E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2022; 10(4): 01-06

www.fisheriesjournal.com
Received: 03-05-2022
Accepted: 04-06-2022

Rosidah
Staff at Fisheries Department, Faculty of Fishery and Marine Science, Universitas, Padjadjaran, Bandung Indonesia, Indonesia

Fittrie Mellianawaty Pratiwy
Staff at Fisheries Department, Faculty of Fishery and Marine Science, Universitas Padjadjaran, Bandung Indonesia, Indonesia

Corresponding Author:
Rosidah
Staff at Fisheries Department, Faculty of Fishery and Marine Science, Universitas Padjadjaran, Bandung, Indonesia, Indonesia

A mini-review: Potential of *Nigella sativa* L. For immune fish against pathogen bacteria attack

Rosidah and Fittrie Mellianawaty Pratiwy

DOI: https://doi.org/10.22271/fish.2022.v10.i4b.2695

Abstract
Improving the immune system in fish as an effort to prevent fish from disease attacks caused by pathogenic organisms, because disease attacks on fish are projected to continue to grow and cause mass death of cultured fish. Giving immunostimulants as additives to feed can be a strategy to improve the fish's immune system. Immunostimulants are compounds that are able to increase the non-specific immune response or the innate immune response. *Nigella sativa* L known as "Black Cumin" or Black Seed is one of the natural ingredients that can be used as medicine, including as an immunostimulant to increase the immune system which can prevent disease attacks. The part of the black cumin plant that is efficacious as a medicine for its seeds. The active compounds contained in *N. sativa* seeds are Thymoquinone, Dithymoquinone, Thymohydriquinone, and Thymol. Nigella sativa can increase the number of white blood cells, such as neutrophils, lymphocytes, and monocytes as an indicator of an increase in the immune system of fish. Several studies have shown that *N. sativa* can be used in the field of fisheries, especially to increase the body's resistance to disease. So the purpose of this article is to explain *N. sativa* as an immunostimulant in cultured fish that can increase the fish's body resistance to disease attacks. Based on the above studies, *N. sativa* L. seeds in powder or oil form can significantly increase the immune response, reduce mortality and increase the survival rate of fish infected with pathogenic organisms. Therefore, *N. sativa* L. seeds have the potential to be used as a dietary supplement immunostimulant to help increase the body's resistance to various types of cultured fish against disease-causing pathogenic organisms. The dose given must be adjusted to the type of fish and the disease.

Keywords: Fish, *nigella sativa* L, immune system, disease.

Introduction
In fish farming, disease problems become the main focus, because it has an impact on decreasing production due to growth retardation and fish death. It is projected that this disease problem will continue to increase until [1]. This situation is very detrimental to cultivators. So far, the use of antibiotics is considered the most appropriate and effective solution to overcome diseases in cultured fish, but if used for a long time with inappropriate doses, it will have an impact on bacterial resistance to the antibiotics given. In addition, the use of antibiotics in large-scale aquaculture is very inefficient because they are relatively expensive, have an impact on pollution of the aquatic environment and can accumulate in fish bodies [2]. Based on the negative impact of the use of antibiotics, it is necessary to make efforts to avoid the use of antibiotics, one way that can be done is to increase the body's resistance of fish to prevent disease.

Immunostimulants can be used to increase the fish's body resistance to disease, because immunostimulants are compounds that can induce the fish's immune system to fight disease attacks that enter the body, so that fish are protected from disease [3, 4]. Several natural ingredients from plants and animals have been used as an alternative to increase the fish's body resistance which is quite effective against disease. In addition, the use of natural materials is relatively cheaper, safe for the environment and also safe for human consumption. One source of natural immunostimulants that are safe for fish and the environment are *Nigella sativa* L. Seeds known as "Black Cumin" or Black Seed. Nigella sativa is a plant from the Ranunculaceae family, originating from West Asian and Mediterranean countries with subtropical climates [5, 6].

