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Effect of Fertilizer Combination Azolla Sp. and Light Intensity on Growth Rate and Microalgae Biomass Dunaliella Sp. PN Laboratory Scale Tests

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A B S T R A C T

Dunaliella sp is one of the green algae that is currently used as fish feed. One of the environmental parameters that support the growth of Dunaliella sp. are light intensity and nutrients. Usually, the cultivators use commercial fertilizers as a source of nutrients, and currently as a substitute for commercial fertilizers they use liquid organic fertilizers from Azolla sp. to increase production yields and ensure the continuous availability of natural feed. The purpose of this study was to determine the best combination dose of liquid organic fertilizer fermented from Azolla sp and light intensity for cell density of Dunaliella sp. The research was carried out at the Aquatic Biology Laboratory, Faculty of Fisheries and Marine Affairs in April 2022. This research used the experimental method, using a completely randomized design, a completely randomized factorial design, with two treatments (liquid organic fertilizer (P1) and different light (P2)). , with 9 levels (T) and 3 replications. Treatment of liquid organic fertilizer derived from the fermentation of Azolla sp with different doses, low level (TP1 1 ml/l), medium level (TP2 1.5 ml/l), and high level (TP3 2 ml/l), and different levels of light intensity (TC), low treatment (TC1 20 watts), medium treatment (TC2 40 watts) and high treatment (TC3 60 watts). Based on the analysis of Dunaliella sp cell density variance, it showed that there was a very significant difference (α 0.01). The highest Dunaliella sp cell density was in the high light fertilizer treatment reaching 1,873,000 cells/L and the lowest was in the high low light fertilizer treatment reaching 803,000 cells/L

Keywords: Dunaliella sp., Azolla sp., light intensity, abundance

INTRODUCTION

Dunaliella sp. is a species of microalgae from the Chlorophyta division that lives in saline waters. Dunaliella sp. is one type of microalgae that is commonly used as natural feed, because it is easy to digest [1]. The nutritional content of Dunaliella sp. is shown in the phytochemicals of Dunaliella sp dry matter, namely protein (57%), carbohydrates (32%), and fat (6%)[2],[3]. Several biological conditions of Dunaliella sp. which are known to reproduce rapidly in their growing conditions (living habitat), are easy to cultivate, produce oxygen through the process of photosynthesis, and contain high protein with the main component of amino acids [4]. Based on the results of research by experts [5], [6] on Scenedesmus sp. which was cultivated in tapioca wastewater with different light intensities, showed that the higher light intensity, the growth pattern of Scenedesmus sp. faster in reaching the peak of growth [7]. This fast growth due to the provision of high light intensity can suppress the photosynthetic activity of Scenedesms sp. thereby accelerating cell reproduction [8].

The growth of Dunaliella sp. in culture media is strongly influenced by the availability of nutrients, especially nitrogen and phosphate, as well as several environmental factors of water quality such as salinity, pH, temperature, and light intensity [9]. This excessive use of inorganic fertilizers produces waste that can pollute and harm organisms in the waters, therefore it is necessary to use organic fertilizer solutions that have macro and micronutrients that are not harmful to the environment at a relatively low cost and are easy to obtain, one of which is using culture media. Organic fertilizer made from Azolla sp. [10]. Azolla sp. contains a variety of essential minerals that can support the growth of phytoplankton [11]. Azolla sp. can be utilized in various forms, especially in the form of liquid organic fertilizer (POC). POC Azolla sp. is a solution for the results of decay or fermentation-derived from the plant Azolla sp. The advantages of this organic fertilizer are that it can quickly overcome nutrient deficiencies, has no problem with nutrient leaching, and can provide nutrients quickly [12].

Utilization of Azolla sp. as POC in microalgae culture Spirulina sp. has been tested [13], [14] which the results showed POC fermented from Azolla sp. can increase the growth of microalgae cells Spirulina sp. on laboratory scale culture with the best dose of 18 ml/l. Then the administration of POC Azolla sp. on Chaetoceros sp. Previous researchers have also done [15]–[17] which showed that POC Azolla sp. had a significantly different effect on the population of Chaetoceros sp. with the best dose of 12 ml/l. The various essential mineral content in Azolla sp. which can support the growth of phytoplankton and the important role of Dunaliella sp. as natural food for fish and shrimp larvae made researchers interested in researching the effect of a combination of organic fertilizers Azolla sp. and light intensity on the abundance of Dunaliella sp.

