

Growth Response and Biochemistry of Red Spinach (*Amaranthus tricolor* L.) with the Application of Liquid Organic Fertilizer *Lemna* sp.

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ABSTRACT

Red spinach is widely cultivated and consumed by the public because it has high. The problem in cultivating red spinach is the uncertain climate due to global warming. This causes a prolonged dry season and drought. The research was carried out at the Biology Laboratory of Ahmad Dahlan University. The design used in this study was RAL (Completely Randomized Design) with 3 replications and 3 treatments. A (control treatment), B (fertilizer treatment 5%), and C (fertilizer treatment 10%)., it can be concluded that the administration of POC *Lemna* sp. With a concentration of 5% can increase the growth of plant height (44.50 cm), stem *spinach* (*A. tricolor* L.), POC, *Lemna* sp. diameter (3.13 mm), and flavonoids (7.86 mg QE/ 100 g).

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1. Introduction

Red spinach is a plant that is widely cultivated and consumed by the community (Adelia et al., 2013). Red spinach is in demand by the public because it has high antioxidants and can cure anemia (Rukmana, 2008). Maccording to Hendro in Manurung (2020) red spinach (*A. tricolor* L.) contains nutrients including vitamins (A, C, E), protein, carbohydrates, fats, minerals, iron, magnesium, manganese, potassium and calcium. In addition, there are anthocyanin pigments (red pigments) which are included in phenolic compounds that function as antioxidants to prevent free radical oxidation in the body (Annisa, 2018).

The problem in cultivating red spinach is the uncertain climate due to global warming. This causes a prolonged dry season and drought (Surmaini and Faqih, 2016). Drought stress is not only caused by high light intensity but can also be caused by a lack of nutrients such as nitrogen from fertilizers. Nitrogen has an important role in the phenylpropanoid pathway for the formation of the PAL (Phenylalanine Ammonia Lyase) enzyme which is useful in producing flavonoids (Yuniarachma et al., 2019). Strisell in Pratiwi (2017) states that high nitrogen can increase plant productivity but sometimes can reduce flavonoid content due to the regulation of primary and secondary metabolism in plants. Flavonoids in some plants can also play a role in attracting pollinators for flower pollination and protecting from UV rays (Manurung, 2019). Drought stress will cause plants to experience stress so that the chlorophyll content will decrease. Plants that lack

water will hamper the absorption of nutrients which will have an impact on the formation of chlorophyll (Ai and Banyo in Nabilla, 2022).

According to research Jomo et al. (2015), Drought stress has an impact on growth reduction in *Amaranthus* spp. red spinach. Handling that can be done to anticipate the worse impact of drought stress is the use of organic materials (Gusta and Kusumastuti, 2017). According to Sarker & Oba (2019), administration of POC can increase red spinach biomass. Nutrients in POC have been decomposed so that they are easily absorbed by plants (Sutanto, 2002). According to Nurhasanah (2015), the *Azolla pinnata* plant can be used as an alternative to organic fertilizer because it has a fairly high nitrogen (N) content and can increase the growth of pakcoy (*Brassica chinensis* L.) such as the number of leaves and the fresh weight of the plant. *Lemna* sp. can be used as organic fertilizer because it has a concentration of nitrogen (N) and phosphorus (P) (Astrid et al., 2013). In addition, nutrients such as nitrogen (N), magnesium (Mg) and iron (Fe) play an important role in the process of leaf chlorophyll formation (Wenno and Sinay, 2019). The content of vitamins C and A is also influenced by the element nitrogen. Nutrients such as Cu, Mg, N, P and K can also increase the production of secondary metabolites such as flavonoid content (Setiawati et al., 2021) liquid organic fertilizer *Lemna* sp. is expected to be able to meet the nutrients of red spinach plants (*A. tricolor* L.) drought-stressed so that they can grow optimally.

