4. RESULT AND DISCUSSION

With increase in global population the demand for excessive high-quality protein particularly from aquatic sources is raising dramatically. A multiplied demand for aquaculture production is definitely needed to meet this demand in the 0.33 millennium. Increased demand for aquaculture production potentially increased the pressure for the development of more efficient manufacturing systems. However, the intensive and wide culturing of fish has paralleled with the emergence of microbial diseases. There is a growing problem about increased fish infection and diseases as indispensable issue influencing aquaculture and leading reason of financial losses.

The usage of chemicals and antibiotics to fight against fish diseases has various drawbacks inclusive of the threat of accumulation of toxins, development resistant species and environmental pollution. World Health Organization supports the usage of plants and herbs as alternatives to chemical compounds. Use of the plant materials could be broadly conventional as feed additives and supplements to enhance feed utilization efficiency and productive performance in animals (Mohamed *et al.,* 2003).

C.aromaticus and *O.basilicum* are economically essential medicinal herbs used to treat variety of diseases and as culinary herbs used as flavoring agent. The current investigation was undertaken to analyze the presence of phytochemical compounds in the leaf extracts of *C.aromaticus* and *O.basilicum* and to evaluate the efficiency of these leaf extracts as supplemented feed on growth characteristics, survival rate, feed utilization efficiencies, hematological and biochemical parameters, physiological and immunological parameters in *O.mossambicus*. The outcomes of present study are presented and discussed below under four phases.

Phase I

Phytochemical evaluation and characterization of bioactive compounds in the leaf extracts of *C.aromaticus* and *O.basilicum*

Qualitative phytochemical analysis

Phytochemicals are bio- active chemical compounds that originated from plants. They are considered as secondary compounds and are naturally synthesized in different parts of the plants (Tiwari *et al.,* 2011).

Phytochemical screening is a simple, quick and inexpensive method that gives the researcher a quick reply to identify the presence of various types of phytochemicals and is an vital tool for identification of bioactive compounds (Sasidharan *et al.,* 2011). Phytochemical analysis helps the scientist with the perception to know how plants are effective and an understanding about the composition of chemicals which helps for the development of new drugs (Nithya *et al.,* 2011).

Qualitative evaluation of phytochemicals namely alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids, in the leaf extracts of *C.aromaticus* and *O.basilicum* have been performed and the leaf extracts showed the presence of phytochemicals by showing effective and positive response with respective test reagent and the results are presented in Table 3 and Plate 13 -14.

Table 3

Phytochemical constituents in the leaf extracts of *C.aromaticus* and *O.basilicum*

Phytochemicals	Test	*C. aromaticus*	*O. basilicum*
Alkaloids	**Dragendroff's test**	+	+
	Mayer's test	+	+
	Wagner's test	+	+
Flavonoids	**Lead acetate Test**	+	+
	Shinoda test	+	+
Glycosides	**Sodium hydroxide test**	+	+
Phenol	**Ferric Chloride Test**	+	+
Saponins	**Froth test**	+	+
Steroids	**Salkowski's test**	+	+
	Libermann Burchard's test	+	+
Tannins	**Lead acetate test**	+	+
	Ferric Chloride Test	+	+

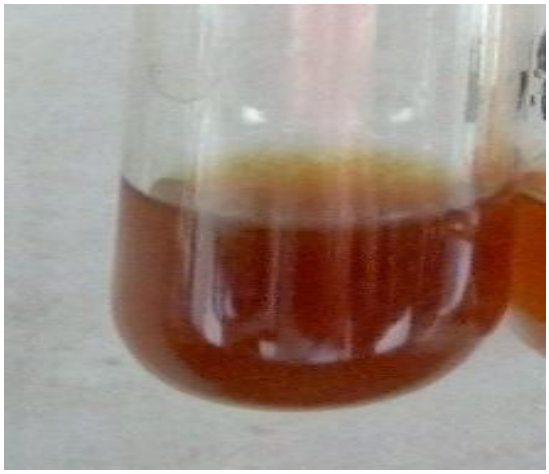
Plate 13

Presence of phytochemical constituents in the leaf extracts of *C.aromaticus*

Tannin

Saponin

Alkaloid



Phenol

Steroid

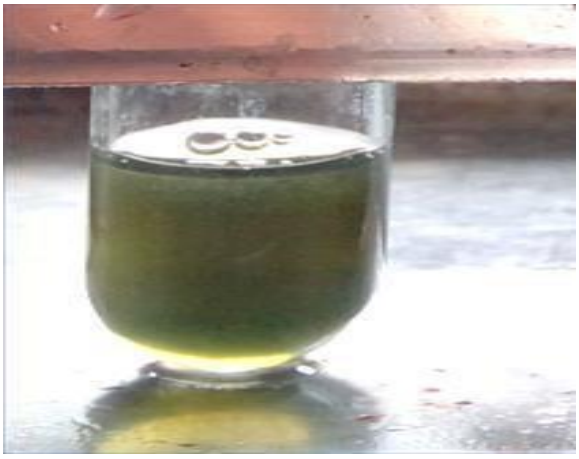


Plate 14

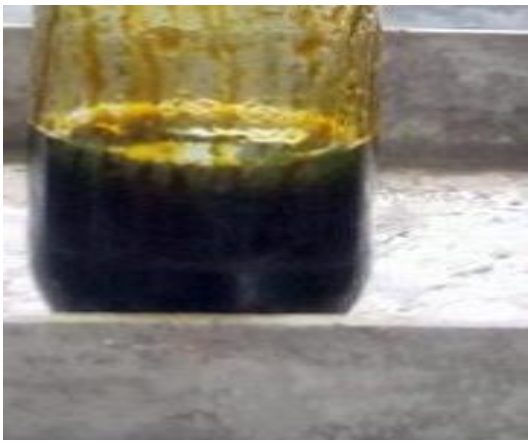
Phytochemical constituents in the leaf extracts of *O.basilicum*

Tannin

Saponin

Alkaloid

Phenol



Steroid

The leaf extracts of *C.aromaticus* and *O.basilicum* confirmed the presence of alkaloids, flavonoids, glycosides, phenol, saponin , steroids and tannin. Magesh *et al* (2015) had suggested the presence of tannins, flavanoids, terpenoids, phenols,and alkaloids in the aqueous extract of *C.aromaticus*. Sanni *et al* (2008) and Fakhroo and Sreerama (2016) stated that the aqueous extract of *O.basilicum* possess phytochemicals like tannins, flavanoids, terpenoids, phenols and alkaloids. Daniel *et al* (2011) cited that the aqueous extract of *O.basilicum* confirmed the presence of saponin, tannin and cardiac glycosides.

Alkaloid is a phytochemical compound identified in the leaf extracts of *C. aromaticus* and *O.basilicum*. It is used in medicinal field for centuries ago. The most important activity of alkaloids are their toxicity in opposition to foreign agents, anti - inflammatory, anti- asthmatic and anti- anaphylactic activities (Gopalakrishnan *et al* .,1979; Ganguly and Sainis, 2001 and Staerk *et al.,* 2002). Alkaloids have nitrogen and they are physiologically active in nature with various properties such as sedative, analgesic properties, etc., It is used as pain reliever, nervousness and melancholy (Jisika *et al.,* 1992).

C.aromaticus and *O.basilicum* leaf extracts confirmed the presence of flavonoids. They are a essential group of phenolic compounds known for their anti-viral activity (Mehrangiz *et al.*,2011), anti-microbial activity (Mari Lysete *et al.*,2009) and spasmolytic property (Julianeli *et al.*,2011). Flavonoids have antioxidant activity. They are transformers which regulate the body to react against carcinogens, certain viruses, and allergens. They exhibit anticancer, anti-inflammatory, antimicrobial and anti-allergic activity (Balch and Balch, 2000; Ekam and Ebong, 2007) and may also be useful in therapeutic treatments (Jisika *et al.,* 1992).

In the present study the leaf extracts of *C. aromaticus* and *O.basilicum* showed the presence of phenol. Plant phenols are effective antioxidants and have anti-bacterial, anti-viral, anti-carcinogenic, anti-inflammatory and vasodilatory actions (Shahidi and Naczk, 1995; Nakatani, 1997; Breinholt, 1999; Duthie *et al.,* 2000 and El-Beshbishy *et al.,* 2010).

The phytochemical compounds like saponin, steroids and tannins were additionally found in the leaf extracts of *C.aromaticus* and *O.basilicum*. Saponins have several pharmacological actions and anti-inflammatory effects. (Just *et al.,* 1998 & Estrada *et al.,* 2000).Steroids have a broad range of therapeutic applications such as cardiotonics, anti-inflammatory and anabolic agents (Tyler *et al.,* 1981). Tannins exert anti-microbial activity with the aid of deprivation of iron, formation of hydrogen bonds or unique interactions with enzymes in these cells of microorganisms. (Njume *et al.,* 2009). Herbs that possess tannins are mordant in nature and are used in the treatment of disorders in the intestine including diarrhea and dysentery (Dharmananda, 2003).Tannins are also used to treat inflammation and ulcer (Motar *et al* .,1985).

Characterization of bioactive compounds in the leaf extracts of *C.aromaticus* and *O.basilicum*

The analytical techniques like High pressure lipid chromatography, ulatraviolet visible spectrophotometer, Fourier transform infrared spectroscopy, nuclear mass spectrometry and GC-MS analysis are the vital and powerful used for separation, identification and structural determination of functional groups found in the phytochemical compounds of the samples.

UV-Vis spectroscopic analysis

UV –visible absorption spectroscopy is one of the vital analytical techniques. This technique has the advantages, encompass simplicity, speed, specificity and sensitivity (Parikh and Karkhanis, 2011). It is used to determine phenolic compounds (280nm), flavons (320nm), phenolic acids (360nm) and complete anthocyanids (520nm). Phenolic compounds along with anthocyanin‟s, tannins, polymer dyes and phenol complex with iron can be detected by using UV spectroscopy (Kemp,1991).

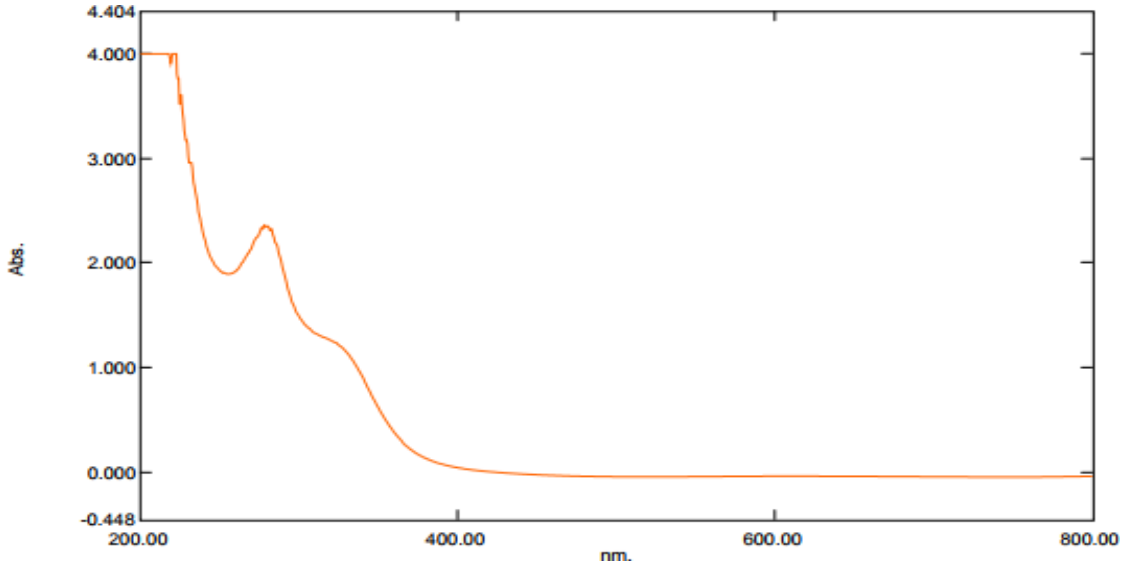
The ultraviolet region of the spectrum is generally typically consider to vary from 200 -400 nm and the visible region from 400-800nm. Based on the absorption bands in ultraviolet region the results are depicted in the figure 1 and 2 .

a)* UV visible absorption spectrum of leaf extract of *C.aromaticus

UV spectrum exhibited absorption band between 200- 800nm which expresses the presence of compounds such as phenol, phenolic acids, flavonoids, anthrocyanides. The values obtained at 278 nm indicated the presence of phenol (Figure 1).

Figure 1

UV-visible spectrum analysis of leaf extracts *C.aromaticus*

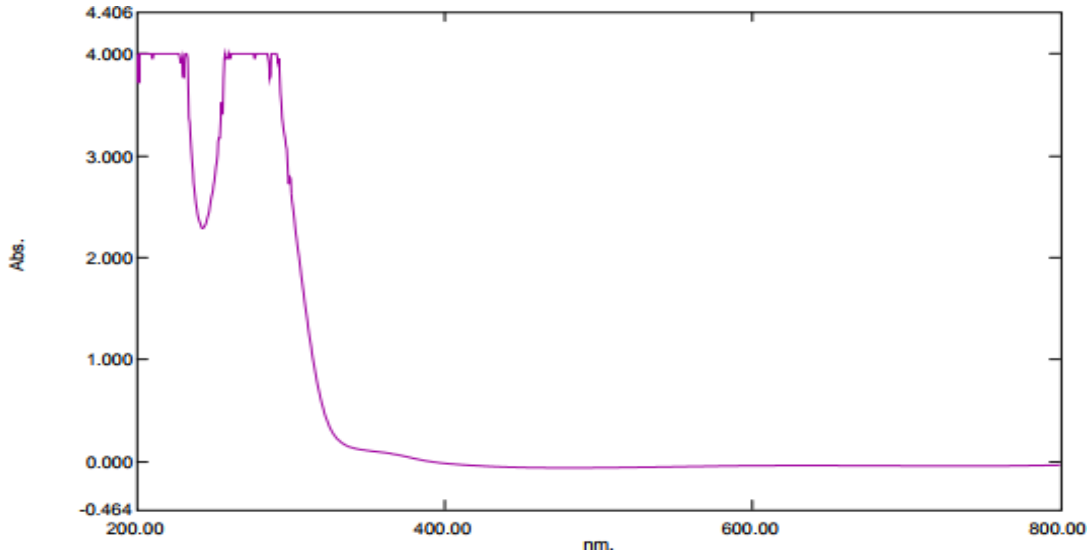


b)* UV visible absorption spectrum of leaf extract of *O.basilicum

The UV visible absorption spectrum showed distinct peaks at 289nm and 272.50nm.This clearly showed the presence of phenol group in the leaf extract of *O.basilicum* (Figure 2).

Figure 2

UV-visible spectrum analysis of leaf extracts *O.basilicum*



Jain *et al* (2016) stated that UV -Visible spectroscopic analysis of methanolic extract of *M.spiculata* confirmed one or more peaks in the regions from 200-400 nm. The UV visible spectral analysis of Citrus reticulate exhibit the peaks at 223.5, 258,284, 303, 226 nm respectively (Showmya *et al.,* 2014). In this study, UV -Visible spectroscopic analysis showed the presence of phenols, flavonoids and anthrocyanides in the leaf extract of *C.aromaticus* and phenol in *O.basilicum* leaf extract.

FT-IR Analysis (Fourier Transform Infrared Spectroscopy)

FTIR spectrum of leaf extracts of *C.aromaticus* and *O.basilicum* were analyzed and the result of FTIR spectrum profile is depicted in Figure 3 & 4 and in Table 4 & 5 .

a) **FTIR spectrum of leaf extracts of *C.aromaticus***

C.aromaticus leaf extracts showed characteristic absorption band at 3425.58 cm-1 (O-H ; alcohols and phenols (H bonded), the peaks at 2920.23 cm^{-1} and 2854.65 cm^{-1} (C- H ; alkanes). The peak at 1616.35 cm^{-1} (C=C;alkynes and asymmetric nitrocompounds) (Figure 3 and Table 4).

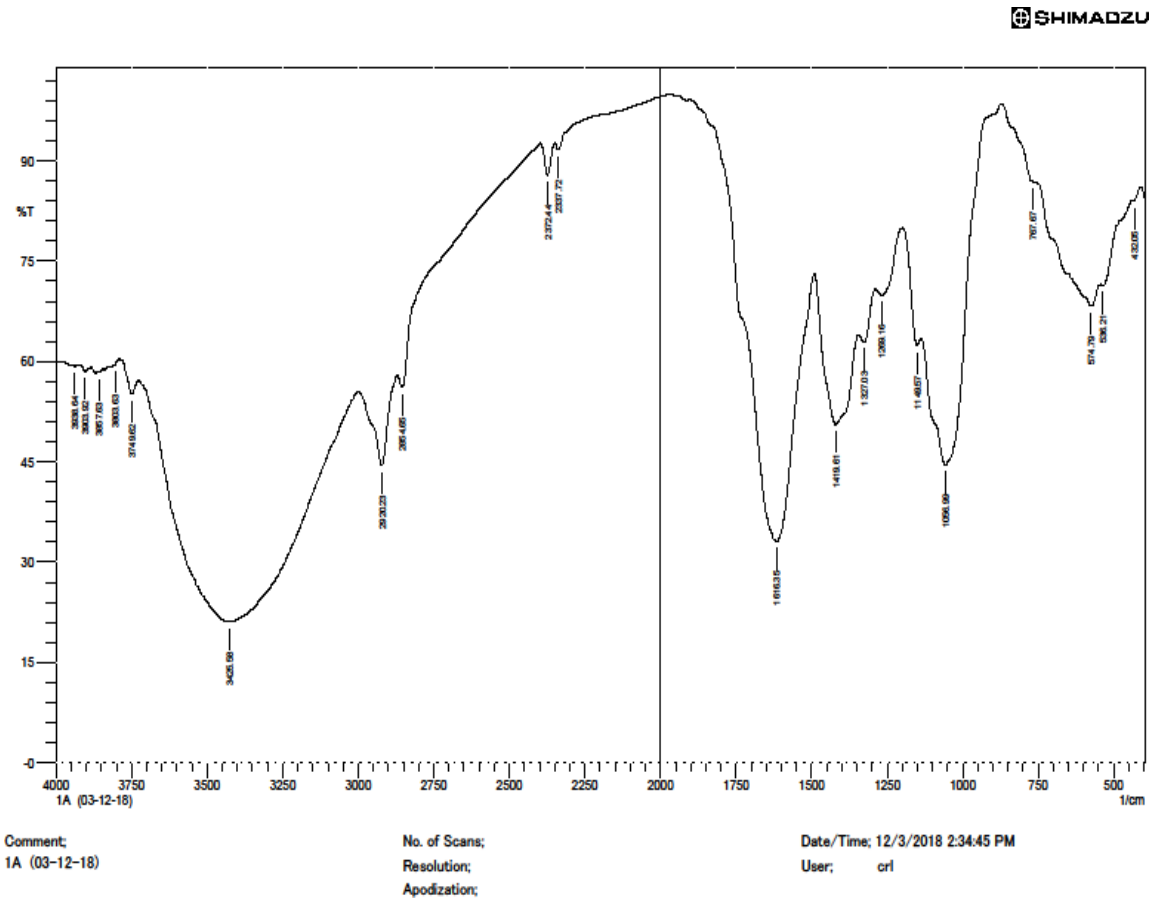
Table 4

Interpretation of FTIR absorptions of leaf extracts of *C.aromaticus*

FTIR peak values of *P.ambonicus* distilled water leaf extract wave number values	**Band stretching**	**Functional group assignment**
1616.35	C=C	Alkynes, nitro compounds
2854.65	C-H	Alkanes
2920.23	C-H	Alkanes
3425.58	O-H	- OH Normal polymeric OH stretch- Alcohols/Phenols Alcohols, Phenols (H bonded)

Figure 3

FTIR absorption spectrum of leaf extracts of *C.aromaticus*



b)* FTIR spectrum of leaf extracts of *O.basilicum

The leaf extracts of *O.basilicum* showed characteristics absorption band at 3745. 76 cm^{-1} (O-H ; alcohol and phenol compounds). The peak at 3367.71 cm^{-1} represents (N-H ; secondary amines and amides). The peak at 2927.44 cm^{-1} showed (C-H ;alkanes). The peak at 1643.35 represents (C= O; aldehydes, ketones ,carboxylic acid, ester) (Figure 4 and Table 5).

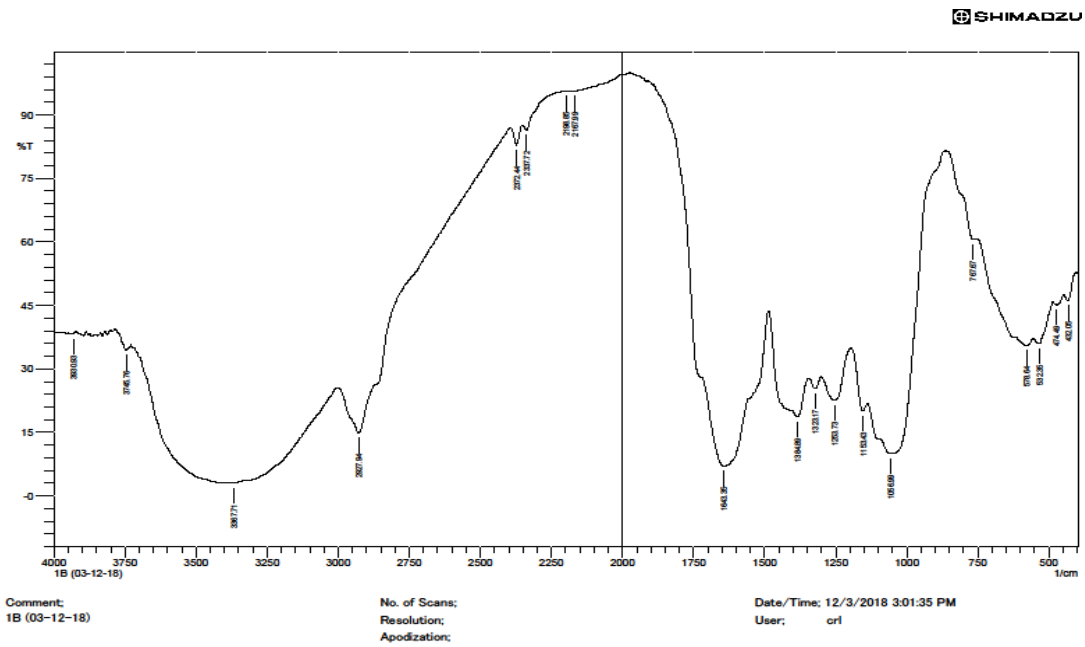
Table 5

Interpretation of FTIR absorptions of leaf extracts of *O.basilicum*

FTIR peak values of *P.ambonicus* distilled water leaf extract wave number values	Band stretching	Functional group assignment
3930.93	O-H	Alcohols, Phenols
3367.71	N-H	Primary, secondary amines, amides
2927.94	C-H	Alkanes
1643.35	C=O	Aldehydes, Ketones, Carboxylic acids, esters (Carbonyls general)
1253.73	C-N	Amines, amide groups

Figure 4

FTIR absorption spectrum of leaf extracts of *O.basilicum*



The existences of paromomycin, steviocycle, campesterol and ascaridole epoxide, aliphatic fluro compounds, OH, ethers, carboxylic acids, esters, nitro compounds, alkynes, H-bonded, H-X group, hydrogen bonded alcohols and phenols was identified by using GCMS and FTIR analysis in the leaf extracts of *Ocimum basilicum* (Kadhim *et al.,* 2016)

Similarly Sangeetha *et al* (2014) stated that aqueous extracts of *Gymnema sylvestre* by revealed the presence of aliphatic and aromatic amines. and plant possessed highest antioxidant activity. Ragavendran *et al* (2011) stated that the different biological activities of *Aerva lanata*. were due to the presence of various compounds such as carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons and halogens.

