



## Hemato-immunological Response and Growth Performance of Ornamental Koi Fish (*Cyprinus carpio* L.) Fed with *Butea monosperma* (Palash) Flower Powder Enriched Diet as an Herbal Additive

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**ABSTRACT:** Ornamental fish are predominantly valued for their body colour, shape, and overall health. In recent times, herbal additives have been increasingly incorporated into aquafeeds to enhance growth and stimulate immune responses. This study evaluated the effects of Palash (*Butea monosperma*) flower powder as a dietary additive on growth performance, feed utilization, survival rate, and hemato-immunological parameters of koi carp (*Cyprinus carpio*). A 60-day feeding trial was conducted using five tanks, each of which was stocked with 10 fish. The control group (T1) received a basal diet, while experimental groups T2, T3, T4, and T5 were supplemented with palash flower powder at 2.5%, 5%, 7.5%, and 10% respectively. Fish were fed 4% of their body weight daily. Among all treatments, T4 (7.5% inclusion) exhibit the highest growth performance. Hematological parameters showed increase ( $p < 0.05$ ) in the experimental groups compared to the control. Serum biochemical values of fish fed the experimental diets remained within normal physiological ranges. Total immunoglobulin levels increased significantly in all experimental groups related to the control. Overall dietary inclusion of 7.5% of palash flower powder positively influenced growth, survival and hemato-immunological responses in Koi carp.

**Keywords:** Koi carp, Palash flower, Growth, Hematology, Ornamental fish.

### INTRODUCTION

India has a vast resource of water bodies (rivers, streams, lagoons, and coral reefs). A wide range of attractive ornamental fishes are found in India. Abundance and suitable habitat result in enriching the resources of ornamental fishes. Freshwater ornamental fish contribute 80%, whereas marine and brackish water fish together contribute 20% to the ornamental fish trade (Raja *et al.*, 2019). India boasts a vast biodiversity, with 374 indigenous freshwater ornamental fish species and 700 indigenous marine ornamental fish species (Mahapatra, 2018). India contributes about 1% of the ornamental fish export in total of the global ornamental fish trade from freshwater resources (Rani *et al.*, 2014).

Koi Carp, also called Nishikigoi, are very well-known ornamental fish that are sometimes reared in a pond or aquarium. Koi's colour and pigmentation are caused by carotenoid uptake and deposition in the body. The Japanese recognize several kinds of koi carps that are identified by scalation, patterning, and colour.

Ayurveda has played a crucial role in India's tradition and culture since ancient times. Many herbs and trees have significant contributions to the well-being of humans and other organisms by treating disease and

strengthening the immune system. *Butea monosperma*, or Palash tree used as a medicinal plant since ancient times. The Palash flower contains multiple bioactive compounds and phytochemicals that have unique efficacy and advantages for healthcare, medical cosmetology, and the adjuvant treatment of numerous illnesses (Ren *et al.*, 2018). Its leaf, bark, root, and mainly the flowers are used since ancient traditional medicine systems like Ayurveda, Unani, and Homeopathy (Saroj and Shah 2023). Palash (*Butea monosperma*) is a deciduous tree, belongs to the Fabaceae family Palash is known as the "THE FLAME OF FOREST" due to its mesmerizing colour and beauty. Flowers are 5 to 10 cm long, having 4 short lobes. The corolla of the flower is 5 to 7 cm long. In India, we can see the four colour varieties of Palash, which are - Red or 'Rakta', Blue or 'Nila', Yellow or 'Pita', and white or 'Shweta'. It was mentioned by Narahari in 'Raj Nighantu'. Due to the endangeredness of blue, white, and yellow colours, mostly red colour can be seen now.

In the aquafeed production sector, medicinal plants in powder, extract, or derivative (essence) form have been regarded as immunostimulants for the past ten years. Immunostimulants have been used more frequently to

enhance fish health and productivity (Mehana *et al.*, 2015). Effective substitutes for antibiotics and chemotherapeutants are medicinal plants. Most herbs can boost the synthesis of blood cells and other hematological indicators, which can boost the immune system both specifically and generally. Haematological parameters of a fish are an important factor to assess their health state. Haematological parameters can be changed or affected by the metabolic activity or other behavioural changes of fish and as well as by the diet composition (Ighwela *et al.*, 2012). High growth indicates a good FCR value and also provides good economic support.

