Probe - Fishery Science & Aquaculture 2024, 6(1), 2208.
https://doi.org/10.18186/fsa.v6i1.2208

Review

Azadirachta indica as a sustainable nutritional alternative in Nile tilapia farming—A mini review

Matheus Berlofa Ferreira[1], Paola Capistrano dos Santos[1], Gracienhe Gomes dos Santos[1], Gabriele Bueno dos Anjos[1], Graziella Vivine Gonçalves de Matos Silva[1], Ana Paula de Souza[1], Giulia Beatrice Ferreira[2], Marco Shizuo Owatari[1,*], José Luiz Pedreira Mouriño[1], Mauricio Laterça Martins[1]

[1] AQUOS—Aquatic Organisms Health Laboratory, Aquaculture Department, Federal University of Santa Catarina (UFSC), Florianópolis 88034-000, SC, Brazil
[2] Biohall Pesquisa e inovação, 88304-430, Itajaí, SC, Brazil
*** Corresponding author:** Marco Shizuo Owatari, owatarimarco@hotmail.com

CITATION

Ferreira MB, dos Santos PC, dos Santos GG, et al. Azadirachta indica as a sustainable nutritional alternative in Nile tilapia farming—A mini review. Probe - Fishery Science & Aquaculture. 2024; 6(1): 2208. https://doi.org/10.18186/fsa.v6i1.2208

ARTICLE INFO

Received: 13 March 2024
Accepted: 17 April 2024
Available online: 31 May 2024

COPYRIGHT

Copyright © 2024 by author(s). *Probe - Fishery Science & Aquaculture* is published by Universe Scientific Publishing. This work is licensed under the Creative Commons Attribution (CC BY) license. https://creativecommons.org/licenses/by/4.0/

Abstract: Nile tilapia *Oreochromis niloticus* is the most cultivated fish species in continental waters and represents the second most important group for global aquaculture. In Brazilian continental fish farming, diseases represent an annual loss of approximately US$84 million. Aiming to minimize these adversities, researchers are constantly investigating viable alternatives to improve fish health, mainly through diet. At present, various products of plant origin are tested in aquaculture as growth and health promoters in fish as an alternative to chemotherapeutics and antibiotics, which represent real concerns for the health of animals, environment, and consumers. Azadirachtin, also known as neem extract, is a bioactive phytocompound extracted from Azadirachta indica, with great microbicidal and immunostimulating potential. In animal feed, neem extract or azadirachtin have already been tested as growth promoters, immunostimulants and modulators of the gastrointestinal microbiota. In recent years, many studies involving the use of azadirachtin and other neem derivatives have presented varied and controversial results in aquaculture, with positive and negative effects on the productive performance and health of animals. Therefore, the present review aimed to systematize information about *A. indica* and its derivatives, considering the viability for use in tilapia farming.

Keywords: tilapia culture; Phyto therapeutics; sustainability; neem extract

1. Introduction

The accelerated growth of the aquaculture industry and the processes of productive intensification imply, among other issues, a greater density of fish in ponds, which consequently contribute to the deterioration of water quality [1]. Additionally, inadequate management of water quality can trigger a cycle of challenging events in the productive sector, as they contribute to the imbalance of the host-pathogen-environment triad, increasing the risks for the emergence of outbreaks of infectious diseases e.g., causing financial losses [2].

In global terms, the annual production of Nile tilapia *Oreochromis niloticus* reached 4.82 million tons in 2021, with Indonesia being the largest producer of the species in the world, with a production of 1.30 million tons, slightly greater than that recorded by China, of 1.24 million tons in 2021. This global tilapia production corresponds to around 3.83% of all global aquaculture production, approximately 126 million tons in 2021, and 9.74% of total freshwater fish production. Although Brazil is ranked sixteenth in the world ranking of aquaculture production—responsible for

0.52% of global aquaculture production—the growth of recent decades in Brazilian tilapiculture has elevated the country to the position of fourth largest producer of tilapia in the world, accounting for 7.48% of world production, which in 2021 was estimated at US$ 9.68 billion [3].

Despite its rusticity and resistance, tilapia is susceptible to diseases and in recent decades an increase in mortality records attributed to pathogens [4] or inadequate nutrition [5] has been evidenced. Considering that nutrition is strongly related to greater animal resistance to pathogens [5] (, it is likely that, in certain reports, disease outbreaks are related to inappropriate diets or inadequate feeding regimes for a given species. Among the most important pathogens in freshwater tilapia farms are the protozoa *Ichthyophthirius multifiliis*, *Epistylis* sp., *Chilodonella* sp., and *Trichodina* spp; the monogenoids *Dactylogyrus* sp.; the crustaceans *Argulus* spp. and *Dolops* spp.; the bacteria *Aeromonas hydrophila*, *Pseudomonas* sp., *Vibrio spp*., *Flavobacterium columnare* and *Streptococcus agalactiae*; the fungus *Saprolegnia parasitica*, and the *Iridovirus* [2].

The use of antibiotics to treat bacteriosis has been routinely applied in aquaculture production for many years, however, indiscriminate use can bring numerous concerns related to the farmed animal, the environment, and humans, in addition to promoting the emergence of resistant strains [6]. For these reasons, the use of antibiotics in aquaculture has been strongly discouraged worldwide, while prophylactic management aimed at preventing illnesses using probiotics, prebiotics, immunostimulants and phytogenic substances have received great attention from research institutions in recent years, which have focused on the development and use of alternative products to antibiotics and synthetic chemotherapeutics.

Feed additives in aquaculture

Feed additives are substances, microorganisms or formulated products that are not normally used as ingredients, intentionally added to animal diets, with or without nutritional value, but affect or improve the characteristics of animal feed or animal products, benefiting the growth performance of animals and health, without compromising nutritional requirements [7]. Additionally, feed additives are differentiated according to their usefulness, such as (1) nutritional additives—those referring to substances used to maintain or improve the nutritional properties of a product [8]; (2) sensory additives—those whose usefulness is to improve or modify organoleptic or visual characteristics of a product [9]; (3) technological additives—substances added to the product intended for animal feed for technological purposes [10]; (4) anticoccidial additives—used to inhibit or eliminate protozoa [11,12]; and (5) zootechnical additives—used to improve animal zootechnical performance [7,8,13].

Among the feed additives commonly used in aquaculture, prebiotics and probiotics are widely studied and frequently found on the market [14]. The microorganisms commonly used for the development of probiotics are from the genera *Lactobacillus*, *Bifidobacterium*, *Enterococcus* and *Bacillus*, while the most important prebiotics are fructooligosaccharides (FOS), mannan oligosaccharides (MOS), mannan oligosaccharides (MOS), yeast cell wall (YCW) [15,16].

