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Influence of Immunomodulators on Growth and Haematology of Nile Tilapia (Oreochromis Niloticus)

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Abstract:
The immunomodulant as agent (Plant extract) or immune therapeutic drug, Which stimulate the nonspecific immune mechanism. Tilapia fishes (Oreochromis niloticus) were obtained from Virudhunagar aqauarium and exposed to dietary administration of two different natural Immunomodulators. In all the groups of Nile tilapia (Oreochromis niloticus) the growth rate was better for the period of 30 and 60 than Control. Almost all the Biochemical, haematological parameters and Protein profile of serum was also gradually increased after 30 and 60 days of treatment period than control.

Keywords: *Immunomodulant, Oreochromis niloticus, Lotus stem, Jamun seed ect.,*

1. Introduction

Aquaculture means "growing aquatic organism in confined waters and harvesting the production for human benefits". 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. The second is through medication. Globe fish (*Oreochromis niloticus*) is a black or a light white color. The fish are mostly collected from river and pond. The *Oreochromis niloticus* fishes are commercial important and also used to the research laboratories for the medicinal purposes. Currently, Egypt is one of the country where aquaculture is growing fastest with Nile tilapia. *Oreochromis niloticus* is the most widely farmed species. (A. Figueras et al., 2005).The immunomodulant as agent (Plant extract) or immune therapeutic drug, Which stimulate the nonspecific immune mechanism. Immunomodulant are mainly natural product. Hence there are no environmental hazards and they have no residual effect on organism. Immunomodulants are useful for control of fish diseases and may be useful in fish culture field. (Lipton et al., 1995). The Immunomodulatory effects of glucan, chitin and lactoferin for fish have been reported. Nutritional factors such as vitamin B and C growth hormones have also been reported as Immunomodulators (Sung et al., 1997).These Immunomodulators also stimulate the activities of the natural killer cells, complement and antibody response of fish.(Rae et al., 1996). Immunomodulant which include both synthetic and biological substances perform different stimulating functions. (Huxley et al., 2002). The addition of immunomodulants to fish feed to stimulate the immune system is becoming very fashionable. When used in the correct circumstances can be a very useful management tool for the fishery managers. The technology has been transferred from the commercial world of aquaculture and the keeping of valuable *Tilapia fish*. (Scang and Hsieh., 1994). Fish are cold blooder and as such as their biological systems are temperatures dependent. At low temperatures around 10°c the immune system in fish is virtually inactive. The metabolism is so slow at these temperatures that there is effectively very little immune system response when the fish is challenged by a pathogen or environmental stressor. (Anderson et al., 1993).The immune system is the body's best defense mechanism against disease. (Soderhall et al., 1992). The best method to ensure a healthy immune system is to provide a stress-free environment and quality nutrition. (Sakai et al., 1999). Prepared diets not only provide the essential nutrients that are required for normal physiological functioning, but also may serve as a medium by which fish can receive other components that may affect their health. (Gatlin et al., 2002). The fish feed composed of soya bean, rice bran, ground nut oil cake, tapioca Some vitamins and probiotics. Immunomodulators such as medicinal plant like Lotus stem (*Nelumbo nucifera*) and Jamun seed (*Syzygium cumini*) were also added to it. It enhances not only the growth rate but also can positively stimulate the non specific defense mechanism of fish. (Gullian.M et al., 2004). The term probiotic inevitably refers to Gram-Positive bacteria associated with genus lactobacillus. However, now a days there has been a renewal of interest in the use of probiotics. In general terms, a group of requirements have been identified as important properties for lactobacilli to be effective probiotic organism. (Reid G. Smeianow et al., 1999). Probiotics have profound effect on potentiating both arms of immune response ie. Cell mediated immunity and humoral immunity. Commercial probiotics are added to the feed for *Oreochromis niloticus* fish. This feed stimulates the growth rate and immune system. (Naidu et al., 1999). The

Lotus stem has many nutritional values such as a rich source of calcium, iron, fiber. Lotus stem is used in human health they are rich in iron, which is required for RBC formation in blood. The chrorophyll present in the stack acts as active antioxidant to fight against cancer producing free radicals. (Dongmei et al., 2007). Jamun is a seasonal fruit. Jamun fruit is a sweety taste. This seed is a round shape and dark violet color. Jamun seed have a nutritional source rich in zinc, iron, calcium, sodium, carbohydrate. It has the ability to reduce the blood sugar levels and glucosuria in diabetic patients. Jamun seed is an antiseptic activity. (Pandey and Pandey 2001). The immunomodulatin effects of lotus stem (*Nelumbo nucifera*) and jamun seed (*Syzygium cumini*) as feed additives for Nile tilapia (*Oreochromis niloticus).* The Influence of such additives on the hematological parameters, non-specific immune response of *Nile tilapia* and their disease resistance abilities will be evaluated. (Dem LW et al., 1986).

