



A REVIEW ON IMMUNOSTIMULANT EFFECTS IN FISHES

R.Thangamani^{1*}, P.Viji², B.Govindarajan³

¹Assistant Professor, P.G & Research department of Zoology, Nehru Memorial College, Puthanampatty, 621007, Tiruchirappalli, India.

²Assistant Professor of Zoology, Virudhunagar Hindu Nadar Senthikumara Nadar College, Virudhunagar, Tamil Nadu, India

³Department of Zoology, Virudhunagar Hindu Nadar Senthikumara Nadar College, Virudhunagar, Tamil Nadu, India.

Article Info

Received 29/09/2014

Revised 16/10/2014

Accepted 19/10/2014

Keywords :-

Mucormycosis, Fungal Infection, Amphotericin B, Uncontrolled Diabetes.

ABSTRACT

Fish culture in India, traditionally have strong export markets, which makes sea food industry a major net earner of foreign exchange for the country. But, in fish culture where the fish is at high challenge with infectious disease caused by bacteria, virus, parasites etc. Most infectious diseases of fish are opportunistic. This means that the simple presence of the pathogen in the environment of the fish is adequate to cause a disease outbreak. Intensive aquaculture practices have led to a growing interest in understanding fish diseases, so that they can be prevented or treated. To overcome such problems immunomodulators or immunostimulators can be used as protective and supportive therapy to promote host resistance. In Fish, non-specific defense system is activated by the immunostimulants. By definition, as immunostimulant is a chemical drug-stressor or activator that elevates the non-specific defense mechanism or the specific immune response. Immunostimulants are being used today both within aquaculture sector and in traditional animal husbandary to reduce mortality due to infections and to improve general performance of animals. Research in fish immunostimulants is developing and many immunostimulating agents are currently in use in the aquaculture industry. This review article mainly considers the recent development in this area to control the fish diseases and enhance the fish production with the help of different types of immunostimulants.

INTRODUCTION

Fisheries can be taken as a major economic activity in the country as it supports nutrition, employment, and foreign exchange [1] and a reliable source of protein for the future [2]. But a variety of microbial agents cause diseases in fish culture. Fish have many non-specific and specific humoral and cell mediated mechanisms to resist bacterial disease. In order to infect the host and multiply in the tissues, bacterial pathogens must overcome or avoid these defenses and in many cases, in order to resist an infectious challenge, the immune response of fish must react in a concerted multifactorial manner.

Protective immune responses can be primed by vaccination so that the use of immunostimulants was

introduced as a prophylactic measure [3,4]. In recent years Immunology has witnessed an explosion on knowledge and experimental skills that has expended our view of the immune system and means of searching for its structures and functions in an impressive way. The basic function of the immune system is to protect against foreign pathogens and infectious agents. The immunostimulatory agents may selectively activate either cell mediated or humoral immunity.

The immunostimulants have several advantages
i) being natural products, there is no environmental hazard.
ii) Unlike vaccines, which give protection to a specific pathogen, immunostimulants provide a wide range of protection against several pathogens.

Several immunostimulants such as glucan [5], Lactoferin [6], Levamisole [7], Chitin [8] EF203 [9], FK565 [10] usually show enhanced phagocytic cell activities. Nutritional factors such as Vit.B, Vit.C, growth hormone and prolactin have also been reported to be

Corresponding Author

R.Thangamani

Email:- pommi_gr@yahoo.com

Review Article



immunostimulators [11]. Immunostimulants are being used today within aquaculture sector to reduce mortality due to infections and to improve general performance of fishes.

Immunostimulant Effects in Fishes

The immune response of *Tilapia mossambica* to bovine serum albumin (BSA) and Sheep red blood cells (SRBC) was characterized in detail in terms of the appearance of hemolysin plaque-forming cells (PFC) in spleen, head-kidney and thymus of immunized fish and the maximum number was observed in these organs. Analysis of histological and smear preparations revealed that there were consistent cellular changes occurring in the spleen as well as head-kidney due to immunization [12].

The immune response of Indian major carps to antigens of motile aeromonads. The Indian major carp, *Labeo rohita* showed immunological memory and secondary response on booster administration. The extent of protection showed good correlation with titers of agglutinating antibody [13].

