

Original Article

The Effect of Black Cumin Seeds, *Nigella sativa*, on the Immune Response of Rainbow Trout, *Oncorhynchus mykiss*

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Abstract

Using of natural plants as immunostimulant in fish is more useful than antibacterial drugs that cause adversely side effects for fish, environment and consumers. Therefore, we investigated the effect of black cumin seeds, *Nigella sativa*, as a herbal medicine plant on some immuno-hematological parameters and specific as well as non-specific defence mechanisms of rainbow trout, *Oncorhynchus mykiss*. Fish were fed with basal diet incorporated with 1, 2.5 and 5% *Nigella sativa* for 21 days. At the end of experiment, the hematocrit (Hct), leukocyte levels, glass-adherent NBT positive cell activation, serum protein and total immunoglobulin level were determined in fish blood. No significant differences ($P > 0.05$) were found in hematocrit levels of fish fed the diets containing 1 and 2.5% black cumin seeds, but it was significantly ($P < 0.05$) increased in fish receiving diet containing 5% black cumin seeds compared to the control. Glass-adherent NBT positive cell activation and leukocyte level in black cumin supplemented groups showed no significant differences ($P > 0.05$) when compared to the control group. The serum protein and total immunoglobulin levels were significantly ($P < 0.05$) higher than those of the control group. In conclusion, based on the current findings together with the low cost and the immunostimulative effect of black cumin seed, it is recommended to be used in fish feed to minimize the mortalities caused by some pathogens.

Key Words: Black cumin seed, *Nigella sativa*, Rainbow trout, Specific immunity, Non-specific immunity.

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Introduction

Fish are exposed to some pathogenic microorganisms since they survive in an unfavorable environment. Hence, defence system of fish is very important against microorganisms. The use of antibacterial drugs in aquaculture is risky due to cross resistance against pathogens and residues in tissues (Gyles, et al. 1977). Therefore, using organic, inorganic and synthetic matters as immunostimulant has increased in recent years. For this purpose, levamisole (Findlay and Munday, 2000 and Cuesta, et al. 2004), glucan (Jeney and Anderson, 1993 and Dalmo and Seljelid, 1995), vitamin C and E (Blazer and Woicke, 1984; Hardie, et al. 1990 and Ortuno, et al. 2001), chitin (Sakai, et al. 1992), FK-565 (Lactoyl tetrapeptide) (Kitao, et al. 1987) FCA (Olivier, et al. 1985 and Kajita, et al. 1992) have been used as immunostimulants and investigations are still being carried out on these substances.

The use of plants as medicines began during the earliest years of human evolution. *Nigella sativa* Linn., commonly known as the black cumin seed, is an annual herb that belongs to the botanical family of Ranunculaceae (Al Jishi and Hozaiifa, 2003). It has been employed for thousands of years as spice and food preservative, as well as a protective and curative remedy for numerous disorders. It is known to have many medicinal properties in traditional medicine (Salem and Hossain, 2000). Wild growth of this plant is widespread in southern Europe, northern Africa and Asia (Salem, 2005). The diuretic, antihypertensive, antibacterial and anthelmintic effects of black cumin seed in human and animals have been investigated (Agarwal, et al. 1979; Hanafy and Hatem, 1991; Zaoui, et al. 2000 and Mahmoud, et al. 2002). Additionally, antidiabetic (Kanter, et al. 2003), antioxidant (El Saleh, et al. 2004) and anti-inflammatory (Zedlitz, et al. 2002) characteristics of *N. sativa* have been proved. Dugenci et al. (2003) studied the influence of mistletoe, *Viscum album*, nettle, *Urtica dioica* and ginger *Zingiber officinale* on immune system of rainbow trout,

O. mykiss. They found that immune activation was achieved in only fish fed with 1% nettle supplemented diet. Increasing proliferative resistance by producing interleukin-3 in human cells by black cumin seeds shows that this plant could be used as immunomodulatory (Haq, et al. 1995). Some researchers have used black cumin seeds as enhancer for performance, growth and immune system of some fish species (Abd Elmonem, et al. 2002; John, et al. 2007 and Diab, et al. 2008). The effect of black cumin seeds on immune system of rainbow trout was very scarce. Therefore, the aim of the present study was to examine if black cumin seeds, *Nigella sativa*, extract would influence some immunohematologic parameters and specific as well as non-specific immune response of rainbow trout.

