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Effect of KW21 and water-extracted horseradish leaf combination on *Nannochloropsis* sp. density in laboratory scale

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Abstract

This study aims to know the effect of water-extracted horseradish *Moringa oleifera* leaf and KW21 fertilizer combination application on the density of *Nannochloropsis* sp. It was conducted in the Natural Food Laboratory of State Polytechnique, Tual. The experiment used complete randomized design with 5 treatments and 3 replications: A (25% horseradish leaf extract + 75% KW21), B (50% horseradish leaf extract + 50% KW21), C (75% horseradish leaf extract + 25% KW21), D (positive control of 100% KW21), and E (negative control of 100% horseradish leaf extract). Results showed that Treatment C yielded the best result, both the highest density of *Nannochloropsis* sp. and suitable harvest time.

Keywords: *Nannochloropsis* sp., Horseradish leaf, KW21 fertilizer, Laboratory scale

Introduction

Fish larval rearing into seeds highly needs appropriate natural food in order to avoid an intake gap of energy at the early larval stage (Kadarini et al., 2013). Therefore, the natural feed supply must be in a suitable amount to the larval need, continuity, and on time (Sari & Manan, 2012).

Feed is one of the important needs to be considered to determine the success of the fish culture. One of the crucial feed types needed at the larval stage is a natural food that consists of phytoplankton and zooplankton (Enzing et al., 2014; Gheysen et al., 2019) and becomes one of the supporting factors in fish culture success. Microalga is a unicellular organism, green-colored, occurs in freshwater and marine environments, and produces high lipid levels, high carotenoid, amino, and rich in various micronutrients (Buono et al., 2014; Matos et al., 2017; Wu et al., 2017).

Microalga is also required as food in zooplankton rearing (Mukhlis et al., 2017). Microalga is a lower-level plant that possesses chlorophyll for photosynthesis (Rismiarti et al., 2016).

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Several species of microalgae in nature are natural food for fish and shrimps. It becomes an important source of nutrition at the early stage of organism development (Mufidah et al., 2017).

Microalga is one of the organisms that have the most varied types and are widely distributed in all aquatic environments, either seawater or freshwater (Gimpel et al., 2015). In general, microalgae produce long-chained fatty acid that has numerous benefits for health (Chitranjali et al., 2015; Qiu et al., 2019; Zuorro et al., 2016). The substances, such as fatty acid, protein, chlorophyll, carotenoid, and several vitamins are very interesting to be developed on a commercial scale (Ali & Watson, 2015; de Jesus Raposo et al., 2013; Qadariyah et al., 2018), besides as biodiesel products (Huerlimann et al., 2010; Zuorro et al., 2016).

Nannochloropsis sp. is one of the important feed types for fish larvae and zooplankton (rotifer and artemia) that has high nutritive content so that it is reared in high numbers by the fish farmers (Sinaga et al., 2020) and contains omega3 (Adam et al., 2012; Qiu et al., 2019). *Nannochloropsis* sp. is an autotrophic organism (capable of producing food for itself) by absorbing carbon dioxide in photosynthesis and producing oxygen. This organism can grow and develop through photosynthesis by taking advantage of sunlight as an energy source and simple inorganic nutrients, such as CO_2, dissolved nitrogen, and phosphate. The presence of chlorophyll makes this phytoplankton capable of photosynthesizing to become a source of protein, carbohydrate, fat, vitamin, and minerals for aquatic organisms (Utami et al., 2012).

Important factors in *Nannochloropsis* sp. rearing besides nutrients are light intensity and length of exposure. The nutrient can be used by each phytoplankton to do the development process (Selvika et al., 2016). Phytoplankton needs light to photosynthesize, and if the light is limited, the growth activity will also be inhibited (Nurdiana, 2017).

