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Effect of Dietary Supplementation of Tootache Plant (*Acmella oleracea)* Extract on the Growth Performance of *Cirrhinus mrigala* Fingerlings

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Authors' contributions

This work was carried out in collaboration among all authors. Material preparation, data collection and analysis were performed by Authors NM and MKY. The first draft of the manuscript was written author NM, and all the authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the efficacy of *Acmella oleracea* extract on the growth performance of *Cirrhinus mrigala* fingerlings. This trial was designed for 45 days. The experiments were designed as T0 (0%) T1 (0.5%), T2 (1.0%), and T3 (1.5%) *A. oleracea* extract with three replications. During the study the growth parameters including net weight gain, percent weight gain, length gain,

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specific growth rate and feed conversion ratio were recorded at 15-days interval. Results indicated a significant improvement ($p < 0.05$) in growth parameters in all treated groups compared to the control, with the best performance was observed at the T1 treatment. Fish fed with 0.5 percent *A. oleracea* extract exhibited the highest net weight gain (32.3 ± 1.15 g), percent weight gain (61.64% ± 1.36), specific growth rate (1.067% ± 0.018), and the most efficient feed conversion ratio (2.62 ± 0.049). Higher concentrations (T2 and T3) showed moderate improvements but were less effective than the T1 treatment, suggesting a dose-dependent response. This study highlights the potential of *A. oleracea* as a natural, cost-effective, and sustainable growth promoter in aquafeed.

Keywords: Intensification; immunostimulatory; formulated feed; aquafeed and sustainable.

1. INTRODUCTION

Aquaculture plays a crucial role in ensuring global food security and economic development, particularly as the global population continues to rise. Sustainable intensification of aquaculture demands eco-friendly alternatives to synthetic feed additives to improve growth performance and overall fish health. In this context, plant-derived additives have emerged as promising candidates due to their bioactive properties. Aquaculture essential to global food security, it accounts for a major portion of high-quality protein and plays a significant role in contributing to economic development (FAO, 2021). The global population is predicted to increase by a little less than 30% by the year 2050 and, therefore, the rise in demand for food will be proportionately exponential. In this regard, it will be essential to guarantee that all people have access (which is both physically and economically feasible) to sufficient amounts of safe and nutritious food, to sustain a healthy and active life; and whether we are capable of meeting this demand will rely greatly on the development of the most sustainable food production systems. Aquaculture is showing itself to be a major vehicle toward this goal. (FAO, 2009; Hassan *et al*., 2021) Nevertheless, aquaculture sustainable intensification necessitates novel feed formulations that improve growth performance and minimize reliance on synthetic additives and antibiotics (Naylor *et al*., 2021). Plant-derived feed additives are increasingly considered for use because of their bioactive nature, which improves fish growth, immunity, and feed efficiency (Van Hai, 2015; Dawood *et al*., 2018). One of such potential plants is *A. oleracea*, or the toothache plant. It contains a high concentration of spilanthol, a bioactive compound that has been shown to exhibit antimicrobial, immunostimulatory, and appetite-stimulating activities (Singh *et al*., 2020). Experiments have proven that bioactive compounds extracted from plants are able to improve the activity of digestive enzymes, increase nutrient absorption, and stimulate improved growth performance in aquaculture species (Reverter *et al*., 2014; Awad & Awaad, 2017). Still, there is little research on the effectiveness of *A. oleracea* as an aquaculture feed additive. The current research assesses the impact of *A. oleracea* extract on the growth performance of *C. mrigala*, a highly valued freshwater fish species used commercially. *C. mrigala* is extensively farmed in South Asia because of its high growth rate, adaptability, and economic value in carp polyculture (Kumar *et al*., 2022). Through the evaluation of major growth indicators like weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and survival rate, this research will seek to identify the potential of *A. oleracea* as a natural growth promoter in aquafeed. Results of this study may help develop sustainable and low-cost feeding programs for aquaculture (Ahmad *et al*. 2010).

2. MATERIALS AND METHODS

2.1 Experiment Trial and Diet Preparation

The experiment was conducted in Aquaculture Laboratory of S.A.G.E University, Bhopal, Madhya Pradesh from December 2024 to January 2025.

2.2 Collection of Toothache Plant (*A. oleracea*)

The toothache plant was collected from Papum Pare District of Arunachal Pradesh. The plant was sundried for one week and grinded into powder form. The extract was obtained through Soxhlet method.

