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Effect of *Ocimum sanctum* (Tulsi) powder on the growth and survival in *Cirrhinus mrigala* fingerlings

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Abstract
The experiments were conducted using feed prepared with supplementation of *Tulsi* powder to know their effect on growth performance and survival rate in *Cirrhinus mrigala* fingerlings (2.65 ± 0.02g). Fingerlings were stocked at a density of 10 nos./experimental tank. Experimental diets were formulated with 35% protein level. In this experiment 10 diets were prepared. T0 diets were considered as control. *Tulsi* were added at the rate of 5g, 10g, 20g and 50g per kg of feed in T1, T2, T3 and T4 respectively. The experimental feed was given at the rate of 10% of body weight of the fish twice daily. After 10 days, feeding was decreased to 4% of body weight. After 60 days, Results showed that mean weight gain (%), Specific Growth Rate, Protein Efficiency Ratio and survival were significantly higher in T3. The significantly lowest Feed Conversion Ratio was recorded in T3 (2.66± 0.07) respectively. The survival rate was higher in T3 (97.75±0.75), but it was not significantly different among treatments. The results of the present investigation revealed that the supplementation of *Tulsi* in the diet of *C. mrigala* fingerlings significantly affected the mean weight gain (%), SGR, PER and survival rate.

Keywords: *Tulsi*, *Cirrhinus mrigala*, growth performance, feed utilization

Introduction
The progress in aquaculture is directly dependent upon the nutritionally balanced feed which provides superior growth within a stipulated time period. The best path for economic farming of various aquatic animals lies in determining the appropriate and precise diet and preparation of low budget artificial feeds from readily available food stuffs. Food and feeding habits play an important role to understand the rate of growth, population density and maturation of gonads and other metabolic activity of aquatic organisms [9]. The objective of feed formulation in aquaculture is to supply the nutrient density for optimal animal production. Feed cost and feed efficiency are the prime factors that control the farm economy. The availability of nutrients from feed ingredients is essential in determining the nutritional value of the feed ingredient. Traditionally, the feed have been based on animal protein.

The shortage of animal protein intake in developing countries can be satisfied with proper development of aquaculture. Fish feed is the most expensive input in aquaculture operations. Most of the high cost of feed arises from extensive reliance on protein sources such as fish meal and shrimp meal. To overcome the high cost input in feed, it would be economical to utilize plant ingredients which will enhance fish production. If plant sources can be used as a supplement to animal protein sources, it will not only reduce the production cost and also increases the growth and production [11].

Now days many biological activities have been recorded for medicinal plants including growth promotion, appetite stimulation, immune stimulation, antimicrobial, and anti-stress in fish. Easy access and the cheap price for many plants are also encouraging factors for their use in large scale in aquaculture to provide better growth and protection at the same time. They have been used in several forms, either as crude, or extracts or active compounds from the plant. Sometimes, they are used in incorporation with a probiotic or with an animal product [3].

In India, the use of various parts of medicinal plants as folk medicine is being practiced since time immemorial [8]. Indeed, more than 800 species of plants have been described as traditional remedies to treat various bacterial, viral, fungal and parasitic diseases [1, 2]. Herbs have long been used for promotion of health, prevention and treatment of diseases. *Ocimum sanctum*, which belongs to the Lamiaceae family, is commonly known as '*Tulsi*', 'holy basil', 'queen of

plants' and 'the mother medicine of nature'. Above names are given to *Tulsi* plant due to its enormous medicinal properties like anti stress activity [4], anti asthmatic effect [16], anti tubercular activity [15], immune modulatory activity [7], antimicrobial activity [14], hypoglycaemic activity [6], anti-inflammatory and antipyretic activity [17] and endocrinological effects [12]. These properties can be utilized for curing and preventing diseases [4].

With this background information, this study was carried out to systematically evaluate the effects of *O. sanctum* (*TULSI*) powder on growth and survival of mrigal (*Cirrhinus mrigala*) fingerlings.

