# Productive of fish larvae feed, *Nannochloropsis* sp. Using Different Fertilizers Media

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## ABSTRACT

*Nannochloropsis* sp., a Microalgae, play an important role in aquaculture as live food for the larval stage of many species of crustacean and fish as well as for all stages of bivalves and as food for the zooplankton. *Nannochloropsis* sp. is easy to cultivate in semi-mass and mass cultivations. The high cost of culture medium used in optimal growth of the algae is one of the main problems related to the large scale culture of *Nannochloropsis* sp. The present study was made to develop a cheap and simple medium for *Nannochloropsis* sp. based on agricultural fertilizers available in the agricultural shop. This research was designed using five different cultural Media A, B, C, D and Conway was a control. Agricultural fertilizers consisted of A Urea 100 ppm, Ammonium Sulphate 2 ppm, Triple Super Phosphate (TSP) 20 ppm, Urea 50, Ammonium Sulphate 20 ppm, Triple Super Phosphate (TSP) 15 ppm, C: Urea 40 ppm, Ammonium Sulphate 20 ppm, Triple Super Phosphate (TSP) 10 ppm, D: Urea 30, Ammonium Sulphate 5 ppm, Triple Super Phosphate (TSP) 20 ppm, and Conway media 1 ppm which has been used in traditional culture. Conway as a control. A total of four replications were prepared for each media. The cell densities of *Nannochloropsis* sp. in different culture media were counted daily through the culture period. The result showed that the maximum population density of *Nannochloropsis* sp. occurred in agricultural fertilizer media A compared to other media. It was concluded that the combination of the agricultural fertilizer media A was the best for the growth rate of *Nannochloropsis* sp.

***Keywords*:** Microalgae, *Nannochloropsis* sp., Agricultural fertilizers media, population density, cultivation of *Nannochloropsis* sp., growth rate of *Nannochloropsis* sp.

## INTRODUCTION

Live food organisms include all plants (phytoplankton) and animals (zooplankton). Most of the fish and shellfish larvae in nature feed on small phytoplanktonic and zooplanktonic organisms

Phytoplankton consists of one-celled marine and freshwater microalgae and other plant-like organisms. Microalgae are the floating micros unicell plant that is generally free-living and pelagic. Marine microalgae are the floating microscopic unicellular plant of the seawater which is generally free-living pelagic and a size range from 2 to 20μm. Microalgae are able to swim in water columns and are constantly available to fish and shellfish larvae are likely to stimulate larval feeding response (Anuraj *et al*., 2015).

The important components of microalgae are the diatoms, dinoflagellates, green algae, blue-green algae, and coccolithophores. Most microalgae have immense value are rich sources of essential fatty acids, pigments, amino acids, and vitamins. Microalgae are used to produce nutritional supplements containing omega-3 long-chain polyunsaturated fatty acids. Microalgae have unique antibacterial and/or immunostimulatory properties (Austin *et al*., 1992).

Microalgae especially phytoplankton should have criteria that are able to be used in aquaculture such as non-toxic, having nutrition value, easy to digest and cultured (Hemaiswarya *et al*., 2010).

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Microalgae play an important role in aquaculture as live food for the larval stage of many species of crustaceans and fish as well as for all stages of bivalves and as food for zooplankton species such as nauplii of *Artemia* sp. and *Brachiomus plicatilis* (Barclay and Zeller, 1996). The hatchery production of fish fingerlings for stocking is largely dependent on the availability of suitable live food for feeding fish larvae, fry, and fingerlings.

Live food organisms contain all the nutrients such as essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids, and fatty. Providing appropriate live food plays a major role in achieving maximum growth and survival of the larval stage of finfish and shellfish.

Several microalgae strains are usually used in aquaculture either individually or in combination, based on the nutrition demands. The most common species are *Nannochloropsis* sp., *Scenedesmus* sp., *Isochrysis* sp., *Pavlova* sp., *Dunaliella* sp., *Spirulina phaeodactylum*, *Chlorella* sp., *Rhodomonas* sp., *Tetraselmis* sp., *Skeletonema* sp., and *Thalassiosira* sp. (Parrish *et al*., 2012).

