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 (T1), 3% Tulsi (T2), 1% Marigold (T3) and 3% Marigold (T4) 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 T2 group. Highest body weight gain, length and percentage length was found in T4 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 T2, T3 and T4 diets increased the total erythrocyte count, haemoglobin and total leucocyte 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 tank agriculture or in cages. Each species has its own optimal parameters 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.

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 al., 1995) [33], glycyrrhizin (Jang et al, 1995) [15], aloes (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, antiinflammatory and lipidemic properties with a wide margin of safety (Ranjana and Tripathi, 2015) [30]. 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 pigments 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 cleaned and sun dried for 5 days. 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 Ikhanuddin (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 Gangajao, 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

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Ingredients</th>
<th>Percent incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fish meal</td>
<td>50</td>
</tr>
<tr>
<td>2.</td>
<td>Wheat meal</td>
<td>25</td>
</tr>
<tr>
<td>3.</td>
<td>Soybean meal</td>
<td>15</td>
</tr>
<tr>
<td>4.</td>
<td>mix. oil</td>
<td>5</td>
</tr>
<tr>
<td>5.</td>
<td>Vitamin and minerals</td>
<td>2</td>
</tr>
<tr>
<td>6.</td>
<td>Cornstarch</td>
<td>3</td>
</tr>
</tbody>
</table>

### Table 2: Composition of Experimental Diet

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Ingredients</th>
<th>Control diet (C)</th>
<th>Treatment diet (T1 and T2) (1%)</th>
<th>Treatment diet (T2/T1) (3%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fish meal</td>
<td>50</td>
<td>49</td>
<td>47</td>
</tr>
<tr>
<td>2.</td>
<td>Wheat meal</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>3.</td>
<td>Soybean meal</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>4.</td>
<td>mix. oil</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>5.</td>
<td>Vitamin and minerals</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>6.</td>
<td>Cornstarch</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>7.</td>
<td>Tulsi (T1/T2)</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>8.</td>
<td>Marigold (T2/T1)</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

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**
  \[ \text{Weight gain (\%)} = \frac{\text{Final weight – Initial weight \times 100}}{\text{Initial weight}} \]
- **Specific growth rate (SGR)**
  \[ \text{SGR (\%)} = \frac{\ln \text{Final Weight} – \ln \text{Initial Weight} \times 100}{\text{Number of Days}} \]
- **Feed conversion efficiency (FCE)**
  \[ \text{FCE} = \frac{\text{Net weight gain (wet weight) \times 100}}{\text{Feed given (dry weight) \times 100}} \]

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

**Water Physio-chemical Parameters**
Water quality parameters including temperature (°C), pH, dissolved oxygen (ppm), free carbon dioxide (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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>T(_1)</th>
<th>T(_2)</th>
<th>T(_3)</th>
<th>T(_4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperature (°C)</strong></td>
<td>25.5±(0.0172)</td>
<td>26.2±(0.017)</td>
<td>26.1±(0.0141)</td>
<td>25.8±(0.021)</td>
<td>26.36±(0.0289)</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>8.03±(0.205)</td>
<td>7.9±(0.295)</td>
<td>7.73±(0.287)</td>
<td>7.8±(0.245)</td>
<td>7.95±(0.367)</td>
</tr>
<tr>
<td><strong>Dissolved Oxygen (ppm)</strong></td>
<td>5.6±(0.295)</td>
<td>5.93±(0.205)</td>
<td>5.8±(0.245)</td>
<td>5.63±(0.249)</td>
<td>5.76±(0.287)</td>
</tr>
<tr>
<td><strong>Free CO(_2) (ppm)</strong></td>
<td>4.53±(0.236)</td>
<td>4.03±(0.125)</td>
<td>4.33±(0.287)</td>
<td>4.5±(0.245)</td>
<td>4.4±(0.249)</td>
</tr>
<tr>
<td><strong>Total alkalinity (ppm)</strong></td>
<td>179.56±(1.759)</td>
<td>179.03±(1.212)</td>
<td>179.8±(1.606)</td>
<td>178.9±(1.639)</td>
<td>178.63±(1.657)</td>
</tr>
<tr>
<td><strong>Ammonia – N (ppm)</strong></td>
<td>0.05±(0.0013)</td>
<td>0.05±(0.0016)</td>
<td>0.04±(0.0016)</td>
<td>0.04±(0.002)</td>
<td>0.05±(0.002)</td>
</tr>
<tr>
<td><strong>Phosphate – P (ppm)</strong></td>
<td>0.038±(0.0016)</td>
<td>0.033±(0.0016)</td>
<td>0.036±(0.002)</td>
<td>0.032±(0.002)</td>
<td>0.037±(0.002)</td>
</tr>
</tbody>
</table>

**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\(_2\) and T\(_3\) as compared to Control group (C). The highest value of the percentage of length increment was 26.97\(1±1.044\) (\%) 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\(_3\). 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\(_3\) treatment group. There was a significant difference (\(p<0.05\)) in FCR values was.