N. sativa seeds contain fatty oils, essential oils, carbohydrates, minerals, saponins, alkaloids and other vitamins. [7]. However, the main active ingredients of *N. sativa* seeds are essential oils consisting of thymoquinone, dithymoquinone, thymohydroquinone, p-cymene, and thymol [7, 8]. On the basis of the active ingredients it contains, besides being an immunostimulant, *N. sativa* also has other pharmacological activities, including antibacterial [4, 9], antioxidant, anticancer and anticholesterol [10]. The pharmacological activity of *N. sativa* seeds as an immunostimulant is related to its ability to increase the number of leukocytes, where leukocytes are part of the immune system that has an important role in fighting disease agents. The main function of leukocytes is to destroy pathogenic organisms that cause disease through the process of phagocytosis and forming antibodies [11, 12].

Based on the pharmacological activity of Nigella sativa, the purpose of writing this article is to reveal the potential of *N. sativa* as an immunostimulant to increase the body's resistance/immunity of cultured fish against diseases caused by pathogenic bacteria.

Classification and morphology of *Nigella sativa* L.

Nigella sativa L. (Figure 1) is a species of the genus Nigella which has approximately 14 plant species belonging to the family Ranunculaceae [13], including *Nigella arvensis*, *Nigella ciliaris*, *Nigella damascena*, *Nigella hispanica*, *Nigella integrifolia*, *Nigella nigellastrum*, *Nigella orientalis*, and *N. sativa* [14]. Nigella sativa L. is known as black cumin (English), habbatussauda (Arabic), kalonji (India). *N. sativa* seeds have been used for hundreds of years by the people of the Middle East and several Asian countries to treat various diseases in humans [15, 16]. The classification of *N. sativa* L. plants is as follows:

Kingdom	:	Plantae
Divisio	:	Magnoliophyta
Kelas	:	Magnoliopsida
Ordo	:	Ranunculales
Famili	:	Ranunculaceae
Genus	:	Nigella
Spesies	:	*Nigella sativa* L [13]

(Source: 17)

Fig 1: Black Cumin *Nigella sativa* L

N. sativa L. is a medicinal plant originating from West Asia and the Mediterranean with a subtropical climate. Black cumin plant stems have an upright shape, green color, soft, grooved, ribbed, and coarse hair. This plant has leaves with an oval shape about 1.5-2 cm long, single leaves with a pointed base and tip and green. The number of petals is generally five, with a bluish-white color. Nigella sativa seeds have an oval shape with blackish brown color [18]. *N. sativa* L seeds are inside the fruit which has an ovoid or slightly rounded shape. Nigella sativa L forms a fruit capsule consisting of several white trigonal seeds. When the fruit capsule is ripe, the capsule will open and the seeds inside will pop out and turn black [19].

The content of *Nigella sativa* L.

N. sativa L. seeds contain various constituents including water, fatty oil 22-38%, essential oil (essential) 0.40-1.50%, protein (eight of the nine essential amino acids) 20.8-31.2%, carbohydrates 24.9-40%, minerals 3.7-7%, saponins 0.013%, alkaloids 0.01% and vitamins 1-4%. However, the percentage of compounds contained in *N. sativa* seeds is determined by geographical location, harvest time, and cultivation method. The composition of the fatty oil is linoleic acid (Omega-6), oleic acid, palmitoleic acid, linolenic acid (Omega-3), myritoleic acid, dihomolionenic acid, stearic acid, eicosadienoic acid, myristic acid, arachidic acid, behanic acid, sterols, tocopherols (α, and) timoquinone, retinol (vitamin A), carotenoids (β-carotene). The composition of the essential oil (essential) consists of thymoquinone, dithymoquinone, thymohydroquinone, p-cymene, thymol carvakrol, -Pinene, -Pinene, longifolenen and t-Anethole. Protein contains compounds of glutamic acid, arginine, aspartic acid, leucine, glycine, valine, lysine, threonine, phenylalanine, isoleucine, histidine, methionine. The composition of carbohydrates consists of glucose, rhamnose, xylose, arabinose. Mineral content consists of calcium, phosphorus, iron, potassium, sodium, zinc, magnesium, manganese, copper, selenium. Saponin compounds consist of Hederagenin (melanthigenin), -Hederin (melanthin) and alkaloids consisting of Nigelicine, Nigellimine, Nigellidine, while the vitamin content consists of vitamin A, Thiamin, Riboflavin, Pyridoxine, Niacin, Folacin, Vitamin C [20, 7, 21, 22, 23, 24]. Compounds that have pharmacological activity contained in *N. sativa* L seeds are mostly contributed by thymoquinone, dithymoquinone, thymohydroquinone, p-cymene, and thymol which includes components of fatty (stable) and essential oils (essential/volatile) [7]. However, the most important bioactive is thymoquinone [25, 26]. The chemical structure of the active compound is presented in Figure 2.