1.1. Haematological Parameters

Oreochromis niloticus fish blood sample were collected for the analysis of haematological parameters such as RBC count, WBC count, platlet count, Macrophage count, Differential count, ESR, Haemoglobin concentration, phagocytic count (Sattari M. 2001). The serum of *oreochromis niloticus* fish was analysed for the biochemical parameters .

2. Materials and Methods

2.1. Collection of Experimental Fish

The experimental animal Jilabe fish, *Oreochromis niloticus* was collected from surya sri aquarium, viruthunagar. The collected fish were taken to the laboratory and were stocked in a recirculating water tanks. They were acclimatized to the ambient laboratory condition prior to starting the experiment.

2.2. Selection of Suitable Medical Plant

Medicinal plant which are having the active principles such as growth promoter, nervine, tonic, antistress, appetizer and immunomodulants were selected for the present study.

The important medicinal plants were selected for the present study such as lotus stem (*Nelumbo nucifera*), jamun seed (*Syzygium cumini*).

2.3. Preparation of Feed

	Control	Test 1	Test 2
Ground nut oil cake	400g	400g	400g
Soya bean	200g	200g	200g
Rice bran	330g	330g	330g
Tapioca	50g	50g	50g
Vitamin A & B	20g	20g	20g
Probiotics	-	1g	1g
Lotus stem powder	-	5g	-
Jamun seed powder	-	-	5g

Table 1

Figure 1: Experimental Setup

Figure 2: Fish Feed

The control, test 1, test 2 diet were prepared by mixing rice bran 330g, ground nut oil cake 400g, soya bean 200g, tapioca 50g. It was sterilized in pressure cooker for 30 minutes and cooled. Then 20g of vitamins B-complex and vitamin A was added. After cooling vitamins and minerals were mixed with the feed thoroughly. The feed was divided in to three equal parts, one part was served as

control and remaining parts were incorporated with 2 different immunomodulants (Test 1-1g Probiotics &5g Lotus stem powder, Test 2 –1g Probiotics & 5g Jamun seed powder) and it was made in the form of noodles. The noodles were sun dried till the moisture content was reduced to less than 10% and were broken manually into very small pieces. The dried noodles were stored in plastic box and used for the present study. The fishes were primarily divided into 3 experimental groups. The experimental group 1 was kept as control group which were fed with control diet. The experimental group 2 was fed with test 1 diet and group 3 was fed with test 2 diet for 60days. The experimental fish *Oreochromis niloticus* were purchased from local fish farm and allowed to accumulate the laboratory conditions for 60days. Then the fishes were weighed individually in a monopan balance. The initial weight of the fishes was ranged from 30g to 31.5g.

2.4. Growth Parameters

The initial and final weight of all experimental group of *Oreochromis niloticus* fish was recorded. The growth rate was calculated from the result obtained.

$$\text{Absolute growth rate (g/body wt/day)} = \frac{\text{Final body weight – Initial body weight}}{\text{Total number of days.}}$$

$$\text{Relative growth rate (\%)} = \frac{\text{Final body weight – Initial body weight}}{\text{Total number of days.}}$$

2.5. Hematological Studies

2.5.1. Fish Blood Sampling

Blood was obtained from the gills using a 25 gauge needl. The blood of *oreochromis niloticus* fish was analysed for the Haematological parameters, Such as RBC, WBC, PLATELET etc.,

2.5.2. Biochemical Analysis

The serum of *oreochromis niloticus* fish was analysed for the biochemical parameters such as

1. Estimation of protein (*Lowrys Method*).
2. Estimation of Albumin (*Lowrys Method).*
3. Separation of Protein (SDS-PAGE)

3. Result and Discussion

3.1. Effect of Immunomodulant on Growth Rate

Group	Initial weight	30 days	60 days
Control	30.5 g	39 g	48 g
Test - 1	30.5	48	59
Test - 2	30.5	45	54

Table 2

In the present investigation the fishes were exposed to T-1 (*Nelumbo nucifera*) and T-2 (*Syzygium cumini*) diet, when compared to control the Growth rate was increased gradually for the period of 30 and 6o days.

