EFFECT OF DIFFERENT CULTURE MEDIA FOR THE GROWTH OF SOME MARINE MICROALGAE

May Thu Rein Oo[1], Cho Cho Thin[2], July Maung Maung[3], Aye Aye Khaing[4], Kyaw Naing Than[5], Saw Soe Moe Thu[6], Kay Lwin Tun[7]

Abstract

Microalgae play an important role in supporting the development of aquaculture because they can be used as natural feed for larvae due to their high nutrient value. The present study was to develop a cost effective and optimal growth of some marine microalgae such as *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. by using two different culture media in laboratory condition. conway media for experiment I A_1, B_1 and C_1, and agricultural fertilizer media for experiment II A_2, B_2 and C_2 were used to culture microalgae. Three replications were prepared for each media. The cell densities of *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. in different culture media were counted daily through the culture period. The results showed that the maximum population densities of *Nannochloropsis* sp. (8.80 x 10^6 cells/ml), *Chlorella* sp. (8.37 x 10^6 cells/ml), and *Tetraselmis* sp. (8.29 x 10^6 cells/ml) occurred on the fourth day with agricultural fertilizer media, while it was observed on the fifth day with conway media. The population densities of *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. started to decrease in agricultural fertilizer media on fifth day while on sixth day in conway media. However, the agricultural fertilizer media had higher density than the conway media. It was concluded that the agricultural fertilizer media was the best for growth rate of *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp.

Introduction

Algae are primary producers of the oceans, rivers, streams and lakes (Stottrup & McEvoy, 2003). Microalgae are microorganism such as diatoms, blue green algae and flagellates and primary producers of the oceans, rivers, streams and lakes that change sunlight, water and carbon dioxide in to algal biomass (Stottrup & McEvoy, 2003). The algae are manufactured for complex nutritive molecules including proteins, starches, fatty acids and oils and used as an excellent diet for early stages of mollusks, farmed shrimp, crustacean and fish (Stahl, 2009).

Micro-algae are cultured intensively for direct or indirect feeding through production of zooplanktons and *Artemia nauplii*. Sea water was supplemented with commercial nitrate and phosphate fertilizers, and a few other essential micro nutrients, are commonly used for growing marine microalgae. The elements required for the growth of green algae are N, P, K, Mg, Ca, S, Fe, Cu, MN+, and Zn and these elements are added in the form of salts (Sen *et al*., 2005).

Certain nutrients in appropriate quantities are needed in culture media for the algae to multiply. All media possess nitrogen, phosphorous and carbonate as major nutrients and lack trace metals, vitamins and other mineral nutrients. Some algae require trace metals and minor nutrients for better growth.

The most common microalgae species are *Nannochloropsis* sp., *Scenedesmus* sp., *Isochrysis* sp., *Pavlova* sp., *Dunaliella* sp., *Spirulina phaeodactylum, Chlorella* sp., *Rhodomonas* sp., *Tetraselmi*s sp., *Skeletonema* sp., and *Thalassiosira* sp. (Parrish et al., 2012).

[1] Department of Zoology (Fisheries and Aquaculture), University of Yangon
[2] Department of Zoology (Fisheries and Aquaculture), University of Yangon
[3] Department of Zoology (Fisheries and Aquaculture), University of Yangon
[4] Department of Zoology (Fisheries and Aquaculture), University of Yangon
[5] Department of Zoology (Fisheries and Aquaculture), University of Yangon
[6] Department of Zoology (Fisheries and Aquaculture), University of Yangon
[7] Department of Zoology, University of Yangon

Among them, *Nannochloropsis* sp. is a single-celled sea microalga that can be used as the live feed for larvae cultivation of shrimp, fish, and shellfish. *Nannochloropsis* sp. is used in aquaculture as a valuable feed, providing polyunsaturated fatty acids, essential vitamins, and amino acids, along with energy. *Nannochloropsis* sp. has high nutrition value, and it is used widely as aquaculture hatchery industry for food of larvae and juvenile of bivalve, rotifer, as well as fish larvae (Tawfiq et al., 1999).

The *Chlorella* sp. is perfect food for shrimps, marine fish and all other ornamental fish, crustacean and also serves as a food for zoo-planktons such as daphnia, moina and rotifer. It is also used in food industry, cosmetics and pharmaceutical industry (Sergejevová and Masojidek, 2011).

Tetraselmis has been one of the microalgae most frequently recommended as a feed for early life stages of shrimp. Providing natural feed usually arises when organisms live in a cultivation environment.

In Myanmar fish farmers have limited experience to cultivate the microalgae such as *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. which is necessary for marine fish and shrimp hatchery. However, fish farmers are facing the high cost of culture medium used in optimal growth of the algae .It is one of the main problems related to the large scale culture of microalgae.

