleost fish *Channa punctatus* (Bloch) using the micronucleus test and comet assay. Arch. Environ. Contam. Toxicol., 58: 123–130.

Kumar R., Sankhla M.S., Kumar R., Sonone S.S. (2021). Impact of pesticide toxicity in aquatic environment. Biointerface Res. Appl. Chem., 11: 10131–10140.

Le Bras M., Clement M.V., Pervaiz S., Brenner C. (2005). Reactive oxygen species and the mitochondrial signaling pathway of cell death. Histol. Histopathol., 20: 205–219.

Mekkawy I.A., Lashein F. (2003). The effect of lead and cadmium on LDH and G-6-PDH isoenzyme patterns exhibited during the early embryonic development of the teleost fish, *Ctenopharyngodon idellus* with emphasis on the corresponding morphological variations. Proc. 26th Annual Fish Larva Conference, Bergen, Norway, Citeseer, pp. 22–26.

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

Mirghaed A.T., Fayaz S., Hoseini S.M. (2019). Dietary 1,8-cinoele affects serum enzymatic activities and immunological characteristics in common carp (*Cyprinus carpio*) exposed to ambient ammonia. Aquac. Res., 50: 146–153.

Mohammadi G., Rashidian G., Hoseinifar S.H., Naserabad S.S., Van Doan H. (2020). Ginger (*Zingiber officinale*) extract affects growth performance, body composition, haematology, serum and

mucosal immune parameters in common carp (*Cyprinus carpio*). Fish Shellfish Immunol., 99: 267–273.

Mohammed E., Kamel M., El Iraqi K., Tawfik A.M., Khattab M.S., Elsabagh M. (2020). *Zingiber officinale* and *Glycyrrhiza glabra*, individually or in combination, reduce heavy metal accumulation and improve growth performance and immune status in Nile tilapia, *Oreochromis niloticus*. Aquac. Res., 51: 1933–1941.

Monteiro D.A., De Almeida J.A., Rantin F.T., Kalinin A.L. (2006). Oxidative stress biomarkers in the freshwater characid fish, *Brycon cephalus*, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol, 143: 141–149.

Mousavi E., Mohammadiazarm H., Mousavi S.M., Ghatrami E.R. (2016). Effects of inulin, savory and onion powders in diet of juveniles carp *Cyprinus carpio* (Linnaeus 1758) on gut micro flora, immune response and blood biochemical parameters. Turkish J. Fish. Aquat. Sci., 16: 831–838.

Omidifar N., Nili-Ahmadabadi A., Nakhostin-Ansari A., Lankarani K.B., Moghadami M., Mousavi S.M., Hashemi S.A., Gholami A., Shokripour M., Ebrahimi Z. (2021). The modulatory potential of herbal antioxidants against oxidative stress and heavy metal pollution: plants against environmental oxidative stress. Environ. Sci. Pollut. Res., 28: 61908–61918.

Ortuno J., Esteban M.A., Meseguer J. (2001). Effects of short-term crowding stress on the gilthead seabream (*Sparus aurata* L.) innate immune response. Fish Shellfish Immunol., 11: 187–197.

Pandey S., Parvez S., Sayeed I., Haque R., Bin-Hafeez B., Raisuddin S. (2003). Biomarkers of oxidative stress: a comparative study of river Yamuna fish *Wallago attu* (Bl. & Schn.). Sci. Total Environ., 309: 105–115.

Parsaei P., Bahmani M., Naghdi N., Asadi-Samani M., Rafieian-Kopaei M. (2016). A review of therapeutic and pharmacological effects of thymol. Pharm. Lett., 8: 150–154.

Patil V.K., David M. (2013). Oxidative stress in freshwater fish, *Labeo rohita* as a biomarker of malathion exposure. Environ. Monit. Assess, 185: 10191–10199.

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

Raissy M., Ghafarifarsani H., Hoseinifar S.H., El-Haroun E.R., Naserabad S.S., Van Doan H. (2022). The effect of dietary combined herbs extracts (oak acorn, coriander, and common mallow) on growth, digestive enzymes, antioxidant and immune response, and resistance against *Aeromonas hydrophila* infection in common carp, *Cyprinus carpio*. Aquaculture, 546: 737287.