Materials and Methods

Fish and management

The experiment was conducted at the Wet Laboratory of Department of Aquaculture, College of Fisheries Science, JAU, Veraval, over a period of 60 days from 14th October, 2017 to 12th December, 2017. Fingerlings of *Cirrhinus mrigala* were collected from Government Fish Hatchery, Ukai, Gujarat and transported in polythene bags by road to Veraval. The fishes were brought to Aquaculture Wet Laboratory of College of Fisheries Science, JAU, Veraval, and were allowed to remain in the plastic tank (500 L) with continuous aeration and feeding for 10 days. Fingerlings with a total weight of 2.64 ± 0.02g to 2.72 ± 0.05g were selected for the experiment. The experiment was conducted in rectangular plastic aquarium tanks of 40 litres capacity with the size of 2x1x1 feet. Aquarium tanks were filled with fresh water up to 30 litres.

The experimental set-up consisted of 20 plastic tanks. In this, 20 plastic tanks were set-up for *Tulsi* powder. The tanks were washed with potassium permanganate solution (4 ppm) thoroughly and cleaned with fresh water. Two hundred (200) fishes were distributed in five distinct experimental groups under each experiment. Each plastic tank containing 30 L chlorine free water was stocked with 10 fishes. Water used for the entire experiment was sourced from bore-well (ground water source). Aeration was provided through the aerators. The aeration pipe in each tank was provided with an air stone and a plastic regulator to control the air pressure uniformly in the entire tank.

Treatment details of Experiment

Table 1: Experiment: *Ocimum sanctum* (*Tulsi*) Powder

Treatment Groups	T0	T1	T2	T3	T4
	Gram of herbal powder per kg feed				
	0	05	10	20	50

Collection of plant materials

Tulsi leaves were collected from the village farm at Sutrapada (Dist. Gir-Somnath). The collected *Tulsi* leaves were then washed thoroughly and kept for sun drying over the plastic sheet. After 3 days of sun drying, the leaves were crushed and ground in a mixture grinder. The powder was sieved and packed in plastic bag.

Composition of experimental diets (%)

Table 2: Composition of diets

Ingredients (%)	Treatments				
	T0	T1	T2	T3	T4
Fish Meal	58	58	58	58	58
Wheat Bran	10	10	10	10	10
Wheat flour	23	22.5	22	21	18
Binder	2	2	2	2	2
Sun Flower Oil	3	3	3	3	3
Fish Oil	3	3	3	3	3
Vitamin & mineral	1	1	1	1	1
Tulsi	0	0.5	1	2	5
Total	100	100	100	100	100

Preparation of experimental diets

The experimental diet was formulated with 35% protein level using locally available ingredients. The *Tulsi* powder was added to other ingredients separately and diets were blended for 40 min to make a paste of each diet. For each treatment, there were four replications. The required quantities of ingredients were collected and weighed accurately as per feed formula as shown in Table 2. The ingredients were mixed well with the required quantity of water in an enamel tray to prepare dough. The prepared dough was thermally processed at 121 ºC and 15 lbs pressure for 10-15 minutes and then cooled at room temperature. After cooling of dough, the vitamin-mineral mixture, sunflower oil, fish oil and feed supplements (*Tulsi*) were added as per the treatment details (Table 2) and mixed well. The feed mixture was then pelletized in the form of pellets using hand pelletizer. The size of the pellets was approximately 1-2 mm. The pellets were spread on a plastic sheet, exposed to sunlight for 2-3 hours every day for 2 days and dried till the moisture content was reduced to less than 10%. The pelleted feed was then packed in marked plastic jars. After that, the composition of 5 Diets was composed (Table 3).