One of the Indonesian plants that are believed to have antioxidant content is horseradish leaf *Moringa oleifera* (Jusnita & Tridharma, 2019; Toripah et al., 2014; Yuliani & Dienina, 2015) which is used as food, drug, fertilizer, etc. (Misra & Misra, 2014; Oluduro, 2012; Zongo et al., 2013). Horseradish leaf is found to contain calcium, iron, protein, vitamin A, vitamin B, and its vitamin and iron content are higher than other vegetables, 17.2 mg/100 g (Yamèogo et al., 2011). Another previous study shows that horseradish plant contains antimicrobial, antifungal, antihypertension, antihyperglycemic, antitumor, anticancer, and anti-inflammation (Toma & Deyno, 2014). The extract can also function as antidiarrhea at the oral dose of 300 mg/kg body weight (Misra et al., 2014).

Horseradish is an important food material for humans, especially those who live in rural areas. It is also believed to have antibiotic, anticancer, anti-inflammatory, hypocholesterolemic, and hypoglycemic effects (Fahey & Fahey, 2005). This plant contains sufficiently good phytochemical content from the root part, wood skin, leaf, fruit, flower, and seed, which are traditionally used to cure various diseases, such as skin infection, anemia, asthma, bronchitis, headache, rheumatic, diarrhea, and etc. (Kumar et al., 2010).

The leaf also contains various amino acids, such as aspartic acid, glutamic acid, alanine, valine, leucine, isoleucine, histidine, lysine, arginine, phenylalanine, tryptophan, cysteine, and methionine, and fresh leaf contains 3.4% phenol and 1.6% in leaf extract (Aminah et al., 2015).

KW21 fertilizer is commonly used by microalga farmers, and it is imported from Japan for microculture on a laboratory scale. However, this fertilizer is expensive but difficult to find, so its application needs to be considered (Arfah et al., 2019). Therefore, looking for a KW21 substitute is crucial to reducing the farmer's expenditures. This is done by benefitting the horseradish leaves that are inexpensive and easily found in great numbers. This study aims to know the effect of KW21 and horseradish leaf extract combination on the growth of *Nannochloropsis* sp.

Materials and Methods

Media preparation of *Nannochloropsis* sp. culture

This experiment used seawater filtered through a filter bag as media. The filtered seawater was boiled so that it is free of germs and sterile. Boiling is intended to remove all living organisms that could disturb the growth of *Nannochloropsis* in culture. According to Purnamawat et al. (2014), sterilization is carried out to make the culture media free of contaminants.

Preparation of water-extracted horseradish leaf as a natural fertilizer and KW21 fertilizer

Horseradish leaves were collected from the planted tree shooting dense leaves in bright green color, cleaned, removed from the branch, and even from the leaf bone so that only leaf sheets were obtained. The leaves were then washed in clean freshwater to remove dirt, parasite, or bacteria attached to the leaf surface up to clean. As much as 100 g was weighed and blended with 500 mL of water, filtered through flannel, and prepared to be

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combined with KW21 for the *Nannochloropsis* sp. growth experiment.

Laboratory culture

Nannochloropsis sp. culture in controlled media used KW21 fertilizer of 100% as positive control and 100% horseradish leaf extract (1 mL/L) *Nannochloropsis* sp. as a negative control in 3 L water-plastic jar and put on the culture cupboard facilitated with 2 units of 40 watt-Philip TL light as source of light and aerated for oxygen supply. *Nannochloropsis* sp. needs light intensity between 2,500 and 5,000 lux. Culture activity was done for 11 days so that the algae could adapt to the new environment. The inoculant used was 30% of the water volume.

The study applied 5 treatments with 3 replications as follows: A (25% horseradish leaf extract + 75% KW21), B (50% horseradish leaf extract + 50% KW21), C (75% horseradish leaf extract + 25% KW21), D (positive control of 100% KW21), and E (negative control of 100% horseradish leaf extract). Each plastic jar contained sterile 24‰ salinity-seawater and 30% of *Nannochloropsis* sp. 30% seed in one-liter volume added with horseradish leaf extract and KW21 fertilizer, except control positive and control negative treatments. Growth observations on *Nannochloropsis* sp. cells were done daily under the microscope supported with a hemocytometer. Water quality parameter measurements were temperature, salinity, and acidity (pH). Room temperature was recorded as well. This observation was carried out until *Nannochloropsis* sp. showed declined growth trends up to mortality.

