Growth performance of marine microalgae *Tetraselmis chuii* in different media for indoor culture system

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By

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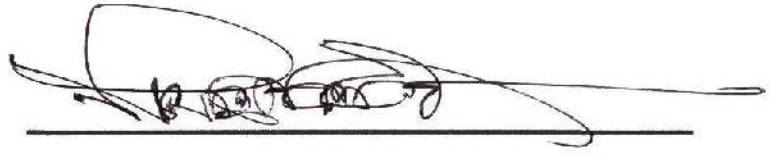

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November, 2015

Declaration

Growth performance of marine microalgae *Tetraselmis chuii* in different media for indoor culture system

The results submitted in this thesis are entirely of the candidate's own investigations and no part of the results has been accepted for any degree, nor is it being concurrently submitted for any degree.

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Dedicated to

My Beloved Parents

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At first I want to admire the Almighty, the supreme creator and ruler of the universe from the deepest corner of my heart for His blessing that keeps me alive and enable me to accomplish this work successfully.

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Abstract

The growth performance of *Tetraselmis chuii*, was studied using three nutrient media viz. (i) Walne's or Conway, (ii) TMRL and (iii) Miquel's, designated as Treatment-1 – 3 (T-1 -3) respectively, at laboratory condition for 41st days at 1600-2000 Lux light intensity. The culture was conducted in 1L transparent plastic bottle with an initial cells density of 10×10^2 cells /ml. with three replicates of each treatment maintaining constant temperature and salinity of 25°C and 30 ppt respectively with continuous aeration and illumination. *T. chuii* cultured in T-2 showed significantly ($P < 0.05$) higher growth with a mean peak density ($169.00 \pm 1.36423 \times 10^2$ cells/ml), on the 22nd day of culture, than that of T-1 ($150.44 \pm .52997 \times 10^2$ cells/ml) and T-3 ($127.11 \pm 1.01986 \times 10^2$ cells/ml) on 16th and 23rd day respectively. Stationary growth phase among the Treatments varied from 16 to 27 days (T-1: 16-27 days; T-2: 16-23 days and T-3: 18-26 days) after which declining phase started and very few number of individual found alive at 41st day of culture. It resembles that TMRL media is suitable for the culture of *Tetraselmis chuii.*

Key Words: *Tetraselmis chuii*, Growth, Nutrient media and Stationary growth phase.

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List of Abbreviations

Abbreviation	Meaning
%	Percentage
et al.	Et alia
°C	Degree Celsius
sp.	Species
L	litre
mL	millilitre
mg	milligram
g	gram

CHAPTER ONE

INTRODUCTION

Live food organisms include all plants (phytoplankton) and animal (zooplankton) lives grazed upon by economically important fishes. Phytoplanktons are generally eaten by zooplankton. Thus, phytoplankton forms the basis of the food chain. Live foods are able to swim in water column and are constantly available to fish and shellfish larvae are likely to stimulate larval feeding response (David, 2003). In an aquatic ecosystem, these live food organisms constitute the most valuable resource for aquaculture. Most of the fish and shellfish larvae in nature feed on small phytoplanktonic and zooplanktonic organisms. However, natural fish food organisms are usually not abundant in clear pond water, but are abundant in ponds having greenish water. The green colour indicates the presence of phytoplankton and other natural food organisms.

In the natural food web, zooplankton constitutes a major part of the diet for marine fish larvae and it is generally believed that copepods can meet the nutritional requirements of fish larvae (Evjemo *et al.* 2003). Microalgae are utilized as live feed for all growth stages of bivalve mollusks, for the larval/early juvenile stages of abalone, crustacean, some fish species and zooplankton in aquaculture food chains. Over the last four decades, several hundred microalgae species have been tested as food, but probably less than twenty have gained wide spread use in aquaculture. The past few years have witnessed considerable activities relating to the production of microalgae for commercial purposes. From a modest beginning in the late fifties, new endeavors have emerged as specialized industries are aiming to produce animal feed, food additives, bio fertilizers and different natural products using microalgae (Danquah *et al.* 2010). Microalgae must possess a number of key attributes to be useful as aquaculture species (Kawamura *et al.* 1998).

In nature, zooplankton is one of the primary foods of fish larvae. Two of the dominant zooplankton groups are Rotifera (rotifers) and Copepoda (copepods). These two groups are the preferred prey for shrimp and fish they are the most widely used live feeds by aquaculturists. The intensive larval culture of most marine fish depends on a large supply of zooplankton. Larvae are first fed on a small strain of rotifers, and as larvae increase in size, a larger strain of rotifers is introduced. Rotifers are regarded as living food capsules for transferring nutrients to fish larvae. These nutrients include highly unsaturated fatty acids (mainly 20: 5 n−3 and 22: 6 n−3) essential for survival of marine fish larvae (Lubzens *et al.* 1998). In addition, rotifers treated with antibiotics may promote higher survival rates. Rotifers have broad nutritional requirements that must be met to produce stable cultures. They are planktonic filter feeders, feeding on organic particles brought to their mouths by the movements of their coronas (Dhert *et al.* 2001). Most production facilities use 10 to 20 ppt

salinity (Dhert *et al.* 2001). The rotifer, *Brachionus plicatilis*, can be mass cultivated in large quantities and is an important live feed in aquaculture. This rotifer is commonly offered to larvae during the first 7–30 days of exogenous feeding (Dhert *et al.* 2001).Live food may enhance the digestive processes of larval predators. A large range of genetically distinct *B. plicatilis* strains with a wide range of body size permit larval rearing of many fish species.

Over the last two decades, shrimp farming has emerged as a major industry in Bangladesh (Alauddin *et al.* 1995 shrimp). Shrimp farming on a large scale in the coastal regions of Bangladesh has created a new employment structure (Alauddin *et al.* 1995). Shrimp farming and related activities contribute significantly to the national economy of Bangladesh. Successful shrimp culture depends mainly on the availability of healthy and quality shrimp seeds (Islam *et al.* 2011 shrimp 3) .The production of quality seeds and their survivability mostly depends on the live feed they are to feed at their larval stage. Since no artificial feed formulation for first feeding of marine larval fish has been developed yet, live prey feeding remains essential in commercial marine hatchery operations (rotifer). Larviculture, more particularly the start feeding of early larval stages, appears to be the major bottleneck for the industrial up scaling of the culture of fish and crustaceans. Evolutionary, larvae of most fish and crustaceans are fixed on the scheme of motile prey organisms and encounter problems to accept inert dry diets. Even if they accept the diets, their poor enzymatic activity and non-functional stomach will not allow them to digest the existing formulated diets (Pedersen *et al.* 1987; Pedersen and Hjelmeland, 1988). Improving the acceptance of dried diets for fish larvae and formulate more digestible and less polluting diets thus still remain a central task for aquaculturists. Before this is achieved, live food (phyto and zooplanktons) will remain an important food source for the start feeding of early larval stages. One of the important starter feeds used in larviculture is the marine rotifer *Brachionus plicatilis*.

The successful development of commercial farms in the Mediterranean area has been made possible by several improvements in the production techniques of this live food (Candreva *et al.* 1996; Dehasque *et al.* 1998).The nutritional aspects of rotifers have received major attention in larviculture and several commercial products have been launched to increase the lipid and vitamin content in rotifers (Coutteau and Sorgeloos, 1997). Although it is technically possible to produce rotifers of high nutritional value, their quality is often far from optimal due to their low hygienic quality and overall condition (low swimming velocity, low reproduction rate, etc.). In this respect, the microbiology involved in rotifer rearing is getting increased attention (Rombaut *et al.* 1999). Microalgae have an important role in aquaculture as a means of enriching zooplankton for feeding fish and other larvae. In addition

to provide protein (essential amino acids) and energy, they provide other key nutrients such as vitamins, essential polyunsaturated fatty acids (PUFA), pigments and sterols, which are transferred through the food chain. Importance of micro algae in aqua hatcheries not only owes its nutritional attributes but more so for its small size ranging from 5 to 25 microns meeting the feed size requirements ideally well for early stages of various aquatic animals. Today, micro algae is used as an essential food source for rearing all stages of marine bivalve molluscs (clams, oysters, scallops), gastropods (abalone, conch), larvae of fishes (cod, halibut, tilapia) and shrimps (*Penaeus* sp.). Micro algae are frequently supplied together with rotifers during first feeding of marine larvae. This technique has normally enhanced survival as well as growth.