3.2. Effect of Immunomodulant on Growth Rate

Group	*Growth Rate*
Control	1.747 ± 0.543058
Test-1	2.5795 ± 0.761554
Test-2	2.1125 ± 0.686601

Table 3

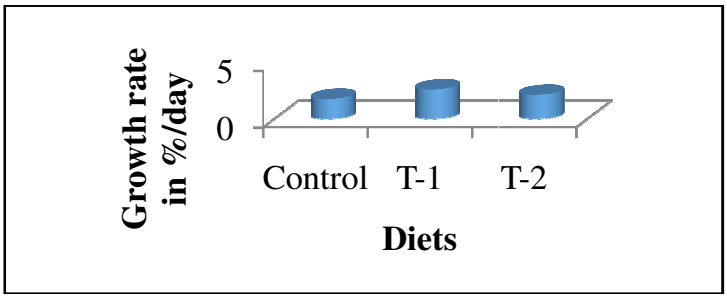


Figure 3

In the present investigation the fishes were exposed to T-1 (*Nelumbo nucifera*) and T-2 (*Syzygium cumini*) diet, when compared to control the Growth rate was increased gradually for the period of 30 and 6o days.

3.3. Effect of Immunomodulant on WBC Count

Group	WBC Count
Control	2.375 ± 0.318198
Test-1	2.75 ± 0.353553
Test-2	2.625 ± 0.318198

Table 4

In the present study the fishes were exposed to T-1 (*Nelumbo nucifera*) and T-2 (*Syzygium cumini*) diet, when compared to control in T1 & T2 the WBC count was increased gradually for the period of 30 and 60 days.

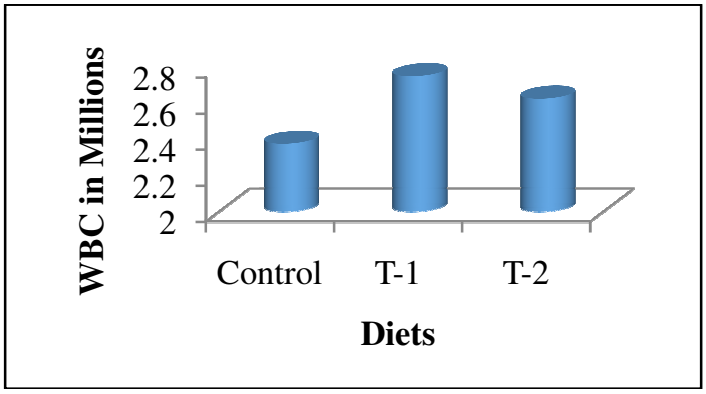


Figure 4

3.4. Effect of Immunomodulant on RBC Count

Group	RBC Count
Control	4.1 ± 0.197990
Test-1	5.46 ± 0.622254
Test-2	4.85 ± 0.212132

Table 5

The RBC content was increased gradually in T-1 (*Nelumbo nucifera*) and T-2 (*Syzygium cumini*) for the period of 30 and 60 days when compared to control.

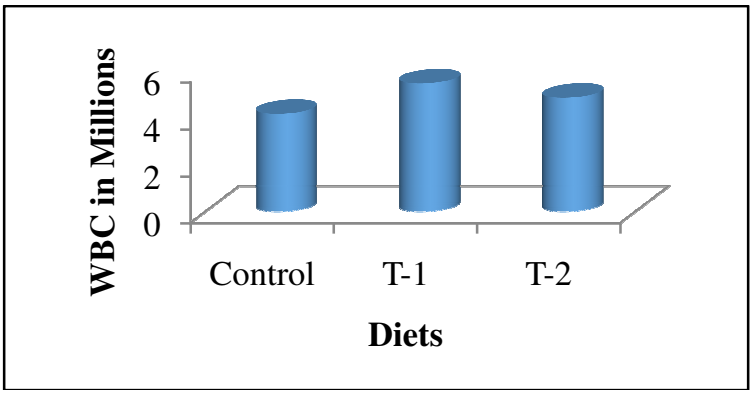


Figure 5

3.5. Effect of Immunomodulant on Differential Count

Group	**Differential Count**		
	Neutrophil	Basophil	Eosinophil
Control	34.5 ± 2.12132	65 ± 1.414214	54.5 ± 4.949747
Test-1	47 ± 11.31371	75.5 ± 4.949747	66 ± 5.656854
Test-2	42 ± 9.899495	73.5 ± 3.5335534	66.5 ±3.533534

Table 6

In the present study it was observed that the differential count (Neutrophil, Basophil, Eosinophil) of T-1 (*Nelumbo nucifera*) T-2 (*Syzygium cumini*) group was increased significantly for both 30 and 60 days when compared to control.