Materials and Methods

The method used in this study is an experimental method using a factorial completely randomized design (CRD). In this study, 2 factors (fertilizer and light) were used, with 9 levels, and 3 replications. Dosage of POC Azolla sp. on each treatment refers to research conducted by other researchers [18]. Meanwhile, the light intensity treatment has been tested by previous researchers [19]. Based on the results of research [20], it can be concluded that the treatment of Azolla sp concentration is high (P1 2 ml), medium (P2 1.5 ml), low (P3 1 ml), and high light intensity (C1 60 watts), medium (C2 40 watts).) and low (C3 20 watts).

Preparation of the research container (experimental unit) consisted of 2 stages, namely the stage of randomizing the numbering of the container and the stage of randomizing the treatment. The stages of randomization of container numbering were carried out by drawing lots where 27 alternative numberings were written on paper, of which one number was taken by lot to determine the numbering and randomization used during the study. Then the second stage is the stage of randomization of treatment. The stages of randomization of treatment were carried out by drawing lots where the 27 treatments used were written on paper and the paper was drawn 27 times, then the paper that came out of the draw was affixed to the container according to the numbering. [21], [22] Counting the number of Dunaliella sp. for natural feed culture using the formula below:

V1 = N2xV2N1

Information:

V1 = Seedling volume for initial stocking (ml)

N1 = density of seeds/ stock Dunaliella sp (units/ml)

V2 = Volume of desired culture medium (ml)

N2 = Density of desired Dunaliella sp seedlings (unit/ml)

The growth rate of Dunaliella sp. was observed every day for 10 days using the formula [10-11]:

$\mu=(\ln\{N_t\}-\ln\{N_0\})/t \times 100\%$

Information:

μ : Specific growth rate (%/day)

N0 : Initial cell density (cells/ml)

Nt : Final cell density (cell/ml)

t : Time (days) from N0 to Nt

To determine the effect of concentration treatment of Azolla sp. and light intensity on population growth of Dunaliella sp. ANOVA statistical test was used (F test with a confidence level of 5%). If the value of the F test is significantly different, then it is continued with Duncan's multiple distance test to determine the relationship between the independent variable (treatment) and the dependent variable (result) [23].

Results and Discussion

Based on the results of the study, the abundance of *Dunaliella* sp. after being cultured for 10 days on a laboratory scale (Table 1). Based on Table 1. Above, it is known that the observations on the abundance of *Dunaliella* sp. the highest was on the 7th day of high light fertilizer treatment which was 1,873,000 cells/L and the lowest abundance was on the 7th day of high low light fertilizer treatment which was 803,000 cells/L. An increase in the abundance of *Dunaliella* sp. is caused by the nutrient activity of liquid fertilizer *Azolla* sp. and also light rays that cause continuous cell division. The average abundance of *Dunaliella* sp. during the study can be seen in Figure 1.

Table 1. Growth of *Dunaliella* sp. due to the application of a combination of different fertilizers and light treatments in laboratory-scale culture

Treatment		Abundance of *Dunaliella* sp during maintenance in a laboratory scale (103) cells/Liter									
		D-1	D-2	D-3	D-4	D-5	D-6	D-7	D-8	D-9	D-10
Low level fertilizer	Low light (CR)	201	323	398	521	630	761	864	683	660	598
	Medium light (CS)	227	315	420	698	876	1140	1256	1110	900	752
	High light (CT)	210	355	431	567	706	800	967	746	654	492
	Sub Total	638	993	1249	1786	2212	2701	3087	2539	2214	1842
	Average	212,7	331	416,3	595,3	737,3	900,3	1029	846,3	738	614
Medium level fertilizer	Low light (CR)	276	420	500	605	875	1057	1289	1002	986	899
	Medium light (CS)	307	456	530	723	900	1155	1315	1158	940	905
	High light (CT)	320	435	524	783	921	1435	**1873**	1200	998	882
	Sub Total	903	1311	1554	2111	2696	3647	4477	3360	2924	2686
	Average	301	437	518	703,7	898,7	1216	1492	1120	974,7	895,33

