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Effect of *Spirulina platensis*, *Chlorella vulgaris* and *Spirogyra maxima* on Population Growth, Egg Production and Nutritional Profiles in *Thermocyclops hyalinus*

[1]Venkatachalam Uthayakumar, [1]Kumar Vidhya, [1]Ramachandran Chandirasekar, [1]Parathattil Rathan Sreedevi, [2]Kannan Mohan, [3]Rajarajeswaran Jayakumar, [4]Dhanabalan Senthilkumar and [1]Ramasubramanian Venkatachalam

[1]Department of Zoology, School of Life Sciences,
Bharathiar University, Coimbatore-641046, Tamilnadu, India
[2]Department of Zoology, Sri Vasavi College, Erode- 636 316, Tamilnadu, India
[3]Department of Molecular Medicine, Faculty of Medicine,
University of Malaya, Kuala Lumpur-50603, Malaysia
[4]Department of Zoology, Kandaswami Kandar's College, Paramathi Velur, 638 182, Tamil Nadu, India

Abstract: This experiment was carried out to evaluate the effect of algal diet on dietary profiles of copepod. The microalgae like *Spirulina platensis*, *Chlorella vulgaris* and *Spirogyra maxima* were mass cultured in artificial medium for the period of 20 days, cells were harvested, dried, powdered and used as feed. The small freshwater cyclopoid *Thermocyclops hyalinus* a species of copepod commonly preferred by most of the fish larvae. It was easily maintained in culture, when fed with algal diet. Biochemical composition, egg production ratio, growth performance, amino acid and fatty acids profile of the *T. hyalinus* were analyzed after the experimental period of 15 days, all the nutritional values found high and statistically variable. On the basis of biochemical composition, egg production ratio, growth performance, amino acids and fatty acids profile it is found high in *T. hyalinus* fed with *S. platensis* was the suitable candidate algae for larval fish diets.

Key words: *Thermocyclops hyalinus* · *Spirulina platensis* · *Chlorella vulgaris* · *Spirogyra maxima*

INTRODUCTION

Aquaculture has become recognized as a growth area of economic importance countries and has attracted the attention of both private and public sectors [1]. It is a rapid developing industry [2] ancient art that has been sophisticated by scientific practices for the need of supplying essential protein foods and economic activity in majority of countries through commercial fishing business [3]. The global aquaculture production will continue to increase through the expansion of semi-intensive, small scale pond aquaculture [4] to reduce the hunger and malnutrition worldwide [5].

Microalgae are the rich source of proteins and other nutrients, similar to higher plants. Micro algae play important roles as primary producers for various consumers such as rotifer, Copepods, Daphina, brine shrimp etc., which are in turn, fed to late larval and juvenile fish and crustaceans Microalgae are rich source of protein, carbohydrates and especially essential fatty acids [6].

Chlorella is unicellular green algae which contain essential amino acids, protein, minerals, vitamins, dietary fiber and a wide range of antioxidants, bioactive substances and chlorophylls etc., Kwak *et al.* [7]. *C. vulgaris* is only about 2 - 10 µm in diameter and it has a high content of nutrient, composed of 51-58% protein, 12-17% carbohydrate and 14-22% lipid [8]. Spirogyra, a free floating macroscopic alga, rich in nutritive value, hence serves as a fish feed ingredient (9). It is an edible filamentous green alga found in freshwater. This alga consists of 20.90% proteins, 59.07% carbohydrate, 4.95% fat, 1.78 ± 0.04% sulfate and 7.66% dietary fiber [10].

Corresponding Author: Venkatachalam Uthayakumar, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore-641046, Tamilnadu, India.

Spirulina is free floating filamentous microalgae. Its nutritional qualities are truly "one-of-a-kind" with 71 % total protein, carotenoids, essential fatty acids, B complex vitamins, vitamin E, copper, manganese, magnesium, iron, selenium and zinc [11]. The optimum temperature for spirulina culture is in the range of 35-38°C [12]. In addition, spirulina requires relatively high pH values between 9.5-9.8 [13]. Live feeds are one of the major inputs in aquaculture. The success of fish farming depends on the adequate quality of nutritionally balanced feeds [14]. Copepods are tiny crustaceans thrive abundantly in most aquatic habitats. The word "Copepod" derives from the Greek "Cope" meaning ore and "Podes" meaning food and refers to their paddle-like paired swimming legs. Three orders dominate in fresh waters: calanoids, harpacticoids and cyclopoids. The calanoids are mainly omnivorous. Most of the cyclopoids are predators. They play an essential role in pelagic food chains and aquatic ecosystems [15].