Enzymes and enzymatic complexes constitute another group commonly used as

feed additives in aquaculture [7]. The interest in the use of exogenous enzymes in diets arose because many ingredients used to formulate feed in aquaculture have a high market value, thus it is increasingly common for alternative ingredients, often of vegetable origin, to be tested and used in the aquafeed industry. In this sense, the use of these enzymes is an alternative to increase feed digestibility and animal performance [17]. Phytase, protease, amylase, pectinase, xylanase and beta-glucanase are among the main enzymes used as feed additives in aquaculture.

Organic acids have also been used as zootechnical additives and play the role of digestive substance, flora balancer and performance improver in the production of numerous aquacultural species [7,18–20]. Acetic, benzoic, propionic, and formic acids are examples of organic acids frequently used and/or studied for use in aquaculture.

Essential oils constitute a group that is currently widely studied in relation to their potential as a functional feed additive for aquaculture [21]. The challenge in using plant extracts for animal nutrition has been the identification and establishment of the effects exerted by the active compounds, present in these plants, on the animal organism, as there is still little knowledge of the action of many of these compounds. In this way, several plant extracts, products based on plant extracts and by-products from other food production sectors have been tested as potential food additives in aquaculture.

For example, Khalil et al. [22] observed improvements in the zootechnical performance of tilapia fed diets enriched with dry leaf extract (3%) or seed extract (2%) of *Eruca sativa* compared to fish fed a basal diet. Tang et al. [23] tested the dietary supplementation of a mixture of Chinese herbs in the diet of *O. niloticus* and observed immunological improvements, in addition to improving the resistance of fish exposed to experimental infection by *Aeromonas hydrophila*. Extracts of *Curcuma longa*, *Rosmarinus officinalis* and *Thymus vulgaris*, *Solanum ferox* and *Zingiber zerumbet* have also been tested as feed additives for tilapia, providing positive results for some hemato-immunological parameters and/or resistance to *A. hydrophila* [24,25], while fenugreek *Trigonella foenum-graecum* and *Aloe vera* increased the resistance of tilapia *O. mossambicus* and *O. niloticus* to *Streptococcus iniae* infection [26,27]; and *Aristolochia debilis*, *Panax ginseng*, *Spatholobus suberectus* and *Aegle marmelos* increased the resistance of *O. niloticus* to *Streptococcus agalactiae* infection [28,29].

It is notable that the use of phytogenic substances is intrinsically connected to the concept of sustainable aquaculture, based on prophylaxis management. However, there are still gaps regarding the application of this knowledge, whether in relation to the substances used, the specificity of species, the stage of development of the animals, and the dose to be applied. Furthermore, supplementation methods with such phytogenic compounds, whether isolated or protected, micro or nanoparticles, have shown great potential, as they have the capacity to increase the stability of the product and enhance the effect on the digestive tract of the target species [30].

2. Indian neem: Ancient uses for application in modern agroindustry

Over the last few decades, agriculture has incorporated the use of bioherbicides, also known as nature-based herbicides, to control pests as an alternative to the use of

chemical pesticides [31], and in this scenario the Meliaceae family has been identified as one of the most promising groups [32]. This family consists of approximately 550 species in 50 genera, with a wide geographic distribution [33]. Among the species, the chinaberry *Melia azedarach*, the cedar *Cedrela fissilis*, the red-cedar *Cedrela odorata*, the Brazilian mahogany *Swietenia macrophylla* and the Indian neem *Azadirachta indica* A. Jussieu stand out in global research and commercially as raw materials for logging industry, cosmetics, and bioinsecticide, pesticide or microbicide products [32,34–37].

The Indian neem *A. indica* is a tree, whose size varies from 15 to 20 meters in height, originating from India and Myanmar, however, as it can develop under the most diverse climatic conditions, especially in tropical climates, it adapts easily in poor soils and with reduced rainfall. Because it has been used as a medicinal plant for centuries, it has spread throughout the world, and is currently present in many countries, including Brazil, where it was introduced in the 1980s for research for medicinal purposes [32,38].

The Indian neem is a species widely studied among the Meliaceae in the agricultural industry [38]. Its bioactive compounds have action on more than 400 species of insects, acting through mechanisms such as repellency, reduction of feeding, repellency of posture, interruption of development and ecdysis, reduction of fertility and fecundity, while presenting low toxicity to humans and other mammals, as well as poultry. Furthermore, compounds from neem also present toxicity against nematodes, which cause major losses in the agricultural industry [32,38–40].

The oil from neem leaves, seeds and bark has a broad spectrum of antibacterial action [41]. Some of the bioactive compounds responsible for the antibacterial property of neem are azadirachtin, nimbidin, nimbin, nimbinin, nimbidinin, nimbidic acid and nimbolide; in addition to margolone, margolonone and isomargolonone, limnoids and tetranotriterpenoids. Among them, the compound found in the highest concentration and mainly in seeds, is azadirachtin [32,41–43]. The concentration of azadirachtin, however, can vary greatly according to harvest, processing and fruit health, tree health and environmental conditions such as temperature and humidity, even varying from tree to tree in the same production, and according to the origin of the seed material [32,38].

Azadirachtin ($C_{35}H_{44}O_{16}$) is a water-soluble, highly oxidized limonoid with a complex structure and rigid conformation due to the presence of intramolecular hydrogen bonds and many reactive functional groups in extremely close positions [44]. The chemical structure of azadirachtin comprises 16 stereogenic centers, of which seven are tetrasubstituted carbon atoms and nine are disubstituted carbon atoms. Additionally, the structure of azadirachtin comprises 16 oxygen atoms in four ester groups, two hydroxy groups and one hemiacetal group; epoxide and dihydrofuran, the latter being mainly responsible for the antifeedant activity of the molecule [38,44].

Agricultural and aquacultural uses of azadirachtin and neem derivatives

The use of azadirachtin in the agricultural industry is not restricted to the purpose of bioinsecticide, moreover, as an effective chemotherapeutic agent in the control of some pest flies in sheep, horses, stables and cattle horns [45–50]. In animal feed, neem

extract or azadirachtin have been recurrently tested in poultry production, both as a growth-promoting supplement [51–53], as well as an immunostimulant and modulator of the gastrointestinal microbiota [32,54–56].

Nevertheless, in aquaculture it is possible to list the research carried out with azadirachtin and other neem derivatives into three distinct groups: i) those focused on investigations of possible toxic effects of neem and its derivatives on aquatic organisms (**Table 1**); ii) those that test neem derivatives, whether the extract, oil or isolated azadirachtin, as substances for therapeutic baths in aquaculture (**Table 2**); iii) research related to the effects of dietary supplementation of azadirachtin and other Indian neem derivatives on the growth performance and health of aquatic organisms (**Table 3**).