High level fertilizer	Low light (CR)	**196**	307	400	454	578	656	803	723	655	630
	Medium light (CS)	213	367	423	600	867	1119	1305	1015	890	840
	High light (CT)	250	333	497	712	807	934	1009	850	761	543
	Sub Total	659	1007	1320	1766	2252	2709	3117	2588	2306	2013
	Average	219,7	336	440	588,7	750,7	903	1039	862,7	768,7	671
Total		2200	3311	4123	5663	7160	9057	10681	8487	7444	6541
Average		733,3	1104	1374	1888	2387	3019	3560	2829	2481	2180,3

Note: D: Day

Dunaliella sp. has increased and decreased during the 10-day maintenance period, based on Figure 1. it can be seen that from day 1 to day 6 in all treatments there was an increase and the peak of the increase was on day 7, then on day 8 started to decrease. This is related to the optimal absorption of nutrient levels and the influence of the given light. The lag phase in each treatment occurred from day 0 to day 1. The lag phase or adaptation phase is a phase when the cell density does not change, but the cell size in that phase increases. Photosynthesis is still actively taking place and organisms undergo metabolism but cell division has not yet occurred so the density has not increased. The lag phase begins with the adjustment of cells to the new environment. At the time of adaptation, cells are deficient in enzymes or coenzymes, so they must be synthesized first to carry out further cell biochemical activities. In the lag phase the cell density did not change, but the cell density in that phase increased [24]. In this phase, each treatment still obtained an abundance that was not much different and still adapted to the treatment medium.

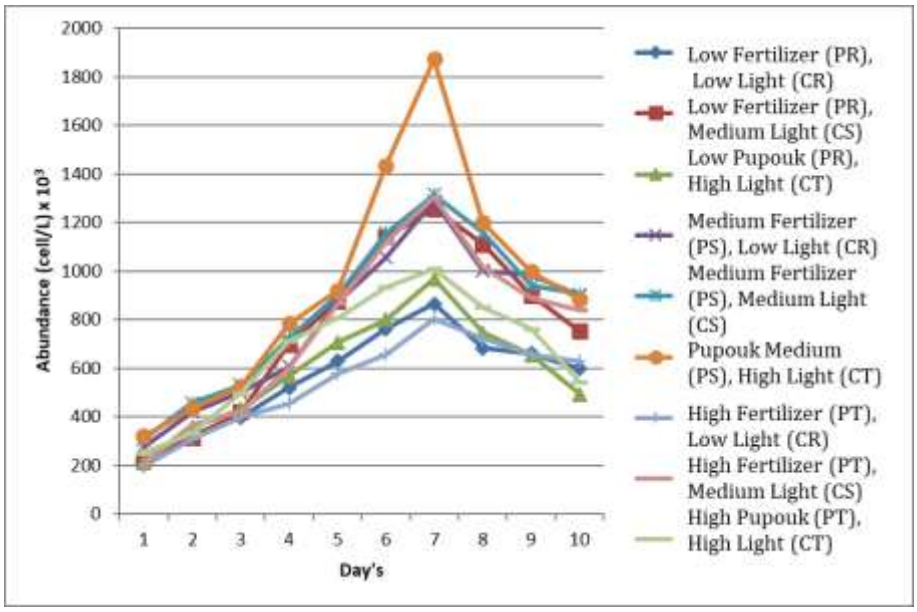


Figure 1. Growth of *Dunaliella* sp. for 10 days

From day 4 to day 7 *Dunaliella* sp. is in the exponential phase. The exponential phase begins with cell division with a continuous growth rate, the exponential phase occurs until the cell density reaches its maximum density. This is the following statement [2], where the exponential phase is marked by the increasing density of *Dunaliella* sp cells until they reach maximum density. The fastest doubling time is usually reached during the exponential phase, i.e. the growth phase when cells divide rapidly and constantly follow a logarithmic curve. This is indicated by a significant improvement in the graph and the acceleration of growth in this phase is comparable.