Growth analysis of *Nannochloropsis* sp. was estimated following Isnansetyo & Kurniastuty (1995):

$$\text{Cell density (cells/mL)} = n \times 4 \times 10^6$$

where n = number of cells counted and 4×10^6 = hemocytometer constant.

The cell growth data of Nannochloropsis sp. were presented in the graphical form and analyzed with one way analysis of variance (ANOVA) using the SPSS program to know whether the culture media affects the growth. The presence of significant difference was then continued with the least significant difference (LSD) test to detect the effect between treatments.

Results

Application of horseradish leaf extract combined with KW21 at different concentration combinations significantly affected the *Nannochloropsis* sp. cell density (Table 1).

The difference between treatment effects was done using the LSD. It indicated that Treatment A (25% horseradish leaf extract + 75% KW21) gave a non-significantly different density of *Nannochloropsis* sp. from other treatment concentration applications. Treatment B (50% horseradish leaf extract + 50% KW21) gave a highly significant difference in *Nannochloropsis* sp. density from that of Treatment C (75% horseradish leaf extract + 25% KW21) ($p < 0.001$), but a non-significantly different effect on *Nannochloropsis* sp. density from that of Treatments A, D, and E. Treatment C (75% horseradish leaf extract + 25% KW21) also yielded a significantly different density of *Nannochloropsis* sp. from Treatment D (100% KW21) ($p < 0.05$) and Treatment E (100% horseradish leaf extract) ($p < 0.05$) (Table 2).

Nannochloropsis sp. cell density changed with time. Treatment A (25% horseradish leaf extract + 75% KW21) yielded an increase in cell density from $2.52 \times 10^7 \pm 4.50 \times 10^6$ to $4.75 \times 10^7 \pm 5.30 \times 10^6$ on day-3, then fell down from $4.14 \times 10^7 \pm 1.95 \times 10^6$ on day-4 to $1.81 \times 10^7 \pm 3.97 \times 10^6$ on day-7, slightly rose on day-8, but declined again to day-10 (Table 3). All *Nannochloropsis* cells died on day-11.

Treatment B (50% horseradish leaf extract + 50% KW21) made the cell density of *Nannochloropsis* sp. increase up to day-3 from $1.84 \times 10^7 \pm 8.67 \times 10^5$ to $4.34 \times 10^7 \pm 2.49 \times 10^6$, then fell down until day-6 to $1.59 \times 10^7 \pm 9.62 \times 10^5$. From day-7 to day-9, the cell density slightly rose to $1.80 \times 10^7 \pm 2.93 \times 10^6$, but all cells died on day-10.

Treatment C (75% horseradish leaf extract + 25% KW21) increased the cell density of *Nannochloropsis* sp. from $2.49 \times 10^7 \pm 4.81 \times 10^6$ on day-1 to $4.42 \times 10^7 \pm 1.12 \times 10^6$ on day-3,

Table 1. Analysis of variance on *Nannochloropsis* sp. density during the study

Source of variance	Sum of squares	*df*	Mean square	*F*-value	*p*-value
Between groups	2,171,740,462,955,047.800	4	542,935,115,738,761.940	3.703	0.007
Within groups	21,261,746,758,640,792.000	145	146,632,736,266,488.220		
Total	23,433,487,221,595,840.000	149			

Table 2. Multiple comparisons of mean difference of cell density between treatments

