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Effect of leaf powder of giloy (*Tinospora cordifolia*) in fish feed on survival and growth of post larvae of *Catla catla*

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Abstract: The study was designed to evaluate the effect of leaf powder of Giloy (*Tinospora cordifolia*) in fish feed on survival and growth of post larvae of *Catla catla.* The experiment consisted of five treatments (T1, T2, T3, T4 and T5) in triplicate. The artificial feed was prepared from de-oiled groundnut cake (49.5%), de-oiled rice bran (49.5%) and vitamin – mineral mixture (1%). The leaf powder of giloy was incorporated into larval feed at various rates. In treatment T1 (control), no leaf powder was incorporated in the feed. In T2, T3, T4 and T5 leaf powder was mixed at the rate of 0.25%, 0.5%, 0.75% and 1%, respectively. One hundred post larvae of *C. catla* of uniform size (7.3 mm length, 1.5 mg weight) were stocked in each of 5×3=15 tanks. The larvae were fed twice in a day. The water quality parameters were regularly monitored. The survival rate of post larvae in T1, T2, T3, T4 and T5 were recorded as 95.3, 94.3, 97.0, 98.6 and 100%, respectively. Post larvae fed with diet incorporated with 1% leaf powder achieved significantly enhanced (P< 0.05) specific growth rate (6.97%), feed conversion ratio (2.41) and gross conversion efficiency (0.415). The study revealed that giloy leaf powder has very good growth promoting potential in raising post larvae of carp. Thus, it is recommended that the leaf powder of giloy may be incorporated in fish feed upto 1% for enhancing growth and survival of post larvae of carp fishes up to fry stage.

Keywords: Feed conversion ratio, Gross conversion efficiency, Specific growth rate, *Tinospora cordifolia*

INTRODUCTION

Aquaculture is one of the fastest growing food producing sectors in the world. It provides -- 44.14% of the world's fish production for human consumption. Asia contributes 88.91% of the total global aquaculture production (FAO, 2016). Major Indian and Chinese carps are main cultivated species contributing more than 70% in world aquaculture production. India is the second largest fish producer in the world. Around 14.49 million people are involved in fisheries sector for livelihood security(DAHDF, 2016).The prime requirement for enhancing aquaculture production is availability of quality seed. Though huge amount of carp seed is being produced in India, the fish hatcheries producing seed do not follow any genetics standards. The selection of brood stock is done in such a way that it has become counterproductive because of the desire to choose late maturing and slow growing brooders (Eknath and Doyle, 1985). By improving the feeding and nutrition of brood stock, improvement in quality of egg and sperm is recorded (Izquierdo *et al.*, 2001).

The larval stages are most critical stages in life cycle of fish as the larvae are defenceless against predators, diseases outbreak, environmental conditions and stress. The average survival rate is very poor mainly due to non-adoption of appropriate larval care protocols. The environmental factors (Jezierska *et al.,* 1979)and feeding mode, mouth size and food particles size(Hartman, 1983)affect the survival of fish larvae. The fish production is increasing significantly over the past few decades mainly due to adoption of the intensive fish culture practices. These practices are the major factors that make the fish susceptible to disease. During the last few years probiotics and herbs have gained attention for their use in fish culture operations for promoting growth and disease resistance. They offer many advantages to overcome the limitations and side effects of antibiotics and other drugs and result into high production through enhanced growth and disease prevention (Das -, 2008; Sahu *et al*., 2008).

Plants are natural sources of cheaper and safer chemicals (Citarasu, 2010). Plant products have various attributes like anti-stress, anti-tumour, anti-pyretic, tonic, antimicrobial, growth promotion, appetite stimulation and immunostimulation in aquaculture practices. In India, many herbal preparations are used for the enhancement of growth in human beings. Some of these herbs have also proved effective as a growth promoter in aquaculture (Olusola *et al*., 2013).

A number of plant biomaterials have been tried for growth promotion and immunity enhancement in fish

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like *Meliaazedarach* in formulated diet of *C. catla* (Rajeshwari *et al*., 2015), *Zingiberofficinale* powder in feed of the Indian major carp *(C. catla)* infected by *Aeromonas hydrophila* (Arulvasu *et al.,* 2013), *Coriandrum sativum* in *C. catla* (Innocent *et al*., 2011), *Glycyrizzha glabra* in *Cirrhinus mrigala* (Kumar *et al*., 2007), *Z. officinale* and *Curcuma longa* in *C. mrigala* (Sivagurunathan *et al*., 2011)and *T.-cordifolia* leaf powder in fingerlings of Amur carp (Anita *et al.*, 2016).