2.3 Experiment Setup

There were three treatments and one control designated as T0 (Control), T1 (0.5%), T2 (1%)

Table 1. Experimental Diet Ingredient Composition

S. No.	Ingredients (g)	T0	T1	T2	T3
1	**Fish Meal**	34.00	34.07	34.08	34.07
2	**Rice Brand**	25.00	24.93	24.92	23.44
3	**Wheat Flour**	15	15	15	15
4	**Vit. Premix**	1.0	1.0	1.0	1.0
5	**MOC**	25.0	25.0	25.0	25.00
6	**Toothache plant**	0	0.5	1	1.5
Total		100.00	100.00	100.00	100.00

and T3 (1.5%) *A. oleracea* extract were fed with experimental diets for 45 days. Each treatment had three replications. The fish was acclimatized for two weeks in the tank before the start of experiment. Each tank consists of five fish with average weight 8.3 g and length 6.8cm and they were fed a basal diet of 28 per cent protein at 3 per cent body weight and feeding was done at two times in a day for 45 days. The ingredients used for formulated feed were fish meal, rice brand, wheat flour, vitamin premix and MOC as shown in (Table 1.) Each tank was provided the aeration facility through aerators.

2.4 Growth Parameters

The growth parameters were calculated on the 15 days gapes.in this study several growth parameters were analysed as Net Weight Gain, Per cent Weight Gain, Net Length Gain, SGR and FCR.

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

1. **Net Weight gain (g)** = Final Weight (g) – Initial weight (g)
2. **Percent Weight Gain**= (Final weight gain – Initial weight gain / Initial weight gain) ×100
3. **Net 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)

2.5 Statistical Data Analysis

The growth performance data, including mean, NWG, PWG, LG, SGR and FCR, and were identified their significant differences among the groups. The data obtained from this study were analysed using the Statistical Package for the Social Sciences (SPSS 16.0). To compare the treatment the Duncan values and the data were presented as mean ± SE. Results will be considered statistically significant at the 5% level ($p < 0.05$).

3. RESULTS AND DISCUSSION

Adding toothache plant (*A. oleracea*) to the diet of mrigal (*C. mrigala*) consistently led to increased net weight gain across all treatment groups throughout the study. By adding (*A. oleracea*) extract at various concentrations and feeding it at 3% of the body weight, the study recorded continuous weight gain across all treatments. At the end of experiment, the relationship between NWG, NLG, PWG, SGR and FCR were significantly ($p<0.05$) impacted by the diet that used Tootache plant extract. NLG, NWG, PWG, SGR and FCR better in T1 (0.5%) followed by T2 (1.0%), and T3 (1.5%), whereas the lowest was control. The highest LG value ($p<0.05$) was observed in fish diet T1 (8.88^{a}±1.154), followed by T2 and T3 and lowest ($p<0.05$) in fish fed control (T0) (Table 2, Fig.1). The highest NWG value ($p<0.05$) was observed in fish diet T1 (32.3^{d}±1.15470), followed by T2 (23.9^{c}±1.15470**)** and T3(20.3^{b}±1.15470), with the lowest ($p<0.05$) in the fish fed diet control T0 (18.8^{a}±1.15470). The highest PWG value ($p<0.05$) was observed in fish diet T1 (61.64^{d}±1.360) followed by T2 (48.09^{c}±1.117) and T3 (41.51^{b}±0.981), with the lowest ($p<0.05$) in the fish fed diet control T0 (35.81^{a}±0.788). The highest SGR value ($p<0.05$) was observed in fish diet T1 (1.067^{d}±0.018) followed by T2 (0.873^{c}±0.017) and T3 (0.772^{b}±0.015) with the lowest ($p<0.05$) being observed fish fed diet control (0.68^{a}±0.012). The better FCR significantly ($p<0.05$) was observed in fish diet

T1 (2.62^{a}±0.049) followed by T2 (3.17^{b}±0.066) and T3 (3.68^{c}±0.075), with the lowest ($p<0.05$) being observed fish fed diet control (4.16^{d}±0.083), all growth parameters data were presented in Table 2. Fig. 1. NWG of experimental fish body showed significant ($p<0.05$) difference during the entire observation of the experiment. PWG of Mrigal carp also showed significant ($p<0.05$) difference during observation of the experiment. FCR of the experimental fish showed significant ($p<0.05$) difference.

The bioactive compounds *of A. oleracea*, especially spilanthol, which were had antimicrobial properties and an appetite stimulant (Rahim *et al*., 2021), was likely responsible for the growth performance seen in the different treatment. *A. oleracea* may increase growth performance by increasing the palatability of feed, as well as nutrient use. *A. oleracea* also contained bioactive compounds, such as glycosides, pyridine alkaloids, and flavonoid (Nabi & Shrivastava, 2016) these compounds stimulated growth performance of treated fish (Asha *et al*. 2015; Ahmad *et al*. 2017).