Two types of live feeds have been used for rotifer production; live algal cells such as *Nannochloropsis oculata, Tetraselmis tetrathele* and *Chlorella vulgaris*, and a supplementary food such as baker's yeast (Maruyama *et al.* 1997). Importance of microalgae (*Tetraselmis* sp.) as larval food is also because it stimulates enzymatic synthesis and on-set of feeding in young larvae. Besides, it also acts as water conditioner by stripping off the nitrogenous substances. *Tetraselmis* sp. biomass is obtained from a renewable source, does not require a large area of land for cultivation, possesses a high growth rate and accumulates a satisfactory amount of nutrient for rotifer production (Danquah *et al.* 2010). So it is highly feasible to study on the growth performance of *Tetraselmis* sp. in aspect of Bangladesh as it is used as one of the most popular feed for the zooplankton (rotifer, copepod etc.) those are used as a larval feed for shrimp culture. Considering the above issue, the present study is inevitable to know the growth performance of *Tetraselmis* sp.

1.1 Objectives of the study

The long-term objective of the study is to gain the optimum growth performance of microalgae (*Tetraselmis* sp.). However, the objective of this study is to:

- Study the growth performance of *Tetraselmis* sp. in different media
- Find out a suitable media for optimum growth of *Tetraselmis* sp.

A considerable works have been done on the culture of microalgae in different parts of the world. In the present study efforts were made to review a few available literatures. The following are some of the literatures relevant to the present study.

The study aimed to evaluate the effect of nutrient depletion during the cultivation of the microalgae *Tetraselmis tetrathele*, and to verify the algal growth and productivity in different culture media (Guillard f/2 and Conway) was done by Coelho *et al.* (2013). The cultivation in Conway medium presented better results concerning microalgae growth, with a more pronounced exponential phase. This was due to the higher nitrate availability, which was reduced more rapidly in the culture medium Guillard f/2.

Growth and multiplication of three algae, *Isochrysis galbana, Tetraselmis chuii* and *Nitzschia dosteritim,* belonging to three different algal classes, was studied in the laboratory, using four culture media. The algae yielding different growth rates in the media, the experiment proved that no available culture medium is uniformly effective in all the cases, unless supplemented with either trace elements or vitamins, according to the specific requirement of the algae according to Gopinathan (2012).

Surface water samples and sediments were sampled during the Brernerhaven Workshop at 9 locations on a transect from the inner German Bight to the Dogger Bank. Two species (*Isochrysis galbana* and *Tetraselmis suecica*) of algae were grown in the water samples and sediment elongates prepared from the sediments. In both type of samples algal growth over 5 day was highest in the inshore samples and a graded response in growth was observed over the 5 inshore stations according to Thain, J. (1992).

Phytoplankton culture is a multi-faceted activity, and the task of designing a large-scale microalgal production system can be complex. The size, location, and engineering specifications should depend on the type of culture to be practiced (e.g., batch, semi-continuous, continuous), the site characteristics (such as water quality and temperature), illumination, the requirements of the target species, and production goals. These considerations are interdependent, and each should be treated as a part of the whole (Creswell, 2010).

Raya *et al.* (2015) estimated on chlorophyll productivity and potency of hydrogen produced by phytoplankton *Chaetoceros calcitrans, Chlorella vulgaris, Dunaliella salina, and Porphyridium cruentum.* Conway medium and vitamin was added with sea water as culture

media on the four species of phytoplankton. The result indicated that phytoplankton *C. vulgaris* produced the most amount of the chlorophyll and the potency of hydrogen are 72.541 µg/ml and 6364 L/kg DW successively from the dry biomass 14187 g and the higher cells density 3.060 x 10^4 cells/ml. The amount of hydrogen indicated that *C. vulgaris* has potential as the source of renewable energy.

Shah *et al.* (2003) discussed on the growth of three microalgae species, *viz., Nannochloropsis oculata, Tetraselmis chuii* and *Chaetoceros muelleri* which are commonly used in aquaculmre using three different inorganic nutrient media: (i) Modified Guillard's f/2 medium (ii) Rix Mix medium and (iii) BFRI medium. Each microalgae species was cultured for 24 days in small- scale with initial inoculation density of 17 × 10^4 cells /ml in the three media with triplicates. *N. oculata* cultured in modified Guillard's f/2 medium showed superior growth with a mean peak density of 221 ± 4.24 × 10^4 cells/ ml. *C. muelleri* attained the highest density of 321 ± 5.41 × 10^4 cells/ml in Rix Mix medium and about same density (319 ± 12.02 × 10^4 cells/ml) at the 24th day of culture in modified Guillard's f/2 medium. However, with an increase in cells density, growth of *T. chuii* (182 ± 6.26 × 10^4 cells/ml) was significantly ($P < 0.05$) higher in BFRI medium.

Chen *et al.* (2012) studied on four species of microalgae evaluating their ability to remove ammonia from intensive marine fish/shrimp culture systems at different temperatures. Total ammonium nitrogen (TAN) uptake efficiencies of these organisms at different temperatures were 10.3 pg dry weights (DWs) per cells for *Nannochloropsis oculata*, 40.4 pg. for *Isochrysis* aff. *galbana*, 39.7 pg for *Chaetoceros muelleri*, and 369 pg for *Tetraselmis chui* at 25°C. Temperatures for maximal biomass production were 30°C for both *Nannochloropsis oculata* and *Isochrysis* aff. *galbana*, 35°C for *Chaetoceros muelleri*, and 20~30°C for *Tetraselmis chui.* Overall, *Tetraselmis chui* is a good choice for removing ammonia from indoor intensive marine culture systems.

Okauchi *et al.* investigated on ammonium N (NH -N) and phosphate P (PO -P) uptake by unicellsular algae as a method for removing excessive nitrogen (N) and phosphorus (p) from larval shrimp rearing water and evaluated the feasibility of using algae to manage the water quality. *Tetraselmis tetrade, Nannochloropsis oculata, Isochrysis* sp. and *Chaetoceros gracilis* were considered to be suitable algae to keep the N and P content of the rearing water low. However, a mixed feeding of *N. oculata* and an artificial diet provided better growth and higher survival rate of larvae than did each of them separately. Although the algae had low

nutritional value for the larvae, adding it to the rearing water was useful in keeping the N and P content low and improving the survival rate of shrimp larvae.

Ritcharoen *et al.* (2014) studied on configurations and modes of airlift photobioreactors used in the cultivation of *Chaetoceros gracilis*. Internal loop and external loop airlifts were cultivated in a batch mode in a controlled indoor environment. The external loop system provided a better performance than the internal loop system due to better light exposure. Outdoor large-scale operation was conducted in a flat-panel airlift photobioreactor. Among the four systems investigated, the continuous culture in airlift photobioreactors-in-series provided the best performance with the highest cells density of 12.12×10^6 cells mL^{-1} incorporated with large productivity.

Lincymol *et al.* (2012) reported that the microalgae (*Chaetoceros calcitrans*) cultured in Walne's medium gave high values of protein and lipid content. Miquel's gave high value of carbohydrate .Although cells count was the highest in f/2 medium, Walne's medium provided the best nutritional quality of algae.

Nezhad *et al.* (2012) showed that maximum growth rate of *C. calcitrans* was observed in 10 ppt salinity in Conway medium and highest cells density was achieved at 27 ppt salinity. The highest level of total carotenoid content was 5.8 mg/g fresh weight and the maximum protein content was at the 10 ppt in 60% of dry weight.

Cordova *et al.* (2006) described the effects of antibiotics on microalgae used as food for scallop larvae. Six different dose levels of chloramphenicol, erythromycin, and furazolidone were added to cultures of *Isochrysis galbana* and *Chaetoceros gracilis*. *C. gracilis* was significantly sensitive to erythromycin and chloramphenicol at doses higher than 0.5 and 3.0 mg/l, respectively. Furazolidone inhibited the growth of both *I. galbana* and *C. gracilis* at all test doses. Chloramphenicol, erythromycin and furazolidone had adverse effect on *I. galbana* and *C. gracilis* microalgae but the positive effect on survival of the scallop larvae, decreasing bacterial population.

It was reported by Hassan *et al.* (2015) that the optimum light intensity, temperature, pH and salinity range for the indoor culture of *Tetraselmis* sp. are about 4000 lux, 24°c to 26°c, 8.2 to 8.7 and 30 ppt.