Copeopods have been recognized as the most suitable feed for early stages of fish larvae because of their nutritional status high essential fatty acids compared with other live feeds, such as rotifer and artemia [16]. When copepod nauplii have been provided as food, larval growth and survival has improved for many of fishes [17]. They are abundant and are distributed in saltwater and freshwater bodies worldwide. There are over 21,000 documented species, 2600 genera, 250 families and 10 orders of copepods [18]. Play an important role in aquatic food webs, as natural prey items of fish larvae, at which typically making up 50 percent are more of their stomach contents [19]. The present trend in Copepodology research is towards utilizing Copepods as a live feed in aqua culture [20]. Biochemical studies have shown that copepods are rich in proteins, lipids, essential amino acids and essential fatty acids [21].

The genus *Thermocyclops* inhabits mainly water bodies within limnetic zone. It was identified by Kiefer, 1927 (Copepoda, Cyclopoida) which is originated in the tropical region, in which it presents a high diversity of species. In many tropical water bodies, this genus represents the most important component of the zooplankton biomass [22]. This genus occurs worldwide, with more than 50 described species and subspecies [23]. The discrimination of most species of Thermocyclops (By some author still treated as a subgenus of Mesocyclops) is based on a series of micro characters which until recently have not been defined in the literature generally available to the non-specialist. In this present study *Thermocyclops hyalinus* is used. The aim of this present study is to evaluate the nutritional profiles, egg production ratio and growth performance of cyclopoid copepod*, T. hyalinus* fed with 3 different algae such as chlorella, spirulina and spirogyra. It also aims to mass culturing and the three different algae *Chlorella vulgaris, Spirulina platensis* and *Spirogyra maxima.*

MATERIALS AND METHODS

Collection and Culture of *T. hyalinus*: Cyclopoid Copepods *T. hyalinus* were collected from the Ukkadam Lake, Ukkadam, Coimbatore, Tamilnadu, India using 100µm mesh dipnet.

Experimental Setup: Experiment I - Control for *T. hyalinus* fed with Baker's yeast, Experiment II - *T. hyalinus* fed with Spirulina, Experiment III *T. hyalinus* fed with Chlorella, Experiment IV - *T. hyalinus* fed with Spirogyra.

Copepod Culture: Stock cultures of copepod were maintained in a temperature-controlled room (27 ± 0.5°C) in 20 Liter culture containers with gentle aeration. Cultures were fed to excess with the microalgae. A 12:12 light–dark cycle was maintained in the culture room. Water quality was continuously monitored; 40-60 % of water was completely replaced every 2 - 3 days.

Egg Production Ratio: Adult female Copepodite with egg sacs was isolated from each experimental setup and kept in different vials with water in triplicate, to determine the egg production ratio. The numbers of eggs produced were identified by counting the number of naupllius hatched.

Growth Performance: Like experimental setup 10 adult Copepods were isolated and introduced into 1 liter beaker, the experimental setup are fed with respective algae and the control are fed with baker's yeast. After the period of 15 days, the number of copepods was counted to know the growth rate performance.

Algae Cultures: All Algae were obtained from Krishna Algal Culture Industry, Aathur, Salem. The three algal species cultured were: *S. platensis*, *C. vulgaris* and *S. maxima.* They were grown under natural sunlight with (28-35°C) temperature, in sterilized containers 40-L Aquarium glass tanks and supplied with filtered tap water. *C. vulgaris* and *S. platensis* are only cultures grown with agitation. *C. vulgaris* was supplied with f/2 medium, *S. platensis* with Zarrouk medium.

Physicochemical Water Quality Parameters: Water quality parameters such as temperature, pH, fluoride, nitrate, nitrite, ammonium phosphate, iron, chloride, residual chloride, total hardness, total alkalinity and calcium hardness were analyzed for pond water, copepod rearing water and algal culture medium using water quality parameter kits.

Biochemical Composition: Protein estimation was done by standard method Lowry *et al*. [24]. Carbohydrates were estimated by Roe [25] and Lipid values were estimated by Folch *et al*. [26] methods.

High Performance Thin Layer Chromatography for Amino acid Analysis: Amino acids profiles were found by high performance thin layer chromatography method Direkbusarakom [27]. Copepods were dried (80°C for 3 hrs), digested with 6(M) HCl and samples were dissolved in deionized water and 5µl of the sample was loaded on 8 mm thick pre-coated Silica gel 60 F254 TLC plate (20cm x 15cm) and processed in CAMAG-LINOMAT 5 instrument - (Germany). The plate was developed in Butane-Ammonia-Pyridine-Water (3.9:1:3.4:2.6) mobile phase. Plate was sprayed with ninhydrin and dried; the developed plate was documented using photo-documentation chamber (CAMAG-REPROSTAR3) at UV 254 nm and UV 366 nm lights. Finally, the plate was scanned at 500 nm using CAMAG-TLC SCANNER 3. The peak area compared with the standard amino-acids and their level was quantified.

