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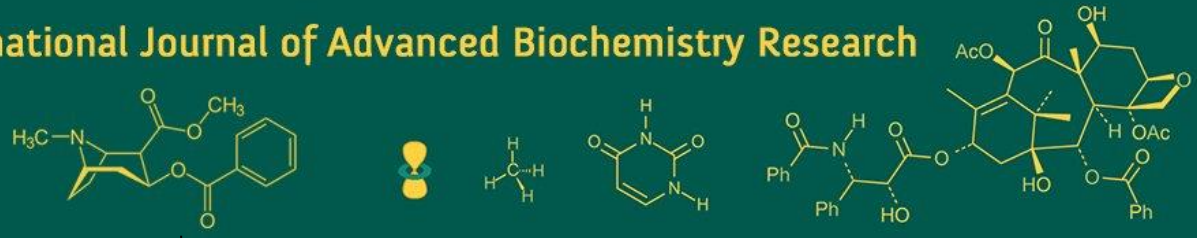

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The effects of Tulsi and marigold as dietary supplement on growth, haematological and biochemical parameters in *Labeo rohita* (Hamilton, 1822)

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Abstract
The efficacy of *Ocimum sanctum* (Tulsi) and *Tagetes erecta* (Marigold) on growth-promotion, haematological and biochemical profiles in fingerlings of *Labeo rohita* was evaluated. Different diets were prepared 0% (control), 1% Tulsi (T_1), 3% Tulsi (T_2), 1% Marigold (T_3) and 3% Marigold (T_4) and fed to the fishes. The sampling of the experiment was done on the 18th, 36th, 54th, 72th and 90th days for growth, haematological, biochemical parameters and water quality parameters analysis. The lowest FCR and significantly higher SGR, FCE, and percentage (%) weight gain were found in T_2 group. Highest body weight gain, length and percentage length was found in T_4 group. At the end of the experiment the biochemical parameters indicated that inclusion of *Ocimum sanctum* and *Tagetes erecta* in feed significantly (p<0.05) increased the serum total protein, albumin and globulin content. Results revealed that administration of *Ocimum sanctum* and *Tagetes erecta* through different diets mainly in T_2, T_3 and T_4 diets increased the total erythrocyte count, haemoglobin and total leukocyte count which enhanced the innate immune response. The physico-chemical parameters to be analysed include pH, temperature, total alkalinity, ammonia–nitrogen, dissolved oxygen and phosphate-phosphorus. The result of the present study indicated that all parameters are optimum for fish culture. The incorporation of Tulsi and Marigold extract @ 3% in the feed may influence the innate immunity, growth performance and resistance of fish.

Keywords: Tulsi, marigold, rohu, biochemical, growth

Introduction
Indian fisheries and aquaculture are major sectors of food production. These sectors also provide livelihood support, nutritional security along with their contribution to economic growth through exports. The globally total fish production during 2021-22 is estimated to be 177.8 MMT, of which nearly 37.23% is from the inland sector and India constitutes about 8% of the global fish production (FAO, 2022) [10].

Indian foremost carps (IMCs), rohu (*Labeo rohita*), catla (*Gibelion catla*), and mrigal (*Cirrhinus mrigala*) are the most common carp species grown in India and other Asian nations, accounting for approximately 87% of total freshwater aquaculture production in India. *Labeo rohita* is a member of the subfamily Cyprininae. It may be bred in tanks and is appropriate for both composite fish culture and single species culture. It is an 'illiophage,' an herbivorous organism that feeds on the column. It can grow to a maximum length of roughly 100 cm, but in the first year, it only develops to around 20 cm and 800 g. Quality seed, feed (quality and quantity) and infectious and non-infectious diseases are the major constraints of aquaculture for fish production. It's demonstrated that the cultured fish are more susceptible to diseases than the wild ones because of over handling, transportation, overcrowding and maintenance of poor water and soil quality etc. (Jobling, 2010; Ali *et al*., 2003) [18, 2].

Water characteristics such as pH, oxygen, and carbon dioxide, among others, play a significant role in the maintenance of homeostasis in aquatic creatures, with pH drops causing problems in acid—base and ion control, as well as ammonia excretion (Jensen and Brahm, 1995) [16].

In case of fish infections, however, chemicals of natural origin which are biodegradable and biocompatible properties have gained much importance and attention. Several plant extracts/material/products such as saponin (Ninomiya et at., 1995) [33], glycyrrhizin (Jang *et al*, 1995) [15], aloe (Kim *et al*., 1999) [22], *O. sanctum* (Logambal *et al*., 2000) [26], Azadirachtin (Logambal *et al*., 2001) [25], *Viscum album*, *Urtica dioica* and *Zingiber officinale* (Dugenci *et al*., 2003) [47], *Astragalus radix* and *Scutellari radix* (Yin *et al*., 2006) [45], and *Achyranthes aspera* (Rao *et al*., 2004) [37] have been documented to increase the immunity response and growth of the fish.