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). Providing natural feed usually arises when organisms live in a cultivation environment. Cultivation of Live food is economically important in hatcheries of marine finned fish and crustaceans. *Nannochloropsis* sp. is easy to cultivate in semi-mass and mass scale cultivations and can be grown in ponds or in more intensive bioreactor systems. The success in the hatchery production of fish fingerlings for stocking in the grow-out production system is largely dependent on the availability of suitable live food.

In the culture, the use of pro-analysis fertilizer (Conway) causes very high cost, so that the alternatives of agricultural fertilizers were applied as sources of nitrogen, micronutrients, and phosphate to reduce the cost in producing microalgae (Lam and Lee, 2012).

In Myanmar fish farmers have limited experience to cultivate the microalgae such as *Nannochloropsis* sp., which is necessary for marine fish and shrimp rearing. Moreover, fish farmers are facing the high cost of culture medium used in optimal growth of the algae is one of the main problems related to the large scale culture of *Nannochloropsis* sp. One of the strategies to resolve this problem is the use of a low cost culture medium. The mass production *Nannochloropsis* sp. by the use of agricultural fertilizer benefits maximum growth and survival of fish larvae, fry and fingerlings.

The aims of the present study were to develop the cheap and simple medium for *Nannochloropsis* sp. culture in Laboratory conditions to reveal the appropriate doses of agricultural fertilizer for the growth of *Nannochloropsis* sp.

## MATERIALS AND METHODS

### Study site

The present study was conducted in the Live food Laboratory of Fisheries and Aquaculture in the Research and Innovation Center, University of Yangon (Fig. 1 and 2).

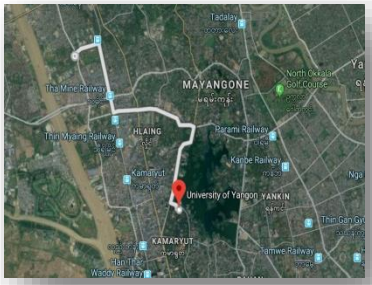


Fig.1 Map of the Study Area


Fig. 2 Live Food Culture Laboratory

**Study period**

The study period lasted from 2020 to 2021.

**Sample collection**

The pure strains of *Nannochloropsis* sp. were purchased from Thailand. Cultivation of *Nannochloropsis* sp. was conducted in Live Food Culture Laboratory of Fisheries and Aquaculture, Center for Research and Innovation, University of Yangon.

All apparatus (beakers and bottles) were covered by aluminum foil and autoclaved at 120 °C for 25 mins to avoid contaminations. The seawater was autoclaved at 120 °C for 20 mins to avoid contamination. The seawater was diluted with distilled water to obtain 25‰ salinity and filtered with Millipore (0.45 μm) filter paper. The solution was then kept in a dark and cold place until used.

**Preparation of different Agricultural Fertilizers Media**

Agricultural fertilizers such as Urea, Ammonium Sulphate, and Triple Super Phosphate (TSP) were weighed by using a digital balance and added to the beaker that contained 1000ml of distilled water. After adding the substances to the beaker, the solution was stirred by using a magnetic stirrer. The solutions were sterile in an autoclave machine for 121°C at 25 mins, the solutions were then taken out when the temperature was dropped to 80°C. Then, the solutions were stored in a sterilized bottle and kept in refrigerator to avoid contamination for further use. The fertilizer ratio of media (A), (B), (C) and (D) are described in (Table 1).

Table.1 Nutrient composition and concentrations of different Fertilizer

| Concentration of Agricultural Fertilizer ppm (milligram/Liter) | | | | |
|---|---|---|---|---|
| Nutrient (Media) | Nitrogen (Urea) | Ammonium Sulphate | Triple Super Phosphate (TSP) | Conway Fertilizer as Control |
| A | 100 | 2 | 20 | |
| B | 50 | 20 | 15 | |
| C | 40 | 20 | 10 | |
| D | 30 | 20 | 5 | |
| Conway | | | | 1 |

**Preparation of Conway media**

For Conway media three types of solution: solution I, II and III were prepared separately. They were then mixed prior to the cultivation of *Nannochloropsis* sp. For solution I, all chemical substances (Disodium Hydrogen Phosphate, Boric Acid, Ferric Chloride, Manganese Chloride, Na2EDTA and Sodium nitrate) were weighed by using a digital balance and added in the beaker that contained 900ml of distilled water. After adding the substances to the beaker, the solution was stirred by using a magnetic stirrer. (Table 2).