CHAPTER THREE

MATERIALS and METHODS

3.1. Study Area

The culture of the microalgae was done in a temperature controlled (about 25°C) microalgae laboratory at Fisheries and Marine Resources Technology (FMRT) discipline of Khulna University, Khulna. The culture was maintained in 1 liter transparent mineral water bottles with sufficient aeration. The brackish water of 30 ppt was treated, for disinfection, with 30 ppm active chlorine by dissolving bleaching powder at the rate of 100 mg/L and kept for 24 hours. Then vigorous aeration for 6 hours was done to eliminate residual chlorine from the water. The treated water was filtered through double rings 102 filter paper 12.5 cm qualitative (New, 2008). This disinfected water was used with different nutrient media (Table-2) to prepare different culture media viz. Walne or Conway medium, TMRL medium and Miquel's medium. The algal cells were inoculated into the bottles with equal initial concentration (10×10^2 cell/ml) for the species of microalgae. Aeration was moderate and the source was aquarium aerators. The bottles were set up approximately 15 cm from the two 'daylight' fluorescent tube light sources with a light intensity of about 1600-2000 lux / m^2/ S.

3.2. Duration of Study Period

The duration of the study period was 41 days.

3.3. Sampling Frequency

Samples were collected every day for three times.

3.4. Sample collection

The Sample (*Tetraselmis sp.*) was collected from the Marine Fisheries and Technology station, Cox's Bazar of Bangladesh Fisheries Research Institute (BFRI). Brine (100 ppt) was collected from salt pan or commercial prawn hatchery.

3.5. Experimental Design

The data were taken very carefully every day. The water sample for measuring plankton abundance was collected at morning (10.00 am). The number of treatment was 3. Each treatment had 3 replications. Each replication had triplicate.

Table 1: Layout of the Experiment.

Treatment	Bottle no	Replication
Conway medium (T1)	1	T1R1
		T1R1
		T1R1
	2	T1R2
		T1R2
		T1R2
	3	T1R3
		T1R3
		T1R3
TMRL medium (T2)	1	T2R1
		T2R1
		T2R1
	2	T2R2
		T2R2
		T2R2
	3	T2R3
		T2R3
		T2R3
Miquel medium (T3)	1	T3R1
		T3R1
		T3R1
	2	T3R2
		T3R2
		T3R2
	3	T3R3
		T3R3
		T3R3

3.6. Preservation of sample

Collected samples were preserved with Lugol Solution (20g potassium iodide (KI) and 10g iodine crystals dissolved in 200 mL distilled water containing 20 mL glacial acetic acid). Lugol solution was added in an amount of 0.7 mL per 100 mL of sample. The sample with solution was preserved at 4^0c in refrigerator.

3.7. Necessary equipment

Light, pH meter, aerator, autoclave, flask (Erlenmeyer), microscope, foil paper etc. (New, 2008).

Table 2: Various prevalented culture media for the stock culture of microalgae.

Ingredients	Amount of Ingredients		
	Miquel's medium	TMRL medium	Walne'so r Conway medium
A. Potassium nitrate	20.2 gm	l0.gm	100 gm
Sodium orthophosphate	-	1.0 gm	20 gm
EDTA (sodium salt)	-	-	45 gm
Boric acid	-	-	33.4 gm
Ferric chloride	-	.3 gm	1.3 gm
Manganese chloride	-	-	0.36 gm
Distilled water	100ml	100ml	1 lit
B. Calcium chloride	2.0 gm	-	-
Ferric chloride	2.0 gm	-	-
Hydrochloric acid	2.0 ml	-	-
Sodium silicate	-	.1 gm	-
Distilled water	100 ml	-	1 lit
Zinc chloride	-	-	4.2 gm
Cobalt chloride	-	-	4.0 gm
Ammonium molybdate	-	-	1.8 gm
Sodium orthophosphate	4.0 gm		
C.1.Vitamin B_1 (Thiamine HCL)	-	-	200mg dissolved in 100ml dist. Water
2.VilamineB_{12} (Cyanacobalamine)	-	-	10 mg dissolved in 100ml dist water

(Reference: Kaladharan and Tara, 1997)

3.8. Counting Cell Preparation and Counting Procedure

The quantitative enumeration of the plankton was carried out with the help of a Sedgewick-Rafter (S-R) counting cell which is 50 mm long, 20 mm wide and 1 mm deep. Before filling the S-R cell with sample, the cover glasses were diagonally placed across the cell and then samples were transferred with a large bore pipette so that no air bubbles in the cell covers were formed. The S-R cell was let stunned for at least 15 minutes to settle plankton. Then plankton on the bottom of the S-R cell was enumerated by electronic microscope. By moving the mechanical stage, the entire bottom of the slide area was examined carefully. To achieve a random sampling, each time 3 fields were examined for each sample and an average of the counts had been recorded. The organisms thus counted, were expressed as cells per liter of the sample. From each sample 30 cells counts in 3 slides have been made to achieve random counts and an average of the counts has been recorded. Number of plankton

(phytoplankton & zooplankton) in the S-R cell was derived from the following formula.

$$No/ml = \frac{C \times 1000\ mm^3}{L \times D \times W \times S}$$

Where, C = Number of Organisms Counted; L = length of each strip (S-R cell length) in mm; D = depth of a strip (whipple grid image width) in mm; W = Width in mm, S = number of strips counted. The number of cells per mm was multiplied by a correction factor to adjust the number of organisms per liter (APHA, 1976).

3.9. Microalgae Identification

Tetraselmis chuii was identified by using checklists of different scientists.

3.10. Statistical analysis

Statistical analysis was performed by using SPSS Software Package Version 16.0 (SPSS Inc., Chicago, IL, USA). The experimental data was not normally distributed. The validity of all the data was tested through different statistical package (Square, Square root, Cubic root, Reciprocal root and Reciprocal Square root) associated with R software. The data was never transformed normally. Then we used Box Cox transformation method to normalize the data. Finally the model and residues of the model showed the distribution of the data normally. All values were expressed as mean ± standard error (SE). Data were analyzed using one-way analysis of variance (ANOVA) and in order to determine the statistical difference between media, the Tukey test was used. A value of $P < 0.05$ was considered statistically significant (Durmaz *et al.* 2007 & Pal *et al.* 2011).

CHAPTER FOUR

RESULTS

4.1. Growth performance of *Tetraselmis chuii* in Conway medium

The total culture period of *Tetraselmis chuii* was forty one days in three culture media viz. Conway, Miquel's and TMRL. The initial cell density was 10×10^2 cells/ml at the time of stocking (inoculation density at initial day). The highest density of *Tetraselmis chuii* in Conway medium was observed $150.44 \pm 0.52997 \times 10^2$ cells/ml at the 16th day of culture period, beginning of the stationary phase and the stationary phase found to continue up to 27th day of culture period (Fig. 1) that showed no significant difference ($P > 0.05$) among these days (day-18 to 27). Finally the declining phase started at 28th day and at 41th day of culture period (declining phase) some species was found still alive ($5.6667 \pm 0.28868 \times 10^2$ cells/ml) although it showed gradually declining trend in growth with significant difference ($P < 0.05$) among the days (day-28 to 41).

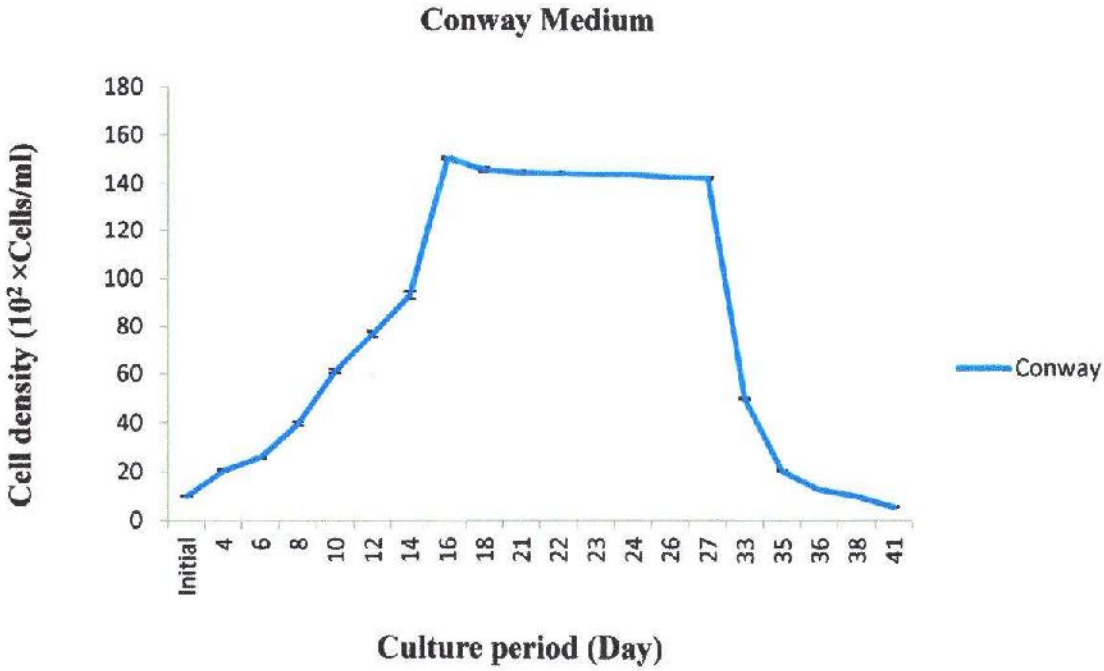

Figure 1: Growth performance of *Tetraselmis chuii* in Conway medium at different time interval.