FTIR spectrum of methanolic extract of leaves of *S.toruvum* showed the presence of alcohols, alkanes and aromatic carboxylic acids (Nithyadevi and Siva kumar, 2015). George and Shanmugam (2014) stated that FTIR spectrum analysis of ethanolic extract of fruit of *Sapindus mukorossi* showed the presence of several compounds namely OH, alkanes, alkenes, esters, ether and alkyne groups. FTIR analysis of ethanolic extract of *Peristrophy bycalyculata* and the FTIR analysis are confirmed the presence of ketones, amines and amides Janakiraman *et al* (2011). *Myristica fragrans* fruit extract showed the presence of phenolic and the alcoholic compounds by FTIR analysis (Joseph and George, 2014).

Phase II

Growth characteristics, feed utilization efficiencies and proximate composition of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts supplemented feed

Growth parameters

The leaf extracts of *C.aromaticus* and *O.basilicum* at three different concentrations (2.5, 5 and 10 gm/kg of feed) as feed supplement to *O.mossambicus* for 45 days. The growth parameters such as body BW, GR, RGR, SGR and SR of *O.mossambicus* in the control and six different treatments were observed initially and on 15^{th}, 30^{th} and 45^{th} days after treatment and the results obtained are presented in Table 6 - 8 and Figure 5 - 22 .

The initially the body weight of *O.mossambicus* fingerlings were weighed before the experimental period. The mean initial body weight was almost same for the control and treatment groups (Table 6). The freshwater fish *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed exhibited higher growth at all levels of supplementation, compared to those fed with control feed on 15th, 30th and 45th days after treatment.

4.2.1.1. 15 (DAT)

Growth characteristics of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed at three different concentrations (2.5, 5 and 10 gm/kg of feed) after 15 days of treatment are presented in Table 6 and Figure 5 - 10. After 15 days of treatment , significant differences (P<0.05%) was noted in the BW of *O.mossambicus* fingerlings treated with leaf extract supplemented feed (T1 –T6). Final body weight of fishes were higher (10.40±0.38 gm) in T2 group when compared to control (8.05±0.05 gm). The weight gain was maximum (3.40 ±0.38 gm) in T2 group whereas control recorded a minimum weight gain of 1.05±0.05 gm. The highest growth rate (1.58±0.18gm/day) was recorded in T2 when compared to control (0.49±0.02 gm/day).Average daily growth rate was maximum (0.23±0.03 gm/day) in T2 group and minimum in control(0.07±0.01 gm/day). RGR% was significantly higher (P<0.05%) (42.94±0.08%) in T2 group when compared to control (14.32±0.03%). Specific growth rate % was high (6.53±0.12%) in T2 group whereas control recorded a minimum value of 5.72±0.02 %.The survival rate during the experimental period was high in all the treatments (T1 – T6) when compared to control. The highest SR (100%) was observed in all treatments whereas control showed 90% survival rate.

An overall, after 15 days of treatment the growth parameters was significantly high (P<0.05) in *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed at three different concentration (2.5,5 and 10 gm/kg of feed) when compared to control group

Figure 5

Final body weight (gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (15 DAT). Data represents mean± SE of 10 fishes.

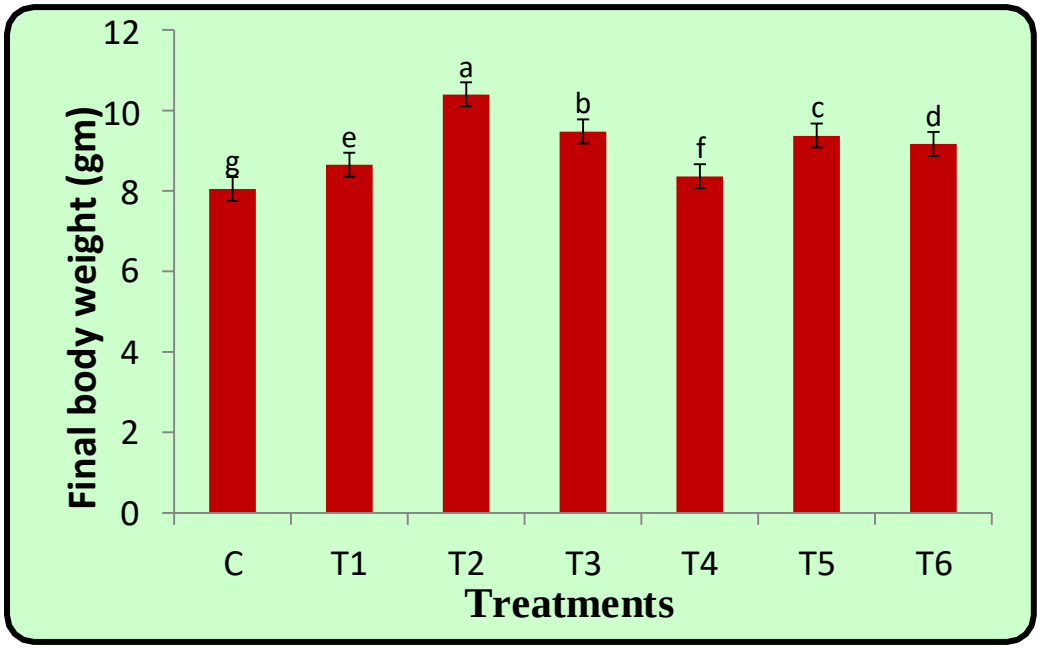


Figure 6

Body weight gain(gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (15 DAT). Data represents mean± SE of 15 fishes.

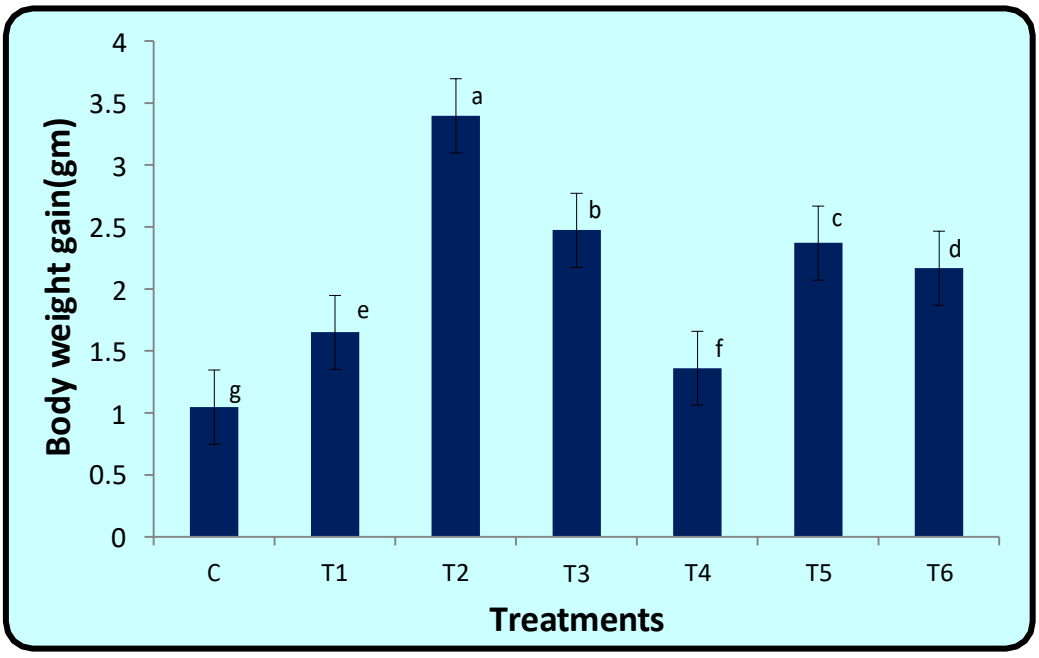


Figure 7

Growth rate (mg.day^{-1}) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (15 DAT). Data represents mean± SE of 15 fishes.

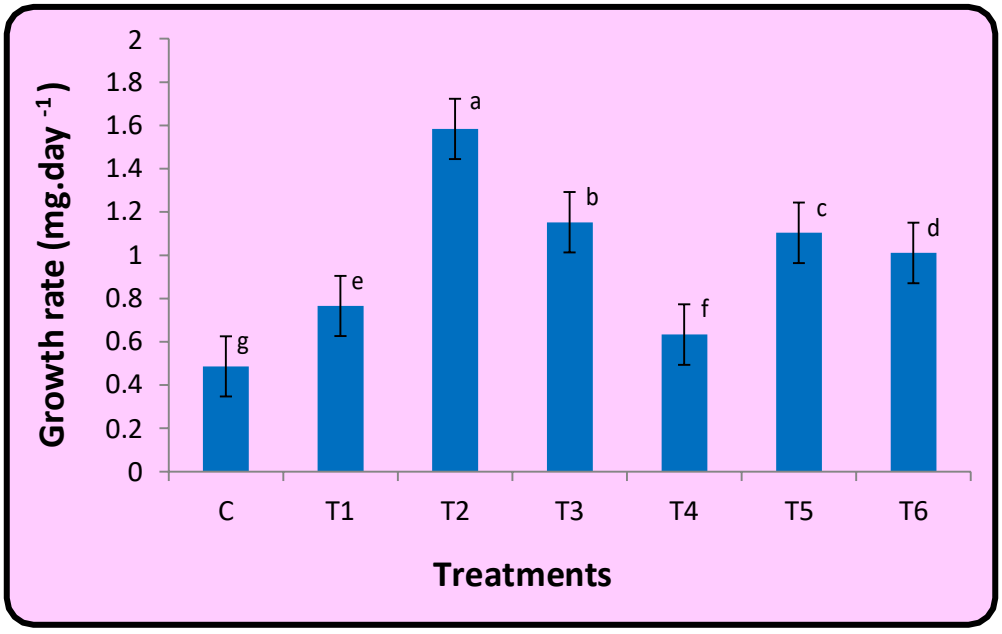


Figure 8

Average daily growth (mg/day)of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (15 DAT). Data represents mean± SE of 15 fishes.

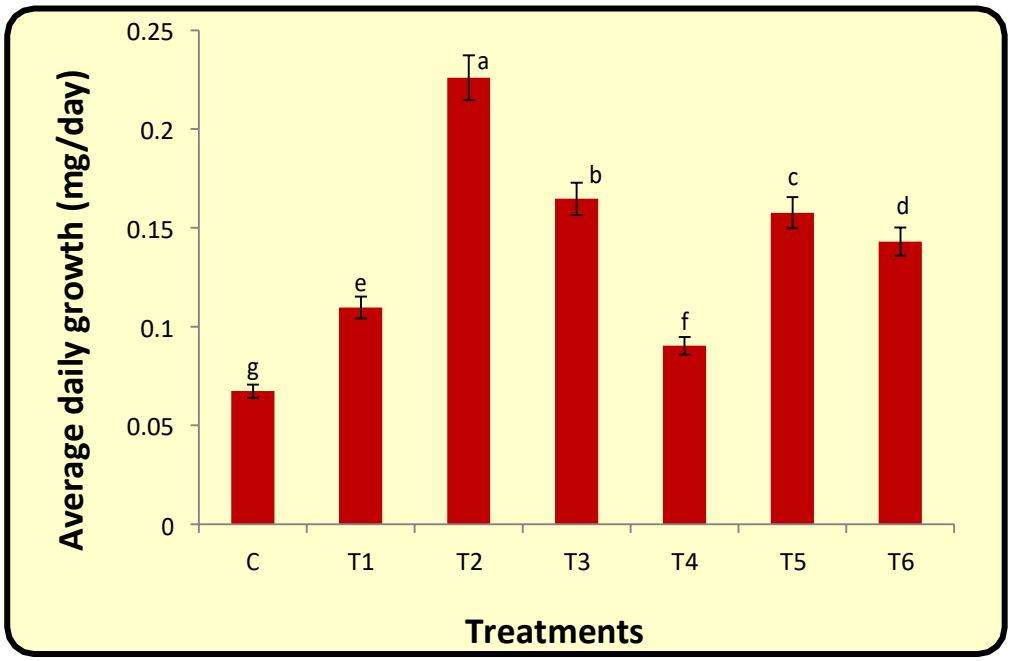


Figure 9

Relative growth rate(%) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (15 DAT). Data represents mean± SE of 15 fishes.

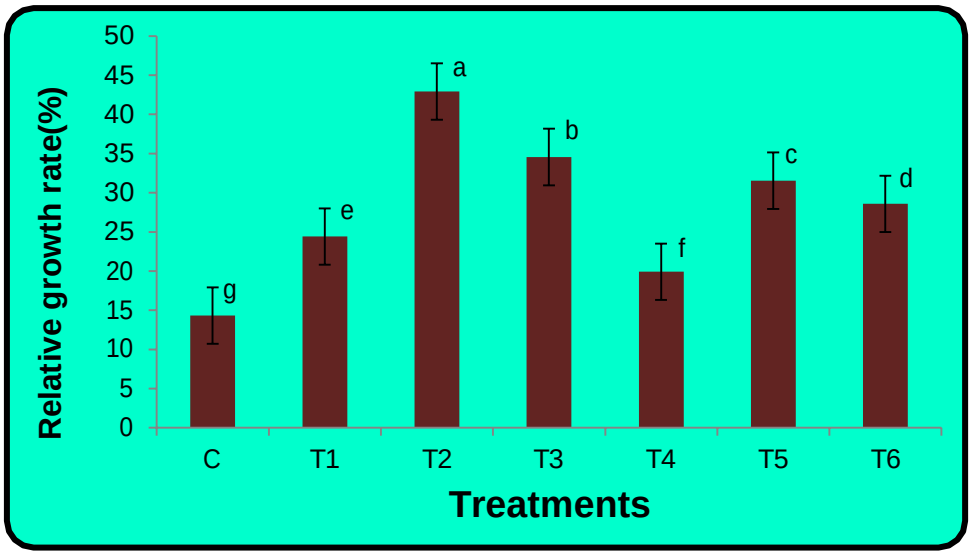
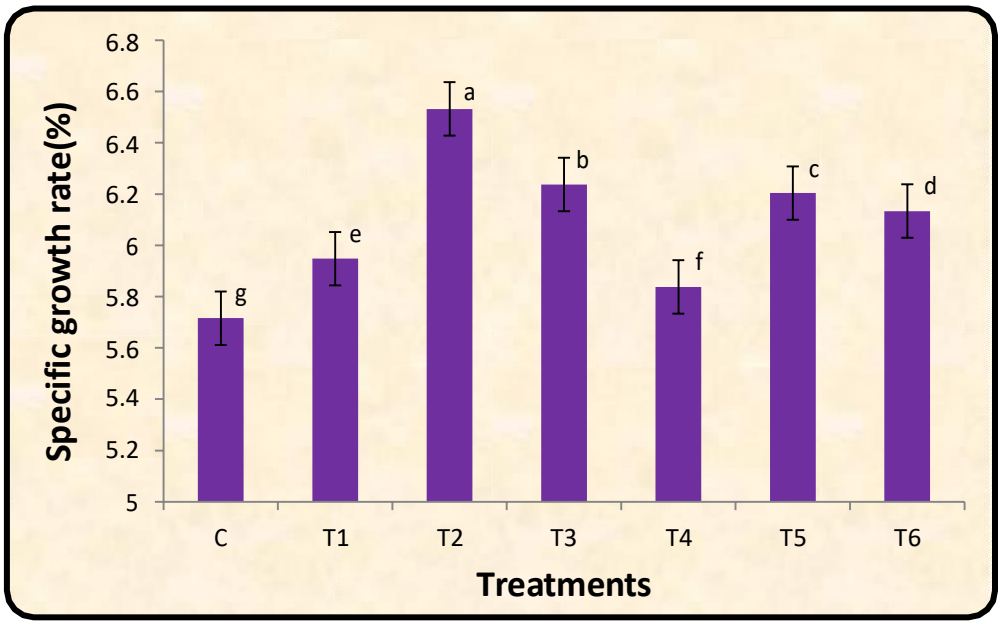


Figure 10

Specific growth rate(%) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (15 DAT). Data represents mean± SE of 15 fishes. 4.2.1.2. 30 (DAT)



Growth characteristics of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed at three different concentrations (2.5, 5 and 10 gm/kg of feed) after 30 days of treatment are presented in Table 7 and Figure 11 to 16. After 30 days, significant differences (P<0.05%) was noted in the BW of *O.mossambicus* treated with leaf extract supplemented feed (T1 –T6). Final body weight of fishes were higher (13.67±0.28 gm) in T2 group when compared to control (8.58±0.05 gm). The weight gain was maximum (6.67 ±0.28 gm) in T2 group whereas control recorded a minimum weight gain of 1.58±0.05 gm. The highest growth rate (1.63 ±3.15 gm/day) was recorded in T2 when compared to control (0.51±0.32 gm/day).Average daily growth rate was maximum (0.22±0.01 gm/day) in T2 group and minimum in control(0.05±0.02 gm/day). RGR % was significantly high (P<0.05%) (94.37±0.10%) in T2 group when compared to control (27.23±0.08%). Specific growth rate % was high (3.69±0.03%) in T2 group whereas control recorded a minimum value of 2.96±0.08%.The survival rate during the experimental period was high in all the treatments (T1 – T6) while compared to control. The survival rate was high (100%) in all treatments whereas control showed 80% survival rate.

An overall, growth parameters was significantly high (P<0.05) in *O.mossambicus* fed with of *C.aromaticus* and *O.basilicum* supplemented feed at three different concentration (2.5, 5 and 10 gm/kg of feed) when compared to control group after 30 days of treatment.

Figure 11

Final body weight (gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (30 DAT). Data represents mean± SE of 10 fishes.

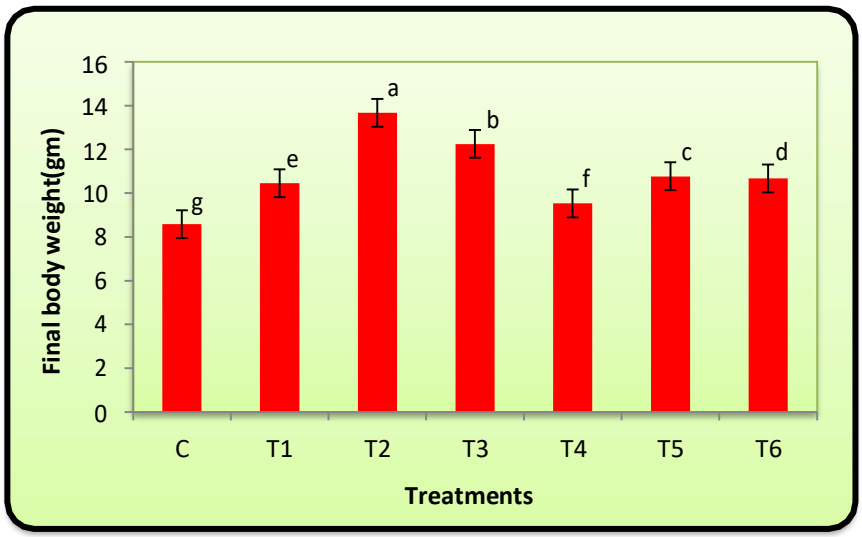


Figure 12

Body weight gain(gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (30 DAT). Data represents mean± SE of 15 fishes.

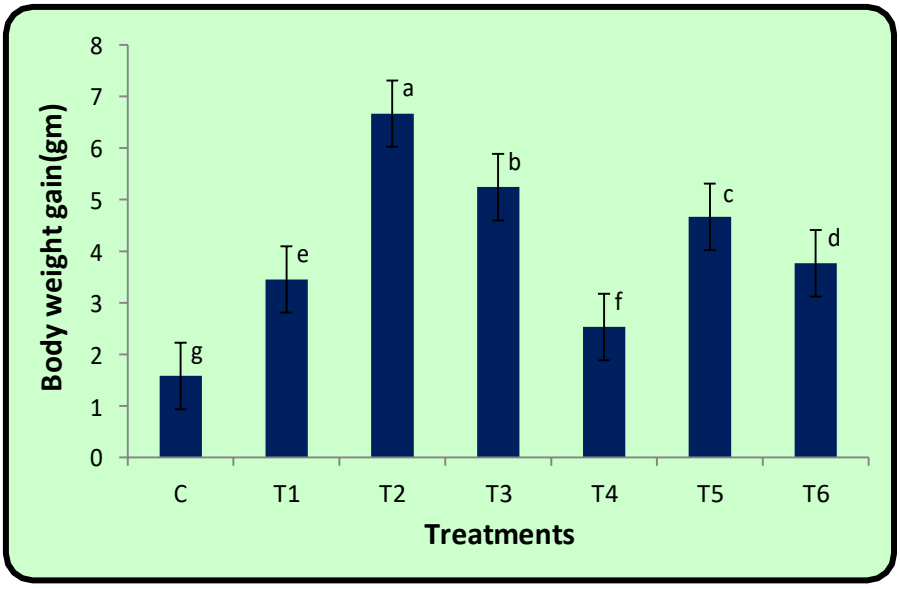


Figure 13

Growth rate (mg.day^{-1}) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (30 DAT). Data represents mean± SE of 15 fishes.