Rajabiesterabadi H., Yousefi M., Hoseini S.M. (2020). Enhanced haematological and immune responses in common carp *Cyprinus carpio* fed with olive leaf extract-supplemented diets and subjected to ambient ammonia. Aquac. Nutr., 26: 763–771.

Ramsden C.S., Smith T.J., Shaw B.J., Handy R.D. (2009). Dietary exposure to titanium dioxide nanoparticles in rainbow trout, (*Oncorhynchus mykiss*): no effect on growth, but subtle biochemical disturbances in the brain. Ecotoxicology, 18: 939–951.

Rehman H., Ali M., Atif F., Kaur M., Bhatia K., Raisuddin S. (2006). The modulatory effect of deltamethrin on antioxidants in mice. Clin. Chim. Acta, 369: 61–65.

Ross N.W., Firth K.J., Wang A., Burka J.F., Johnson S.C. (2000). Changes in hydrolytic enzyme activities of naive Atlantic salmon *Salmo salar* skin mucus due to infection with the salmon louse *Lepeophtheirus salmonis* and cortisol implantation. Dis. Aquat. Org., 41: 43–51.

Rota M.C., Herrera A., Martínez R.M., Sotomayor J.A., Jordán M.J. (2008). Antimicrobial activity and chemical composition of Thymus vulgaris, *Thymus zygis* and *Thymus hyemalis* essential oils. Food Control., 19: 681–687.

Rudiansyah M., Abdelbasset W.K., Jasim S.A., Mohammadi G., Dharmarajlu S.M., Nasirin C., Jalil A.T., Opulencia M.J.C., Abid M.K., Naserabad S.S. (2022). Beneficial alterations in growth performance, blood biochemicals, immune responses, and antioxidant capacity of common carp (*Cyprinus carpio*) fed a blend of *Thymus vulgaris*, *Origanum majorana*, and *Satureja hortensis* extracts. Aquaculture, 738254.

Samadi L., Zanguee N., Mousavi S.M., Zakeri M. (2016). Effect of dietary Garlic extract on growth, feeding parameters, hematological indices and body composition of *Litopenaeus vannamei*. J. Persian Gulf, 7: 29–42.

Santana M.S., de Melo G.D., Sandrini-Neto L., Di Domenico M., Prodocimo M.M. (2022). A meta-analytic review of fish antioxidant defense and biotransformation systems following pesticide exposure. Chemosphere, 291: 132730.

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

Shayeghi M., Shahtaheri S., Selseleh M. (2001). Phosphorous insecticides residues in Mazandaran river waters, Iran (2000). Iran. J. Public Health, 30: 115–118.

Singh R.N., Pandey R.K., Singh N.N., Das V.K. (2009). Acute toxicity and behavioral responses of common carp *Cyprinus carpio* (Linn.) to an organophosphate (Dimethoate). WJZ, 4: 70–75.

Siwicki A.K. (1993). Nonspecific defense mechanisms assay in fish. ii. potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs and total immunoglobulin level in serum. In: Fish disease diagnosis and prevention methods, Siwicki A.K., Anderson D.P., Waluga J. (eds). Olsztyn, Poland, pp. 105–112.

Smith D.R., Padilla W.J., Vier D.C., Nemat-Nasser S.C., Schultz S. (2000). Composite medium with simultaneously negative permeability and permittivity. Phys. Rev. Lett., 84: 4184.

Sönmez A.Y., Bilen S., Alak G., Hisar O., Yanık T., Biswas G. (2015). Growth performance and antioxidant enzyme activities in rainbow trout (*Oncorhynchus mykiss*) juveniles fed diets supplemented with sage, mint and thyme oils. Fish Physiol. Biochem., 41: 165–175.

Topal A., Atamanalp M., Uçar A., Oruç E., Kocaman E.M., Sulukan E., Akdemir F., Beydemir Ş., Kılınç N., Erdoğan O., Ceyhun S.B. (2015). Effects of glyphosate on juvenile rainbow trout (*Oncorhynchus mykiss*): Transcriptional and enzymatic analyses of antioxidant defence system, histopathological liver damage and swimming performance. Ecotoxicol. Environ. Saf., 111: 206–214.