The present study has been conducted to produce the three marine microalgae such as *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. using Conway and agriculture Fertilizers media.

Materials and Methods

Study site and Study period

The present study was conducted in the Live Food Laboratory of Fisheries and Aquaculture in the Research and Innovation Center, University of Yangon .The microalgae was collected from Department of Fisheries (DoF) and cultured in the laboratory from January 2022 to September 2023.

Microalgae collection

The pure strains of *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. were purchased from Department of Fisheries (DoF). The cultivation of *Nannochloropsis* sp. *Chlorella* sp. and *Tetraselmis* sp. were conducted in Live Food Culture Laboratory of Fisheries and Aquaculture, Center for Research and Innovation, University of Yangon.

Preparation of apparatus

All apparatus (beakers, bottles, measuring spoons and sea water) were covered by aluminum foil and autoclaved at 120 °C for 25 mins to avoid contaminations. The seawater was measured and diluted with distilled water to obtain 25‰ salinity. They were then filtered with Millipore (0.45 μm) filter paper. The solution was then kept in dark and cold place.

Preparation of Conway media

For conway media, three type of solution: solution A, B and C were prepared separately. They were then mixed prior to cultivation of *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. For solution A, all chemical substances (Disodium Hydrogen Phosphate, Boric Acid, Ferric Chloride, Manganese Chloride, Na2EDTA and Sodium nitrate) were weighed by using digital balance and added in the beaker that contained 900ml of distilled water.

After adding the substances in the beaker, the solution was stirred by using a magnetic stirrer. (Table1).

Table 1 Composition of conway media (Solution A)

Chemical	Amount
Disodium Hydrogen Phosphate (NaH_2PO_4)	20 g
Boric Acid	33.6 g
Ferric Chloride	1.3 g
Manganese chloride	0.36 g
Na2 EDTA	40 g
Sodium nitrate	100 g
Distilled water	900 ml

For solution B, all chemical Substances (Zinc Chloride, Calcium chloride Ammonium paramolybdate tetrahydrate and Copper II sulfate) were weighed by using digital balance and added in the beaker that contained 100ml of distilled water. The solution was stirred by using a magnetic stirrer (Table 2).

Table 2 Composition of conway media (Solution B)

Chemical	Amount
Zinc Chloride	2.1 g
Calcium chloride	2.1 g
Ammonium paramolybdate tetrahydrate	2.1 g
Copper II sulfate	2 g
Distilled water	100 ml

For solution C, 3mg of Vitamin B1 and 10mg of B12 were added in the beaker that contained 200ml of distilled water.

All the solutions (A, B and C) in Conway media were prepared separately. 900 mL of Solution I was mixed 1 mL of Solution II (Trace metal) into a 1000 mL beaker. Then, the solutions were autoclaved in an autoclave machine for 121°C at 25 mins, the solutions were taken out when the temperature dropped until 80°C. The solutions were added Solution III (Vitamin) and stirred. Then, the solution was stored in a sterilized bottle and kept in refrigerator.

Preparation of agricultural Fertilizer Media

Agricultural fertilizers such as 100 g of Urea, and 20 g of Triple Super Phosphate (TSP) were weighed by using digital balance and added in the beaker that contained 1000ml of distilled water. After adding the substances in the beaker, the solution was stirred by using a magnetic stirrer. The solutions were sterilized in an autoclave machine at 121°C for 25 mins. The solutions were then taken out when the temperature dropped until 80°C. Then, the solutions were stored in a sterilized bottle and kept in refrigerator to avoid contaminations for further use.