However, in one of the first reports of the use of azadirachtin in aquaculture health, Logambal and Michael [57] tested the effects of injectable doses (0.526 to 526 ng) of azadirachtin in *O. mossambicus* combined with injection of bovine serum albumin (BSA) antigens and sheep erythrocytes (SRBC). Fish that received injections of azadirachtin and BSA antigen showed an increase in primary and secondary antibody responses to BSA and an increase in leukocyte counts, regardless of the dose and time of application. On the other hand, an inverse relationship was observed between the dose of azadirachtin administered and the degree of stimulation of the immune response when SRBC were used as antigen, indicating that azadirachtin could produce different and even conflicting results for different types of stimuli.

Another potential use of azadirachtin in aquaculture is the influence of this substance on controlling the reproduction of fish in captivity. Obaroh and Achionye-Nzeh [58] tested the effects of dietary inclusion of neem leaf extract on the reproduction of groups of 180 *O. niloticus*, 90 females and 90 males per tank, for 56 days. Fish that did not receive the diet with the addition of neem had reproduction ranging from 63 to 89 larvae in a period of 3 weeks, while the administration of 0.5 g kg diet^{-1} of neem extract resulted in 35 to 51 larvae in a period of 5 weeks. Doses greater than 0.5 g kg feed^{-1} have not recorded reproduction.

Table 1. Assembly of research conducted in recent decades on the toxicity of azadirachtin and its neem derivatives to aquacultured fish.

Compound	Species	Main goal	Posology	Main findings	Key references
Azadirachtin	*Cyprinus carpio*	Investigated effects of azadirachtin on thyroid, stress hormone and some cytokines levels in freshwater common carp.	Acute and sub-chronic trials, lasting 4 and 30 days, respectively, and doses of 1.0, 2.0 and 2.4 ppm for both periods.	Significant worsening in hormone levels compared to control; significantly higher cortisol levels compared to control in both trials regardless of dose.	Korkmaz and Örün [59]
Azadirachtin	*Oncorhynchus mykiss*	Effects of azadirachtin on markers of oxidative damage and antioxidant enzymes in the brain of *O. mykiss*.	Exposure to azadirachtin doses of 0.12 and 0.24 ppm for 21 days.	0.24 ppm azadirachtin significantly increased the expression of oxidative damage markers; while 0.12 and 0.24 ppm significantly decreased the activity of antioxidant enzymes.	Alak et al. [60]
Azadirachtin	*Labeo rohita*	Effects of azadirachtin on the hematological profile of *L. rohita.*	Exposure for 96 h to increasing levels of azadirachtin from 30 to 60 mg L^{-1}.	$LC_{50}^{96\ h}$ = 44.61 ppm. Azadirachtin decreased total erythrocyte and blood hemoglobin concentration and MCHC index; immune-toxicological effects were identified in relation to leukocyte subpopulations.	Maitra et al. [61]
Azadirachtin	*Danio rerio*	Toxicity of azadirachtin on markers of oxidative stress in the brain and muscle of *D. rerio*.	Exposure to azadirachtin at concentrations of 0.025, 0.17 and 0.35 µg L^{-1} for 16 days.	Increased levels of markers of oxidative damage in the brain and muscle for all concentrations throughout the period; significant decrease in catalase activity in both tissues after 16 days.	Sharma and Ansari [62]
A. indica (oil)	*Glossogobius giuris*	Effects of neem oil on gill tissues and azadirachtin accumulation in *G. giuris*.	Exposure to 1.0 ppm neem oil for 96 h.	HPLC analysis identified 1.9 ppm of azadirachtin in gill tissue after 96 h of treatment; 0 ppm after 90 days; increased histological changes and ventilation rate of the opercula.	Mamatha and Mohan [63]
Azadirachtin	*Heteropneustes fossilis*	Evaluate the acute toxicity of azadirachtin to *H. fossilis*.	Baths lasting 96 h in increasing concentrations of 25 to 250 mg L^{-1} of azadirachtin.	LC_{50}^{24h} = 173.06 mg L^{-1}; LC_{50}^{48h} = 80.69 mg L^{-1}; LC_{50}^{72h} = 58.57 mg L^{-1}; LC_{50}^{96h} = 52.35 mg L^{-1}.	Kumar et al. [64]
A. indica (Leaf extract)	*O. niloticus*	Evaluate lethal levels and effects of sub-lethal doses of neem leaf extract for *O. niloticus.*	Extended 2-day immersion bath in concentrations of 0.5, 1.5, 2.0 and 2.5 mg L^{-1} of extract.	2.5 mg L^{-1} of neem extract caused 90% mortality of *O. niloticus* within 48 h. LC50 = 1.64 g L^{-1}. Sub-lethal doses provided effects of stress and anemia.	Fafioye [65]
Azadirachtin	*O. niloticus*	Toxicity of azadirachtin to *O. niloticus* post-larvae	Tested exposure to azadirachtin at concentrations of 0, 0.59, 1.18, 1.77 and 2.36 mg L^{-1} for 96 h.	Animals exposed to azadirachtin showed sub-lethal behavior in the first 12 h. Estimated lethal dose in 96 h = 1.28 mg L^{-1}.	Lima et al. [66]
A. indica (seed oil)	*O. niloticus*	Evaluated effects of the oil on oxidative stress and erythrocytes of *O. niloticus* and the potential of lupine seeds in mitigating these effects.	Fish exposed to doses of 56 and 112 ppm of neem oil for 1, 2 and 3 weeks; dietary supplementation with 5% lupine powder for 3 weeks.	Neem oil increased concentrations of markers of oxidative stress and glycemia; Lupine powder supplementation significantly mitigated the stressful effects of neem oil.	El-Badawi and Al-Salahy [67]
Azadirachtin	*Heteropneustes fossilis*	Evaluate the effects of azadirachtin on blood electrolytes of *H. fossilis*.	Short-term exposure = 41.89 mg L^{-1} over 96 h; Long-term exposure = 10.47 mg L^{-1} over 28 days.	Both exposure times caused a significant decrease in *H. fossilis* serum calcium and phosphate levels over time.	Kumar et al. [68]

Table 1. (*Continued*).