Materials and Methods

A total of 120 rainbow trout of 34.43 ± 3.56 g mean initial body weight were obtained from the Freshwater Fish Production Unit of the IX. Regional Directorate of State Hydraulic Works (Keban/Elazığ). The fish were transferred to the laboratory of the Fisheries Faculty of Firat University. The fish were maintained in 400-L water recirculated tanks for adaptation for one week. Then, the healthy fish were divided into four groups (One is the control and three experimental groups) of 30 fishes each. *N. sativa* was added to experimental diets at levels 0, 1, 2.5 and 5% and fed to fish groups (1-4) respectively at a rate of 2% of fish body weight per day for 21 days. At the end of the study period, fish were anaesthetised with Benzocaine (50 ppm) and blood was collected by caudal vein puncture with heparinized syringes. Hematocrit and leukocyte levels were determined according to Konuk (1981), leukocrit, glass-adherent nitroblue tetrazolium (NBT) (+) cell activation, total protein and total immunoglobulin levels were measured according to Siwicki and Anderson (1993).

All data are expressed as mean±SD and Student's-t test was applied by using Minitab Software Release 14.

Results and Discussion

The effects of black cumin seeds, *Nigella sativa*, on hematocrit, leukocrit, leukocyte levels and glass-adherent NBT (+) cell activation were given in (Table 1), and on serum protein, total immunoglobulin levels are shown on (Fig. 1, 2). Hematocrit and leukocrit levels in the fish fed with 2.5% black cumin seeds added diet were found to be significantly ($P < 0.05$) higher than

the control group. However, leukocyte level in experimental groups was not significantly different ($P > 0.05$) comparing with the control group. In neutrophils of experimental fish group, there were higher numbers of NBT (+) cells fixed on lamellas than those of the control group, but it was not statistically significant ($P > 0.05$). Cells were not shiny, stained cloudy, blue light circle around cytoplasm and looked like polymorphonuclear leukocytes. Serum protein and total Ig levels in experimental groups showed significantly ($P < 0.05$) higher levels than the control group (Fig. 1, 2).

Table 1: Hematocrit, leukocrit, leukocyte levels and glass-adherent NBT (+) cell activation of fish fed on diet incorporated with *Nigella sativa*:

Group/Diet	Hematocrit level (%)	Leukocrit level (%)	Leucocyte number (10^3)	NBT (+) cell activation (number)
The control Only basal diet	22.2±3.12	1.9±0.56	43.60±6.80	27.0±1.41
Basal diet + 1% <i>Nigella sativa</i>	23.0±2.94	1.6±0.69	44.20±4.32	33.0±4.24
Basal diet + 2.5% <i>Nigella sativa</i>	23.5±2.27	2.0±0.47*3	40.40±4.57	29.5±3.54
Basal diet + 5% <i>Nigella sativa</i>	25.8±2.25* ^{k,1,2}	1.4±0.51	38.80±3.96	29.5±0.70

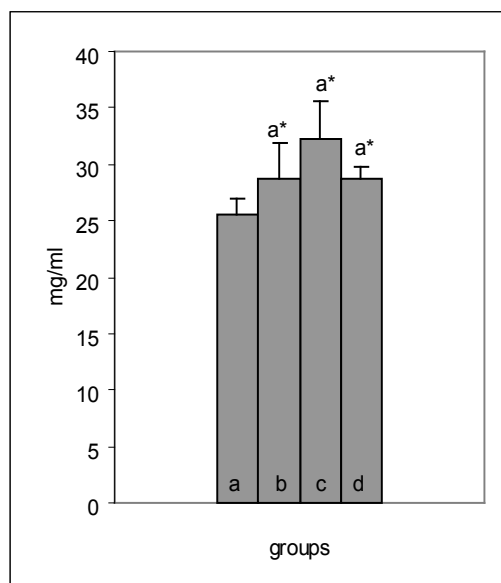


Fig. 1: Serum protein levels in rainbow trout fed with *N. sativa*.

a: the control, **b:** the group fed with basal diet+1% *Nigella sativa*, **c:** the group fed with basal diet+2.5% *Nigella sativa*, **d:** the group fed with basal diet+5% *Nigella sativa*. * $p < 0.05$.