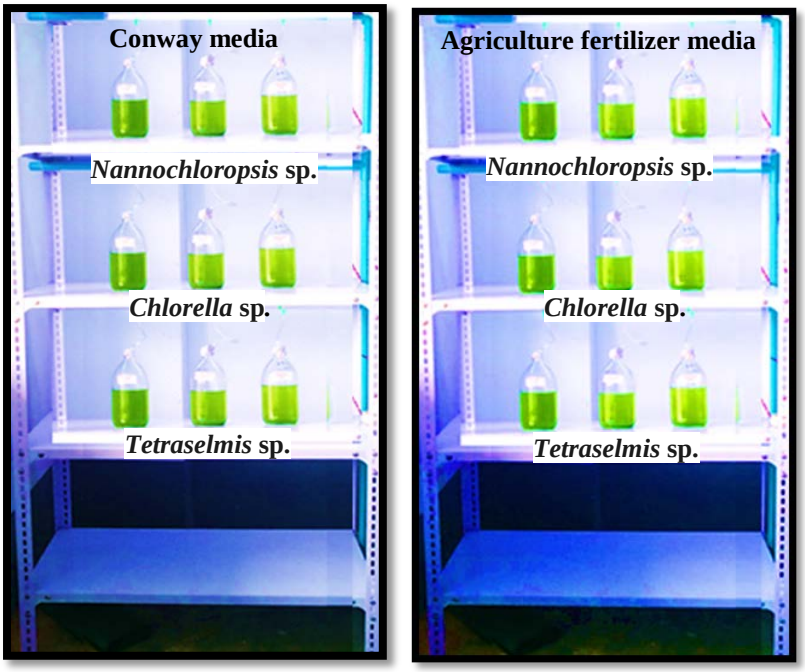
Microalgae Inoculant Preparation

Inoculant preparation of *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. were started by using the sterilized plankton culture glass bottle (2000 mL). The bottle was filled with 1500 mL of sterilized 25% seawater and add 300 mL of pure strain *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. A total of 3ml of the nutrient agriculture fertilizers media was added for each experiment. Each culture bottle was sealed with aluminum foil and labeled with

date and time. All culture bottles were kept at air-conditions room at 25°C with light by fluorescent tubes and cultured for 4 days so that the microalgae were ready to be used.

Cultivation of *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. using conway and agricultural fertilizer media

Nannochloropsis sp., *Chlorella* sp. and *Tetraselmis* sp. were treated with conway and agricultural fertilizer media. The sterilized plankton culture glass bottle (1000 mL**)** was filled with 500 mL of sterilized 25% seawater and 100 mL of pure strain *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. were added into each glass bottle. Then 1ml of conway media was added to each glass bottle for experiment I A_1, B_1 and C_1, while 1ml of the agricultural fertilizer media was added to experiment II A_2, B_2 and C_2. Three replications were prepared for each experiment (Plate 2). Each culture bottle was sealed with aluminum foil and labeled date and time. The Plankton culture bottles were arranged on cultivation shelf and aerated with blower. All culture bottles were kept at air-conditions room at 25°C with light by fluorescent tubes. The experiments were extended for 10 days. The population density of *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. were calculated every day by collecting 1ml of subsample from each bottle.



(A) Conway media (B) Agriculture fertilizer media

Plate 2 Cultivation of *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. using conway and agricultural fertilizer media

Determination of growth conditions and cell density of *Nannochloropsis sp., Chlorella sp., and Tetraselmis sp.*

The growth of *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp.were estimated by counting cell density using hemocytometer. The subsamples of 1-mL from each bottle were collected without replacement. One drop cell suspension was placed in the central counting chamber of hemocytometer (Thoma, Grmany) and covered with cover glass (22 mm) carefully to avoid the formation of bubbles between the cover glass and hemocytometer. The chamber was placed under light microscope (CX 31, Olympus) at 100× magnification. The counting of cell

density was started from the first day of culture period until the 10^{th} days and calculated using the formula (Taw, 1990).

$$\text{Cell count (cells/mL) for 25 squares} = \frac{\text{total number of cells counted}}{\text{Number of blocks}} \times 4 \times 10^{6}$$

The growth of culture of *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. was characterized by five phases (Creswell, 1993). The detail descriptions of phases are;

1. Lag phase or induction phase*:* After the addition of stock culture inoculum for the subculture of micro algae, there was no cell division phase for few hours which was known as lag or induction phase.

2. Exponential phase*:* after the lag phase, the cells were acclimatized and started dividing, grow fast by utilizing nutrients, aeration and light. This growth phase was called exponential phase and reaches maximum cell concentration during this period.

3. Declining phase*:* After reaching the growth phase, the cells showed less growth or slow growth. This stunted growth stage was known as declining phase.

4. Stationary phase*:* The declining phase continued for few days without any cell division and this period was known as stationary phase. Sometimes, the culture might divide the cells with suitable conditions.

5. Death phase*:* Prolonged stationary phase would lead to the death phase, where algal cells would lose their viability and the cells died. This phase was called death phase (Fig. 3).

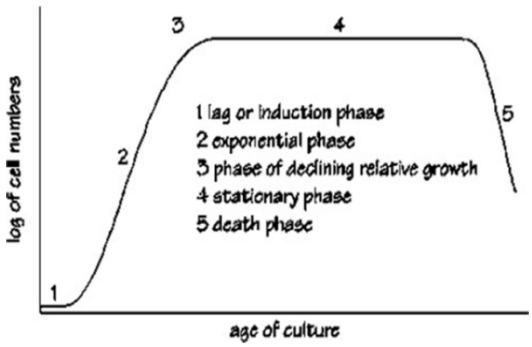


Figure 3 Growth phase of Algae (Creswell, 1993)

Determination of water quality

Water quality analysis was conducted for temperature and salinity at the early stage and at the end of culture.