Tukmechi A., Andani H.R.R., Manaffar R., Sheikhzadeh N. (2011). Dietary administration of beta-mercapto-ethanol treated Saccharomyces cerevisiae enhanced the growth, innate immune response and disease resistance of the rainbow trout, *Oncorhynchus mykiss*. Fish Shellfish Immunol., 30: 923–928.

Valladão G.M.R., Gallani S.U., Kotzent S., Assane I.M., Pilarski F. (2019). Effects of dietary thyme essential oil on hemato-immunological indices, intestinal morphology, and microbiota of Nile tilapia. Aquac. Int., 27: 399–411.

Vasseghian Y., Almomani F., Moradi M., Dragoi E.N. (2022). Decontamination of toxic Malathion pesticide in aqueous solutions by Fenton-based processes: Degradation pathway, toxicity assessment and health risk assessment. J. Hazard. Mater., 423: 127016.

Veisi S., Johari S.A., Tyler C.R., Mansouri B., Esmaeilbeigi M. (2021). Antioxidant properties of dietary supplements of free and nanoencapsulated silymarin and their ameliorative effects on silver nanoparticles induced oxidative stress in Nile tilapia (*Oreochromis niloticus*). Environ. Sci. Pollut. Res., 28: 26055–26063.

Xiong D., Fang T., Yu L., Sima X., Zhu W. (2011). Effects of nano-scale TiO_2, ZnO and their bulk counterparts on zebrafish: acute toxicity, oxidative stress and oxidative damage. Sci. Total Environ., 409: 1444–1452.

Xue M., Luo L., Wu X., Ren Z., Gao P., Yu Y., Pearl G. (2006). Effects of six alternative lipid sources on growth and tissue fatty acid composition in Japanese sea bass (*Lateolabrax japonicus*). Aquaculture, 260: 206–214.

Yano T. (1992). Assays of hemolytic complement activity. Tech. Fish. Immunol., pp. 131–141.

Yonar S.M., Ural M.Ş., Silici S., Yonar M.E. (2014). Malathion-induced changes in the haematological profile, the immune response, and the oxidative/antioxidant status of *Cyprinus carpio carpio*: Protective role of propolis. Ecotoxicol. Environ. Saf., 102: 202–209.

Yousefi M., Hoseini S.M., Vatnikov Y.A., Nikishov A.A., Kulikov E.V. (2018). Thymol as a new anesthetic in common carp (*Cyprinus carpio*): Efficacy and physiological effects in comparison with eugenol. Aquaculture, 495: 376–383.

Yousefi M., Ghafarifarsani H., Hoseinifar S.H., Rashidian G., Van Doan H. (2021). Effects of dietary marjoram, *Origanum majorana* extract on growth performance, hematological, antioxidant, humoral and mucosal immune responses, and resistance of common carp, *Cyprinus carpio* against *Aeromonas hydrophila*. Fish Shellfish Immunol., 108: 127–133.

Yousefi M., Ghafarifarsani H., Hoseini S.M., Hoseinifar S.H., Abtahi B., Vatnikov Y.A., Kulikov E.V., Van Doan H. (2022). Effects of dietary thyme essential oil and prebiotic administration on rainbow trout (*Oncorhynchus mykiss*) welfare and performance. Fish Shellfish Immunol., 120: 737–744.

Yousefian M., Sheikholeslami A., Kor Davood M. (2011). Serum biochemical parameter of male, immature and female Persian sturgeon (*Acipencer persicus*). Aust. J. Basic Appl. Sci., 5: 476–481.

Zadmajid V., Mohammadi C. (2017). Dietary thyme essential oil (*Thymus vulgaris*) changes serum stress markers, enzyme activity, and hematological parameters in gibel carp (*Carassius auratus gibelio*) exposed to silver nanoparticles. Iran. J. Fish. Sci., 16: 1063–1084.

Zargar A., Rahimi-Afzal Z., Soltani E., Taheri Mirghaed A., Ebrahimzadeh-Mousavi Soltani M., Yuosefi P. (2019). Growth performance, immune response and disease resistance of rainbow trout (*Oncorhynchus mykiss*) fed *Thymus vulgaris* essential oils. Aquac. Res., 50: 3097–3106.

Zarzuelo A., Crespo E. (2002). The medicinal and non-medicinal uses of thyme. In: Thyme. CRC Press, pp. 277–306.

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