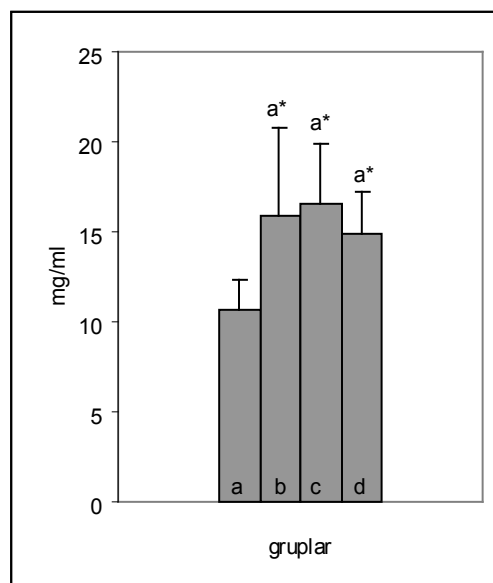


Fig. 2: Total Ig levels in rainbow trout fed with *N. sativa*.

a: the control, **b:** the group fed with basal diet+1% *Nigella sativa*, **c:** the group fed with basal diet+2.5% *Nigella sativa*, **d:** the group fed with basal diet+5% *Nigella sativa*. * $P < 0.05$.

The results showed that feeding with 1%, 2.5% and 5% black cumin seeds supplemented diets for three weeks stimulated the specific defence mechanisms of rainbow trout. Different immunostimulatic agents had no influence on hematocrit and leukocrit although it is accepted that they exist in immuno-haematology not in immune system (Dörücü, et al. 2005). Hematocrit level is an indicator for fish health which gives clue on fish health and explains abnormalities caused by immunostimulants. Statistically insignificant differences ($P > 0.05$) between experimental groups and the control group were recorded in this study confirming previous findings. The increase of hemoglobin and hematocrit levels have been reported in rats fed with black cumin seeds (Nair, et al. 1991 and Zaoui, et al. 2002a, b). In this study, there were no differences in hematocrit levels between 1% and 2.5% black cumin seed groups ($P > 0.05$), but there was a statistically significant increase ($P < 0.05$) in hematocrit level in 5% black cumin seeds group comparing with the control and the other experimental groups. It is well known that leukocrit level, which is an indicator of percentage leukocyte level in blood, is not affected by immunostimulants. There was no significant differences ($P < 0.05$) in leukocrit levels between experimental groups and the control group. Leukocyte levels in fish fed with black cumin seeds added diet were not significantly different ($P > 0.05$) comparing with control group. A significant decrease was reported in serum cholesterol, triglycerides, glucose levels, leukocyte and thrombocyte numbers of rats fed with black cumin seeds added diet (Nair, et al. 1991 and Zaoui, et al. 2002a, b). Our findings showed no changes in the leukocyte levels of experimental groups when compared to the control group.

The peripheral blood neutrophils take an important role in non-specific defence mechanisms. These cells adhere on tissue surface by producing adhesive proteins that facilitate migration in problematic zones (Anderson, et al. 1992). Neutrophils play an important role in producing O_2^- - and OH^- - oxygen radicals

by respiratory burst which is a substantial mechanism for eliminating the pathogenic microorganisms.

Glass-adherent NBT (+) cell activation has been reported to increase after immunostimulation by an immunostimulant (Jeney and Anderson, 1993). Although a steady increase was recorded in NBT (+) cell activation in the treated groups, the difference was not statistically significant compared with the control group ($P > 0.05$).

The serum protein level is an important indicator of humoral defence system of fish and increases especially in the fish fed with plant extracts. As a matter of fact, Dugenci et al. (2003) reported that serum plasma protein level was high in fish fed with 1% ginger added diet. The effects of ginger on human serum protein have also been detected (Haq, et al. 1995). High serum protein levels in blood of ginger applied experimental fish groups compared with the control group ($P < 0.05$) agreed with the results of previous studies. The specific immunity is being led by humoral releasing of antigen-antibody by B-cells, cellular by auxiliary T ($CD4^+$) and cytolytic T ($CD8^+$) cells. Serum immunoglobulin is an important part of humoral immune system of vertebrates (Salem, 2005). In this study, a significant increase ($P < 0.05$) was seen in total immunoglobulin levels of experimental fish groups. Previous studies have found that, IgM levels differ according to fish species, even between individuals of the same fish species. This difference could be related to size and age of fish (Klesius, 1990 and Picchiatti, et al. 2001), environmental factors (Klesius, 1990 and Magnadottir, et al. 1999) and diseases (Nielsen, 1999). Immunomodulators affect production of circulating IgM. Cuesta et al. (2004) reported that IgM production in *Sparus aurata* fed with vitamin A and levamisole added diet started in second week and increased continuously during six weeks. In this study, the increase of total immunoglobulin level after three weeks period of feeding agreed with the results of other studies.

Conclusion

Although black cumin seed is known as an effective immunomodulator, it exhibit a moderate activity in this study. However, considering its low cost and immunostimulative effect, black cumin seed could be recommended to be used for farmed fish to decrease mortalities caused by pathogenic microorganisms.

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