**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) [43]. Cytochrome oxidase 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, LiquiGluce kit (BLT120235), Liquiux total protein kit (BLT00054), Liquiux 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.

\[ \text{Serum globulin (g/dl)} = \text{Serum total protein (g/dl)} - \text{Serum albumin (g/dl)} \]

**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.
observed between T1 and T3 treatment groups (Table 5). The most perfect value of FCE was 0.396±0.007 which was found in T2 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)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Initial length</th>
<th>Final length</th>
<th>Length increment</th>
<th>% length increment</th>
<th>Initial weight</th>
<th>Final weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>14.45±0.0408</td>
<td>17.072±0.0906</td>
<td>2.622±0.149</td>
<td>18.149±1.085</td>
<td>18.802±0.035</td>
<td>33.166±0.0266</td>
</tr>
<tr>
<td>T1</td>
<td>14.408±0.221</td>
<td>17.359±0.2439</td>
<td>2.951±0.174c</td>
<td>20.489±1.329b</td>
<td>17.725±0.102</td>
<td>35.953±0.3896</td>
</tr>
<tr>
<td>T2</td>
<td>14.297±0.0386</td>
<td>17.737±0.097</td>
<td>3.440±0.112c</td>
<td>24.062±7.889c</td>
<td>18.222±0.125</td>
<td>37.689±0.268</td>
</tr>
<tr>
<td>T3</td>
<td>14.387±0.126</td>
<td>17.547±0.113</td>
<td>3.106±0.285bc</td>
<td>21.98±2.209b</td>
<td>18.5167±0.072</td>
<td>36.656±0.062</td>
</tr>
<tr>
<td>T4</td>
<td>14.121±0.049</td>
<td>17.93±0.065</td>
<td>3.808±0.132c</td>
<td>26.971±1.044c</td>
<td>19.0916±0.145</td>
<td>38.806±0.339</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Wt. gain</th>
<th>% WT. GAIN</th>
<th>SGR</th>
<th>FCR</th>
<th>FCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>14.363±0.082a</td>
<td>76.389±0.575a</td>
<td>0.631±0.004a</td>
<td>3.535±0.027a</td>
<td>0.283±0.003a</td>
</tr>
<tr>
<td>T1</td>
<td>18.228±0.314b</td>
<td>102.835±1.439b</td>
<td>0.786±0.008b</td>
<td>2.626±0.037b</td>
<td>0.381±0.005b</td>
</tr>
<tr>
<td>T2</td>
<td>19.467±0.268c</td>
<td>106.836±1.904c</td>
<td>0.808±0.011b</td>
<td>2.528±0.046b</td>
<td>0.396±0.007b</td>
</tr>
<tr>
<td>T3</td>
<td>18.139±0.125b</td>
<td>97.962±1.046c</td>
<td>0.759±0.006c</td>
<td>2.756±0.029c</td>
<td>0.363±0.004c</td>
</tr>
<tr>
<td>T4</td>
<td>19.714±0.373c</td>
<td>103.268±2.398b</td>
<td>0.788±0.0131b</td>
<td>2.616±0.059b</td>
<td>0.383±0.009b</td>
</tr>
</tbody>
</table>

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/mm3) level of *Labeo rohita* during the experimental period of different groups.](image1)

![Fig 2: Total Leucocyte Count (per cu.mm) level of *Labeo rohita* during the experimental period of different groups.](image2)
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, T₁, T₂, T₃ and T₄) on each sampling day (18th, 36th, 54th, 72nd and 90th). No significant difference \((p>0.05)\) was observed in globulin content among the respective sampling days.

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

**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.
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. 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 T2 followed by T4 and T1 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 an 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) [25]. The T4 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) [3] 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-stimulant diets. Increased in total protein, 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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