Many scientific studies have found *Ocimum sanctum* to have anti-stress, antioxidant, hepatoprotective, immunomodulating, anti-inflammatory, antibacterial, antiviral, antifungal, antidiuretic, and hypolipidemic properties with a wide margin of safety (Ranjana and Tripathi, 2015) [36]. *Tagetes* species, which are members of the Asteraceae family, are the most prevalent in the plant kingdom and are utilized in a variety of applications such as cosmetic preparation, medicinal, and ornamentals. *Tagetes erecta* contains carotenoids which is one of the main pigment and major constituent and also have a very strong aromatic target oil (essential oil), syringic acid, quercetagetin, phenolics, a glucoside of quercetagetin, quercetin, ethyl gallate, terpenes, methyl-3, 5- dihydroxy-4-methoxy benzoate, thienyl and many other important phytochemical substances in different plant parts (Priyanka *et al*., 2013) [34]. The *Ocimum sanctum* (Tulsi) and *Tagetes erecta* (Marigold) effects when supplemented through feed to *Labeo rohita* has not been evaluated to our best understanding. Therefore, the present study was aimed to the effects of plant extracts on growth, haematological and biochemical parameters in *Labeo rohita* (Hamilton, 1822) [48].

Materials and Methods

Experimental Setup

The experiment was carried out in 15 rectangular fiberglass reinforced plastic (FRP) tanks of 500 L each. The tanks were cleaned and sun-dried for 5 days. They were filled with tap water up to 200 L which was maintained throughout the experimental period. The exchange of water was done after every week.

Stocking and Rearing of Experimental Fish

The healthy fingerlings of *Labeo rohita* were acquired from Sonarpur Fish Hatchery, Kolkata, India and were carefully brought to the experimental location, Faculty of Fishery Sciences in good condition. Before reaching the wet laboratory, as a prophylactic measure for 3 to 4 minutes the fish were treated with 2% Potassium Permanganate ($KMnO_4$) solution. Then they were kept in a 500 L rectangular FRP tank and fed with the formulated diet for 12 days prior to the experiment. Feeding was stopped 24 hours before the experiment and the fishes were kept in starvation. The average weight 18±1 (g) and length 14±1 (cm) of the fishes were measured and distributed in tanks according to the experimental protocol. 12 fishes were stocked in each experimental tank and every treatment group consisted as triplicate with continue adequate aeration.

Collection of Plant Material

Fresh two-kilogram leaves of *Ocimum sanctum* were collected from the local market and *Tagetes erecta* leaves were collected from the campus of the Faculty of Fishery Sciences, Chakgaria, Kolkata. The leaves were washed, shade-dried and pulverized by a grinder, passed through a mesh sieve and stored in a sealed container.

Feed Preparation

The diet was prepared following the method of Talpur and Ikhwanuddin (2013) [42] with slight modifications. Commercial ingredients such as Fish meal, Wheat meal, Soybean meal, Fish oil, Vitamins and minerals (pre mixture) and Corn starch were procured from a local market present at Gangajoar, South 24 Pargana, West Bengal. The composition of different feed ingredients and of formulated diets is given in Table (1) and (2) respectively. Three types of feed were prepared which were as follows: Tulsi (1% and 3%), Marigold (1% and 3%) and control feed (without Tulsi and Marigold powder). All the ingredients were weighed properly as per the requirement and kept in a big plastic container. In a plastic container all the properly weighed ingredients are taken and by adding required amount of water dough is prepared. To that dough calculated amount of oil was added and properly mixed. The mixed dough was cooked for 30 minutes in a pressure cooker. The steamed and cooked dough was cooled by taking it out and added with the required amount of vitamin and mineral mixture. Then this dough was pressed using a hand pelletizer to prepare uniform size pellets. These pellets were fan dried initially and then later dried completely under sunlight. Later these pellets stored in airtight polythene bags and were labelled accordingly.

