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Effect of leaf powder of giloy (*Tinospora cordifolia*) in fish feed on survival and growth of post larvae of *Cyprinus carpio* fingerlings

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Abstract
This study investigated the effect of dietary supplementation with Giloy (*Tinospora cordifolia*) stem powder on the growth performance of common carp (*Cyprinus carpio*) fingerlings. A 150-day feeding trial was conducted using *C. carpio* fingerlings (average initial weight 10.5±0.2 g) randomly distributed into four tanks, representing four dietary treatments in triplicate. The treatments consisted of a basal control diet (T_0) and three experimental diets supplemented with *T. cordifolia* stem powder at 3% (T_1), 5% (T_2), and 8% (T_3) of the feed. Fish were fed twice daily at 3% of their body weight. Growth parameters including weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR), along with survival rate, were assessed. Water quality parameters (temperature, pH, dissolved oxygen, total ammonia nitrogen, nitrite-N, and nitrate-N) were monitored regularly. Results indicated that supplementation with *T. cordifolia* stem powder significantly ($P<0.05$) improved growth performance and feed utilization. The highest WG (30.25 g) and SGR (2.05%/day), and the best FCR (1.52) were observed in fish fed the diet supplemented with 8% *T. cordifolia* stem powder (T_2), followed by the T_3 (1.5%) and T_1 (0.5%) groups, all of which performed significantly better than the control group (T_0). Survival rates were high and not significantly different among treatments. Water quality parameters remained within the acceptable ranges for common carp culture throughout the experimental period in all treatments. Total ammonia nitrogen and nitrite-N levels were found to be marginally lower, though not always statistically significant, in tanks with higher Giloy supplementation, corresponding with improved feed utilization. The study concludes that dietary supplementation with *Tinospora cordifolia* stem powder at an inclusion level of 5% can effectively enhance the growth performance and feed utilization efficiency of *Cyprinus carpio* fingerlings without adversely affecting water quality, suggesting its potential as a beneficial natural feed additive in common carp aquaculture.

Keywords: Giloy, environmental, sustainability, ecosystems and aquatic resources

Introduction
Aquaculture continues to drive growth, with global production soaring to 130.9 million tons in 2022, representing a significant 6.6% increase from 122.8 million tons in 2020. (FAO, 2024) [6]. With the wild fisheries resources continuing to decline aquaculture have become a primary source of global food fish production. To address the rising demand for protein driven by population growth, the aquaculture sector has witnessed substantial expansion. However, this growth has been accompanied by a rising use of synthetic chemicals—such as pesticides to treat parasites, fungal infections, and unwanted organisms in culture ponds, aiming to prevent disease outbreaks, enhance fish health, and improve environmental conditions, ultimately impacting production and farmer incomes. Fish is key provider of inexpensive protein and essential fatty acids and maintaining fish health is paramount to human nutrition and food security. Global aquatic and fisheries resources, spanning from inland waters (lakes, reservoirs, ponds, and rivers) to marine ecosystems (seas and oceans), yield numerous long-term benefits for human well-being, including, sustainable food sources, economic growth, environmental sustainability and social welfare. Despites its abundant provision, human induced-stressors, including pesticides disrupts the delicate balance of aquatic ecosystems harm fish populations, impair fish health and degrade environmental quality. This disruption threatens the long-term sustainability of aquatic resources, undermining the benefits they provide to human well-being. Consumption of pesticide-contaminated animals and their by-products can transfer toxicity to humans and

wildlife, including carnivorous fish species that feed on contaminated aquatic insects, potentially propagating harmful effects up the food chain (Maurya & Singh (2021) [10].