Data Analysis

Cell densities were expressed as the average number of cell.ml^{-1} ± standard deviation. Growth curves for each experiment were prepared by plotting the average cell density *vs* corresponding cultivation time. Curves were prepared by using EXCEL computer program.

Results

The systematic position of marine microalge *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. studied in the present research were as follow.

Nannochloropsis sp.

Phylum: Ochrophyta
Class: Eustigmatophyceae
Order: Eustigmatales
Family: Monodopsidaceae
Genus: *Nannochloropsis* D.J.Hibberd, 1981

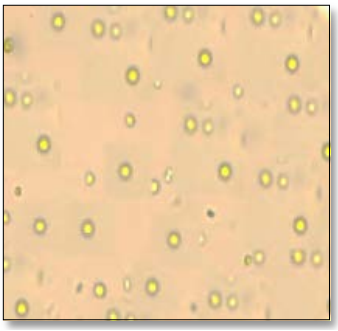


Plate 3 *Nannochloropsis* sp. (400 x magnifications)

Chlorella sp.

Phylum: Chlorophyta
Class: Trebouxiophyceae
Order: Chlorellales
Family: Chlorellaceae
Genus: *Chlorella* M.Beijerinck, 1890

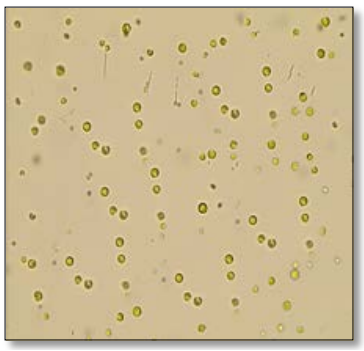


Plate 4 *Chlorella* sp. (400 x magnifications)

Tetraselmis sp.

Phylum: Chlorophyta
Class: Chlorodendrophyceae
Order: Chlorodendrales
Family: Chlorodendraceae
Genus: *Tetraselmis* F.Stein, 1878

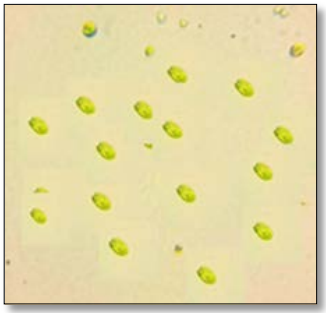


Plate 5 *Tetraselmis* sp. (400 x magnifications)

Population density of *Nannochloropsis* sp. *Chlorella* sp. and *Tetraselmis* sp. with conway and agricultural fertilizer media

The present experiments were performed to produce the *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. in laboratory condition using agricultural fertilizers media and conway media. The initial stocking density of *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. were (1.5 x 10^6 cell/ml), (1.38 x 10^6 cell/ml) and (1.42x 10^6 cell/ml) in first day, respectively. The cell density of *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. were described in table 6.

The population density of *Nannochloropsis* sp. cultured with conway media started to increase from the second day to fifth day. However, it decreased in sixth day. In agricultural fertilizeral media, the population density of *Nannochloropsis* sp. started to increase from the second day to the fourth day. And it decreased on fifth day.

The maximum population density of cultured *Nannochloropsis* sp. occurred in conway media (8.47 x 10^6 cell/ml) while (8.8 x 10^6 cell/ml) in agricultural fertilizer media (Table 3 and Fig.1).

Table 3 Population densities of *Nannochloropsis* sp. with conway and agricultural fertilizer media

Time (Day)	Conway media	Agricultural fertilizer media
Day 1	1.50±0.01	1.50±0.01
Day 2	3.02 ± 0.25	3.20 ± 0.18
Day 3	5.39 ± 0.15	6.53 ± 0.13
Day 4	7.83 ± 0.30	**8.80 ± 0.25**
Day 5	**8.47 ± 0.07**	7.93 ± 0.11
Day 6	7.56 ± 0.2	6.56 ± 0.2
Day 7	5.54 ± 0.25	5.18 ± 0.10
Day 8	3.46 ± 0.10	2.46 ± 0.10
Day 9	1.78 ± 0.10	1.41 ± 0.06
Day 10	0.99 ± 0.26	0.69 ± 0.01

