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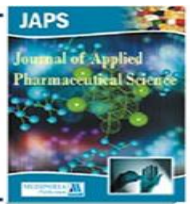

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Immunomodulatory Effect of Dietary *Nelumbo Nucifera (Lotus)* in Growth and Haematology of *Cirrhinus Mrigala* Challenged With *Pseudomonas Aeruginosa*

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ABSTRACT

The present study was carried out to evaluate the immunostimulant potential of *Nelumbo nucifera* (Lotus). Formulated diets with different concentrations of ethanolic extract of *N.nucifera* (D1=0%, D2=1% and D3=2%) were fed to *Cirrhinus mrigala* for 40 days, the Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) were calculated significant increase was observed in SGR &FCR. Then the experimental fishes were infected with *Pseudomonas aeruginosa* and after 5 days the haematological parameters like Total Erythrocyte count (TEC), Haemoglobin (Hb), Total leucocyte count (TLC), Differential leucocyte count (DLC), serum total protein, serum albumin and globulin levels were analyzed. The TEC, Hb, TLC, Lymphocytes and monocyte counts increased significantly in D3 diet fed fishes. Highly significant increase was observed in serum total protein and albumin levels only. Globulin levels remain unchanged. Basophil and Eosinophil counts decreased significantly. Thus *N.nucifera* in fish feed preparations may be included as growth promoter and immunostimulator.

Keywords: *Nelumbo nucifera*, *Cirrhinus mrigala*, *Pseudomonas aeruginosa*, haematology, Immunostimulator

INTRODUCTION

Aquaculture remains a growing, vibrant and important production sector for high protein food. The growth of intensive aquaculture production has led to a growing interest in treating or preventing fish diseases. Protecting the fishes from disease can be done through two ways. One is by strengthening the Immune power of the organism to fight the invasion of pathogens, and the second is through medication (Stephen *et al.*, 2006). Traditionally, antibiotics have been used in aquaculture for the prevention and treatment of bacterial diseases. However, the use of antibiotics in aquaculture poses threats such as development of bacterial strains that are resistant to antibiotic treatment, or the occurrence of antibiotic residues in fish farmed for human consumers (FAO 2002). Based on these two considerations, the potential uses of existing antibiotics and approval of new ones for aquaculture are limited. Alternatively, vaccines against specific pathogens have been developed with varying degree of success. The wide range of pathogens in fish farming also limits vaccines' effectiveness (Jayakumari and Sahoo, 2006).

Immunostimulants seem to represent a useful alternative to vaccination and chemotherapy in the control of fish diseases as they can enhance the non-specific immune response (Sakai 1999). The Immunostimulants also have additional advantages, such as growth enhancement and increase in the survival rates of the fishes under stress (Hoe *et al.*, 2004).

Herbs have been used as medicine and an immune booster for humans for thousands of years throughout the world, as many of them are rich sources of volatile oils, saponins, phenolics, tannins, alkaloids, polysaccharides and polypeptides which are responsible for various activities like anti-stress, appetizer, tonic, anti-microbial and immunostimulant. (Citarasu *et al.*, 2002, 2003). Recently, in aquaculture scores of plant extracts have been tested and used with good results in the control of bacterial and viral diseases.

Rao *et al.*, (2006) described that dietary supplementation of *Achyranthes aspera* seed stimulated immunity and enhanced resistance to *Aeromonas hydrophila* infection in *Labeo rohita*, similar results were also observed after feeding Tilapia (*O.niloticus*) with *Psidium guajava* (ethanol extract) incorporated diets. Similarly Sivagurunathan *et al.*, (2011) observed that feed incorporated with *Zingiber officinale* and *Curcuma longa* has enhanced the nonspecific immune response in *Cirrhinus mrigala* exposed to *Pseudomonas aeruginosa.*

Nelumbo nucifera (Lotus) is a perennial aquatic plant with yellow flowers. It is utilized as a dietary staple and also for a variety of medical purpose worldwide. The seeds of the lotus are utilized in the management of a variety of conditions including tissue inflammation, poisoning, cancer and leprosy. The plumule alleviates acute systemic inflammation in vivo (Lin *et al.*, 2007) and the rhizomes have been shown to have antioxidant properties (Dongmei *et al.*, 2007).The leaves are utilized to stanch bleaching in traditional Chinese medicine, and have also been shown to ameliorate hyperlipidemia in rodents (Lacour *et al.*, 1995). Palmitic acid is the predominant component of Lotus plumule oil and nuciferine is the major alkaloid isolated from the leaves (Bi *et al*., 2006). Kulkarni & Juvekar 2008 observed that *Nelumbo nucifera* has significant antistress potential.