4.2. Growth performance of *T. chuii* in TMRL medium

In TMRL medium the initial cell density of *T. chuii* was same cells/ml as like as other two tested media at the zero day in the total forty one day of culture period. In this medium cell density of *T. chuii* has increased within short time than the cell density of above media and stationary phase was started at 16th day of culture period that was extended to 23rd day that showed no significant difference ($P > 0.05$) among these days (day-16 to 23). The highest cell density 169.00 ± 1.36423 × 10^2 cells/ml was found at the 22nd day of culture period for *T. chuii* (Fig: 2). Finally the declining phase started at 24th day and at 41st day of culture period (declining phase) some individual was found still alive (6.7778 ± 0.36430 × 10^2 cells/ml) although it showed gradually declining trend in growth with significant difference ($P < 0.05$) among the days (day-24 to 41).

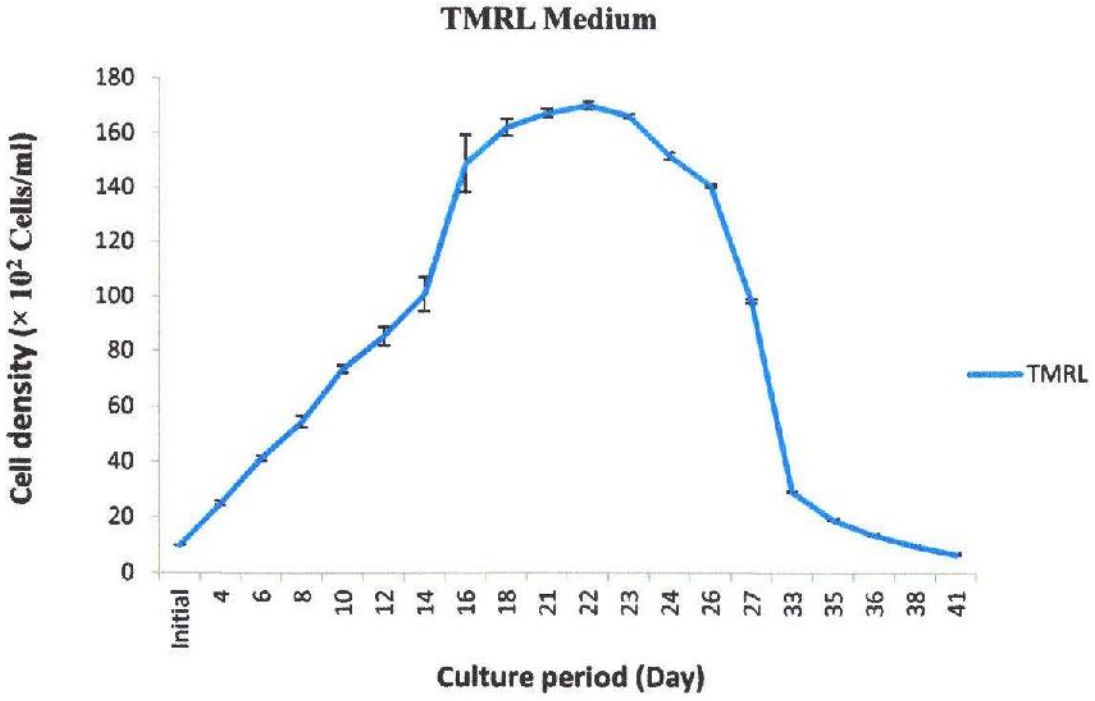

Figure 2: Growth performance of *T. chuii* in TMRL medium at different time interval.

4.3. Growth performance of *T. chuii* in Miquel's medium

In Miquel's medium the initial cell density of *T. chuii* was 10×10^2 cells/ml at the initial day in the total forty nine of culture period. The peak density ($127.11 \pm 1.01986 \times 10^2$ cells/ml) of *T. chuii* in Miquel's medium was found at the 23rd day of culture period after stationary phase and the stationary phase (lowest cell density $123.11 \pm 0.56383 \times 10^2$ cells/ml and highest cell density $127.11 \pm 1.01986 \times 10^2$ cells/ml) was found from 18th to 26th days of culture period (Fig. 3) that showed no significant difference ($P > 0.05$) among these days. Finally the decline phase started at 27th days and at 41st day of culture period (declining phase) some individual was found still alive ($4.0000 \pm 0.28868 \times 10^2$ cells/ml) although it showed gradually declining trend in growth with significant difference ($P < 0.05$) among the days (day-27 to 41).

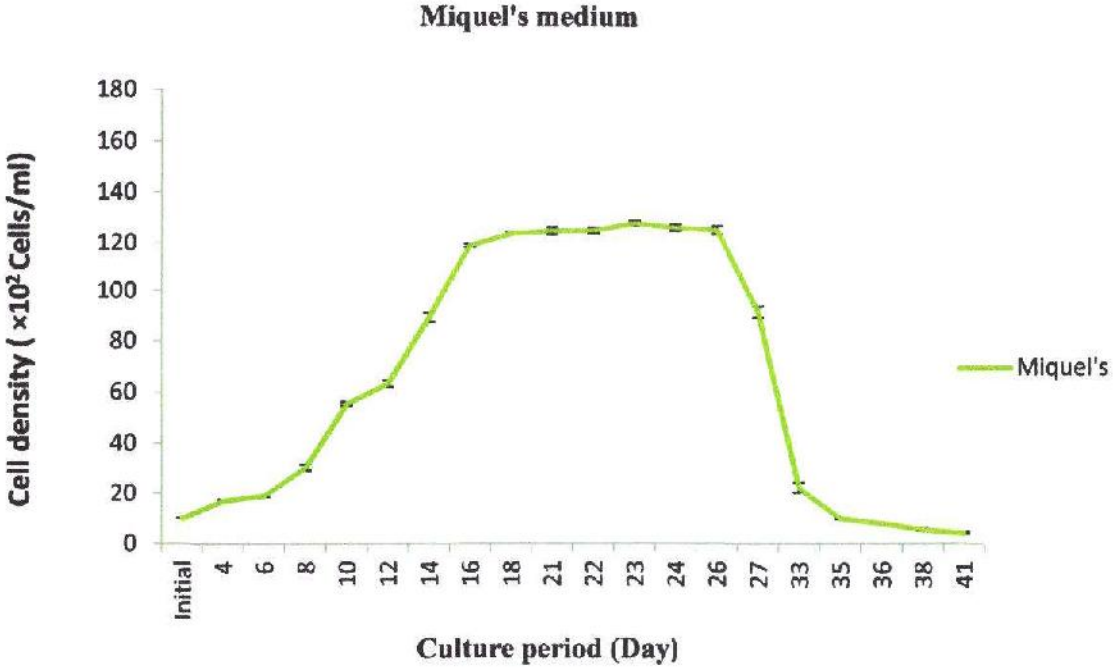

Figure 3: Growth performance of *T. chuii* in Miquel's medium at different time interval.

4.4. Growth performance *Tetraselmis chuii* in three culture media (viz. Conway, Miquel's and TMRL).

The stationary growth phase of the species among the treatments was observed to start at different days of stocking and the duration of the phase among the treatments also varied. The earliest stationary phase was observed to achieve in TMRL and Conway media that started at the 16th day of culture and sustained for 8 and 12 days in respect of respective medium. The longest duration of the stationary phase of the species was observed in Conway medium that started at 16th day and sustained for 12 days (27th day) of culture period. The shortest stationary phase was observed in TMRL medium that started at 16th day of culture and sustained for 8 days (23rd day). The stationary phase of the species was observed in Miquel's medium that started at 18th day and sustained for 9 days (26th day) of culture period. At 41st day of culture period, at the end of the experiment in the declining phase, a considerable number of *T. chuii* was found alive irrespective of any media.