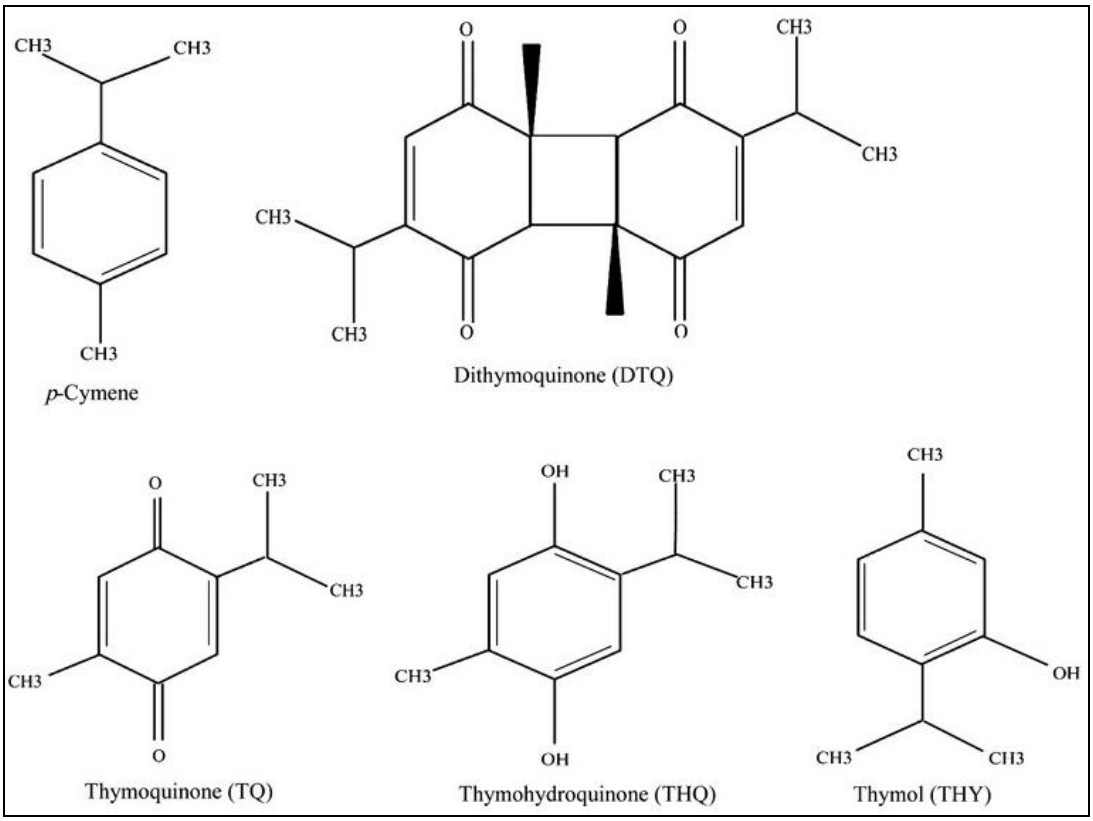

Fig 2: Chemical structures of major compounds from *N. sativa* L. Seed [7]

The benefits of *Nigella sativa* L

Traditionally in Arab countries, Far East Asia, Europe, and Africa *N. sativa* has long been used to treat various diseases [27], among others, to treat respiratory disorders, chronic headaches, back pain, diabetes, paralysis, infections, inflammation and hypertension. In addition, it is also used to treat nasal inflammation, orchitis, eczema and joint swelling [28]. In addition, consumption of *N. sativa* is important to maintain kidney, bile, liver function, and also to improve the immune system [15, 16]. Another benefit of *N. sativa* L. is that it is used as a seasoning for various processed foods, such as cakes, yogurt, pickles, sauces, and salads [29].