Table 3: Proximate composition of experimental diets containing *Tulsi* powder

Sr. No.	Composition	Treatments				
		T0	T1	T2	T3	T4
1	Protein	35.79	34.96	35.09	35.54	34.76
2	Fat	15.36	15.17	15.73	15.81	15.26
3	Moisture	5.90	4.86	5.45	5.91	5.20
4	Ash	21.36	21.45	21.82	22.31	22.19

Analysis of Physio-Chemical Water Parameters

Water quality parameter such as temperature, pH, dissolved oxygen and total hardness were measured on weekly basis throughout the experimental period. The temperature of each tank was measured by using mercury thermometer and pH was measured by pH meter, respectively. Dissolved oxygen and total hardness was measured using wrinkler's method and EDTA method respectively.

Statistical Analysis

One-way Analysis of Variance (ANOVA) was applied to test the significance of the treatments at 5% error level. The data analysis was undertaken at Department of Agricultural Statistics, Junagadh Agricultural University, Junagadh.

Results

The Effect of *Tulsi* (*O. Sanctum*) Incorporated Diet on Growth of *C. Mrigala* Fingerlings

Mean weight gain (%)

The mean weight was calculated for each tank at each fortnight interval. The initial mean weight recorded were 2.64± 0.02g, 2.65± 0.02g, 2.72± 0.05g, 2.65± 0.05g and 2.65± 0.03g in treatment T0, T1, T2, T3 and T4 respectively. At the end of experiment the final mean weight recorded were 5.64±0.11g, 6.01±0.05g, 5.9±0.014g, 6.20±0.034g and 5.72±0.034g in treatment T0, T1, T2, T3 and T4 respectively. The highest final mean weight (g) was observed in T3 and lowest in T0 treatment. Mean weight of *C. mrigala* fingerlings at each fortnight interval for 60 days of culture period is presented in Table 4.

Table 4: Mean weight (g) of *C. mrigala* fed with *Tulsi* supplemented diets during experimental period (n=5 fish, Mean± SE)

Treatments	Days				
	0	15	30	45	60
T0	2.64 ± 0.02	3.17 ± 0.04	4.29 ± 0.04	4.65 ± 0.03	5.64 ± 0.22
T1	2.65 ± 0.02	3.18 ± 0.05	4.35 ± 0.06	4.97 ± 0.13	6.01 ± 0.05
T2	2.72 ± 0.05	3.26 ± 0.05	4.32 ±0.08	4.81 ± 0.03	5.90 ± 0.01
T3	2.65 ± 0.05	3.42 ± 0.05	4.47 ± 0.03	5.22 ± 0.08	6.20 ± 0.03
T4	2.65 ± 0.03	3.37 ± 0.05	4.32 ± 0.02	4.64 ± 0.05	5.72 ± 0.03

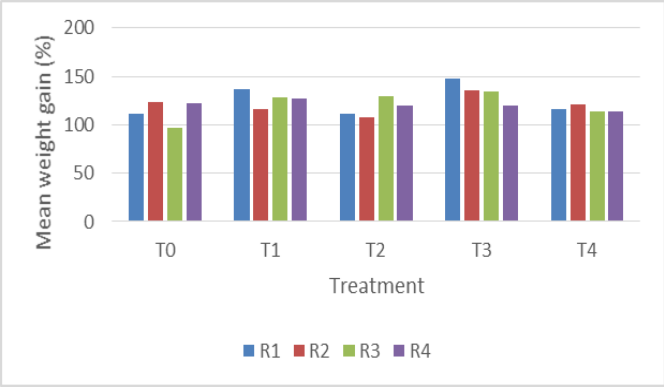

Fig 1: Mean weight gain (%) of *C. mrigala* fed with *Tulsi* supplemented diets at the end of experimental period

Specific growth rate (%)

The specific growth rate (SGR) of *C. mrigala* fingerlings in different treatments is given in Table 5. At the end of the experiment, specific growth rate recorded were 4.995±0.22, 5.612±0.12, 5.299±0.10, 5.924±0.12 and 5.129±0.02 in treatment T0, T1, T2, T3 and T4 respectively. The maximum specific growth rate was observed in T3 and minimum in T0. The statistical analysis revealed significant difference in specific growth rate among the treatment (P>0.05, Table 5, Fig. 2). Treatment T3 showed significantly higher SGR compared to other treatments.