The immunostimulatory effect of the leaf extract of *Phyllanthus niruri* to SRBC (Sheep red blood cells) in *Oreochromis mossambicus*. The results showed significant enhancement of primary and secondary antibody response and non-specific immune response [14].

The effect of azadiractin, a triterpenoid derived from *Azadiracta indica* on the immune response in *Oreochromis mossambicus* to Bovine Serum Albumin (BSA) and SRBC. In general, azadiractin significantly enhanced the antibody response and leucocyte count in a dose dependent manner. Timing of azadiractin administration in relation to immunization revealed that the maximum enhancement of antibody response was observed when the stimulant was given two days prior to immunization [15].

The effect of leaf extracts of *Ocimum sanctum* on the specific and non-specific immune response and disease resistance against *A. hydrophila* in *O. mossambicus*. The stimulatory effect of the leaf extract of *O. sanctum* when administered through intraperitoneal and oral routes was obvious. Leaf extract of *O. sanctum* when administered intraperitoneally, stimulated both antibody response and neutrophil activity. Dietary intake of *O. sanctum* also enhanced the antibody response and disease resistance against *A. hydrophila* [16].

The immunity in Indian major carps *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* against the potent bacterial fish pathogen, *A. hydrophila* by the intraperitoneal route, in field conditions. Two different polyvalent antigen preparations namely, whole cell and extracellular products (ECP) were used. Immunization schedule consisting of a single booster dose given 28 days after priming resulted in a good agglutinating antibody response in the carps. Kinetics of the response was similar in the three carp species with both whole cell and ECP. Upon challenge with virulent strains, relative percent survival as high as

80-90% was recorded [17].

The immunomodulatory effect of Vitamin-A on common carp, *Cyprinus carpio* infected with *A. hydrophila*. In vitamin treated fish, there was no mortality and increase in the weight was observed. Vit.A containing retinol promotes the magnitude of antibody production in response to heat killed *A. hydrophila*. Vit.A showed immunostimulatory effect at the very low dose and immunosuppressive effect at high dose [18].

The immunomodulatory effects of high dietary ascorbic acid (vitamin C) on growth, serum concentration, non-specific immune response and disease resistance of a commercially important Asian catfish, *Clarias batrachus*. Growth, serum concentration of ascorbic acid (AA), Oxidative respiratory burst, lysozyme and natural hemolytic complement activities, myeloperoxidase (MPO) content and natural haemagglutination titre were found to be high. Fish fed with AA-supplemental diets showed significantly higher specific growth rate. The superoxide production was enhanced at a supplemented dose level of 2000 mg/kg. Similarly, MPO content, haemagglutination titre and alternative complement activity in serum enhanced with the increase of dietary AA levels at different duration of feeding. On the other hand, feeding of AA at all concentrations significantly increased percent survival against *A. hydrophila* challenge [19].

The immunostimulatory effect of *Tinospora cordifolia* miers leaf extract in *Oreochromis mossambicus* infected with *A. hydrophila*. Both ethanol and petroleum ether extracts administered, prolonged the peak primary antibody titers upto 1-3 weeks. Ethanol extract at the dose of 8mg/kg enhanced the secondary antibody response. All the doses of ethanol extract significantly enhanced neutrophil activity. Fish injected with petroleum ether or ethanol extract at a dose of 8mg/kg were protected against experimental infection with virulent *A. hydrophila* [20].

The Mushroom glucan and bovine lactoferrin (LF), known for their immunostimulatory potential, were used as adjuvant in conjunction with a formalin-killed *A. hydrophila* vaccine in *Catla catla*. Antigen-specific proliferation, macrophage activating factor (MAF) production and antibody production were significantly higher in glucan adjuvanted vaccine. LF adjuvanted preparations showed a weak proliferation response and MAF production, although the antibody production was significantly higher. A good degree of production was achieved with the glucan adjuvanted vaccine. However, in spite of producing significant anti-*A. hydrophila* antibody, LF adjuvanted vaccine did not confer any protection following challenge with *A. hydrophila* [21].