Table 2. Composition of Conway media (Solution I)

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

For solution II, 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 3).

Table 3. Composition of Conway Media (Solution II)

| Chemical | Weight (g) |
|---|---|
| Zinc Chloride | 2.1g |
| Calcium chloride | 2.1g |
| Ammonium paramolybdate tetrahydrate | 2.1g |
| Copper II sulfate | 2g |
| Distilled water | 100ml |

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

All the solutions (I, II and III) in Conway media were prepared separately. 900 mL of Solution I was mixed with 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 was dropped to 80°C.The solutions were added Solution III (Vitamin) and stirred. Then, the solution was stored in a sterilized bottle and kept in refrigerator.

**Cultivation of *Nannochloropsis* sp. using different media**

*Nannochloropsis* sp. was treated with different agricultural fertilizer media A, B, C, D and E (Conway). The sterilized plankton culture glass bottle (2000 mL**)** was filled with 800 mL of sterilized seawater and 200 mL of pure strain *Nannochloropsis* sp. was added into this glass bottle and then add 1ml of the nutrient different agricultural fertilizers media for each experiment (A, B, C, D) and Conway . Four replications were prepared for the experiment (Plate 1). Each culture bottle was sealed with aluminum foil and labeled with the date and time. The Plankton culture bottles were arranged on the cultivation shelf and aerated with the blower. All culture bottles were kept at air-conditions room at 25°C with light. All glass bottles were supplied with light illuminated by fluorescent tubes. The nutrient agriculture fertilizer concentrations are presented in Table 1. The experiments were extended for 10 days and the population density of *Nannochloropsis* sp. was calculated every day.


Plate 1. Cultivation of *Nannochloropsis* sp. using different media

**Determination of growth conditions and cell density** of ***Nannochloropsis* sp.**

The growth of *Nannochloropsis* sp. was estimated by using hemocytometer to count the cell density. The subsamples of 1-mL from each bottle were collected without replacement. One drop of cell suspension was placed in the central counting chamber of hemocytometer (Thoma, Tokyo) and covered with cover glass (22 mm) carefully to avoid the formation of bubbles between the cover glass and the 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. 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 will be a no cell division phase for few hours which is known as lag or induction phase.
2. Exponential phase**:** after the lag phase, the cells are acclimatized and start dividing, grow fast by utilizing nutrients, aeration and light. This growth phase is called exponential phase and reaches maximum cell concentration during this period.

3. Declining phase*:* After reaching the growth phase, the cells will be showing less growth or slow growth. This stunted growth stage is known as declining phase.
4. Stationary phase*:* The declining phase will be continuing for few days without any cell division and this period is known as stationary phase. Sometimes, the culture may divide the cells with suitable conditions.
5. Death phase*:* Prolonged stationary phase will lead to the death phase, where algal cells will lose their viability and the cells dies. This phase is called death phase (Fig. 3).

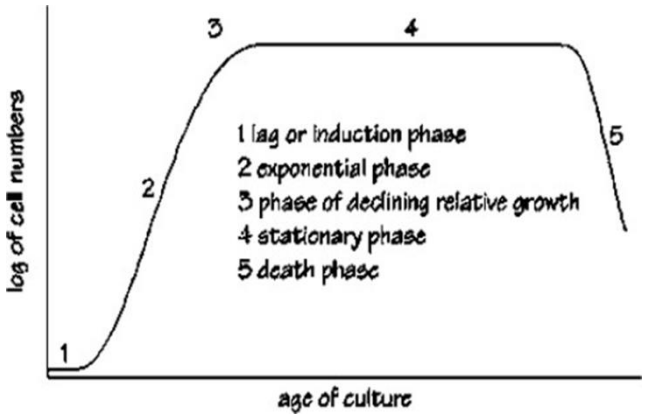


Fig. 3 Growth phase of Algae (Creswell, 1993)

**Determination of water quality**

Water quality analysis was estimated by DO (dissolved oxygen), pH, temperature, nitrate, and ammonia and was conducted at the early and the end of culture.

