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Research Article

Effects of *Nigella sativa* Oil on Immune Mechanisms in Rainbow Trout, *Oncorhynchus mykiss*

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Abstract: The purpose of this study was to evaluate the efficacy of *Nigella sativa* oil on serum responses in rainbow trout. Fish were injected intraperitoneally (i.p.) with 1% and 10% doses of NSO. Serum bactericidal activity, lysozyme activity, myeloperoxidase, total protein and total immunoglobulin levels were analyzed 3, 7, 14 and 21 days after injection. The same procedure was performed in a control group. There were significant changes in all immunological parameters among the treatment groups except for total protein and immunoglobulin levels. In conclusion, the present study showed that the immune parameters of rainbow trout were increased. Therefore, injection of *Nigella sativa* oil leads to a positive effect on the immune response of rainbow trout.

Keywords: Rainbow trout; *Oncorhynchus mykiss*; *Nigella sativa* oil; immune response

Araştırma Makalesi

Nigella sativa Yağının Gökkuşağı Alabalığı (*Oncorhynchus mykiss*)'nın Bağışıklık Mekanizmaları Üzerindeki Etkileri

Özet: Bu çalışmanın amacı, Nigella sativa yağının gökkuşağı alabalığında serum yanıtı üzerindeki etkinliğini değerlendirmektir. Balıklara %1 ve %10 dozlarında NSO intraperitoneal (i.p.) olarak enjekte edilmiştir. Serum bakterisidal aktivite, lizozim aktivitesi, miyeloperoksidaz, total protein ve total immünoglobulin seviyeleri enjeksiyondan 3, 7, 14 ve 21 gün sonra analiz edilmiştir. Aynı prosedür bir kontrol grubunda da gerçekleştirilmiştir. Tedavi grupları arasında total protein ve immünoglobulin seviyeleri hariç tüm immünolojik parametrelerde anlamlı

değişiklikler olmuştur. Sonuç olarak, bu çalışma gökkuşağı alabalığının immün parametrelerinin arttığını göstermiştir. Bu nedenle, Çörek otu yağı enjeksiyonu gökkuşağı alabalığının immün yanıtı üzerinde olumlu bir etkiye yol açmaktadır.

Anahtar Kelimeler: Gökkuşağı alabalığı; *Oncorhynchus mykiss*; *Nigella sativa* yağı; bağışıklık yanıtı

1. Introduction

Intensive culture systems, characterized by associated stress and various husbandry risks, significantly increase the risk of infection. In addition, the high density of fish in aquaculture exacerbates the spread of disease [1]. A significant number of plants have been used in traditional medicine for the treatment and control of a wide range of diseases, including [2]. Many medicinal plants possess the ability to enhance the immune system has been demonstrated including *Nigella sativa* [3]; black cumin oil [4]; *Allium sativum* [5], *Muscari comosum* [6], *Zingiber officinale*, *Urtica dioica* and *Viscum album* [7] in several fish species. The results indicate that the plants studied have significant potential as alternative therapies for bacterial fish diseases.

Nigella sativa is an annual herbaceous plant of the Ranunculacceae family, commonly known as black cumin seed. It has been traditionally used for centuries in the world for the treatment of various diseases [8]. *Nigella sativa* and its oil showed antioxidant [9], bronchodilatatory and anticholinergic [10] anticancer [11], hepatoprotective [12], anti-ulcer [13], antiviral [14], anti-inflammatory [12], anti-diabetic [15] improves growth performance [16] and has immunomodulatory properties [3].

The supplementation of black seed oil derivatives in fish has been demonstrated to significantly enhance growth performance and immune system function [17]. The addition of black cumin to fish feed has been demonstrated to positively influence the growth rate of fish, while simultaneously reducing microbial activity during storage [18]. It has been demonstrated that black cumin has the capacity to diminish the production of ammonia and biogenic amines during the storage of fish meat [19].

Black seed oil boosts the immune system thanks to its thymoquinone [20] and antioxidant [21] compounds. Black cumin oil added at 1% in the diet significantly improved fish growth, hemato-biochemical parameters, and histopathologic tissues [22].

It was established that the nutritional attributes of black cumin oil were efficacious enough to elevate protein levels even when employed at minimal ratios in groups administered via the feeding method [23].

Adding black cumin oil to fish feed is recommended to increase the growth rate of fish and to protect fish health [24].