Table 1: Composition of Feed Ingredients

Sl. No.	Ingredients	Percent incorporated
1.	Fish meal	50
2.	Wheat meal	25
3.	Soybean meal	15
4.	mix. oil	5
5.	Vitamin and minerals	2
6.	Cornstarch	3

Table 2: Composition of Experimental Diet

Sl. No.	Ingredients	Control diet (C)	Treatment diet (T_1 and T_3) (1%)	Treatment diet (T_2/T_4) (3%)
1.	Fish meal	50	49	47
2.	Wheat meal	25	25	25
3.	Soybean meal	15	15	15
4.	Mix. oil	5	5	5
5.	Vitamin and minerals	2	2	2
6.	Cornstarch	3	3	3
7.	Tulsi (T_1/T_2)	0	1	3
8.	Marigold (T_3/T_4)	0	1	3

Experimental Design

One hundred eighty advanced healthy *Labeo rohita* fingerlings were distributed randomly into three experimental groups. Each experimental treatment had three replicates which are arranged using completely randomized design (CRD), design. In each experimental tank twelve fishes were stocked and the experiment was performed for a duration of 90 days. The fishes were fed @ 2-3% of their body weight and feeding was done at 09.00 h and 17.00 h by equally dividing the daily feed ration into two parts. Fish were sampled on the 18th, 36th, 54th, 72th and 90th days for analysis of blood, serum and growth. For analysing

haematological and biochemical parameters blood and serum samples were collected. The animal ethical guidelines of West Bengal University of Animal and Fishery Sciences, Kolkata, India were followed strictly.

Growth Indices

At 18 days interval sampling was performed to assess the growth performance of the fish. Length and weight were measured using scale and electric weighing balance respectively. Growth indices were evaluated using the below described formulae.

Weight gain = Final weight – Initial Weight

Length gain = Final length – Initial Length

Feed conversion ratio (FCR)

$$FCR = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}}$$

Percentage Weight gain

$$\text{Weight gain (\%)} = \frac{\text{Final weight} - \text{Initial weight x 100}}{\text{Initial weight}}$$

Specific growth rate (SGR)

$$\text{SGR (\%)} = \frac{\text{Ln Final Weight} - \text{Ln Initial Weight x 100}}{\text{Number of Days}}$$

Feed conversion efficiency (FCE)

$$FCE = \frac{\text{Net weight gain (wet weight) x 100}}{\text{Feed given (dry weight)}}$$

The calculated value indicates the average increase (%) of body weight per day.

Water Physio-chemical Parameters

Water quality parameters including temperature, pH, dissolved oxygen, free carbon dioxide and alkalinity were recorded during the experimental period was determined according to the methods of APHA (2005) [4].

Determination of Haematological Parameters

White blood cells (WBC) or total leucocyte count (TLC) were counted in haemocytometer's Neubauers counting chamber using leucocyte diluting fluid (Qualigens) using the method described by Shaw (1930) [41]**.** Cyanmethemoglobin method was used for determining Haemoglobin percentage (Van-Kampen *et al*., 1961) [43].

Serum Biochemical Assay

Blood glucose was estimated by using Commercial assay kits, Liquixx Glucose kit (BLT120235), Liquixx total protein kit (BLT00054), Liquixx total albumin kit (BLT0001) which were procured from Erba® Mannheim. Blood glucose level was evaluated in mg dl^{-1}. Calculation of Serum globulin was done using the formulae.

Serum globulin (g/dl) =Serum total protein (g/dl) - Serum albumin (g/dl)

Statistical analysis

Data obtained were analysed, using the statistical package SPSS 16.0 computer program. Differences in the mean value of the parameters of the test concentrations and controls were subjected to one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to determine the level of significance at 5% probability level. Results were expressed as mean ± standard deviation.

Results

Physico-chemical Parameters of Water

Water's different physico-chemical parameters like temperature (°C), pH, free carbon dioxide (ppm), dissolved oxygen (ppm), alkalinity, ammonia (mg L^{-1}), phosphate (ppm), Nitrate (ppm) during the experimental period is presented in Table 3. All the physico-chemical parameters observed were within the optimum range required for the culture of the fish.

Table 3: Physio –chemical parameters of water during the experiment period.

Parameters	Experimental Groups				
	C	T_1	T_2	T_3	T_4
Temperature (°C)	25.5±2.0172	26.2±2.017	26.1±2.041	25.83±2.281	26.367±2.289
pH	8.03±0.205	7.9±0.295	7.733±0.287	7.8±0.245	7.95±0.367
Dissolved Oxygen (ppm)	5.6±0.295	5.93±0.205	5.8±0.245	5.63±0.249	5.76±0.287
Free CO_2 (ppm)	4.53±0.236	4.033±0.125	4.33±0.287	4.5±0.245	4.4±0.249
Total alkalinity (ppm)	179.56±1.759	179.03±1.212	179.8±1.606	178.9±1.639	178.633±1.657
Ammonia –N (ppm)	0.051±0.0013	0.05±0.0016	0.048±0.0016	0.047-0.002	0.05±0.002
Phosphate –P (ppm)	0.038±0.0016	0.033±0.0016	0.036±0.002	0.032±0.002	0.037-0.002