Gas Chromatography -Mass Spectrum for Fatty Acid Analysis: Gas chromatographic (GC) analyses of fatty acids were performed with a THERMO GC-TRACE ULTRA VER: 5.0, THERMO MS DSQ II equipped with an HP-5 cross-linked methyl silicone fused silica capillary column. Helium was the carrier gas. Following addition of methy nonadecanoate and methyl tricosanoate internal standards, samples were injected in a splitless mode at an oven temperature of 50° C. Peaks were quantified with version 1.10 beta, shimadzu software. Individual components were identified using mass spectral data and by comparing retention time data with laboratory standards.

Statistical Analysis: Values of each parameter were measured and expressed in Arithmetic mean ± Standard deviation (SD). Algal fed Copepod groups biochemical parameters, amino acid profiles, fatty acid profiles, growth rate and egg production ratio were tested using one way ANOVA and comparison of mean values was done using Duncan's multiple range tests at 0.05% level of significance using the software program SPSS (Ver: 14) for windows was used for the analysis.

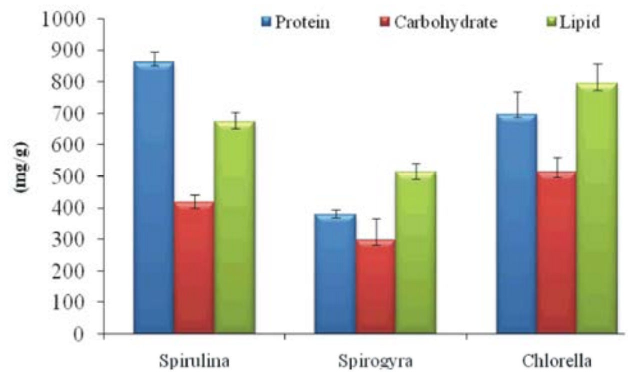
RESULTS

Biochemical Composition: In the present study the Biochemical composition of *S. platensis*, *C. vulgaris* and *S. maxima* were analyzed (Graph-1). The protein content was high in Spirulina as 862.692 (mg/g), 697.26 (mg/g) in Chlorella and low in Spirogyra 379.08 (mg/g). The Carbohydrate was found high in Chlorella as 514.05(mg/g) compared to Spirulina 417.08 (mg/g) and low in Spirogyra 297.12 (mg/g). Even as the lipid value were recorded as high in Chlorella 794.03 (mg/g), 674.09 (mg/g) in Spirulina and 512.72 (mg/g) in Spirogyra.

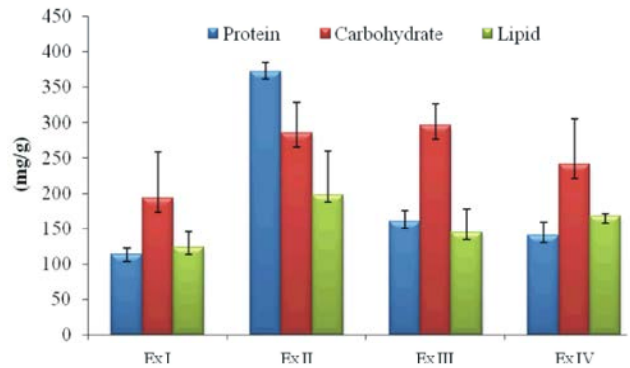
In Graph -2, high protein values were recorded in Experiment- II with 372.07 (mg/g) compared to Experiment- III (159.94 mg/g), Experiment- IV (139.81 mg/g) and low in Experiment-I (113.06 mg/g). The Carbohydrates found high in Experiment -III (296.02 mg/g) compared to (285.12 mg/g) Experiment - II followed by (241.19 mg/g) Experiment - IV and low in Experiment-I (193.09 mg/g). The lipid content was found high in Experiment-II (197.63 mg/g) compared to (167.01mg/g) Experiment - IV, 143.08 (mg/g) Experiment - III and low in Experiment -I (123.02 mg/g). All the biochemical values of Algal groups are significantly different at $p<0.05$ when compared to Control group feed was insignificant ($p>0.05$).

Egg Production Ratio: gg production ratio of Copepod were performed after 15 days with adult females from experimental organisms and the values were mentioned in Graph -3, it shows the high Egg production ratio in Experiment- II (42.17%) compared to Experiment- III (40.21%), Ex- IV (37.01%) and low in Experiment- I with 28.33%. All the egg production ratio of algal feed groups were significantly different at $p<0.05$ compared to control group ($p>0.005$).