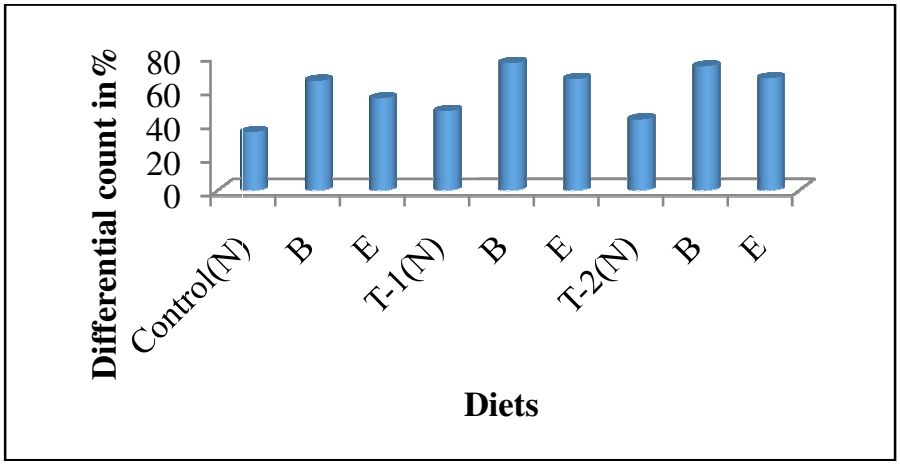


Figure 6

3.6. Effect of immunomodulant on Platlet Count

Group	Platlet Count
Control	3.8 ± 0.141421
Test-1	4.35 ± 0.070711
Test-2	4 ± 0.282843

Table 7

In the present investigation the fishes were exposed to T-1 (*Nelumbo nucifera)* and T-2 *(Syzygium cumini)* when compared to control the Platlet count was increased gradually for the period of 30 and 60 days.

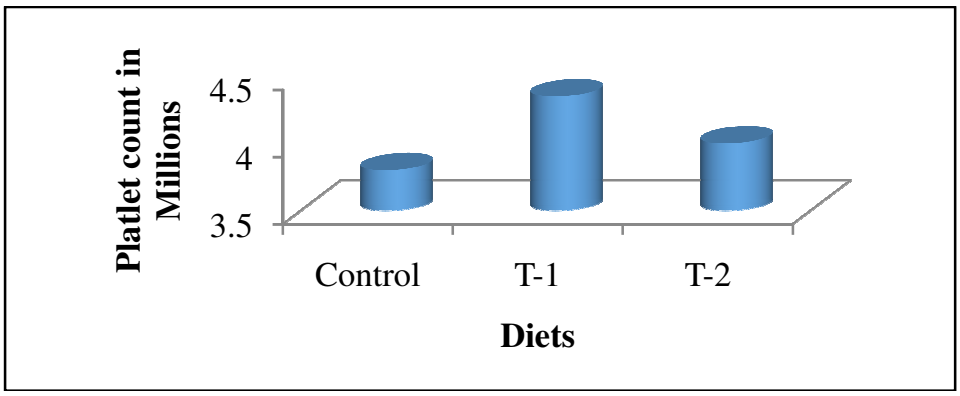


Figure 7

3.7. Effect of immunomodulant on Macrophage Count

Group	Macrophage Count
Control	84 ± 5.656854
Test-1	94.5 ± 7.778175
Test-2	86 ± 4.242641

Table 8

The Macrophage count was increased gradually in T-1 (*Nelumbo nucifera*) and T-2 (*Syzygium cumini*) for the period of 30 and 60 days when compared to control.

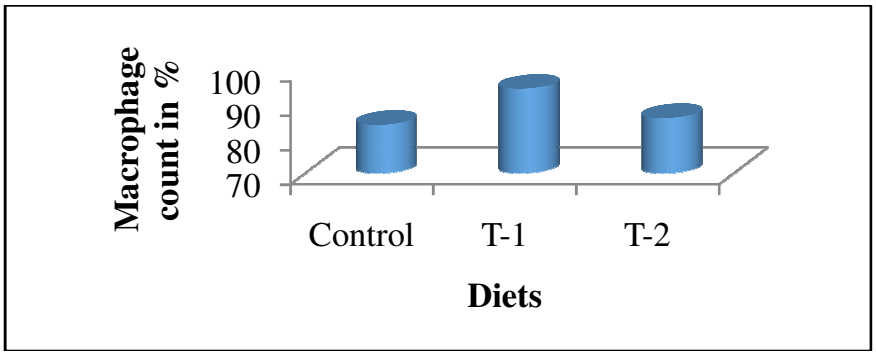


Figure 8

3.8. Effect of immunomodulant on Phagocytic Count

Group	Phagocytic Count
Control	42 ± 5.656854
Test-1	53 ± 8.485281
Test-2	45.5 ± 4.949747

Table 9

In the present study it was observed that the Phagocytic count of T-1 (*Nelumbo nucifera*) and T-2 (*Syzygium cumini*) group was increased significantly for both 30 and 60 days when compared to control.