2. Methods

The research was carried out at the Biology Laboratory of Ahmad Dahlan University. The design used in this study was RAL (Completely Randomized Design) with 3 replications and 3 treatments. A (control treatment), B (fertilizer treatment 5%), and C (fertilizer treatment 10%). The research variables included plant height, number of leaves, stem diameter, leaf area, vitamin A, chlorophyll, and flavonoids. The tools used in this research include polybags, rulers, calipers, analytical or digital scales, cuvettes, spectrophotometers, blenders, vortexes.

Red spinach seedlings aged 2 weeks were transplanted and after 1 week or 1 MST (weeks after planting) were given POC *Lemna* sp. with concentrations of 0%, 5%, and 10%. Measurements of plant height, number of leaves, stem diameter, and leaf area were carried out once a week. Meanwhile, vitamin A, chlorophyll, and flavonoids were tested when the spinach plants had been harvested or were 6 weeks old.

2.1. Plant height

Measurement of plant height using a ruler affixed to the base of the stem to the end of the stem with a line 0 parallel to the base of the spinach plant stem attached to the ground (Jomo et al., 2015).

2.2. Number of leaves

The leaves that are included in the calculation are those that grow perfectly, do not tear, and do not curl. Leaves that have fallen are not included in the calculation (Astuti et al., 2020).

2.3. Rod diameter

The diameter of the rod was measured using a caliper. Measurements were made at the base of the stem \pm 5 cm from the soil surface (Jomo et al., 2015).

2.4. Leaf area

Leaf area is measured by drawing the leaf in millimeters block. Leaf area was measured from the box in the millimeter block. The leaves measured are number 3 and 4 from the bottom.

2.5. Vitamin A

Analysis of vitamin A content using the spectrophotometer method refers to research (Sani, 2019):

Weighing 1 gram of fresh leaves are crushed, then the results are placed into filter paper and added 1 ml of PE + 1 ml of Acetone. The tube is immediately closed tightly. The obtained filtrate was put into a cuvette and measured with an absorbance of 450 nm. Beta-Carotene Standard: Beta-carotene was made to a concentration of 500 ppm (2.5 mg beta-carotene in 5 ml PE). Dilution from a concentration of 500 ppm to a concentration of 0, 6,8,10,12,14 ppm as much as 25 ml. Total of 0; 0.3; 0.4; 0.5; 0.6 and 0.7 mL were pipetted and put into a volumetric flask up to 25 mL. Measured with absorbance of 450 nm and concentration of 0 ppm as blank

2.6. Chlorophyll

Analysis of chlorophyll content using the spectrophotometric method refers to research (Prastyo and Laily, 2015):

Weighing 1 gram of fresh leaves crushed, then added 10 ml of 70% alcohol and filtered. The obtained filtrate was put into a cuvette and measured with absorbances of 645 and 663 nm. Calculation of chlorophyll content is determined by the formula:

Chlorophyll a = $12.7 (D-663) - 2.69 (D-645)$ (mg/l) (1)

Chlorophyll b = $22.9 (D-645) - 4.68 (D-663)$ (mg/l) (2)

2.7. Flavonoids

The analysis of flavonoid content was carried out using the spectrophotometer method referring to the study (Pratiwi, 2017):

The leaves were blended into powder and then macerated with methanol (1:10). The filtrate was evaporated and a thick extract was obtained. 0.5 mL of extract (1 mg/mL, dissolved in methanol) plus 1.5 mL of methanol; 0.1 mL $AlCl_3$ 10%; 0.1 mL of CH_3COOK 1 M and 2.8 mL of distilled water and then voted. Measured with absorbance 415 nm

2.8. Quercetin Standard

Quercetin is made as much as 10 mg plus methanol to 100 ml. Dilution to concentration 0; 0.1; 0.2; 0.3; 0.4; 0.5 mg/ml (but the result is still too high so it is lowered to 0; 0.02; 0.04; 0.06; 0.08; 0.1 mg/ml). A total of 0; 0.02; 0.04; 0.06; 0.08; and 0.1 mL of the quercetin solution was pipetted and put into a test tube to 5 mL (aquadest, 0.1 mL of 10% $AlCl_3$; 0.1 mL of CH_3COOK). It was measured by absorbance of 415 nm and concentration of 0 ppm as blank.