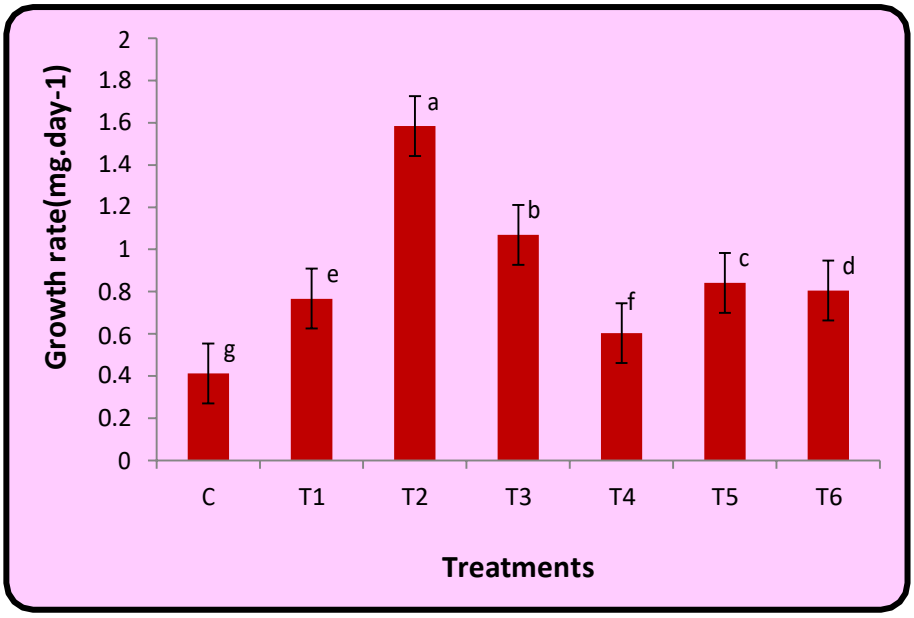


Figure 14

Average daily growth (mg/day)of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (30 DAT). Data represents mean± SE of 15 fishes.

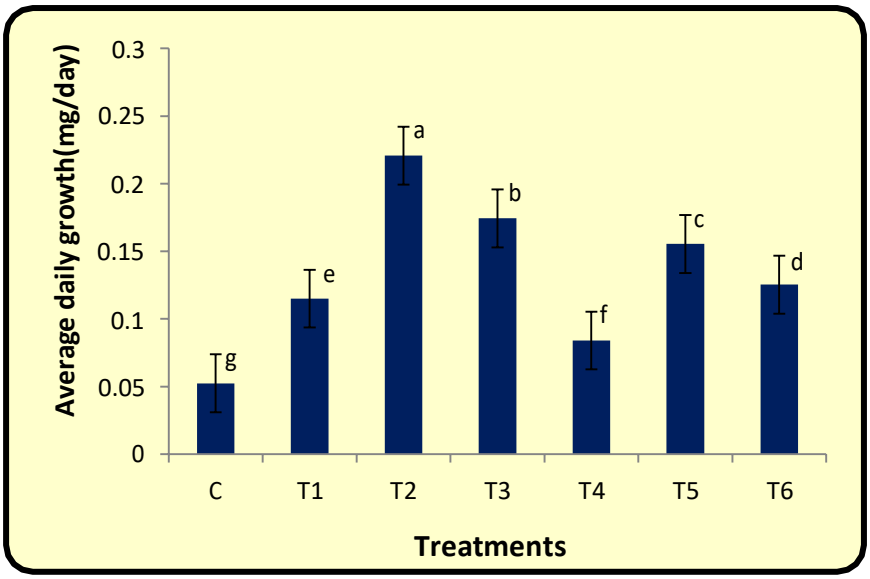


Figure 15

Relative growth rate(%) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (30 DAT). Data represents mean± SE of 15 fishes.

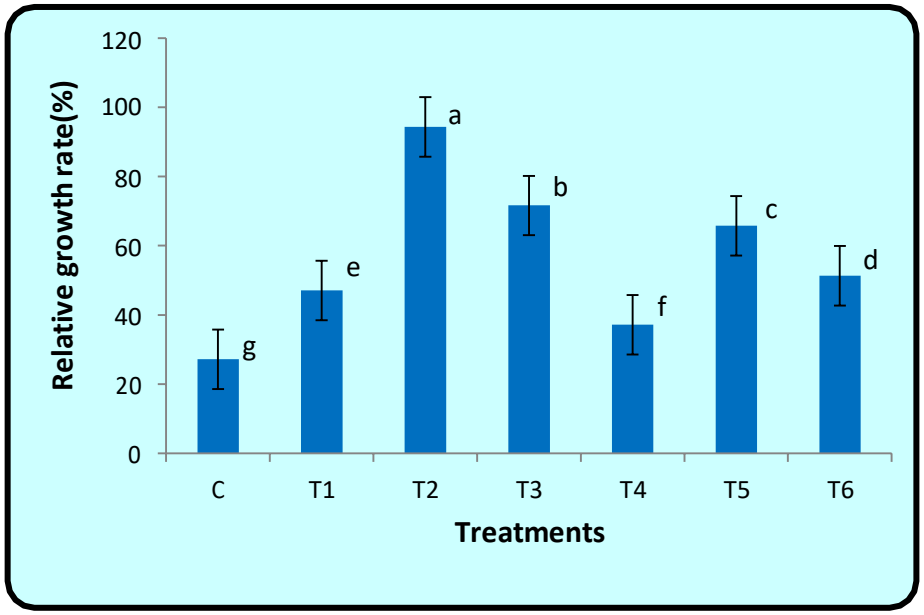
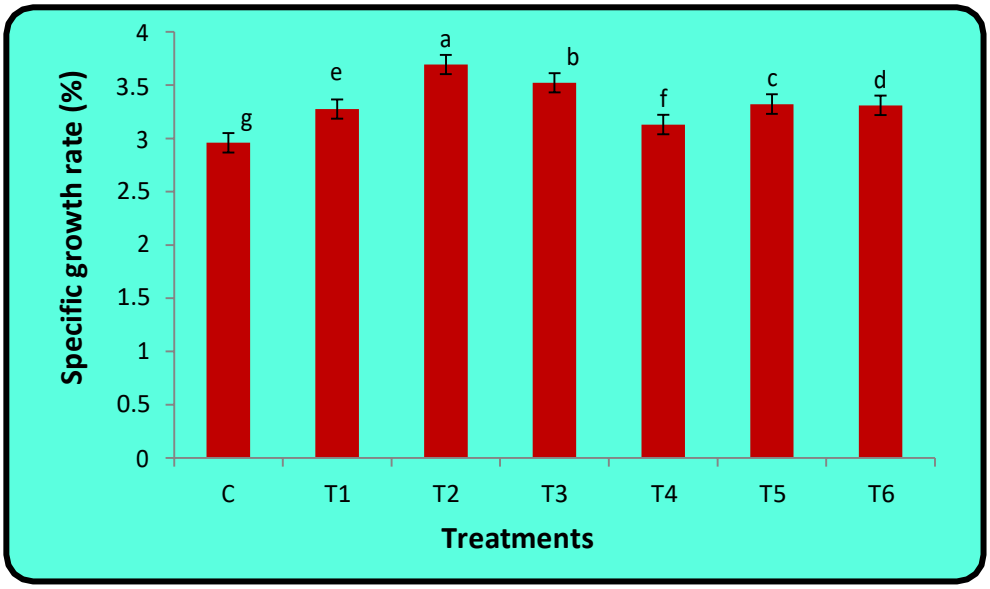


Figure .16

Specific growth rate (%) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (30 DAT). Data represents mean± SE of 15 fishes.



4.2.1.3. 45 (DAT)

Growth characteristics of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed at three different concentrations (2.5, 5 and 10 gm/kg of feed) after 45 days of treatment are presented in Table 8 and Figure 17 to 22. After 45 days, significant variations (P<0.05%) was noted in the BW of *O.mossambicus* treated with leaf extract supplemented feed (T1 –T6). Final body weight of fishes were higher (17.19±0.17gm) in T2 group when compared to control (9.65±0.06 gm). The weight gain of was maximum (10.19 ±0.17 gm) in T2 group whereas control recorded a minimum weight gain of 2.65±0.06gm. The highest growth rate (1.58 ±0.03 gm/day) was recorded in T2 when compared to control (0.41±0.01 gm/day).Average daily growth rate was maximum (0.23±0.0 gm/day) in T2 group and minimum in control(0.06±0.0 gm/day). RGR% was significantly (P<0.05%) higher (142.48±0.44%) in T2 group when compared to control (37.14±0.04%). Specific growth rate % was high (2.69±0.01%) in T2 group whereas control recorded a minimum value of 2.10±0.01%.The survival rate during the experimental period was high in all the treatments (T1 – T6) while compared to control. The survival rate was high (100%) in all treatments whereas control showed 60% survival rate.

An overall,growth parameters was significantly high (P<0.05) in *O.mossambicus* fed with of *C.aromaticus* and *O.basilicum* supplemented feed at three different concentration (2.5, 5 and 10 gm/kg of feed) when compared to control group after 45 days of treatment.

Figure 17

Final body weight (gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 10 fishes.

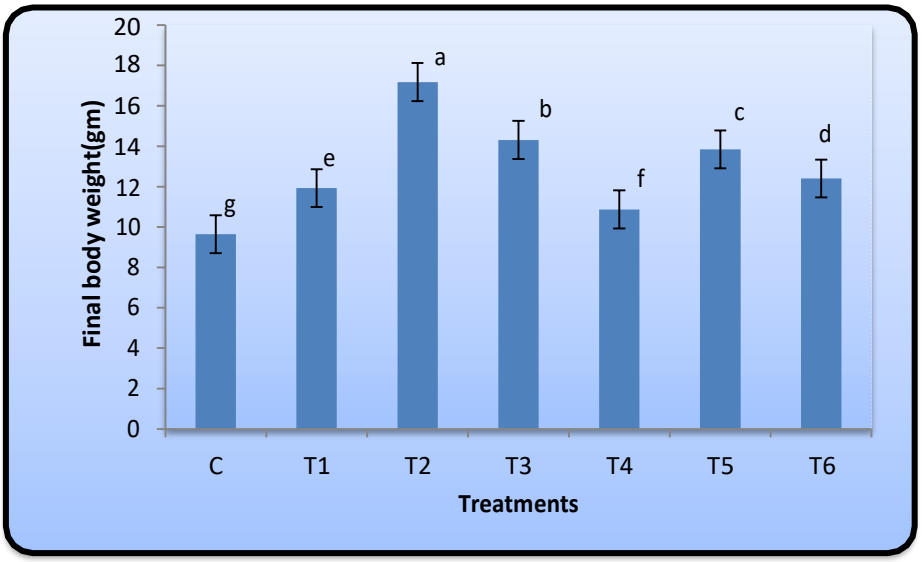


Figure 18

Body weight gain(gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.

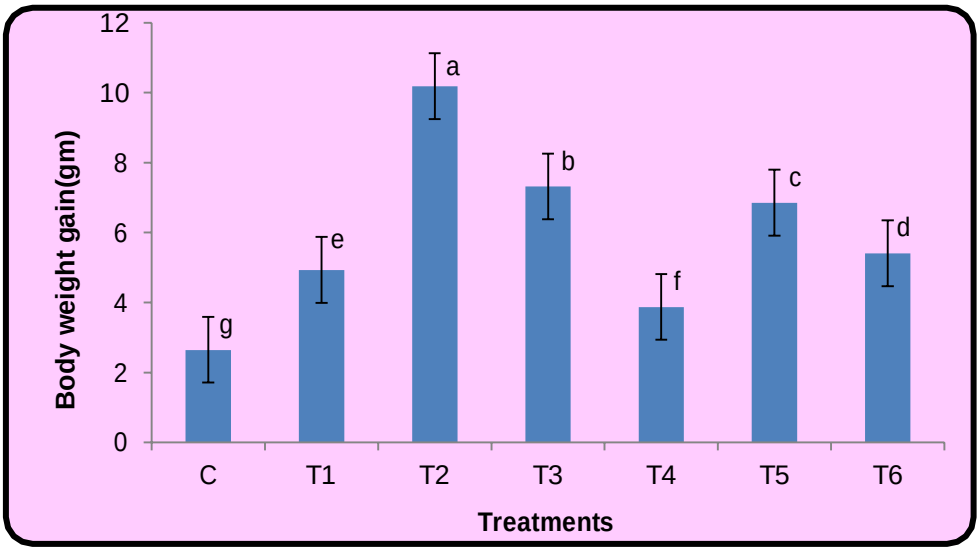


Figure 19

Growth rate (mg.day^{-1})of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.

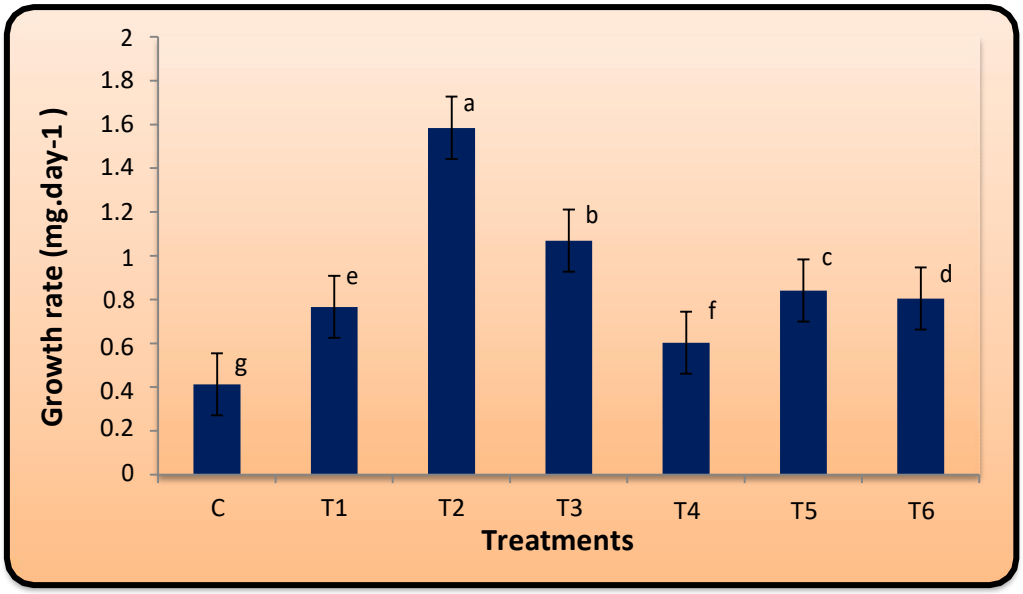


Figure 20

Average daily growth (mg/day)of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.

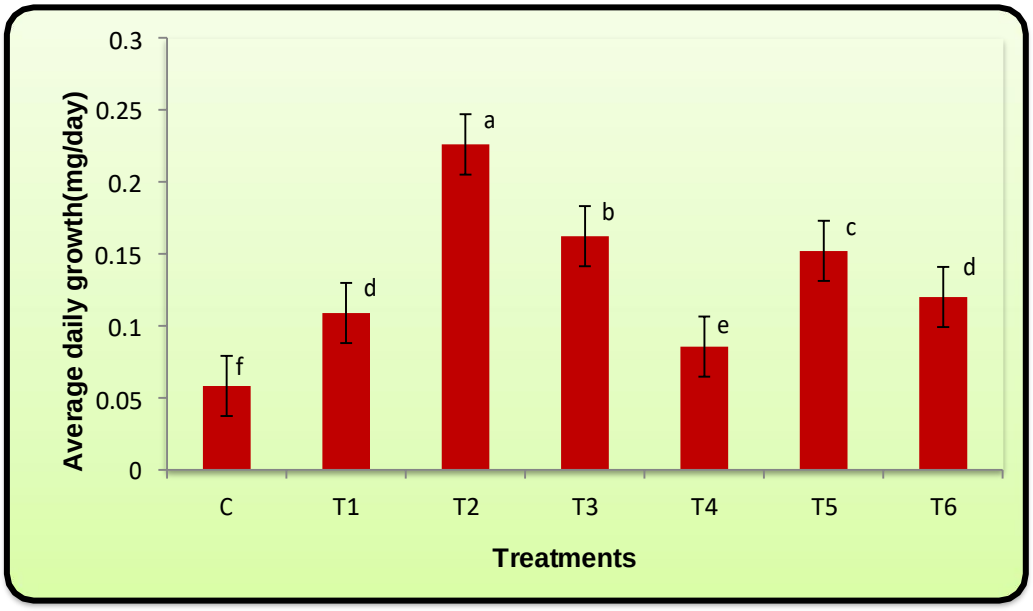


Figure 21

Relative growth rate(%) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.

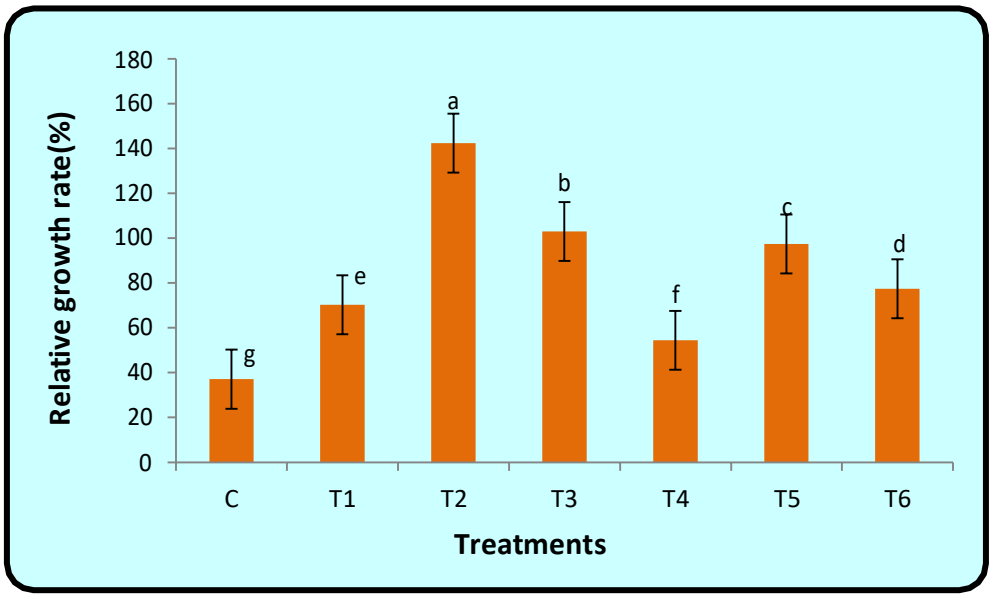
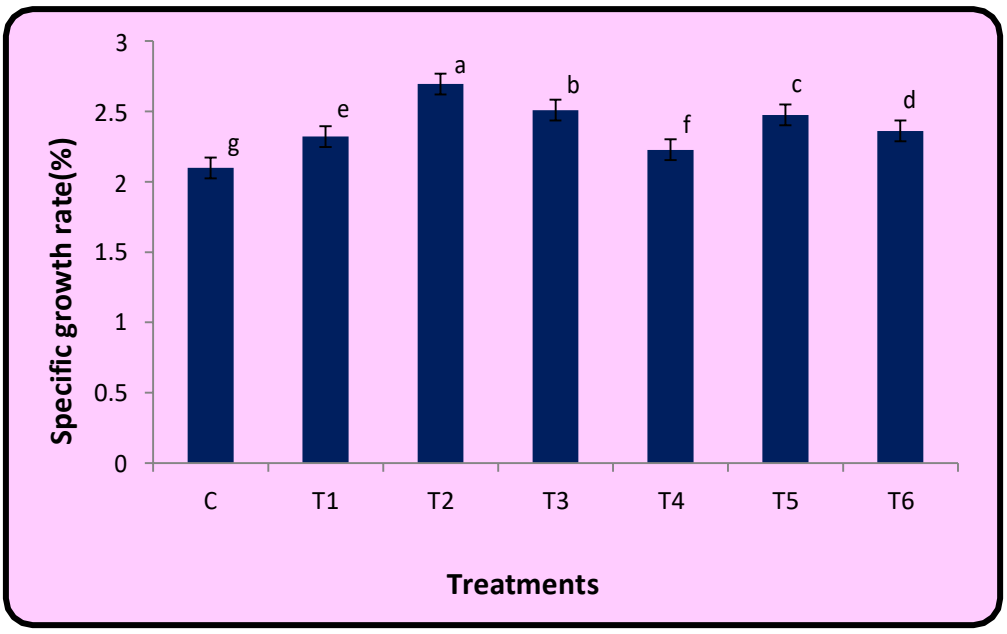


Figure 22

Specific growth rate (%) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.



In the present phase of the study, the significant increase in the growth parameters of treated fishes might reflect the influence of leaf extracts of *C.aromaticus* and *O.basilicum* as feed supplement in experimental feeds Lee and Gao,2012 reported that the initial activity of feeding was performed by herbs as flavoring agent and thereby influencing the feeding pattern, secretion of digestive enzymes and total intake of feed.

Medicinal herbs are desirable products for stimulation of digestion and had highest stimulatory influence, particularly on secretion of bile juices an enzymatic activity in pancreas (Patel *et al.,* 2002). Similarly the commercial herbal growth promotor, Livol (IHF- 1000) significantly improved the digestion of feed, which resulted in improved growth rate , production and health condition of edible fishes (Shadakshari 1993; Unnikrishnan 1995; Jayaprakas and Euphrasia 1996).Maheshappa (2002) observed that medicinal herbs incorporated with feeds stimulated the digestive enzyme activity and resulted in increased feed consumption. Frankic *et al* (2009) inferred that herbs stimulate the secretion of pancreatic enzymes, an important factor for nutrient digestion and assimilation.

Kleerekoper (1969) and Hara (1973) reported that the performance of hybrid tilapia has markedly improved by adding plant powders in their diets was due to its olfactory effect, which plays vital role in the orientation of food in fishes. Olfactory feed ingredients were found to stimulate growth by their ability to act as enhancers of feed. This pattern is similar to that observed by several researchers (Takei 1967; Harada 1990; AbouZied 1998; Sakr, 2003 and El- Dakar *et al.,* 2004) who stated that appetite is a good criterion for testing effectiveness of feed attractants.