The aim of this study was to evaluate the effect of *Nigella sativa* oil (NSO) on serum bactericidal activity, lysozyme activity, myeloperoxidase (MPO), total protein and total immunoglobulin levels of rainbow trout.

2. Materials and Methods

2.1. Experimental fish

The research was conducted at the Fish Disease Laboratory of Kahramanmaras University, Faculty of Agriculture, and Fisheries Department. Fish were obtained from a commercial fish farm in Kahramanmaras and brought alive to the laboratory where the research was conducted. Approximately 200 rainbow trout with an average weight of 40±1.44 g were used. The fish were kept in 600 L fiberglass tanks with dimensions of 120x10x50 cm. Prior to the experimental study, the fish were acclimated for two weeks to adapt to the study conditions. During the acclimatization period, the fish were fed commercial trout feed (Abalıoğlu Blueaq, Denizli, Türkiye) twice a day until satiation.

2.2. *Nigella sativa* oil (NSO)

The black cumin (*Nigella sativa* L.) oil employed in the experiment was procured from a commercial enterprise and produced via cold pressing. The dose of black cumin oil employed in the study was determined in accordance with the findings of previous studies [23]. Solutions were prepared with 1% and 10% of black cumin oil.

2.3. Experimental fish groups

The experiment was maintained in 600 L tanks. The fishes were divided into four groups (two is the control and two experimental groups) of 50 fish each groups.

- Group I (negative control): Control fish (without *Nigella sativa* oil and sunflower oil)
- Group II (positive control): Control fish (Fish were intraperitoneal injection with 100µl of sunflower oil)
- Group IV (*Nigella sativa* oil, 1.0%): Fish were intraperitoneal injection with 100µl of 1.0% *Nigella sativa* oil.
- Group V (*Nigella sativa* oil, 10.0%): Fish were intraperitoneal injection with 100µl of 10.0% *Nigella sativa* oil.

2.4. Water quality parameters

During the research period, water temperature was measured regularly with a 0.1 precision thermometer (WTW, Monoline Oxi 3310), pH with a 0.003 precision digital pH meter (HACH, HQ11d) and dissolved oxygen levels with a 0.01 precision oxygen meter (WTW, Monoline Oxi 3310). Throughout the experiment, dissolved oxygen, average water temperature, and pH were measured as 8.5±0.14 ppm, 12±0.22 °C, and 7.8±0.09 respectively.

2.5. Collection of Samples

At the end of the experimental period of 3, 7, 14, and 21 days, the fish were sedated with an anesthetic substance (2- phenoxyethanol). Analyses were performed on seven fish from each group at 3, 7, 14, and 21 days of exposure. Fish were not fed on sampling days. Blood was collected from the tail. For serum separation, blood was transferred to serological tubes. The tubes were kept at room temperature for two hours and then overnight at 4ºC. The samples were centrifuged at 2500 rpm for 10 minutes. Serum was collected and stored at -20ºC.

2.6. Immunology study

Serum antibacterial activity against *Yersinia ruckeri* was determined according to Zhang et al. Lysozyme activity was determined according to Zhang et al [25] with slight modifications. Total serum myeloperoxidase activity was determined according to Quade and Roth [26] and Sahoo et al. [27] Total protein levels were determined by the Biuret method [28, 29]. Total immunoglobulin levels were determined by the method previously published by Siwicki et al [28].

2.7. Statistical analysis

All experiments were performed in triplicate, and the means and standard deviations of the immune parameter data were calculated from the experimental data obtained. The mean significance of the immune parameters for the experimental groups was analyzed by analysis of variance (ANOVA). Differences between means were considered significant when $P < 0.05$.

3. Results and Discussion

Serum bactericidal activity and lysozyme activity were significantly increased in fish injected with 10.0% NSO (Fig. 1 and Fig. 2). Lysozyme activity on days 3 and 7 was similar in the experimental group. There was a significant increase in serum lysozyme activity ($P < 0.05$) compared to the control group. On days 14 and 21, serum lysozyme activity had decreased but increased significantly compared to control values ($P < 0.05$). In lysozyme activity, the maximum level was 2.06 ± 0.23 ml/mg (in the 1.0% NSO group, day 7) and the minimum level was 1.29 ± 0.40 ml/mg (in the 10.0% NSO group, day 3) in the experimental group (Fig. 2).