From day 4 to day 7 the highest abundance was found in high light fertilizer treatment reaching 1,873,000 cells/L, while at the same time the lowest abundance was in high light fertilizer treatment reaching 803,000 cells/L. Experts [3],[4] stated that the growth of *Dunaliella* sp. can increase if it has high light, but after doing this research and observing Figure 1. The combination treatment of medium-light which had significant growth while high light obtained the lowest abundance when combined with low fertilizer. He [5], [6] stated that when using a dose of liquid organic fertilizer from *Azolla* sp. exceeding 1.7 ml will cause excess nutrients and interfere with growth. This is also supported by the results of this study.

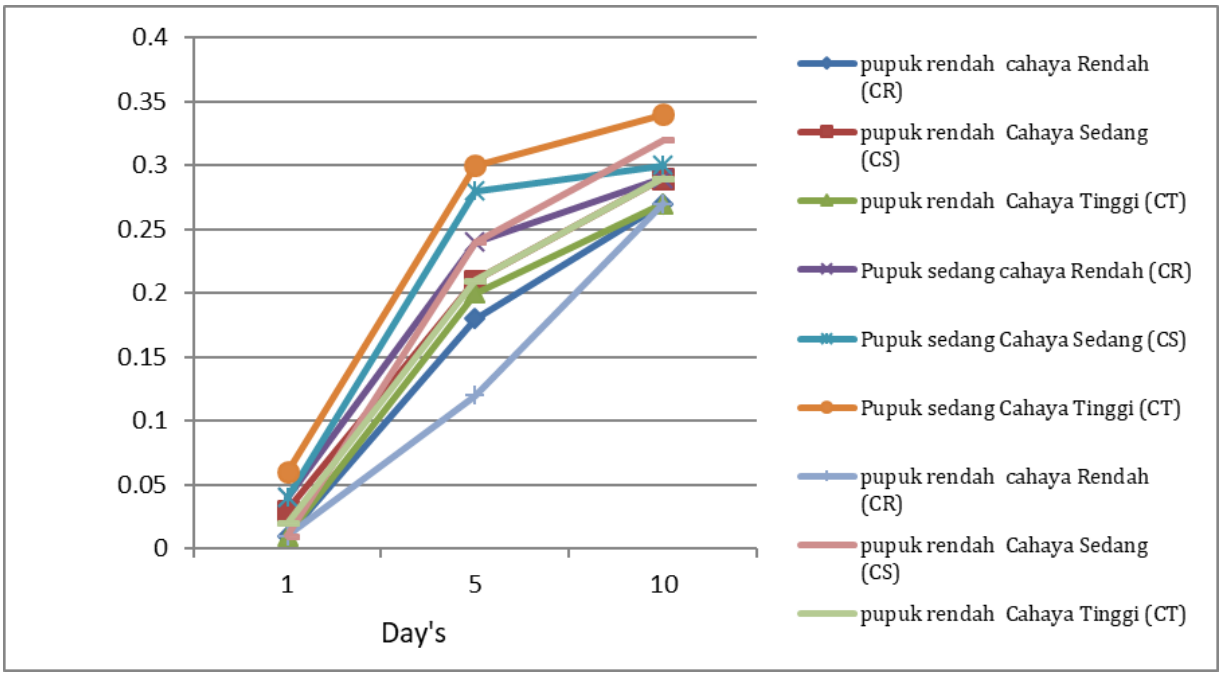
While on day 8 to day 10 the abundance of *Dunaliella* sp. began to decrease, some treatments decreased rapidly and slowly. The high light-medium fertilizer treatment, which had the highest abundance, decreased rapidly; in the other treatments, it decreased slowly. The decrease in growth was caused by reduced nutrients in the medium and greater competition for nutrients, as well as living space [7]. The treatment of high-low light fertilizer decreased drastically due to the abundance of nutrients with the lowest abundance of *Dunaliella* sp. so there was no great competition between *Dunaliella* sp cells. The abundance of *Dunaliella* sp. per day also affects the production of biomass per day, from this abundance the biomass data is obtained in Table 2.

