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Immunostimulatory and growth promoting potential of *Tinospora cordifolia* (Thunb.) Miers on fingerlings of Amur carp

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The enhancement of immunity of fish through application of natural immunostimulants would help in minimizing the risk of occurrence of diseases in aquaculture. The present study was designed to evaluate the growth promoting and immunostimulatory effect of Guduchi, *Tinospora cordifolia* (Thunb.) Miers ex Hook F. leaf powder in fingerlings of Amur carp (*Cyprinus carpio haematopterus* Martens). The fingerlings were divided into four treatment groups, T_1, T_2, T_3 and T_4. Experimental diets were prepared by mixing rice bran, deoiled mustard cake, soybean meal and vitamin mineral mixture. The leaf powder of *T. cordifolia* was incorporated into diets D_2, D_3 and D_4 @ 0.25%, 0.5% and 0.75%, respectively. In control diet D_1, leaf powder of *T. cordifolia* was not incorporated. T_1 group fishes were fed with D_1 diet, T_2 with D_2, T_3 with D_3 and T_4 with D_4 @ 5% body weight per day for 90 days. Fingerlings fed with diet D_4 achieved significantly improved Specific Growth Rate (1), Feed Conversion Ratio (2.76), and Gross Conversion Efficiency (0.361) as compared to the control and all other treatments (P <0.05). Hematological and biochemical parameters, total leuckocyte count (53.849×10^3/μL), total erythrocyte count (3.50×10^6/μL), hemoglobin concentration (17.17%), total serum protein, albumin and globulin (12.35, 1.56 and 10.78 g/dL) in D_4 diet fed fishes showed increase as compared to the control and all other treatments (P <0.05). The above research revealed that *Tinospora cordifolia* leaf powder has significant growth promoting and immunostimulatory potential in Amur carp raising.

Keywords: *Cyprinus carpio haematopterus*, Giloe, Guduchi, Heart-leaved moonseed, Hematological parameters, Koi carp

Aquaculture is a surging industry in India, and India ranks as the second largest fish producer in the world (6.08% of world's fish production) after China[1]. However, this fast growing sector suffers from various bacterial, viral, fungal and parasitic fish diseases. Extensive use of antibiotics has led to the emergence of antibiotic resistance. Use of immunostimulants, a substance that elevates non specific defense mechanism as well as specific immune response, as a prophylactic measure has gained some importance in the last one decade[2]. It also acts as a good eco-friendly growth promoter to the fishes. In aquaculture, the quality of supplementary feeds determines the yield and profit. From economic point of view, it is feasible to use cheaper, easily available and formulated diets which are palatable and have high growth efficiency.

Many ingredients have been used successfully in fish feeds for growth promotion as enhancement of specific and non specific immune mechanisms. *Plambago rosea* which contains pharmacologically active component plumbagin (2-methoxy-5-hydroxy-1-4-napthoquinone) mixed with rice bran, wheat flour, soya flour, dry fish meal, groundnut oil cake, tapioca flour is used as immunostimulant in *Catla catla* post challenged with *Aeromonas hydrophila*[3]. *Glycyrrhiza glabra* containing glycyrrhizin, an immunologically active component, is used as growth promoter in supplementary feed of Indian major carp *Cirrhinus mrigala* (Ham.) with significant result[4]. *Zingiber officinale* which contains gingerols, shogaols and zingerone as immunologically active compounds and *Curcuma longa* containing yellow bioactive compound demethoxycurcumin and bisdemethoxycurcumin possessing antioxidant and immunomodulatory properties are used in *Cirrhinus mrigala* exposed to *Pseudumonas aeruginosa*[5]. *Zingiber officinale* has shown increased growth performance, survival and immune response in Indian major carp *Catla catla* (Ham.) as well[6]. Guduchi (*Tinospora cordifolia)* is a common plant of tropical forests growing over hedges and small trees belonging to the family Menispermaceae which is rich in

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alkaloids and terpenes. It is commonly called '*amrita*', '*gurcha*' '*giloe*', '*guduchi or jewantika* in Hindi and *amrita*, *guluchi*' or '*jwarari*' in Sanskrit[7].