**Data Analysis**

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

## RESULTS

The present experiments were performed to produce the *Nannochloropsis* sp. in laboratory conditions using five different agricultural fertilizers media (A, B, C, D) and Conway media. The cell density of *Nannochloropsis* sp. using five different agricultural fertilizers media (A, B, C, D) and Conway media were daily investigated. The initial stocking density of *Nannochloropsis* sp. in Day 1 was (1.80 x $10^6$ cell/ml).

The growth of *Nannochloropsis* sp. cultured with agricultural fertilizer media A started to increase from the second day to fourth day and fifth day was started to decline. In the other fertilizers media B, C, D and Conway media, the growth progress was started to increase from the second day to fifth day and then sixth day was started to decrease. The peak of *Nannochloropsis* sp. population with the agricultural fertilizers media A was found in the fourth day while it was found in fifth day in fertilizers B, C, D and Conway.

The maximum population density of culture *Nannochloropsis* sp. was occurred in agricultural fertilizers medium A (9.04 x$10^6$ cell/ml) and was followed by medium B (8.79 x$10^6$ cell/ml), then medium C (8.41 x$10^6$ cell/ml) and then by D (8.19 x$10^6$ cell/ml) and finally Conway media (8 x$10^6$ cell/ml).

The highest population density of culture *Nannochloropsis* sp. was occurred in the agricultural Fertilizers media A compared to other agricultural fertilizers medium and Conway media. At the end of cultivation period, the minimum population density of culture *Nannochloropsis* sp. was observed in agricultural fertilizers medium A (1.15 x$10^6$ cell/ml), followed by medium B (1.24 x$10^6$ cell/ml), medium C (1.77 x$10^6$ cell/ml) and then by D (1.74 x$10^6$ cell/ml) and finally Conway media (1.82 x$10^6$ cel$^l$/ml) in Table 4. The population density of *Nannochloropsis* sp. in different agricultural Fertilizers B, C, D and E Conway media were illustrated in Figure 4.

Table 4. Population density of *Nannochloropsis* sp. in the different culture media during the period of 10 days cultivation

| **Cell Densities of *Nannochloropsis* sp. (nx$10^6$cell/ml) (mean ± SD)** | | | | | |
|---|---|---|---|---|---|
| **Time (Day)** | **Media A (cell/mL)** | **Media B (cell/mL)** | **Media C (cell/mL)** | **Media D (cell/mL)** | **Conway media (cell/mL)** |
| **Day 1** | 1.80±0.01 | 1.80±0.01 | 1.80±0.01 | 1.80±0.01 | 1.80±0.01 |
| **Day 2** | 4.06 ± 0.06 | 3.75 ± 0.21 | 3.6 ± 0.11 | 3.5 ± 0.22 | 3.47 ± 0.47 |
| **Day 3** | 8.28 ± 0.04 | 6.54 ± 0.03 | 6.10 ± 0.11 | 5.8 ± 0.15 | 4.9 ± 0.13 |
| **Day 4** | **9.04 ± 0.05** | 7.57 ± 0.21 | 7.23 ± 0.28 | 7.13 ± 1.36 | 7.04 ± 0.38 |
| **Day 5** | 7.51 ± 0.16 | **8.79 ± 0.21** | **8.41 ± 0.17** | **8.19 ± 0.12** | **8.00 ± 0.36** |
| **Day 6** | 6.59 ± 0.12 | 6.72 ± 0.12 | 6.47 ± 0.08 | 6.31± 0.05 | 6.6 ± 0.33 |
| **Day 7** | 5.68 ± 0.22 | 5.64 ± 0.12 | 5.17 ± 0.10 | 5.02 ± 0.13 | 4.48 ± 0.40 |
| **Day 8** | 4.54 ± 0.08 | 4.51± 0.21 | 4.14 ± 0.12 | 4.08 ± 0.11 | 3.10 ± 0.22 |
| **Day 9** | 2.19± 0.13 | 2.21± 0.16 | 2.74± 0.16 | 2.49 ± 0.14 | 2.25 ± 0.01 |
| Day **10** | 1.15 ± 0.10 | 1.24 ± 0.21 | 1.77 ± 0.22 | 1.74± 0.09 | 1.82 ± 0.01 |