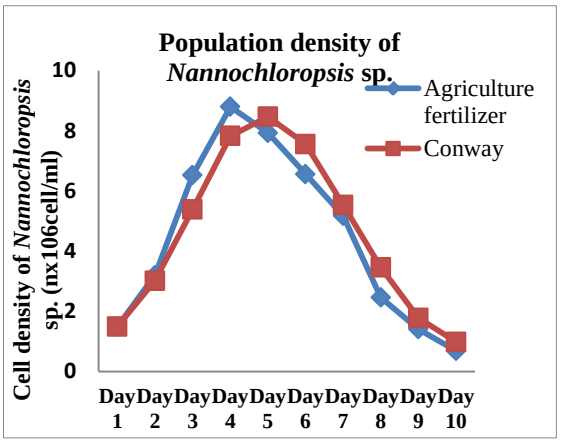


Figure 1 Population density of *Nannochloropsis* sp.

The population density of *Chlorella* sp. cultured with conway media started to increase from the second day to fifth day and it started to decrease in the sixth day. In agricultural fertilizer media, the population density of *Chlorella* sp. started to increase from the second day to the fourth day and then it started to decrease in the fifth day.

The maximum population density of cultured *Chlorella* sp. occurred in conway media (8.12 x 10^6 cell/ml) while ((8.37 x 10^6 cell/ml) in agricultural fertilizer media (Table 4 and Fig.2)

Table 4 Population densities of *Chlorella* sp. with conway and agricultural fertilizer media

Time (Day)	Conway media	Agriculture fertilizer media
Day 1	1.38±0.10	1.38±0.10
Day 2	2.55 ± 0.10	2.88 ± 0.05
Day 3	4.72 ± 0.30	5.43 ± 0.06
Day 4	6.74 ± 0.21	**8.37 ± 0.12**
Day 5	**8.12 ± 0.18**	7.54 ± 0.10
Day 6	7.00 ± 0.42	6.65 ± 0.3
Day 7	5.31 ± 0.15	4.68 ± 0.15
Day 8	3.74± 0.21	2.61± 0.36
Day 9	2.34± 0.06	1.48± 0.22
Day 10	1.32 ± 0.17	0.42 ± 0.36

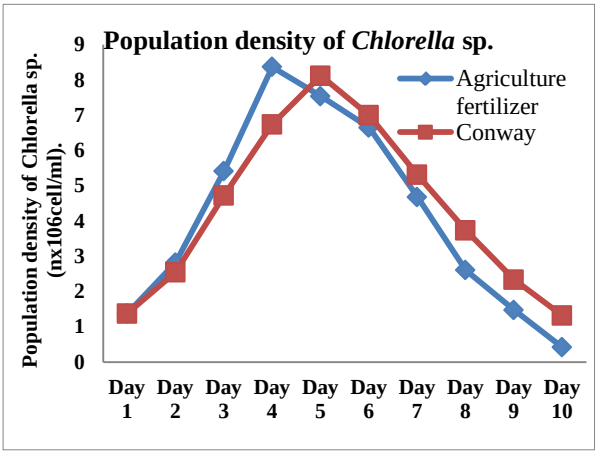


Figure 2 Population density of *Chlorella* sp.

The population density of *Tetraselmis* sp cultured with conway media started to increase from the second day to fifth day while it decreased in sixth day. In agricultural fertilizer media, the population density of *Tetraselmis* sp. started to increase from the second day to the fourth day then it decreased on fifth day.

The maximum population density of cultured *Tetraselmis* sp. was found in conway media (8.06 x 10^6 cell/ml) while it was (8.29 x 10^6 cell/ml) in agricultural fertilizer media. (Table 5 and Fig.3).

Table 5 Population densities of *Tetraselmis* sp. with conway and agricultural fertilizer media

Time (Day)	Conway media	Agriculture fertilizer media
Day 1	1.42±0.02	1.42±0.02
Day 2	3.38 ± 0.07	3.37 ± 0.07
Day 3	5.35 ± 0.11	6.02 ± 0.08
Day 4	6.95 ± 0.06	**8.29 ± 0.10**
Day 5	**8.06 ± 0.08**	7.65 ± 0.3
Day 6	7.02 ± 0.01	6.80 ± 0.2
Day 7	5.27 ± 0.16	4.61 ± 0.4
Day 8	4.01 ± 0.43	2.87 ± 0.06
Day 9	2.07± 0.38	1.74± 0.21
Day 10	1.08 ± 0.10	0.84 ± 0.06

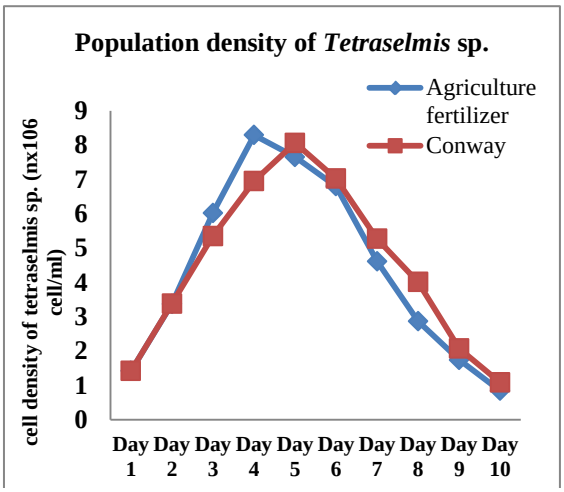


Figure 3 Population density of *Tetraselmis* sp.