The main aim of this experiment was to find out the effectiveness of Palash flower powder additives diet on fish hemato-immunology and growth as no such study previously conducted from best of our knowledge. By feeding the experimental diet of Koi carp, the effectiveness on the health state can be known, and the well-being of *Cyprinus carpio* can also be recognized.

## MATERIAL AND METHODS

### A. Preparation of palash flower powder

Palash flower (*Butea monosperma*) collected from the local area of Midnapore-721102, West Bengal, India. After the collection of flowers, the petals were separated from the sepals. Then petals were dried in a dryer machine at 40° C. After drying, petals were ground into powder form and kept in air-tight container for preparation of experimental feed.

### B. Experimental diet

Experimental diets were formulated using Pearson's square method at a 35% desired protein percentage. Fish meal, soyabean, rice bran, wheat flour, and vegetable oil were used as main ingredients in the basal feed, which is called the control feed (T1). Four additional experimental diets were prepared by adding palash flower powder at varying percentages to the basal feed, namely 2.5% (T2), 5% (T3), 7.5% (T4), and 10% (T5). The proximate composition of experimental diets was analysed by standard methods (AOAC, 2007). The basal feed contains 35.85% carbohydrate, 34.40% protein, 10.45% lipid, and 8.60% ash.

### C. Experimental design

The experiment was conducted in the Department of Fishery Sciences, Vidyasagar University, Midnapore-721102, West Bengal, India, for the period of 60 days (August-September, 2025). Koi carp, *Cyprinus carpio*, fingerlings were used in this experiment which were procured from an ornamental fish farm located in Howrah, West Bengal, India. Before starting the experiment, the fish were acclimated for 2 weeks, and then they were distributed evenly in five aquariums (T1-T5). Fishes were fed twice a day with 4% of their body weight. Growth performance and hematological parameters data were collected after 60 days of the experiment.

### D. Growth, feeding performance and survivability

The following parameters were used to evaluate the growth and survivability of experimental fishes-

- Percentage of weight gain (WG%)  

$$= \frac{\text{Mean final weight (g)} - \text{Mean initial weight (g)}}{\text{Mean initial weight (g)}} \times 100$$
- Specific Growth Rate (SGR)  

$$= \frac{\ln \text{ final weight} - \ln \text{ initial weight}}{\text{Experimental periods (days)}} \times 100$$
- Feed conversion Ratio (FCR) =  $\frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$
- Protein Efficiency Ratio (PER)  

$$= \frac{\text{Weight gain of fish}}{\text{Protein intake}}$$
- Condition Factor (CF) =  $\frac{\text{Final weight (g)}}{\text{Final length}^3 \text{ (cm)}} \times 100$
- Survival rate (%) =  $\frac{\text{Final numbers of fish}}{\text{Initial numbers of fish}} \times 100$

### E. Hematological analysis

Blood was collected from the caudal vein of the fish by using a 2.5 ml syringe fitted with a 24-gauge needle and immediately transferred into EDTA (Anticoagulant) vials. Blood was collected 60 days after feeding experimental feed. Individual fish were sampled only once to avoid bleeding and stress on that particular fish. The total count of RBC, WBC was done by using a Haemocytometer (Improved Neubauer Chamber) (Hesser, 1960). Haemoglobin concentration was determined by using the 'Haemoglobinometer. PCV was determined by using macro Wintrobe pipette.

### F. Serum Biochemistry

After blood collection, the collected blood was transferred into a microtube (without anticoagulant) from the syringe and kept undisturbed for 10 minutes to clot. After clotting, it was centrifuged at 3500 rpm for 15 min at 4°C. Following centrifugation, serum was collected by using a pipette and kept in a new microtube and stored at -20°C for biochemical analysis. In this experiment, serum protein, serum glucose, and serum cholesterol were analysed by using the Erba Kit.

### G. Total Immunoglobulin assay

Following Siwiki and Anderson (1994) method total immunoglobulin (IG) activity of blood serum was determined. In brief, 0.1 mL of serum was mixed with an equal amount of 12% polyethylene glycol (PEG) and incubated at room temperature for 2h under continuous mixing to precipitate the immunoglobulin. After incubation, the solution was centrifuged at 3000g for 15 min, the supernatant was removed and measured the protein content. The total immunoglobulin content was calculated by subtracting the protein content in the supernatant from the total protein content in the serum.