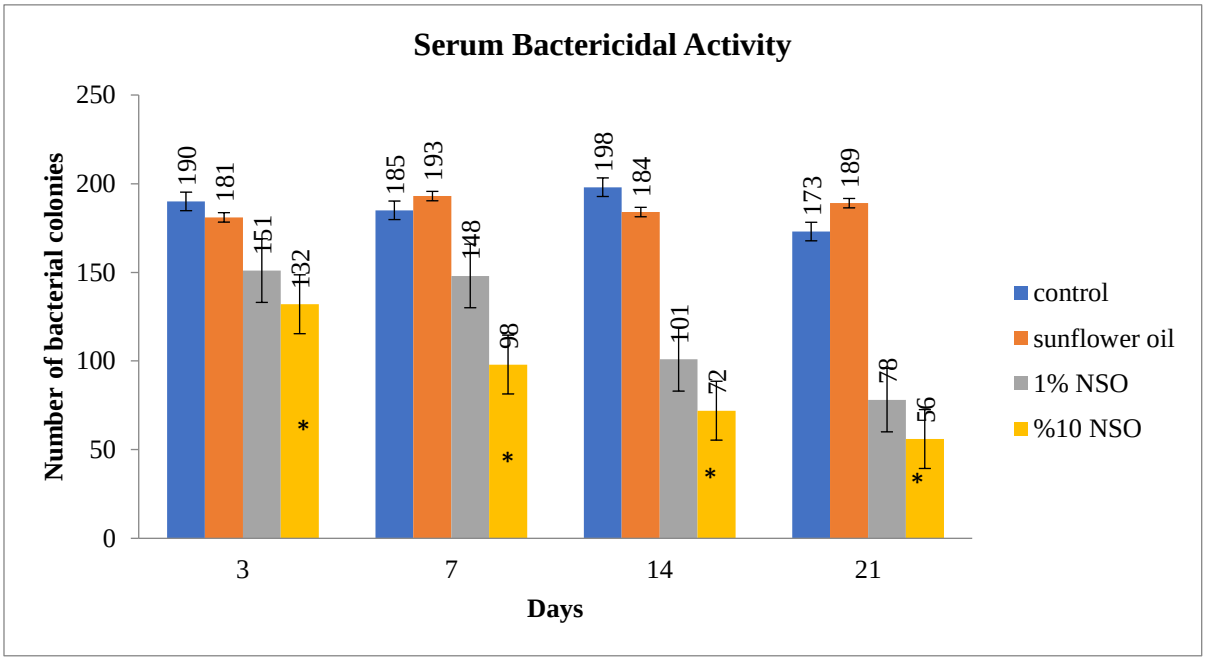

Figure 1. Changes in serum bactericidal activity of rainbow trout on the days after injection with NSO

* Significant difference compared to the control groups $P < 0.05$.