T. cordifolia, also known as the queen of all herbs, is a climbing shrub with a number of coiling branches. The whole plant has been divided into different parts i.e., stem, leaves, flower, and fruits. Other parts, like aril roots, lamina, and seeds, are present as well. Tetra-to penta-arch structures are the characteristics of aerial roots. Other than that, the root cortex has an outer, thick wall and an inner parenchymatous zone. The lamina is ovate, 10-20 cm long and up to 15 cm broad; the base is deeply cordate, membranous, pubescent, and whitish tomentose with a prominent reticulum beneath. Seeds are curved in shape. The embryo turned into acurve automatically. However, the endocarp is well ornamented and confers vital taxonomic characters. Another important part of the plant, its root possesses anti-ulcer and anti-activity. Both the stem and root of *T. cordifolia*, together with other medicinal drugs, are prescribed as an anti-dote against scorpion sting and snakebite. Dry barks of *T. cordifolia* have anti-inflammatory, antiallergic, antipyretic, antispasmodic, and anti-leprotic properties.Herbal immune stimulants are promising in aquaculture since they boost growth performance, fish immunity, and antimicrobial properties, making them viable alternatives to chemicaltreatments and antibiotics (Elumalai *et al.*, 2021) [5]. *Tinospora cordifolia*is a well-knownmedicinal plant in the Menispermaceae family and is a significant supplier of newmedications. It has a wide range of therapeutic applications and phytochemicals responsible for its pharmacological effects (Sajith & Farhan, 2022) [11]. Antibiotics and therapies (Elumalai *et al.*, 2021) [5], one well-known medicinal plant in the Menispermaceae family, *Tinosporacordifolia*, is a major source of novel drugs. Its pharmacological effects are caused by phytochemicals and a variety of medicinal uses (Sajith & Farhan, 2022) [11]. Tinospora leaves are heart-shaped, 5-10 cm long, and high in protein, calcium, and phosphorus (Sinha *et al.*, 2004; Kushwaha, (2020) [12, 8]. In catfish *Pangasius sutchi* fingerlings, it has been documented that diets enriched with *T. cordifolia* leaf extract enhanced growth performance, survival, and haemato-biochemical profiles (Latha *et al.*, 2020) [9]. Likewise, El Basuini *et al.*, (2022) [4] discovered that dietary *T.cordifolia* improves growth performance, antioxidative capacity, immune response, and resistance against hypoxia stress in the Nile tilapia. The current study aimed to evaluate the effects of *T. cordifolia* stem powder on growth performance, and antioxidant-enzyme activities in *C. carpio*, an economically important farmed fish species in India.

2. Materials and Methods

2.1 Experiment Design

The feeding trial was conducted at the aquaculture research facility of the, Sinha fish farm village-Jargaon Block-Chhura District-Gariabandh The experiment was carried out for a total duration of 150 days.

2.2 Procurement of Experimental Fishes

The healthy common carp fingerlings were collected from Sinha fish farm with average body weighing 10.5 g. Then these fishes were transfer in acclimatization tank for 15 days.

2.3 Procurement of Experimental Plant and Collection of Ingredients

Giloy (*Tinospora cordifolia*) stem powders were collected from the Sinha fish farm village-Jargaon, Gariabandh, Chhattisgarh, India. The collected giloy were washed with tap water to remove debris and air dried under shade. After complete drying, the giloy was powdered using a warring blender. A plastic container was use for storing the leaves powder for further use. Remain other ingredients (Rice bran, MOC, wheat flour, fish meal) and the vitamin premixes were purchased from the local market of Gariabandh, Chhattisgarh. The feeding ingredient and their composition were mentioned in Table no1.

Table 1: Experimental Diet and Ingredient Proximate Composition

S. No	Ingredient	Percentage (%)
1.0	Fishmeal (60% CP)	25.0
2.0	Soybean Meal (45% CP)	30.0
3.0	Wheat Flour	25.0
4.0	Rice Bran (De-oiled)	12.0
5.0	Fish Oil	4.0
6.0	Vitamin Premix	1.5
7.0	Mineral Premix	1.5
8.0	Carboxy methyl cellulose (Binder)	1.0
	Total	100.0
Proximate Analysis (Calculated)		
1.0	Crude Protein (%)	33.5
2.0	Crude Lipid (%)	6.8
3.0	Crude Fiber (%)	5.2
4.0	Ash (%)	8.5
6.0	Moisture (%) (as fed)	9.0
7.0	Nitrogen-Free Extract (NFE) (%)	37.0
8.0	Gross Energy (kcal/kg) (Calculated)	~3500