Growth Indices

The results reveal that no significant difference (p>0.05) was observed in the length increment among the different treatment groups. But changes occurred significantly (p<0.05) in between T_2, T_3 and T_4 as compared to Control group (C). The highest value of the percentage of length increment was 26.971±1.044 (%) which was found in T_4 treatment group. There were significant (p<0.05) differences in all treatment groups (Table 4). Body weight gain (g) and body weight gain percentage of the fish were given in Table (5). There were significant (p<0.05) differences in the body weight gain among the various treatment groups C, T_1, and T_2. But no significant difference (p>0.05) was observed among the treatment T_2 and T_4. The inclusion of Tulsi and Marigold extract in the diet exhibited a significant (p<0.05) effect on the body weight gain percentage of *Labeo rohita* in treatment C and T_3. But no significant difference (p>0.05) was found among T_1, T_2 and T_4. The inclusion of *Ocimum sanctum* and Marigold leaf extract in the diet exhibited a significant (p<0.05) effect on the SGR of *Labeo rohita*. But no significant difference (p>0.05) was observed among T_1, T_2 and T_4 (Table 5). The most perfect value of FCR was 2.528±0.046 which was found in T_2 treatment group. There was a significant difference (p<0.05) in FCR values was

observed between T_1 and T_3 treatment groups (Table 5). The most perfect value of FCE was 0.396±0.007 which was found in T_2 treatment group. All treatment groups showed a significant difference (p<0.05) in the value of FCE (Table 5).

Table 4: Growth study of *Labeo rohita* during the experiment period for different groups (Data expressed as Mean ± SD)

Treatments	Growth study (1st day to 90th days					
	Initial length	Final length	Length increment	% length increment	Initial weight	Final weight
C	14.45±0.0408	17.072±0.0906	2.622±0.149^{a}	18.149±1.085^{a}	18.802±0.035	33.166±0.0266
T_1	14.408±0.221	17.359±0.2439	2.951±0.174^{ac}	20.489±1.329^{ab}	17.725±0.102	35.953±0.3896
T_2	14.297±0.0386	17.737±0.097	3.440±0.112^{bd}	24.062±.7889^{bc}	18.222±0.125	37.689±0.268
T_3	14.387±0.126	17.547±0.113	3.106±0.285^{bc}	21.98±2.209^{b}	18.5167±0.072	36.656±0.062
T_4	14.121±0.049	17.93±0.065	3.808±0.132^{d}	26.971±1.044^{c}	19.0916±0.145	38.806±0.339

Different alphabetical superscripts indicate there is significant difference (p<0.05).

Table 5: Growth study of *Labeo rohita* during the experiment period for different groups (Data expressed as Mean ± SD)

Treatments	Growth study (1st day to 90th day				
	Wt. gain	% WT. GAIN	SGR	FCR	FCE
C	14.363±0.082^{a}	76.389±0.575^{a}	0.631±0.004^{a}	3.535±0.027^{a}	0.283±0.003^{a}
T_1	18.228±0.314^{b}	102.835±1.439^{b}	0.786±0.008^{b}	2.626±0.037^{b}	0.381±0.005^{b}
T_2	19.467±0.268^{c}	106.836±1.904^{b}	0.808±0.011^{b}	2.528±0.046^{b}	0.396±0.007^{b}
T_3	18.139±0.125^{b}	97.962±1.046^{c}	0.759±0.006^{c}	2.756±0.0295^{c}	0.363±0.004^{c}
T_4	19.714±0.373^{c}	103.268±2.398^{b}	0.788±0.0131^{b}	2.616±0.0599^{b}	0.383±0.009^{b}

Different alphabetical superscripts indicate there is a significant difference (p<0.05).

Haematological Assay

The outcomes indicated that TEC and TLC content increased significantly among the different treated groups in comparison with the control represented in Figures (1) and (2) respectively. The result of the current study showed that haemoglobin content increased significantly in different treatments in comparison to the control group (figure 3).