The dietary dosages of garlic on the immune response and disease resistance against infections against infections due to the opportunistic pathogen *A. hydrophila* in *Labeo rohita* fingerlings. Superoxide anion production, lysozyme, serum bactericidal, serum protein and albumin were enhanced in garlic treated groups and also fish



challenged with *A. hydrophila* recorded upto day 10 after infection, 85% survivability in the 1g garlic kg⁻¹ and 5g garlic kg⁻¹ and 71% survivability in the 10g garlic kg⁻¹ respectively [22].

The efficacy of dietary dose of *Magnifera indica* (mango) kernal on the immune response and disease resistance of *Labeo rohita* fingerlings against the bacterial pathogen *A. hydrophila* infections. The results demonstrate that fish fed with mango kernal showed enhanced superoxide anion production, lysozyme, serum bactericidal, serum protein albumin. The survivability was higher (98%) in 5g kernal kg⁻¹ fed group. These results indicate that mango kernal stimulates the immunity and makes *L. rohita* more resistance to *A. hydrophila* infection [23].

The effect of water and hexane soluble fractions of the Indian medicinal plant, *Solanum trilobatum* on the nonspecific immune mechanisms and disease resistance in *O. mossambicus* challenge with live *A. hydrophila*. Almost all the doses of both water and hexane soluble fractions enhanced the serum lysozyme activity. All the doses of water soluble fraction significantly enhanced the ROS (Reactive Oxygen Species) production. The leaf fraction administration preceding the challenge with live *A. hydrophila*, decreased the percentage mortality in the experimental group with the consequent increase in RPS (Relative Percent Survivability) values [24].

The oral administration of Bovine Lactoferrin (100mg kg⁻¹) in rainbow trout, *Oncorhynchus mykiss* (Walbaum) for three days prior to an intraperitoneal challenge with *V. anguillarum* resulted in increased survival rates, and enhanced resistance against streptococcus sp., although to a lesser extent. In lactoferrin-treated fish, an increase in phagocytic and chemiluminescent (CL) activities of pronephros cells against streptococcus sp., were also significantly increased [25]. The stimulatory effect of fermented vegetable products (FVP) upon the phagocytic and superoxide generation of leucocytes in Japanese flounder *Paralichthys olivaceus*. The phagocytic activities, lysozyme activity and superoxide generation of peritoneal induced leucocytes were significantly higher [26].

The impact of different levels of the dietary β -carotene on immune function in rainbow trout. The total immunoglobulin and serum complement activity was significant for the 200mg and 400mg β -carotene fed group than unsupplemented group. An increasing trend in lysozyme activity was observed, however, the differences among diet groups were not significant. Phagocytic activity was similar among diet groups except at highest level of supplementation where it was the maximum. Oxygen radical production by peripheral blood leucocytes appeared to be lower at higher levels of carotenoid supplementation [27].

The effect of levamisole on the specific and non-specific immune mechanism in rainbow trout, *O. mykiss*

(Walbaum). There was considerable increase in the glass-adherent Nitroblue tetrazolium (NBT), positive cell activation, NBT activities, phagocytic activity, potential killing activities of neutrophil and monocytes, myeloperoxidase production (MPO) and significant differences were detected. However there were no differences in the levels of hematocrit, leucocrit or immunoglobulin [28].

The antibody response of water extract of *Achyranthes aspera* leaves to heat killed *Aeromonas hydrophila* in *Oreochromis mossambicus* using Bacterial agglutination assay. Generally, the lower doses of leaf extracts administered groups significantly stimulated the antibody response and the high dose of leaf extract did not showed any significant differences from that of control. The results revealed the enhancement of the antibody response when compared with control especially using the lower doses of *Achyranthes aspera* leaf extracts [29].

The immunomodulatory effect of dietary levamisole in Asian cat fish *Clarias batrachus* against *A. hydrophila*. Levamisole supplement at the lower level (50mg kg⁻¹) significantly enhanced oxidative radical production and serum MPO content. Hemolytic complement and haemagglutination titre were significantly raised. At the highest level of levamisole feeding (450mg kg⁻¹) there was significant decrease in superoxide production and complement activity. The result supports the use of levamisole at 50 mg kg⁻¹ feed for 10 days as an immunostimulant in Asian catfish farming [30].