2.4 Experimental Feed Preparation

For the preparation of experimental diets all the ingredients were (Rice bran, MOC, wheat flour, soybean meal, fish meal) finely powdered and it were mixed all together according to the percentage amount calculation of the ingredient with water, vitamin was added in all the three feeds including the control tank. The feed was formulated with 35 per cent protein content that was 3, 5 and 8 percent of Giloy (*Tinospora cordifolia*) stem powders added. After complete mixing the dough the different treatment feed was put in an autoclave for 30 minutes to kill the microorganism. The autoclaved dough was added with vitamin premix and after complete mixing the dough feed were put in a feed pelletizer and extruded according to the required size for the fingerling fish mouth. Extruded pelleted feed are then dried at 40 °C for four hours to remove the moisture from the feed. The control feed used contains 0 per cent Giloy powders, for the three treatment tanks, three different percentages of Giloy powders were used to prepare the other three experimental diets, for the control experiment, feed containing fish meal, wheat flour, mustard oil cake (MOC) and rice barn as the ingredients was used. The feeding was done at the rate 3 per cent of their body weight.

2.5 Water quality analysis

Various water quality parameters viz. water temperature (°C), pH, dissolved oxygen (mg/l), nitrite, nitrate and ammonia (APHA, 2005) [3].

2.6 Growth Parameters

The following formulas were used to calculate the fish's growth performance.

1. Weight gain (g) = Final Weight (g)-Initial weight (g)

2. Percent Weight Gain = (Final weight gain-Initial weight gain / Initial weight gain) ×100

3. Length gain (g) = Final length gain(g)-Initial Length gain (g)

4. Specific Growth Rate (SGR)

$$SGR = \frac{(\ln Wt - \ln Wo)}{D} x100$$

Where,
In = log
W_0 = Initial weight of live fish (gm)
Wt = Final weight of live fish (gm)
D = Duration of feeding (days)

5. Feed Conversion Ratio (FCR)

FCR = Feed given (g) / Weight gain (g)

2.7 Statistical Data Analysis

The data obtained from this study were analysed using the Statistical Package for the Social Sciences (SPSS) 2019. The data were presented as Mean ± SE. Results will be considered statistically significant at the 5 per cent level ($p< 0.05$).

3. Results and Discussion

The growth performance and feed utilization parameters of common carp (*Cyprinus carpio*) fingerlings fed diets supplemented with different levels of *Tinospora cordifolia* stem powder for 150 days are presented in Table 1.

Table 2: Growth Performance and Feed Utilization of Common Carp

Parameter	T_0 (0%)	T_1 (3%)	T_2 (5%)	T_3 (8%)
Initial Body Weight (g)	10.52 ^{a}±0.05	10.48^{a}±0.05	10.55^{a}±0.02	10.50 ^{a}±0.02
Final Body Weight (g)	31.25 ^{b}±0.3	36.80 ^{b}±0.03	40.80 ^{b}±0.3	38.50 ^{b}±0.05
Weight Gain (g)	20.73 ^{a}±0.05	26.32 ^{a}±0.05	30.25 ^{c}±0.02	28.00 ^{c}±0.3
Percent Weight Gain (%)	197.05 ^{a}±0.3	251.14 ^{b}±0.03	286.73^{ab}±0.3	266.67^{ab}±0.3
Specific Growth Rate (%/day)	1.81^{a}±0.05	1.95 ^{b}±0.03	2.05 ^{c}±0.05	1.99^{a}±0.05
Feed Intake (g/fish)	37.31 ^{a}±0.02	38.16 ^{c}±0.03	38.70^{c}±0.3	38.36^{c}±0.02
Feed Conversion Ratio (FCR)	1.80 ^{a}±0.02	1.64 ^{c}±0.05	1.52 ^{c}±0.05	1.58^{b}±0.3
Survival Rate (%)	95.56 ^{a}±0.05	97.78 ^{b}±0.05	97.78 ^{c}±0.02	97.78 ^{a}±0.3