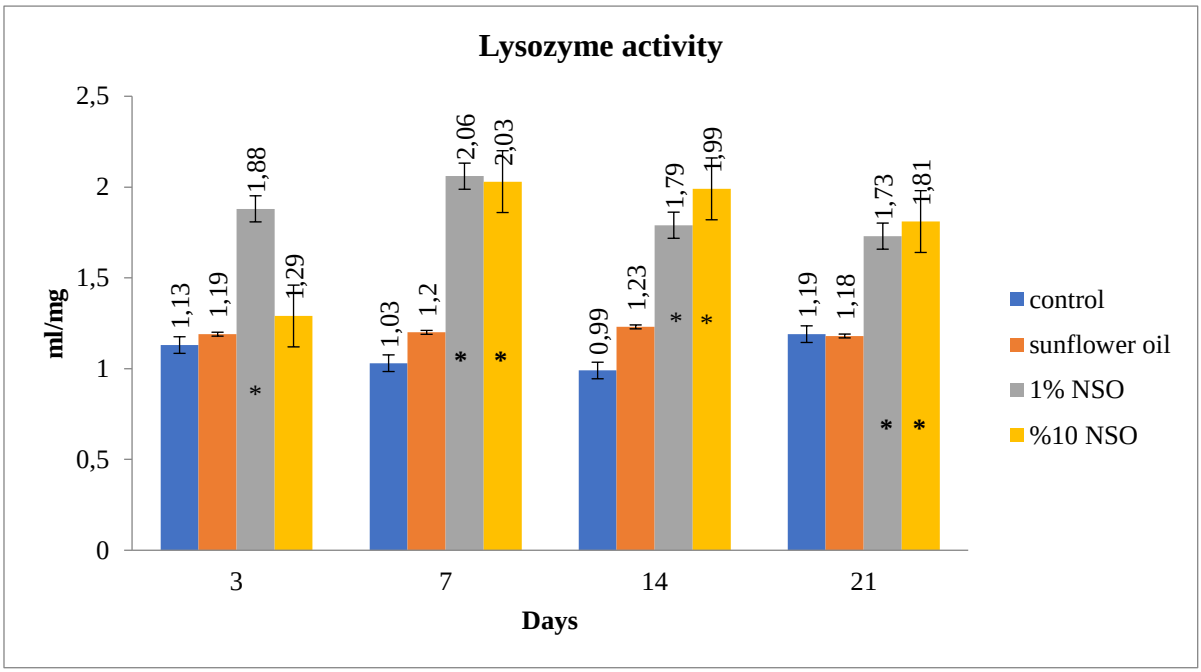

Figure 2. Changes in lysozyme activity of rainbow trout on the days after injection with NSO.

* Significant difference compared to the control groups P < 0.05.

Myeloperoxidase levels in rainbow trout were affected by NSO. MPO levels were significantly increased when rainbow trout were injected with NSO at all times tested. The maximum values of myeloperoxidase activity were 0.813, 0.818, 0.238, and 0.203 (OD at 450 nm) for the experimental and control groups, respectively (Fig. 3).

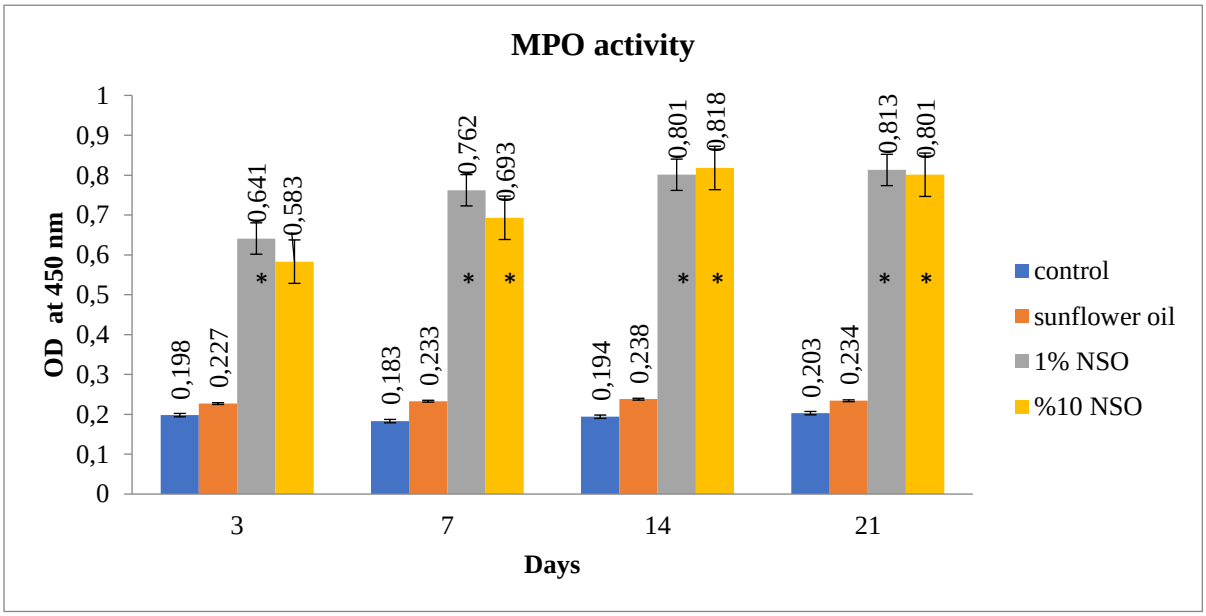

Figure 3. Changes in myeloperoxidase content of rainbow trout on the days after injection with NSO

* Significant difference compared to the control groups P < 0.05.

In the experimental group, total protein and total Ig levels at 3, 7, 14 and 21 days after injection are shown in Fig. 4 and Fig. 5. The total protein and total Ig levels in the NSO-injected group were not significantly different (P > 0.05) from those in the control group.