Treatment comparison	Mean difference	SE	*p*-value
Treatment A			
B	6,174,999.99800	3,126,582.11968	0.050
C	–5627777.77967	3,126,582.11968	0.074
D	2,074,999.99933	3,126,582.11968	0.508
E	602,777.77700	3,126,582.11968	0.847
Treatment B			
A	–6174999.99800	3,126,582.11968	0.050
C	–11802777.77767*	3,126,582,11968	0.000
D	–4099999.99867	3,126,582.11968	0.192
E	–5572222.22100	3,126,582.11968	0.077
Treatment C			
A	5,627,777.77967	3,126,582.11968	0.074
B	11,802,777.77767*	3,126,582.11968	0.000
D	7,702,777.77900*	3,126,582.11968	0.015
E	6,230,555.55667*	3,126,582.11968	0.048
Treatment D			
A	–2074999.99933	3,126,582.11968	0.508
B	4,099,999.99867	3,126,582.11968	0.192
C	–7702777.77900*	3,126,582.11968	0.015
E	–1472222.22233	3,126,582.11968	0.638
Treatment E			
A	–602777.77700	3,126,582.11968	0.847
B	5,572,222.22100	3,126,582.11968	0.077
C	–6230555.55667*	3,126,582.11968	0.048
D	1,472,222.22233	3,126,582.11968	0.638

Treatment A, 25% horseradish leaf extract + 75% KW21; Treatment B, 50% horseradish leaf extract + 50% KW21; Treatment C, 75% horseradish leaf extract + 25% KW21; Treatment D, 100% KW21; Treatment E, 100% horseradish leaf extract.
* The asterisk indicates a significant difference ($p < 0.05$).

and reached the highest, $4.76 \times 10^7 \pm 2.60 \times 10^6$, on day-4. Afterward, the cell density started declining to $4.10 \times 10^7 \pm 4.17 \times 10^5$ on day-5, and $3.57 \times 10^7 \pm 4.96 \times 10^6$ on day-7, then the cell density looked stable, but on day-10, there were no *Nannochloropsis* sp. cells alive.

Treatment D (100% horseradish leaf extract) yielded a *Nannochloropsis* sp. density of $1.77 \times 10^7 \pm 3.37 \times 10^5$ cells. They grew to $2.65 \times 10^7 \pm 2.32 \times 10^6$, cells on day-2, $2.69 \times 10^7 \pm 6.26 \times 10^5$ on day-3, and reached the peak of $3.75 \times 10^7 \pm 7.71 \times 10^6$ on day-5. The cell density of *Nannochloropsis* sp. fell down to $2.89 \times 10^7 \pm 5.53 \times 10^6$, then went up and down until day-10. The cell mortality occurred on day-11.

Treatment E (100% KW21) revealed a cell density of $1.34 \times 10^7 \pm 8.66 \times 10^5$ on day-1, continuously rose, and reached the peak of $4.42 \times 10^7 \pm 1.43 \times 10^6$ cells on day-5. The *Nannochloropsis* sp. density started declining from day-6 to day-7 at the density of $3.14 \times 10^7 \pm 2.33 \times 10^6$ cells, but rose again to $4.07 \times 10^7 \pm 3.50 \times 10^6$ cells, then continuously fell down until day-10. There were no lived cells recorded on day-11.

Water quality parameters

Water quality conditions in the culture media of *Nannochloropsis* sp. during a 10-day culture experiment are presented in Table 4.

Discussion

ANOVA revealed that each treatment of horseradish leaf extract and KW21 concentration combinations influenced the cell density of *Nannochloropsis* sp. ($p < 0.01$). The highest growth at the exponential phase was recorded in Treatment C with a density of $4.76 \times 10^7 \pm 2.60 \times 10^6$ cells/mL. The highest growth recorded in Treatment C could result from the correct concentration of the horseradish leaf extract and KW21 fertilizer combination so that the nutrients could be better absorbed to support the growth of the cell density of *Nannochloropsis* sp. than Treatments B, A, D, and E.