3.1 Growth Parameters

The present study investigated the effect of dietary supplementation with varying levels of *Tinospora cordifolia* (Giloy) stem powder on the growth performance and feed utilization of *Cyprinus carpio* fingerlings. The growth performance of *Cyprinus carpio* fed diets supplemented with varying levels of *Tinospora cordifolia* (Giloy) was summarized in Table 2. The initial body weights of fish were comparable across all treatments, ranging from 10.48 g (T_1) to 10.55 g (T_2), and confirming uniformity at the start of the experiment. A significant improvement in growth parameters was observed in fish fed diets containing Giloy. The highest final body weight (40.80 g) was recorded in the T_2 group (5% Giloy), followed by T_3 (8%) with 38.50 g, T_1 (3%) with 36.80 g, and the lowest in the control group T_0 (31.25 g). Similarly, weight gain was significantly higher in T_2 (30.25 g), demonstrating the most effective growth promotion, while the control group showed the lowest gain (20.73 g). The percent weight gain followed a similar trend: T_2 (286.73%) > T_3 (266.67%) > T_1 (251.14%) > T_0 (197.05%). This pattern indicates that the incorporation of Giloy in the diet enhances growth, particularly at a 5% inclusion level. The Specific Growth Rate (SGR) was also highest in the T_2 group (2.05%/day), indicating faster biomass accumulation compared to other treatments. The SGR in the control group was the lowest at 1.81%/day. This result supports the conclusion that Giloy supplementation positively affects metabolic and anabolic processes, possibly due to its bioactive compounds like alkaloids and diterpenoid lactones known for immunostimulant and growth-promoting effects. Feed intake across the treatments was relatively consistent, ranging from 37.31 g (T_0) to 38.70 g (T_2). The Feed Conversion Ratio (FCR), an important index of feed efficiency, improved with increasing Giloy supplementation up to 5%. The lowest FCR was observed in T_2 (1.52), indicating better feed utilization efficiency. In contrast, the control group had the highest FCR (1.80), suggesting less efficient feed conversion into body mass. FCR slightly increased in T_3 (1.58), suggesting that a further increase beyond 5% may not yield additional benefits and could be less efficient. The survival rate remained high across all groups, ranging from 95.56% (T_0) to 97.78% (T_1, T_2, and T_3). The lack of significant differences in survival rate among treatments indicates that Giloy supplementation up to 8% does not adversely affect the health or survivability of *C. carpio*. Elumalai *et al.*, 2021; Sajith & Farhan, 2022 [5, 11] they suggested that the Giloy is safe and well-tolerated by fish even at higher concentrations. The results clearly demonstrate that 5% Giloy supplementation (T_2) provides optimal enhancement of growth performance and feed conversion in common carp. The improved weight gain, SGR, and FCR in this group may be attributed to the bioactive phytochemicals present in *Tinospora cordifolia*, which are known to enhance nutrient absorption, digestive enzyme activity, and immune response in fish. Elumalai *et al.*, 2021 [5]; Sinha *et al.*, 2004 [12] and Sajith & Farhan, 2022 [11] they were suggested that the consistent with earlier studies where herbal additives improved fish performance by enhancing gut health and boosting immunity. The lowest FCR was observed in T5 (1%) which was significantly different with T_1, T_2, T_3 and T_4 ($p<0.05$). The mean value of different treatments shown that best FCR was recorded in T5 (2.41) followed by T_4 (2.74), T_3 (5.35), T_2 (6.87) and control T_1 (6.51). Kour *et al.* (2004) [7] lead a feeding trial

on *C. mrigala* to measure the impact of Bala (*Sida cordifolia*) herb diverse with ground nut oil cake and rice bran with ratio 1:1. Though the 8% Giloy group (T_3) too presented better growth comparative to the control, its act was slightly lower than T_2, suggesting a threshold effect beyond which no additional benefits occur, and excessive inclusion may even hinder feed efficiency. Therefore, inclusion levels beyond 5% may not be economically justified.

3.2 Water Quality Parameters Analysis

The mean values of water quality parameters monitored in the experimental tanks throughout the 150-day culture period were presented in Table 3.

. **Table 3:** Profile of water quality parameters in different treatments

Parameter	T_0	T_1	T_2	T_3
Temperature (°C)	26.5	26.6	26.4	26.5
pH	7.45	7.40	7.38	7.42
Dissolved Oxygen (mg/L)	6.2	6.3	6.4	6.3
Total Ammonia Nitrogen (TAN, mg/L)	0.28	0.25	0.22	0.24
Nitrite-Nitrogen (NO_2-N, mg/L)	0.055	0.048	0.040	0.045
Nitrate-Nitrogen (NO_3-N, mg/L)	1.85	1.70	1.65	1.75