Several studies have reported that *N. sativa* has a number of therapeutic compounds and has pharmacological activity, including as an effective antioxidant both *in vitro* and *in vivo* [30], antidiabetic agents demonstrated histologically in the pancreas of rats had increased pancreatic cell degeneration after being given *N. sativa* [31], antihypertensive [32], has proven neuroprotective effect in managing depression [33], and other neurological disorders including Alzheimer's disease [34], Parkinson's disease [35] and Epilepsy [36].

Other pharmacological activities are anti-inflammatory and analgesic, wherein essential oil and thymoquinone at various doses have dose-dependent anti-inflammatory activity [37] and show substantial pain-relieving effects in wriggling acetic acid, formalin, and tail fick test [38], antimicrobials which include antibacterial [39], antifungal [41, 42], antiviral [43, 44] and antiparasitic [45, 46]. The tymoquinone compound contained in *N. sativa* seeds has antibacterial activity with a broad spectrum against several strains of gram-positive and gram-negative bacteria, including Bacillus, Listeria, Enterococcus, Micrococcus, Staphylococcus, Pseudomonas, Escherichia, Salmonella, Serovar, and Vibrio parahaemolyticus. besides that it can inhibit the formation of bacterial biofilms [39], anticancer [47] and have an effect on Male Infertility [48]. Another opinion states that *N. sativa* contains fatty acids (Omega-3 and Omega-6) which function to help the formation of new cells. The zinc present in *N. sativa* can help the wound healing process by forming collagen [49]. Other pharmacological activities of *Nigella sativa* L apart from being antimicrobial anticancer, spasmolytic, hepatoprotective, and antioxidant, it also has activity as an immunomodulator [50]. *N. sativa* L. works as an immunomodulator by stimulating and strengthening the body's immune system by increasing the number, quality, and activity of the body's immune cells and also by modulating (repairing) the immune system [51] especially the content of thymoquinone is able to increase the proliferation and differentiation of lymphocyte cells which are immune cells [52]. Increased proliferation of lymphocyte cells as an indicator of increased activity of the immune system against infection [53]. Consumption of 1 gram of *N. sativa* seed twice a day can increase human immune function significantly [54].

***Nigella sativa* as immunostimulant for fish disease prevention**

As described above, *N. sativa* L seeds can act as an immunostimulant that can increase the body's immune system, with the aim of preventing disease attacks. As according to (Hendrik (2007) [51] *N. sativa* L seeds can increase and strengthen the fish's immune system against pathogenic bacterial attacks. *N sativa* L. contains nigellon and glutathione components which function to protect the body from various harmful foreign substances [55]. Several studies have proven that *N. sativa* L seeds have an immunostimulant effect for several types of cultured fish, so that they are prevented from attack by pathogenic bacteria. As the results of research that have been carried out, it shows that *Pangasianodon hypophthalmus* measuring 8+0.5 cm, experienced an increase in the number of leukocytes after being given *N. sativa* L seed flour at a dose of 15% through feed for 14 days, the number of leukocytes produced was