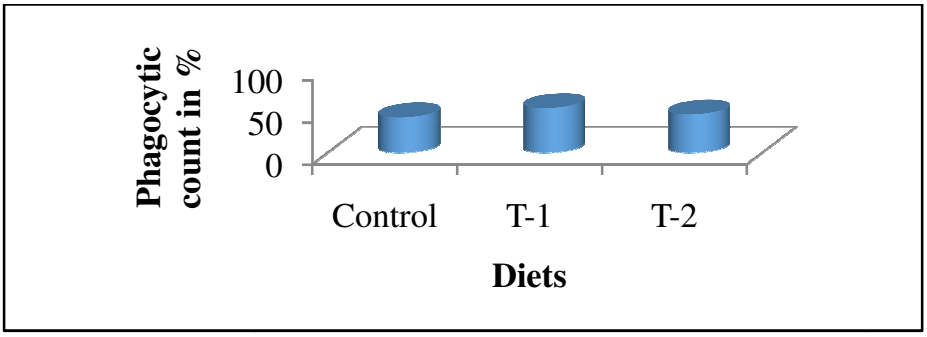


Figure 9

3.9. Effect of Immunomodulant on Haemoglobin Concentration

Group	Haemoglobin concentration
Control	8 ± 1.272792
Test-1	12.9 ± 1.13171
Test-2	11 ± 0.141421

Table 10

The Haemoglobin concentration was increased gradually in T-1 (*Nelumbo nucifera*) and T-2 (*Syzygium cumini*) for the period of 30 and 60 days when compared to control.

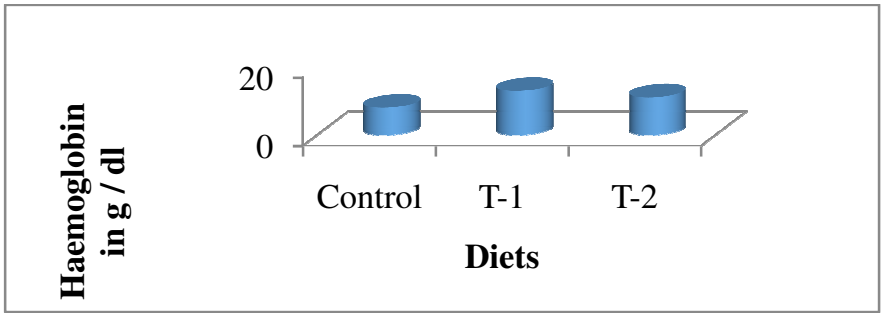


Figure 10

3.10. Effect of immunomodulant on Erythrocyte Sedimentation Rate

Group	ESR
Control	6.25 ± 0.777817
Test-1	6.9 ± 0.565685
Test-2	6.5 ± 0.565685

Table 11

In the present study it was observed that the ESR contration of T-1 (*Nelumbo nucifera*) and T-2 (*Syzygium cumini*) group was increased significantly for both 30 and 60 days when compared to control.

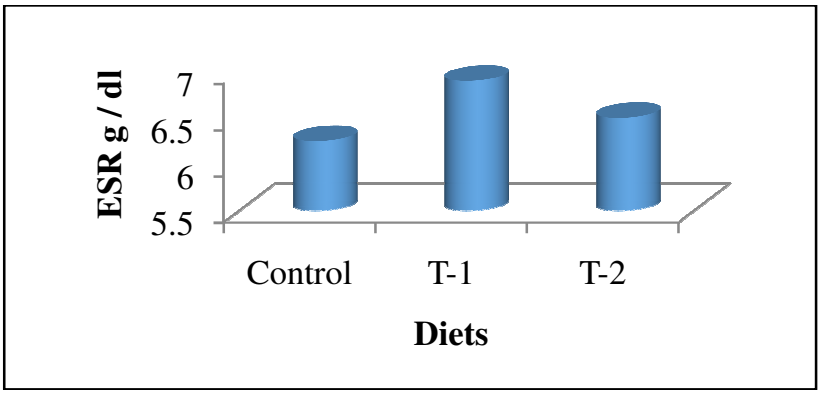


Figure 11

3.11. Effect of immunomodulant on Serum Protein

Group	Serum Protein
Control	6.25 ± 0.777817
Test-1	6.9 ± 0.565685
Test-2	6.5 ± 0.565685

Table 12

Highly significant increase in serum total protein levels were observed in fishes fed T-1 (*Nelumbo nucifera*) and T-2 (*Syzygium cumini*) diet for period of 30 and 60 days when compared to control.