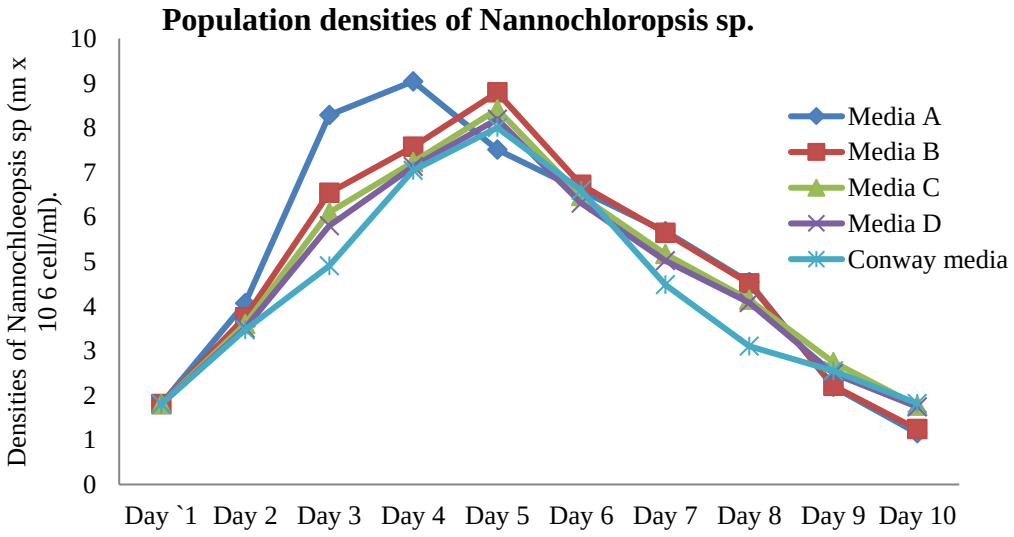


Fig. 4 Population density of *Nannochloropsis* sp. during the culture period

Mean cell density in each media during the cultivated period was calculated and described in Table 5. The highest mean cell density of *Nannochloropsis* sp. was found in Media A while the lowest one was in Conway.

Table 5. The maximum cell density of *Nannochloropsis* sp. in the different culture media.

| **Nutrient Fertilizer Media** | **Mean cell densities of *Nannochloropsis* sp. (nx10$^6$ cell/ml (mean ± SD) )** | | |
|---|---|---|---|
| Media A (cell/mL) | 9.04 | ± | 0.05 |
| Media B (cell/mL) | 8.79 | ± | 0.21 |
| Media C (cell/mL) | 8.41 | ± | 0.17 |
| Media D (cell/mL) | 8.19 | ± | 0.12 |
| Conway media(cell/mL) | 8.00 | ± | 0.36 |

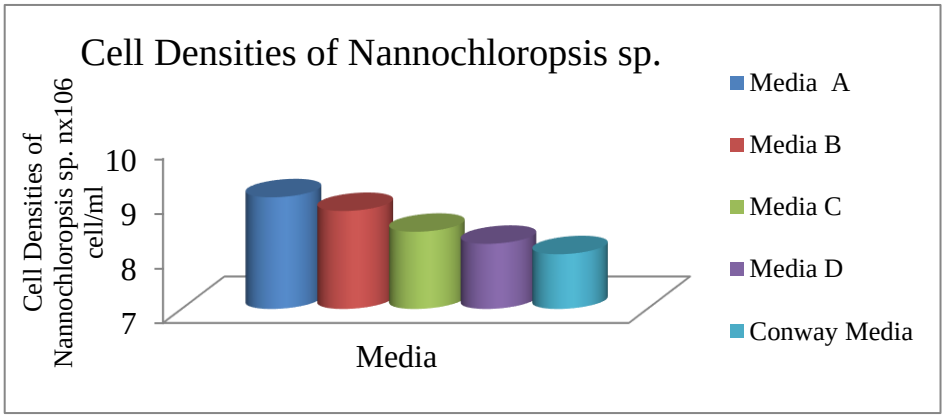


Fig. 5 Maximum cell densities of *Nannochloropsis* sp. in different culture media

The water quality observation of the culture period was described in Table 6. Concentrations of DO (dissolved oxygen), pH, temperature, nitrate, and ammonia in the media culture were around approximately 5.51 – 5.56 mg/L, 8.1- 8.2, 27.8 - 28.2 °C, 7.02 – 5.04 mg/L and 2.52 -2.47 mg/L respectively.