Ashraf and Goda, 2008 stated that the growth, efficiency of feed utilization and hematology of the Nile tilapia, *Oreochromis niloticus,* were greatly enhanced by using dietary ginseng herb (Ginsana_ G115) . Dietary wood betony, *Stachy lavandulifolia* vahl extract fed to common carp showed positive effect on growth performance and some immunostimulation activities (Babrami Babaheydari *et al.,* 2014). Xie *et al.,* 2008 observed growth promotion in common carp by using Z.*officinale* extract. increased growth rate, muscle protein content and polyunsaturated fatty acids in juvenile pikeperch *(Sander lucioperca)* when fed with formulated diet containing medicinal herbs, A*stragalus radix* and *Lonicerajaponica* (Zakes *et al*.,2008)

Feed utilization efficiency

The leaf extracts of *C.aromaticus* and *O.basilicum* at three different concentrations (2.5, 5 and 10 gm/kg of feed) were given as feed supplement to *O.mossambicus* for a period of 45 days. FI, FER, FCR, PI, PER, LI and LER were noted in *O.mossambicus* in the control and six different treatments every 15 days once and the results obtained are presented in table 9 to 11 and figure - 23 to 43.

4.2.2.1. 15 (DAT)

Feed utilization efficiency of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed at three different concentrations (2.5, 5 and 10 gm/kg of feed) after 15 days of treatment are presented in Table 9 and Figure 23 to 29. After 15 days , significant variation (P<0.05%) in the feed intake of *O.mossambicus* fingerlings treated with leaf extract supplemented feed (T1 –T6). Feed intake of fishes was higher (3.70±0.27gm) in T2 group when compared to control (3.26±0.05 gm). Feed efficiency ratio was high (0.95 ±0.08 gm) in T2 group and low (1.05±0.05 gm) in control fishes. Feed conversion ratio was maximum (3.40± 0.12 gm) in control fishes and minimum (1.15± 0.04) in T2 group of fishes. Protein intake of fishes was high (1.32±0.07gm) in T2 group when compared to control (1.19±0.03 gm). Protein efficiency ratio was maximum (2.37 ±0.05 gm) in T2 group and minimum (0.88±0.04 gm) in control fishes. Lipid intake was high (24.49±0.32gm) in T2 group of fishes and low (23.34±0.11gm) in control. Lipid efficiency ratio was maximum (0.14±0.01 gm) in T2 fishes and minimum (0.05±0.01 gm) in control fishes.

An overall, feed utilization efficiencies, was significantly high (P<0.05) in *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed at three different concentration (2.5, 5 and 10 gm/kg of feed) when compared to control group after 15 days of treatment.

Figure 23

Feed intake (gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (15 DAT). Data represents mean± SE of 15 fishes.

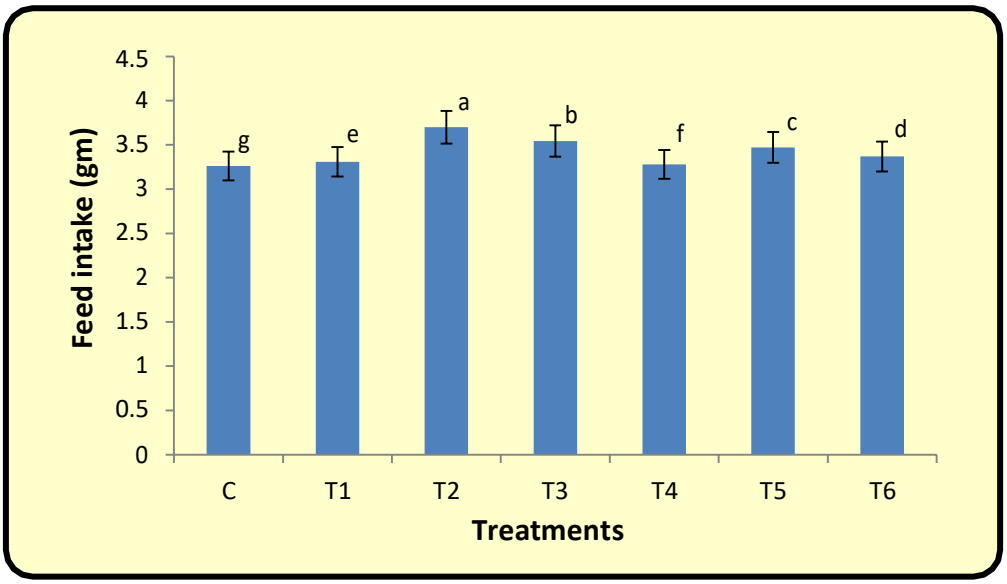


Figure 24

Feed conversion ratio (gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (15 DAT). Data represents mean± SE of 15 fishes.

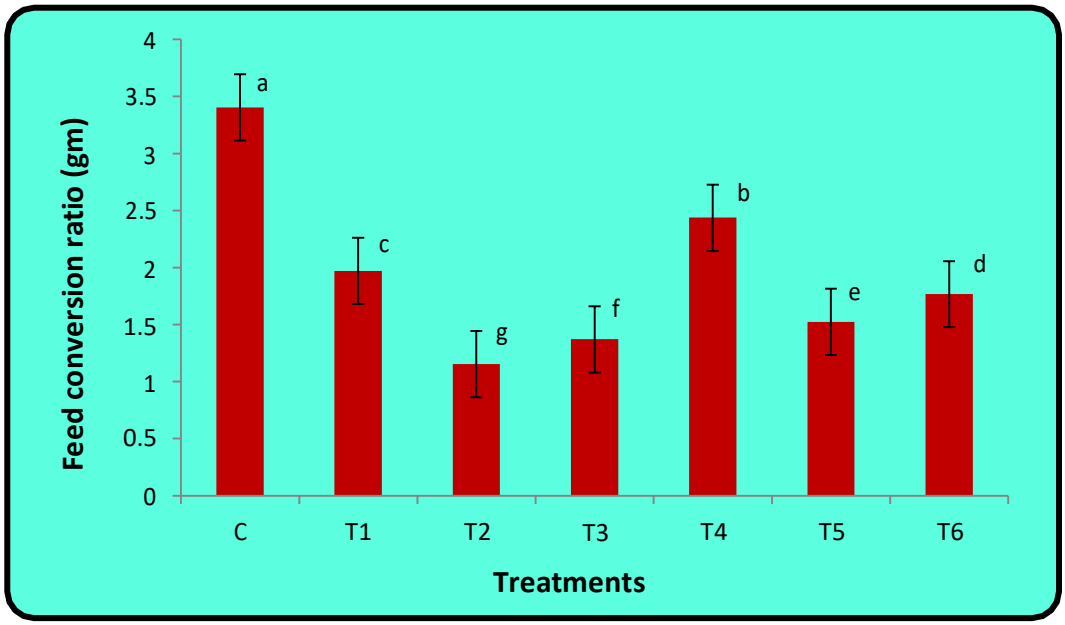


Figure 25

Feed efficiency ratio(gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (15 DAT). Data represents mean± SE of 15 fishes.

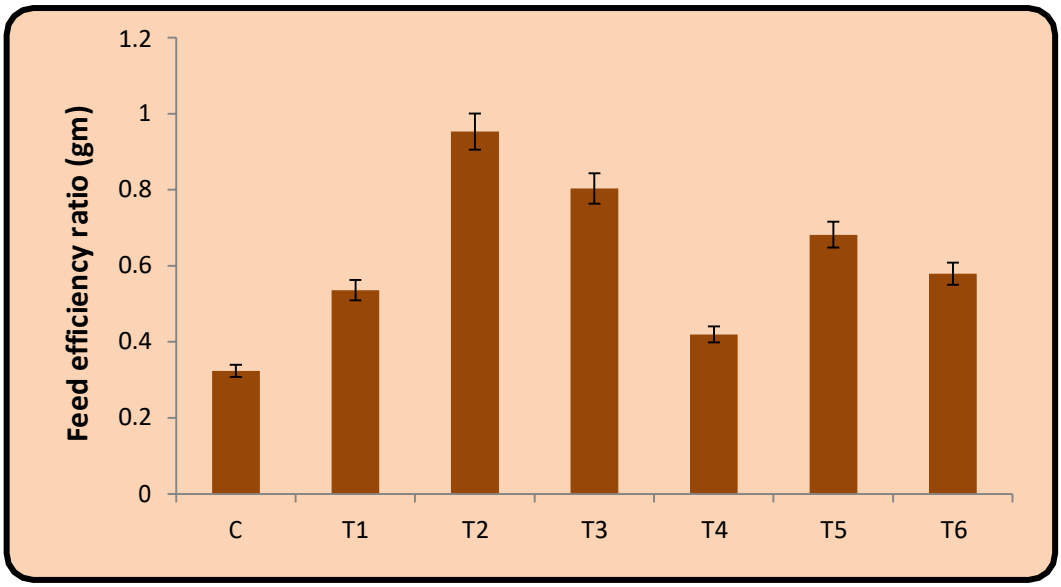


Figure 26

Protein intake (gm%) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (15 DAT). Data represents mean± SE of 15 fishes.

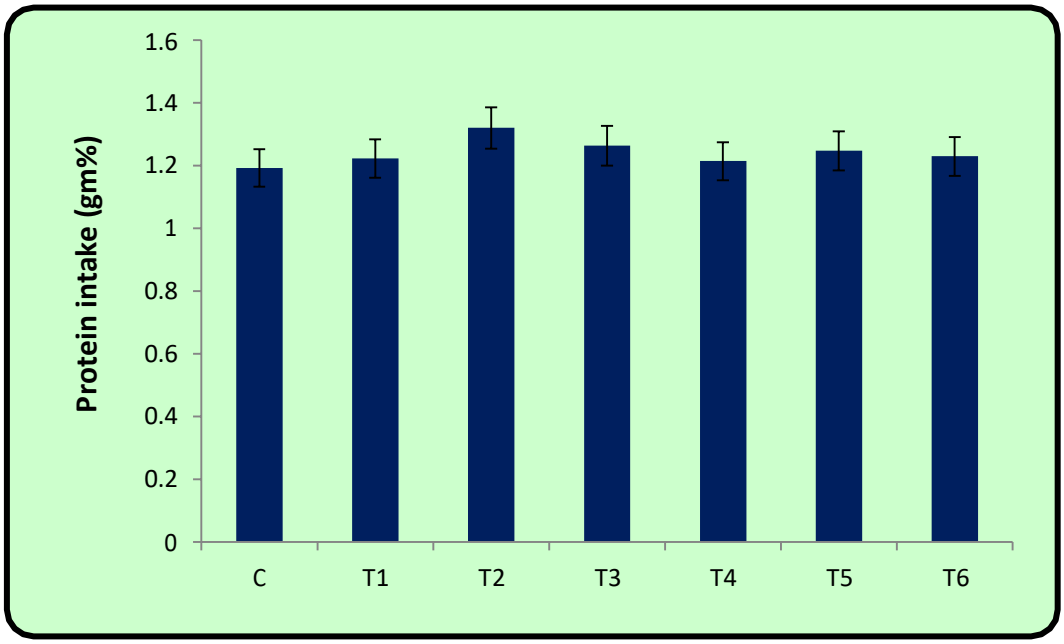


Figure 27

Protein efficiency ratio (gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (15 DAT). Data represents mean± SE of 15 fishes.

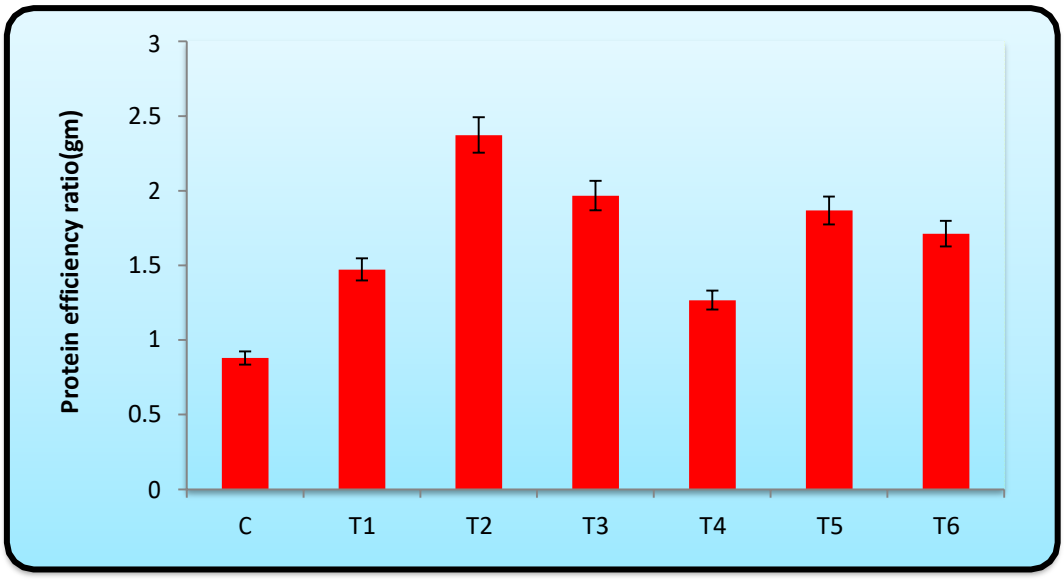


Figure 28

Lipid intake (gm%) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (15 DAT). Data represents mean± SE of 15 fishes.

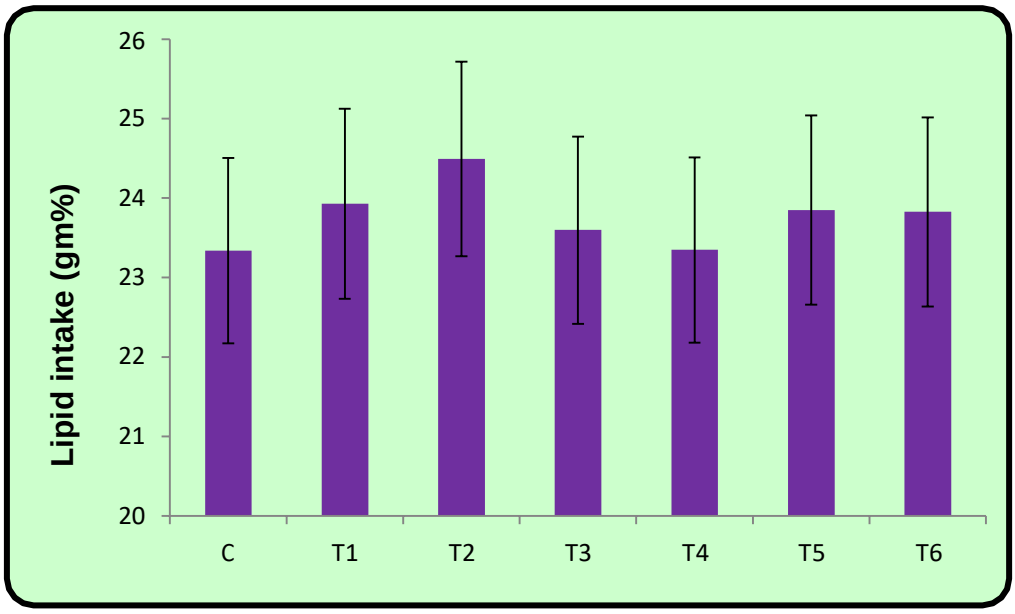
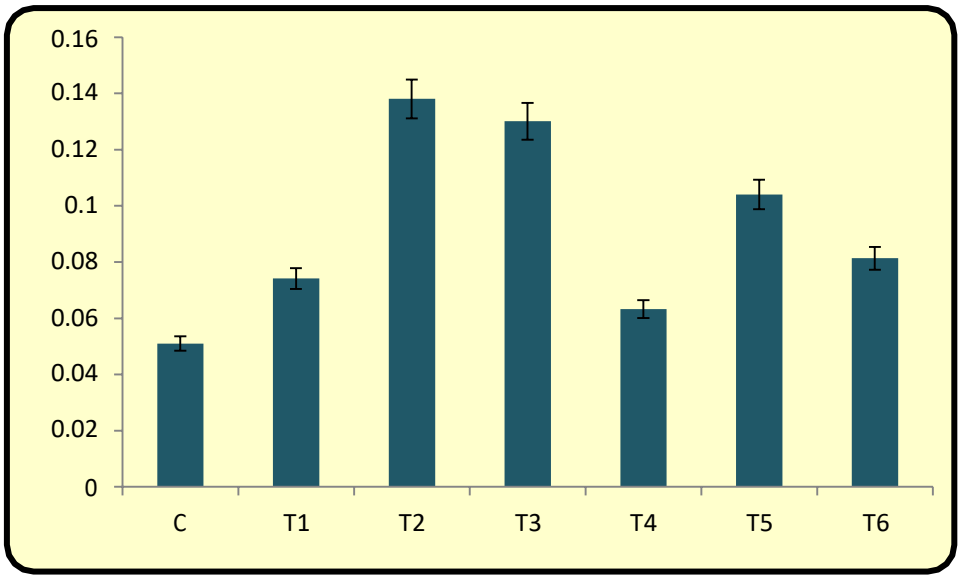


Figure 29

Lipid efficiency ratio (gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (15 DAT). Data represents mean± SE of 15 fishes.



4.2.2.2. 30 (DAT)

Feed utilization efficiency of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed at three different concentrations (2.5, 5 and 10 gm/kg of feed) after 15 days of treatment are presented in Table 10 and Figure 30 to 36. After 30 days , significant variation (P<0.05%) in the feed intake of *O.mossambicus* fingerlings treated with leaf extract supplemented feed (T1 –T6). Feed intake of fishes was higher (4.85±0.19gm) in T2 group when compared to control (3.74±0.24gm). Feed efficiency ratio was high (1.31 ±0.04 gm) in T2 group and low (0.54±0.04 gm) in control fishes. Feed conversion ratio was maximum (2.28± 0.43 gm) in control fishes and minimum (0.83± 0.07) in T2 group of fishes. Protein intake of fishes was high (1.85±0.07gm) in T2 group when compared to control (1.31±0.04gm). Protein efficiency ratio was maximum (3.73 ±0.15 gm) in T2 group and minimum (1.43 ±0.04 gm) in control fishes. Lipid intake was high (34.76 ±0.12gm) in T2 group of fishes and low (26.69±0.11gm) in control. Lipid efficiency ratio was maximum (0.21 ±0.02 gm) in T2 fishes and minimum (0.08±0.01 gm) in control fishes.

An overall feed utilization efficiencies was significantly high (P<0.05) in *O.mossambicus* fed with of *C.aromaticus* and *O.basilicum* supplemented feed at three different concentration (2.5, 5 and 10 gm/kg of feed) when compared to control group after 30 days of treatment.

Figure 30

Feed intake (gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (30 DAT). Data represents mean± SE of 15 fishes.

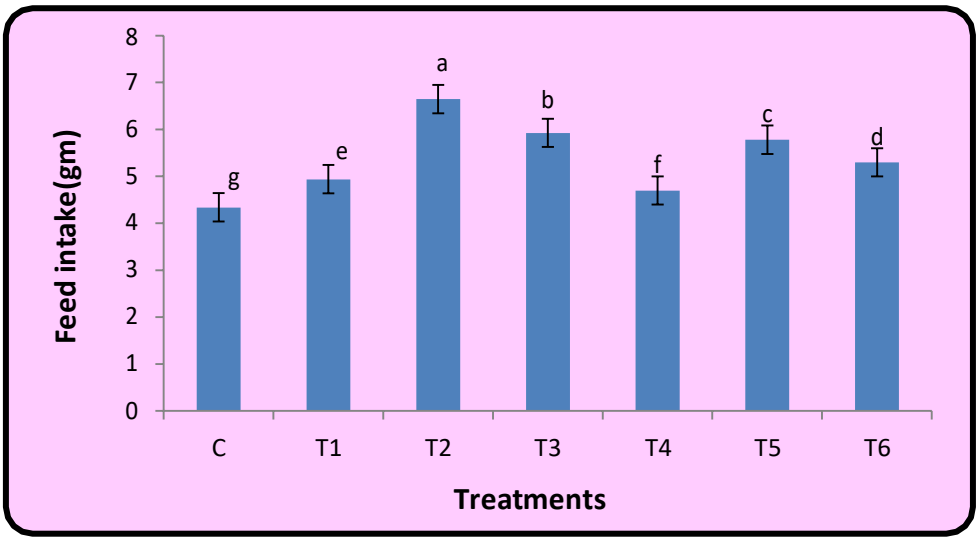


Figure .31

Feed conversion ratio(gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (30 DAT). Data represents mean± SE of 15 fishes.

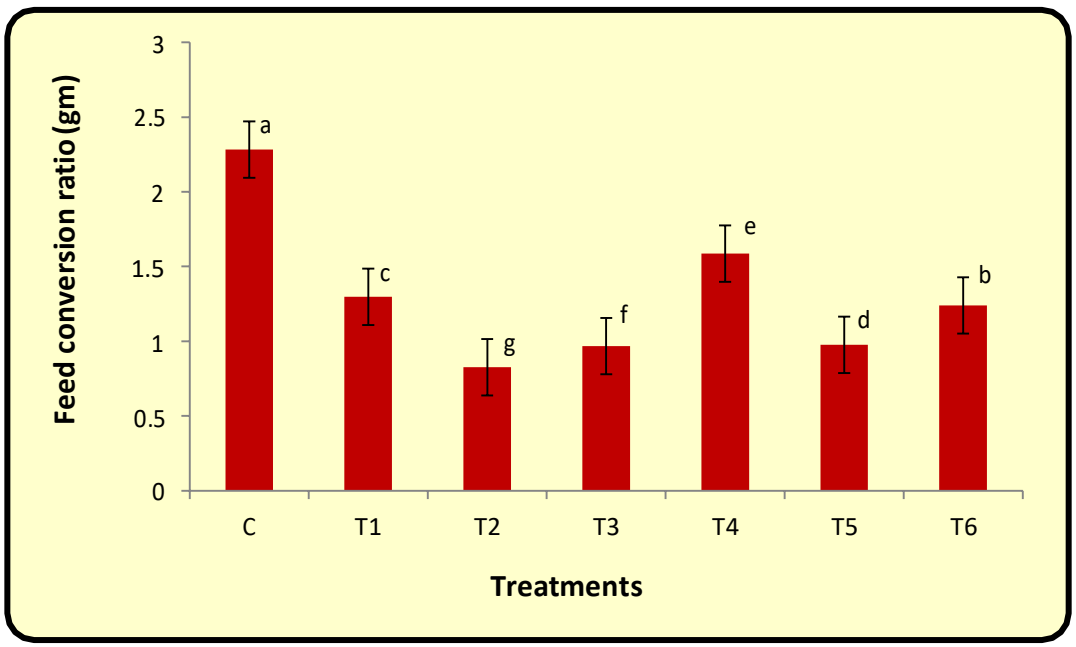


Figure 32

Feed efficiency ratio(gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (30 DAT). Data represents mean± SE of 15 fishes.