The relationship between NWG, NLG, PWG, SGR and FCR were significantly ($p<0.05$) impacted by the diet that used Tootache plant extract. NLG, NWG, PWG, SGR and FCR better in T1 (0.5%) followed by T2 (1.0%), and T3 (1.5%). The more similar results were reported regarding use of herbal supplements in experimental fish diet Abidin *et al*. 2022 and other study that use of neem (*Azadirachta indica)* leaf extract 7 per cent in feed significantly improved growth performance in rainbow trout (*Oncorhynchus mykiss).* Abdel-Tawwab *et al*. (2010) reported that incorporating 0.5 g/kg of *Camellia sinensis* (green tea) extract into Nile tilapia diets significantly improved weight gain and feed conversion ratios. Additionally, (Kumar *et al*., 2017) reported study on *C. mrigala* fingerlings demonstrated that dietary supplementation with anthraquinone extract at 1% enhanced growth performance and immune responses. (Lee *et al*. 2012) reported that garlic extract in the diet of Starlet sturgeon exhibited better FCR and PER. growth parameters data were presented in Table 2. Fig. 1. NWG of experimental fish body showed significant ($p<0.05$) difference during the entire observation of the experiment. PWG of Mrigal carp also showed significant ($p<0.05$) difference during observation of the experiment. FCR of the experimental fish showed significant ($p<0.05$) difference. This study was supported and more similar finding were found by (Ragunath & Ramasubramanian, 2024).

Table 2. Overall Growth parameters of different treatments (means ± standard error)

Treatment	NWG	PWG	SGR	FCR	NLG
T0	18.2^{a}±1.15470	35.81^{a}±0.788	0.68^{a}±0.012	4.16^{d}±0.083	6.1^{a}±1.154
T1	32.2^{d}±1.15470	61.64^{d}±1.360	1.067^{d}±0.018	2.62^{a}±0.049	8.88^{a}±1.154
T2	23.9c±1.15470	48.09^{c}±1.117	0.873^{c}±0.017	3.17^{b}±0.066	6.78^{a}±1.154
T3	20.3^{b}±1.15470	41.51^{b}±0.981	0.772^{b}±0.015	3.68^{c}±0.075	6.2^{a}±1.154

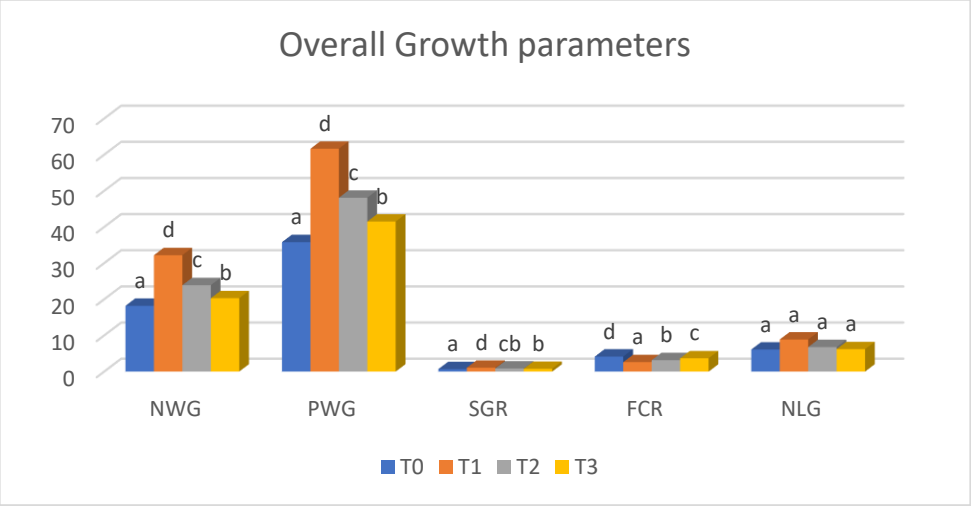

Fig. 1. Comparative analysis of growth performance

4. CONCLUSION

The growth performance of *C. mrigala* was considerably improved by the dietary addition of *A. oleracea* extract in the feed; the 0.5 per cent supplementation (T1) produced the best results. Comparing this trail to greater inclusion levels and the control group, the T1 showed the significant gains in NWG, PWG, SGR, and FCR. These findings demonstrate the promise of *A. oleracea* as a sustainable alternative to other herbs in aquaculture as a natural growth stimulant. However, its efficacy is dose-dependent, highlighting the importance of adjusting inclusion levels to maximize good benefits and minimize negative ones.

ETHICAL APPROVAL

In the present study, silver 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 in strict agreement with all the recommendations India.

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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 writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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