Blood forms an integrated and inevitable part in all immune system and the changes in these parameters can be correlated to the response of the organism to the changing environmental condition and therefore can be used to screen the health status of the fish submitted to the exposed toxicant (Pandey and Pandey 2001).

The present work was carried out to evaluate the immunostimulant potential of *Nelumbo nucifera* (Lotus) by incorporating it with formulated diet and feeding to the fish *Cirrhinus mrigala*, followed by infection with *P.aeruginosa* and analyzing the haematological parameters.

MATERIALS & METHODS

The experimental fish *Cirrhinus mrigala* (weight 45±5g) were purchased from local fish farm and allowed to acclimate to laboratory conditions for 15 days. During acclimatization they were fed with rice bran and groundnut oil cake *ad libitum.* During the experimental period the water quality variables: temperature (28±1^{o}C), pH (7.4±0.2), salinity (10±2) and dissolved Oxygen (>5mg/l) were recorded. The water was changed daily in order to maintain the fishes in healthy state.

The dry powder of Lotus was purchased from the local market (Manufacturers: Agasthiyar Herbal Products, Madurai). 10 gram of the powder was dissolved in 100ml ethanol, shaken intermittently for 48hours and filtered through filter paper. The filtrate was evaporated in water bath and redissolved in 10ml ethanol. This formed the extract.

Feed preparation

The Control diet (D1) was prepared by mixing Rice bran 10g, Wheat bran 10g, Soya flour 23g, dry fish meal 24g, Ground nut oilcake 23g and Tapioca flour 10g made as a dove, sterilized in pressure cooker for 30 minutes, cooled, 2% of vitamins & minerals premix were added and made in the form of noodles by adding a little amount of sunflower oil which are then shade dried, broken into small desirable sized pieces. D2 diet was prepared by adding 1% of ethanolic extract of *Nelumbo nucifera* (Lotus) to the control diet and D3 diet was prepared by adding 2% of ethanolic extract of *Nelumbo nucifera* (Lotus). The fishes were primarily divided into three experimental groups. The experimental group I was kept as control group which were fed with control diet (D1). The experimental group II was fed with D2 diet and group III was fed with D3 diet for 40 days.

Growth parameters were calculated using the following formulae

$$\text{Specific Growth Rate (SGR)} = \frac{\text{Final weight} - \text{initial weight}}{\text{Time (days)}} \times 100$$

$$\text{Feed Conversion Ratio} = \frac{\text{Feed given (dry weight)}}{\text{weight gain (wet weight)}}$$

All the fishes (D1, D2, D3 diet fishes) were challenged Intra muscularly with the bacteria *Pseudomonas aeruginosa (*10^{-1} dilution) previously grown in nutrient broth for 24 hours. After 5 days the fishes in each group were subdivided into 3 subgroups with 5 fishes each and hematological study was conducted.

Haematological Analysis

After the experimental period blood was collected from the fishes by cutting the caudal peduncle and the blood was collected in heparinized tubes (to count TEC, TLC, Hb and DLC) and non heparinized tubes (to estimate serum total protein and albumin levels). All analysis was performed on pooled blood samples. Total Erythrocyte Counts (TEC), Total Leucocyte Counts (TLC) were counted using Haemocytometer with improved Neubaurer ruling chamber (Weber & sons, England), Haemoglobin (Hb) content was measured using cyanohaemoglobin method using Drabkins fluid. Blood smears stained with May-Grunewald's Giemsa's stain was used for differential leucocytes count. The serum total protein and serum albumin levels were analysed using the kits (CPC diagnostics, Chennai) and globulin levels were

calculated by subtracting albumin values from plasma total protein. The data were analyzed statistically and students "t" test was used to test their significance.