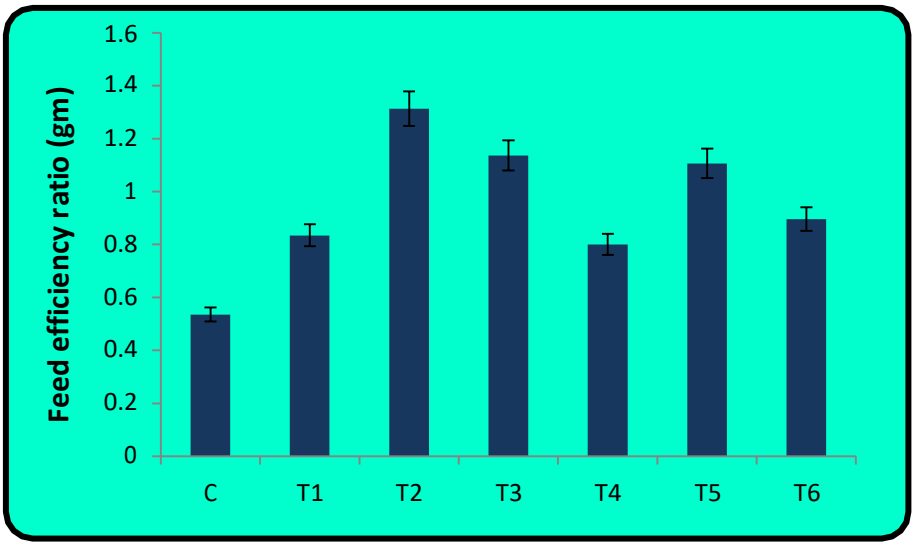


Figure 33

Protein intake (gm%) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (30 DAT). Data represents mean± SE of 15 fishes.

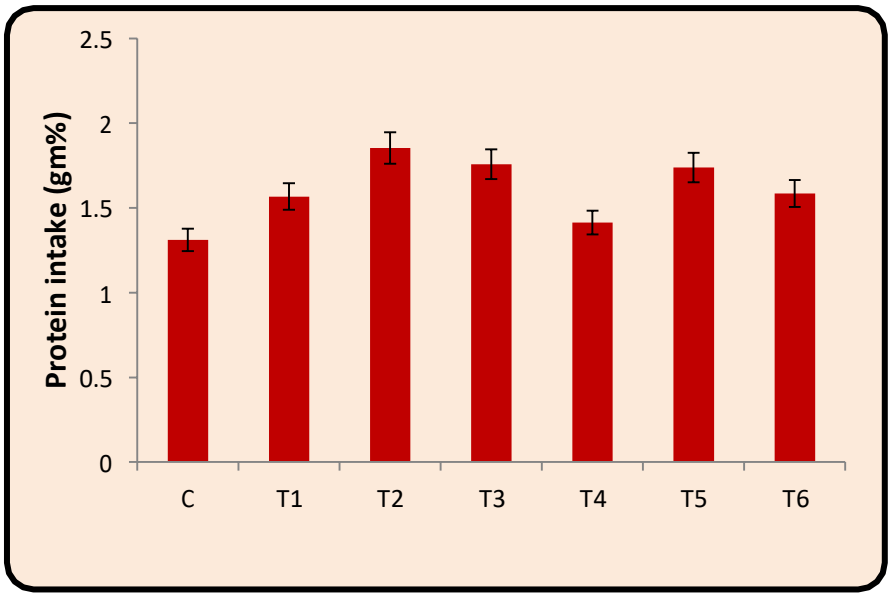


Figure 34

Protein efficiency ratio (gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (30 DAT). Data represents mean± SE of 15 fishes.

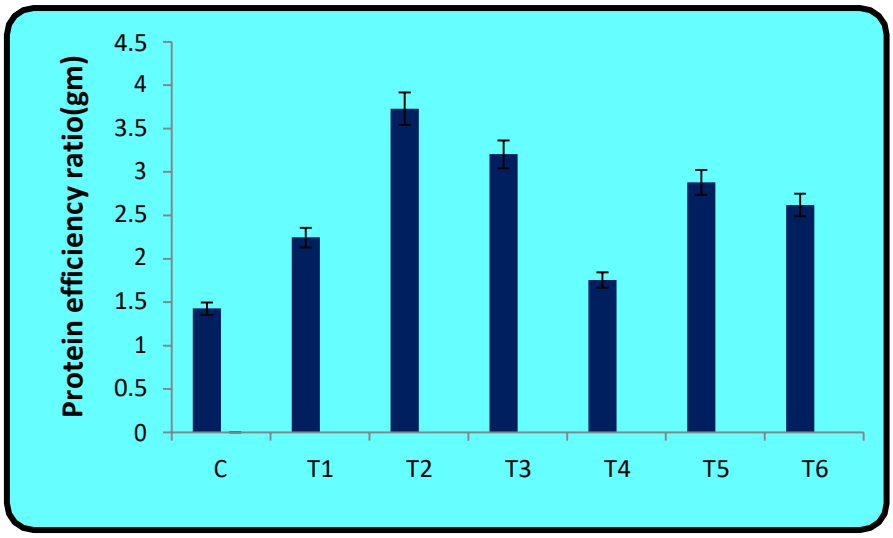


Figure 35

Lipid intake (gm%) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (30 DAT). Data represents mean± SE of 15 fishes.

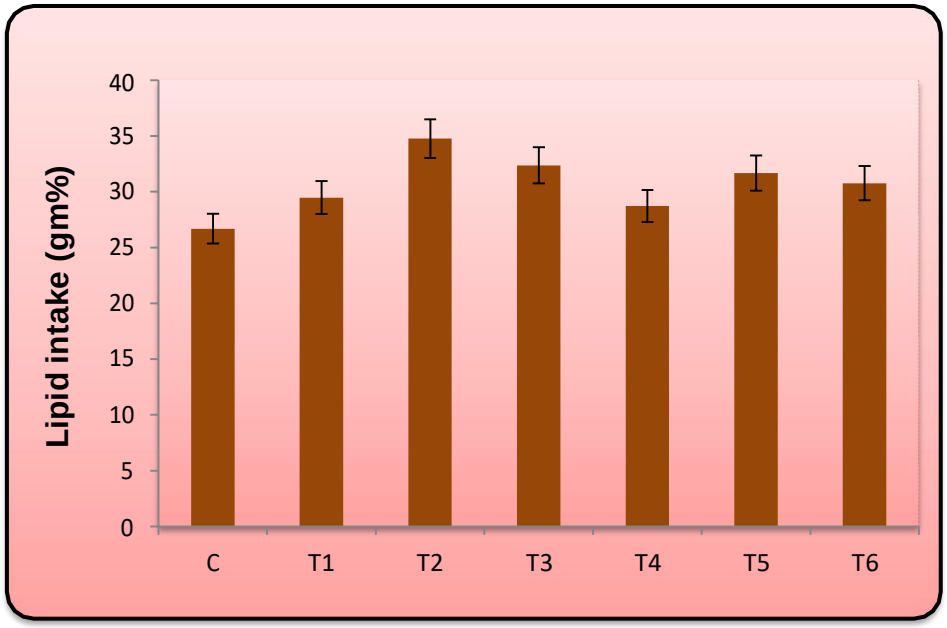
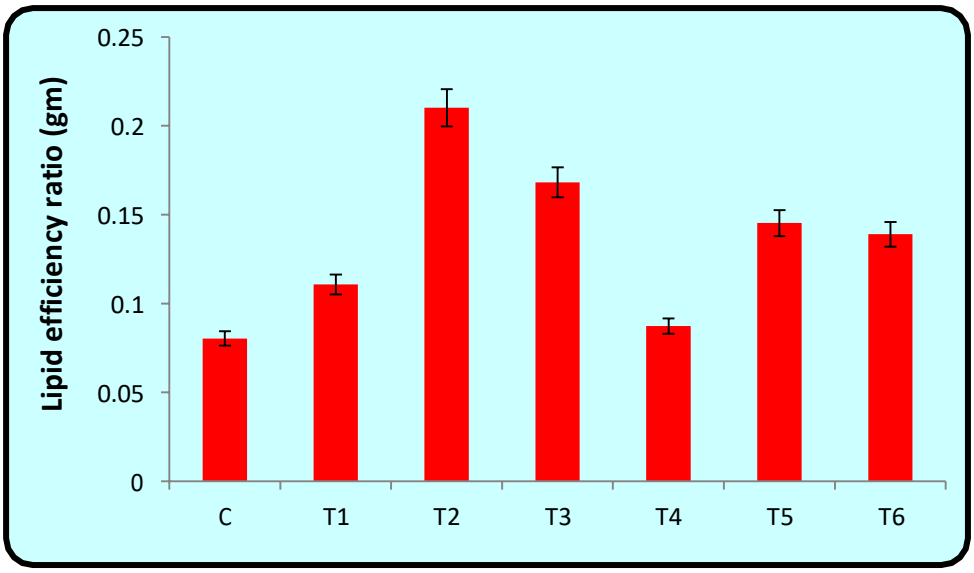


Figure 36

Lipid efficiency ratio (gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (30 DAT). Data represents mean± SE of 15 fishes.



4.2.2.3. 45 (DAT)

Feed utilization efficiency of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed at three different concentrations (2.5, 5 and 10 gm/kg of feed) after 45 days of treatment are presented in Table 11 and Figure 37 to 43 . After 45 days , significant variation (P<0.05%) in the feed intake of *O.mossambicus* fingerlings treated with leaf extract supplemented feed (T1 –T6). Feed intake of fishes was higher (6.64±0.11gm) in T2 group when compared to control (4.34±0.08 gm). Feed efficiency ratio was high (1.53 ±0.06 gm) in T2 group and low (0.83±0.30 gm) in control fishes. Feed conversion ratio was maximum (1.66±0.03 gm) in control fishes and minimum (0.77± 0.09) in T2 group of fishes. Protein intake of fishes was high (2.55±0.12gm) in T2 group when compared to control (1.51±0.03 gm). Protein efficiency ratio was maximum (4.24 ±0.13 gm) in T2 group and minimum (1.83±0.14 gm) in control fishes. Lipid intake was high (47.39±0.16 gm) in T2 group of fishes and low (29.85 ±0.11gm) in control. Lipid efficiency ratio was maximum (0.24±0.22 gm) in T2 fishes and minimum (0.14±0.11 gm) in control fishes.

An overall, feed utilization efficiencies, was significantly high (P<0.05) in *O.mossambicus* fed with of *C.aromaticus* and *O.basilicum* supplemented feed at three different concentration (2.5, 5 and 10 gm/kg of feed) when compared to control group after 45 days of treatment.

Figure 37

Feed intake (gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.

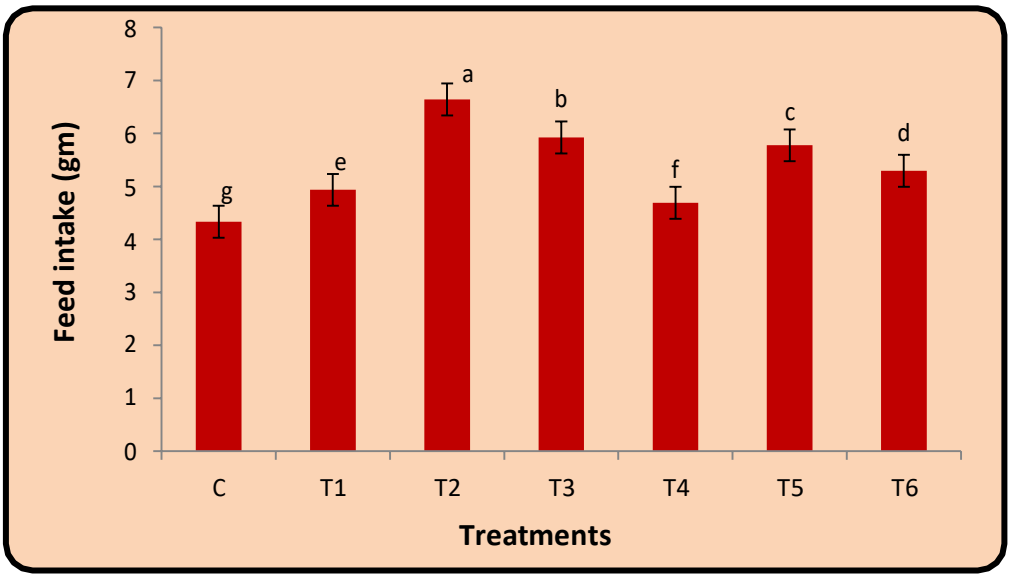


Figure 38

Feed conversion ratio(gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.

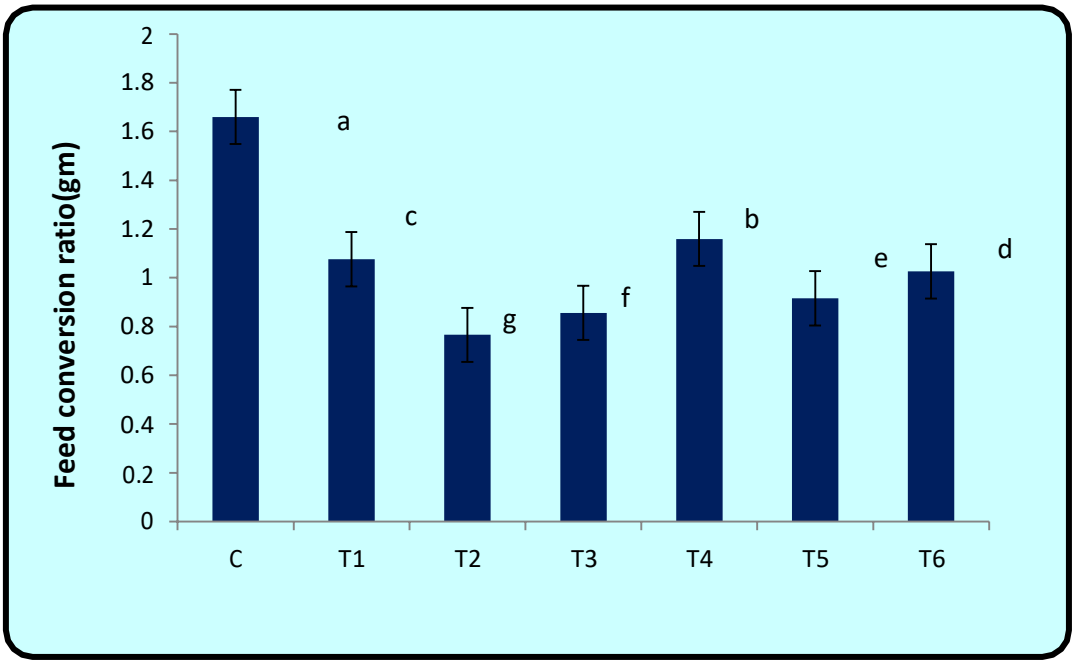


Figure 39

Feed efficiency ratio(gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.

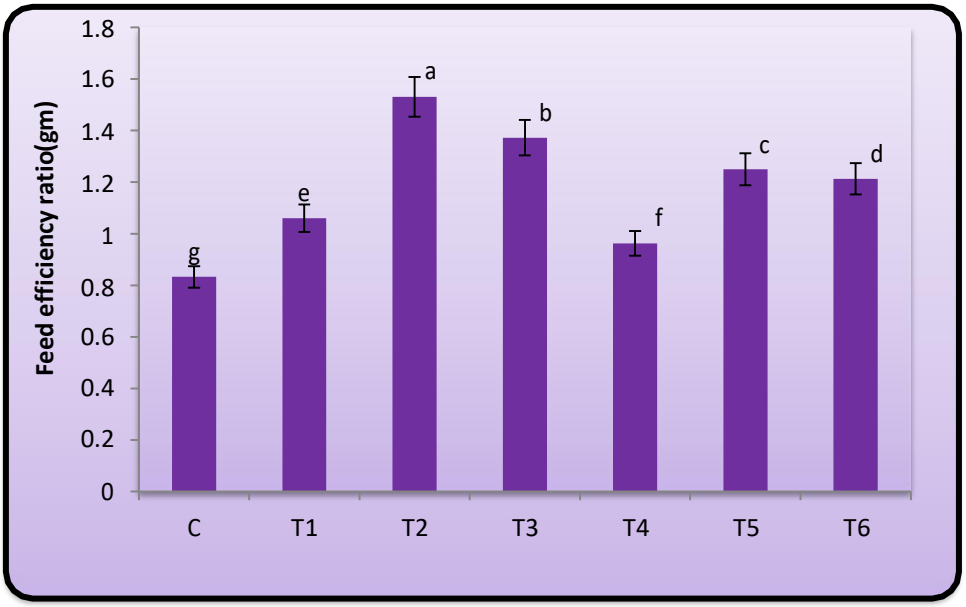


Figure 40

Protein intake (gm%) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.

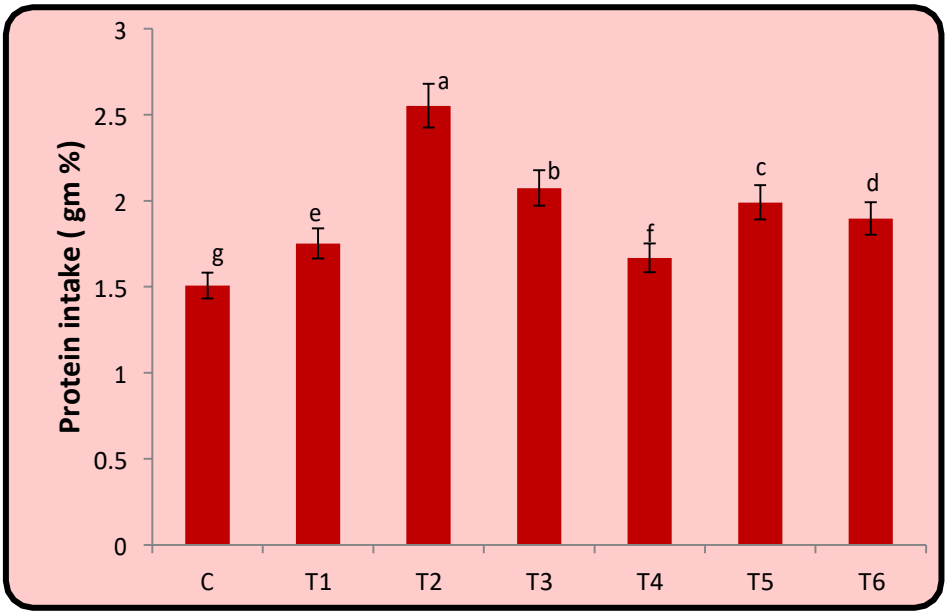


Figure 41

Protein efficiency ratio (gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.

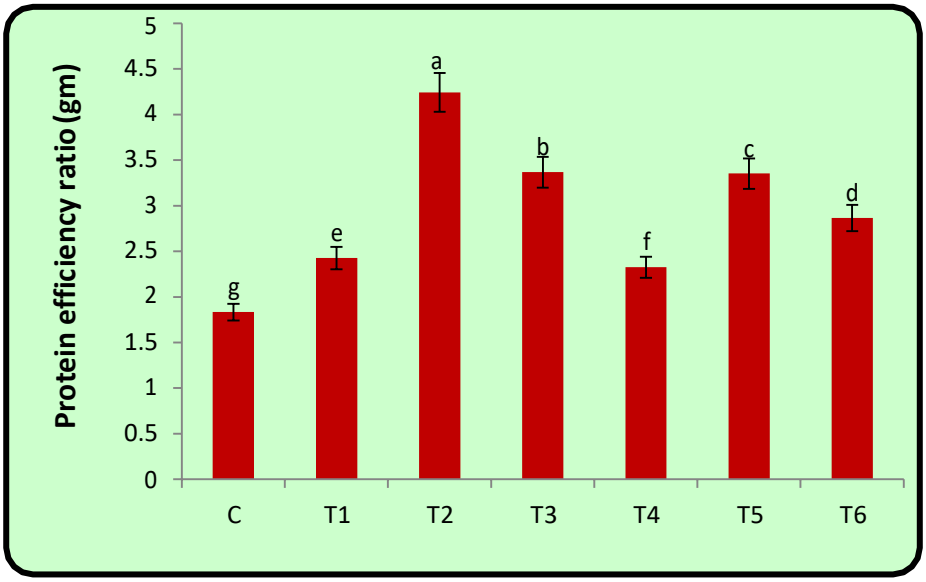


Figure 42

Lipid intake (gm%) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.