The population density of *Nannochloropsis* sp., *Chlorella* sp., and *Tetraselmis* sp. cultured with conway media were shown in Table 6. The peaks of *Nannochloropsis* sp. (8.47 x 10^6 cell/ml), *Chlorella* sp. (8.12 x 10^6 cell/ml) and *Tetraselmis* sp. (8.06 x 10^6 cell/ml) populations with conway media occurred on fifth day. The cell densities of *Nannochloropsis* sp. (7.56 x 10^6 cell/ml), *Chlorella* sp, (7.00 x 10^6 cell/ml), and *Tetraselmis* sp. (7.02 x 10^6 cell/ml) decreased on sixth day (Table 6 and Fig.4).

Table 6 Population densities of *Nannochloropsis* sp., *Chlorella* sp.and *Tetraselmis* sp.in conway media during the period of 10 days cultivation

Time (Day)	*Nannochloropsis* sp.	*Chlorella* sp.	*Tetraselmis* sp.
Day 1	1.50±0.01	1.38±0.10	1.42±0.02
Day 2	3.02 ± 0.25	2.55 ± 0.10	3.38 ± 0.07
Day 3	5.39 ± 0.15	4.72 ± 0.30	5.35 ± 0.11
Day 4	7.83 ± 0.30	6.74 ± 0.21	6.95 ± 0.06
Day 5	**8.47 ± 0.07**	**8.12 ± 0.18**	**8.06 ± 0.08**
Day 6	7.56 ± 0.2	7.00 ± 0.42	7.02 ± 0.01
Day 7	5.54 ± 0.25	5.31 ± 0.15	5.27 ± 0.16
Day 8	3.46 ± 0.10	3.74± 0.21	4.01 ± 0.43
Day 9	1.78 ± 0.10	2.34± 0.06	2.07± 0.38
Day 10	0.99 ± 0.26	1.32 ± 0.17	1.08 ± 0.10

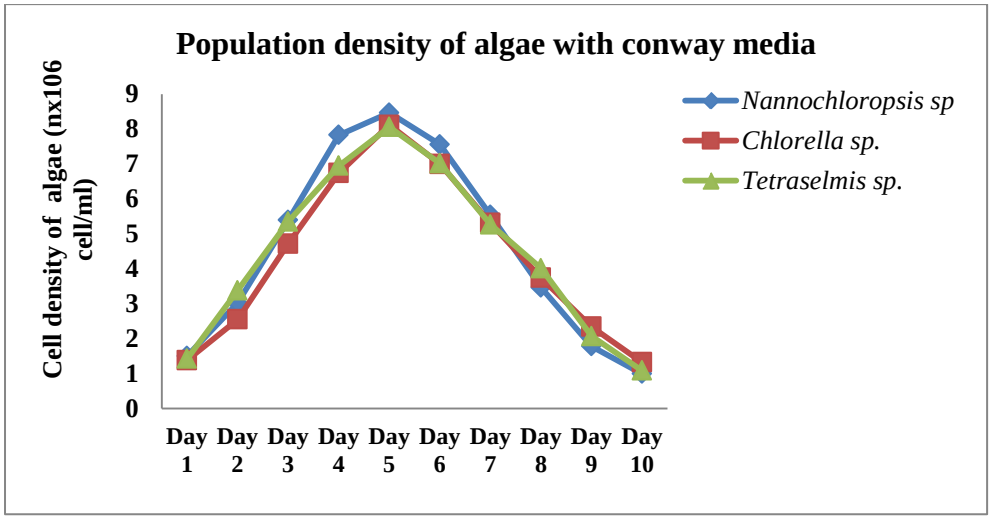


Figure 4 Population densities of *Nannochloropsis* sp., *Chlorella* sp.and *Tetraselmis* sp.in conway media

The population densities of *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. cultured with agricultural fertilizer media was shown in Table 7. The maximum population densities of *Nannochloropsis* sp.(8.80 x 10^6 cell/ml), *Chlorella* sp. (8.37 x 10^6 cell/ml) and *Tetraselmis* sp. (8.29 x 10^6 cell/ml) with agricultural fertilizer media occurred on fourth day. The cell densities of *Nannochloropsis* sp. (7.93 x 10^6 cell/ml), *Chlorella* sp, (7.54 x 10^6 cell/ml), and *Tetraselmis* sp. (7.65 x 10^6 cell/ml) decreased on fifth day (Table 7 and Fig.5).