RESULTS & DISCUSSION

Growth Performance

The specific growth rate and Feed Conversion Ratio exhibited an increasing trend in all the three experimental groups; however it was significantly higher in D3 diet fed fishes (Fig.1 & 2). Thus it is evident that dietary incorporation of Lotus acts as growth promoter. Aly and Mohamed (2010) observed that Nile Tilapia (*Oreochromis niloticus*) fed with *Echinacea purpurea* and *Allium sativum* fortified diet exhibited significantly higher specific growth rate, Mohsen Abdel-Tawwab *et al.,* (2010) also observed significant increase in specific growth rate in Nile Tilapia fed with Green Tea (*Camellia sinensis*) incorporated diet evenwhen infected with *Aeromonas hydrophila.* Incorporation of Lotus in the diet might have improved palatability, digestion and absorption of nutrients**.**

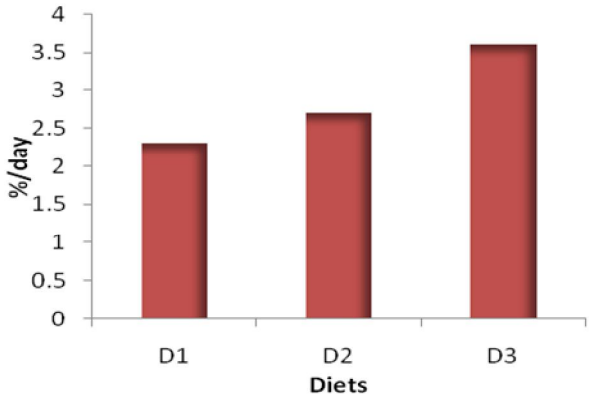

Fig. 1: Specific Growth Rate.

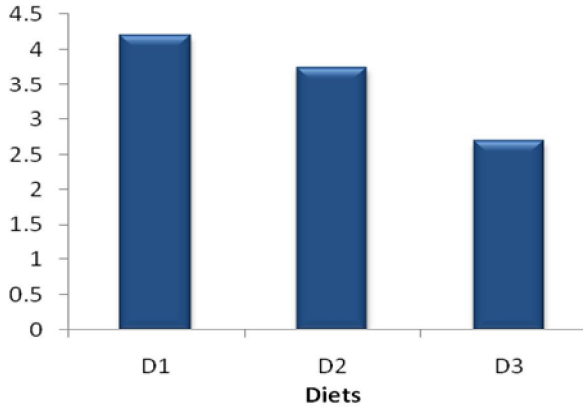

Fig. 2: Feed Conversion Ratio.

Erythrocytic Parameters

In the present study it was observed that the Total Erythrocyte Count (TEC) and Haemoglobin concentration levels were higher in both D2 & D3 diet fed fishes over D1 diet fed fishes. However the increase in the infected fishes was insignificant in D2 diet but highly significant in D3 diet fed fishes (Fig. 3 & 4). Similar increase in RBCs was observed by Mohsen Abdel-Tawwab *et al.,* (2010) in Nile Tilapia fed with Green Tea supplemented diet and infected with *Aeromonas hydrophila*, Sudagar and Hajibeglou (2010) also observed similar result in common carp fed with feed incorporated with plants like *Inula helenium, Tussilago farfaro, Brassica nigra, Echinacea purpurea* & *Chelidoniume majus* and infected with *Aeromonas hydrophila.* Goda (2008) observed that RBCs, Ht and Hb of Nile Tilapia increased significantly with increasing dietary ginsana levels compared to control fish.