The effect of each treatment on *Nannochloropsis* culture was known from day-1 to day-3 with the highest *Nannochloropsis* density in Treatment A (25% leaf extract + 75% KW21), 2.52×10^7, on day-3 and the lowest in the positive control (KW21), 2.08×10^7. An increase in cell density is indicated with a color change from mild green to dark green. Other treatments still showed clear color since *Nannochloropsis* sp. started to adapt to KW21 and horseradish leaf extract application so Treatments B, C, and control did not clearly show cell development. The lag phase or adaptation phase occurs in which *Nannochloropsis* sp. grows slowly at the beginning of rearing due to adaptation to a new living environment (Isnansetyo & Kurniastuty, 1995).

Treatment C (75% leaf extract + 25% KW21) also gave a significant effect on the cell density of *Nannochloropsis* on day-4, in which the cell density rose to $4.76 \times 10^7 \pm 2.60 \times 10^6$ and reached the highest on the day-6, $4.06 \times 10^7 \pm 8.33 \times 10^5$. The cell density increased from day-4 to day-8. Positive control (100% KW21) showed the density increment on day-2 and reached the highest density on day-5, $4.42 \times 10^7 \pm 1.43 \times 10^6$, then declined from the day-6 to day-10. This condition shows that *Nannochloropsis* has entered the exponential phase from day-4 to day-6 in Treatment C and day-5 in the positive control

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Table 3. *Nannochloropsis* sp. cell density condition during the study

Day	*Nannocloropsis* sp. density (cells/mL)				
	Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
1	$2.52 \times 10^7 \pm 4.50 \times 10^6$	$1.84 \times 10^7 \pm 8.67 \times 10^5$	$2.49 \times 10^7 \pm 4.81 \times 10^6$	$1.77 \times 10^7 \pm 3.37 \times 10^5$	$1.34 \times 10^7 \pm 8.66 \times 10^5$
2	$4.40 \times 10^7 \pm 1.38 \times 10^6$	$4.01 \times 10^7 \pm 2.29 \times 10^6$	$4.00 \times 10^7 \pm 1.21 \times 10^6$	$2.65 \times 10^7 \pm 2.32 \times 10^6$	$1.89 \times 10^7 \pm 4.17 \times 10^6$
3	$4.75 \times 10^7 \pm 5.30 \times 10^6$	$4.34 \times 10^7 \pm 2.49 \times 10^6$	$4.42 \times 10^7 \pm 1.12 \times 10^6$	$2.69 \times 10^7 \pm 6.26 \times 10^5$	$2.08 \times 10^7 \pm 5.18 \times 10^6$
4	$4.14 \times 10^7 \pm 1.95 \times 10^6$	$3.87 \times 10^7 \pm 5.63 \times 10^6$	$4.76 \times 10^7 \pm 2.60 \times 10^6$	$3.05 \times 10^7 \pm 1.98 \times 10^6$	$1.79 \times 10^7 \pm 4.02 \times 10^6$
5	$2.81 \times 10^7 \pm 7.48 \times 10^6$	$1.78 \times 10^7 \pm 3.36 \times 10^6$	$4.10 \times 10^7 \pm 4.17 \times 10^5$	$3.75 \times 10^7 \pm 7.71 \times 10^6$	$4.42 \times 10^7 \pm 1.43 \times 10^6$
6	$2.08 \times 10^7 \pm 5.02 \times 10^6$	$1.59 \times 10^7 \pm 9.62 \times 10^5$	$4.06 \times 10^7 \pm 8.33 \times 10^5$	$2.89 \times 10^7 \pm 5.53 \times 10^6$	$3.48 \times 10^7 \pm 1.58 \times 10^6$
7	$1.81 \times 10^7 \pm 3.97 \times 10^6$	$1.79 \times 10^7 \pm 6.70 \times 10^6$	$3.57 \times 10^7 \pm 4.96 \times 10^6$	$3.15 \times 10^7 \pm 1.46 \times 10^6$	$3.14 \times 10^7 \pm 2.33 \times 10^6$
8	$2.22 \times 10^7 \pm 5.34 \times 10^6$	$1.78 \times 10^7 \pm 6.03 \times 10^6$	$3.63 \times 10^7 \pm 2.50 \times 10^6$	$2.98 \times 10^7 \pm 4.08 \times 10^6$	$4.07 \times 10^7 \pm 3.50 \times 10^6$
9	$1.49 \times 10^7 \pm 3.15 \times 10^6$	$1.80 \times 10^7 \pm 2.93 \times 10^6$	$3.59 \times 10^7 \pm 1.63 \times 10^6$	$3.38 \times 10^7 \pm 1.07 \times 10^6$	$3.49 \times 10^7 \pm 9.66 \times 10^6$
10	$2.77 \times 10^7 \pm 3.67 \times 10^6$	0.00	0.00	$2.10 \times 10^7 \pm 1.91 \times 10^7$	$1.21 \times 10^7 \pm 2.10 \times 10^7$