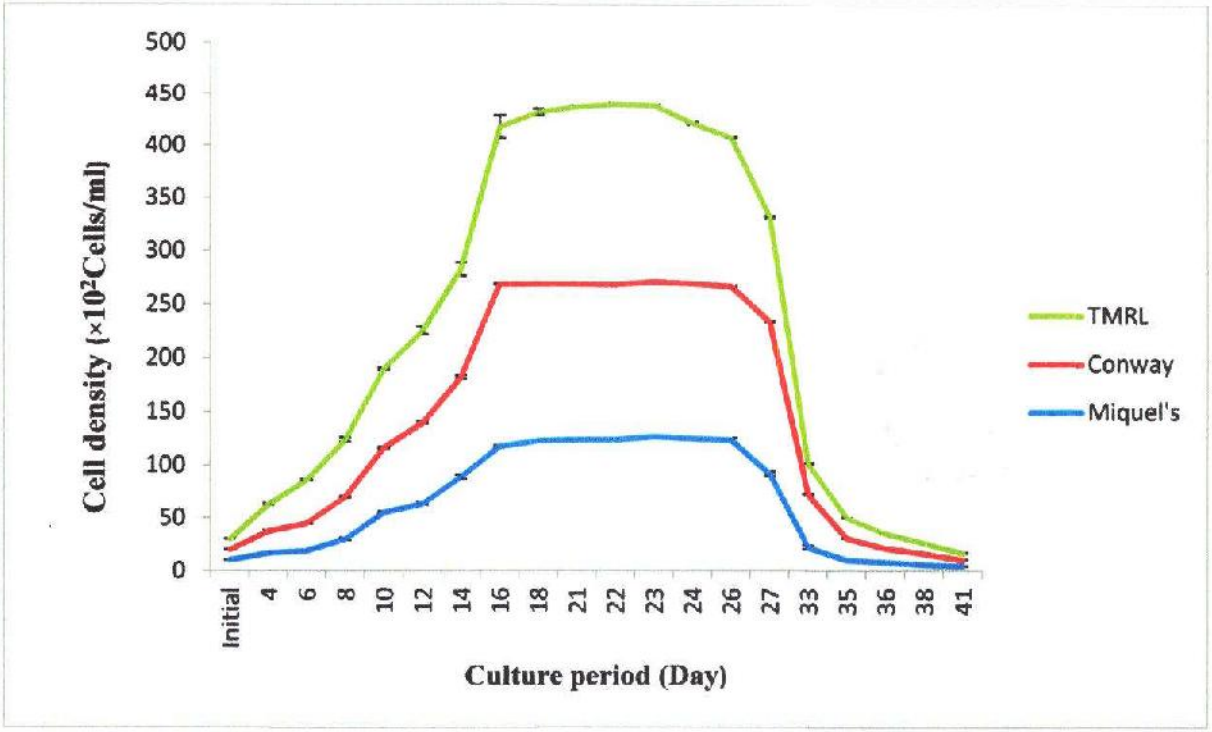

Figure 4: Growth performance *Tetraselmis chuii* of in three culture media (viz. Conway, Miquel's and TMRL) at different time interval.

CHAPTER FIVE

DISCUSSION

Table 3: Growth performance of *Tretraselmis chuii* in different media at different time interval (× 10^2 cells/ml).

Day	Treatment (Media)		
	Treatment-1 (Conway)	Treatment-2 (TMRL)	Treatment-3 (Miquel's)
	Mean ± Std. Error	Mean ± Std. Error	Mean ± Std. Error
Initial	10± 0	10±0	10±0
Day 4	20.7778^{b} ± .46481	25.1111^{a} ± .71578	17.0000^{c} ± .50000
Day 6	26.2222^{b} ±.43390	41.3333^{a} ± .91287	19.0000^{c} ± .40825
Day 8	40.0000^{b} ±.79931	54.4444^{a} ± 2.06902	30.2222^{c} ± 1.21081
Day 10	61.3333^{b} ± .78174	73.4444^{a} ± 1.31351	55.5556^{c} ± .78371
Day 12	76.7778^{b} ± 1.1758	85.1111^{a} ± 3.36008	63.4444^{c} ± 1.3028
Day 14	93.1250^{a} ± 1.57477	100.67^{a} ± 6.28490	89.1111^{a} ± 1.93968
Day 16	150.44^{b} ± .52997	168.44^{a} ± .60093	118.11^{c} ± .71578
Day 18	145.78^{b} ± .99691	161.89^{a} ± 3.14221	123.11^{c} ±.56383
Day 21	144.67^{b} ± .74536	167.11^{a} ± 1.56742	124.11^{c} ± 1.56742
Day 22	144.22^{b} ± .81271	169.00^{a} ±1.36423	126.22^{c} ± 1.30998
Day 23	143.78^{b} ± .75971	166.11^{a} ± .71578	127.11^{c} ± 1.01986
Day 24	143.89^{b} ± .51220	151.33^{a} ± 1.29099	125.22^{c} ±1.39222
Day 26	142.67^{a} ± .28886	140.22^{a} ± .52116	124.00^{b} ± 1.69148
Day 27	142.22^{a} ± .46481	98.0000^{b} ± .78174	91.4444^{c} ± 2.10232
Day 33	50.5556^{a} ± .29397	29.2222^{b} ± .36430	21.8889^{c} ± 1.90354
Day 35	20.7778^{a} ±.27778	19.3333^{b} ± .28868	10.0000^{c} ±.33333
Day 36	13.2222^{a} ± .40062	1.20185^{a} ± .40062	8.0000^{b} ± .28868
Day 38	10.3333^{a} ± .16667	9.8889^{a} ± .38889	5.5556^{b} ± .68943
Day 41	5.6667^{b} ± .28868	6.7778^{a} ±.36430	4.0000^{c} ± .28868

Values are mean ±SE (n= 9); different letter superscripts in the same row show significant difference ($P < 0.05$).

Table 4: The Stationary phase of *T. chuii* in different media at different time interval (× 10^2 cells/ml).

Days	Stationary phase		
	Conway	TMRL	Miquel's
Day-16	150.44^{b} ± .52997	168.44^{a} ± .60093	
Day-18	145.78^{b} ± .99691	161.89^{a} ± 3.14221	123.11^{c} ± .56383
Day-21	144.67^{b} ± .74536	167.11^{a} ± 1.56742	124.11^{c} ± 1.56742
Day-22	144.22^{b} ± .81271	169.00^{a} ± 1.36423	126.22^{c} ± 1.30998
Day-23	143.78^{b} ± .75971	166.11^{a} ± .71578	127.11^{c} ± 1.01986
Day-24	143.89^{b} ± .51220	151.33^{a} ± 1.29099	125.22^{c} ± 1.39222
Day-26	142.67^{a} ± .28886	140.22^{a} ± .52116	124.00^{b} ± 1.69148
Day-27	142.22^{a} ± .46481		

Table 5: The duration of Stationary phase of *T. chuii* in different media.

Media	Stationary Phase	Total Day
Conway	16^{th} to 27^{th}	12
TMRL	16^{th} to 23^{rd}	8
Miquel's	18^{th} to 26^{th}	9

In this experiment, *Tetraselmis chuii* showed the best growth performance in TMRL medium ($169.00^{a} \pm 1.36423 \times 10^{2}$ cells/ml) at the 22nd day of the culture period. *T. chuii* has shown highest growth performance at BFRI medium among the three medium respectively Modified Guillard's f/2 medium , Rix Mix medium and BFRI medium according to Shah *et al.* (2003). *T. chuii* is less sensitive to environmental stress (Liao *et al.*, 1991), which could be responsible for the extended exponential growth phase of this species in all the media. This experiment was conducted at 30 ppt salinity, with 1600 to 2000 lux light intensity, 25^{0}c temperature and at 7.9 pH although the optimum light intensity, temperature, pH and salinity for *Tetraselmis* sp. are about 4000 lux, 25^{0}c, 8.2 to 8.7 and 30 ppt for the indoor culture of *T. chuii* (Hassan *et al*, 2015).

In this study three different media viz. Conway, TMRL and Miquel's were used for the culture of *T. chuii* where Treatment-2 (TMRL) showed significantly higher ($P < 0.05$) growth performance than the rest two at 4th to 12th day of culture period (Table-3). At the day of 14th of culture period, there were no significance differences among the three media for the growth performance of *T. chuii* (Annex-3). The maximum cell density of $169.00^{a} \pm 1.36423 \times 10^{2}$ cells/ml was with TMRL medium at the 22nd day of culture period where for Conway and Miquel's media the highest cell density were $150.44^{b} \pm 0.52997 \times 10^{2}$ cell /ml and $127.11^{c} \pm 1.01986 \times 10^{2}$ cell /ml respectively for 16th & 23rd day (Table-3).