Table 7 Population densities of *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. in agricultural fertilizer media during the period of 10 days cultivation

Time (Day)	*Nannochloropsis* sp.	*Chlorella sp.*	*Tetraselmis sp.,*
Day 1	1.50±0.01	1.38±0.10	1.42±0.02
Day 2	3.20 ± 0.18	2.88 ± 0.05	3.37 ± 0.07
Day 3	6.53 ± 0.13	5.43 ± 0.06	6.02 ± 0.08
Day 4	**8.80 ± 0.25**	**8.37 ± 0.12**	**8.29 ± 0.10**
Day 5	7.93 ± 0.11	7.54 ± 0.10	7.65 ± 0.3
Day 6	6.56 ± 0.2	6.65 ± 0.3	6.80 ± 0.2
Day 7	5.18 ± 0.10	4.68 ± 0.15	4.61 ± 0.4
Day 8	2.46 ± 0.10	2.61± 0.36	2.87 ± 0.06
Day 9	1.41 ± 0.06	1.48± 0.22	1.74± 0.21
Day 10	0.69 ± 0.01	0.42 ± 0.36	0.84 ± 0.06

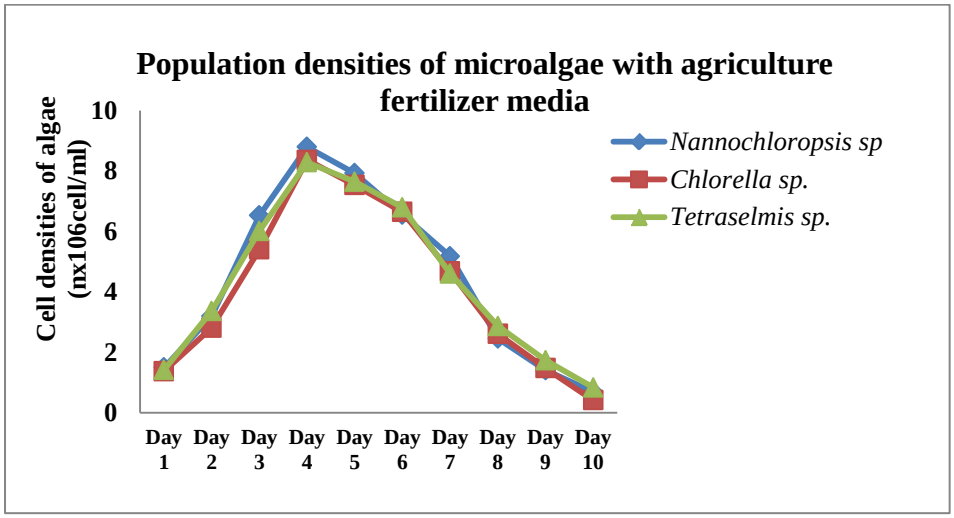


Figure 5 Population densities of *Nannochloropsis* sp., *Chlorella* sp.and *Tetraselmis* sp.in agricultural fertilizer media

Determination of water quality

In conway media, the water temperature in culture bottles ranged from 26.4 -27.6 ^{o}C while salinity ranged from 25-27.3 ppt. In agricultural fertilizer media, it ranged from 26.4 -27. 86 ^{o}C while salinity ranged from 25-27.1 ppt. Similar water quality was maintained for the culture (Table 8).

Table 8 Water salinity and temperature during the culture period

		Conway media		Agriculture fertilizermedia	
No.	**Algae**	**Temparature (^{o}C)**	**Salinity (ppt)**	**Temparature (^{o}C)**	**Salinity (ppt)**
1.	***Nannochloropsis* sp.**	26.4 - 27.6	25-27	26.4 - 27.9	25-27
2.	***Chlorella* sp.**	26.6 - 27.23	25-27.3	26.6 - 27.86	25-27.1
3.	***Tetraselmis* sp.**	26.6 -27.3	25-27	26.6 -27.76	25-27

Discussion

The variation of cell densities of *Nannochloropsis* sp., *Chlorella* sp., and *Tetraselmis* sp. was observed using the conway media and agricultural fertilizer media. The growth rates of *Nannochloropsis* sp., *Chlorella* sp., and *Tetraselmis* sp. were estimated by population density.