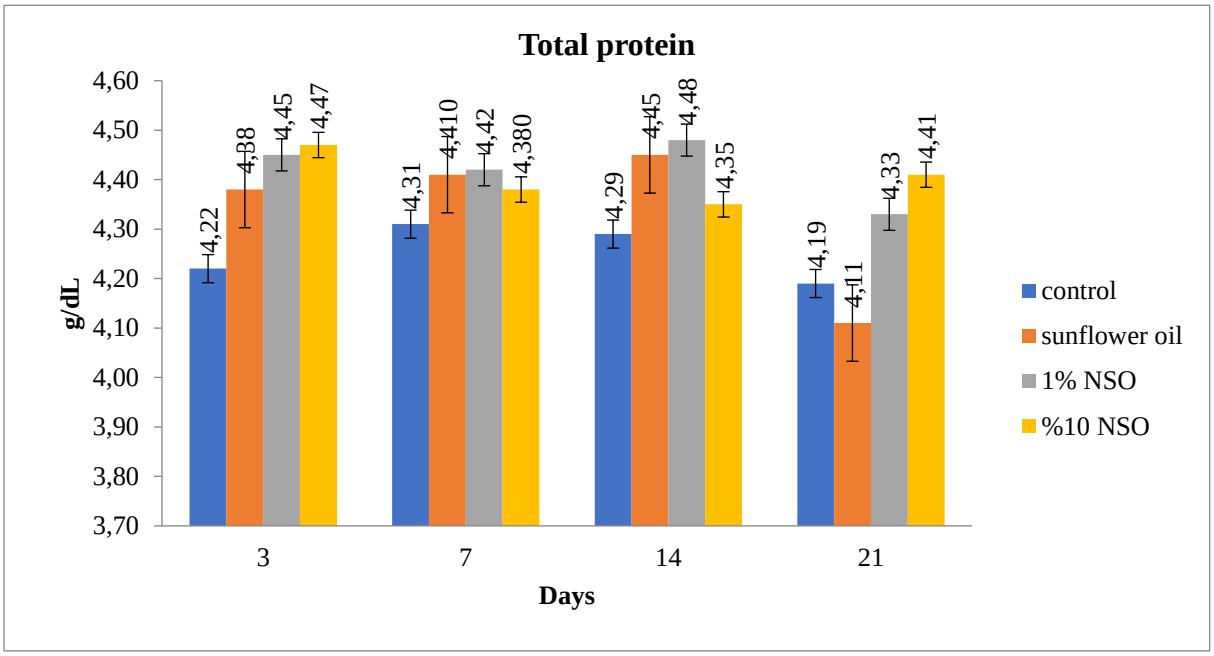

Figure 4. Changes in total protein levels of rainbow trout on the days after injection with NSO