Although Conway medium showed highest cell density at 16th day but it showed significant difference ($p < 0.05$; Annex-3) with the cell density ($168.44^{a} \pm 0.60093 \times 10^{2}$ cells/ml) of TMRL medium at 16th day. According to Shah *et al.* (2003) the maximum cell density of *T. chuii* $182 \pm 6.26 \times 10^{4}$ cells/ml was with BFRI medium at the 24th day of culture where the highest density of the species with Rix Mix medium was $169 \pm 4.48 \times 10^{4}$ cells/ml at the 24th day, which was close to the maximum value with BFRI medium at the same day.

The cultivation of *Tetraselmis tetrathele* in Conway medium presented better results concerning microalgae growth, with a more pronounced exponential phase that was due to the higher nitrate availability, which was reduced more rapidly in the culture medium Guillard f/2 according to Coelho *et al.* (2013).

The stationary phase in Conway medium started at 16th day and continued up to 27th day of culture (Table-5 and Annex-4). The duration of this phase existed for 12 days continuously for *T. chuii*. Where For TMRL medium stationary phase existed for 8 day from 16th to 23rd and for Miquel's medium this phase existed for 9 days from 18th to 26th (Table: 5 & Annex-4). The stationary phase in BFRI medium started at 12th day and continued upto 24th day of culture for *T. chuii* where for Modified TMRL medium it was continued upto 16th day of culture period (Shah *et al.*, 2003).

The growth performance in the TMRL medium show significantly highest performance from 18th to 24th day of the culture period among the three media ($P < 0.05$; Table-4). Although the declining phase started at 24th days of culture period for TMRL medium but it still showed statistically the highest growth performance of *T. chuii* among the three media ($P < 0.05$; Annex-3). The declining phase started in Conway and Miquel's media at 27th and 26th days of culture period.
No significant difference ($P > 0.05$; Appendix) in cell density within the same treatment at different days in the stationary phase was observed. However, it showed difference ($P < 0.05$) in growth among the treatments.

From the experiment of 41th day of culture period it was observed that TMRL medium showed the highest growth performance for *T. chuii* among the three media. *T. chui* gave reduced growth in Miquel's medium during the entire culture period. At 41st day of culture period very few numbers of cells were still alive.

CHAPTER SIX

CONCLUSION

Conclusion

The growth performance of *Tetraselmis chuii* was studied in three different nutrient media viz. Conway, TMRL and Miquel's in laboratory condition. Depending on the findings of this study it might be concluded as follows:

1. *Tetraselmis chuii* can grow well at 25° C.
2. *Tetraselmis chuii* can be found to be alive at 41st day of culture irrespective of any media. However, the stationary growth phase of the species sustains for 8-12 days (16th -27th day of culture).
3. TMRL media is suitable for the culture of *Tetraselmis chuii.*
4. Harvesting time of *Tetraselmis chuii* for further uses would be 16 – 23 days of culture period.

Recommendation

- Further work on optimization of light intensity, photoperiod, air flow, pH and salinity could be done.
- Mass culture study should be done both in indoor and outdoor system.
- Further media development study could be taken where TMRL would be used as a reference media.

CHAPTER SEVEN

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Appendix

Annex- 1

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Day0	Between Groups	.000	2	.000	.	.
	Within Groups	.000	24	.000		
	Total	.000	26			
Day4	Between Groups	296.519	2	148.259	50.511	.000
	Within Groups	70.444	24	2.935		
	Total	366.963	26			
Day6	Between Groups	2337.852	2	1168.926	327.906	.000
	Within Groups	85.556	24	3.565		
	Total	2423.407	26			
Day8	Between Groups	2672.889	2	1336.444	69.761	.000
	Within Groups	459.778	24	19.157		
	Total	3132.667	26			
Day10	Between Groups	1500.222	2	750.111	84.741	.000
	Within Groups	212.444	24	8.852		
	Total	1712.667	26			
Day12	Between Groups	2150.000	2	1075.000	24.936	.000
	Within Groups	1034.667	24	43.111		
	Total	3184.667	26			
Day14	Between Groups	615.407	2	307.704	2.267	.125
	Within Groups	3256.889	24	135.704		
	Total	3872.296	26			
Day16	Between Groups	11800.074	2	5900.037	1.704E3	.000
	Within Groups	83.111	24	3.463		
	Total	11883.185	26			
Day18	Between Groups	6831.185	2	3415.593	101.789	.000
	Within Groups	805.333	24	33.556		
	Total	7636.519	26			
Day21	Between Groups	8325.852	2	4162.926	253.722	.000
	Within Groups	393.778	24	16.407		
	Total	8719.630	26			
Day22	Between Groups	8303.630	2	4151.815	326.581	000
	Within Groups	305.111	24	12.713		
	Total	8608.741	26			
Day38	Between Groups	125.407	2	62.704	31.943	.000
	Within Groups	47.111	24	1.963		
	Total	172.519	26			
Day23	Between Groups	6892.667	2	3446.333	539.426	000
	Within Groups	153.333	24	6.389		
	Total	7046.000	26			
Day24	Between Groups	3256.963	2	1628.481	140.364	.000
	Within Groups	278.444	24	11.602		
	Total	3535.407	26			
Day26	Between Groups	1852.741	2	926.370	96.015	.000
	Within Groups	231.556	24	9.648		
	Total	2084.296	26			
Day27	Between Groups	13730.889	2	6865.444	436.158	.000
	Within Groups	377.778	24	15.741		
	Total	14108.667	26			
Day33	Between Groups	3992.000	2	1996.000	173.147	.000
	Within Groups	276.667	24	11.528		
	Total	4268.667	26			
Day35	Between Groups	616.074	2	308.037	378.045	.000
	Within Groups	19.556	24	.815		
	Total	635.630	26			
Day36	Between Groups	182.889	2	91.444	75.389	.000
	Within Groups	29.111	24	1.213		
	Total	212.000	26			
Day41	Between Groups	35.185	2	17.593	19.588	.000
	Within Groups	21.556	24	.898		
	Total	56.741	26			