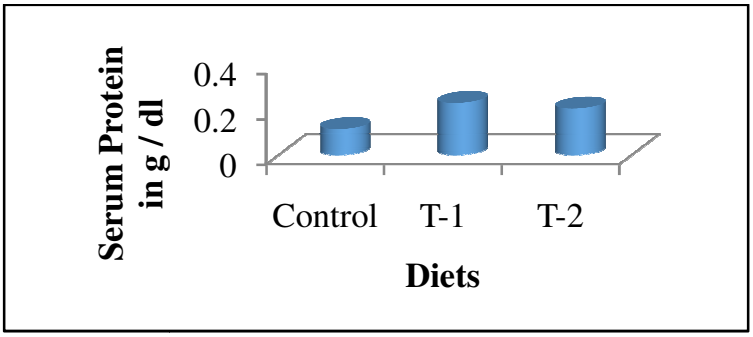


Figure 12

3.12. Effect of immunomodulant on Serum Albumin

Group	Serum Albumin
Control	0.115 ± 0.021213
Test-1	0.17 ± 0.56569
Test-2	0.175 ± 0.077782

Table 13

Highly significant increase in serum total Albumin levels were observed in fishes fed T-1 (*Nelumbo nucifera*) and T-2 (*Syzygium cumini*) diet when compared to control for the period of 30 and 60 days.

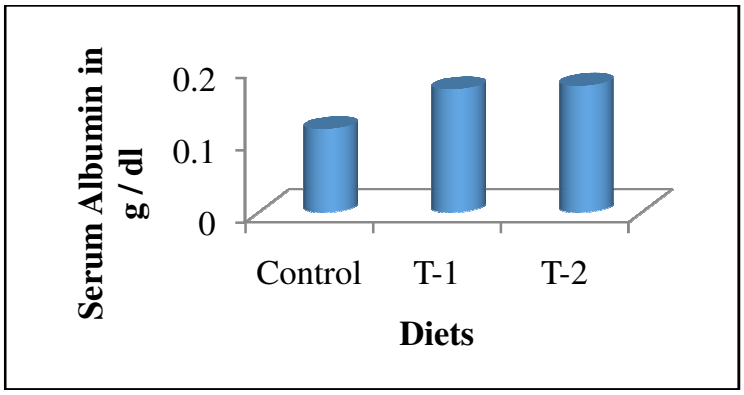
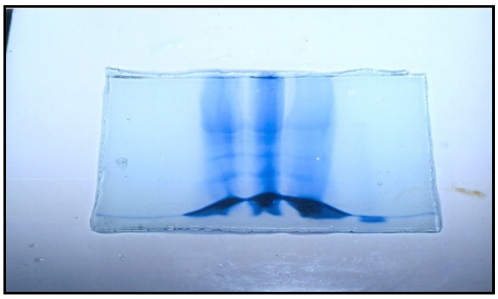


Figure 13

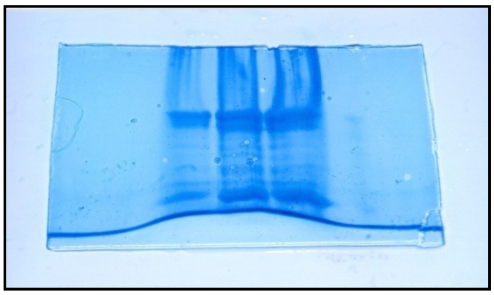
3.13. Effect of immunomodulant on SDS-PAGE

Three experimental fishes were subjected to SDS-PAGE after 30 and 60 days.The changes in the protein profile were recorded as follows.

The protein profile of *Oreochromis niloticus* fish after exposing the two different diet T-1 *(Nelubo nucifera)*, T-2 *(Syzygium cumini)*, the protein fraction was increased gradually in T-1, and then T-2 for the period of 30 and 60 days when compared to control.



