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### Animal and plant originated immunostimulants used in aquaculture

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#### ABSTRACT

*The use of immunostimulants as an alternative to the drugs, chemicals and antibiotics currently being used to control fish diseases in fish culture is attracting the attention of many researchers. In this context, many have focused on the use of medicinal plant and animal originated products as potential therapeutic measures for modulating the immune response to prevent and control fish diseases. The possible use of naturally available herbal extract such as Ocimum sanctum (Tulsi), Phyllanthus emblica (Amla), Azadirachta indica (Neem), Solanum trilobatum (Purple Fruited Pea Eggplant), Eclipta alba (Bhringraj), Zingiber officinale (Ginger), Echinacea (Purple coneflowers), Allium sativum (Garlic), Camellia sinensis (Green tea), Aloe vera, Cynodon dactylon (Bermuda Grass), Achyranthes aspera (Prickly Chaff Flower), Nyctanthes arbortristis (Night-flowering Jasmine), Tinospora cordifolia (Guduchi) and Picrorhiza kurooa (Kutki) and animal originated product like Chitin, chitosan and Fermented products of chicken egg (EF203) etc. has been discussed in this article. The aim of this paper is to review research currently being carried out on the herbal extracts and animal originated products that have been shown to modulate the immune system of fish.*

**Key words** –Aquaculture, Fish culture, Immunostimulants, Indian herbs and immunity

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#### INTRODUCTION

World wide fish and shellfish culture are subjected to many diseases that lead to great losses and decrease in fish production. The lack of effective disease control has the potential of being the chief limiting factor of the realization of highly stable fish production. The use of immunostimulants in aquaculture for prevention of diseases is a promising new development. Immunostimulants may be defined as the agent, which stimulate the non-specific immune mechanisms on their own or specific immune mechanism when coupled with an antigen. In general, immunostimulants comprise a group of biological and synthetic compounds that enhance the non-specific defense mechanisms in animals, thereby imparting generalized protection. This protection may be particularly important for fish that are raised in or released into environments where the nature of pathogen is unknown and immunization by specific vaccine may be futile. Immunostimulants promote a greater and more effective sustained immune response to those infectious agents (viruses, bacteria, fungi, and parasites), producing subclinical disease without risks of toxicity, carcinogenicity or tissue residues. They help to hastening the maturation of non-specific and specific immunity in young susceptible animals.

The immunostimulants enhance the level of duration of specific immune response, both cell-mediated and humoral, following vaccination. Immunostimulant supports to overcome of immunosuppressive effects of stress and of those infectious agents that damage or interface with the functioning of cells of immune system. A variety of substance

have been shown to have the immunostimulatory effects which are microbial derivatives, plants or animal extracts, vitamins, hormones and synthetic chemical but Herb extracts and animal originated product have a potential application as an immunostimulant in fish culture, primarily because they can be easily obtained, are not expensive and act against a broad spectrum of pathogens. Most of the herbs and herb extracts can be given orally, which is the most convenient method of immunostimulation.

### PLANT ORIGINATED IMMUNOSTIMULANTS

Natural plant product promote various activities such as Antistress, Growth promotion, Appetite stimulation, Immunostimulation, Aphrodisiac and Antimicrobial properties Due to the active principles such as alkaloids, flavanoids pigments, phenolics, terpenoids, steroids and essential oils.

#### 1. *Ocimum sanctum* (Tulsi)

Leaves of *Ocimum sanctum* contain water-soluble phenolic compounds and various other constituents, such as eugenol, methyl eugenol and caryophyllene that may act as an immunostimulant. In tilapia (*Oreochromis mossambicus*), the acetone extract of *O. sanctum* was found to enhance the anti-sheep red blood cell (SRBC; sheep erythrocytes) antibody response. Leaves extract of *Ocimum sanctum* affected both specific and non-specific immune responses and disease resistance against *Aeromonas hydrophila*. It stimulated both antibody response and neutrophil activity [10].

#### 2. *Phyllanthus emblica* (Amla)

*Phyllanthus emblica* has antioxidant activity, anti-fungal activity, antimicrobial activity and anti-inflammatory activity. Amla fruit pulp contains large proportion of vitamin C, which has also been identified as an immunostimulant. An acetone extract of *P. emblica* enhanced the anti-SRBC antibody response in tilapia [10], while both crude extract and a water-soluble fraction of *P. emblica* fruit had a stimulatory effect on the immune response of tilapia<sup>4</sup>.