Treatment A, 25% horseradish leaf extract + 75% KW21; Treatment B, 50% horseradish leaf extract + 50% KW21; Treatment C, 75% horseradish leaf extract + 25% KW21; Treatment D, 100% KW21; Treatment E, 100% horseradish leaf extract.

Table 4. Water quality parameters during the study

Treatment	Parameters		
	Salinity (‰)	Temperature (℃)	pH
A	27–30	22–25	7.1–8.3
B	28–30	22–25	8.2–8.6
C	28–30	22–25	8.1–8.3
D	29–30	22–25	8.1–8.6
E	28–31	22–25	8.0–8.2

Treatment A, 25% horseradish leaf extract + 75% KW21; Treatment B, 50% horseradish leaf extract + 50% KW21; Treatment C, 75% horseradish leaf extract + 25% KW21; Treatment D, 100% KW21; Treatment E, 100% horseradish leaf extract.

treatment, in which the development of cell amounts is very high. The exponential phase makes the cell structure be in normal condition and nutrient equilibrium in the medium and cell.

This high cell density could result from a sufficiently high abundance of nutrients in the culture media, the ability of *Nannochloropsis* to benefit from the available nutrients, and the effect of horseradish leaf extract and KW21 combination at the right dose to be able to be absorbed by *Nannochloropsis* sp. to accelerate the growth. In this phase, the microalgae grow very fast because of increased photosynthetic activity and yield high biomass (Madigan et al., 2010). It is in agreement with Wahyuni et al. (2019) who use horseradish leaf extract and Walne fertilizer combination to stimulate the growth of *Dunaliella salina* that optimal nutrient utilization could result in a high number of microalga cells so that they can accumulate all the carotenoid content.

On day-9, the cell density began to decline, but Treatment C was still the highest followed by control Treatments E, A, and B. On day-10, cell mortality occurred in Treatments B and C, while the cell density in the control Treatment D, A, and B were in very low numbers. On day-11, no *Nannochloropsis* cells were found alive. This phase occurs from day-7 to day-11 in all treatments. The cell density declines with the availability of nutrients in the media, in which level of nutrient concentration in the media highly influences the density of *Nannochloropsis* sp. (Sari & Manan, 2012). The nutrient limitation can also inhibit the metabolism.

The present results indicated that Treatments A and B had increased growth from day-1 to day-3, then declined from day-5 to day-10, whereas Treatments C and control had increased cell density from day-4 to day-6, then started declining from day-7 to day-9. Treatments D and control had an unstable increase and decline in the number of cells due to uneven nutrient absorption in the culture media resulting in unstable growth as well. The number of *Nannochloropsis* sp. cells rises every day due to the stimulation of KW21 fertilizer and horseradish leaf extract. The growth of *Nannochloropsis* sp. seems to be not the same among the treatments. It could result from different adaptations of *Nannochloropsis* sp. cells to the new medium. The present finding indicated that the addition of horseradish leaf extract at 75% concentration and 25% KW21 highly affected the density of *Nannochloropsis* sp. ($p < 0.05$).