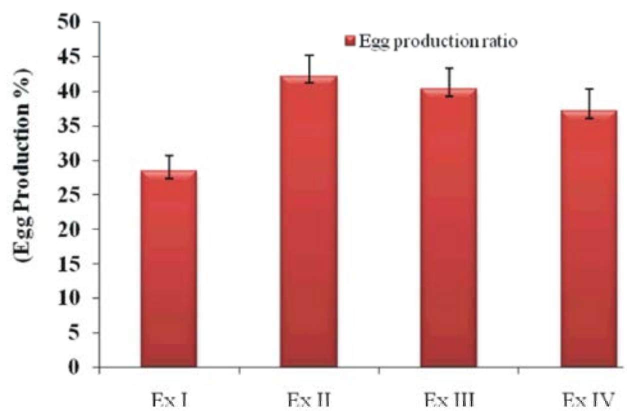
Growth Rate: In the present study growth performance was analyzed in experimental organism at 3^{rd,} 6th, 9th, 12th and 15th day. Graph- 4 shows the growth performance on 15th day it was founded high in Experiment- II (31.27%) compared to 25.04% in Experiment- III, 23.74% in Experiment- IV and low in Experiment- I (17.57%). Copepod growth rate were significantly different at $p<0.05$ when compared to control group (p>0.05).



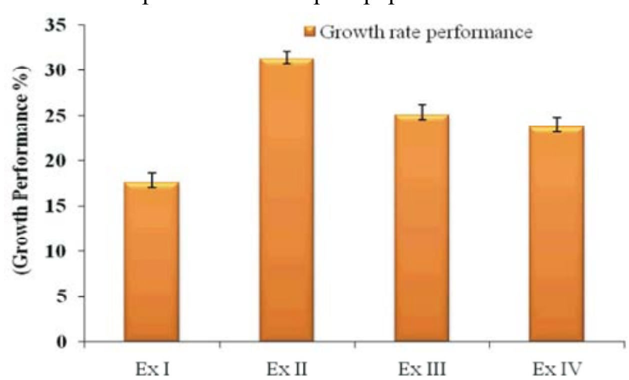
Graph 1: The Biochemical Composition of Commercial Algae



Graph 2: The Biochemical Composition of Experimental Animals



Graph 3: The Egg Production Ratio of Experimental Group Copepods



Graph 4: The Growth Rate Performance in Experimental Copepods

Table 1: The Amino acid Composition (%) of Control and Experimental Copepods

Amino acids	Ex-I	Ex-II	Ex-III	Ex-IV
Arginine ARG	5.02±0.22^{d}	7.62±0.059^{c}	6.77±0.086^{c}	6.32±0.072^{b}
Histidine HIS	2.19±0.095^{g}	3.88±0.377^{g}	4.53±0.062^{f}	3.40±0.116^{e}
Isoleucine ILE	6.09±0.111^{bc}	9.82±0.139^{a}	8.52±0.129^{a}	7.73±0.104^{a}
Leucine LEU	6.23±0.009^{b}	8.88±0.014^{b}	7.65±0.008^{b}	7.63±0.044^{a}
Lysine LYS	5.43±0.05^{d}	8.85±0.051^{b}	6.57±0.095^{c}	6.95±0.047^{ab}
Methionine MET	1.26±0.05^{h}	1.98±0.043^{j}	1.82±0.067^{g}	1.87±0.014^{g}
Phenylalanine PHE	1.01±0.08^{h}	2.62±0.042^{h}	1.35±0.016^{g}	1.68±0.015^{g}
ThreonineTHR	4.30±0.180^{e}	5.74±0.102^{e}	6.47±0.097^{c}	4.49±0.059^{d}
TyrosineTYR	2.28±0.09^{g}	3.46±0.385^{g}	4.85±0.168^{f}	2.66±0.090^{ef}
TryptophanTRY	5.49±0.018^{d}	7.50±0.036^{c}	8.52±0.092^{a}	7.56±0.063^{a}
Valine VAL	0.43±0.015^{i}	0.72±0.035^{k}	0.60±0.047^{h}	0.60±0.055^{h}
Alanine ALA	6.0±0.078^{c}	7.13±0.114^{c}	7.32±0.130^{b}	6.82±0.049^{a}
Aspartic acid ASP	4.26±0.073^{e}	6.26±0.121^{d}	5.53±0.093^{e}	4.42±0.040^{d}
Glutamic acid GLU	6.54±0.012^{a}	8.24±0.105^{b}	7.93±0.036^{b}	7.63±0.093^{a}
Glysine GLY	6.02±0.269^{c}	6.31±0.301^{d}	6.53±0.086^{c}	6.06±0.104^{b}
Serine SER	2.31±0.043^{g}	3.08±0.035^{g}	5.03±0.016^{e}	4.05±0.352^{d}
Proline PRO	4.36±0.099^{e}	6.32±0.215^{d}	6.38±0.103^{c}	5.13±0.154^{c}
Cystine CYS	0.43±0.026^{j}	2.90±0.052^{h}	0.82±0.105^{h}	1.27±0.481^{g}
Glutamine GLA	3.01±0.150^{f}	4.08±0.028^{f}	5.33±0.150^{e}	4.17±0.057^{d}
Asparagine ASN	0.27±0.012^{j}	1.29±0.067^{i}	1.61±0.069^{g}	0.66±0.012^{h}
Total Amino acids	72.93	106.68	104.13	91.1