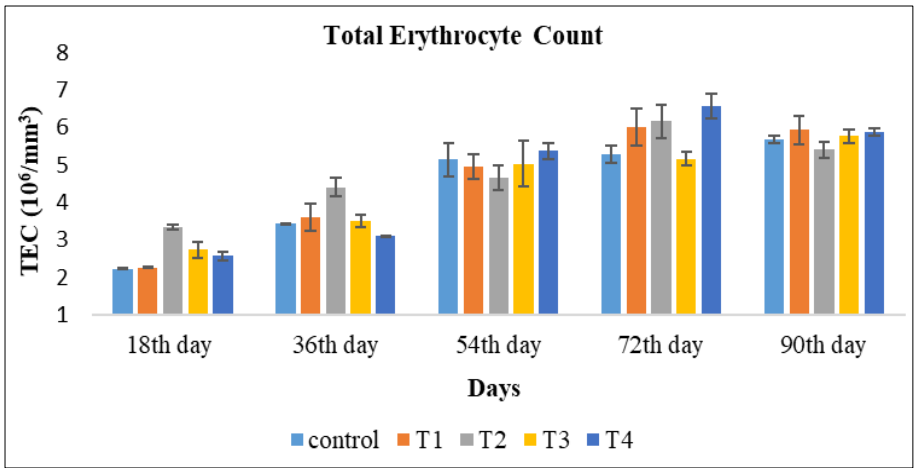

Fig 1: Total Erythrocyte Count (x10^6/mm^3) level of *Labeo rohita* during the experimental period of different groups.

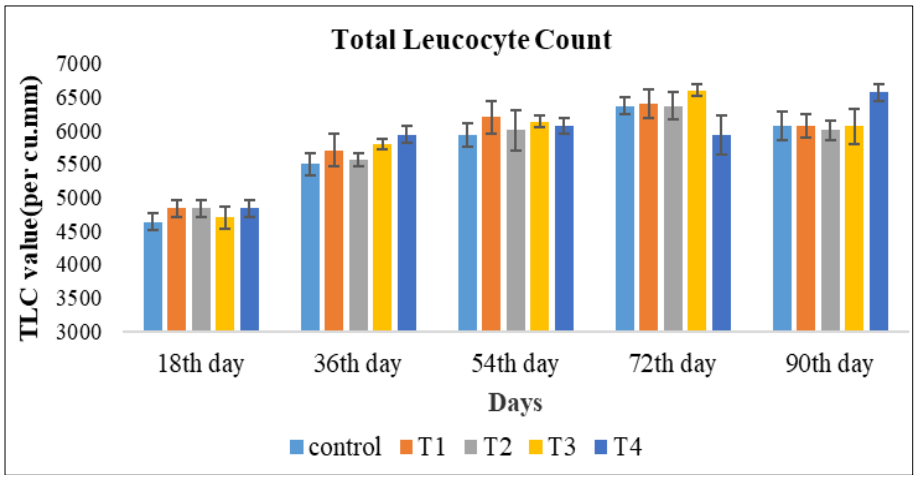

Fig 2: Total leucocyte Count (per cu.mm) level of *Labeo rohita* during the experimental period of different groups.

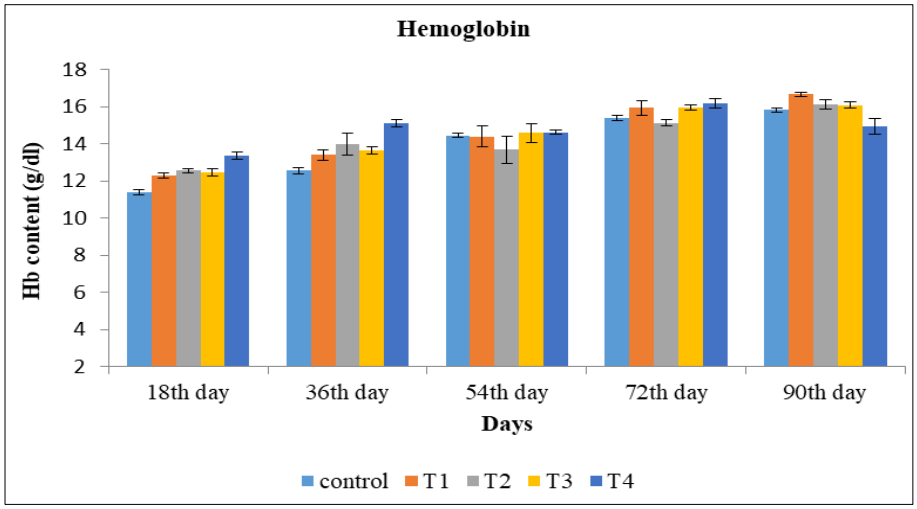

Fig 3: Hemoglobin (g/dl) level of *Labeo rohita* during the experimental period of different groups.

Biochemical Assay

The findings of this study indicated that the blood glucose content decreased significantly ($p<0.05$) in different treatments as compared with the control (figure 4). The results of serum total protein, albumin and globulin level in fingerlings of *L. rohita* of different experimental groups are presented in figure (5), (6) and (7) respectively. The findings of this present study indicated that protein, albumin and globulin content increased in different treatments as compared with the control group. There were significant differences ($p<0.05$) in glucose level, total serum protein and albumin content among the various treatment groups (C, T_1, T_2, T_3 and T_4) on each sampling day (18^{th}, 36^{th}, 54^{th}, 72^{nd} and 90^{th}). No significant difference ($p>0.05$) was observed in globulin content among the respective sampling days.