According to Sari & Manan (2012), good growth conditions will yield good quality *Nannochloropsis* sp. cells to be used as natural food for the fish larvae. The success of culture is indicated by high phytoplankton abundance. *Nannochloropsis* sp. cells in the culture media increase in cell size and numbers. It is also highly influenced by contaminant-free culture media con-

dition, rearing time, seed quality, initial stocking density, and light absorption condition.

On day-11, cell mortality occurred in all treatments, and it is shown by a color change from dark green to clear color meaning the mortality of *Nannochloropsis* cells because there is no *Nannochloropsis* sp. cell found in the culture media. The mortality could also occur from water quality changes to poor conditions so that the nutrients in the culture media decline, the metabolism ability of the microalgae is low due to insufficient nutrient availability, and the limited culture media. Thus, the cell division tends to be restricted by the ability of *Nannochloropsis* cells in benefitting from the nutrients, and the cell division will stop when the nutrient availability in the culture media is not enough. According to Sari & Manan (2012), the type of nutrients and concentration in the media highly influence the growth of *Nannochloropsis* sp. It is in agreement with Nurfadillah et al. (2012) that the decline in phytoplankton growth is caused by photosynthesis, sufficient nutrient availability, and turbidity.

Water quality measurements showed that water salinity, temperature, and pH were in the good range for microalga growth during the study (Table 4).

The optimum water salinity for microalga growth ranges from 25‰ to 35‰. The salinity of the culture media increases from evaporation caused by the temperature of the light used during the culture experiment (Fachrullah, 2011). Optimum salinity for the growth of *Nannochloropsis* sp. ranges from 25‰ to 30‰ (Isnansetyo & Kurniastuty, 1995; Jadid et al., 2017; Sahira et al., 2017). *Nannochloropsis oculata* cultured at the salinity of 25‰ for 10 days has the highest dry biomass, whereas *N. oculata* cultured at the salinity of 35‰, the highest biomass is recorded from day-14 to day-19 (Gu et al., 2012). Khatoon et al. (2014) stated that the density of *Nannochloropsis* sp. cells highly increases at the salinity of 30‰. Temperature observation during the culture up to day-10 ranged between 22℃ and 25℃. According to Sari & Manan (2012), the optimum temperature for phytoplankton growth ranges from 25℃–30℃, whereas the maximum density of *N. oculata* is found at 25℃–30℃ (Cho et al., 2007). However, Malakootian et al. (2016) stated that *N. oculata* has slow specific growth at 20℃ and maximum biomass production at 25℃. The present study revealed that the pH range was still at optimum condition for the growth of *Nannochloropsis* sp. cells, 7.1–8.6. *Nannochloropsis* sp. cell density rose at a pH of 7.5–8.5 (Khatoon et al., 2014). A pH of 7–8 is the optimum pH range for the growth of *Nannochloropsis* sp. cells (Sahira et al., 2017), and the best growth occurs at a pH of 9 (Zaher & Helal, 2020).

Conclusion

The treatment of KW21 fertilizer and water-extracted horseradish leaf combination significantly influenced the population growth of *Nannochloropsis* sp. with the best concentration of 25% KW21 and 75% horseradish leaf extract that could increase the population of *Nannochloropsis* sp. at the laboratory scale. Treatment A (25% horseradish leaf extract + 75% KW21) did not give a significantly different effect from other treatments. Treatment C yielded highly significant different cell density from Treatments B, D, and E. All water quality parameter measurements during the study were in the suitable range for the growth of *Nannochloropsis* sp. The present study has revealed that the use of natural materials as a nutrient source could be considered for microalga cultivation development.

Competing interests

No potential conflict of interest relevant to this article was reported.

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Availability of data and materials

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate

This article does not require IRB/IACUC approval because there are no human and animal participants.

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