Lane 3 – Control Lane 4
Test-1 *(Nelumbo nucifera*
Lane 5 – Test-2 *(Syzygium cumini)*



Lane 3 – Control
Lane 4 – Test-1 *(Nelumbo nucifera)*
Lane 5 – Test-2 *(Syzygium cumini)*

Figure 14: PROTEIN PROFILE OF Oreochromis niloticus ; SDS-PAGE AFTER 3O & 60 DAYS

In the present study the dietary administration of 2 different Immunomodulators were fed to Tilapia fish to asses their impart on growth and haematological studies. Panigrahi and Misra, (1978) Observed that reductions in haemoglobin percentage and red blood cell RBC count of the fish Anabas scandens treated with mercury. Decrease in haemoglobin, RBC count and Hct was observed in fish *Tinca tinca* exposed to mercuric chloride and lead . Shah and Alindag, (2004). The reduction of RBC is mainly due to development of hypoxic condition during the treatment which inturn leads to increased in destruction of RBC or decrease in rate of formation of RBC due to non availability of Hb content in cellular medium. X.Yin et al., (2007).The result of the present investigation showed that the Lotus stem and Jamun seed treatment increased the total count of RBC. The immunomodulant has been already reported for glucon, lactoferin, chitoson in fishes.saakai, 1999). This method is non stressful and allows mass administration, regardless of the size of the fish. The immune-potentiation effects of black cumin and/or cloSTAT were investigated after 30 days of successive dietary supplementation of *Nile tilapia.* In fish, most of the beneficial effects of dietary supplementation of medicinal herbs or probiotics as weight gain, improved immunity, and disease resistance have been recorded within a feeding regime of 1-10 weeks (C.Heo et al., 2010).The time course for optimum induction of immune response differs with respect to probiotic strain, type of medicinal plant, and type of the immune parameter. S.K.Nayak, (2010). In the present study, 2 different plant extracts were mixed with the T-1 *(Nelumbo nucifera) &* T-2 *(Syzygium cumini)* diet fed to Nile filapia fish for 60 days. Result showed that the fish fed with T-1*(Nelumbo nucifera)*, T-2 *(Syzygium cumini)* diet and control diet significantly increased the growth rate. Prit benny et al., (2010) also observed an increase in WBC and lymphocyte count in *clarias batrachus* fed with musa acuminate peel extract. Similar increase in WBC, Lymphocyte and monocyte were observed in cirrhinus mrigala fed with feed supplemented with Ginger and Turmeric infected with *pseudomonas aeruginosa* by Sivagurunathan et al., (2010). White blood cells (WBC's) of fish play a crucial role in the cellular immunity and resistance to infectious disease S.K.Whyte et al.,(2010). The significant increase in WBC's counts in fish fed with the nigella or combination diets may be due to the activation of the hamopoietic tissues by the black cumin SC.Nair et al., (1991). In the present study, WBC values also increased after exposure of T-1 *(Nelumbo nucifera)* and T-2 *(Syzygium cumini)* when compared to control. The effect of metrifonate on the immunity of the exposed fish evaluated through the determination of the antibody titier and