The efficacy and immunoreversal effect of the 4 dietary immunomodulators, viz. lactoferrin, beta-1,3 glucan, levamisole and vitamin C, on disease resistance of a commercially important catfish, *Clarias batrachus*. The results demonstrate that all four immunomodulators were capable of significantly enhancing the specific immune response. Similarly, all four substances significantly raised the survival rates in immunocompromised and healthy non-vaccinated fish. The results support the introduction of these substances into the diet of fish grown in farms under immunosuppressive/stressful conditions in order to enhance protection against infection and offer economic benefits [31].

The neutrophil activity of water extract of *Achyranthes aspera* leaves to heat killed *Aeromonas hydrophila* in *Oreochromis mossambicus* using NitroBlue Tetrazolium (NBT) assay. Generally, the lower doses of leaf extracts administered groups significantly stimulated the neutrophil activity and the high dose of leaf extract did not showed any significant differences from that of control. The results revealed the enhancement of the number of activated neutrophils when compared with control especially using the lower doses of *Achyranthes aspera* leaf extracts [32].

The impact of polyherbal immunomodulatory formulation 'ImmuPlus' (AquaImmu) on growth, immunity and disease resistance of rohu (*Labeo rohita*), one of the



Indian major carp at different stages of growth. The ImmuPlus fed fish showed enhanced non-specific immunity and resistance against *A. hydrophila* challenge in non-vaccinated fish as well as specific immunity levels. Incorporation of ImmuPlus at 1 g/kg level in the diet of rohu may be beneficial for enhancing disease resistance [33].

The effect of probiotic strains on the cellular and humoral immune responses of rainbow trout (*Oncorhynchus mykiss*), and their capacity to prevent furunculosis. Probiotic strains (*Lactococcus lactis* ssp. *lactis* CLFP 100, *Leuconostoc mesenteroides* CLFP 196, and *Lactobacillus sakei* CLFP 202) were administered orally to fish at $10(6) \text{ CFU g}^{-1}$ of feed. The phagocytic activity of head kidney leucocytes and the alternative complement activity in serum were significantly greater in all probiotic groups. With the exception of the group fed with *Lactobacillus sakei*, superoxide anion production was also significantly increased in the probiotic groups. The fish supplemented with probiotics exhibited survival rates ranging from 97.8% to 100% whereas survival was 65.6% in fish not treated with the probiotics [34].

The effect of three immunostimulants in *Oreochromis niloticus* fingerlings (Black seed 1.0, 2.0 & 3.0%; crushed garlic 1.0, 2.0 & 3.0% and Biogen 0.5, 1.0 & 1.5%). A significant increase in the hematocrit value and NBT test were seen in garlic groups & total protein, albumin and globulins was noticed in all treatments. The mortality rate of challenged group was lower in garlic group than other two treatments. The growth performance of fish revealed a significant increase in the body gain with garlic 1.0% and in the condition factor with garlic 2.0% and Biogen 1.5%. Also, a significant decrease in the mortality rate was observed in all treatments except Biogen 0.5% [35].

The neutrophil activity of water extract of *Aegle marmelos* leaves to heat killed *Aeromonas hydrophila* in *Oreochromis mossambicus* using NitroBlueTetrazolium (NBT) assay. Generally, the lower doses of leaf extracts administered groups significantly stimulated the neutrophil activity and the high dose of leaf extract did not showed any significant differences from that of control. The results revealed the enhancement of the number of activated neutrophils when compared with control especially using the lower doses of *Aegle marmelos* leaf extracts [36].

The effects of sex ratio of the population on the immune system of *Oreochromis mossambicus*. The results showed that antibody response and numbers of antibody producing cells were increased in fish in the equal male and female sex ratio group compared to fish in monosex ratio groups. Similar enhancement was also observed in nonspecific serum lysozyme level and the ROS and RNS production. The host resistance test revealed that enhanced immunity in equal male and female sex ratio group was protective against *Aeromonas hydrophila* infection. The study clearly reveals positive and negative effect of sex

ratio the immune system of *Oreochromis mossambicus* [37].