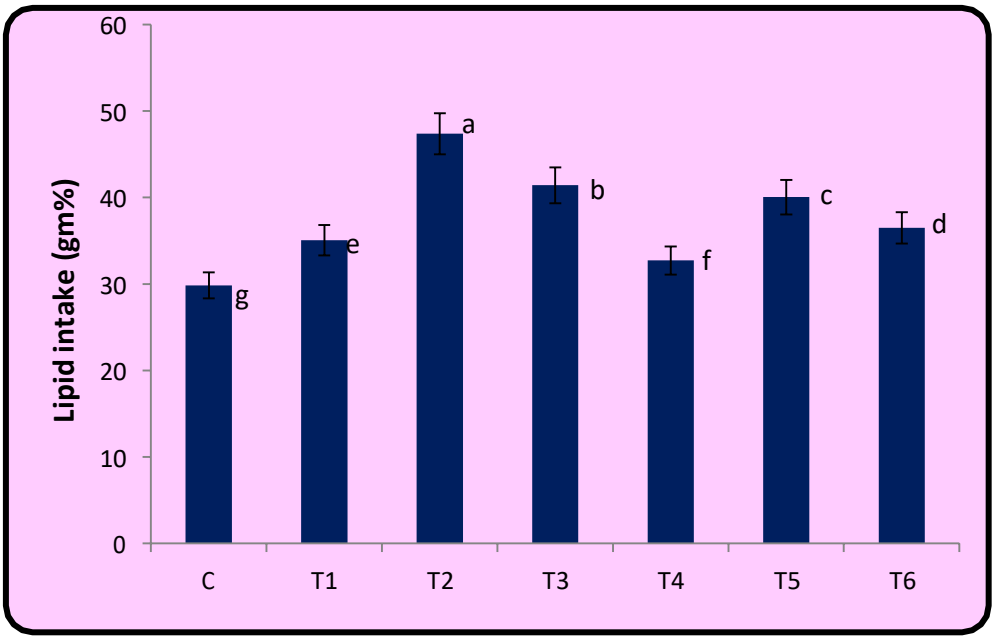
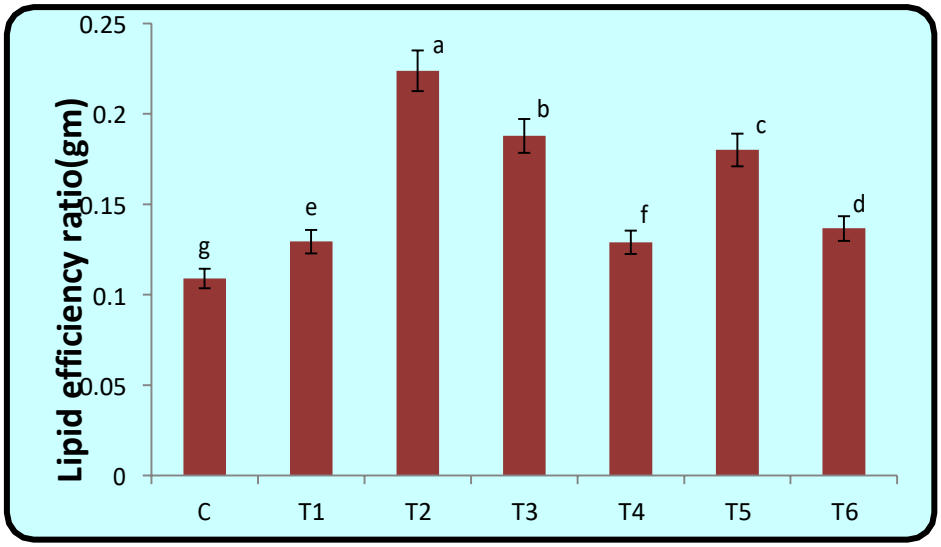


Figure 43

Lipid efficiency ratio (gm) of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.



The improved feed intake and utilization efficiencies of *O.mossambicus* might be due to the influence of *C.aromaticus* and *O.basilicum* in stimulating secretion and activity of enzymes and resulted in increased metabolism. Medicinal herbs supplementation improved the activity of digestive enzymes such as protease and amylase in hepatopancrease and intestines, which led to enhance digestion and absorption of feed, this in turn could contribute to the improved growth in *L.vannamei* (Yu *et al.*, 2008).

Citrarasu, 2010 reported that extracts from herbs and spices have the ability to improve the performance of animals by stimulating the secretion in the gut region or by showing its bactericidal action directly on the micro flora of gut and furthermore the herbal bioactive compounds in the diets stimulate the secretion of the digestive enzymes and protein synthesis .

Herbal growth promoter Livol (IHF-1000) significantly improved the digestion of feed which resulted in increased growth rate, production and health condition of edible fishes (Jayaprakas and Euphrasia 1996). Maheshappa (1993) stated that, the feed incorporated with Livol stimulated the activity of digestive enzymes which led to increased consumption in *L. rohita* .Ceulemans *et al.*, 2009 reported that the dietary supplementation in Nile tilapia, with a mixture of plants extracts, with digestive properties, natural emulsifying agents and co-factors of digestion, generated a higher feed conversion ratio and protein efficiency.

Proximate composition of *O.mossambicus*

The leaf extracts of *C.aromaticus* and *O.basilicum* at three different concentrations (2.5, 5 and 10 gm/kg of feed) were given as feed supplement to *O.mossambicus* for 45 days and the proximate composition such as moisture, protein, carbohydrate, fat and ash content were analyzed initially before the experiment and after 45 days of treatment. The data obtained for proximate composition of *O.mossambicus* are tabulated in table 12 and illustrated in figure 44 to 48.

After 45 days of treatment , significant variation (P<0.05) in the proximate composition of *O.mossambicus* treated with *C.aromaticus* and *O.basilicum* supplemented feed at three different concentrations (2.5, 5 and 10 gm/kg of feed). The moisture content (72.55±0.45%),protein content (21.02±0.54%),carbohydrate content (7.71± 0.20%), fat content (5.82±0.37%) and ash content (3.49±0.43%) was high in T2 fishes and low (moisture- 70.5±0.5%,protein – 12.76±0.41%, carbohydrate – 4.94±0.21%,fat – 3.49±0.53%,ash – 2.32±0.11%) in control fishes.

An overall, increase in the proximate composition was significantly high (P<0.05) in *O.mossambicus* fed with of *C.aromaticus* and *O.basilicum* supplemented feed at three different concentration (2.5, 5 and 10 gm/kg of feed) when compared to control group after 45 days of treatment.

Figure .44

Moisture content in the muscle of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.

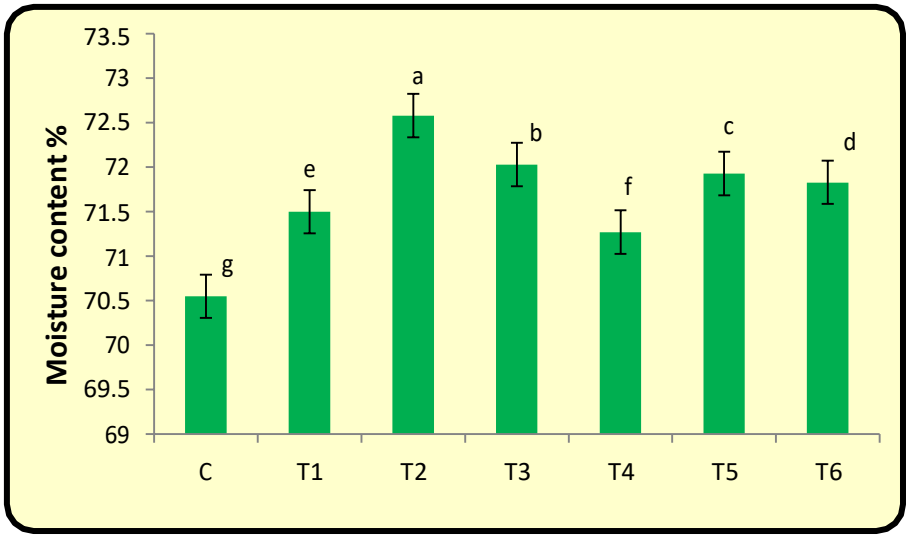


Figure 45

Protein content in the muscle of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.

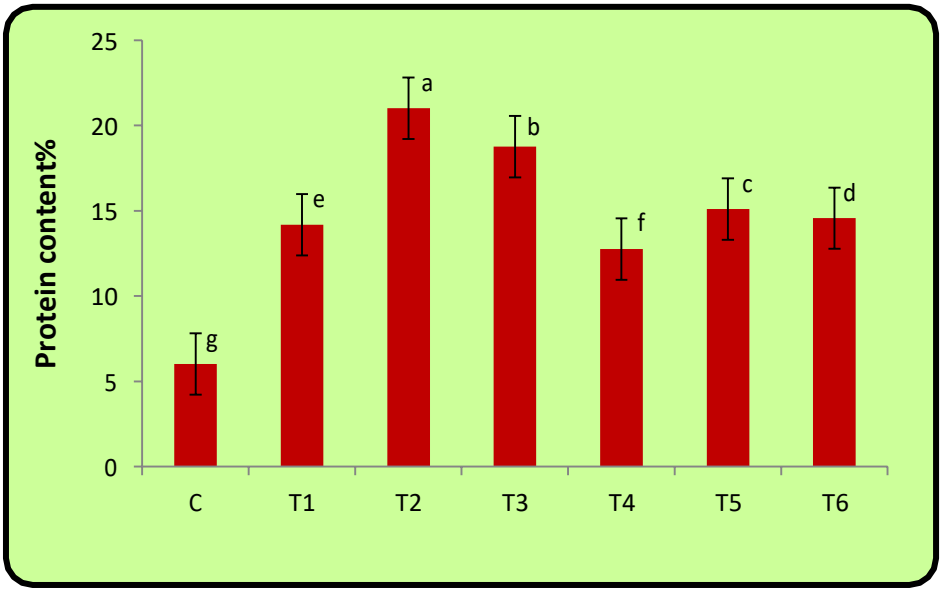


Figure 46

Carbohydrate content in the muscle of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.

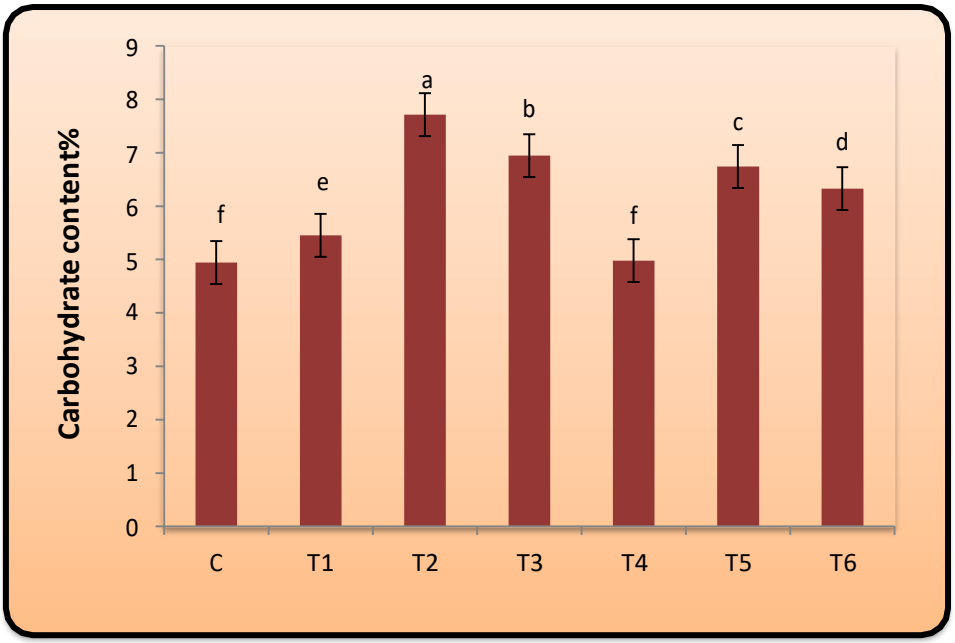


Figure 47

Fat content in the muscle of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 10 fishes.

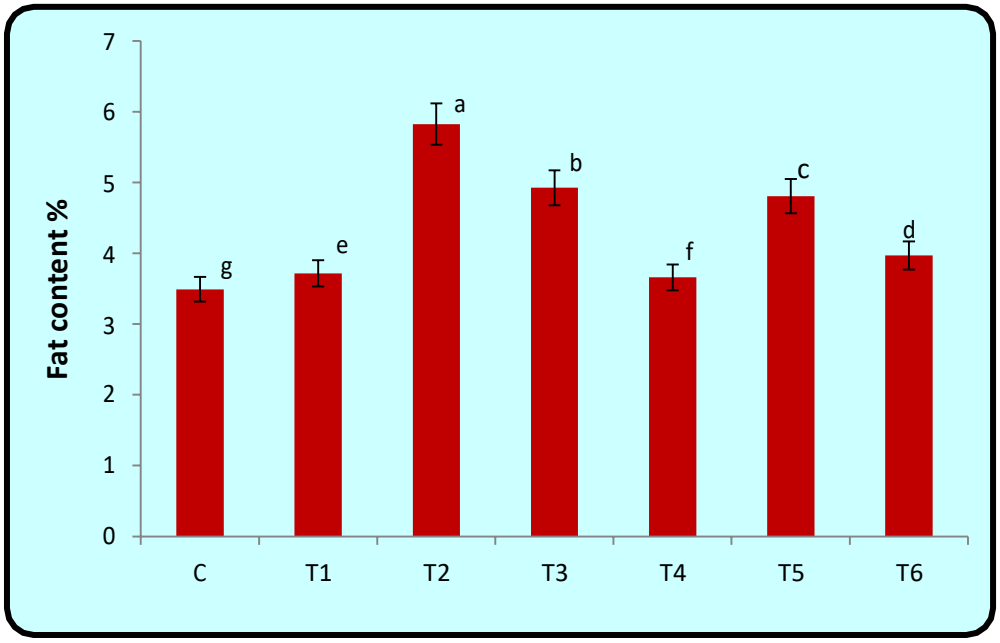
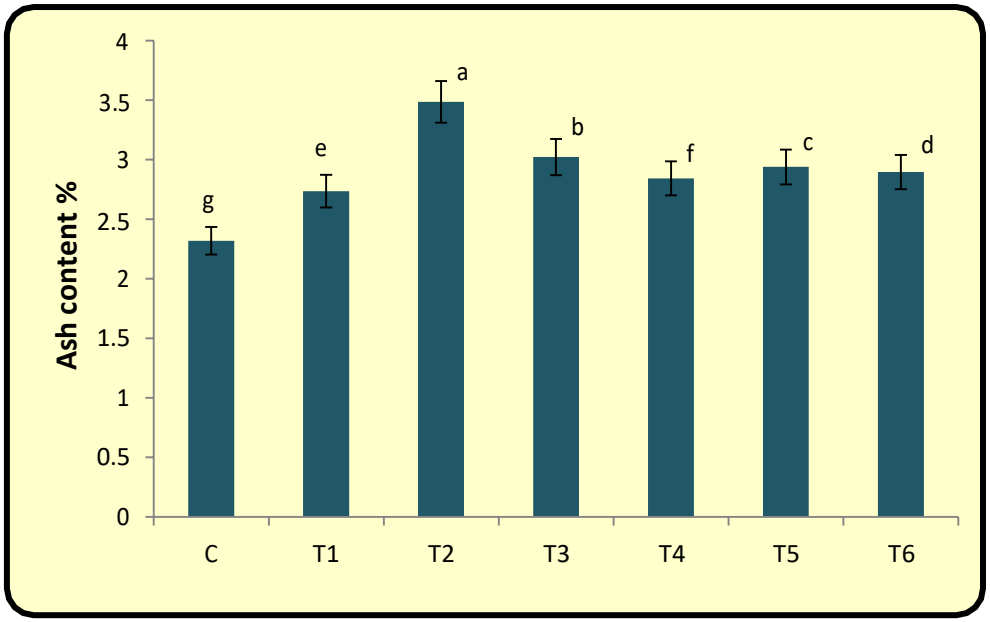


Figure 48

Ash content in the muscle of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 10 fishes.



Increase in the proximate composition might be due to influence of *C.aromaticus* and *O.basilicum* in the supplemented feed enhanced the enzyme activity which positively influenced the protein, carbohydrate and fat digestion and absorbtion resulted in increased feed utilization and muscle proximate composition in *O.mossambicus* .

The percentage of moisture content in the fish is a good indicator of the relative energy, protein and fat. (Aberoumad and Pourshafi, 2010; Barua *et al.,* 2012). The proportion of moisture content in fish vary widely between 65-90%. The normal range of moisture content is 70- 75%.

Citarasu (2010) stated that increased protein content in the muscle of fish might be due to the presence of active principles of *P.niruri*. Zheng *et al*., 2009 reported that increased protein content in the muscle of channel catfish (*Ictalurus punctatus*) supplemented with Greek Marjoram (*Origanum heracleoticum*). It also agrees with the results obtained by Khattab *et al.,* (2004), and Shalaby *et al* (2006), who reported that addition of Biogen in the feed increased fish protein content. Narendra *et al*., 2012 stated that dietary supplementation of *P.niruri*, increased the carbohydrate content in fish which might indicate the unstressful condition and good nutritive value of herbs in the diet.

Maryam Kamgar *et al.*, 2013 stated that the increase in total lipid has been attributed to enhanced biochemical processes in the digestive tract and enhanced the absorption and assimilation of the ingested diet. Also, the enhanced total lipid in body tissues has been reported in *Tilapia zilli* by El-Sayed and Garling, (1988) fed on herbal supplemented diets.

The overall results on the body composition of the present study correlates with the findings of Unnikrishnan (1995) who stated that herbal product Livol (IHF-1000) increased the body composition of Indian major carp, *C.catla* . The dietary herbal powder (superliv®) supplemention in *Oreochromis niloticus* increase the growth and body composition *by* Adekunle Ayokanmi(2012).

Phase III

Hematological and biochemical parameters of *O.mossambicus* supplemented with the leaf extracts of *C.aromaticus* and *O.basilicum*.

Hematological parameters

Hematological parameters of fish are used as indicators of their physiology and stress condition (Logambal *et al.*, 2000; Adigozel *et al.*, 2005 and Ardo *et al.*, 2008).Hematological techniques, including RBC, WBC, Hb concentration, PCV,MCV, MCH and MCHC have provide an understanding and knowledge for the biologist in the field of aquaculture in assessing the health status of fishes (Blaxhall, 1972; Munkittrick and Leatherland,1983) and to observe response of fishes towards stress (Soivio and Oikari, 1976).It is also used to evaluate the oxygen carrying capacity and defense system of blood which stimulate immunity . (Chinabut *et al.*, 1995).

The leaf extracts of *C.aromaticus* and *O.basilicum* at three different concentrations (2.5, 5 and 10 gm/kg of feed) were given as feed supplement to *O.mossambicus* for a period of 45 days and the hematological parameters such as RBC, WBC, Hb concentration, PCV,MCV, MCH and MCHC were analyzed initially before the experiment and after 45 days of treatment. The data obtained for hematological parameters are tabulated in table 13 and illustrated in figure 49 to 54.

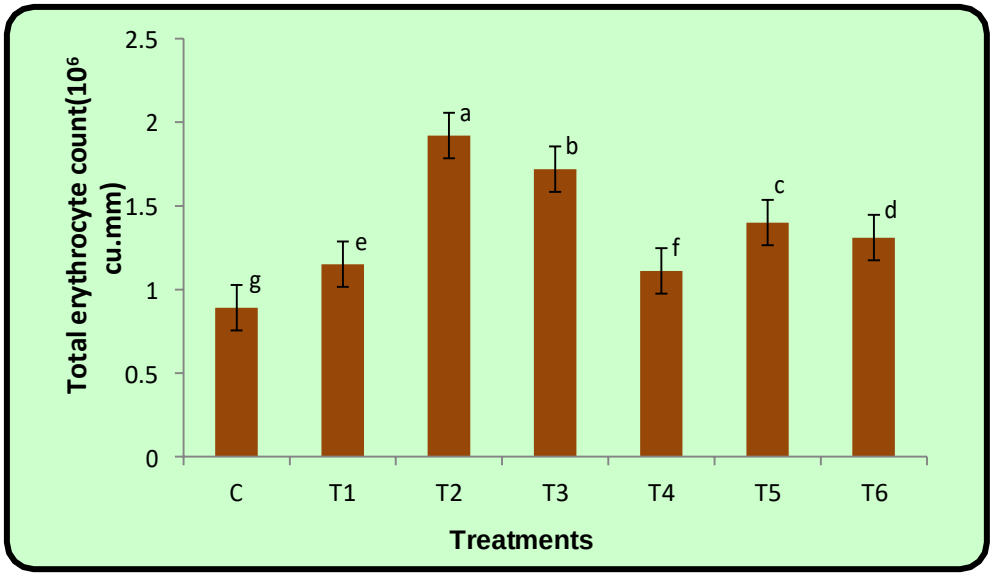
Total erythrocyte count

Table 13 and figure 49, illustrate the total erythrocyte count of *O.mossambicus* supplemented with leaf extracts of *C.aromaticus* and *O.basilicum* at three different concentrations (2.5, 5 and 10 gm/kg of feed).

Maximum erythrocyte count of 1.92 ± 0.06 $x10^6$ million cells/cu.mm was noticed T2 fishes fed with leaf extract of *C.aromaticus* at 5 gm/kg of feed whereas control showed a minimum value of 0.89± 0.08 $x10^6$ million cells/cu.mm. The values of erythrocyte count were significantly higher ($P<0.05$) in treated fishes.

Figure 49

Total erythrocyte count of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.



This increase in the number of RBC might be due to presence and action of phytochemicals in the leaf extracts. Phytochemicals such as terpenoid, saponin and tannin act as a vital role in the blood cell constitution in the organ and in stimulating the immune system. These chemicals can secrete erythropoietin in stem cells. The stimulation of this hormones increases expeditious synthesis of red blood cells (Ohlesson and Aher, 2014).

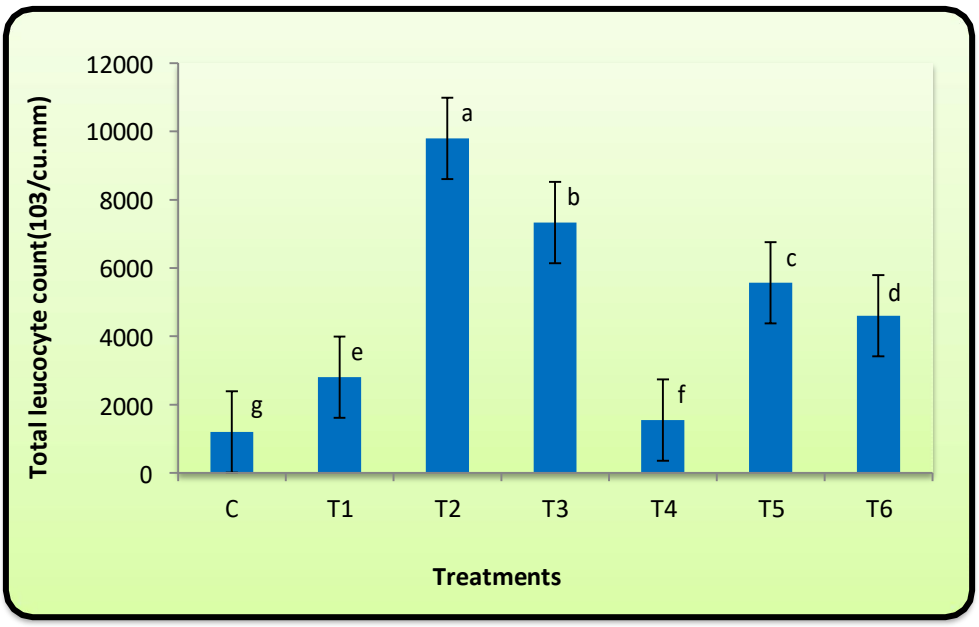
These results were supported by Pratheepa and Sukumaran (2014) who reported that *E.hirta* induced erythropoesis in *Cyprinus carpio*. The results were also in accordance with Sahu *et al.,* 2007 a,who stated that erythrocyte count were significantly higher in *L.rohita* fingerlings fed with *M.indica* kernel. An increased erythrocyte count in *C.catla* fed with *C.dactylon* mixed diet at 0.5% and 5% concentration . (Kaleeswaran *et al.,* 2012b). Similar results were reported in *O.mossambicus* fed with *Cynodon dactylon* for 30 days (Aruldoss *et al*., 2014).