The rejuvenating property of *Tinospora cordifolia* is well-known and reported in ayurvedic and other ancient literature as well as in tribal knowledge[7-10]. Tribals from Aravalli, Gujarat used *T. cordifolia* as an ethnoveterinary herb to treat foot and mouth disease in cattle[11]. Recently, various researchers investigated and reviewed the medicinal properties of *T. cordifolia*[8-13]. Ayurveda refers to *T. cordifolia* as a constituent of various preparations used in general debility, dyspepsia fever and urinary diseases or a multiple medicinal plant[7-10]. Various parts of *T. cordifolia* are used as monoherbal and polyherbal preparations used for the treatment of Anaemia, skin diseases, jaundice diabetes and hepatitis in human[14,15]. *T. cordifolia* has been shown to be the source of diverse phytochemicals such as alkaloids, glycosides, polyphenols, steroids, tannins, etc., and its leaves, in particular showed the maximum concentration of sugar, starch, flavonoids, phenolic, and tannin content as compared to aerial roots and stem[8]. While Khosa & Prasad[16] reported 11.2% protein, considerable amount of calcium and phosphorus in Guduchi leaves, Sankhala *et al*.[17]and others[7] have observed various biologically active components *viz*. alkaloids, diterpenoids, lactones, glycosides, steroids, sesquiterpenoids, phenolics, aliphatics and several polysaccharides. Aerial parts of *T. cordifolia* has been reported to act as antistress agent[18]. The dry stem along with bark is known to exhibit antiperiodic, antispasmodic, anti-inflammatory and antipyretic properties[7]. Some researchers successfully utilized leaf extract of *T. cordifolia* for synthesis of antibacterial, pediculocidal and larvicidal silver nanoparticles[19] and their characterization[20]. Researchers have also studied the comparative immunomodulation potential of *T. cordifolia* (Wild), *T. sinensis* (Lour.) Merrill and also *T. cordifolia* growing on *Azadirachta indica* and found that the latter (neem-guduchi) possessed higher immunomodulatory potential[10]. The leaf extract of *T. cordifolia* is also known to be used as immunostimulator for *Oreochromis mossambicus*[21].

Amur carp (*Cyprinus carpio haematopterus* Martens) is Hungarian strain of common carp native to Amur River basin South Chine, grows about 27 % faster than common carp and tolerate low temperature regime[22]. In the present study, we evaluated the growth promoting and immunostimulatory potential of leaf powder of *Tinospora cordifolia* in fingerlings of Amur carp *Cyprinus carpio haematopterus* by analyzing their growth, haematological and biochemical parameters.

Materials and Methods

Animal collection and maintenance

Healthy *Cyprinus carpio haematopterus* fingerlings, weighing average 2.0±0.1 g were selected from Fish seed Hatchery, College of Fisheries, GB Pant University of Agriculture and Technology Pantnagar and kept in disinfected FRP (Fibreglass Reinforced Plastic) tanks in oxygenated water and acclimatized for 5 days. During acclimatization, the fingerlings were fed with normal diet rice bran, de-oiled mustard cake, soybean meal and vitamin mineral pre-mixture. Water was changed on daily basis.

Feed preparation

Control diet was prepared by mixing rice bran, de-oiled mustard cake, soybean meal sterilized or semi cooked in pressure cooker for 15 min, cooled and filled in pelletizer adding little amount of wheat flour as binder and 1% Agrimin Forte (Vitamin mineral mixture). The pellets were then dried in shade and stored for further use. Proximate composition analysis of the experimental feed was also performed in the laboratory (Table 1).

Experimental diets were prepared by adding leaf powder of *Tinospora cordifolia* with conventional fish feed @ 0.25 % in D_2, 0.5% in D_3 and 0.75% in D_4 diets. Leaves of *T. cordifolia* collected from local forest of Pantnagar were identified at Medicinal Plant Research and Development Centre (MRDC), Pantnagar, properly washed, dried in shade and finely ground in the laboratory. The plant can easily be identified as it is large deciduous climbing shrub, creamy white to grey bark; leaves of the plant are exstipulate, alternate, pulvinate, lobed, membranous, chordate and heart shaped, also called heart leaved

Table 1—Proximate composition of experimental diet

Contents	Percentage %
Moisture	11
Ash	7
Crude protein	28
Crude fat	7.5
Crude fiber	6

Moonseed plant and found almost all parts of India ascending to an altitude of 300 m[7,9].

Experimental design

The experiment consists of 4 treatment groups, each with 25 fingerlings of Amur carp. The control group (Gr I) (T_1) fishes were fed with normal diet (D_1) and others (Gr II-IV) with the diet incorporated with leaf powder of *Tinospora cordifolia* T_2/D_2 (0.25%), T_3/D_3 (0.5%) and T_4/D_4 (0.75%) @ 5% body wt. per day for 90 days. The growth parameters were analyzed in every 15th day, haematological and biochemical analysis was done at the end of the experiment (90th day).