Mean ± SD (n=5) Experiment I- Control, Experiment II- *T. hyalinus* fed with *S. platensis*, Experiment- III -*T. hyalinus* fed with *C. vulgaris*, Experiment-IV- *T. hyalinus* fed with *S. maxima.* Mean values within the same row sharing the same superscript are significantly different (*$p<0.05$*) when compared to Experiment-I group (*$p>0.05$*)

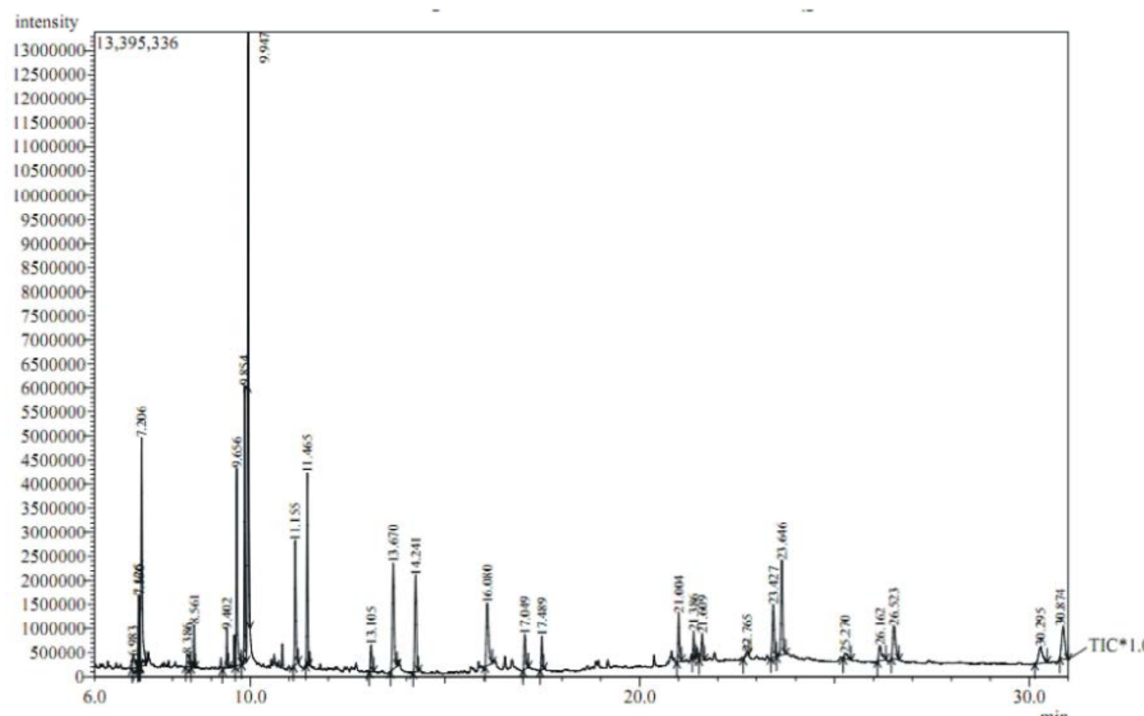


Fig. 1: Fatty acid Profiles of Experiment-I Control (*Thermocyclops hyalinus)* fed with Baker's yeast

Amino Acid Profiles: The amino acid profiles of Copepods were analyzed and presented in Table 1. The essential amino acids like Histidine were found high in Experiment- III (4.53 %) and low in Experiment – I (2.19%). High values of Isoleucine were reported in Experiment - II (9.82%) and low in Experiment- I (6.09%). Leucine values were found high in Experiment II with 8.88% compared to Experiment- IV (6.55%) very low. Lysine was noted high