Total leucocyte count

Total leucocyte count of *O.mossambicus* supplemented with leaf extracts of *C.aromaticus* and *O.basilicum* at three different concentrations (2.5, 5 and 10 gm/kg of feed) are shown table 12 and figure 50.

Figure 50

Total leucocyte count of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.



The leaf extracts of *C.aromaticus* and *O.basilicum* increased the number of leucocytes when administered as feed supplement. Total leucocyte count was maximum 9800± 0.05 $x10^3$ thousand cells/cu.mm in T2 fishes and minimum of 1200± 0.03 $x10^3$ thousand cells/cu.mm was noted in control.

This increase in leucuocyte count might be due to the presence of alkaloid and phenol .(Rajaram and Janardhanan ,1991). A significant increase ($P < 0.05$) in WBC of cat fish brood stock treated with ethanolic extract of *G. cambogia* was observed by (Dada and Ikuerowo, 2009). Similar increase in leucocyte count was observed in common carp fed with feed incorporated with plants like and infected with *Aeromonas hydrophila* (Absali and Mohammad,2010)

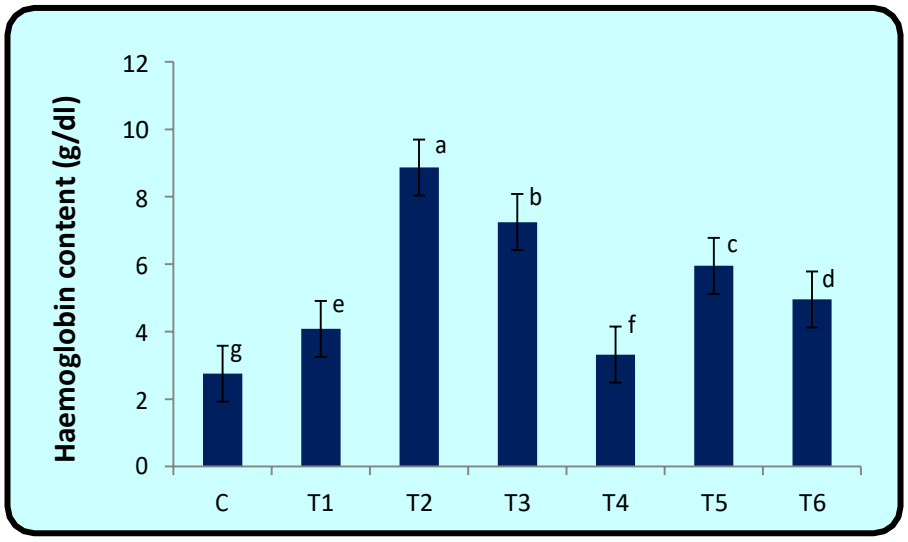
Hemoglobin content

Table 12 and figure 51 illustrate the hemoglobin content of *O.mossambicus* supplemented with leaf extracts of *C.aromaticus* and *O.basilicum* at three different concentrations (2.5, 5 and 10 gm/kg of feed).

The leaf extracts of *C.aromaticus* and *O.basilicum* elevated the hemoglobin content when administered as feed supplement. After 45 days of treatment the hemoglobin content was high (8.86±0.20 g/dl) in T2 fishes and low(2.75±0.24 g/dl) in control fishes. Overall, statistical analysis revealed that the hemoglobin content has significantly increased($P<0.05$) in *O.mossambicus* supplemented with leaf extracts of *C.aromaticus* and *O.basilicum* at three different concentrations when compared to control after 45 days of experiment .

Figure 51

Haemoglobin content of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.



Significant increase ($P<0.05\%$) in the level of hemoglobin might be due to the presence and action of chemical constituents in the leaf powders of experimental feeds which influenced the binding of iron in the blood. Similar increase in haemoglobin content was observed in common carp fed with feed incorporated with plants materials and infected with *Aeromonas hydrophila* (Absali and Mohammad,2010). Shalaby *et al* (2006) reported that an increased hemoglobin content (Hb) in Nile tilapia after administration of garlic. Feed containing lupin, mango and stinging nettle showed increased hemoglobin content in rainbow trout, *Oncorhynchus mykiss* (Awad, 2010).

PCV, MCH and MCHC

The PCV,MCV, MCH and MCHC are corpuscular indices which indicates the anemic condition in animals and used as diagnostic tool. The low PCV would indicate anemia or oligohaemia (Wepener *et al* ., 1992).

PCV, MCH and MCHC of *O.mossambicus* supplemented with leaf extracts of *C.aromaticus* and *O.basilicum* at three different concentrations (2.5, 5 and 10 gm/kg of feed) are shown in table 12 and figure 52 to 54 .Hematological study on *O.mossambicus* shows that the PCV, MCH and MCHC value was maximum (12.39±0.35 %, 47.42±0.38 gm/dl, and 71.76±1.13 gm/dl) in T2 fishes and minimum(5.24±0.04 %, 28.40±0.11 gm/dl ,49.13±1.07 gm/dl) values was recorded in control fishes. The values of PCV, MCH and MCHC were significantly higher($P<0.05$) in treated fishes. The overall results revealed that the PCV,MCH and MCHC has significantly increased($P<0.05$) in *O.mossambicus* supplemented with leaf extracts of *C.aromaticus* and *O.basilicum* at three different concentrations when compared to control after 45 days of experiment .

Figure 52

Packed cell volume of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.

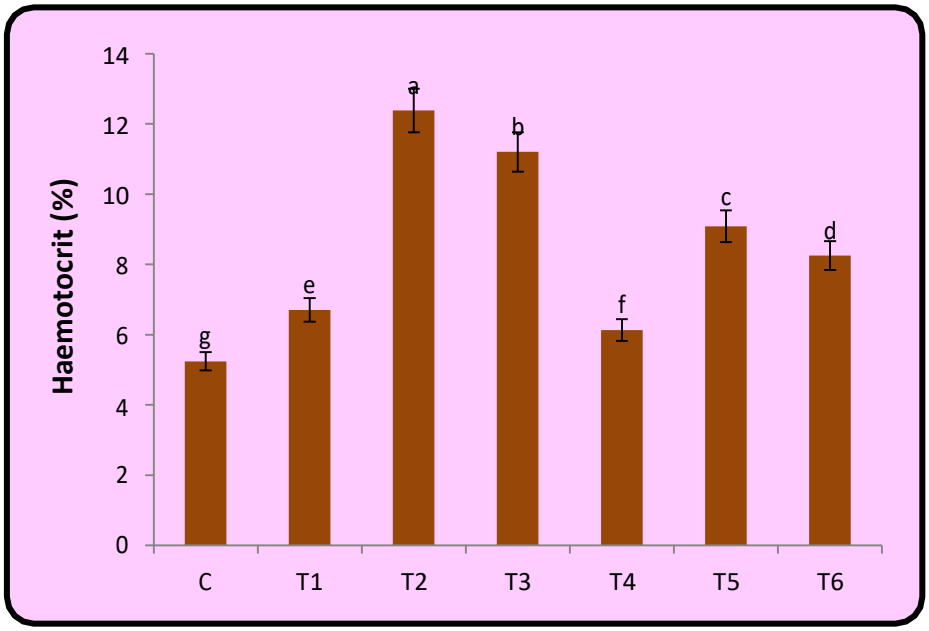


Figure 53

Mean cell haemoglobin of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.

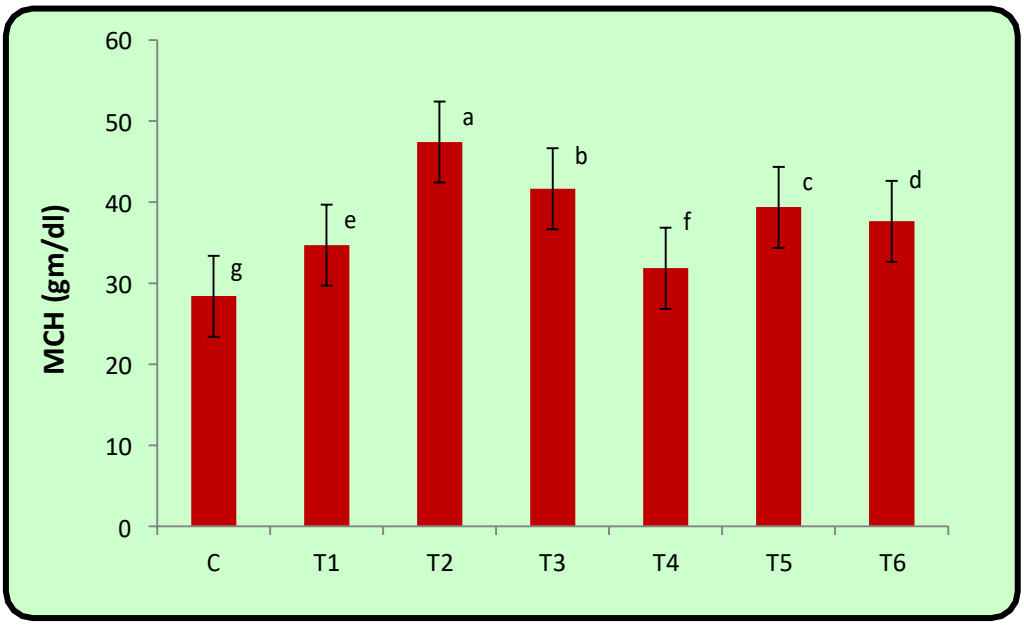
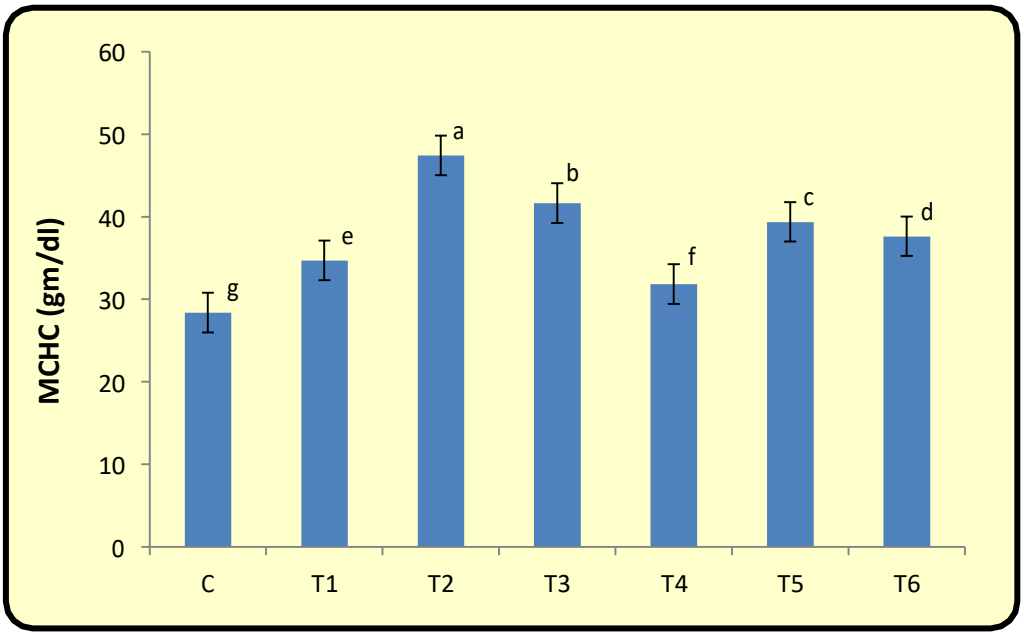


Figure 54

Mean cell haemoglobin concentration of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.



The present results were similar to the findings of Aruldoss *et al* (2013) who stated that PCV , MCH and MCHC were significantly increased in fishes which were treated with *C.dactylon* plant extract supplemented feed. Farahi *et al* (2010) stated that the fish diets containing garlic (*A.sativum*) at 20g and 30g /kg of diet showed significant increase in PCV, MCV,MCH and MCHC values of *O. mykiss* . The maintenance of optimum level or increase of PCV, MCH and MCHC might indicate the improvement of health condition in fish (Suresh and Amolkumar, 2009).

Biochemical parameters

Serum biochemical data are very important in observing the health condition of fishes, especially in management programs (Dias and Moraes , 2007),

The leaf extracts of *C.aromaticus* and *O.basilicum* at three different concentrations (2.5, 5 and 10 gm/kg of feed) were given as feed supplement to *O.mossambicus* for a period of 45 days and the serum biochemical parameters such as protein, glucose and cholesterol content were analyzed initially before the experiment and after 45 days of treatment. The data obtained for serum biochemical parameters are presented in table 14 and illustrated in figure 55 -57.

Serum protein

Proteins are the important constituents of serum that form defensive molecules and help the body against infection. Serum protein is a labile biochemical molecule that reflects the condition of organism and changes under the influence of internal and external factor (Metwally *et al.*, 2009).

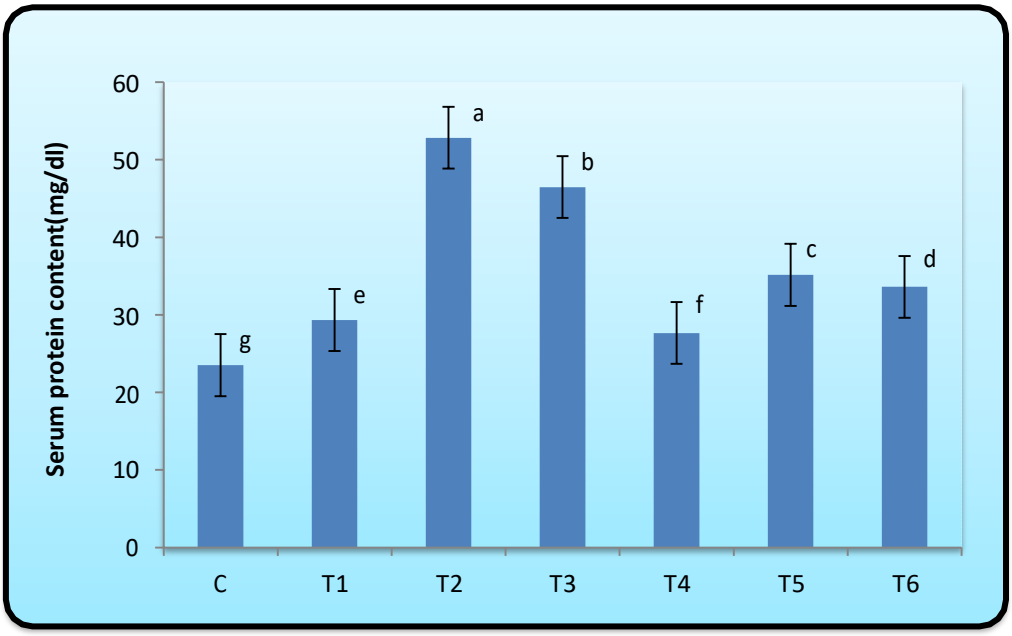
Table 14 and figure 55, illustrate the serum protein content of *O.mossambicus* supplemented with leaf extracts of *C.aromaticus* and *O.basilicum* at three different concentrations (2.5, 5 and 10 gm/kg of feed).

After 45 days of treatment, the serum protein content was high (52.84±1.11 mg/dl) in T2 fishes fed with leaf extract of *C.aromaticus* at 5 gm/kg of feed whereas control showed a low value of 23.51±0.06 mg/dl. The values of serum protein content were significantly higher($P<0.05$) in treated fishes.The serum protein content has

significantly increased (P<0.05) in *O.mossambicus* supplemented with leaf extracts of *C.aromaticus* and *O.basilicum.*

Figure 55

Serum protein level in *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.



Misra et al., 2006 stated that increase in total protein might be due to elevated white blood cell counts (WBC) as a major source of serum protein . High protein and globulin in blood plasma is indicant of high level of safety in fish that is result of stimulation of leukocytes and secretion of immunoglobulins (Nayak et al., 2014).

The present findings were supported by Mohamad and Abasali (2010) who reported that common carp treated with different doses of extracts of a herbal mixture for 20, 40 and 60 days showed increased level of total protein, albumin and globulin. Supplementation of yellow leader (A.membranaceus) and Japanese honeysuckle (Lonicera japonica) at 0.1% showed highest serum protein level in Nile tilapia (Ardó et al., 2008).

Also, phytochemicals such as tannin, saponin, flavonoid, steroid, terpenoid, eugenol, cardiophylline and cardioglyceroid present in plant were responsible for enhanced serum protein that act as immunostimulant in C.batrachus (Nahak and Sahu,

2014). Flavonoids, glycosides and alkaloid present in Mimosa pudica were responsible for increasing total serum protein (Rajendran et al., 2009).

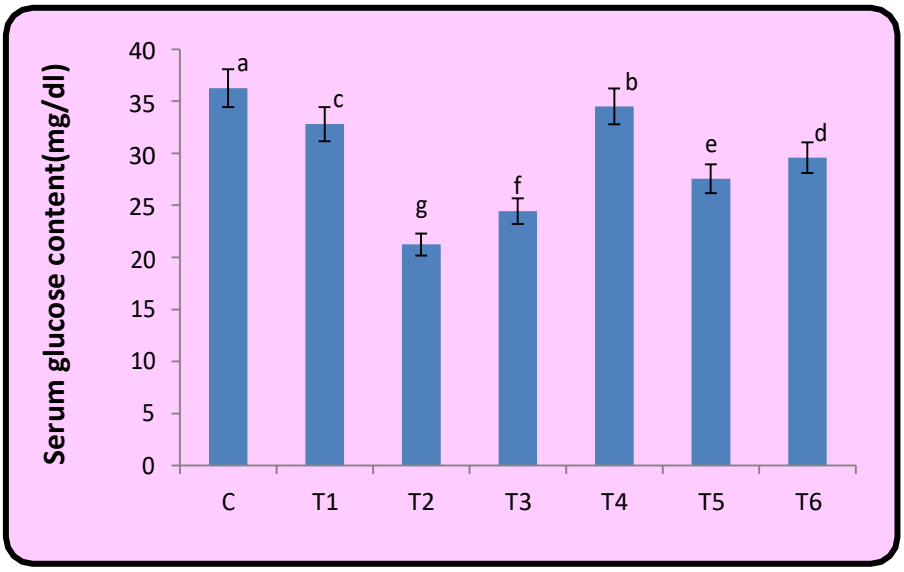
Serum glucose

Serum glucose content of *O.mossambicus* supplemented with leaf extracts of *C.aromaticus* and *O.basilicum* at three different concentrations (2.5, 5 and 10 gm/kg of feed) are shown in table 14 and figure 56 .

The leaf extracts of *C.aromaticus* and *O.basilicum* decreased the level of glucose when administered as feed supplement. Serum glucose level was high (35.09±1.02 mg/dl) in control fishes and low (21.25±0.80 mg/dl) in T2 fishes. The values of serum glucose level were significantly lower (P>0.05) in treated fishes. Statistical analysis revealed that, the serum glucose level has significantly decreased (P>0.05) in *O.mossambicus* supplemented with leaf extracts of *C.aromaticus* and *O.basilicum* at three different concentrations when compared to control after 45 days of experiment .

Figure 56

Serum glucose level in *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.



The decrease in the level of plasma cortisol and glucose is recognized as the main hormonal response to stressors and is widely used as an indicator of stress response indicator (Barton and Iwama, 1991 ; Morgan and Iwama, 1997). Metwally,2009 stated

that there was decrease in the level of glucose in the serum of fishes fed on diets containing different sources of *Allium sativum.* This condition might be due to improved the antioxidant system in cells of pancreas to produce insulin . Glucose level was reduced in *L.rohita* fingerlings after feeding with mango kernel and garlic (Sahu *et al.,* 2007 a&b). Bhaskar *et al*., (2008) reported that aqueous extract of the seeds of *Mucuna pruriens* significantly reduced the blood glucose level after an oral glucose load and oral administration of seed extract.

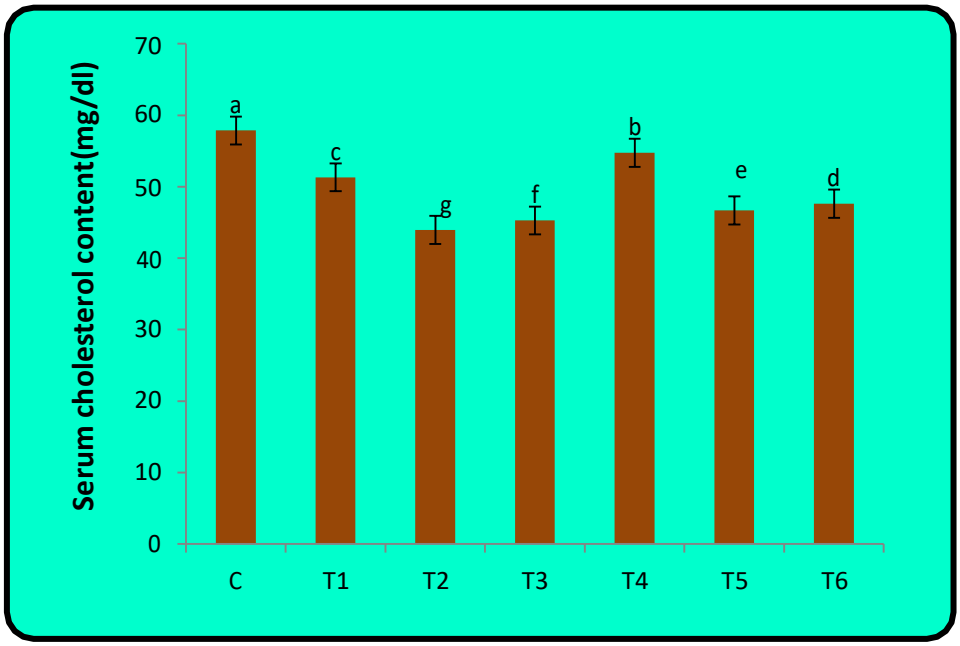
Serum Cholesterol

Serum cholesterol content of *O.mossambicus* supplemented with leaf extracts of *C.aromaticus* and *O.basilicum* at three different concentrations (2.5, 5 and 10 gm/kg of feed) are shown in table 14 and figure 57.