14.88 cells/mm3 . This dose was quite effective against the attack of *Aeromonas hydrophila* bacteria with a density of 10^6 CFU.mL-1 which was infected in catfish through immersion [56]. *Anabas testudineus* fish after being fed for 30 days enriched with 30% *N. sativa* oil showed the highest increase in immune response compared to other treatments, as seen from the significant increase in phagocytic activity ($P < 0.05$) the highest compared to other treatments. After being challenged with *A. hydrophila* bacteria containing 3.2×10^6 CFU ml 1 via intraperitoneal injection, it significantly resulted in the lowest fish mortality and the highest survival rate [57]. *N. sativa* seed powder at a dose of 2.5 g / kg of feed given for 15 days, showed the best results on immune parameters (leucocyte, lymphocyte, monocyte, granulocytes, mean cell hemoglobin concentration) in rainbow trout (*Oncorhynchus mykiss* Walbaum) sized 100-250 gram/individual both before and after being challenged with the bacteria of *Yersinia ruckeri* with a density of $1x10^7$ CFU mL^{-1} [58]. Another study reported that *N. sativa* L seeds with a dose of 3.0% and the addition of 0.04% *Bacillus subtilis* PB6 (cloSTAT) experienced a significant increase in the number of tilapia (*Oreochromis niloticus*) leukocytes, the number of leukocytes reached 44.53 ± 5.11 cells/mm^3 [59]. *Channa striata* measuring 10-12 cm fed with 3.5% *N. sativa* L. seed flour mixed for 14 days could prevent *A. hydrophila* bacteria attack (with a density of 10^8 CFU mL^{-1}) being infected, as evidenced by good survival. Reached 93.33% [49]. Tilapia (*Oreochromis niloticus*) fry fed with a mixture of 3.5% *N. sativa* seed flour for 14 days were resistant to *Streptococcus agalactiae* bacteria at a density of 10^7 CFU mL^{-1}, as evidenced by a fairly high survival rate post-infection, which is 90%, [60].

The results of another study showed that the addition of 2.5-5% *N. sativa* L seeds into the feed combined with the whole cell vaccine *A. salmonicida* was able to increase the imonogenicity of carp, indicated by the leukocyte cell count with the highest value, namely 51,883 cells/mm^3 [61]. Another study showed that *N. sativa* extract could be used as an immunostimulant and had a positive effect on increasing the resistance of common carp (*Cyprinus carpio*) to disease attacks caused by the bacterium *Pseudomonas fluorescens*, especially at a dose of 4%. This dose produced the highest phagocytic activity, namely 4.47%, a significant increase in bactericidal activity and also resulted in the highest survival (80%) after challenge with *P. fluorescens* bacteria at a density of 2.9 × 10-6 CFU ml^{-1} [62]. From the description above, it proves that *N. sativa* has an immunostimulant effect that can increase the fish's body resistance to disease attacks caused by pathogenic bacteria, indicated by an increase in the number of leukocytes. Leukocytes play a role in the immune response, if there are foreign substances that enter the body, the leukocytes will make antibodies. Antibodies will be used by the immune system to provide stimulation, identify and neutralize foreign objects (antigens) that enter, such as bacteria [63]. *N. sativa* can increase the body's resistance to several types of fish against attacks from different types of pathogenic bacteria. So each type of fish has a different response to the concentration of *N. sativa* given to increase the body's resistance to attack pathogenic organisms that cause disease. The mechanism of action of leukocytes and the components in them against pathogenic (infectious) organisms by forming antibodies, T lymphocytes and B lymphocytes. The function of T lymphocytes is responsible for the formation of activated lymphocytes that can form immunity. In the formation of immunity, T lymphocytes produce helper T cells, cytotoxic T cells and suppressor T cells. Helper T cells are the main regulators of all immune functions and suppressor T cells function to inhibit the activation and action of T cells and B cells [64]. The compounds thymoquinone (THQ) and thymol (THY) contained in N. sativa are efficacious in inducing a specific immune system that is able to increase levels of T helper cells, suppressor T cells and natural killer cells, all of which are lymphocytes. Lymphocyte activity is also influenced by chemical mediators produced by the immune response such as interleukin 1 (IL-1) produced by macrophages and IL-2 produced by activated helper T lymphocytes [65, 50]. B and T lymphocytes, helper T cells, suppressor T cells and cytotoxic T cells that have been formed will then phagocytose antigens (pathogens) that enter the body [66].

Conclusion

Based on the results of research and testing on several fish that have been described above, it appears that *Nigella sativa* seeds in powder or oil form can significantly increase the immune response, reduce mortality and increase the survival rate of fish infected with pathogenic organisms. Therefore, *N. sativa* seeds have the potential to be used as an immunostimulant supplement to help increase the body's resistance to various types of cultured fish against attacks by pathogenic organisms that cause disease. The dose given must be adjusted to the type of fish and the disease.

Competing Interests

Author has declared that no competing interests exist.

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