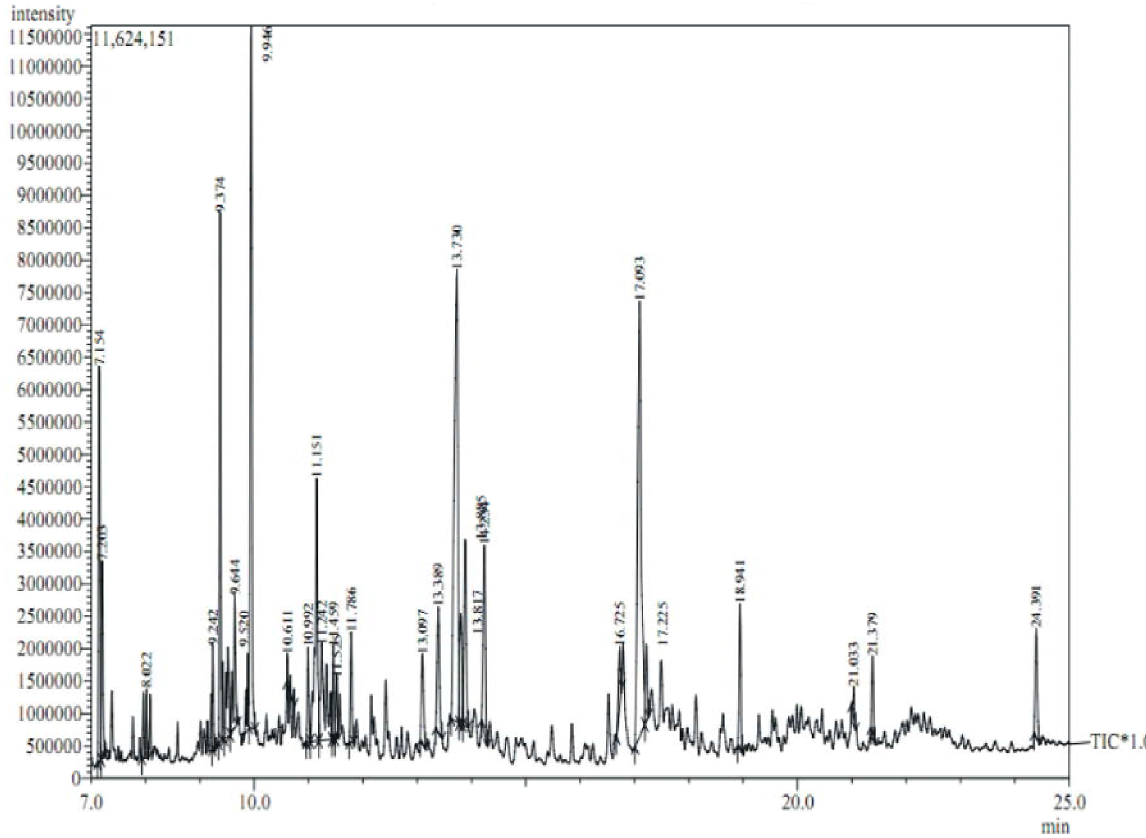


Fig. 2: Fatty acid Profiles of Experiment II (*Thermocyclops hyalinus* fed with *S. platensis*)

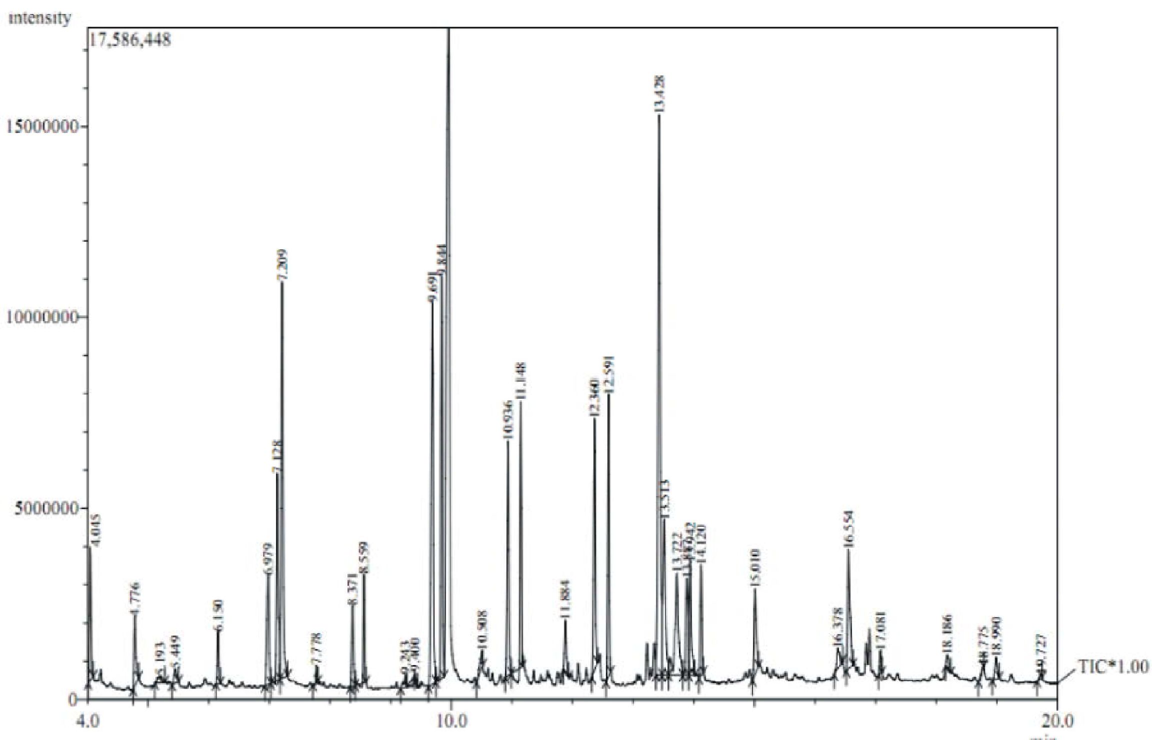


Fig. 3: Fatty acid Profiles of Experiment III (*Thermocyclops hyalinus* fed with *C. vulgaris*)