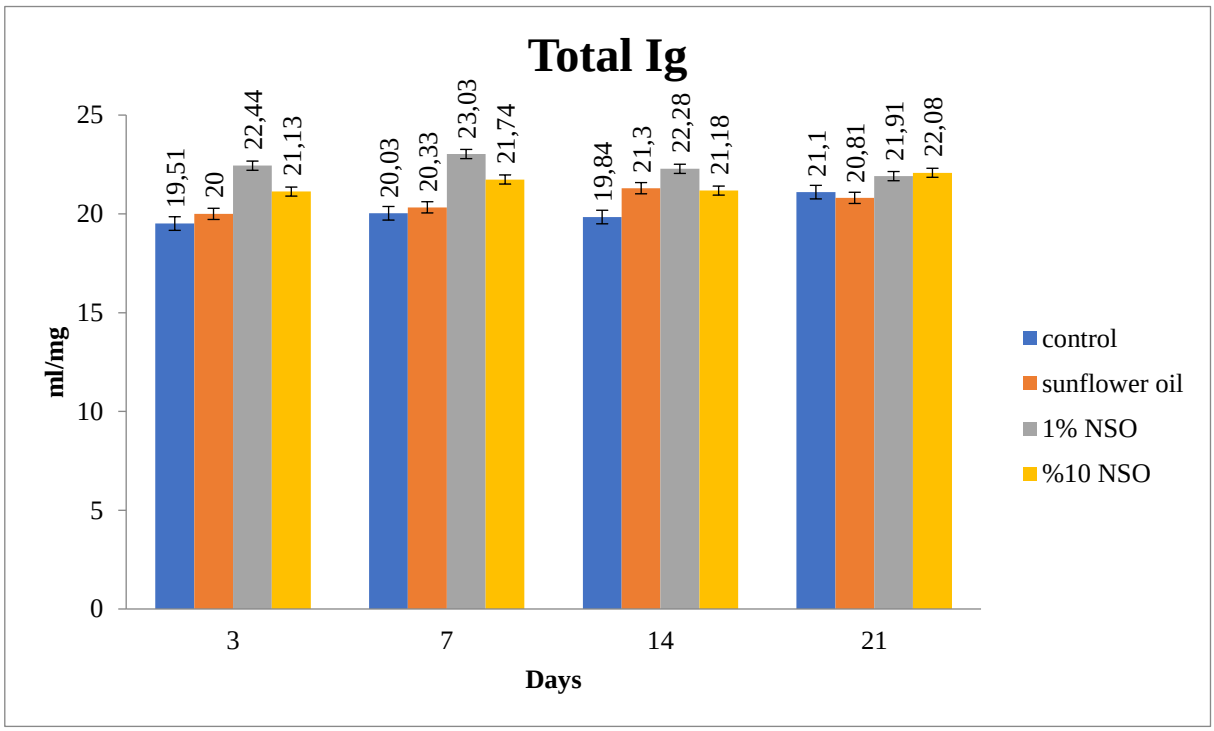

Figure 5. Changes in total Immunoglobulin levels of rainbow trout on the days after injection with NSO

Influence of intraperitoneal injection administration of *Nigella sativa* oil at doses of 1% and 10% was evaluated on some immunological factors (Bactericidal activity, Myeloperoxidase activity and Lysozyme activities; Total protein and Total immunoglobulin) of rainbow trout.

Serum bactericidal activity represents a mechanism that enables the resistance of pathogen growth [30]. Climbing perch (*Anabas testudineus*) of average weight 25±5 g were fed for 1, 2 and 4 weeks with diet supplemented 20 ml (20%), 30 ml (30%) and 40 ml (40%) 100 g-1 of *Nigella sativa* Treatment groups fed the dose 30% *Nigella sativa* oil showed a significant enhancement in bactericidal activity [31]. Immunological parameters including bactericidal activity action of test fish increased after treatment [32]. Serum bactericidal activity in *Nigella sativa* administered groups increased in non-immunized fish on days 20 and 30 of treatment ($P < 0.05$) [33]. The effects of *Nigella sativa* were examined at doses of 2%, 4%, and 6% on immunological parameters, including bactericidal activity. The results of the present study revealed a significant increase in the bactericidal activity in day 14 with 4% *Nigella sativa* seed oil supplemented diet compared to the control group [34]. The objective of this study was to investigate the effect of bactericidal activity black cumin seed oil (*Nigella sativa*) at a rate of 1%, 2%, and 3% on the enhancement of immunity in rainbow trout (*Oncorhynchus mykiss* Walbaum). The results of this study revealed significantly higher serum bactericidal activity in treatment groups, especially in higher doses, i.e. and 3% of *Nigella sativa* oil [4]. In this study, serum bactericidal activity was significantly increased in fish injected with *Nigella sativa* oil at levels of 10.0% ($P < 0.05$). It is likely that the increase in their functions led to the improvement of serum protein levels.

The immune system comprises numerous components, and the activity of lysozyme is a significant indicator of innate immunity in fish. This enzyme is ubiquitous among living organisms [35]. Lysozyme has been demonstrated to function as an opsonin in fish, activating the complement system and phagocytes [36]. Awad et al. [4] studied Effect of black cumin seed oil (*Nigella sativa*) in a rate of 1%, 2% and 3% on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum) and investigated Humoral immune parameters including lysozyme. Groups fed the highest doses, i.e. 3% *Nigella sativa* oil, showed a highly significant difference ($P<0.05$) in lysozyme. The impact of *Nigella sativa* (0.5; 1.0; 2.5; 5.0; 10.0 and 20.0 g/kg) on the nonspecific defence mechanisms of rainbow trout (*Oncorhynchus mykiss*) was examined. With regard to lysozyme activity, the control group exhibited similarities across all groups in accordance with lysozyme activity. However, notable differences were observed between the experimental groups. The mathematical analysis revealed that the lysozyme activity was significantly elevated at the doses of 1.0 and 2.5 g/kg. In the 25-day feeding experiment, the highest lysozyme activity was observed in the 0.5 g/kg treatment group [37]. Çelik Altınoğlu et al. [38] determined significant increase in lysozyme in 0.1 and 0.5 g/kg dose group. Serum lysozyme activity increased significantly in immunized fish fed *Nigella sativa* supplemented diets for 20 and 30 days [33]. In this study, the lysozyme activity was similar on days 3 and 7 in the experimental group (1.0% and 10.0%). There was a significant increase in serum lysozyme activity ($P < 0.05$) compared to the control group. On days 14 and 21, serum lysozyme activity had decreased but increased significantly compared to control values ($P <0.05$).