### H. Statistical Analysis

For statistical analysis, SPSS version 27.0 was used to perform analysis of variance (ANOVA) and significance testing at a confidence level of  $p < 0.05$ . To compare the groups, a post hoc Duncan multiple range test was used.

## RESULTS AND DISCUSSION

### A. Growth, feeding performance and survivability

To improve aquaculture species ecological adaptability, growth performance, and survivability, it is essential to optimize nutritional factors (Adel *et al.*, 2021). The stimulation of intestinal flora, bile acid secretion, and

digestive enzymes is most likely the cause of the supplemented feeds enhanced growth performance and better feed utilization (Platel *et al.*, 2002; Lin *et al.*, 2006; Lee and Gao 2012). According to the current study, growth performance, feeding efficiency, and survival of *Cyprinus carpio* fed diets containing different levels of palash flower powder along with the basal diet (T1- T5) are presented in Table 1. Significant differences ( $p < 0.05$ ) among growth parameters were

observed between experimental groups compared with the control group. Fish fed with T4 diet exhibited the highest weight gain, protein efficiency, condition factor and survival rate. Feed conversion ratio was significantly lower in T4 compared to the other groups. Survival differed among groups, with T1 showing 70% Survival, whereas all experimental diets (T2-T5) achieved 100% survival.

**Table 1: Growth, feeding performance and survival rate of koi carp in different tanks (T1-T5).**

Parameters	T1	T2	T3	T4	T5
Weight gain (%) g	51.00±1.43 <sup>a</sup>	56.61±1.63 <sup>b</sup>	68.71±1.41 <sup>c</sup>	80.87±1.10 <sup>d</sup>	69.47±1.35 <sup>c</sup>
SGR	0.29±0.00 <sup>a</sup>	0.32±0.00 <sup>b</sup>	0.37±0.00 <sup>c</sup>	0.42±0.00 <sup>d</sup>	0.38±0.00 <sup>c</sup>
FCR	4.74±0.13 <sup>a</sup>	4.27±0.13 <sup>ab</sup>	3.50±0.07 <sup>b</sup>	2.97±0.04 <sup>ab</sup>	3.47±0.06 <sup>ab</sup>
PER	0.60±0.01 <sup>a</sup>	0.67±0.01 <sup>b</sup>	0.81±0.01 <sup>c</sup>	0.96±0.01 <sup>d</sup>	0.82±0.01 <sup>c</sup>
CF	1.09±0.03 <sup>a</sup>	1.23±0.03 <sup>bc</sup>	1.25±0.02 <sup>bc</sup>	1.30±0.04 <sup>c</sup>	1.27±0.02 <sup>c</sup>
survival rate (%)	70±0.00	100±0.00	100±0.00	100±0.00	100±0.00

**Footnote-** Values are expressed as (means ± standard error of mean; N=10); significant differences ( $p < 0.05$ ) among the experimental groups are indicated by different superscript letters.

#### B. Hematological parameters

The hematological parameters of *C. carpio* fed different levels of Palash flower powder for 60 days are presented in Table 2. Significant differences ( $p < 0.05$ ) were observed in all measured blood parameters in the experimental groups compared with the control. The target fish species, age, sexual maturity, health, nutritional value, and environmental factors all affect hematological parameters (Bielek and Strauss 1993). RBC levels increased when palash flower powder was added to the fish diet. In the current study, the rise in RBC can be regarded as a beneficial effect of palash flower powder. RBC in teleost is related to the oxygen requirement (Zanjani *et al.*, 1967). From T1 to T4, RBC count in this study gradually increased, and a modest decline was found in T5. The notable rise in RBC across treatments suggests increased erythropoiesis, which could be triggered by erythropoietic stimulants or improved nutritional status. WBC, the immune system competent cells, are essential for both infectious and non-infectious illnesses (Magnadottir, 2006). All treatments in this investigation showed significant differences, with the WBC count increasing progressively from T1 to T5. This implies a stronger immunological response, which immunostimulants or