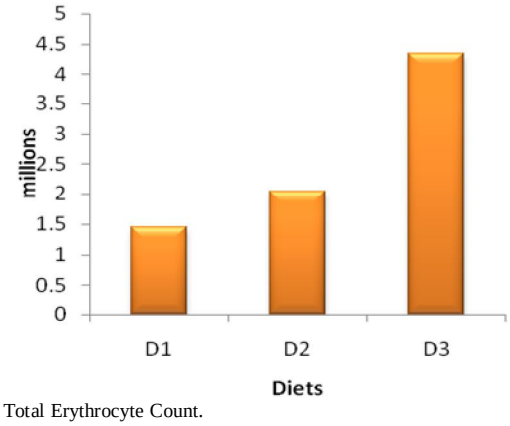

Fig. 3: Total Erythrocyte Count.

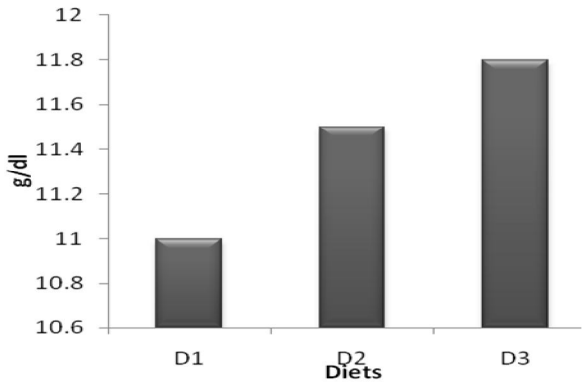

Fig. 4: Haemoglobin Content.

Erythrocytes are a major and reliable indicator of various sources of stress. RBCs transport Heamoglobin that in turn transports O_2, and the amount of O_2 received by tissues depends on the maturity of RBCs and Amount of Hb (Rehulka 1989, 2000 & 2002). Thus highly significant increase in RBC and Hb levels in D3 diet fed fishes is a response to tolerate stress or on the other hand is a measure to maintain general health.

Leucocytic Parameters

Highly significant increase in total leucocyte counts were observed in both D2 and D3 diet fed fishes over D1 diet fed fishes. In Differential leucocyte counts, in all the three experimental groups it was observed that the Lymphocyte counts were higher followed by Neutrophil, Monocyte, Eosinophil and Basophil (Table-1). The Lymphocytes, Neutrophils and Monocytes exhibited an increasing trend in both D2 &D3 diet fed fishes; however the lymphocyte increase was significant in both D2 & D3 diet fed fishes and monocyte increase was significant only in the D3 diet fed fishes, whereas insignificant increase was observed in

neutrophil counts when compared to infected fishes fed with D1 diet. On the other hand Eosinophil and Basophil counts exhibited highly significant decrease in both D2 & D3 diet fed fishes than their control counterpart. Mohsen Abdel-Tawwab *et al.,* (2010) observed an increase in WBC and Lymphocyte counts in Nile Tilapia fed with feed incorporated with Green Tea and infected with *Aeromonas hydrophila.* Similarly Sudagar and Hajibeglou (2010) also observed increase in WBC in common carp fed with feed incorporated with plants like *Inula helenium, Tussilago farfaro, Brassica nigra, Echinacea purpurea* & *Chelidoniume majus* and infected with *Aeromonas hydrophila*. Sahu *et al.,* (2007) reported that the WBC & RBC counts were higher in *Labeo rohita* fingerlings fed with *Magnifera indica* kernel. Gopalakrishnan and Arul (2006) also reported that there was an increase in WBC counts after feeding common carp with 'Chitin'.

Table . 1: Comparison of Total and Differential Leucocytes in *C.mrigala* fed with 0%, 1% and 2% of Lotus incorporated diet and challenged with *P.aeruginosa.*

Parameters	Diets		
	D1	D2	D3
TLC (x 10^4)	4.2±0.5	6.5±0.6**	10.1±0.8**
Lymphocyte(%)	45±3.1	51±2.5*	52±2.2*
Neutrophil (%)	30±2.8	32±1.9	33±2.1
Monocyte (%)	10±1.8	11±0.9	13±1.1*
Eosinophil (%)	10±1.2	3±0.4**	1±0.3**
Basophil (%)	5±0.4	3±0.3**	1±0.1**

*=significant, **=highly significant

Prit Benny *et al.,* (2010) also observed an increase in WBC and lymphocyte counts in *Clarias batrachus* fed with *Musa acuminate* peel extract. Similar increase in WBC, neutrophil, lymphocyte and monocyte were observed in *Cirrhinus mrigala* fed with feed supplemented with Ginger and Turmeric and infected with *P.aeruginosa* by Sivagurunathan *et al.,* (2011).