Table 5: Specific growth rate (%) of *C. mrigala* fed with *Tulsi* supplemented diets at the end of experimental period (Mean ± SE)

Days	Treatments				
	T0	T1	T2	T3	T4
60	4.99^{a}± 0.22	5.6^{bc}± 0.12	5.29^{ab}± 0.10	5.92^{c}± 0.12	5.12^{a}± 0.02

Mean values with different superscripts in the same row are significantly different (P<0.05)

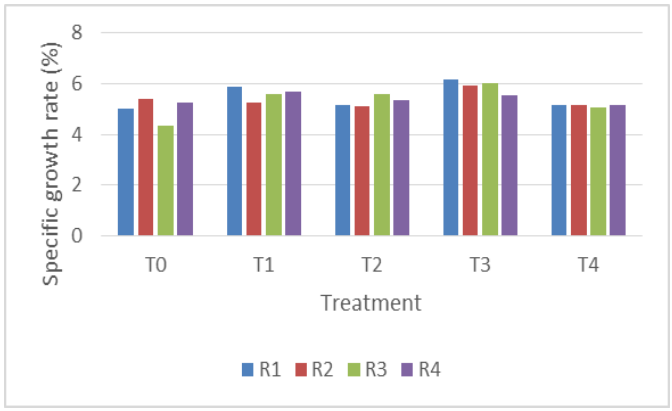

Fig 2: Specific growth rate (%) of *C. mrigala* fed with *Tulsi* supplemented diets at the end of experimental period

Feed conversion ratio (FCR)

The feed conversion ratio (FCR) of *C. mrigala* fingerlings in different treatments is given in Table 6. At the end of the experiment, feed conversion ratio recorded were 2.97±0.15, 2.75±0.06, 2.85±0.05, 2.66±0.07 and 2.92±0.02 in treatment T0, T1, T2, T3 and T4 respectively. The lowest feed conversion ratio was observed in T3 and highest in T0. The statistical analysis revealed that there was No significant difference in feed conversion ratio of fishes fed with different feeds (P<0.05, Table 6, Fig 3).

Table 6: Feed conversion ratio (FCR) of *C. mrigala* fed with *Tulsi* supplemented diets at the end of experimental period (Mean ± SE)

Days	Treatments				
	T0	T1	T2	T3	T4
60	2.97^{b}± 0.15	2.75^{ab}± 0.06	2.85^{ab}± 0.05	2.66^{a}± 0.07	2.92^{ab}± 0.02

Mean values with different superscripts in the same row are significantly different (P<0.05)

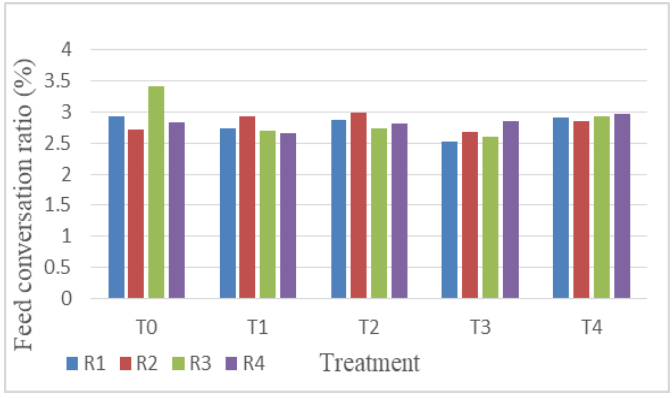

Fig 3: Feed conversion ratio (FCR) of *C. mrigala* fed with *Tulsi* supplemented diets at the end of experimental period

Protein efficiency ratio (PER)

The result on protein efficiency ratio of *C. mrigala* fingerlings under different experiments are given in Table 7. At the end of the experiment, protein efficiency ratio recorded were

0.94±0.04, 1.05±0.03, 0.99±0.01, 1.05±0.02 and 0.98±0.008 in treatment T0, T1, T2, T3 and T4 respectively. The highest protein efficiency ratio was observed in T3 and lowest in T0. The statistical analysis revealed that there was significant difference in protein efficiency ratio of fishes fed with different feeds (P<0.05, Table 7, Fig 4). The higher level of significant difference in T3 treatment compare with other, T2 and T4 showed no significant difference at the end of experimental period.