The effect of hexavalent Chromium on carp *Cyprinus carpio* derived immune cells. In vitro exposure of carp leukocytes to hexavalent chromium induced cytotoxicity, decreased mitogen-induced lymphocyte activation and phagocyte functions. Neutrophils responded to chromium challenge by changes in cell shape together with reduced nitric oxide and reactive oxygen production. Altered lymphocyte and neutrophil functions are considered to be responsible for decreased resistance to pathogens observed in fish under chronic chromium challenge [38]. The effect of intraperitoneal injection of polysaccharides (2-10mg) (Schizophyllan, Scleroglucan and Lentinan) to carp 6 and 3 days prior to intraperitoneal challenge with *Edwardsiella tarda* ($5 \times 10^7 \text{ CFU/100g}$) resulted in a significantly increased survival rate. In all polysaccharide-treated groups, the rapid elimination of the challenge bacteria from the blood was observed and phagocytic activity of pronephros cells against baker's yeast was significantly elevated.

Furthermore, it was shown that these polysaccharides activated the alternative complement pathway of carp. These results indicate that Schizophyllan, Scleroglucan and Lentinan enhance the resistance of carp [39] to bacterial infection through the activation of the non-specific immune system.

The immunostimulatory effect on *Catla catla* ($150 \pm 20 \text{ g}$) were fed a diet containing seed of *Achyranthes aspera* (0.5%) and control diet without *A. aspera* for four weeks prior to and after intraperitoneal injection with chicken erythrocytes. Fish were sampled for four consecutive weeks after immunization. Hemagglutination antibody titers were significantly higher in the test group of fishes compared with the control group. Serum globulin levels were significantly ($P_{t\text{-test}} < 0.05$) higher in the test group than control group on days 14 and 21. Anti-trypsin activity due to total serum protease inhibitors and $\alpha 1$ -antiprotease was also significantly ($p_{t\text{-test}} < 0.05$) higher in the test group of fishes than the control. RNA/DNA ratio of spleen and kidney was also significantly ($P_{t\text{-test}} < 0.05$) higher in test group than the control group. All these results confirm that *A. aspera* enhances the immunity of catla [40].

The effect of *Achyranthes aspera* seed was incorporated in the diets (at 0.01%, 0.1% and 0.5%) of *Labeo rohita*, rohu fingerlings ($3.0 \pm 0.4 \text{ g}$). After 2 weeks, the fish were immunized with heat-killed *A. hydrophila*, and after a further 2 weeks the rohu were experimentally infected with *A. hydrophila* (ATCC 49140). After 7 days blood and serum were sampled to determine superoxide anion production, bactericidal activity, lysozyme, serum protein, albumin, globulin, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP). Superoxide anion production, serum bactericidal activity,



lysozyme, ALP, serum protein, albumin:globulin ratio (A/G) were enhanced in *Achyranthes* treated groups compared to the control group. SGOT and SGPT levels were elevated in control group, but in *Achyranthes* treated groups the levels were similar to the uninfected-control group. Higher cumulative mortalities were observed in the control group (77%) up to day-9 after infection. This gradually decreased with increasing dose of *Achyranthes*, 66% mortality in 0.01% group, 57% mortality in 0.1% group and 28% mortality in 0.5% group. These results indicate that *A.aspera* stimulates immunity and increases resistance to infection in *L. rohita* [41].

The antibody response of water extract of *Aegle marmelos* leaves to heat killed *Aeromonas hydrophila* in *Oreochromis mossambicus* using Bacterial agglutination assay. Generally, the lower doses of leaf extracts administered groups significantly stimulated the antibody response and the high dose of leaf extract didn't showed any significant differences from that of control. The results revealed the enhancement of the antibody response when compared with control especially using the lower doses of *Aegle marmelos* leaf extracts [42].

The methanol extract of *Adhatoda vasica* leaves was tested for immunomodulatory effect on the primary and secondary antibody response and also neutrophil activity in *Oreochromis mossambicus* using direct haemagglutination assay and NitroBlueTetrazolium assay, respectively. Intraperitoneal route was followed for the administration of Sheep Red Blood Cells (SRBC) as antigen. The results showed, the optimal dose of antigen (SRBC) injected to the fish, prolonged the primary antibody response upto 30th day and also it was found that there is significant enhancement in secondary response when compared to the

primary response. The various doses of extract showed significant enhancement of neutrophil activity when compared with control [43].