Myeloperoxidase (MPO) is a critical enzyme that reflects the cellular immune response in fish and indicates antimicrobial activity [39, 40]. Awad et al. [4] investigated the impact of black cumin seed oil (*Nigella sativa*) at concentrations of 1%, 2%, and 3% on the enhancement of immunity in

rainbow trout. Additionally, they examined the effects on humoral immune parameters, including myeloperoxidase. All doses of *Nigella sativa* led to the highest significant myeloperoxidase ($P<0.05$), compared to the control which recorded the lowest value at all. Kumar et al. [41] It is abundantly present in fish neutrophils. In the current study, myeloperoxidase levels in rainbow trout were affected by *Nigella sativa* oil. The Myeloperoxidase content was significantly increased when rainbow trout were injected with *Nigella sativa* oil for all the times tested. In agreement with this study, the activity of Myeloperoxidase increased significantly in common tilapia fed with diets supplemented with different levels of aqueous extract of false daisy leaf for one week. However, no significant increase was observed when the feeding period was extended to two or three weeks [42].

Total serum protein is a key indicator of the general health of fish. [43]. The study conducted by Awad et al. [4] demonstrated that the administration of *Nigella sativa* oil resulted in an elevation of total protein levels, particularly in groups that received higher doses, which exhibited the most significant increase compared to the control group. A notable elevation in total serum protein levels was observed in rainbow trout fed a basal diet supplemented with 1, 2.5, and 5% annual flowering plant (*Nigella sativa*) compared to the control group. The present study demonstrated that the total protein levels in the *Nigella sativa* oil injected group were not significantly different ($P > 0.05$) from those observed in the control group [3]. These results are different from Awad et al. [4] and Dorucu et al. [3]. The total protein concentration in fish serum varies depending on a number of factors, including the diet of the animal, its species, the time of year, its degree of sexual maturity, and the temperature of the water in which it lives [44].

Serum immunoglobulin is an important part of humoral immune system of vertebrates [45]. Total Ig levels in the *Nigella sativa* oil injected group was not significantly different ($P > 0.05$) than that in the control group. The findings presented here differ from those reported by Dorucu et al. [3], who observed a significant elevation in total immunoglobulin levels in rainbow trout fed a basal diet supplemented with 1, 2.5 and 5% of the annual flowering plant *Nigella sativa*, in comparison to the control group. It has been previously reported that there is considerable individual variation in serum Ig levels among fish. Some changes may be related to environmental conditions [46, 47] or disease status [48, 49], size and/or age [50, 51].

4. Conclusions

Herbal extracts have a potential application as immunostimulants in fish culture mainly because they are readily available, inexpensive, and effective against a wide range of pathogens. Most herbs and herbal extracts are the most convenient method of immunostimulation. However, the effect is dose-dependent and there is always a potential for overdose, so dosage optimization is strongly recommended. The use of such plant products as immunostimulants in fish culture systems may also be of environmental value due to their biodegradability. Due to their beneficial properties, we conclude that herbal extracts and animal-derived products can be used in fish culture as alternatives to vaccines, antibiotics or chemotherapeutic agents. The results of the present study revealed that the administration of apricot oil (1% and 10%) by the injected route is a potential method in rainbow trout culture to enhance the resistance of rainbow trout. Further studies are needed to elucidate the bioavailability of apricot kernel oil and its role in immune response and disease resistance in fish.

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Ethical approval

All fish studies were approved by the Experimental Ethics Committee of the Faculty of Agriculture, Sütcü Imam University (KSÜZİRHADYEK) (Protocol number: 2015/5-1).

Conflict of Interest

The authors declare that they have no conflicts of interest regarding this article.

Statement of Research and Publication Ethics

The authors declare that this study complies with research and publication ethics.

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