#### 3. *Azadirachta indica* (Neem)

*Azadirachta indica* is a highly esteemed “wonder” tree of India that is widely dispersed throughout the country. Biomedical research has revealed that neem possesses anti-human immunodeficiency virus, anti-tumor and antimicrobial activities. Azadirachtin, a triterpenoid derived from *A. indica*, enhanced respiratory burst activities, the leukocyte count and the primary and secondary antibody response against SRBC in tilapia [13, 14].

#### 4. *Solanum trilobatum* (Purple Fruited Pea Eggplant)

The herbal extract of *Solanum trilobatum* contains compound like Sobatum, b-solamarine, solaine, solasodine, glycoalkaloid, diosogenin and tomatidine<sup>17</sup>. *Solanum trilobatum* possesses a broad spectrum of antibiotic, antibacterial and anticancer activity. A study aimed at assessing the effects of the water- and hexane-soluble fractions of *S. trilobatum* on the nonspecific immune mechanisms and disease resistance of tilapia found that all doses of the water soluble fraction significantly enhanced the production of reactive oxygen and decreased the percentage mortality following a challenge with *Aeromonas hydrophila* [8].

#### 5. *Eclipta alba* (Bhringraj)

*Eclipta alba* a herb belonging to Asteraceae, is widely available and distributed throughout India. This plant has been reported to possess several medicinal properties. The methanol extracts of the whole plant of *Eclipta alba* significantly increased the phagocytic index, antibody titer and WBC count in mice [11]. Oral administration of *Eclipta alba* leaf aqueous extract to *Oreochromis mossambicus* indicate that dietary intake of *E. alba* aqueous leaf extract enhances the non-specific immune responses and disease resistance of *O. mossambicus* against *A. hydrophila* [6].

#### 6. *Zingiber officinale* (Ginger)

Roots and the obtained extracts of *Zingiber officinale* contain polyphenol compounds (6-gingerol and its derivatives), which have a high antioxidant activity. The use of Ginger @0.5g/110g of feed reduced mortalities to 0% compared with the controls (64%). Moreover, there was a significant increase in growth, feed conversion and protein efficiency. There was proliferation in the number of neutrophils, macrophages and lymphocytes, and enhanced phagocytic, respiratory burst, lysozyme, bactericidal and anti-protease activities compared with the controls [9].

#### 7. *Echinacea* (purple coneflowers) and *Allium sativum* (garlic)

*Echinacea* and *Allium sativum* improve the gain in body weight, survival rate and resistance against challenge infection of *Aeromonas hydrophila*. Both compounds showed extended effects after withdrawal and improved resistance to cold stress during the winter season [2].

### 8. *Camellia sinensis* (Green tea)

Green tea (GT) extracts contain a unique set of catechins that possess biologic activity in antioxidant, antiangiogenesis, and antiproliferative assays that are potentially relevant to the prevention and treatment of various forms of cancer. The inclusion of green tea in fish diet up to 0.5 g/kg diet enhanced the protein contents in fish body, while the lowest lipid contents were obtained at 0.0–0.5 g GT/kg diet. Hematological and biochemical parameters were improved in fish fed 0.25–2.0 g GT/kg diet, while the lowest values were obtained in the control. The survival of fish challenged with *A. hydrophila* increased with increasing GT level in fish diets. These results indicate that GT supplement is promising immunostimulant, which could improve fish performance, health [18].

### 9. *Aloe vera*

Oral administration of aloe vera in common carp can enhance some of specific and non specific immune responses. This appears to be achieved primarily by increasing lysozyme activity, serum bactericidal power and the total protein and IgM levels. *Aloe vera* supplementation (0.5%) per feed can increase the resistance to *Aeromonas hydrophila* and *A. septicemia*. The relative percent survival (RPS) was found to be increased in the fish fed with *Aloe vera* [1].

### 10. *Cynodon dactylon* (Bermuda Grass)

The antiviral activity of a large scale produced plant extract of *Cynodon dactylon* was examined on white spot syndrome virus (WSSV) in black tiger shrimp *Penaeus monodon* by in vivo testing after administration through oral route. The results of the study showed that the plant extract of *C. dactylon* was found to be highly effective in preventing WSSV infection with no mortality and no signs of WSD (White spot disease) at 2% and 40% mortality at 1% in *P. monodo*, respectively [5].