Total and differential leucocyte counts are important indices of non-specific defence activities in fish (Pedro *et al.,* 2005), as leucocytes are centrally involved in phagocytic and immune responses to parasitic, bacterial, viral and similar challenges (Houstan 1990). Similarly neutrophilia and monocytosis can be contributed to acute inflammatory response due to infection, as monocytes undergo transformation into macrophages and may be involved in phagocytosis and killing of pathogens upon first recognition and subsequent infections. Neutrophil is the first cell to respond to infection within 24 hours, increases during bacterial infections to phagocytose them, but they die after having phagocytosed a few pathogens as they cannot renew their lysosomes used in digesting microbes. Whereas monocytes phagocytose the pathogens not only more efficiently as it is long lived and able to replace lysosomes but also presents the antigens to lymphocytes.

Thus increase in the TLC, Neutrophil and Monocytes in Lotus extract incorporated diet fed fishes can be attributed to the non-specific immune response and increase in Lymphocytes may be a specific pathogen induced Immune response.

Serum Total protein, albumin & Globulin

Highly significant increase in serum total protein and serum albumin levels were observed in fishes fed with D3 diet, however the globulin level remained unchanged in all D1, D2 & D3 diet fed fishes (Table 2). Mohsen Abdal-Tawwab *et al.,* (2010) observed an increase in serum protein, albumin and globulin levels in Tilapia fed with Green tea incorporated diet and infected with A.hydrophila. Similarly results were also observed by Sudagar and Hajibeglou (2010) observed in *C.carpio* fed with feed incorporated with mixed plant extracts (*Inula helenium, Tussilago farfaro, Brassica nigra, Echinacea purpurea* & *Chelidoniume majus*) for 60 days and infected with *A.hydrophila.* There is a close relationship between the level of protein synthesis in liver tissue and plasma protein pools, total protein levels in plasma may be elevated due to the increased levels of protein synthesis in liver tissue (Asadi *et al.,* 2012). Commonly, increases in the levels of plasma total protein, albumin and globulin in fish are thought to be associated with a stronger innate immune response (Wiegertjes *et al.,* 1996). The increase in serum protein content might be in part due to an increase in the WBC, which is a major source of serum protein production such as lysozyme, complement factors and bactericidal peptides (Misra *et al*., 2006). Citarasu *et al.,* (2006) reported that serum proteins include various humoral elements of the non-specific immune system and increase in serum total protein, globulin and albumin are likely to be a result of the enhancement of the non-specific immune response of fishes. Serum albumin not only maintains osmotic pressure needed for proper distribution of body fluids between intravascular compartments and body tissues but also acts as plasma carrier protein to transport steroid hormones, hemin, fatty acids and also compounds like drugs (Asadi *et al.,* 2012, Wikipedia). Thus increase in serum Total protein and albumin levels in D3 diet fed fishes in the present experiment may be an indication to increased levels of non-specific immunity and the increased albumin levels may facilitate the transport of more humoral compounds as well as lotus extract in the blood.

Table. 2: Comparison of Serum parameters in *C.mrigala* fed with 0%, 1% and 2% of Lotus incorporated diet and challenged with *P.aeruginosa.*

Diet	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
D1	3.2±0.4	2.2±0.3	1.0±0.3	2.2±0.4
D2	3.1±0.5	2.0±0.4	1.0±0.2	2.0±0.3
D3	4.7±0.7**	3.7±0.6**	1.0±0.4	3.7±0.5*

*=significant, **=highly significant

CONCLUSION

In the present study it was observed that the Specific Growth Rate and Feed Conversion Ratio of Lotus diet fed fishes were significantly higher. Anaemic organisms are prone to infection as the healthy functioning of any cell requires adequate supply of oxygen. As the TEC and Hb values has increased in the Lotus diet fed fishes indicating improved health status to combat the pathogens. Increased levels of neutrophils and monocytes in Lotus diet fed fishes is a sign of Lotus induced improvement of non specific immune response and increase in the lymphocyte counts can be considered as an acute specific immune response. Similarly increased levels of serum total protein can be correlated to the increased levels of TLC both playing a vital role in

improving the non specific immunity. Thus from the present study it was observed that incorporation of Lotus in fish feed formulations not only acts as Immunostimulator but also as Growth Promoter.