dietary changes could bring on. Because they are closely linked to the blood's ability to bind oxygen, Hb and Hct are physiologically essential to fish life (Blaxhall and Daisley 1973). From T1 to T3, the hemoglobin concentration rose dramatically; it remained constant in T4 and T5. This implies an increase in oxygen-carrying capacity, which could be brought about by improved erythropoiesis or iron usage. PCV increased at T1 to T4 before marginally declining at T5, following a similar pattern to that of Hb. This suggests increased erythropoietic activity and oxygen transport efficiency. With the greatest value in T1 and the lowest in T5, MCV displayed a declining trend. This points to a change in RBCs from macrocytic to normocytic, which may be brought on by improved iron consumption and a decrease in the number of immature RBCs in circulation. T1 and T3 had considerably higher MCH values than T2 and T4, indicating potential differences in hemoglobin production. Similar trends were seen in the MCHC values, which were greater in T3 and T5. Overall, in the experiment, dietary treatments significantly influenced hematological parameters, with fish in T3 and T4 exhibiting improved RBC count, Hb, PCV, and WBC compared to the control group.

**Table 2: Hematological parameters of koi carp in different tanks (T1-T5).**

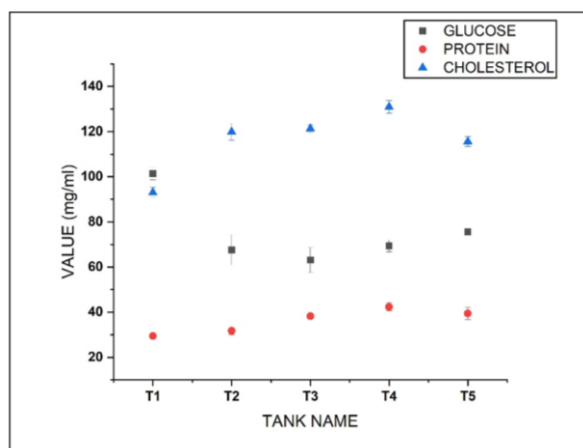
Parameters	T1	T2	T3	T4	T5
RBC (10 <sup>6</sup> /μL)	1.21±0.01 <sup>a</sup>	1.52±0.02 <sup>b</sup>	1.69±0.00 <sup>c</sup>	1.82±0.01 <sup>d</sup>	1.69±0.01 <sup>c</sup>
WBC (10 <sup>3</sup> /μL)	22.04±0.03 <sup>a</sup>	23.63±0.06 <sup>b</sup>	23.98±0.03 <sup>c</sup>	24.37±0.04 <sup>d</sup>	24.65±0.07 <sup>c</sup>
Hb% (g/dL)	4.20±0.05 <sup>a</sup>	4.80±0.05 <sup>b</sup>	5.80±0.05 <sup>c</sup>	5.70±0.05 <sup>c</sup>	5.70±0.05 <sup>c</sup>
PCV (%)	27.00±0.57 <sup>a</sup>	30.66±0.33 <sup>b</sup>	34.66±0.33 <sup>c</sup>	37.00±0.57 <sup>d</sup>	33.66±0.33 <sup>c</sup>
MCV (fl)	221.72±3.26 <sup>d</sup>	200.82±1.74 <sup>bc</sup>	204.59±0.89 <sup>c</sup>	202.34±1.59 <sup>bc</sup>	198.53±0.82 <sup>ab</sup>
MCH (pg)	34.49±0.34 <sup>b</sup>	31.43±0.11 <sup>a</sup>	34.23±0.23 <sup>b</sup>	31.18±0.49 <sup>a</sup>	33.61±.25 <sup>b</sup>
MCHC (%)	15.56±0.11 <sup>a</sup>	15.65±0.09 <sup>a</sup>	16.73±0.08 <sup>b</sup>	15.41±0.35 <sup>a</sup>	16.93±0.08 <sup>b</sup>

**Footnote-** Values are expressed as (means ± standard error of mean; N=3); significant differences ( $p < 0.05$ ) among the experimental groups are indicated by different superscript letters.

### C. Serum biochemistry

Vital information on the internal environment of the body can be obtained from blood biochemical profiles. Examining fish biochemical traits has become a crucial way to comprehend both healthy and unhealthy processes as well as the effects of toxicity (Borges *et al.*, 2007; Sudova *et al.*, 2009).

In the current study, biochemical parameters of blood serum in different treatments are presented in Fig. 1. Serum Protein, Glucose, and Cholesterol show significant variations ( $p < 0.05$ ) among experimental groups with the control group.