Compound	Species	Main goal	Posology	Main findings	Key references
Azadirachtin	*Piaractus mesopotamicus*	Evaluate LC_{50} and toxic effects of azadirachtin for alevins and juveniles of *P. mesopotamicus.*	Exposure of alevins and juveniles to six increasing doses of azadirachtin, from 0 to 1.77 mg L^{-1} for 96 h.	LC_{50} = 1.18 mg L^{-1} for alevins; 1.20 mg L^{-1} for juveniles; 0.29 and 0.59 mg L^{-1} safe for alevins and juveniles, respectively.	Cruz et al. [69]
A. indica (Aqueous extract of leaves)	*O. niloticus*	Assessment of the piscicidal potential of neem extract in *O. niloticus.*	96-h exposure to extract doses of 0, 2.0, 4.0, 6.0, 8.0 and 10 mL L^{-1}.	LC_{50}^{24h} = 6.40 ml L^{-1}; LC_{50}^{48h} = 3.22 ml L^{-1}; LC_{50}^{96h} = 2.57 ml L^{-1}	Cagauan et al. [70]
A. indica (Aqueous extract of leaves)	*Gambusia affinis*	Assessment of the piscicidal potential of neem extract in *G. affinis*.	96-hour exposure to extract doses of 0, 2.0, 4.0, 6.0, 8.0 and 10 mL L^{-1}.	LC_{50}^{24h} = 6.00 ml L^{-1}; LC_{50}^{48h} = 3.43 ml L^{-1}; LC_{50}^{96h} = 3.00 ml L^{-1}	Cagauan et al. [70]

Table 2. Assembly of research conducted in recent decades on therapeutic baths with azadirachtin and neem derivatives for aquacultured fish.

Compound	Species	Main goal	Posology	Main findings	Key references
Azadirachta indica (leaf powder)	*O. niloticus*	Health effects of tilapiaexposed to lead.	Bath for 2 weeks with 1.0 g L^{-1} of leaf powder, concomitantly with contamination of the water with 5.0 or 10 mg L^{-1} of lead.	Mitigated oxidative stress and suppressed antioxidant enzymes in animals exposed to lead at a concentration of 5.0 mg L^{-1}.	Abu-Elala et al. [71]
Azadirachta indica (leaf powder)	*Anabas testudineus*	Treatment of lesions caused by hexamitid protozoa.	36-h baths in concentrations of 5.0; 10.0 and 30.0 mg L^{-1}.	LC_{50}^{96h} for *A. testudineus* = 6.2 g L^{-1}; positive results for the three treatments in relation to the control, for total erythrocytes, total leukocytes, antioxidant enzyme activity and reduction in lipid peroxidation.	Mondal et al. [72]
Azadirachta indica (oil)	*Lates calcarifer*	Control of caligid copepod infestation	Prolonged baths in different concentrations.	LC_{50} of 2 ppm and 20 ppm for copepods and sea bass, respectively; 10 ppm bath = 100% efficacy against the pathogen in 96 h.	Khoa et al. [73]
Azadirachtin	*Carassius auratus*	Antiparasitic activity against *Argulus* spp.	72-h bath; doses of 1.0; 5.0; 10.0; 15.0 and 20.0 mg L^{-1}.	100% efficacy within 72 and 48 h for doses of 15.0 and 20.0 mg L^{-1}, respectively.	Kumar et al. [74]
Azadirachtin	*C. auratus*	Effects on hemato-biochemical parameters of fish treated against *Argulus* spp.	72-h bath; doses of 1; 5; 10; 15 and 20 mg L^{-1}.	Generalized improvement in hematological parameters and activity of antioxidant enzymes in relation to the control; however, the dose of 20 mg L^{-1} resulted in higher concentrations of glucose and LDH, indicating stress.	Kumar et al. [42]
Azadirachta indica (aqueous extract of leaves)	*C. carpio*	Effect on hematological parameters of fish infected by the fungus *Aphanomyces invadans*.	Daily baths of 5 min for 24 days with a dose of 1.0% aqueous extract of the leaves, 12 days after experimental infection.	Induced lesions were healed and hematological parameters were restored to values close to the pre-infection period.	Harikrishnan et al. [75]

Table 3. Assembly of research conducted in recent decades on dietary supplementation with azadirachtin and neem derivatives for aquacultured fish.

Compound	Species	Main goal	Posology	Main findings	Key references
Hydrolyzed *A. indica* seed protein	*O. niloticus*	Verified effects on performance, general health and resistance of *O. niloticus* to *A. veronii*.	Replacing fishmeal with rates of 10.0; 20.0; 30.0 and 40.0% hydrolyzed *A. indica* protein for 60 days.	Higher survival rates post-infection with *A. veronii* compared to the control; general improvement in zootechnical performance, immunological parameters of blood serum and activities of antioxidant enzymes.	Rahman et al. [76]
Azadiractina	*Salmo salar*	Verified the effectiveness of dietary inclusion with azadirachtin to control *Lepeophtheirus salmonis* infestation.	Dietary inclusion of azadirachtin at concentrations of 0.24, 0.47, 0.64 and 1.78 mg g^{-1} of feed for 104 days.	Lower feed consumption and lower condition factor in diets of 0.64 and 1.78 compared to 0.24 and 0.47 mg g^{-1}; all inclusion levels resulted in lower parasite counts compared to the control after 57 days of treatment.	Kim and Walker [77]
A. indica (leaf extract)	*O. mykiss*	Effects of dietary supplementation of neem extract on the zootechnical performance and proximate composition of *O. mykiss.*	Dietary inclusion of neem extract at concentrations of 5.0; 7.0 and 10.0% for 90 days.	7.0% inclusion promoted greater weight gain and greater feed efficiency in relation to all treatments and control; highest condition factor of all levels in relation to control; 7.5% estimated as ideal dose.	Abidin et al. [78]
A. indica (leaf extract)	*C. carpio*	Hematological response of *C. carpio* to dietary supplementation with neem extract.	Inclusions of 0.25, 0.50, 1.00, 1.50 and 2.00 g kg $feed^{-1}$ for 180 days.	Increase in total erythrocytes and leukocytes; increase in hemoglobin concentration and MCHC index; increase in total proteins and serum globulins.	Kaur et al. [79]
A. indica (leaf powder)	*O. niloticus*	Zootechnical performance and general health.	1.0; 2.0; 4.0 and 8.0 g kg $feed^{-1}$ for 3 months.	Significant decrease in reproduction; worsening in growth and feed conversion; decreased hemoglobin concentration, lower MCHC index; lymphocytosis.	Kapinga et al. [80]
Azadiractina	*C. auratus*	Immunostimulation and resistance to *A. hydrophila.*	Dietary inclusion in concentrations of up to 1.0 g kg^{-1}.	Significantly improved immunological parameters and survival post-infection by *A. hydrophila* compared to the control group.	Kumar et al. [42]
Azadiractina	*Cirrhinus mrigala*	Effects of azadirachtin on hemato-biochemical parameters of *C. mrigala* challenged with *Aphanomyces invadans.*	Dietary inclusion of 0.2% azadirachtin for 30 days post-infection with *A. invadans.*	Increase in total erythrocytes, hemoglobin and hematocrit; lymphocyte, eosinophil and neutrophil count; serum proteins, glucose, calcium and cholesterol compared to untreated infected fish.	Harikrishnan et al. [81]
A. indica + *Ocimum sanctum* + *Curcuma longa* (leaf powder in proportion 1:1:1)	*C. auratus*	Immunostimulation and resistance to *A.hydrophila.*	Dietary inclusion at a concentration of 2.5 g kg^{-1} 30 days post-infection.	Increased production of superoxide anion, phagocytic activity, complement activity and lysozyme; 100% survival to bacterial challenge *versus* 5% for untreated fish.	Harikrishnan et al. [82]
Azadiractina	*C. auratus*	Immunostimulation and resistance to *A. hydrophila.*	Dietary inclusion at a concentration of 2.5 g kg^{-1} 30 days post-infection.	Increased production of superoxide anion, phagocytic activity, complement activity and lysozyme; 90% survival to bacterial challenge *versus* 5% for untreated fish.	Harikrishnan et al. [82]