In the present study, the maximum population densities of *Nannochloropsis* sp., *Chlorella* sp., and *Tetraselmis* sp. cultured with agricultural fertilizer media were found on the fourth day of cultivation while it reached their maximum densities on the fifth day during the cultivation period using Conway media.

Creswell, (1993) stated that the Growth phases of plankton consist of 4 phases namely; lag phase, exponential phase, stationary phase, and death phase. Tugiyono, (2018) stated that the growth phase of *Nannochloropsis* sp. with agricultural fertilizer media occurred from second day to the fourth day as called the exponential growth phase. The population densities of microalgae *Nannochloropsis* sp. and *Tetraselmis* sp. showed exponential phase on fourth day.

In the present study, the population densities of *Nannochloropsis* sp.,*Chlorella* sp., and *Tetraselmis* sp. cultured with agricultural fertilizer media showed the exponential phase on fourth day. Therefore, the present findings agreed with the previous findings of Tugiyono, (2018).

The population densities of *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis* sp. with conway media started to decrease on sixth day while cell density of *Nannochloropsis* sp., *Chlorella* sp, and *Tetraselmis* sp. cultured with agricultural fertilizer media decreased on fifth day. Rahardini *et al.*, (2018) revealed that the growing population of *Chlorella* sp. occurred when nutrients concentration was enough, and the cell division occurred rapidly (exponential phase). The cell population decreased when the nutrients run out.

In this study, the decline was caused by the decreasing of nutrients in the culture medium and it was eventually lost so that the cells deprived nutrients for growth. The present finding was agreed with the previous finding by Rahardini *et al.*, (2018).

Sivakumar and Rajendran, (2013) stated that microalgae require nutrients for their growth because nitrogen is a major nutrient for microalgal cultivation. The principal nutrients for algae are nitrogen (N), potassium (K), carbon (C), and phosphorus (P). In addition, microalgae growth also requires appropriate temperature, adequate sun rays, and an optimal combination of NPK. In the present study, the agricultural fertilizer media composed of nitrogen (N) and phosphorus (P) for microalgal cultivation.

Rahardini *et al.*, (2018) described that temperature is an important limiting factor for organism life, because every organism has limiting ability to tolerate temperature change in environment. The temperature and salinity in the present study were 26.4 -27.6 ^{o}C and 25-27.3 ppt respectively .Therefore, water quality in the present study was appropriate for marine algae culture.

Agriculture fertilizers mainly composed of macro-elements as nitrogenous and phosphorus and some of other elements such as iron, sodium, and potassium. However, they do not contain, in general, all micro-nutrients and vitamins necessary for the growth of microalgae.

Acién *et al.*, (2018) stated that the *Tetraselmis* sp. consumed nitrogen and phosphate in the fertilizers based-media during the culture period.

Elnabris (2012) also described that agricultural fertilizer such as urea, calcium superphosphate, ammonium sulfate, micronutrient, and vitamin solutions greatly supported the

growth of *Nannochloropsis* sp. and confirmed that using agricultural grade fertilizers could substitute the F/2 media which was commonly used for culture in commercial aquaculture.

In the present study, the population densities of *Nannochloropsis* sp., *Chlorella* sp., and *Tetraselmis* sp. with agricultural fertilizer media were higher than those of conway media. Nitrogen contained urea (fertilizer Media) which is a more dominant factor in stimulating *Nannochloropsis* sp., *Chlorella* sp., and *Tetraselmis* sp. growth than in conway media.

According to the Canter *et al* ., (2015) several studies have investigated the performance of low-cost culture media based on commercial fertilizers for the cultivation of marine microalgae. The preparation of pure chemical media for mass algae culture was very expensive. Therefore, the cheaper commercial fertilizers were used for microalgae culture, this could contribute to the further development of large scale microalgae culture for different fields.

The results of the present study indicated that agricultural fertilizer media was cost effective than the conway media.

Conclusion

The best growth and cell densities of *Nannochloropsis* sp., *Chlorella* sp., and *Tetraselmis* sp. were found in agricultural fertilizer media when compared to conway media. The combination of agricultural fertilizers such as urea and triple superphosphate strongly supported the growth of of *Nannochloropsis* sp., *Chlorella* sp., and *Tetraselmis* sp.

The success of fingerlings production of fish in the hatchery for stocking in the grow-out production system is largely dependent on the availability of suitable microalgae. The mass production of *Nannochloropsis* sp., *Chlorella* sp., and *Tetraselmis* sp. using agricultural fertilizer will benefit to get maximum growth and survival of marine fish larvae, fry, and fingerlings.

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