mortality rate during the challenge trial. The determination of some blood parameters revealed a significant decrease of WBC as there were Neutrophil, Basophie &Eosinophil during the chronic exposure to metrifonate. [G.Mohamed et al ., 2005] . The haematological parameters in a fish are reflected of by the physic-chemical conditions of its habitate. Lesser values of haematological parameters were observed in slow moving, sedentary species than predacious and pelagic species. In *chann a gughua* and *mystus gulia* there were more eosinophil cell in females than in males. In the present study was observed that the differential count (Neutrophil, Basophil, Eosinophil) of T-1*(Nelumbo nucifera)* and T-2*(Syzygium cumini)* group was also increased significantly. Hrubec et al., (2006) expressed age of factors effective on hematocrit concentration and number of RBC and reported that level of haemoglobin and RBC in male *cirrhinus mrigala* is higher than female. Barnharte et al., (2003) reported age and sex are effective on haematological parameters in the oncorhynchus mykiss. Haematological studies done by Mc Kim et al., (1976) reported that mercury accumulants in the fish blood decline in Haemoglobin and haematocrit was observed in *channa punctatus* exposed to mercury Sastry and Kanna Sharma,(1980). Takeda et al., (1958) showed that when ethyl mercuric chloride was administered subcutaneously to rats, the metal appeared primarily in red blood cells as 5-methylmercuric cyteine in the haemoglobin molecule. Haematocrit decreased significantly in the mercuric chloride treated fish when compared with the control fish.The disturbed haemoglobin synthesis due to an effect of lead on ALA-D may result in anemia. Santos and Hall,(1990). In the present study it was observed that haemoglobin concentration was also increased gradually in T-1*(Nelumbo nucifera)* & T-2 *(Syzygium cumini)* when compared to control. Regarding to the effect of biogen which used as a feed additives, in group 2 to overcome the drastic effect of metrifonate, a positive result were obtained in phagocytic cells, serum enzymes and protein increasing the immune status of fish leading to decrease the mortality rate among the treated fish. faisal et al.,(1988). Probiotic bacteria have been shown to influence immune responses by enhancing phagocytosis of pathogens as well as modifying cytokine production. Jennifer et al., (2010). In one study, a strain of Lacto bacillus acidophilus isolated from human newborn was inoculated into germ free and conventional mice and phagocytosis of E.coil was assessed in vivo Newmann et al., (1998). The mono association of germ free mice with these lactic acid bacteria for 7 days improved macrophage, phagocytosis capacity as demonstrated by the clearance of E.coil inoculated intravenously and similar to our previous studies. Randhawa et al., (2011); Bhatia et al., (2007); pawan et al., (2007) which observed immunomodulation by different probiotics such as L.delbrueckii, L.casei, Streptococcus thermophilus. In the present investigation the fishes were to exposed T-2 *(Syzygium cumini)* & T-1 *(Nelumbo nucifera)* when compared to control. The phagocytic cells were gradually increased for the period of 30 & 60 days. The time dependent effects of ethanolic extract of salmonalla treatment changes of macrophagas in mice showed significant increase in the number of macrophage cells on the 15th day of drug administration. Thus it significantly activated macrophages and enhanced their function as compared to control. RV.Savadi et al.,(2005) The plant extract of T-1*(Nelumbo nucifera)* & T-2 *(Syzygium cumini)* treatment on functional changes of macrophages in fish (Nile tilapia) showed significant increase in the number of macrophage cells on the 30 & 60 days when compared to control. The effect of cypermethrin on the haematological parameters of *cirrhinus mrigala* fish were subjected to haematological investigation.The haematological analysis showed that the RBC, HB, PCV, Platlet count were significantly increased in the fish *cirrhinus mrigala*, cardiospermum halicacabum plant supplementary feed influence to recover the immunological effects. M.Meenambicai et al.,(2007) In present study was observed that the Platlet count was increased gradually in T-1 *(Nelumbo nucifera)* & T-2*(Syzygium cumini)* when compared with control for the period of 30 and 60 days. Mohsen - 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 Aeromonas hydrophila. Similar results were also observed by Sudagar and Hajibelou (2010) observed in *Clarias carpio* fed with feed incorporated with mixed plant extracts (Inula holenium, Brassica nigra) for 60 days and infected with *aeromonas hydrophila.* The blood glucose, total plasma protein, uric acid and urea levels (76.74 ± 4.98, 4.08 ± 0.74, 1.57 ± 0.38 & 22.5 ± 3.45 respectively) of studied *Clarias garipinus* were similar to these reported by Tarares Dias (2000). However these levels were significantly higher than the levels of channel cattish, Ictalus metas. Neal and Weirich (2001). In the present study it was observed that the highly significant increase in serum total protein and serum albumin levels were observed in fishes fed with T-1 *(Nelumbo nucifera)* and T-2 *(Syzygium cumini)* diet for period of 30 & 60 days. Katherine.L et al.,1998 reported that this paper shows that proteins display an unexpectedly widerange of behaviors in buffers containing moderate capillary electrophoresis provides a convenient method of examining these behaviors. Examination of the dynamics of the response of proteins to SDS offers a way to differentiate and characterize proteins. Based on a survey of 18 different proteins, result showede that proteins differ in the concentrations of SDS at which they denature, in the rates of unfolding in SDS, and in the profiles of the denaturation pathways. The protein profile of *Oreochromis niloticus* fish after exposing the two different diet T-1 *(Nelubo nucifera)*, T-2 *(Syzygium cumini)*, the protein fraction was increased gradually in T-1, and then T-2 for the period of 30 and 60 days when compared to control.

4. Conclusion

Tilapia fishes *(Oreochromis niloticus)* were obtained from Virudhunagar aqauarium and exposed to dietary administration of 2 different Immunomodulators. It is also interesting to find out that in all the groups of Nile tilapia *(Oreochromis niloticus)* the growth rate was better for the period of 30 and 60 days. Blood collected were subjected to Haematological parameters such as WBC, RBC, Differential count, platelet count, hemoglobin concentration, ESR, Macrophage count, Phagocytic count after 30 and 60 days. Almost all the haematological parameters gradually increased after 30 and 60 days of treatment period. Serum collected were subjected to Biochemical studies such as Total Protein, Total Albumin after 30 and 60 days. Almost all the Biochemical Parameters gradually increased after 30 and 60 days of treatment period. Protein profile of serum exposed to Immunomodulator diet on PAGE indicated increased number of protein fraction were observed in the period of 30 and 60 days. The above study clearly indicates that Test-1 *(Nelumbo nucifera)* showed maximal activity than Test-2 *(Syzygium cumini)* and control.

5. References

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