Kapinga et al. [83] tested the effects of neem leaf powder supplementation at doses of 1.0, 2.0, 4.0 and 8.0 g kg feed^{-1} for 3 months on *O. niloticus* and found that neem supplementation, even at the lowest dose, significantly decreased the absolute fecundity and gonadosomatic index of the fish. The authors recommended 2.0 g kg feed^{-1} of the diet to control unwanted reproduction of tilapia in mixed population cultures, without degeneration of the individuals' gonadal tissue. However, during this review we realized that there are still few studies of this nature on fish farming. The screening of different protocols for the administration of azadirachtin in the fish diet and the effects of this supplementation on different aspects of animal health, mainly regarding the histomorphological effects caused in animals, as well as on the antimicrobial action of this compound against a wider range of important pathogens for aquaculture remain scarce.

3. Conclusions

Azadirachtin is a relatively little-explored bioactive compound as a dietary supplement for Nile tilapia *O. niloticus*. Despite this, azadirachtin as an immunostimulant can improve the immune system and reducing infectious diseases in fish. The use of Azadirachtin in aquaculture is viable considering the dosages, application forms and regulatory standards for each species and farming systems. Azadirachtin is a natural product that can contribute to more sustainable and healthier aquaculture. However, aquaculture is concerned with the lack of research related to the inclusion of azadirachtin in the tilapia diet. Thus, the incorporation of the active biocompound derived from azadirachtin as a food additive could become a sustainable strategy in tilapia farming.

Funding: This research was funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior grant number 001 and the APC was funded by National Council for Scientific and Technological Development (CNPq).

Acknowledgments: The authors would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq Brazil and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—CAPES Brazil.

Conflict of interest: The authors declare no conflict of interest.

References

1. Campanati C, Willer D, Schubert J, et al. Sustainable Intensification of Aquaculture through Nutrient Recycling and Circular Economies: More Fish, Less Waste, Blue Growth. Reviews in Fisheries Science & Aquaculture. 2021; 30(2): 143-169. doi: 10.1080/23308249.2021.1897520
2. Tavares-Dias M, Martins ML. An overall estimation of losses caused by diseases in the Brazilian fish farms. Journal of Parasitic Diseases. 2017; 41(4): 913-918. doi: 10.1007/s12639-017-0938-y
3. FAO. Fishery and Aquaculture Statistics. Global aquaculture production 1950-2021 (FishStatJ). Available online: www.fao.org/fishery/en/statistics/software/fishstatj (accessed on 2 January 2024).
4. Shinn AP, Avenant-Oldewage A, Bondad-Reantaso MG, et al. A global review of problematic and pathogenic parasites of farmed tilapia. Reviews in Aquaculture. 2023; 15(S1): 92-153. doi: 10.1111/raq.12742
5. Wang B, Thompson KD, Wangkahart E, et al. Strategies to enhance tilapia immunity to improve their health in aquaculture. Reviews in Aquaculture. 2022; 15(S1): 41-56. doi: 10.1111/raq.12731