The effect of dietary supplementation of seed of *Achyranthes aspera* on the immune system of *Labeo rohita* (rohu) fry (0.547 ± 0.01 g) were fed one of four diets containing 0 (control), 0.1, 0.5, or 1.0% *Achyranthes aspera* seed. After 30 days, the fry were immunized with chicken red blood cells (c-RBC). Blood samples were collected 7, 14, and 21 days after immunization. Significantly ($P < 0.05$) higher average weight (2.565 ± 0.02 g) and SGR were obtained in fry fed the 1.0% diet compared to others. The increase in average weight was directly related to the increasing dose of seed. FCR was significantly ($P < 0.05$) lower in fry fed the 1.0% diet while total serum protein, albumin, and globulin were higher in treated groups than in the unsupplemented control. The antigen-specific antibody titer level was significantly ($P < 0.05$) lower in rohu fed the control diet than in rohu fed the supplemented diets. Titer levels were 32 - 128, 128 - 256, 256 - 1024, and 256 - 1024 in the 0, 0.1, 0.5, and 1.0% diets, respectively. Serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) were significantly ($P < 0.05$) higher in rohu fed the control diet than in rohu fed the supplemented diets while myeloperoxidase was lower in the former. Among the treated groups, myeloperoxidase was significantly ($P < 0.05$) higher in rohu fed the 1.0% diet than in those fed other diets. The present study documents the immunostimulatory properties of *A. aspera* seed and finds that a dose of 1.0% might be suitable for rohu fry [44].

REFERENCES

1. Meenakumari B. (2002). Problems and prospects of Indian fisheries. *SDMRI, Research Publication*, 28, 85-91.
2. Landau. (1992). An introduction to aquaculture, 3rd edn John Wiley and Sons. in Newyork,1-5.
3. Anderson DP. (1992). Immunostimulants,adjuvants and vaccine carriers in fish, Applications of aquaculture. In, Annual Rev.
4. Mulero V, Esteban MA, Munoz J and Meseguer J. (1998). Dietary intake of levamisole enhances the immune response and disease resistance of the marine teleost gill head sea bream (*Sparus aurata*) *J Fish and Shellfish immunol*, 8, 49.
5. Jorgensen JB, Lunde H, Robertsen B. (1993). Peritoneal and head kidney cell response in intraperitoneally injected yeast glucan in Atlantic salmon, *Salmo salar* L. *J Fish Dise*, 16,313-325.
6. Sakai M, Otubo T, Atsuta S and Kobayashi M. (1993). Enhancement of resistance to bacterial infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum) by oral administration of Bovine lactoferrin. *J Fish Dise*, 16, 239-247.
7. Kajita Y, Sakai M, Atsuta S and Kobayashi M. (1990). The immunomodulatory effects of levamisole on rainbow trout, *Oncorhynchus mykiss*. *Fish Pathol*, 23, 93-98.
8. Sakai M, Kamiya H, Ishii S, Atsuta S, Kobayashi M. (1992). Immunostimulating effects of chitin in rain bow trout, *Oncorhynchus mykiss*. In,Shariff.M, Subasighe RP, Arthur J.R(Eds). *Dise. Asian Aquaculture* (1). Fish health section. Asian Fisheries Society, Manila, Philippines, 413-417.
9. Yoshida T, Sakai M, Kitao T, Khlil SM, Araki S, Saitoh R, Ineno T and Inglis V. (1993). Immunostimulatory effects of the fermented products of chicken egg, Ef203, on rainbow trout. *Oncorhynchus mykiss*. *Aquaculture*, 109, 207-214.
10. Kitao T and Yoshida. (1986). Effect of an immunopotentiator on *Aeromonas salmonicida* infection in rainbow trout (*Salmo gairdnesi*). *Vet Immunopathol*, 12, 287-291.
11. Sakai M. (1999). Current research status of fish immunostimulants. *Aquaculture*, 172, 63-92.
12. Sailendri K, Muthukkaruppan VR. (1975). The immune response of teleost, *Tilapia mossambica* to soluble and