### 11. *Achyranthes aspera* (Prickly Chaff Flower)

When Catla *catla* was fed with *Achyranthes aspera* (0.5%), both specific and non-specific immunity were enhanced compared with the control fish fed with the normal diet [15]. This was revealed by higher serum antibody levels and higher serum anti-proteases in the test group fish than control groups. Serum globulin level and RNA/DNA ratio of the spleen were also significantly enhanced in the fish fed with the *A. aspera* containing diet.

### 12. *Nyctanthes arbortristis* (Night-flowering Jasmine)

*Nyctanthes arbortristis* (L) is widely used plant in the traditional medicinal systems of India. It possesses hepatoprotective, antileishmanial, antiviral and antifungal activities. Feeding tilapia for 2 weeks with selected doses of chloroform extract of *Nyctanthes arbortristis* seeds significantly enhanced serum lysozyme, alternate complement activities and cellular ROS (cellular reactive oxygen species), RNI (reactive nitrogen intermediate) and MPO production. It was evident from the disease resistance test that feed supplemented with *Nyctanthes arbortristis* seed extract at 0.1% or 1% level significantly reduced the mortality of *O. mossambicus* and a 3-week feeding with 0.1% extract-supplemented diet appears to be the optimal regimen for maximal disease resistance [12].

### 13. Fermented vegetable product (FVP)

The phagocytic activities and superoxide generation of peritoneal induced leukocytes were significantly higher in fish Japanese flounder (*Paralichthys olivaceus*) fed the FVP supplemented diet than fish fed the control diet. FVP feeding in fish had a significantly higher ( $P < 0.05$ ) activity of lysozyme than in the control fish [3].

### 14. Other plant extract

Plant extract of *Cyanodon dactylon*, *Aegle marmelos*, *Tinospora cordifolia*, *Picrorhiza kurooa* and *Eclipta alba* were used to increase the immunity of shrimps against the WSSV. The mixed methanolic extracts of above plants was supplemented with various concentrations viz. 100 (A), 200 (B), 400 (C), and 800 (D) mg kg<sup>-1</sup> through artificial diets individually [7]. The shrimps fed on diet D (800 mg kg<sup>-1</sup>) significantly ( $P < 0.0001$ ) had more survival (74%) and reduction in the viral load compare to other diet. It also showed better performance of haematological, biochemical and immunological parameters.

## ANIMAL ORIGINATED IMMUNOSTIMULANTS

### 1. Chitin and chitosan:

Both chitin and chitosan have a major role in aquaculture. They are non-specific immunostimulators which are effective on a short term basis. Chitosan is a deacetylation product of chitin. The influence of chitosan on immune response of healthy and cortisol treated rohu was demonstrated. After treatment with chitosan sufficient higher responses in almost all assays of non-specific immunity were observed in comparison to their healthy control [16]. Chitosan added in feed of brook trout has a higher degree of protection against *A. salmonicida* infection for a short duration. It also gave protection when feeding was done @ 0.5 gm/100gm feed for one week. *Litopenaeus vannamei* who received chitin at 6 mg g<sup>-1</sup> or received chitosan at 2 mg g<sup>-1</sup> and 4 mg g<sup>-1</sup>, increased its immune ability by increasing its phenoloxidase activity, and respiratory burst [20].

## 2. Fermented products of chicken egg (EF203)

Fermented products of chicken egg (EF203) contain immunoactive peptides which showed Immunomodulatory effects when administered orally to rainbow trout, *Oncorhynchus mykiss* [19]. The chemiluminescent responses of kidney phagocytes after treatment with EF203 were significantly increased. Fish administered EF203 showed high phagocytic activities as compared to controls, and immunomodulatory effects were found to be dose dependent. Fish treated with EF203 displayed an increased resistance to both natural and experimental beta-haemolytic streptococcal infection

## CONCLUSION

Herbal extracts and animal originated product have a potential application as an immunostimulant in fish culture, primarily because they can be easily obtained, are not expensive and act against a broad spectrum of pathogens. Most of the herbs and herbal extracts can be given orally, which is the most convenient method of immunostimulation. However, the effect is dose-dependent, and there is always a potential for overdosing consequently, dosage optimization is strongly recommended. The use of such plant products as immunostimulants in fish culture systems may also be of environmental value because of their biodegradability. Due to their beneficiary attributes, we conclude that herbal extracts and animal originated product can be used in fish culture as alternatives to vaccines, antibiotics or chemotherapeutic agents.

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