Table 7: Protein efficiency ratio (PER) of *C. mrigala* fed with *Tulsi* supplemented diets at the end of experimental period (Mean ± SE)

Days	Treatments				
	T0	T1	T2	T3	T4
60	0.94^{a} ± 0.04	1.05^{b} ± 0.03	0.99^{ab}± 0.01	1.05^{b}± 0002	0.98^{ab}±0.008

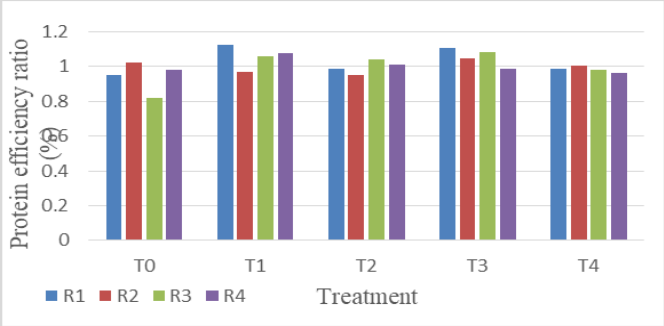

Fig 4: Protein efficiency ratio (PER) of *C. mrigala* fed with *Tulsi* supplemented diets at the end of experimental period

Effect of *O. sanctum* incorporated diets on survival of *C. mrigala* fingerlings

Survival (%) of *C. mrigala* fingerlings in the various treatments at the end of experiment is detailed in Table 8. The highest survival was observed in the T3. However, there was found significant difference among the treatments (P>0.05, Table 8). Survival as observed in respective treatments is shown in Fig. 5.

Table 8: Mean survival (%) of *C. mrigala* fed with *Tulsi* supplemented diets at the end of experimental period (Mean ± SE)

Days	Treatments				
	T0	T1	T2	T3	T4
60	92.5^{a}± 1.65	95^{ab}± 0.40	95^{ab}± 0.40	97.75^{b}±0.75	93.75^{a}± 0.47

Mean values with different superscripts in the same row are significantly different (P>0.05)

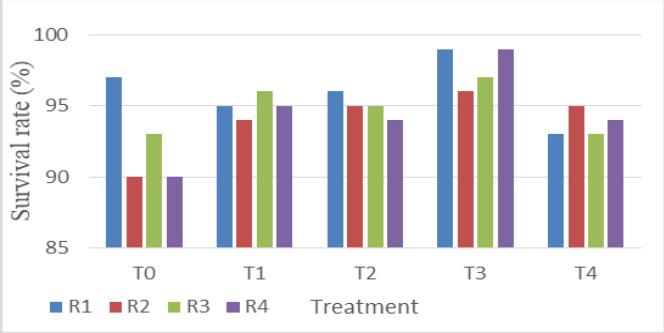

Fig 5: Survival (%) of *C. mrigala* fed with *Tulsi* supplemented diets at the end of experimental period

Physico-chemical water parameters

The water quality parameter such as temperature, pH, dissolved oxygen (DO) and total hardness were analyzed during this experiment on weekly basis.

Temperature

Water temperature in different experimental tanks was recorded weekly. The mean data of temperature (°C) during study period are shown in Table 9. During the whole experimental period, water temperature ranged from 21.75±0.47 to 26.25±0.47 °C. The temperature was found within the optimum range throughout the experimental period.