Growth parameters

Growth measurements such as weight and length of the fingerlings were recorded individually after every 15th day and amount of feed to be fed was calculated according to the body weight of fishes. At the end of 90th day, specific growth rate (SGR), feed conversion ratio (FCR) and gross conversion efficiency (GCE) was calculated as given below:-

$$\text{SGR } \% = \frac{[\log(\text{final weight}) - \log(\text{initial weight})}{\text{t (time intervalin days)}] } \times 100$$

$$\text{FCR} = \frac{\text{Dry weight of feed given in gram}}{\text{Wet weight gain in gram.}}$$

$$\text{GCE} = \frac{\text{Wet weight gain in gram}}{\text{dry weight of feed given in gram.}}$$

Collection of blood samples

Each fish was individually caught by scoop net, weighed and blood was collected from caudal peduncle using 2 mL syringe. Blood was collected in sterilized Ependorf tubes adding a pinch of anticoagulant EDTA; and for serum protein, without adding anticoagulant. Total erythrocyte count (TEC) and total leucocyte count (TLC) estimation were carried out by haemocytometer with improved Neubaurer ruling chamber (Weber & Sons England). Haemoglobin content was estimated by acid-haematin method using Sahli's haemoglobinometer, and the value expressed in g%. The biochemical parameters, total serum protein was measured by modified Biuret Reactions and albumin levels by Bromocresol Green Method. Globulin level was estimated by subtracting the value of albumin from total serum protein.

Water quality parameters

Quality of experimental water remained congenial throughout the experimental period[23]. The temperature of experimental water varied from 13.7 to 30°C. Water pH ranged 7.2-7.8. Total Alkalinity range was 80-119 mg/L. Range of dissolved oxygen was 7.2-8.0 mg/L during experimental period, and free CO_2 of the waters of FRP tanks ranged 0.3-0.9 mg/L.

Statistical analysis

Analysis of variance (ANOVA) was used at 5% significance level to test for significant differences between the various treatments means obtained for the growth, biochemical and haematological parameters[24].

Results

The results suggest that leaf powder of *Tinopospora cordifolia* in all concentrations promoted the growth of *Cyprinus carpio haematopterus* fingerlings. These results showed that leaf powder of *T. cordifolia* in treatment enhanced nutrient utilization as reflected by improved weight gain, food Conversion Ratio, gross conversion efficiency and specific growth rate (Table 2).

The treatments means revealed that the best SGR was recorded in group IV [Treatment T_4 (1%)], followed by group III [T_3 (0.866%)], and group II [T_2 (0.800%)], and minimum with group I [T_1 control (0.666%)]. The best FCR was also recorded in group IV (2.763) followed by Gr III, T_3 (2.854), Gr II, T_2 (3.226) and minimum with Gr I, T_1 control (3.578). The highest GCE was found in treatment T_4/D_4 (diet with 0.75% leaf powder of *Tinospora cordifolia*) after the 90th days which was significantly different from T_1 and T_2 (P <0.05) but not significant from T_3, followed by T_3/D_3 (0.352), T_2/D_2 (0.309) and minimum with T_1/D_1 control (0.279), (P <0.05).

Haematological parameters showed remarkable improvement in all experimental feed fed groups (Fig. 1 a-h). The best Total leukocyte count was

Table 2—Growth parameter observations

Parameters	T_1/D_1(Control)	T_2/D_2 (0.25%)	T_3/D_3 (0.5%)	T_4/D_4 (0.75%)
Initial mean weight (gm)	2.130	2.140	2.150	2.050
Final mean weight (gm)	9.309	12.020	14.150	16.544
SGR (%)	0.666±0.033	0.800±0.028	0.866±0.033	1.00±0.028
FCR	3.578±0.087	3.226 ±0.078	2.854 ±0.181	2.763± 0.028
GCE	0.279± 0.006	0.309 ±0.007	0.352± 0.021	0.361± 0.003