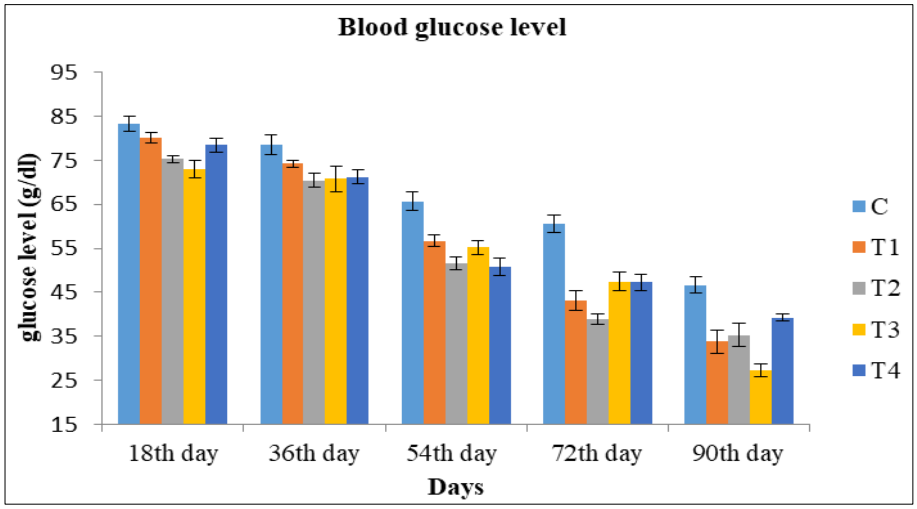

Fig 4: Blood glucose (mg/dl) level of *Labeo rohita* during the experimental period of different groups.

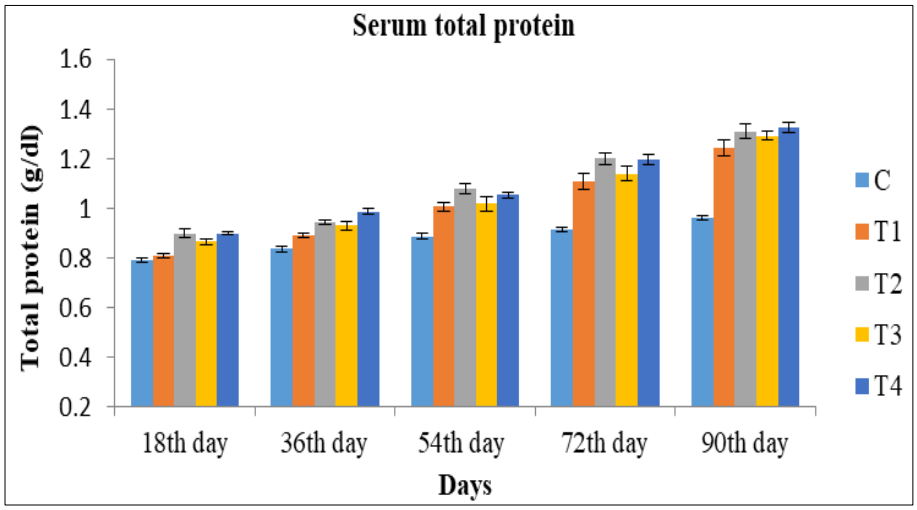

Fig 5: Serum Total Protein (g/dl) level of *Labeo rohita* during the experimental period of different groups.

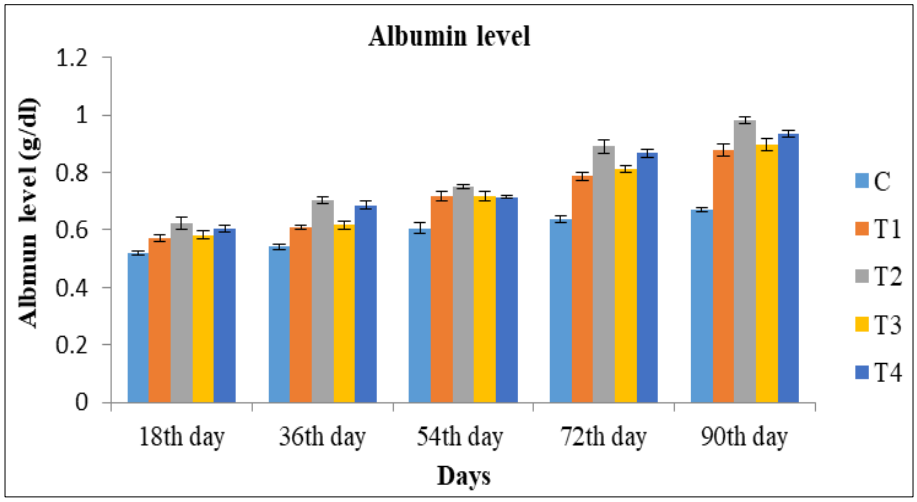

Fig 6: Albumin (g/dl) level of *Labeo rohita* during the experimental period of different groups.