Table 9: Mean water temperature (°C) during the experimental period

Weeks	Treatments (Mean ± SE)				
	T0	T1	T2	T3	T4
1st	26.25± 0.25	26.5± 0.28	26.5± 0.28	26± 0.40	26.25± 0.47
2nd	26.5± 0.28	26± 0.40	26.5± 0.28	25.75± 0.25	26.25± 0.47
3rd	25.5± 0.28	26.5± 0.288	25.75± 0.47	26.5± 0.5	26± 0.40
4th	24.5± 0.28	24.25± 0.62	24.75± 0.47	24.75± 0.47	25± 0.40
5th	24.75± 0.47	24.5± 0.288	25± 0.57	24.75± 0.47	24.5± 0.5
6th	24.5± 0.28	25.25± 0.47	24.75± 0.25	25.5± 0.28	25.5± 0.28
7th	22.5± 0.28	22.25± 0.47	22.75± 0.25	21.75± 0.47	22.5± 0.28
8th	22.5± 0.28	22.25± 0.25	22.25± 0.25	22.25± 0.25	22.5 ± 0.28

pH

The water pH in all experimental tanks was analyzed weekly. The mean values pH during the experimental period is shown in Table 10. During the whole experimental period, water pH ranged from 7.23±0.00 to 8.10±0.05. The pH was within the optimum range throughout the experiment period.

Table 10: Mean water pH during the experimental period

Weeks	Treatments (Mean ± SE)				
	T0	T1	T2	T3	T4
1st	7.69±0.02	7.65±0.02	7.67±0.02	7.72±0.01	7.70±0.01
2nd	7.70±0.03	7.65±0.01	7.59±0.03	7.75±0.01	7.69±0.01
3rd	7.63±0.01	7.65±0.01	7.48±0.02	7.68±0.01	7.71±0.00
4th	7.72±0.07	8.00±0.00	7.44±0.04	7.70±0.04	7.72±0.07
5th	7.96±0.04	8.02±0.02	7.43±0.00	8.00±0.04	7.65±0.05
6th	8.02±0.04	8.07±0.04	7.39±0.02	8.10±0.07	7.77±0.04
7th	8.08±0.02	8.10±0.05	7.31±0.05	8.03±0.04	7.69±0.06
8th	8.06±0.03	8.07±0.04	7.23±0.00	7.99±0.00	7.68±0.02

Dissolved Oxygen (DO)

The dissolved oxygen (DO) in all experimental tanks was analyzed weekly. The mean data of dissolve oxygen during experimental period are shown in Table 11. During the experimental period, dissolved oxygen ranged from 5.00±0.11 to 7.30±0.12 ppm. The dissolved oxygen was found within the ideal range throughout the experimental period.

Table 11: Mean dissolved oxygen (ppm) during the experimental period

Weeks	Treatments (Mean± SE)				
	T0	T1	T2	T3	T4
1st	5.55±0.14	4.90±0.19	5.40±0.14	5.55±0.14	5.10±0.10
2nd	5.60±0.16	5.00±0.11	5.60±0.00	5.60±0.16	5.40±0.11
3rd	5.70±0.10	5.30±0.10	5.70±0.10	5.70±0.10	5.70±0.10
4th	5.80±0.11	5.80±0.11	5.80±0.11	5.80±0.11	5.60±0.16
5th	6.10±0.10	6.00±0.16	6.00±0.16	6.10±0.10	5.80±0.11
6th	6.88±0.13	5.90±0.10	6.00±0.16	6.88±0.13	6.00±0.16
7th	6.00±0.10	6.80±0.08	6.15±0.08	6.00±0.10	6.25±0.05
8th	6.07±0.04	6.22±0.08	7.30±0.12	6.07±0.04	6.20±0.24

Total Hardness

The total hardness was analyzed weekly. The mean data of total hardness (ppm) during experimental period are shown in Table 12. During the whole experimental period, water total

hardness ranged from 220.00±1.63 to 325.00±7.39 ppm. It was found within the optimum range throughout the experimental period.