Annex-2

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Day0	conway	9	10.0000	.00000	00000	10.0000	10.0000	10.00	10.00
	TMRL	9	10.0000	00000	.00000	10.0000	10.0000	10.00	10.00
	Miqual	9	10.0000	00000	00000	10.0000	10.0000	10.00	10.00
	Total	27	10.0000	00000	00000	10.0000	10.0000	10.00	10.00
Day4	conway	9	20.7778	1.39443	46481	19.7059	21.8496	19.00	23.00
	TMRL	9	25.1111	2.14735	71578	23.4605	26.7617	22.00	28.00
	Miqual	9	17.0000	1.50000	50000	15.8470	18.1530	15.00	19.00
	Total	27	20.9630	3.75686	.72301	19.4788	22.4491	15.00	28.00
Day6	conway	9	2.6222E1	1.301708	433903	25.22184	27.22260	25.000	26.000
	TMRL	9	4.1333E1	2.738613	912871	39.22825	43.43842	38.000	45.000
	Miqual	9	1.9000E1	1.224745	408248	18.05858	19.94142	17.000	20.000
	Total	27	2.8851E1	9.654428	1.857996	25.03269	32.67102	17.000	45.000
Day8	conway	9	40.0000	2.39792	79931	38.1568	41.8432	38.00	45.00
	TMRL	9	54.4444	6.20707	2.06902	49.6733	59.2156	45.00	60.00
	Miqual	9	30.2222	3.63242	1.21081	27.4301	33.0143	25.00	36.00
	Total	27	41.5556	10.97667	2.11246	37.2133	45.8978	25.00	60.00
Day10	conway	9	61.3333	2.34521	78174	59.5306	63.1360	59.00	65.00
	TMRL	9	73.4444	3.94053	1.31351	70.4155	76.4734	68.00	78.00
	Miqual	9	55.5556	2.35112	78371	53.7483	57.3628	53.00	60.00
	Total	27	63.4444	8.11614	1.56195	60.2338	66.6551	53.00	78.00
Day12	conway	9	76.7778	3.52767	1.17589	74.0662	79.4894	69.00	80.00
	TMRL	9	86.1111	10.08023	3.36008	77.3628	92.8595	76.00	100.00
	Miqual	9	63.4444	3.90868	1.30289	60.4400	66.4489	59.00	69.00
	Total	27	75.1111	11.06739	2.12992	70.7330	79.4892	59.00	100.00
Day14	conway	9	93.3333	4.21307	1.40436	90.0949	96.5718	89.00	100.00
	TMRL	9	1.0067E2	18.85471	6.28490	86.1737	115.1597	80.00	150.00
	Miqual	9	89.1111	5.81903	1.93968	84.6382	93.5840	80.00	95.00
	Total	27	94.3704	12.20387	2.34964	89.5427	99.1981	80.00	150.00
Day16	conway	9	1.5044E2	1.58990	52997	149.2223	151.6665	148.00	153.00
	TMRL	9	1.6867E2	1.80278	.60093	167.2809	170.0524	165.00	170.00
	Miqual	9	1.1811E2	2.14735	.71578	116.4605	119.7617	115.00	120.00
	Total	27	1.4574E2	21.37862	4.11432	137.2636	154.1978	115.00	170.00
Day18	conway	9	1.4578E2	2.99073	.99691	143.4789	148.0767	142.00	150.00
	TMRL	9	1.6189E2	9.42662	3.14221	154.6429	169.1348	150.00	172.00
	Miqual	9	1.2311E2	1.69148	56383	121.8109	124.4113	120.00	125.00
	Total	27	1.4359E2	17.13804	3.29822	136.8130	150.3722	120.00	172.00
Day21	conway	9	1.4467E2	2.23607	74536	142.9479	146.3855	142.00	148.00
	TMRL	9	1.6711E2	4.70225	1.56742	163.4966	170.7256	160.00	173.00
	Miqual	9	1.2411E2	4.70225	1.56742	120.4966	127.7256	119.00	130.00
	Total	27	1.4530E2	18.31312	3.52436	138.0519	152.5407	119.00	173.00
Day22	conway	9	1.4422E2	2.43812	81271	142.3481	146.0963	142.00	148.00
	TMRL	9	1.6900E2	4.09268	1.36423	165.8541	172.1459	165.00	175.00
	Miqual	9	1.2822E2	3.92994	1.30998	123.2014	129.2430	123.00	132.00
	Total	27	1.4648E2	18.19630	3.50188	139.2833	153.6797	123.00	175.00
Day38	conway	9	10.3333	.50000	.16667	9.9490	10.7177	10.00	11.00
	TMRL	9	9.8889	1.16667	.38889	8.9921	10.7857	8.00	12.00
	Miqual	9	5.5556	2.06828	.68943	3.9657	7.1454	4.00	10.00
	Total	27	8.5926	2.57591	.49573	7.5736	9.6116	4.00	12.00
Day23	conway	9	1.4378E2	2.27913	.75971	142.0259	145.5297	140.00	147.00
	TMRL	9	1.6811E2	2.14735	.71578	164.4605	167.7617	163.00	170.00
	Miqual	9	1.2711E2	3.05959	1.01986	124.7593	129.4629	125.00	132.00
	Total	27	1.4567E2	16.46208	3.16813	139.1545	152.1788	125.00	170.00
Day24	conway	9	1.4389E2	1.53659	.51220	142.7078	145.0700	142.00	146.00
	TMRL	9	1.5133E2	3.87298	1.29099	148.3563	154.3104	148.00	158.00
	Miqual	9	1.2522E2	4.17665	1.39222	122.0118	128.4327	120.00	130.00
	Total	27	1.4015E2	11.66093	2.24415	135.5352	144.7611	120.00	158.00
Day26	conway	9	1.4267E2	86603	.28868	142.0010	143.3324	142.00	144.00
	TMRL	9	1.4022E2	1.56347	.52116	139.0204	141.4240	138.00	142.00
	Miqual	9	1.2400E2	5.07445	1.69148	120.0994	127.9006	118.00	130.00
	Total	27	1.3563E2	8.95350	1.72310	132.0877	139.1715	118.00	144.00
Day27	conway	9	1.4222E2	1.39443	.46481	141.1504	143.2941	140.00	144.00
	TMRL	9	98.0000	2.34521	.78174	96.1973	99.8027	95.00	100.00
	Miqual	9	91.4444	6.30698	2.10232	86.5965	96.2924	86.00	100.00
	Total	27	1.1056E2	23.29466	4.48308	101.3405	119.7706	86.00	144.00
Day33	conway	9	50.5556	.88192	.29397	49.8777	51.2335	50.00	52.00
	TMRL	9	29.2222	1.09291	.36430	28.3821	30.0623	27.00	30.00
	Miqual	9	21.8889	5.71061	1.90354	17.4993	26.2785	17.00	30.00
	Total	27	33.8889	12.81325	2.46591	28.8201	38.9576	17.00	52.00
Day35	conway	9	20.7778	.83333	.27778	20.1372	21.4183	20.00	22.00
	TMRL	9	19.3333	.86603	.28868	18.6676	19.9990	18.00	20.00
	Miqual	9	10.0000	1.00000	.33333	9.2313	10.7687	8.00	12.00
	Total	27	16.7037	4.94442	.95155	14.7478	18.6597	8.00	22.00
Day36	conway	9	13.2222	1.20185	.40062	12.2984	14.1460	12.00	15.00
	TMRL	9	13.7778	1.20185	.40062	12.8540	14.7016	12.00	15.00
	Miqual	9	8.0000	.86603	.28868	7.3343	8.6657	7.00	9.00
	Total	27	11.6667	2.85549	.54954	10.5371	12.7963	7.00	15.00
Day41	conway	9	5.6667	.86603	.28868	5.0010	6.3324	5.00	7.00
	TMRL	9	6.7778	1.09291	.36430	5.9377	7.6179	5.00	8.00
	Miqual	9	4.0000	.86603	.28868	3.3343	4.6657	3.00	5.00
	Total	27	5.4815	1.47727	.28430	4.8971	6.0659	3.00	8.00

Annex-3

Post Hoc

Homogeneous Subsets

Day4

Tukey HSD

1,2,3	N	Subset for alpha = 0.05		
		1	2	3
Miqual	9	17.0000		
conway	9		20.7778	
TMRL	9			25.1111
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day6

Tukey HSD

1,2,3	N	Subset for alpha = 0.05		
		1	2	3
Miqual	9	1.9000E1		
conway	9		2.6222E1	
TMRL	9			4.1333E1
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day8

Tukey HSD

1,2,3	N	Subset for alpha = 0.05		
		1	2	3
Miqual	9	30.2222		
conway	9		40.0000	
TMRL	9			54.4444
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day10

Tukey HSD

1,2,3	N	Subset for alpha = 0.05		
		1	2	3
Miqual	9	55.5556		
conway	9		61.3333	
TMRL	9			73.4444
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day12

Tukey HSD

1,2,3	N	Subset for alpha = 0.05		
		1	2	3
Miqual	9	63.4444		
conway	9		76.7778	
TMRL	9			85.1111
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day14

Tukey HSD

1,2,3	N	Subset for alpha = 0.05
		1
Miqual	9	89.1111
conway	9	93.3333
TMRL	9	100.6667
Sig.		.110

Means for groups in homogeneous subsets are displayed.

Day18

Tukey HSD

1.2.3	N	Subset for alpha = 0.05		
		1	2	3
Miqual	9	1.2311E2		
conway	9		1 4578E2	
TMRL	9			1.6189E2
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day21

Tukey HSD

1.2.3	N	Subset for alpha = 0.05		
		1	2	3
Miqual	9	1.2411E2		
conway	9		1.4467E2	
TMRL	9			1.6711E2
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed

Day22

Tukey HSD

1.2.3	N	Subset for alpha = 0.05		
		1	2	3
Miqual	9	1.2622E2		
conway	9		1.4422E2	
TMRL	9			1.6900E2
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day23

Tukey HSD

1.2.3	N	Subset for alpha = 0.05		
		1	2	3
Miqual	9	1.2711E2		
conway	9		1.4378E2	
TMRL	9			1.6611E2
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day24

Tukey HSD

1.2.3	N	Subset for alpha = 0.05		
		1	2	3
Miqual	9	1.2522E2		
conway	9		1.4389E2	
TMRL	9			1.5133E2
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day26

Tukey HSD

1.2.3	N	Subset for alpha = 0.05	
		1	2
Miqual	9	124.0000	
TMRL	9		140.2222
conway	9		142.6667
Sig.		1.000	.237

Means for groups in homogeneous subsets are displayed.

Day27

Tukey HSD

1.2.3	N	Subset for alpha = 0.05		
		1	2	3
Miqual	9	91.4444		
TMRL	9		98.0000	
conway	9			1.4222E2
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day33

Tukey HSD

1.2.3	N	Subset for alpha = 0.05		
		1	2	3
Miqual	9	21.8889		
TMRL	9		29.2222	
conway	9			50.5556
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day35

Tukey HSD

1.2.3	N	Subset for alpha = 0.05		
		1	2	3
Miqual	9	10.0000		
TMRL	9		19.3333	
conway	9			20.7778
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Day36

Tukey HSD

1.2.3	N	Subset for alpha = 0.05	
		1	2
Miqual	9	8.0000	
conway	9		13.2222
TMRL	9		13.7778
Sig.		1.000	.541

Means for groups in homogeneous subsets are displayed.