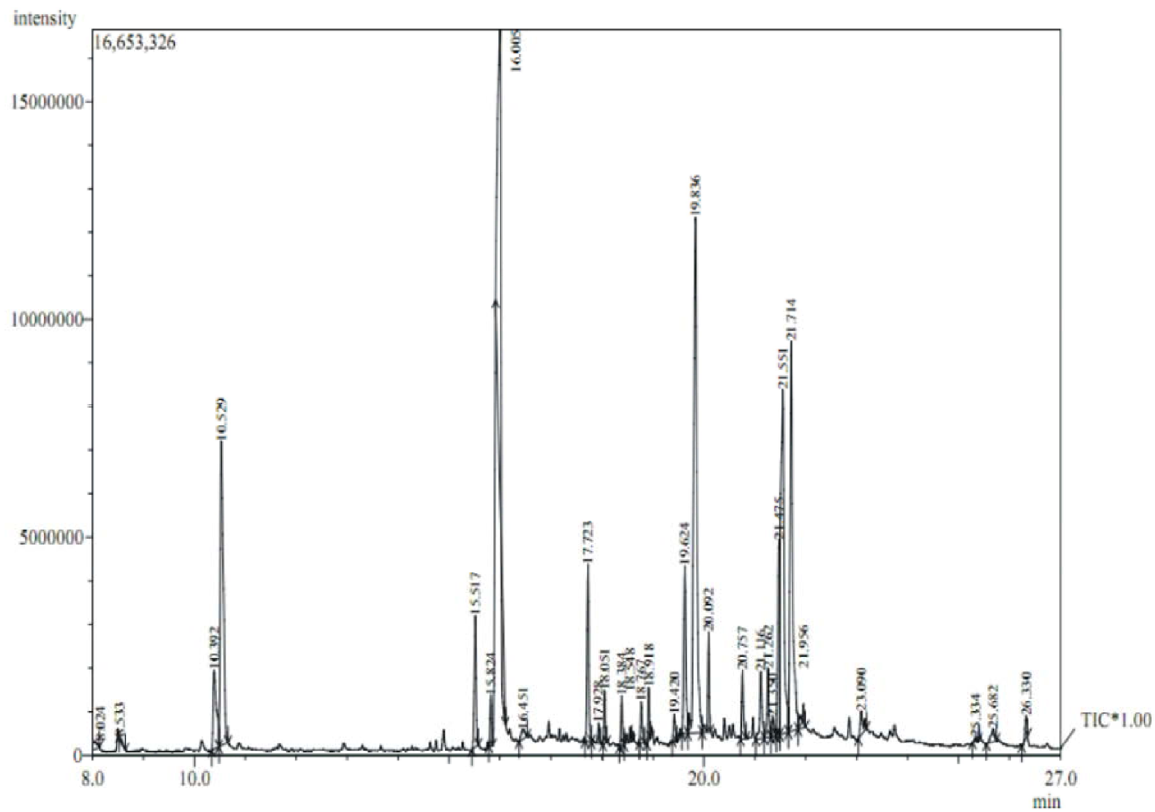


Fig. 4: Fatty acid Profiles of Experiment IV (*Thermocyclops hyalinus* fed with *S. maxima*)

in Experiment-II (98.85%) and low in Experiment-I (5.43%). Methionine was found same level in all the experiments. The level of Phenylalanine found high in Experiment-II (2.62%) and low in Experiment - I (1.32%). Threonine values were high in Experiment- III (6.47 %) compared to Experiment- IV (4.49%). Tryptophan are highly noted in Experiment - III (8.52%) and low in Experiment I (5.49%) respectively. The Valine values found meagerly same in all the experiments.