Table 2. Biomass Production of *Dunaliella* sp

Treatment	Biomass treatment on day to		
	D-1	D-5	D-10

Low fertilizer	Low Light (CR)	0,01	0,180,18	0,27
	Medium Light (CS)	0,03	0,21	0,29
	Cahaya Tinggi (CT)	0,01	0,20	0,27
Medium fertilizer	Low Light (CR)	0,04	0,24	0,29
	Medium Light (CS)	0,04	0,28	0,30
	High Light (CT)	0,06	0,30	0,34
High fertilizer	Low Light (CR)	0,01	0,012	0,27
	Medium Light (CS)	0,01	0,24	0,32
	High Light (CT)	0,02	0,21	0,29

Biomass production is strongly influenced by the abundance of *Dunaliella* sp. the higher the abundance produced, the more biomass that will be made, based on Table 2. This results in a graph of the biomass production of *Dunaliella* sp. for 10 days. Biomass production of *Dunaliella* sp. has increased from day to day according to the rate of growth it produces. The lowest biomass production was in low light fertilizer treatment, with the lowest abundance. Biomass production on day 5 begins to increase and after going through the peak growth period, biomass production will also decrease if the microalgae biomass is not immediately calculated.



Previous researchers stated [24]–[26], that *Dunaliella* sp. is a green microalga that is rich in beta carotene and chlorophyll, is euryhaline, and has been widely used for fish and shrimp farming. One of the strategies used to increase the productivity of microalgae is to optimize environmental conditions. Salinity is an essential factor affecting the growth and biochemical content of *Dunaliella* [8], [25]. In their study [26], [27] described the effect of different salinities and determined the best salinity for growth, biomass production, and chlorophyll-a *Dunaliella* sp. Different salinities of 5 ppt, 15 ppt, 25 ppt, and 35 ppt were used in this study. The results of this study indicate that different salinity affects the growth, biomass production, and chlorophyll-a of *Dunaliella* sp. ($p<0.05$). The best salinity for growth, biomass, and chlorophyll-a production *Dunaliella* sp. obtained at 15 ppt salinity which produces a maximum cell concentration of 19.86 x 106 cells mL-1, biomass 0.52 g l-1, and chlorophyll-a 11.26 mg/l. The high growth rate was related to the biomass and chlorophyll-a content of *Dunaliella* sp. It can be stated that different salinity had a significant effect on the growth, biomass production, and chlorophyll-a of *Dunaliella* sp. It is recommended to use a salinity of 15 ppt to produce optimal growth, biomass production, and chlorophyll-a [5], [22], [28].

In another study, the use of the microalgae *Dunaliella* salina has been studied as a food fortification material, which is expected to meet the community's food and nutrition adequacy with a marine bioeconomic approach. *Dunaliella* salina belongs to a type of phytoplankton in the class Chlorophyceae (green algae) called green unicellular flagellate (green unicellular flagellate) [29]–[32]. D. salina is known as a microalga that is rich in natural sources of -carotene content and excellent nutrition so that it can be used for fortification of food that can be consumed by the community [33]–[35]. In future trends, it is predicted that Indonesia can produce microalgae in large enough quantities, at lower costs, and the resulting products can be applied in the daily lives of Indonesian people, especially as food fortification ingredients [36]–[39].

The use of cultivation techniques under stress conditions can be manipulated specifically to optimize the production of carotenoid pigments and cultivation under optimal conditions can increase the biomass production of the microalgae *Dunaliella* salina [12], [17], [40], [41]. The use of microalgae D. salina as a food fortification material can be applied in the form of powders, tablets, capsules, canned drinks, candies, and mixed ingredients that are added to other foods such as instant noodles and other main commodities to increase the nutritional value in people's lives [2], [24]. Bioeconomic analysis of *Dunaliella* salina, which is calculating the production costs and feed needs of fish larvae by producing 100 liters of *Dunaliella* salina culture, costs Rp. 60,000 using seawater and fertilizer formulations [7], [8], [26].

4. Conclusion

The peak growth of Dunaliella sp. was on the 8th day of treatment with medium fertilizer (1.5 ml) high light (60 watts) with an average of 1.873,000 cells/L while the lowest treatment was high fertilizer (2 ml) low light (20 watts) with an average -an average of 803,000 cells/L. A combination of Azolla sp. and different light intensities showed different effects between treatments.

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