Table 6. Water parameter of cultured *Nannochloropsis* sp. during a period of cultivation

| **No** | **Parameters** | **Unit** | **Media A** | **Media B** | **Media C** | **Media D** | **Media E** |
|---|---|---|---|---|---|---|---|
| **1** | **DO (dissolved oxygen)** | **mg/L** | 5.51 ±0.02 | 5.52±0.01 | 5.54±0.02 | 5.56 ±0.01 | 5.52±0.02 |
| **2** | **pH** | | 8.2 ±0.06 | 8.15 ±0.02 | 8.1 5±0.01 | 8.12 ±0.07 | 8.11 ±0.1 |
| **3** | **Temperature** | **°C** | 28.2 ±0.25 | 28.2 ±0.01 | 27.8 ±0.1 | 27.8 ±0.1 | 27.9 ±0.34 |
| **4** | **Nitrate** | **mg/L** | 7.02 ±0.01 | 6.52 ±0.01 | 6.62 ±0.01 | 6.03 ±0.08 | 5.04 ±0.02 |
| **6** | **Ammonia** | **mg/L** | 2.50±0.02 | 2.49 ±0.02 | 2.52 ±0.02 | 2.51 ±0.01 | 2.47 ±0.01 |

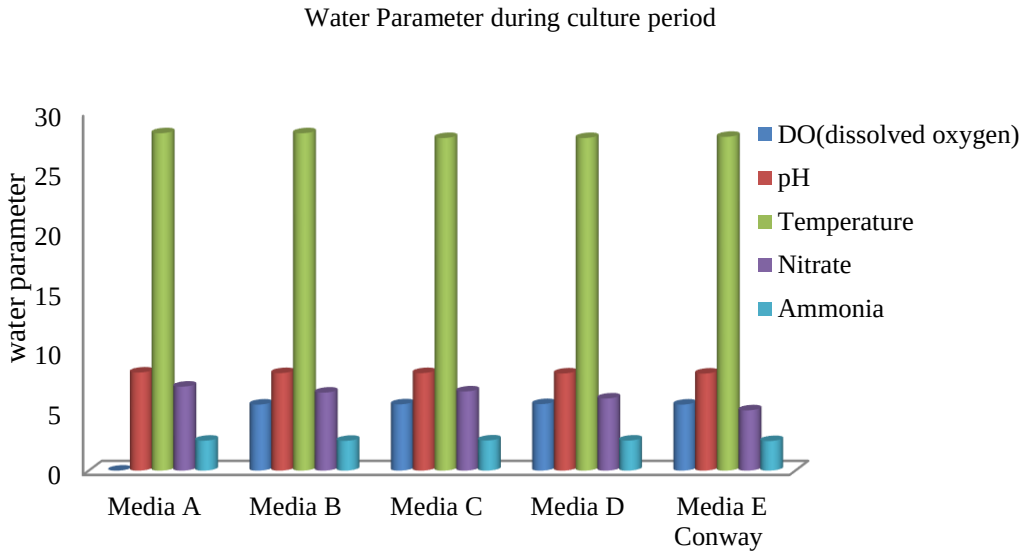


Fig. 6 . Water parameter of cultured *Nannochloropsis* sp. during a period of cultivation

## DISCUSSION

The present study was indicated the variation of cell density of *Nannochloropsis* sp. using the different agricultural Fertilizers Media. The growth of *Nannochloropsis* sp. was estimated by population density. The variation of mean cell density of *Nannochloropsis* sp. was counted daily during the cultured period.

In agricultural fertilizers, media A, the maximum population density of *Nannochloropsis* sp. was occurred on the fourth day of cultivation while other agricultural fertilizers media B, C, D, and Conway media was reached their maximum densities by the fifth day during the cultivation period.