Fatty Acids Profile: The fatty acid profiles of Copepods were analyzed by GC-MS. The saturated fatty acids like Behenic acid C22:0 were found high in Experiment- II with 3.20% and low in Experiment- I (1.60%). The Capric acid C8:0 were high in Experiment- III (2.86%) and low in Experiment- I (1.77%). The Caproic acid C8:0 presented high in Experiment- II with 2.25 % and 0.47 in Experiment- I. The high amount of Caprylic acid C8:0 were recorded in Ex- II with 4.57% and low in 1.79 % in Experiment- I. Myristic acid C14:0 recorded high in Experiment- II (5.87%) and low in Experiment- I with 4.78% respectively. The level of Palmitic acid C16:0 found high in Experiment- II (6.23%) and low in Experiment- I (3.72%). The values of Stearic acid C18:0 was high in Experiment- III (3.74%) and (1.60%) low in Experiment- I. The Lauric acid C12:0 shows high in Experiment- II and (10.53%) low in Experiment- IV (Figures 1-4).

Monounsaturated fatty acid like Palmitolic acid C16:1 is highly found in Experiment- II with 5.55% and low in Experiment- I with 3.72%. The Oleic acid values were high in Experiment- III 3.74 % and low in Experiment- I with 1.34%. Polyunsaturated fatty acids like Linoleic acid C18:2 (n-6) found high in Experiment - II with 9.69% and low in Experiment- I (7.97%). Likewise the Arachidonic acid C20:4 values were high in Experiment- II with 8.885 and very low in Experiment- I (3.93%). The Linolenic acid C18:3 were presented high amount in Experiment- III with 7.12% and low in Experiment- I (3.92%).

DISCUSSION

Aquaculture have vital role in many countries by offering better nutrition and source of income [28]. Copepods can meet the nutritional requirements of fish larvae [29]. Hernandez Molejo and Alvarez-Lajonchere [30] suggested that the Copepods offer a great variety of sizes, species and qualities and have high levels of protein, highly unsaturated fatty acids (HUFA), carotenoids and other essential compounds.

Joaquin macias- Sancho *et al.* [31] stated that Microalgae possess high protein content and amino acid profiles comparable to those of other reference food protein. In the present study the biochemical properties of Spirulina were found to be high in protein when compared to Chlorella and Spirogyra. Bab Capelli *et al.* [32] suggested that Spirulina contains about 60% complete digestible protein, contains all essential amino acids, more beta-carotene than any other whole food. Chlorella contains the highest amount of chlorophyll of any known plant. It is a nutrient-dense super food that contains 60% protein, 18 amino acids, vitamins and minerals. Chlorella had a better activity in inhibiting lipid peroxidation as compared to glutathione and has antioxidant properties [33].

Copepods are nutritionally suitable for marine fish larvae [34] and constitute a large percentage of the natural diet of fish larvae [35]. When compared to Artemia nauplii and rotifers, various species of copepods offer different size ranges of nauplii suitable for the first feeding of many marine finfish larvae, which have small mouth gape. Copepods are the only acceptable sized prey for small larvae of ornamental fish species [36].

Carbohydrate is a cheap source of energy in the diet of animals, including fishes. If a large percentage of the metabolic energy requirements of the animal can be met from the carbohydrate, it have the potential for delivering a low cost source of energy that could spare protein for growth. Lipids are essential components of cell membranes, serve as carriers for fat soluble vitamins and are the source of essential fatty acids. Lipid deposition during maturation is crucial to reproduction and early larval development because lipids are known to play several essential roles in metabolism of crustaceans [37].

In the present study the nutritional profiles of Copepod were increased by providing algal diets. Fatty acids are important components of biomembranes in fish, as well as providing a source of energy [38]. Microalgae are the primary producers of FAs, while zooplanktons form a central link between them and higher trophic levels. Joseph *et al.* [39] mentioned, certain omega 3 and 6 Polyunsaturated fatty acids (PUFAs) are nutritionally essential because all vertebrates and most invertebrates lack the enzymes necessary to desaturate Oleic acid to the analogous omega3 and omega6 fatty acids.

From the present study the biochemical composition and nutritional profiles were analyzed in Copepod *Thermocyclops hyalinus*. From this study we found that biochemical properties, fatty acid profile, amino acids, egg production ratio and growth performance all these were found high in *Thermocyclops hyalinus* fed with algae when compared to control. So from this study it is concluded that *Thermocyclops hyalinus* fed with Spirulina were the suitable species for larviculture.

CONCLUSION

In this study, the biochemical composition, egg production ratio, growth performance, amino acid profiles and fatty acid contents were significantly analyzed in all experimental organisms. The elevations of all these components were high in copepods fed with algal diet. Within the experimental setup all the nutritional profiles, growth performance, egg production found very high in *T. hyalinus* fed with *S. platensis*. Hence, the present results revealed that the *T. hyalinus* fed with *S. platensis* is a best suitable candidate for practical aquaculture. Number of trials is required to improve the nutritional profiles of cyclopoid copepods with different algae to improve successful freshwater larviculture and various species of cyclopoids are yet to be studied to analyze its nutritional potential as live feeds.

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Conflict of Interest Statement: We declare that we have no conflict of interest.

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