REFERENCE

Aly SM., Mohamed MF. *Echinacea purpurea* and *Allium sativum* as Immunostimulants in fish culture using Nile Tilapia (*Oreochromis niloticus*). J. of Ani. Phy & Ani. Nutr. 2010; 94: e31-39.

Asadi MS., Mirvaghefei AR., Nematollahi MA., Banaee M., Ahmadi K. Effects of Watercress (Nasturtium nasturtium) extract on selected immunological parameters of rainbow trout (Oncorhynchus mykiss). Open Veterinary Journal. 2012; 2: 32-39.

Bi Y., Yang G Li H., Zhang G Gou Z. Characterization of the chemical composition of lotus plumule oil. J. Agri. Food. Chem. 2006; 54: 7672-7.

Citarasu T., Babu MM., Sekar RJR., Marian PM. Developing Artemia enriched herbal diet for producing quality larvae in *Penaeus monodon*, Fabricius. Asian Fish Sci. 2002;15: 21-32.

Citarasu T., Sivaram., Immanuel G., Rout N., Murugan V. Influence of selected Indian immunostimulant herbs against white spot syndrome virus (WSSV) infection in black tiger shrimp, Penaeus monodon with reference to haematological, biochemical and immunological changes. Fish shellfish Immunol. 2006; 21: 372-384.

Citarasu T., Venket Ramalingam K., Raja Jeya Sekar R., Micheal Babu M., Marian MP. Influence of the antibacterial herbs, *Solanum trilobatum*, *Andrographis paniculata* and *Psoralea corylifolia* on the survival, growth and bacterial load of *Penaeus monodon* post larvae. Aquac. Int. 2003; 11: 581-595.

Dongmei Y., Qiushuang W., Lequin K., Jianmei J., Tiejin Y. Vitamin A Status of the minority ethnic group of Karen hill tribe children aged 1-6 years in Northern Thailand. Asia Pac J Clin Nutr. 2007;16: 158-62.

En.wikipedia.org/wiki/serum_albumin.

FAO. Antibiotics residue in Aquaulture products. The State of World Fisheries & Aquaculture 220, Rome, Italy (2002) 74-82

Goda AMAS. Effect of dietary ginseng herb supplementation on growth, feed utilization and haematological indices of Nile Tilapia, *Oreochromis niloticus* (L), fingerlings. J. of the World Aquaculture Society. 2008; 39: 205-214.

Gopalakannan A., Arul V. Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of *Cyprinus carpio* and control of *Aeromonas hydrophila* infection in ponds. Aquaculture. 2006; 225: 79-187.

Heo GJ., Kim JH., Jeon BG., Park KY., Ra JC. Effects of FST-Chitosan mixture on cultured rockfish (*Sebastes schlegeli*) and Olive flounder (*Paralichthys olivaceus*). Kor J Vet Pub Health. 2004; 25: 141-149.

Houstan, A. H. (1990). Blood and Circulation. In: Methods for fish Biology, Schreck. CB and P.B Moyle (Eds).American Fisheries Society, USA., ISBN:0-913235-98 X:273-334.

Jaya Kumari ., Pramoda K Sahoo. Dietary levamisole modulates the Immune response and disease resistance of Asian catfish *Clarias batrachus* (Linnaeus). Aquaculture Research. 2006; 37: 500-509.

Kulkarni M P., Juvekar A R. Attenuation of Acute and Chronic Restraint stress-induced perturbations in Experimental Animals by *Nelumbo nucifera* Gaertn. Indian J Pharm Sci. 2008; 70: 327-332.