Water temperature ranged from 26.4°C to 26.6°C and pH ranged from 7.38 to 7.45 across treatments. These values remained stable and within the acceptable range (25-30°C for temperature and 6.5-8.5 for pH) for optimal carp growth and physiological functions. No significant fluctuation in temperature or pH was observed due to Giloy supplementation, suggesting that dietary treatments did not alter these basic water conditions. Dissolved oxygen concentrations were slightly higher in treatment groups receiving Giloy, with the maximum DO recorded in T_2 (6.4 mg/L) and the minimum in the control group T_0 (6.2 mg/L). These differences, although minor, may be attributed to improved water quality as a result of enhanced feed digestion and reduced metabolic waste, particularly in T_2. All values remained above 5.0 mg/L, indicating a well-oxygenated environment suitable for carp culture. The levels of nitrogenous waste products—TAN, NO_2-N, and NO_3-N showed a decreasing trend with increasing Giloy supplementation up to T_2. The lowest TAN (0.22 mg/L) and NO_2-N (0.040 mg/L) were recorded in T_2, while the control group showed the highest levels (TAN: 0.28 mg/L, NO_2-N: 0.055 mg/L). Nitrate levels also followed this trend, with the lowest NO_3-N in T_2 (1.65 mg/L). These findings suggest that Giloy supplementation, particularly at 5%, may have contributed to better nitrogen metabolism or reduced nitrogenous excretion. The lower concentrations of TAN and NO_2-N in the Giloy-supplemented groups could be attributed to improved nutrient assimilation, potentially reducing the amount of nitrogenous waste excreted into the water. This may also indicate that Giloy has a role in enhancing gut health or supporting beneficial microbial activity, which could contribute to the detoxification of ammonia and nitrite in the culture system. Maintaining optimal water quality was critical in aquaculture, as deviations can adversely affect fish health and growth. The results indicate that the inclusion of Giloy stem powder in the diet did not negatively impact water quality. In fact, there was a slight improvement in parameters such as DO and nitrogen compounds in the T_2 treatment, which also corresponded with the best growth performance observed in fish. This correlation supports the idea that 5% Giloy supplementation not only enhances fish growth but may also contribute indirectly to better environmental conditions in the culture system. These findings align with studies reporting that the use of herbal feed additives can lead to improved nutrient utilization and reduced organic and nitrogenous waste output in aquaculture systems. The stabilization of water parameters across all treatments also indicates good management practices and experimental reliability. (Anita *et al.* 2016) [2] reported significant improvements in SGR and FCR in Amur carp (*C. carpio*) fed diets with 0.75% *T. cordifolia* leaf powder.25 similarly, (Yadav *et al.*, 2023; Al-Turaihi *et al.* 2023) [13, 1] found that 6 g/kg (0.6%) *T. cordifolia* leaf powder enhanced growth in common carp. The present study, using stem powder, identified 1.0% as optimal, which falls within a comparable range of effective dosages. Also found that 6 g/kg (0.6%) of *T. cordifolia* stem powder (as well as root powder) significantly enhanced growth performance in common carp 9, further supporting the efficacy of the stem part.

Conclusion

In light of these findings, it is recommended that the incorporated into the diets of common carp fingerlings at a 5 percent inclusion level of *T. cordifolia*. This quantities suggestions optimal growth improvement, feed efficiency, and water quality maintenance, making it a valuable and natural alternative to synthetic feed additives in sustainable aquaculture systems. This research also help in making the strategy for fish culture in mainly grow out stage hence we suggesting some filed trial also.

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Competing Interests

The authors have no relevant financial or nonfinancial interests to disclose.

Author Contributions

All the authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by Revati Raman Tarak and M.K. Yadav. The first draft of the manuscript was written Revati Raman Tarak, and all the authors commented on previous versions of the manuscript. All the authors have read and approved the final manuscript.

Availability of data and Materials

The data will be provided upon request to the journal.

Ethical Statement

In the present study, Common carp were collected from the School of School, Sanjeev Agrawal Global Educational (SAGE) University, and Bhopal India). Ethical approval, specimen collection, and maintenance were performed carp in strict agreement with all the recommendations India.

Conflict of Interest
The authors state that there are no conflicts of interest regarding the publication of this research paper.

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Disclaimer (Artificial Intelligence)
Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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