Data analysis

The data obtained were analyzed using SPSS software. Before being tested with one way ANOVA at the 5% level, the data were tested for normality and homogeneity with the condition that the significance level was > 0.05 . The data from the significantly different ANOVA test results were then tested for DMRT (Duncan Multiple Range Test) with a level of 5%.

3. Findings and Discussion

Plant height occurs due to the activity of the meristem tissue at the end of the stem which continues to divide and increase in size. This meristem tissue activity is called primary growth. Therefore, the plant stem will increase in length (Sastramihardja, 1990). The process of increasing plant height can be stimulated using nitrogen (N), phosphorus (P), and potassium (K). Elemental nitrogen (N) is used for the formation of plant cells, tissues, and organs. The element phosphorus (P) participates in cell division. While the element potassium (K) stimulates the growth point of plants (Haryadi, 2015).

The ANOVA results showed that POC *Lemna* sp. gave significantly different results to the height of red spinach plants.

Table 1. Average plant height and red spinach plants

Treatment	Plant height (cm)
A (POC <i>Lemna</i> sp. 0%)	28.66b
B (POC <i>Lemna</i> sp. 5%)	44.50a
C (POC <i>Lemna</i> sp. 10%)	42.83a

Note: Numbers followed by the same letter show no significant difference based on DMRT level 5%.

Based on Duncan's test, the B and C treatment showed significantly different values. This shows that POC *Lemna* sp. contain nitrogen which can increase plant growth due to apical meristem elongation. This is in accordance with the statement of Istiqomah et al. (2016), that apical meristems located at the ends of stems or branches will produce cells to grow lengthwise. The growth of the apical meristem is influenced by the element nitrogen (N). According to Sari et al. (2018), treatments that were not significantly different or the same were suspected of the growing media still having the availability of nutrients, especially nitrogen (N) so as to produce the same growth in each treatment.

Stem diameter is one of the parameters observed in this study. The results from Anova showed significantly different results on red spinach plants given POC *Lemna* sp.

Table 2. Average stem diameter of red spinach plants

Treatment	Rod diameter(mm)
A (POC <i>Lemna</i> sp. 0%)	4.58a
B (POC <i>Lemna</i> sp. 5%)	3.13b
C (POC <i>Lemna</i> sp. 10%)	4.45a

Note: Numbers followed by the same letter show no significant difference based on DMRT level 5%.

Based on Duncan's test, the B treatment showed significantly different values. The average stem diameter of red spinach showed that A treatment had the largest diameter. According to Suharti et al. (2017) that the growth of small stem diameter is due to the slow differentiation of lateral meristems due to suboptimal nutrient and mineral transport. The small diameter of the stem can also be caused by a decrease in turgor pressure so that the root elongation process is hampered, as a result the flow of water from the xylem to the cells undergoing elongation and dilation will be disrupted.

Leaves function to carry out the process of photosynthesis (Oktafani et al., 2017). According to Haryadi (2015) explained that the nutrient content of nitrogen (N), phosphorus (P), and potassium (K) will affect leaf growth. Phosphorus (P) is an element that forms sugar phosphate which is needed by plants during photosynthesis. Photosynthesis will produce photosynthate which is used for plant growth and development if the phosphorus (P) content is sufficient. Based on the results of Anova showed that the administration of POC *Lemna* sp. gave results that were not significantly different from the number of leaves of red spinach plants.