T. cordifolia is a large deciduous, climbing shrub distributed throughout the tropical Indian subcontinent. It is commonly known as Guduchi, Giloy, Amrita and Gurcha. "Amrita" which is a hindi term refering to elixir that delay aging in humans and keep them young. All parts of this plant like fruits, leaves, stem and seeds are useful. A variety of chemicals have been isolated from *T. cordifolia* belonging to different classes such as alkaloids, lactones, steroids, diterpenoids and glycosides (Sinha *et al*., 2004).

Indian aquaculture has been growing at a fast pace with freshwater culture contributing over 95% of the production. The Indian major carp, *C. catla*, is a promising species for aquaculture exploitation with its rapid growth and good market potential. It is economically important South Asian freshwater fish. On the basis of above mentioned points, the present study was made with the prime objective of evaluation of growth and survival of post larvae of Indian major carp *C.catla* fed with *T- cordifolia* leaf powder incorporated diet.

MATERIALS AND METHODS

Experiment Design: The experiment consisted of 5 treatments (T1, T2, T3, T4 and T5) in triplicate. In total, 15 plastic tubs were properly arranged and aerators were setup therein. Each tub was stocked with 100 post larvae of *C. catla*. The experiment lasted for 21 days which is normal period for growth of post larvae upto fry stage in carps.

Collection and maintenance of post larvae: Healthy post larvae of *C. catla,* weighing 1.5 ±0.01mg were collected from Fish Seed Hatchery, College of Fisheries, G.B. Pant University of Agriculture and Technology, Pantnagar and kept in oxygenated water in the plastic tubs of 60 litre water capacity. Fifty percent water was exchanged daily. The tubs were cleaned every alternate day.

Feed preparation and feeding of post larvae: The feed for the post larvae of *C. catla* was prepared using finely powdered de-oiled groundnut cake (49.5%), de-oiled rice bran (49.5%) and vitamin-mineral mixture (1.0%). The experimental diets were prepared by adding *T. cordifolia* leaf powder at the rate of 0.25% in D2 diet, 0.5% in D3 diet, 0.75% in D4 diet and 1% in D5 diet while control diet D1 was with no supplementation of leaf powder. The proximate analysis of the formulated feed is presented in Table 1.In control treatment T1, the fishes were fed with normal diet whereas in other treatments the larvae were fed with diet having supplementation of leaf powder of *T. cordifolia* @ 0.25% in T2, 0.5% in T3, 0.75% in T4 and 1% in T5. The rate of feeding of post larvae has been detailed in Table 2. The feed was given in two equal instalments daily, during morning and afternoon. In addition, post larvae were also fed with natural food i.e. plankton (2 ml) in each tub every alternate day.

Water quality analysis: Various water quality parameters viz. water temperature (^{0}C), pH, dissolved oxygen (mg/l), free carbon dioxide (mg/l) and total alkalinity (mg/l) were monitored regularly. Water temperature was measured using mercury thermometer while pH was recorded by digital pH meter. Dissolved oxygen, free carbon dioxide and total alkalinity were estimated as per methods of American Public Health Association (2012).

Survival and growth parameters: The rate of survival of post larvae in each experimental tub was determined by comparing the total larvae stocked initially to the larvae recovered at the end of the experiment. The net weight gain (NWG), specific growth rate (SGR), feed conversion ratio (FCR) and gross conversion efficiency (GCE)were calculated as given below:

NWG = Average final body weight (g) – Average initial body weight (g)

SGR % = [log {final weight} – log {initial weight} / t (time interval in days) × 100

FCR = Dry weight of food given in g / Wet weight gain in g

GCE = Wet weight gain in g / dry weight of feed given in g

Statistical analysis: The observations of the present study were analyzed using statistical package (SPSS, version 16.0 for windows, SPSS Inc., Chicago, USA). The differences among the different treatment were determined for significance using ANOVA (Analysis of variance). The level of significance was set at $p<0.05$ and results recorded as mean ± S.E.

RESULTS AND DISCUSSION

Water quality parameters: The physico–chemical parameters like water temperature, pH, dissolved oxygen, free carbon dioxide and total alkalinity play a vital role in the survival and growth of the aquatic organisms. These affect the physiology and other metabolic activities of the fishes. The summarised observations on various water quality parameters have been recorded in Table 3. The ranges of temperature (26.0-

Table 1. Proximate composition of experimental feed.

Sl. No.	Contents	%
1	Moisture	11.9
2	Crude protein	25.8
3	Crude fat	7.8
4	Crude fibre	6.3
5	Ash	7.0

Table 2. Feeding rate of post larvae of *Catla catla* by experimental feed.