La Cour B., Molgaard P., Yi Z. Traditional Chinese medicine in treatment of hyperlipidemia. J. Ethnopharmacol. 1995;46: 125-9.

Lin JY., Lai YS., Liu CJ., Wu AR. Effects of lotus Plumule supplementation before and following systemic administration of lipopolysaccharide on the splenocyte response of BALB/c mice. Food Chem Tox.2007; 45: 486-93.

Misra CK., Das BK., Mukherjee SC., Meher PK. The Immunomodulatory effects of tuftsin on the non-specific immune sstem of Indian Major Carp. Labeo rohita. Fish shellfish Immunol. 2006; 20: 728-738.

Mohsen Abdel-Tawwab., Mohammad H. Ahmad., Medhat E A., Seden ., Saleh FM., Sakr. Use of Green Tea, *Camellia sinensis* L, in Practical Diet for growth and production of Nile Tilapia, *Oreochromis niloticus* (L) against *Aeromonas hydrophila* Infection. *J. of the World Aquaculture* Society. 2010; 41: 203-213.

Pandey AK., Pandey GC. Thiram and Ziram Fungicides induced alterations on some haematological parameters of fresh water cat fish *Heteropneustes fossilis*. Indian. J. Environ. & Ecoplan. 2001; 5(3): 437-442.

Pedro N., Guijarro AI., Patioo MAL., Alvarez RM., Delgado MJ. Daily and seasonal variations in haematological and blood biochemical parameters of Nile Tilapia *Oreochromis niloticus* (L). Braz. Arch. Biol. Technol. 2005; 47: 945-953.

Prit Benny., Geetha Viswanathan., Sunitha Thomas Aruna Nair. Investigation of Immunostimulatory behavior of *Musa acuminate* peel extracts in *Clarias batrachus*. The IIOAB. Journal. 2010; vol1, 2: 39-43. www.IIoab.org/vol1july2010.htm.

Rao YV., Das BK., Pradhan J., Chakrabarthi R. Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. Fish Shellfish Immunol. 2006; 20: 263-273.

Rehulka J. Determining the optimum doses of Kurasan (ethoxyquinolin) and butylhydroxytoluol (BHT) in dry pellets: effect of both anti-oxidants on rainbow trout, *Salmo gairdneri* Richardson. Aquaculture and Fisheries Management.1989; 20: 295-310.

Rehulka J. Influence of astaxanthin on growth rate, condition and some blood indices of rainbow trout *Oreochromis mykiss*. Aquaculture. 2000; 190: 27-47.

Rehulka J. Aeromonas causes severe skin lesions in rainbow trout *Oreochromis mykiss*: clinical pathology, haematology and biochemistry. Acts Veterinaria Brno. 2002;71: 351-360.

Sahu S., Das BK., Pradhan J , Mohapatra BC., Mishra BK., Sarangi NN. Effect of *Magnifera indica* kernel as a feed additive on immunity and resistance to *Aeromonas hydrophila* in Labeo rohita fingerlings. Fish Shellfish Immunol. 2007; 23: 109-118.

Sakai M. Current research status of fish Immunostimulants. Aquaculture.1999; 172: 63-92.

Sivagurunathan A., Amila Meera K., Xavier Innocent B. Investigation of Immunostimulant Potential of *Zingiber officinale* & *Curcuma longa* in *Cirrhinus mrigala* exposed to *P.aeruginosa*-Haematological Assessment. IJRAP. 2011; 2(3): 899-904.www.ijrap.net.

Stephen Sampath Kumar J., Anantharaja K. Herbal Health Care in Aquaculture-the Indian experience. Aquaculture. 2006: Infofish International.

Sudagar Mohamad., Hajibeglou Abasali. Effect of Plant Extracts Supplemented Diets on Immunity and Resistance to *Aeromonas hydrophila* in Common Carp (*Cyprinus carpio*). Agri. J. 2010; 5(2): 119-127.

Wiegertjes GF., Stet RJ., Parmentier HK., Van Muiswinkel WB. Immunogenetics of disease resistance in fish; a comparable approach. Dev. Comp. Immunol. 1996; 20: 365-381.