- cellularantigens. *J Exp Zool*, 191, 371-382.
13. Karunasagar I, Ali A, Ota SK, Karunasagar. (1997). Immunization with bacterial antigens, Infections with motile aeromonads. *Dev Biol Stand*, 90, 135-141.
 14. Muthulakshmi M. (1997). Immunosimulatory effect of the leaf extract of *Phyllanthus niruri* on the immune response in *Oreochromis mossambicus* (Peters) M.Phil dissertation, The American college, Madurai.
 15. Logambal SM and Dinakaran Micheal R. (2000). Immunostimulatory effect of azadiractin in *Oreochromis mossambicus* (Peters). *Ind J Exp Biol*, 38, 1092-1096.
 16. Logambal SM, Venkatalakshmi S and Dinakaran Micheal R. (2000). Immunostimulatory effect of leaf extract of *Ocimum santum*, Linn in *Oreochromis mossambicus* (Peters). *Hydrobiologia*, 430, 113-120.
 17. Chandran MR, Aruna BV, Logambal SM, Micheal RD. (2002). Immunization of Indian major carps against *Aeromonas hydrophila* by intraperitoneal injection. *Fish Shellfish Immunol*, 13(1), 1-9.
 18. Balasubramanian V and Kaliraj P. (2006). Immunostimulatory effect of Vitamin-C on common carp *Cyprinus carpio*. *J Aqua Biol*, 21(1), 202-205.
 19. Jeyakumari and Sahoo PK. (2005). High dietary Vitamin-C affects growth, non-specific immune responses and disease resistance in Asian catfish, *Clarias batrachus*. *Mol Cell Biochem*, 280(1-2), 25-33.
 20. Samuvel Sudhakaran D, Srirekha P, Devasree LD, Preamsingh and Dinakaran Micheal R. (2006). Immunostimulatory effect of *Tinospora cordifolia* Miers leaf extract in *Oreochromis mossambicus*. *Ind J Exp Biol*, 44(9), 726-732.
 21. Kamilya D, Maiti TK, Joardar SN, Mal BC. (2006). Adjuvant effect of mushroom glucan and bovine lactoferrin upon *Aeromonas hydrophila* vaccination in catla, *Catla catla* (Hamilton). *J Fish Dise*, 29(6), 331-337.
 22. Sahu S, Das BK, Mishra BK, Pradhan J and Sarangi N. (2007). Effect of *Allium sativum* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *J App Ichthyol*, 23, 80-86.
 23. Swagatika Sahu, Basantha kumar dDas, Jyotirmayee pradhan, Monapetra BC, Mishra BK and Niranjana Sarangi. (2007). Effect of *Magnifera indica* kernal as a feed additive on immunity and resistance to *Aeromonas hydrophila* in *Labeo rohita* fingerlings. *Fish and shellfish immunol*, 23(1), 109-118.
 24. Divyagnaneswari M, Christyabapita D, Dinakaran Micheal R. (2006). Enhancement of non-specific immunity and disease resistance in *Oreochromis mossambicus* by *Solanum tribatum* leaf fractions. *Fish Shellfish Immunol*, 11, 1744-8687
 25. Sakai M, Otubo T, Atsuta S and Kobayashi M. (1993). Enhancement of resistance to bacterial infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum) by oral administration of Bovine lactoferrin. *J Fish Dise*, 16, 239-247.
 26. Ashida T and Okimasu E. (2005). Immunostimulatory effects of fermented vegetable products on the non-specific immunity of Japanese flounder *Paralichthys olivaceus*. *Fisheries science*, 71(2), 257-262.
 27. Edgar C Amar, Viswanth Kiron, Shuichi Satch, Nobuaki Okamoto and Takeshi Watanble. (2000). Effects of dietary β -Carotene on the immune response of rainbow trout *Oncorhynchus mykiss*. *Fisheries Science*, 66(6), 1068-1075.
 28. Mustafa RD. (2005). A study on the effects of levamisole on the immune system of rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Turk J Vet Anim Sci*, 29, 1169-1176.
 29. Thangamani R, Viji P, Govindarajan B. (2014). Antibody response of *Achyranthes aspera* Linn to *Aeromonas hydrophila* in *Oreochromis mossambicus*. *Asian Journal of Pharmaceutical Science & Technology*, 4(4), 173-177.
 30. Jeyakumari and Pranoda K Sahoo. (2006). Dietary levamisole modulates the immune response and disease resistance of Asian catfish *Clarias batrachus* (Linnaeus) *J Aqua Research*, 37(5), 500.
 31. Jeyakumari and Sahoo PK. (2005). High dietary Vitamin-C affects growth, non-specific immune responses and disease resistance in Asian catfish, *Clarias batrachus*. *Mol Cell Biochem*, 280(1-2), 25-33.
 32. Thangamani R, Viji P, Govindarajan B. (2014). Effect of water extract of *Achyranthes aspera* Linn. leaves on the neutrophil activity to heat killed *Aeromonas hydrophila* in *Oreochromis mossambicus* (Peters) *Int J Phytopharmacy research*. (In Press).
 33. Jeyakumari, Sahoo PK and Giri. (2007). Effects of polyherbal formulation 'ImmuPlus' on immunity and disease resistance of Indian major carp, *Labeo rohita* at different stages of growth. *Indian J Exp Biol*, 3, 291-298.
 34. Jose Luis Balcazar, Ignacio de Blas, Imanol Ruiz-Zarzuola, Daniel Vendrell, Olivia Girones, Jose Luis Muzquiz. (2007). Enhancement of the immune response and protection induced by probiotic lactic acid bacteria against furunculosis in rainbow trout (*Oncorhynchus mykiss*) FEMS. *Immunol Med Microbiol*, 23(17), 645-738.
 35. Ahmed Said Diab, Salah Mesalhy Aly, George John, Jasser Mohamed Adde-Hadi and Mohamed Fathi Mohamed. (2006). Effect of some local immunostimulants on the survival, growth performance and challenge infection of *Oreochromis niloticus*. *Mol Cell Biochem*, 1, 25-33.
 36. Thangamani R, D Thommi Arockia gasper, B Govindarajan, P Nagarajan and P Viji. (2012). Neutrophil activity of *Aegle marmelos* to *Aeromonas hydrophila* in *Oreochromis mossambicus*. *J Bio Sci Res*, 3(3), 203-206.
 37. Binuramesh C, Prabakaran M, Dieter Steinhagen and Dinakaran Micheal R. (2005). Effect of sex ratio on the immune system of *Oreochromis mossambicus* (Peters), *Brain Behave Immune*, 24.
 38. Dieter Steinhagen, Tobias Helmus, Stephan Maurer, Dinakaran Micheal R, Wolfgang Leibold, Jorn P Scharsack, Andreas