6. Preena PG, Swaminathan TR, Kumar VJR, et al. Antimicrobial resistance in aquaculture: a crisis for concern. Biologia. 2020; 75(9): 1497-1517. doi: 10.2478/s11756-020-00456-4
7. Encarnação P. Functional feed additives in aquaculture feeds. In: Aquafeed formulation. Academic Press; 2016.
8. Dawood MAO, Koshio S, Esteban MÁ. Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Reviews in Aquaculture. 2017; 10(4): 950-974. doi: 10.1111/raq.12209
9. Clouard C, Val-Laillet D. Impact of sensory feed additives on feed intake, feed preferences, and growth of female piglets during the early postweaning period1. Journal of Animal Science. 2014; 92(5): 2133-2140. doi: 10.2527/jas.2013-6809
10. Pandey AK, Kumar P, Saxena MJ. Feed additives in animal health. Nutraceuticals in Veterinary Medicine. Springer; 2019.
11. Hady MM, Zaki MM. Efficacy of Some Herbal Feed Additives on Performance and Control of Cecal Coccidiosis in Broilers. APCBEE Procedia. 2012; 4: 163-168. doi: 10.1016/j.apcbee.2012.11.028
12. Sánchez-Hernández C, Castañeda-Gómez del Campo J, Trejo-Castro L, et al. Evaluation of a Feed Plant Additive for Coocidiosis Control in Broilers Herbals for Coccidiosis Control. Brazilian Journal of Poultry Science. 2019; 21(1). doi: 10.1590/1806-9061-2018-0846
13. Owatari MS, da Silva LR, Ferreira GB, et al. Body yield, growth performance, and haematological evaluation of Nile tilapia fed a diet supplemented with *Saccharomyces cerevisiae*. Animal Feed Science and Technology. 2022; 293: 115453. doi: 10.1016/j.anifeedsci.2022.115453
14. Dawood MAO, Kari ZA. Editorial special issue: Friendly nutritional strategies for sustainable aquaculture. Aquaculture and Fisheries. 2024; 9(1): 1-2. doi: 10.1016/j.aaf.2023.01.003
15. Dawood MAO. Nutritional immunity of fish intestines: important insights for sustainable aquaculture. Reviews in Aquaculture. 2020; 13(1): 642-663. doi: 10.1111/raq.12492
16. Wee W, Abdul Hamid NK, Mat K, et al. The effects of mixed prebiotics in aquaculture: A review. Aquaculture and Fisheries. 2024; 9(1): 28-34. doi: 10.1016/j.aaf.2022.02.005
17. Liang Q, Yuan M, Xu L, et al. Application of enzymes as a feed additive in aquaculture. Marine Life Science & Technology. 2022; 4(2): 208-221. doi: 10.1007/s42995-022-00128-z
18. Jesus GFA, Pereira SA, Owatari MS, et al. Protected forms of sodium butyrate improve the growth and health of Nile tilapia fingerlings during sexual reversion. Aquaculture. 2019; 499: 119-127. doi: 10.1016/j.aquaculture.2018.09.027
19. Pereira SA, Jesus GFA, Pereira GV, et al. The Chelating Mineral on Organic Acid Salts Modulates the Dynamics and Richness of the Intestinal Microbiota of a Silver Catfish *Rhamdia quelen*. Current Microbiology. 2020; 77(8): 1483-1495. doi: 10.1007/s00284-020-01962-z
20. Libanori MCM, Santos GG, Pereira SA, et al. Organic benzoic acid modulates health and gut microbiota of *Oreochromis niloticus*. Aquaculture. 2023; 570: 739409. doi: 10.1016/j.aquaculture.2023.739409
21. Mariappan B, Kaliyamurthi V, Binesh A. Medicinal plants or plant derived compounds used in aquaculture. In: Recent advances in aquaculture microbial technology. Academic Press; 2023.
22. Khalil FF, Mehrim AI, Refaey MM. Impact of Dietary Rocket (*Eruca sativa*) Leaves or Seeds on Growth Performance, Feed Utilization, Biochemical and Physiological Responses of *Oreochromis niloticus*, Fingerlings. Asian Journal of Animal Sciences. 2015; 9(4): 134-147. doi: 10.3923/ajas.2015.134.147
23. Tang J, Cai J, Liu R, et al. Immunostimulatory effects of artificial feed supplemented with a Chinese herbal mixture on *Oreochromis niloticus* against *Aeromonas hydrophila*. Fish & Shellfish Immunology. 2014; 39(2): 401-406. doi: 10.1016/j.fsi.2014.05.028
24. Hassan AAM, Yacout MH, Khalel MS, et al. Effects of Some Herbal Plant Supplements on Growth Performance and the Immune Response in Nile Tilapia (*Oreochromis Niloticus*). "Agriculture for Life, Life for Agriculture" Conference Proceedings. 2018; 1(1): 134-141. doi: 10.2478/alife-2018-0020
25. Hardi EH, Nugroho RA, Kusuma IW, et al. Borneo herbal plant extracts as a natural medication for prophylaxis and treatment of *Aeromonas hydrophila* and *Pseudomonas fluorescens* infection in tilapia (*Oreochromis niloticus*). F1000 Research. 2018; 7: 1847.
26. Yılmaz S, Ergün S, Soytaş N. Herbal supplements are useful for preventing streptococcal disease during first-feeding of tilapia fry, *Oreochromis mossambicus*. Israeli Journal of Aquaculture. 2013; 833(1): 195-204.
27. Gabriel NN, Qiang J, Ma XY, et al. Dietary Aloe vera improves plasma lipid profile, antioxidant, and hepatoprotective enzyme activities in GIFT-tilapia (*Oreochromis niloticus*) after *Streptococcus iniae* challenge. Fish Physiology and Biochemistry. 2015; 41(5): 1321-1332. doi: 10.1007/s10695-015-0088-z

28. Guo WL, Deng HW, Wang F, et al. In vitro and in vivo screening of herbal extracts against *Streptococcus agalactiae* in Nile tilapia (*Oreochromis niloticus*). Aquaculture. 2019; 503: 412-421. doi: 10.1016/j.aquaculture.2019.01.024
29. Wangkahart E, Wachiraamonloed S, Lee PT, et al. Impacts of Aegle marmelos fruit extract as a medicinal herb on growth performance, antioxidant and immune responses, digestive enzymes, and disease resistance against *Streptococcus agalactiae* in Nile tilapia (*Oreochromis niloticus*). Fish & Shellfish Immunology. 2022; 120: 402-410. doi: 10.1016/j.fsi.2021.11.015
30. Valente LMP, Batista S, Ribeiro C, et al. Physical processing or supplementation of feeds with phytogenic compounds, alginate oligosaccharide or nucleotides as methods to improve the utilization of *Gracilaria gracilis* by juvenile European seabass (*Dicentrarchus labrax*). Aquaculture. 2021; 530: 735914. doi: 10.1016/j.aquaculture.2020.735914
31. Campos EVR, Ratko J, Bidyarani N, et al. Nature-Based Herbicides and Micro-/Nanotechnology Fostering Sustainable Agriculture. ACS Sustainable Chemistry & Engineering. 2023; 11(27): 9900-9917. doi: 10.1021/acssuschemeng.3c02282
32. Martinez SS. The neem: *Azadirachta indica*: Nature, multiple uses, production (Portuguese). In: O NIM: *Azadirachta indica*: Natureza, usos múltiplos, produção. Instituto Agronômico do Paraná—IAPAR; 2011
33. Lin M, Yang S, Huang J, et al. Insecticidal Triterpenes in Meliaceae: Plant Species, Molecules and Activities: Part I (Aphanamixis-Chukrasia). International Journal of Molecular Sciences. 2021; 22(24): 13262. doi: 10.3390/ijms222413262
34. Jebril S, Khanfir Ben Jenana R, Dridi C. Green synthesis of silver nanoparticles using Melia azedarach leaf extract and their antifungal activities: *In vitro* and *in vivo*. Materials Chemistry and Physics. 2020; 248: 122898. doi: 10.1016/j.matchemphys.2020.122898
35. Lopez YM, Paes JB, Gustave D, et al. Production of wood-plastic composites using Cedrela odorata sawdust waste and recycled thermoplastics mixture from post-consumer products-A sustainable approach for cleaner production in Cuba. Journal of Cleaner Production. 2020; 244: 118723.
36. Salomón YL, Georgin J, Franco DS, et al. Application of seed residues from *Anadenanthera macrocarpa* and *Cedrela fissilis* as alternative adsorbents for remarkable removal of methylene blue dye in aqueous solutions. Environmental Science and Pollution Research. 2021; 28: 2342-2354.
37. Borah A, Selvaraj S, Holla SR, et al. Analysis of the bioactive components in *Swietenia macrophylla* leaves for their antibacterial and antioxidant capabilities. Bioresource Technology Reports. 2023; 22: 101411. doi: 10.1016/j.biteb.2023.101411
38. Islas JF, Acosta E, G-Buentello Z, et al. An overview of Neem (Azadirachta indica) and its potential impact on health. Journal of Functional Foods. 2020; 74: 104171. doi: 10.1016/j.jff.2020.104171
39. Akhtar M. Nematicidal potential of the neem tree *Azadirachta indica* (*A. Juss*). Integrated Pest Management Reviews. 2020; 5(1): 57-66. doi: 10.1023/A:1009609931223
40. Prates HT, Viana PA, Waquil JM. Atividade de extrato aquoso de folhas de nim (*Azadirachta indica*) sobre *Spodoptera frugiperda*. Pesquisa Agropecuária Brasileira. 2003; 38(3): 437-439. doi: 10.1590/s0100-204x2003000300014
41. Wylie MR, Merrell DS. The Antimicrobial Potential of the Neem Tree Azadirachta indica. Frontiers in Pharmacology. 2022; 13. doi: 10.3389/fphar.2022.891535
42. Kumar S, Raman RP, Pandey PK, et al. Effect of orally administered azadirachtin on non-specific immune parameters of goldfish *Carassius auratus* (Linn. 1758) and resistance against *Aeromonas hydrophila*. Fish & Shellfish Immunology. 2013; 34(2): 564-573. doi: 10.1016/j.fsi.2012.11.038
43. Ali E, Islam MdS, Hossen MdI, et al. Extract of neem (*Azadirachta indica*) leaf exhibits bactericidal effect against multidrug resistant pathogenic bacteria of poultry. Veterinary Medicine and Science. 2021; 7(5): 1921-1927. doi: 10.1002/vms3.511
44. Fernandes SR, Barreiros L, Oliveira RF, et al. Chemistry, bioactivities, extraction and analysis of azadirachtin: State-of-the-art. Fitoterapia. 2019; 134: 141-150. doi: 10.1016/j.fitote.2019.02.006
45. Gaaboub IA, Hayes DK. Biological Activity of Azadirachtin, Component of the Neem Tree1 Inhibiting Molting in the Face Fly, *Musca autumnalis* De Geer (Diptera: Muscidae). Environmental Entomology. 1984; 13(3): 803-812. doi: 10.1093/ee/13.3.803
46. Miller AJ, Chamberlain WF. Azadirachtin as a Larvicide Against the Horn Fly, Stable Fly, and House Fly (Diptera: Muscidae). Journal of Economic Entomology. 1989; 82(5): 1375-1378. doi: 10.1093/jee/82.5.1375
47. Schmutterer H. Properties and Potential of Natural Pesticides from the Neem Tree, *Azadirachta Indica*. Annual Review of Entomology. 1990; 35(1): 271-297. doi: 10.1146/annurev.en.35.010190.001415
48. Singh S, Singh RP. Neem (*Azadirachta indica*) seed kernel extracts and azadirachtin as oviposition deterrents against the melon fly (*Bactrocera cucurbitae*) and the oriental fruit fly (*Bactrocera dorsalis*). Phytoparasitica. 1998; 26(3): 191-197.