**Fig. 1.** Serum biochemical parameters of koi carp fed with palash flower incorporated diet in different tanks (T1-T5).

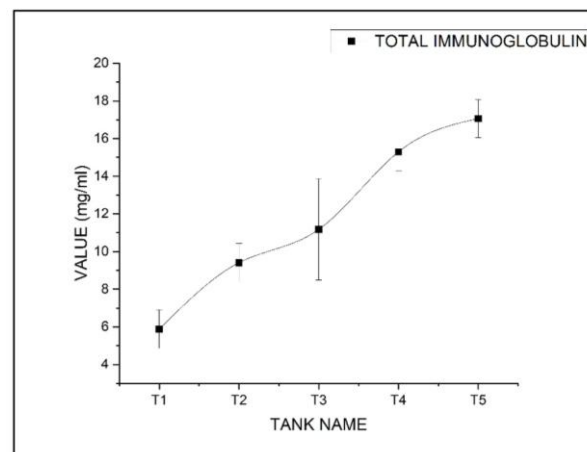
One typical indicator of stress is an increase in blood glucose levels (Luskova *et al.*, 2002). Prior research indicates that fish often have blood glucose levels between 40 and 90 mg/dL (Patriche, 2009). Serum glucose levels ranged from  $58.66 \pm 1.53$  mg/dL (T3) to  $100.44 \pm 0.88$  mg/dL (T1) in the current investigations. The higher T1 glucose levels point to a stress response or a high-carb diet. It is noteworthy that experimental diets T2, T3, T4, and T5 showed moderate glucose levels, suggesting balanced metabolic activity. This could be attributed to ambient factors or dietary formulations with palash flower powder because flowers have antistress factor. Proteins are the most significant and prevalent macromolecules found in living things, and they are essential to cellular metabolism, physiology, and architecture (Mommensen and Walsh 1992). Silva *et al.* (2004) state that 3.5–5 g/dl is the usual range for serum protein levels. Serum protein levels in each treatment in this investigation are within the optimum range. The liver plays a vital role in the production and elimination of cholesterol. Thus, increased serum total cholesterol levels will result from any kind of liver obstacle, whether intrahepatic or extrahepatic (Okechukwa and Auta 2007). The highest values of cholesterol in this study were recorded in the T4 group within optimum range, while the lowest values occurred in T1. Palash flower contains carotenoids, which are lipid-soluble compounds; higher inclusion levels in the diet result in higher carotenoid availability. Consequently, the T4 diet showed elevated cholesterol levels, whereas T1, which lacked Palash

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flower supplementation, exhibited the lowest values. Gain and Maity (2025) reported that Koi carp fed a diet containing 7.5% Palash flower exhibited the highest carotenoid deposition in their body.

### D. Total immunoglobulin

In the present study, dietary inclusion of Palash flower powder had a positive effect on the total immunoglobulin levels of Koi carp. Immunoglobulin concentrations increased progressively with increasing dietary doses (Fig. 2), with the highest levels observed in the T5 group (10%). Similar findings have been reported in other studies, where dietary supplementation with medicinal mushrooms enhanced immune cell activity (Abdullah *et al.*, 2017).



**Fig. 2.** Total immunoglobulin of koi carp fed with palash flower incorporated diet in different tanks (T1-T5).

## CONCLUSIONS

To the best of our knowledge, this is the first study to examine how the diet of Palash flower (*Butea monosperma*) powder promotes the hemato- immune system and growth of Koi (*Cyprinus carpio*) fingerlings. Based on the results of this investigation, T4 tank with inclusion level of 7.5% palash flower powder in the diet was found to be beneficial for growth, hematological parameters, immunological state, and survival rate.

## FUTURE SCOPE

Palash flower enriched diets can be further explored as a promising herbal additive and immunostimulant in cultured fish feed sector. Future research should evaluate their applicability in edible carp species, focusing on potential benefits such as enhanced antioxidant levels in fish meat, safety and acceptable limits of herbal residues, and overall improvement in nutritional quality. These studies will help determine the broader commercial and nutritional value of *Butea monosperma* as a sustainable feed additive in aquaculture.

**Author contributions.** This work, including data collection, data analysis, and paper writing, was carried out in

collaboration among all authors. All authors read and approved the final manuscript.

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**Conflict of interest.** Authors have declared that no competing interests exist.

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