Day38

Tukey HSD

1.2.3	N	Subset for alpha = 0.05	
		1	2
Miqual	9	5.5556	
TMRL	9		9.8889
conway	9		10.3333
Sig.		1.000	.781

Means for groups in homogeneous subsets are displayed.

Day41

Tukey HSD

1.2.3	N	Subset for alpha = 0.05	
		1	2
Miqual	9	4.0000	
conway	9		5.6667
TMRL	9		6.7778
Sig.		1.000	.051

Means for groups in homogeneous subsets are displayed.

Annex-4

Conway

Tukey HSD

0,4,6,8,10, 11,14,16,1 8,21,22	N	Subset for alpha = 0.05											
		1	2	3	4	5	6	7	8	9	10	11	12
Day_41	9	5.67											
Day_initial	9		10.00										
Day_38	9		10.33										
Day_36	9		13.22										
Day_4	9			20.78									
Day_35	9			20.78									
Day_6	9				26.22								
Day_8	9					40.00							
Day_33	9						50.56						
Day_10	9							61.33					
Day_11	9								76.78				
Day_14	9									93.33			
Day_27	9										142.22		
Day_26	9										142.67	142.67	
Day_23	9										143.78	143.78	
Day_24	9										143.89	143.89	
Day_22	9										144.22	144.22	
Day_21	9										144.67	144.67	
Day_18	9											145.78	
Day_16	9												150.44
Sig		1.000	.095	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.536	.130	1.000

Means for groups in homogeneous subsets are displayed.

TMRL

Tukey HSD

0,4,6,8,10, 11,14,16,1 8,21,22...	N	Subset for alpha = 0.05										
		1	2	3	4	5	6	7	8	9	10	11
Day_41	9	6.78										
Day_38	9	9.89	9.89									
Day_initial	9	10.00	10.00									
Day_36	9	13.78	13.78									
Day_35	9		19.33	19.33								
Day_4	9			25.11								
Day_33	9			29.22								
Day_6	9				41.33							
Day_8	9					54.44						
Day_10	9						73.44					
Day_11	9							85.11				
Day_27	9								98.00			
Day_14	9								100.67			
Day_26	9									140.22		
Day_24	9										151.33	
Day_18	9											161.89
Day_23	9											166.11
Day_21	9											167.11
Day_16	9											168.67
Day_22	9											169.00
Sig		.561	.087	.054	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.531

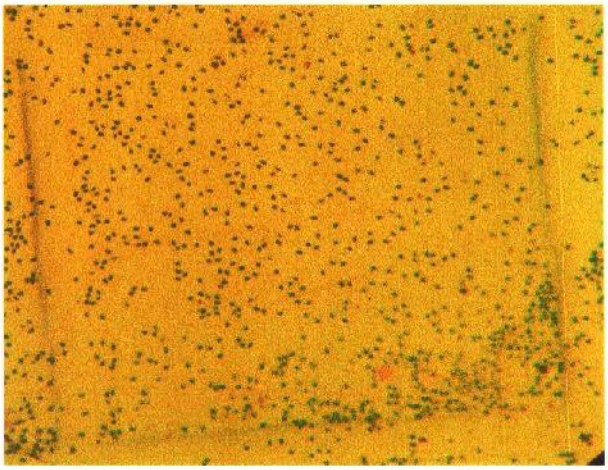
Means for groups in homogeneous subsets are displayed.

Miquel

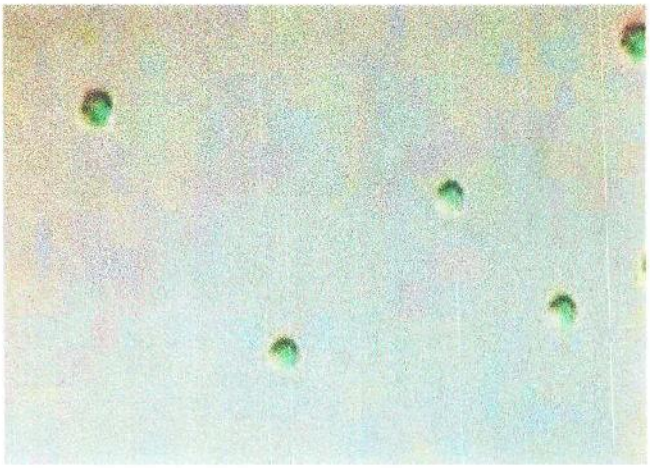
Tukey HSD

0,4,6,8,10, 11,14,16,1 8,21,22...	N	Subset for alpha = 0.05								
		1	2	3	4	5	6	7	8	9
Day_41	9	4.00								
Day_38	9	5.56	5.56							
Day_36	9	8.00	8.00							
Day_initial	9		10.00							
Day_35	9		10.00							
Day_4	9			17.00						
Day_6	9			19.00						
Day_33	9			21.89						
Day_8	9				30.22					
Day_10	9					55.56				
Day_11	9						63.44			
Day_14	9							89.11		
Day_27	9							91.44		
Day_16	9								118.11	
Day_18	9								123.11	123.11
Day_26	9								124.00	124.00
Day_21	9									124.11
Day_24	9									125.22
Day_22	9									126.22
Day_23	9									127.11
Sig.		.655	.454	.277	1.000	1.000	1.000	.997	.060	.655

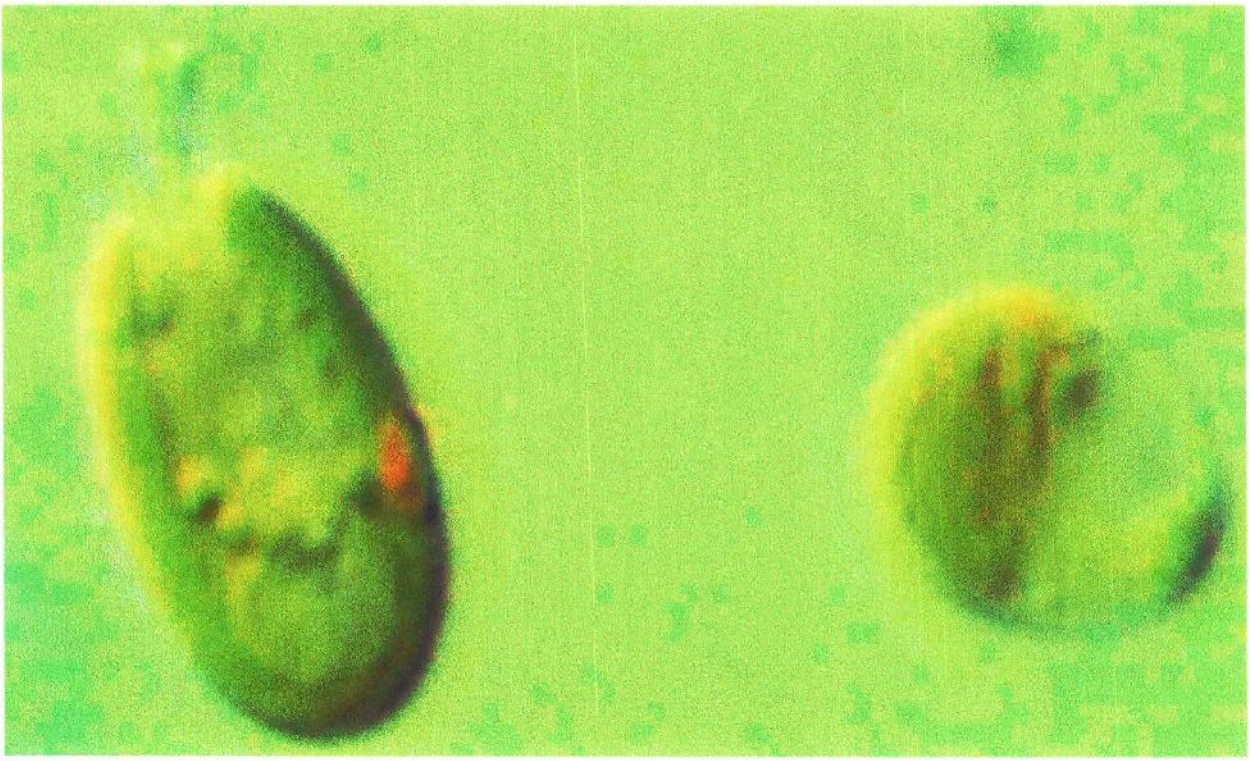
Means for groups in homogeneous subsets are displayed.



4. Microscopic view of *Tetraselmis chuii*



5. Microscopic view of *Tetraselmis chuii*



6. Microscopic view of *Tetraselmis chuii*