Feeding rate	Feeding days
Equal to the total initial weight of post larvae	First 2 days
1.5 times of total initial weight of post larvae	3-4 days
2 times of total initial weight of post larvae	5-6 days
2.5 times of total initial weight of post larvae	7-8 days
3 times of total initial weight of post larvae	9-10 days
3.5 times of total initial weight of post larvae	11-12 days
4 times of total initial weight of post larvae	13-14 days
4.5 times of total initial weight of post larvae	15-17 days
5 times of total initial weight of post larvae	18 – 21 days

Table 3. Profile of water quality parameters (mean±S.E.) in different treatments

Parameters	Treatments				
	T1/D1Range (mean±SE)	T2/D2 Range (mean±SE)	T3/D3 Range (mean±SE)	T4/D4 Range (mean±SE)	T5/D5 Range (mean±SE)
Temperature (˚C)	26.0-29.0 (27.97±0.01)	26.0-29.5 (27.99±0.03)	26.5-29.5 (27.98±0.04)	26.5-29.0 (28.01±0.03)	26.0-29.0 (27.98±0.03)
pH	7.0-8.0 (7.48±0.02)	7.0-8.2 (7.40±0.02)	6.8-7.8 (7.32±0.02)	6.8-8.0 (7.35±0.03)	7.0-8.0 (7.49±0.01)
Dissolved oxygen (mg/l)	6.0-8.0 (7.03±0.01)	6.2-7.8 (7.04±0.02)	6.2-8.0 (7.17±0.01)	6.4-8.0 (7.22±0.03)	6.1-8.0 (7.10±0.05)
Free CO_2 (mg/l)	Nil	Nil	Nil	Nil	Nil
Total alkalinity (mg/l)	98-265 (168.0±0.4)	108-260 (167.8±0.2)	110-264 (164.03±0.6)	115-255 (161.95±0.9)	110-265 (165.2±1.5)

T1/D1- Treatment 1 where post larvae were fed with feed without leaf powder of *T. cordifolia,* T2/D2- Treatment 2 where post larvae were fed with feed containing 0.25% leaf powder of *T. cordifolia,* T3/D3- Treatment 3 where post larvae were fed with feed containing 0.50% leaf powder of *T. cordifolia,* T4/D4- Treatment 4 where post larvae were fed with feed containing 0.75% leaf powder of *T. cordifolia,* T5/D5- Treatment 5 where post larvae were fed with feed containing 1.0% leaf powder of *T. cordifolia.*

Table 4. Survival, growth and feed conversion in *Catla catla* post larvae fed with *Tinospora cordifolia* added diets

	Treatments				
Parameters	**T1/D1 (control)**	**T2/D2 (0.25%)**	**T3/D3 (0.5%)**	**T4/D4 (0.75%)**	**T5/D5 (1%)**
Initial body weight (g)	0.0015±0.01	0.0015±0.01	0.0015±0.01	0.0015±0.01	0.0015±0.01
Final body weight (g)	0.0122±0.001	0.0139±0.001	0.0210±0.01	0.039±0.001	0.044±0.001
NWG (g)	0.011±0.001	0.012±0.001	0.02±0.01	0.037±0.001	0.043±0.002
SGR (%)	4.30±0.20	4.82±0.11	5.29±0.44	5.79±0.30	6.97±0.10
FCR	6.51±0.20	6.87±0.41	5.35±0.11	2.74±0.17	2.41±0.18
GCE	0.17±0.02	0.15±0.01	0.18±0.02	0.36±0.01	0.415±0.02
Survival (%)	95.3±0.8	94.3±3.1	97.0±1.1	98.6±0.2	100.0±0.0

T1/D1- Treatment 1 where post larvae were fed with feed without leaf powder of *T. cordifolia,* T2/D2- Treatment 2 where post larvae were fed with feed containing 0.25% leaf powder of *T. cordifolia,* T3/D3- Treatment 3 where post larvae were fed with feed containing 0.50% leaf powder of *T. cordifolia,* T4/D4- Treatment 4 where post larvae were fed with feed containing 0.75% leaf powder of *T. cordifolia,* T5/D5- Treatment 5 where post larvae were fed with feed containing 1.0% leaf powder of *T. cordifolia.*

29.5^{o}C), pH (6.8-8.2), dissolved oxygen (6.0-8.0 mg/l), free carbon dioxide (Nil) and total alkalinity (98-265 mg/l) in the present study were within the favourable limits for carp rearing as also reported by Jhingaran (1991) for Indian major carps (water temperature 18.3 – 37.8^{o}C and pH 6.5 – 9.0), Chauhan (2001) for carps (pH 7.5 -9.0 and dissolved oxygen 6.5 – 10.5 mg/l), Chauhan (2014) for rearing of *Cyprinus carpio* (water temperature 16.0 – 21.2^{o}C and dissolved oxygen little above 5 mg/l), Nazir *et al.* (2015) for *Labeo rohita* fingerlings (water temperature 19.7 – 20.6^{o}C, dissolved oxygen 6.8 – 7.5 mg/l, free CO_2 0.28 - 0.55 mg/l and total alkalinity 103.8 – 113.9 mg/l) and Arya *et al.* (2016) for *Labeo rohita* (water temperature 19.9 – 20.9^{o}C, dissolved oxygen 7.44 – 7.63 mg/l, free CO_2 0.29 -0.4 mg/l and total alkalinity 106.6 – 110.7 mg/l). The analysis of variance revealed that the differences in water temperature, pH, dissolved oxygen, free carbon dioxide and total alkalinity amongst different treatments were statistically non-significant ($P<0.05$).