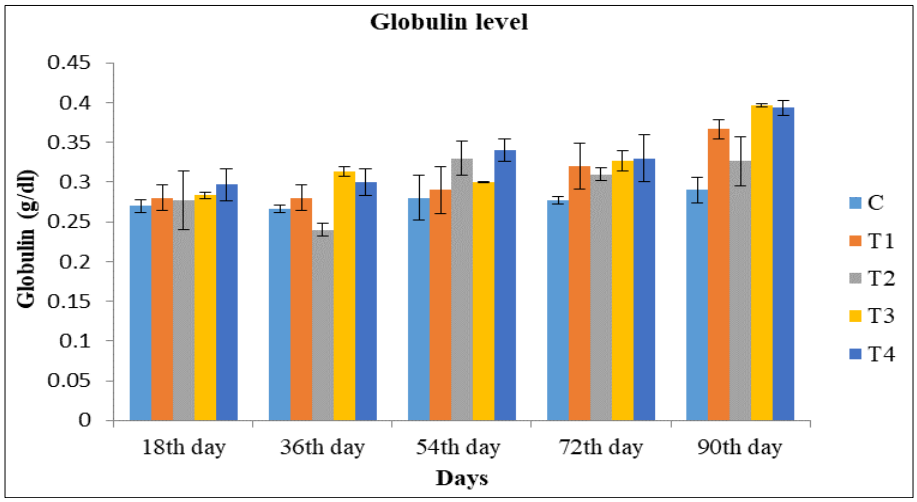

Fig 7: Globulin (g/dl) level of *Labeo rohita* during the experimental period of different groups.

Discussion

The growth indices of this present study are similar to the study of Rao *et al*. (2006) [38] who used *Achyranthes aspera* extract in the growth study of *Labeo rohita*. Equivalent results have also been observed in *Oreochromis mossambicus*, by administering *Ocimum basilicum* extract in feed for growth as reported by Karpagam and Krishnaveni (2014) [20]. Ngugi *et al*. (2015) [32] found an increase in growth rate and SGR compared to control in *Labeo rohita* fed with stinging nettle (*Utrica dioica*). In the present study, the value of the weight gain and SGR was found to be increased which was correlated with the study of Abdel-Tawwab *et al*. (2018) [1] in *Clarias gariepinus* fed with dietary supplement of Clove basil leaf extract for 12 weeks. The present study showed that inclusion powder form of *Ocimum sanctum* leaf in the diet has positive effect on the growth performance of *L. rohita* which is in agreement with the study of Shalaby *et al*. (2006) [40] in Nile tilapia when fed with garlic extract supplemented diet for 12 weeks. The present study showed that the inclusion powder form of *Tagetes erecta* leaf in the diet has positive effects on the growth performance and the FCR value of *Labeo rohita* which was observed to be correlated with the study of Jagadeesh *et al*. (2014) [14] in *Etroplus maculatus* when fed with *Marigold oleoresin* with supplemented feed for 45 days. Mamta *et al*. (2017) [28] justified that an increasing growth performance was observed in fish due to the antioxidant properties of Tulsi leaf which acts as a growth promoter. They also found that Tulsi leaf extract reduced the oxidative stress in the fish body as a result of its proper growth and more body weight gain. Kelm *et al*. (2000) [21] reported that *O. sanctum* contains several compounds like carnosol, ursolic acid, rosmarnic acid, apigenin, cirsimaritin and all of them were found to have potent redox/anti-oxidant properties as well as anti-inflammatory activity.

In a number of species, the haematological parameters are used as an index of fish health to detect various physiological changes in different stress conditions (Alishahi *et al*., 2010) [3]. The current study was similar with Sahu *et al*. (2007) [39] that in *Labeo rohita* fingerlings fed *Magnifera indica* the RBC counts were higher when compared to control. Due to stress and environmental temperature total erythrocytes number varies with species, ranging between 1.05 (10^6/mm^3) and 3.0 (10^6/mm^3). In the present study, significantly higher counts were shown in T_2 followed by T_4 and T_1 due to *Ocimum sanctum* a bioactive principle to protect murine peritoneal macrophage from the deleterious effect of nicotine to help restore their normal functions simultaneously (Nahak and Sahu, 2014) [31].