In this present study, serum cholesterol level were significantly high (P<0.05%) in control group than treated group of fishes. The level of cholesterol in the serum of control fish was maximum (57.86±1.12 mg/dl) and minimum in T2 group (43.94 mg/dl). Overall, the serum cholesterol content has significantly decreased (P>0.05) in *O.mossambicus* supplemented with leaf extracts of *C.aromaticus* and *O.basilicum* three different concentrations when compared to control after 45 days of experiment .

Figure 57

Serum cholesterol level in *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (45 DAT). Data represents mean± SE of 15 fishes.



The results of present work were supported by the findings of Nahak and Sahu (2014) who stated that *Clarias batrachus* treated with 2.5% and 5% *Ocimum basilicum* incorporated diet for 15 and 30 days of treatment had lower cholesterol content than control. Ji *et al.*, 2007 stated that feeding aquatic organisms with feed containing phytochemical compounds might effect the fat metabolism.

Thus the results of present phase of study revealed that the leaf extracts incorporated experimental feeds helped to heighten the cellular and humoral elements in blood of *O.mossambicus*.

Phase IV

***Invitro* antibacterial activity of leaf extract**

Figure 58 and Plate 15 represents the *invitro* antibacterial activity of leaf extracts of *C.aromaticus* and *O.basilicum* against *A.hydrophila* by agar well diffusion assay.

The leaf extracts of *C.aromaticus* and *O.basilicum* when subjected to antibacterial activity against the fish pathogen *A.hydrophila* showed varied inhibitory zones. The leaf extract of *C.aromaticus* inhibited the growth of *A.hydrophila* by producing maximum zone of inhibition of 20 mm, followed by *O.basilicum* (18mm) whereas the positive control chloramphenicol showed 22mm of inhibition.

Figure 58

***Invitro* antibacterial activity of leaf extracts of *C.aromaticus* and *O.basilicum* against *A.hydrophila* by agar well diffusion assay**

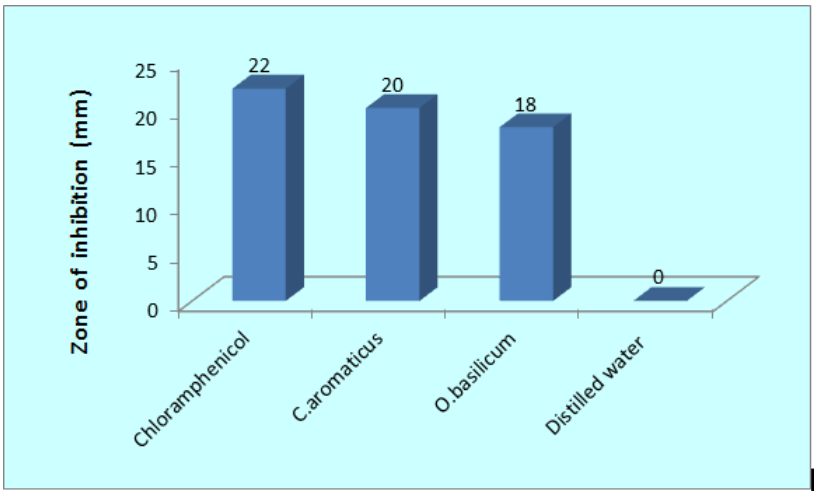
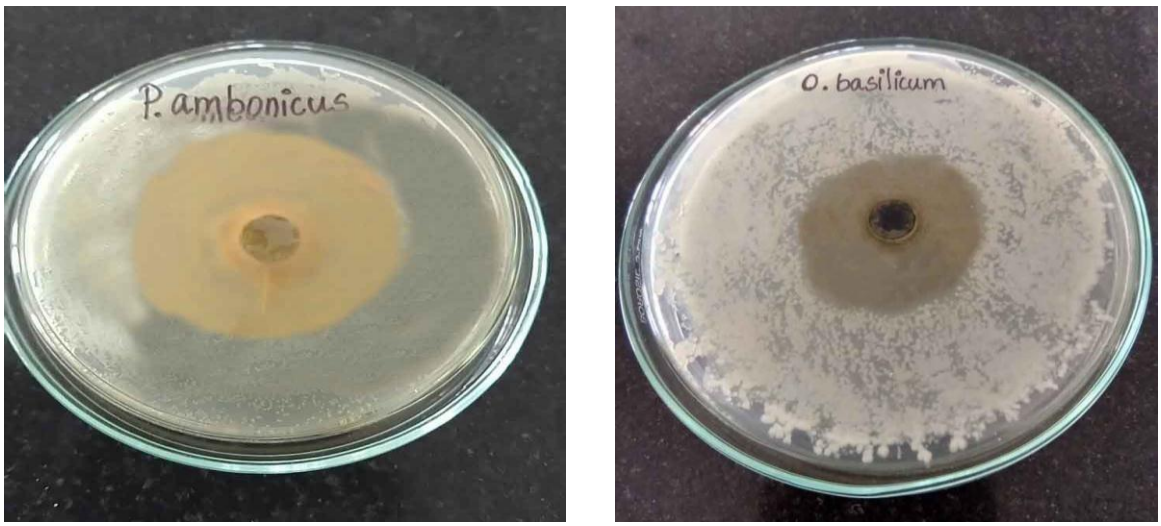


Plate 15

Antibacterial activity of selected leaf extracts against *A. hydrophila*



The antibacterial activity exhibited by leaf extracts might be due to the *invitro* antibacterial activity of leaf extracts of *C.aromaticus* and *O.basilicum* against *A.hydrophila* by agar well diffusion assay presence of bioactive compounds or secondary metabolites, which interacted with the bacteria *A.hydrophila* and resulted in disruption of cell growth, structure, physiological processes and finally leads to damage and death of the pathogen.

Ahmed *et al.,* 2010, stated that alkaloids isolated from plants have antimicrobial properties. Bornemann (1989) reported that the secondary metabolites like phenol, flavanoids, tannins and steroids in the leaf extracts were responsible for antibacterial activity by precipitating the bacterial proteins. Polyphenols have been reported to exhibit antibacterial activities with distinguished characteristics in their reactivity with proteins related polyamides polymer (Haslam,1996).The inhibition of microorganisms by phenolic compounds may be due to iron deprivation or hydrogen bonding with vital proteins such as microbial enzymes (Scalbert,1991).

Wink (2010) stated that bacterial growth could be also inhibited by the presence of phenolic compounds like sterols, hydroxyl chavicol, eugenol. Xu *et al.,* 2008 reported that the essential oils were able to disrupt the lipid structure of bacterial cell, destruct the cell membrane, cytoplasmic leakage, lysis of cell and ultimately cell death. The decrease in pH occurs which might be due to disruption of cell membrane resulted in loss of control of cellular process such as ATP biosynthesis, DNA transcription and protein synthesis .

The present findings were similar to the results of Haniffa and Shanthi (2012) who stated that *C. aromaticus* leaf extracts possessed potent antimicrobial properties against *A. hydrophila*.. Hanniffa and Kavitha (2012) proved that the methanolic extract of *C.aromaticus* was found to have the most effective antagonistic agent against *A.hydrophila* . Girish (2016) stated that the leaf extracts of *C.aromaticus* shows potential effect on *A.hydrophila*. with negligible side effects.

Harikrishnan and Balasundaram (2008) who reported that the extracts of *C.longa, O.sanctum* and *A.indica* were effective against *A. hydrophila*. The aqueous extract of *R. damascena* petals exhibited highest antibacterial activity against the fish *A. hydrophila* with the inhibition zone of 17.33± 1.15 at 100 ppm, 16.33 ± 0.58 at 200 ppm and 16.0±1.0mm at 300 ppm of rose petal extract (Vasanthakumar *et al.,* 2015). Britoo *et al.,*2011., stated that highly significant antibacterial activity was observed in *S. surattense (*16.00±0.00mm) followed by *S. torvum (*11.33±1.15), *S.trilobatum* (11.00±1.00mm), *S.melonagena* and *S.nigrum.*

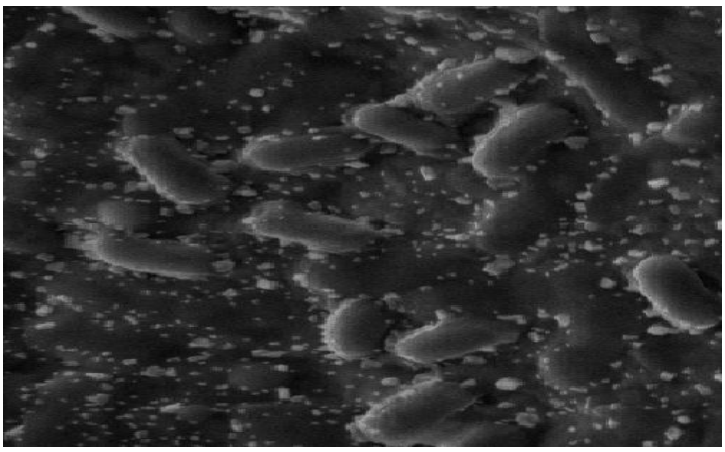
Characterization of molecular structure by SEM

To observe the morphological changes, untreated bacterial samples and the bacterial samples treated with *C.aromaticus* and *O.basilicum* leaf extracts were subjected SEM analysis . The result obtained are depicted in Plate 16 .

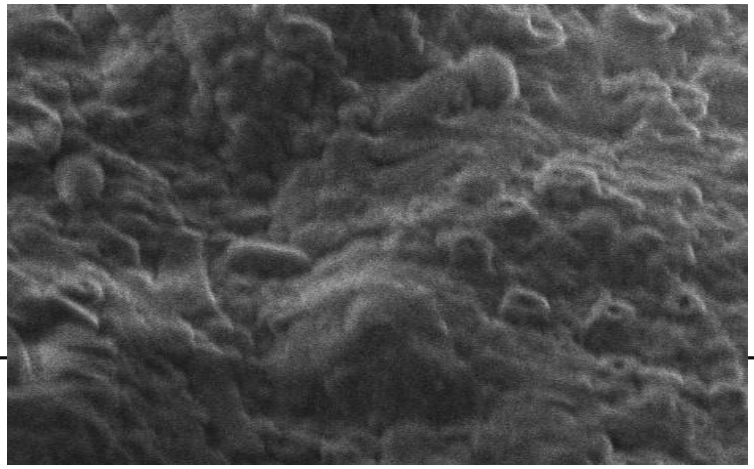
Bacterial cells in the control appeared to be straight rods with rounded ends and the presence of polar flagella.In treatments bacterial cells showed significant differences their structure .The leaf extracts targets cell membrane and cytoplasm and induced potential and deleterious changes in bacteria cell when compared to control. Plate clearly illustrates that treated cells appeared to be shrinking, cell membrane damage, partial disappearance of cytoplasm.

Plate 16

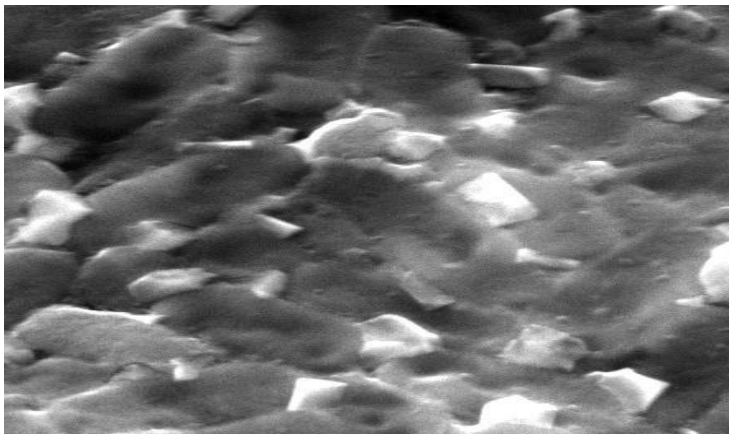
Molecular structure of untreated and treated *A.hydrophila* by selected leaf extracts using SEM



a) **Untreated *A.hydrophila***



b) A.hydrophila* treated with *C.aromaticus



c) A.hydrophila* treated with *O.basilicum

Burt and Reinders (2003) explained the mechanism of action of essential oil components in bacterial cells. The mechanism of action was taught to be degradation of cell wall, damage to cytoplasmic membrane proteins, binding of proteins, leakage of cell contents and coagulation of cytoplasm and depletion of proton motive force.

Similarly Kaya *et al.,* 2008 stated that the bacterial cells treated with plant extracts of Ocimum basilicum appeared to be shrinking and there is a degradation of cell wall. Tang *et al.,* 2016 stated that the extract of *Scutellaria barbata* produced injuries and structural changes in the cell membrane of *Salmonella typhimurium* .This damage is an indication of ROS generation in bacterial cell.

Burt and Reinders (2003) who reported that oregano and thyme essential oil exhibited strong antimicrobial properties against *E.coli* and the observed cells were damaged when treated with essential oil . Gupta *et al.,* 2015 reported that bacteria treated with *Curcuma longa* extracts showed morphological deformity with partial lack of cytoplasmic membrane which leads to distruction.

Physiological parameters

The fishes were fed with *C.aromaticus* and *O.basilicum leaf extract* supplemented feed at three different concentrations (2.5, 5 and 10 gm/kg of feed) for 30 days.. After 30 days, the fishes were fed with only control feed and were injected intramuscularly with 0.1ml of 10^5 cfu/ml of *A.hydrophila* on 30th and 37th day. The fishes were monitored regularly for changes in behavior and mortality. The data obtained for survival rate, mortality and relative level of protection are tabulated in Table 15 to 16.

Cumulative percentage mortality, survival rate and relative level of protection

Table 15 to 16 represents the cumulative percentage mortality and survival rate of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed (2.5, 5 and 10 gm/kg of feed).

On 37th day T2, T3, T5 and T6 have 0% mortality, 100% survival and 100% relative level of protection whereas control caused 13.4% mortality,86.6% survival and 0% relative level of protection.

A dose of 0.1ml of 10^5 CFU/ml of *A. hydrophila* inoculation inflicted an increase in cumulative percentage mortality, as the increase in days. On 45th day , T2,T3,T5 and T6 showed 0% mortality, 100% survival and 100% whereas the control caused 26.67% mortality and 73.33% survival.

Table 15

Cumulative mortality, survival rate and relative level of protection in *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (2.5, 5 and 10 gm/kg of feed) on 37th day.

Treatments	No. of fishes introducing	No. of fishes dead	% mortality	No.of fishes survived	% survivial	% Relative level of protection
Control	15	2	13.4±0.08	13	86.6±11.57	0±0.00
T1	15	1	6.67±0.38	14	93.3±5.57	50±10.00
T2	15	0	0±0.00	15	100±0.00	100±0.00
T3	15	0	0±0.00	15	100±0.00	100±0.00
T4	15	1	6.67±0.38	14	93.3±5.57	50±10.00

T5	15	0	0±0.00	15	100±0.00	100±0.00
T6	15	0	0±0.00	15	100±0.00	100±0.00

Table 16

Cumulative mortality, survival rate and relative level of protection in *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (2.5, 5 and 10 gm/kg of feed) on 45th day.

Treatments	No. of fishes introducing	No. of fishes dead	% mortality	No.of fishes survived	% survivial	% Relative level of protection
Control	15	4	26.67±7.64	11	73.33±	0±0.00
T1	15	1	6.67±0.38	14	93.3±5.57	75±10.00
T2	15	0	0	15	100±0.00	100±0.00
T3	15	0	0±0.00	15	100±0.00	100±0.00
T4	15	2	13.4±0.08	14	86.66±11.57	50±10.00
T5	15	0	0±0.00	15	100±0.00	100±0.00
T6	15	0	0±0.00	15	100±0.00	100±0.00

The fishes in the different treatments revealed 100% survival rate when compared to control group. When the fishes were injected with *A. hydrophila* the immuno stimulatory effect might be produced by the leaf extracts in the experimental feed against the pathogen. This shows that the leaf extracts were able to stimulate the resistance and inhibit the growth of *A. hydrophila*. Wassom and Kelly, 1990 reported that the development of resistance in fish to a particular disease can be determined from the survival rate after bacterial infection.

The results were supported by Sahu *et al* (2007 a & b) who reported that *L. rohita* treated with garlic and mango kernel showed increased level of resistance and survival against *A. hydrophila*. Abutbul *et al.*, 2004, inferred that *O.mossambicus* fed with *R.officinialis* increased the survival rate against *A. hydrophila*. Yin *et al.,* 2009 reported that common carp fed with *Astragulas* showed enhanced survival rate against *A.hydrophila* when compared to control.

Immunological parameters – Antigen antibody titer

Antibody response to *A. hydrophila* by various experimental groups fed with *C.aromaticus* and *O.basilicum* at different concentrations (2.5, 5 and 10 gm/kg of feed) *)* were found out by using antibody titre plate and showed in the Table 17 and Plate 16 .

In this study significant enhancement of specific antibody by all the six treatments in a dose dependent manner was noted, When *C.aromaticus* and *O.basilicum* leaf extracts were given as feed supplement to *O.mossambicus*.

The antibody titre (log 2 values) was higher (1.041) in T2,T3, T5 and T6 followed by T1 (0.954), T4(0.845) whereas control had a low value of 0.698 on both 37th day. On 45th day the antibody titre (log 2 values) was high (1.041) in T2,T3,T5 and T6 followed by T1 and T4 as 1.000 whereas control showed a minimum value of 0.698.

On overall view, the antibody titer value has significantly increased ($P<0.05$) in *O.mossambicus* supplemented with leaf extracts at different concentration when compared to control on 37th and 45th day of treatment. A comparison of antibody response of fish supplemented with leaf extracts at higher concentrations on different days of treatment was significantly higher ($P<0.05$).

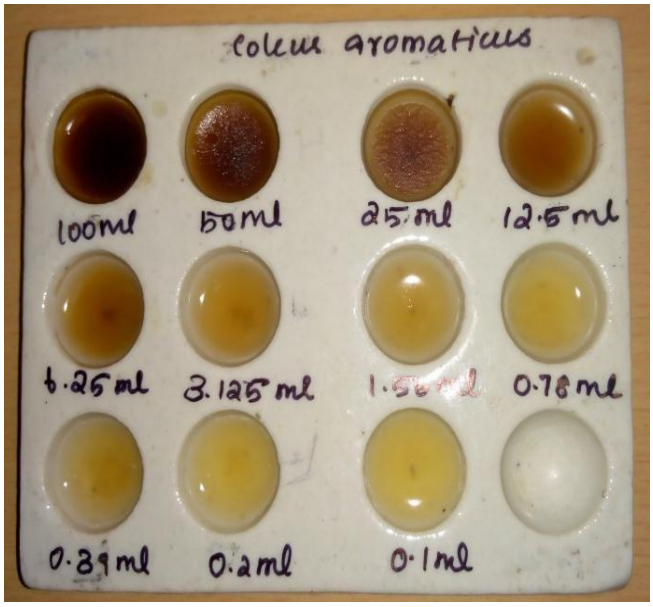
Table 17

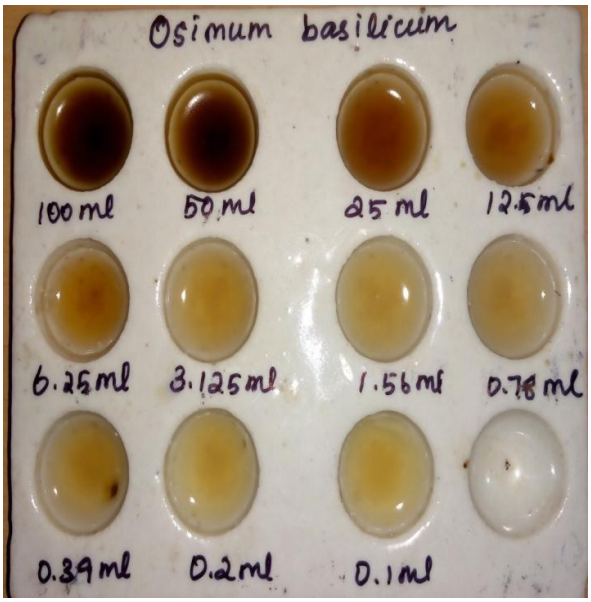
Antibody titer in *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed (2.5, 5 and 10 gm/kg of feed) on 37th and 45th day.

Treatments	Antibody titer (37th day)	Antibody titer (45th day)
Control	0.698	0.698
T1	0.954	1.000
T2	1.041	1.041
T3	1.041	1.041
T4	0.845	1.000
T5	1.041	1.041
T6	1.041	1.041

Plate 16

Antibody titer of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf extracts as supplemented feed





The antibody titer was significantly higher in treated group of fishes when compared to control. Antigen antibody titer can be used to measure the humoral immunity an organism. The significant increase in the production of antibody might be due to immunostimulatory effect of leaf extracts in the experimental feeds. The phytochemical components in the leaf extracts were able to resist the growth of bacteria which resulted in enhancement in the production of antibodies against *A.hydrophila*.

Mercy (2006)stated that a significant increase in haem agglutination antibody titer was observed in *C.mrigala* treated with *P.emblica*. Pratheeba and Sukumaran (2014) reported that leaf extracts of *Euphorbia hirta* at all the concentrations of produced antibody response, but at higher concentration of 25.5g antibody response was high against *A. hydrophila* in *C.carpio*.

The leaf extract of *O.sanctum* produced a significant stimulatory effect on both primary and secondary responses against *A.hydrophila* in *O.mossambicus* (Logambal *et al.*, 2000 and Venkatalakshmi and Michael, 2001). Behera *et al.*, 2011 stated that

increased bacterial agglutination activities in *A.hydrophila* infected fishes primed with low dose (15mg) curcumin. Kumar *et al.,* 2007 inferred that that there was an enhanced antibody response in *L.rohita* fed with polyherbal formulation when challenged against *A.hydrophila*. Lokesh *et al.,* 2012 reported that the herbal extracts and animal originated product have a probable application as an immuno stimulant because application is easy, not expensive and act against a wide variety of pathogen.