Table 12: Mean total hardness (ppm) during the experimental period

Weeks	Treatments (Mean ± SE)				
	T0	T1	T2	T3	T4
1st	266.0±0.81	266.0±1.41	276.5±5.67	269.5±3.30	272.0±2.16
2nd	227.5±0.95	225.0±1.73	229.0±3.31	221.0±1.73	227.5±0.95
3rd	274.0±1.25	221.5±1.50	222.0±1.82	220.0±1.63	225.0±1.29
4th	265.5±1.50	277.5±1.89	275.0±4.20	280.0±5.47	274.5±0.95
5th	279.0±0.50	266.0±0.81	263.0±1.29	263.0±1.29	263.0±1.91
6th	292.0±1.00	278.0±0.81	281.0±1.73	279.0±0.57	280.0±1.82
7th	299.0±2.42	280.0±5.18	289.1±4.15	300.0±4.09	300.0±2.38
8th	300.0±4.28	313.0±2.34	307.0±6.01	325.0±7.39	310.0±4.33

Discussion

In present experiment, the fishes fed with the *Tulsi* supplemented diet showed significantly higher growth compare to control. The growth rate increased with the increase in concentration of *Tulsi* leaf supplement similarly, in the present experiment, growth rate increased with the increase of concentration of *Tulsi* powder in diet. Panprommin *et al.* (2015) reported that the Nile tilapia (*Oreochromis niloticus*) fed with *Tulsi* supplemented diets showed a significant increase in weight gain. Compared to the fishes fed with control diet. Similarly, in present experiment, the fishes fed with the *Tulsi* and onion supplemented diet showed significantly higher growth compare to control. The growth rate increased with the increase in concentration of *Tulsi* leaf supplement similarly, in the present experiment, growth rate increased with the increase of concentration of *Tulsi* powder in diet. Immanuel *et al.* (2009) also reported similar findings when they used four medicinal plants extract supplementation on *Oreochromis mossambicus* fish.

Panprommin *et al.* (2015) reported that the Nile tilapia (*Oreochromis niloticus*) fed with *Tulsi* supplemented diets showed a significant increase in SGR. Compared to the fishes fed with control diet. Similarly, in present experiment, the fishes fed with the *Tulsi* and supplemented diet showed significantly higher growth compare to control. The SGR increased with the increase in concentration of *Tulsi* leaf supplement similarly, in the present experiment, the Nile tilapia (*Oreochromis niloticus*) fed with *Tulsi* supplemented diets showed a significantly improved FCR compare to control diet. Sivaram *et al.* (2004) recorded lower FCR value when *E. tauvina* fed diet containing *Tulsi* supplementation at the rate of 100-200mg per kg of diet. In the present study, the fishes fed with *Tulsi* incorporated diet showed minimum FCR compare to the control diet. Panprommin *et al.* (2015) reported that the Nile tilapia (*Oreochromis niloticus*) fed with *Tulsi* supplemented diets showed a significantly improved FCR compare to control diet.

Sivaram *et al.* (2004) studied the effect of *O. sanctum* on growth performance of *E. tauvina* at different concentration. At the end of experiment, the *Tulsi* Incorporated diet showed higher PER in fishes than control one. Panprommin *et al.* (2015) reported that the Nile tilapia (*Oreochromis niloticus*) at different doses and found that the *Tulsi* supplemented diet gave more effective PER as compare to control diet.

Bhavan *et al*. (2011) studied the effect of *Tulsi* powder supplemented diet in *M. rosenbergii* and recorded highest survival rate of the prawn fed with diets containing *Tulsi* powder as compare to prawn in the control diet (84%). The results of present study were similar to this. The rate of survival increased with increase in concentration of *Tulsi* in the diet.

Conclusions

The results obtained under the present study revealed that mean weight gain (%), SGR, PER and survival rate were significantly higher in T3 (*Tulsi* supplemented diet). The significantly lowest FCR was also recorded in T3 (*Tulsi* supplemented diet). The survival rate was found to be higher in T3 (*Tulsi* supplemented diet). Based on these results it can be concluded that the addition of 20.00g *Tulsi* powder/ kg diet of *C. mrigala* fingerling is better for the higher mean weight gain (%), SGR, PER, FCR and better survival rate of *C. mrigala* fingerlings.

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