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. occurred from the first day to the second day as called the lag phase. The fastest generating time (doubling time) of *Nannochloropsis* sp. started on the second day to the fourth day as called the exponential growth phase.

In the present study the growth phase of *Nannochloropsis* sp. was occurred on the first day and the second day of the cultured period is the lag phase and the fastest generating time (doubling time) of *Nannochloropsis* sp. started on the second day to the fourth day is exponential phase growth phase. The growth of *Nannochloropsis* sp. progress started to decrease in the sixth day.

Sivakumar and Rajendran, (2013) stated that microalgae require nutrients for their growth because nitrogen is a major nutrient for microalgal cultivation and the marine environment. The principal nutrients are nitrogen (N), potassium (K), carbon (C), and phosphorus (P). The source of carbon comes from carbon dioxide or sodium bicarbonate and NPK from the commercial NPK fertilizer. In addition, microalgae growth also requires appropriate temperature, adequate sun rays, and an optimal combination of NPK.

Daefi *et al*., 2017 described that Nitrogen contained in urea (fertilizer Media) is a more dominant factor in stimulating *Nannochloropsis* sp. growth than in Conway Media. Nitrogen contained in urea fertilizer at doses of 50 ppm is a more dominant factor in stimulating *Nannochloropsis* sp. growth than doses of 40 ppm and 30 ppm in laboratory-scale cultures.

Becerra-Dórame *et al.,* (2010) reported the cultivation of *Dunaliella* sp. in which a positive correlation was found between nutrient concentration and cell density, with a high cell density with the richer medium. Golz *et al.,* (2015) stated that the composition of elements in culture media affects the growth of *Nannochloropsis* sp. as a major producer in the aquatic ecosystem food chain.

In the present study, the composition of nitrogen (urea) in agricultural Fertilizers media A was higher than other agricultural Fertilizers media B, C, and D. Therefore, the highest population density of culture *Nannochloropsis* sp. was occurred in the agricultural Fertilizers media A compared to other agricultural Fertilizers media B, C, D and Conway media. So, the present finding was agreed with the previous finding by Daefi *et al*., 2017.

Alsull and Omar, (2012) described that *Nannochloropsis* sp. shows a significant decrease in growth rate, chlorophyll content, and dry weight when it was cultivated in the limited nitrogen condition and nitrogen starving. Water parameters also support the growth of phytoplankton especially *Nannochloropsis* sp. for optimum conditions (Creswell, 1993).

On the other hand, Renaud and Parry (1994) stated that the most important parameters nitrate ($NO_3$) and phosphate $(PO4)^{-3}$ influencing algae growth, biochemistry composition and physiological activity includes quality and amount of nutrients, pH, light, salinity, turbulence, and temperature. In this study, the highest density was found in media A in which the highest nitrogen content was found.

Kamal Elnabris, (2012) indicated that increasing the concentration of ammonia (NH3) which has a toxic effect on alga growth and consequently, decreases the algal density.

The result of the present study the amount of ammonia ($NH_3$) in fertilizers medium A was lower than the media B, C, D. and Conway amount. However, the amount of urea in media A was higher than in the other media. Algal density was increased in agricultural media A compared to others. Urea is agricultural fertilizer and it enhances the formation of chlorophyll.

Kamal Elnabris, (2012) described that agricultural fertilizer such as urea, Calcium superphosphate, ammonium sulfate, micronutrient, and vitamin solutions strongly supported the growth of *Nannochloropsis* sp. and confirmed that using agricultural grade fertilizers can substitute the F/2 media which is commonly used for culture in commercial aquaculture.

The agricultural fertilizer media were eight times cheaper than the F/2 medium (Simental &Sánchez-Saavedra, 2003). The result of the present study observed that the cell density of *Nannochloropsis* sp. in agriculture fertilizer media was higher and the prices of agricultural fertilizer were cheaper than the Conway media.

## CONCLUSION

The best growth of *Nannochloropsis* sp. was found in agricultural fertilizer medium A when it was compared to other media B, C, D., and Conway media. It is indicated that combinations of agricultural fertilizers such as urea, Triple superphosphate, ammonium sulfate, strongly support the growth of *Nannochloropsis* 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. using agricultural fertilizer will benefit maximum growth and survival of fish larvae, fry, and fingerlings.

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