doi: 10.1007/bf02981434
49. Kumar ARV, Jayadevi HC, Ashoka HJ, Chandrashekara K. Azadirachtin use efficiency in commercial neem formulations. Current Science. 2003; 84(11): 1459-1464
50. Morgan ED. Azadirachtin, a scientific gold mine. Bioorganic & Medicinal Chemistry. 2009; 17(12): 4096-4105. doi: 10.1016/j.bmc.2008.11.081
51. Girotto VD, Santos GB. Performance of broiler chickens from 1 to 42 days subjected to different levels of Neem (*Azadirachta indica*) cake inclusion in feed (Portuguese). RETEC-Revista de Tecnologias. 2012; 5(2).
52. Sarker S, Mostofa M, Akter F, et al. Effects of aqueous extract of Neem (*Azadirachta indica*) leaves as growth promoter and anti-colibacillosis in broilers. Bangladesh Journal of Animal Science. 2014; 43(2): 138-141. doi: 10.3329/bjas.v43i2.20715
53. Mahejabin N, Mostofa M, Akter F, et al. Effects of Neem, turmeric and papaya leaf extract mixture on growth performance of broilers. International Journal of Natural and Social Sciences. 2015; 2(2): 17-21.
54. Durrani FR, Sultan A, Jan M, et al. Immunomodulatory and growth promoting effects of Neem (Azadirachta indica) leaves infusion in broiler chicks. Sarhad Journal of Agriculture. 2008; 24(4): 655-659.
55. Landy N, Ghalamkari G, Toghyani M. Performance, carcass characteristics, and immunity in broiler chickens fed dietary neem (*Azadirachta indica*) as alternative for an antibiotic growth promoter. Livestock Science. 2011; 142(1-3): 305-309. doi: 10.1016/j.livsci.2011.08.017
56. Odoh LI, Bratte L. Effects of varying levels of neem (*Azadirachta indica*) leaf meal in layer diets on the haematological and serological indices, and faecal bacterial counts of layers. Journal of Natural Sciences Research. 2015; 5(4): 37-44.
57. Logambal SM, Michael RD. Immunostimulatory effect of azadirachtin in Oreochromis mossambicus (Peters). Indian Journal of Experimental Biology. 2000; 38: 1092-1096.
58. Obaroh IO, Achionye-N GC. Effects of Crude Extract of Azadirachta indica Leaves at Controlling Prolific Breeding in Oreochromis niloticus (Linnaeus, 1758). Asian Journal of Agricultural Research. 2011; 5(5): 277-282. doi: 10.3923/ajar.2011.277.282
59. Korkmaz N, Örün İ. Effects of pesticide NeemAzal-T/S on thyroid, stress hormone and some cytokines levels in freshwater common carp, *Cyprinus carpio* L. Toxin Reviews. 2021; 41(2): 496-505. doi: 10.1080/15569543.2021.1895841
60. Alak G, Ucar A, Çilingir Yeltekin A, et al. Neurophysiological responses in the brain tissues of rainbow trout (*Oncorhynchus mykiss*) treated with bio-pesticide. Drug and Chemical Toxicology. 2018; 42(2): 203-209. doi: 10.1080/01480545.2018.1526180
61. Maitra B, Sen S, Homechaudhuri S. Flow cytometric analysis of fish leukocytes as a model for toxicity produced by azadirachtin-based bioagrocontaminant. Toxicological & Environmental Chemistry. 2014; 96(2): 328-341. doi: 10.1080/02772248.2014.934026
62. Sharma DK, Ansari BA. Toxicity of azadirachtin on some biomarkers of oxidative stress in zebrafish, *Danio rerio*. Journal of Biology and Earth Science. 2014; 4(2): 160-167.
63. Mamatha P, Mohan MR. Detection of biopesticide Azadirachtin and analysis of gill tissues of the fish *Glossogobius giuri* using HPCL. Advances in Forestry Science. 2014; 1(3): 83-88.
64. Kumar A, Prasad M, Mishra D, et al. Acute toxicity of azadirachtin to a teleost, *Heteropneustes fossilis*. Acta Scientiarum Biological Sciences. 2012; 34(2). doi: 10.4025/actascibiolsci.v34i2.9455
65. Fafioye OO. Acute and sub-acute toxicities of five plant extracts on white tilapia, *Oreochromis niloticus* (Trewavas). International Research Journal of Agricultural Science and Soil Science. 2012; 2(13): 525-530.
66. Lima A, Barbosa JM, Silva AA, et al. Toxicity of neem extract for post-larvae tilapia. Available online: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/46396/1/aqua3.pdf (accessed on 2 January 2024).
67. El-Badawi AA, Al-Salahy MB. Physiological studies on gill and corpuscular oxidative stress in the fish *Oreochromis niloticus* exposed to Neem oil in response to antioxidant power of lupine seeds. In: Proceedings of the 4th Global Fisheries and Aquaculture Research Conference, the Egyptian International Center for Agriculture.
68. Kumar S, Raman RP, Kumar K, et al. In vitro and in vivo antiparasitic activity of Azadirachtin against Argulus spp. in Carassius auratus (Linn. 1758). Parasitology Research. 2011; 110(5): 1795-1800. doi: 10.1007/s00436-011-2701-0
69. Cruz C, Machado-neto JG, DE Menezes ML. Acute Toxicity of The Insecticide Methyl Parathion and The Biopesticide Azadirachtin from Neem Leaves (*Azadirachta indica*) To Pacu Fry and Juveniles (*Piaractus mesopotamicus*) (Portuguese). Pesticidas: Revista de Ecotoxicologia e Meio Ambiente. 2004; 14. doi: 10.5380/pes.v14i0.3127
70. Cagauan AG, Galaites MC, Fajardo LJ. Evaluation of botanical piscicides on Nile tilapia *Oreochromis niloticus* L. and