Survival: The observations on survival, growth and feed conversion of post larvae of *C. catla* in different treatments have been included in Table 4.At the end of the experiment, 100% survival was observed in T5 treatment with 1 % supplementation of *T. cordifolia* whereas minimum average survival of 94.3 ± 3.1% was seen in T2 treatment with 0.25% supplementation of giloy leaf powder. This shows that the herb *T. cordi-*

folia is helpful in enhancing the survival of the fish. The difference between the survival percentage of different treatments were statistically non – significant. The results are in conformity with the findings of Arulvasu *et al.* (2013) who got relative percentage survival of 100% in *C. catla* fed with *Z. officinale* (1000 mg/kg) incorporated diet.

Specific growth rate: The highest net weight gain (0.043g) and specific growth rate (6.97%) were recorded in treatment T5 followed by T4, T3, T2 and T1. The values of NWG and SGR recorded in T5 were significantly ($p<0.05$) higher in comparison to T1 (control) and other treatments. The results are in accordance with Kumar *et al.* (2007) who observed that fingerlings of *C. mrigala* when fed with 0.3 % mulethi achieved highest weight gain of 24 % as compared to other treatments in 60 days culture period. Anita *et al.* (2016) also recorded significantly higher SGR in *Cyprinus carpio haematopterus* fed with *T. cordifolia* incorporated feed (0.25%, 0.5% and 1%).

Feed conversion ratio: The minimum FCR was seen in T5 (Table 4) (with 1 % leaf powder supplementation) which was significantly different from T1, T2, T3 and T4 ($p<0.05$). The mean of treatments display that best FCR was recorded in T5 (2.41) followed by T4 (2.74), T3 (5.35), T2 (6.87) and control T1 (6.51). Kour *et al.* (2004) conducted a feeding trial on *C. mrigala* to assess the impact of Bala (*Sida cordifolia*) herb mixed with ground nut oil cake and rice bran (1:1). They concluded that the value of FCR was better in fishes fed with herb supplemented feed in comparison with other treatment in 60 days trial.

Gross conversion efficiency: The highest GCE was observed in T5 treatment (Table 4) (with 1 % leaf powder supplementation) which was significantly ($P < 0.05$) different from all other treatments. Kumari *et al.* (2007) observed the enhanced survival and growth of larvae of rohu fed with diet containing polyherbal mixture (ImmuPlus) of *Withania somnifera* (Ashwagandha), *T. cordifolia* (Giloy), *Ocimum sanctum* (Tulsi) and *Emblica officinalis*(Amlaki) at the rate of 0.5 g ImmuPlus/kg feed. Punitha *et al.* (2008) also got significantly ($P<0.05$) increased survival and growth rate in grouper *Epinephelus tauvina* juveniles raised on diet containing extracts (400 mg/kg diet) of herbs like *Cynodon dactylon, Tridax procumbens, Phyllanthus niruri, Piper longum* and *Zingiber officinale* mixed in equal proportions of the all- plant extracts. The results of the present study support the findings of Anita *et al. (*2016) who got enhanced growth in amur carp (*Cyprinus carpio haematopterus*) fingerlings fed with *T. cordifolia* incorporated (0.75%) diet.

The possible reason of enhanced survival and growth of *C. catla* post larvae may be growth promoting and immunostimulatory potential of *T. cordifolia* leaf powder as also reported by Anita *et al*. (2016) who recorded significantly ($P<0.05$) increased values of SGR (1%), total leukocyte count ($53.84x\ 10^3/\ \mu l$) and total serum protein (12.35 g/dL) in *C. carpio haematopterus* fingerlings fed with *T. cordifolia* (0.75%) incorporated diet.

Conclusion

On the basis of the present investigation, it could be summarized that inclusion of leaf powder of medicinal herb Giloy (*T. cordifolia)* in carp feed at the rate of 1.0 % has potential for enhancing the survival and growth performance of the post larvae of carp fish *C. catla*. It can be inferred from the study that leaf powder of Giloy (*T. cordifolia)* can be safely incorporated up to 1% level in feed of post larvae of carp. Development of better feed technologies need to be developed for utilisation of herbal / medicinal plants extracts so that farmers can easily purchase such cheap herbal growth promoters and immunostimulators and use them for enhanced sustainable fish production.

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