Leucocytes (WBC) which are known as the first line of defence plays a important role in non-specific immunity in fish and shellfish (Fawole *et al*., 2016) [11]. The higher WBC count in the extract treated groups indicates the activation of non-specific immune system. This is in agreement with the

similar findings of Kumar *et al*. (2013) [23] and Sahu *et al*. (2007) [39] in *L. rohita* fingerlings fed I-carrageenan and *M. indica* kernel respectively. The results obtained by Dugenci *et al*. (2003) [47] were similar when tested various medicinal plant extracts for the immune stimulatory effect, such as ginger (*Zinger officinale*), mistletoe (*Viscum album*), nettle (*Urtica dioica*), in rainbow trout.

During the study an increasing trend in haemoglobin content in concern with the RBC count in conformity with the findings of Harikrishnan *et al*. (2003) [12], in *C. carpio* with a diet containing *A. indica.* The haemoglobin content in the blood and oxygen consumption increases when fishes are under stress. Under such conditions there will be an increase in the release of immature RBCs from the haemopoietic organs, which in turn elevate haemoglobin concentration in blood (Misra, 2004) [30].

The most important source of energy in animal body is said to be glucose. It is generally considered as a stress indicator by physical factors (Manush *et al*., 2005) [29]. The anti-stress factor present in *Ocimum sanctum* may show a significant decrease in serum glucose level (Citarasu *et al*., 2006) [7]. The levels of serum glucose level got low in number during the respective days which is in conformity with the findings of Sahu *et al*. (2007) [39] in *Labeo rohita* fed with *Magnifera indica* supplemented, *Lates calcarifer* fed with neem supplemented diet, Talpur and Ikhwanuddin (2013) [42] in Asian sea bass. Similar to the present observation was found in *Labeo rohita* fingerlings (Sahu *et al*., 2007) [39] and black tiger shrimp, *Penaeus monodon* (Citarasu *et al*., 2006) [7] that glucose levels were reduced after feeding with herbal Immuno-stimulant diets.

The albumin and globulin constitute the total protein in the serum and these major proteins play a significant role in immune responses (Kumar *et al*., 2007) [24]. The T_4 group was found with the highest protein concentration being supplemented with Marigold than the other groups. This is persistent with the outcomes of Basha *et al.* (2013) [5] and Kumar *et al*. (2013) [23] in rohu (*Labeo rohita)*. In a similar vein, *C. carpio* given diets containing 0.5% and 1% Chinese herbal medication showed a significant increase in total protein (Yuan *et al*., 2007) [46]. An Increases in total protein, albumin, and globulin levels in fish is believed to be linked to a more robust non-specific immune response (Wiegertjes *et al*., 1996; Magnadottir, 2006) [44, 27]. The albumin and globulin content were observed to be increased in all treatment groups according to the current study. Fish when treated with different immuno-stimulants showed an increased value of serum albumin then those of control groups (Choudhury *et al*., 2005) [6]. In this study, Tulsi and Marigold extract treated fishes showed a significant increase in serum albumin values in comparison with control groups. According to Kaleeswaran *et al*. (2012) [10], *C. dactylon* ethanol extract fed C. catla had significantly higher serum protein, albumin, and globulin levels than the control group which is in support to our findings. In globulin the largest portion is made of gamma fraction Das *et al*. (2015) [8] justified that immune response is enhanced due to the large portion of globulin made up of gamma fraction. Rakus *et al*., (2003) [35] reported that elevations in globulin levels are believed to be related with a stronger innate immune response of fish and are vital fractions for sustaining the strongest immune system (Jha *et al*., 2007) [27]. Since serum proteins include various humoral elements of the non-specific immune system, measurable total protein, albumin and globulin levels suggest that high concentrations are like to be a result of the enhancement of the non-specific immune response of fishes (Citarasu *et al*., 2006) [7].

Conclusion

In India, herbs have long been used for the promotion of health, prevention and treatment of diseases. Tulsi plants have enormous medicinal properties like anti-inflammatory and antipyretic activity, antimicrobial activity, antitubercular activity, immunomodulatory activity, endocrinological effects, hypoglycaemic activity, antistress activity, and antiasthmatic effect. *Tagetes* species, commonly known as Marigold, are grown as ornamental plants and thrive in varied agroclimatic zones. The genus has been recognized as a potential source of very interesting biologically active products i.e. carotenoids that are used as food colorants, feed additives and possess anticancer and anti-aging effects, essential oil known for antimicrobial and insecticidal properties, thiophenes with marked biocidal activity and flavonoids having pharmacological properties. Thus, the current study led us to the conclusion that plant extract enhances fish growth and immunity.

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