mosquito fish *Gambusia affinis* Baird and Girard. Available online: https://ag.arizona.edu/azaqua/ista/ista6/ista6web/pdf/179.pdf (accessed on 2 January 2024).

71. Abu-Elala NM, Khattab MS, AbuBakr HO, et al. Neem leaf powder (Azadirachta indica) mitigates oxidative stress and pathological alterations triggered by lead toxicity in Nile tilapia (Oreochromis niloticus). Scientific Reports. 2023; 13(1). doi: 10.1038/s41598-023-36121-4
72. Mondal P, Garai P, Chatterjee A, et al. Toxicological and therapeutic effects of neem (*Azadirachta indica*) leaf powder in hole-in-the-head (HITH) disease of fish *Anabas testudineus*. Aquaculture Research. 2020; 52(2): 715-723. doi: 10.1111/are.14928
73. Khoa TND, Mazelan S, Muda S, et al. Use of neem oil (*Azadirachta indica*) to control caligid copepod infestation on Asian seabass (*Lates calcarifer*). Aquaculture Research. 2019; 50(7): 1885-1892. doi: 10.1111/are.14074
74. Kumar S, Raman RP, Kumar K, et al. Effect of azadirachtin on haematological and biochemical parameters of Argulus-infested goldfish *Carassius auratus* (Linn. 1758). Fish Physiology and Biochemistry. 2012; 39(4): 733-747. doi: 10.1007/s10695-012-9736-8
75. Harikrishnan R, Balasundaram C, Bhuvaneswari R. Restorative effect of *Azadirachta indica*b aqueous leaf extract dip treatment on haematological parameter changes in *Cyprinus carpio* (L.) experimentally infected with *Aphanomyces invadans* fungus. Journal of Applied Ichthyology. 2005; 21(5): 410-413. doi: 10.1111/j.1439-0426.2005.00614.x
76. Rahman ANA, Amer SA, Masoud SR, et al. Neem seed protein hydrolysate as a fishmeal substitute in Nile tilapia: Effects on antioxidant/immune pathway, growth, amino acid transporters-related gene expression, and *Aeromonas veronii* resistance. Aquaculture. 2023; 573: 739593. doi: 10.1016/j.aquaculture.2023.739593
77. Kim KS, Walker GC. Efficacy of neem extract against sea lice (*Lepeophtheirus salmonis*) infestations: A potential biopesticide for Atlantic salmon (*Salmo salar*). Aquaculture. 2022; 560: 738453. doi: 10.1016/j.aquaculture.2022.738453
78. Abidin ZU, Hassan HU, Masood Z, et al. Effect of dietary supplementation of Neem, *Azadirachta indica* leaf extracts on enhancing the growth performance, chemical composition and survival of rainbow trout, *Oncorhynchus mykiss*. Saudi Journal of Biological Sciences. 2022; 29(4): 3075-3081. doi: 10.1016/j.sjbs.2022.01.046
79. Kaur Y, Dhawan A, Holeyappa SA, Thammegowda NB. Hematological responses of common carp *Cyprinus Carpio* administered with Neem (*Azadirachta indica*) leaf extraction. Indian Journal of Animal Research. 2019; 53(2): 161-167.
80. Kapinga IB, Limbu SM, Madalla NA, et al. *Aspilia mossambicensis* and *Azadirachta indica* medicinal leaf powders modulate physiological parameters of Nile tilapia (*Oreochromis niloticus*). International Journal of Veterinary Science and Medicine. 2018; 6(1): 31-38. doi: 10.1016/j.ijvsm.2018.03.003
81. Harikrishnan R, Balasundaram C. Supplementation Diet Containing Probiotics, Herbal and Azadirachtin on Hematological and Biochemical Changes in *Cirrhina mrigala* Against *Aphanomyces invadans*. Fisheries and Aquaculture Journal. 2010; 01(06). doi: 10.4172/2150-3508.1000004
82. Harikrishnan R, Balasundaram C, Heo M. Effect of chemotherapy, vaccines and immunostimulants on innate immunity of goldfish infected with *Aeromonas hydrophila*. Diseases of Aquatic Organisms. 2009; 88: 45-54. doi: 10.3354/dao02143
83. Kapinga IB, Limbu SM, Madalla NA, et al. Dietary Aspilia mossambicensis and *Azadirachta indica* supplementation alter gonadal characteristics and histology of juvenile Nile tilapia (*Oreochromis niloticus*). Aquaculture Research. 2018; 50(2): 573-580. doi: 10.1111/are.13931