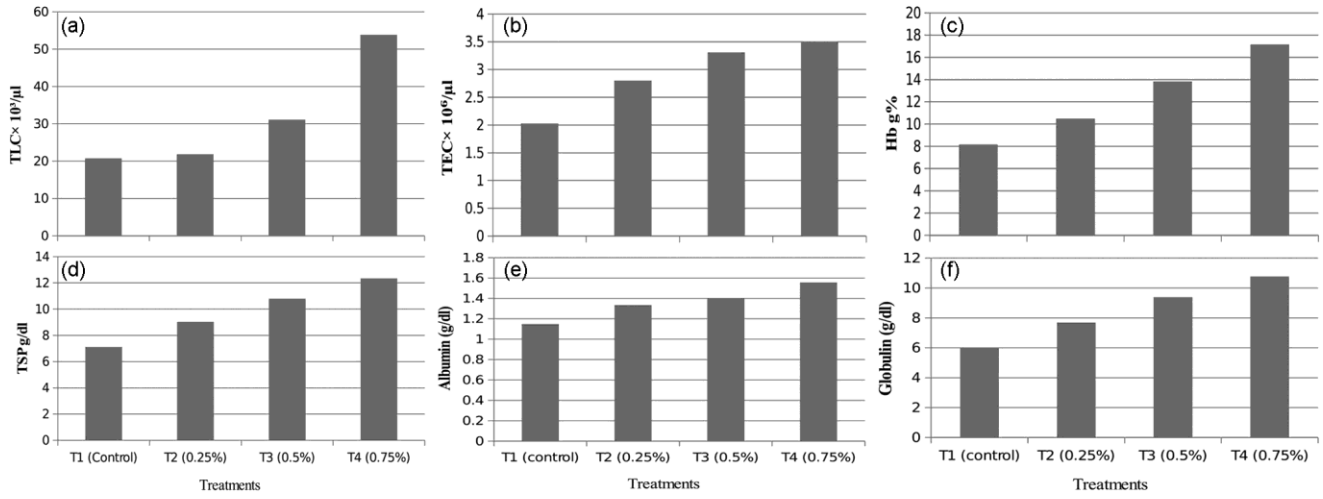

Fig. 1—Haematological and biochemical observations. [Profile of (a) total leucocyte count; (b) total erythrocyte count; (c) haemoglobin; (d) total serum protein; (e) albumin; and (f) globulin of Amur Carp blood]

recorded in T_4 group (53.849×10^3/µL), followed by T_3 (31.10×10^3/µL), T_2 (21.850×10^3/µL) and minimum with T_1 control (20.75×10^3/µL), (P <0.05). Highest total erythrocyte count was also recorded in T_4 group (3.50×10^6/µL), followed by T_3 (3.10×10^6/µL), T_2 (2.80×10^6/µL) and minimum with T_1 control (2.03×10^6/µL) (P <0.05).

Best Hb level was recorded in T_4 group (17.166 g %), followed by T_3 (13.833 g %), T_2 (10.50 g %) and minimum with T_1 control (8.166 g %), (P <0.05). The biochemical analysis of fish blood (Fig. 1d) showed the best total serum protein level (12.35 g/dL) in group T_4, T_3 treatment fishes showed 2nd best TSP level (10.796 g/dL) high as compared to control (7.117 g/dL) and T_2 (9.028 g/dl) treatment (P <0.05). The best albumin level (1.556 g/dL) was found in T_4 group, T_3 showed second best albumin level (1.404 g/dL) followed by T_2 with albumin level of (1.336 g/dL) and minimum in T_1 control treatment (1.148 g/dL) (P <0.05). Highest globulin level was found in Treatment T_4 (10.777 g/dL), followed by treatment T_3 (9.391 g/dL), treatment T_2 (7.692 g/dL) and minimum in T_1 control treatment (5.969 g/dL) (P <0.05).

Discussion

The present study focused to evaluate the growth promoting and immunostimulatory potential of leaf powder of *Tinospora cordifolia* in *Cyprinus carpio haematopterus*. Fingerlings fed with *T. cordifolia* leaf powder incorporated diet exhibited improvement in palatability, digestion and absorption of nutrients and thereby remarkable improvement in specific growth rate, food conversion ratio and gross conversion efficiency. Rainbow trout (*Oncorhynchus mykiss*) fed with dietary *Aloe vera* showed that *Aloe vera* at 0.1% and 1% administration resulted in improved specific growth rate (SGR) and feed conversion ratio (FCR)[25].

Cirrhinus mrigala (Ham.) fed with herb Bala (*Sida cordifolia* Linn.) showed FCR, GCE and SGR were also superior with herb supplemented diets[26]. Thus, it is an evident that incorporation of leaf powder of *Tinospora cordifolia* in fish diet plays a significant role as growth promoter.

RBC is reliable indicator of stress, and WBC, good health. Thus, highly significant increase in RBC and WBC and Hb levels in D_4 incorporated diet fed fishes could be a response to tolerate stress and measure to maintain good health. Rainbow trout (*Oncorhynchus mykiss*) fed with 1 % watercress extract in fish enhanced hematological and immunological parameters including Hb and MCHC, lysozyme and complement activities, total protein and globulin levels[27]. *Tilapia mossambicus* fed with dietary Amla (*Phyllanthus emblica*) showed significantly increased TEC, Hb, TLC, lymphocyte, neutrophils and monocyte counts in D_3 diet (2 % ethanolic extract of *P. emblica*) fed fishes. Highly significant increase was observed in serum, total protein and albumin levels in fishes fed with D_2 diet (1% ethanolic extract of *P. emblica*) and increase in globulin level were significant[28].

Oreochromis mossambicus administered with ethanol and petroleum leaf extracts of *Tinospora cordifolia* showed that all the dose of ethanol extract significantly enhanced neutrophil activity[21]. Fishes injected with petroleum ether or ethanol extract

@ 8 mg/kg were protected against experimental infection with virulent *Aeromonas hydrophila*. It shows immunoprophylactic or immunostimulatory nature of *Tinospora cordifolia*[3]. Thus, it is evident that incorporation of leaf powder of *Tinospora cordifolia* in fish diet plays a significant role in enhancing the adaptive immunity in fingerlings of Amur carp.

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