- Skouras, Hans-Joachim Schuberth. (2004). Effect of hexavalent carcinogenic chromium on carp, *Cyprinus carpio* immune cells. *Dis Aquat Organ*, 62, 155-61.
39. Tomoki Yano, Remy Mangindaan EP and Hiroko Matsuyama. (1989). Enhancement of resistance of carp *Cyprinus carpio* to experimental *Edwardsiella tarda* infection, by some β -1,3-Glucans. *Nippon Suisan Gakkaishi*, 55(10),1815-1819.
40. Rao VY and Chakrabarti R. (2005). Stimulation of immunity in Indian major carp *Catla catla* with herbal feed ingredients. *Fish & Shellfish Immunology*, 18, 327-334.
41. Vasudeva Rao Y and Chakrabarti R. (2005). Dietary incorporation of *Achyranthes aspera* seed influences the immunity of common carp *Cyprinus carpio*. *Indian Journal of Animal Sciences*, 75, 1097-1102.
42. Thangamani R, Viji P, Govindarajan B. (2014). Antibody response of *Aegle marmelos* Correa to *Aeromonas hydrophila* in *Oreochromis mossambicus*. *J Phar Bio*, 4(3), 127-130.
43. Kasthuri N, Thangamani R and Govindarajan B. (2012). Immunomodulatory effect of *Adhatoda vasica* in *Oreochromis mossambicus*. *J BioSci Res*, 2012, 3(2), 90-99.
44. Srivastava PK and Chakrabarti R. (2012). Effect of dietary supplementation of seed of *Achyranthes aspera* on the immune system of *Labeo rohita* fry. *Journal of Aquaculture Bamidgeh*, (IJA, 64.2012.786).