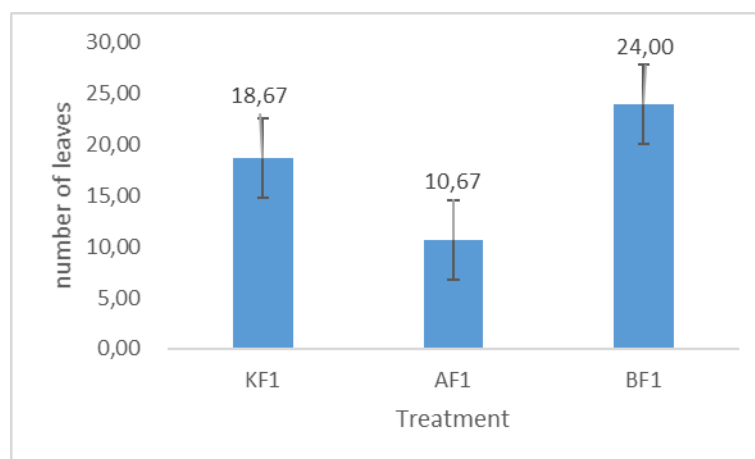


Fig. 1. The average number of leaves of red spinach (*A. tricolor* L.)

Based on the average number of leaves on red spinach plants in Figure 1 shows the growth in the number of leaves the most in the BF1 treatment. The number of leaves is influenced by the element nitrogen (N) absorbed by plants for the formation of chlorophyll. According to an analysis from BPTP (2020), POC *Lemna* sp. contains nitrogen (N) a total of 0.06%, phosphorus (P) a total of 0.02%, potassium (K) a total of 0.02% where the value is still below the quality standard of 2-6%, but can still support the increase in the amount red spinach leaves. Leaf area affects the rate of plant growth. Based on the results of Anova administration of POC *Lemna* sp. did not give significantly different results.

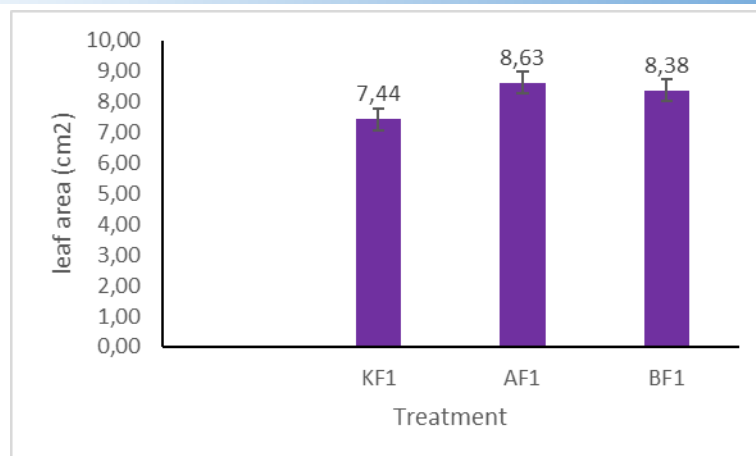


Fig. 2. Leaf area of red spinach (*A. tricolor* L.)

The B treatment had the largest leaf area when compared to treatment A and treatment C. According to Hidayat (2020), the wider the leaves, the more photosynthetic process will increase because the light captured by the leaves will be more. The narrowing of leaf area can be caused by insufficient nutrients in the soil. POC *Lemna* sp. has a nitrogen content below the quality standard but can expand the leaf area so that photosynthesis can be maximized. As stated by Satria et al. (2015) that nitrogen serves to expand the area of Duan so that photosynthesis will increase.

The content of flavonoids in red spinach leaves (*A. tricolor* L.) is known to have a role as an antioxidant for plants during water deficit conditions, besides that it also acts as a red pigment (betasianin) in plants. In line with Halim's statement in Pratiwi (2017) that flavonoids as antioxidants are able to inhibit the formation of free radicals by donating hydrogen. Based on the results of Anova, the administration of POC *Lemna* sp. give significantly different results.

Table 3. Average flavonoid content in red spinach (*A. tricolor* L.) leaves

Treatment	Flavonoids (mg QE/ 100 g)
A (POC <i>Lemna</i> sp. 0%)	4.89b
B (POC <i>Lemna</i> sp. 5%)	7.86a
C (POC <i>Lemna</i> sp. 10%)	4.21b

Note: Numbers followed by the same letter show no significant difference based on DMRT level 5%.

Based on Duncan's test, B treatment showed the most significant results. The content of nutrients, especially nitrogen contained in POC *Lemna* sp. more easily absorbed by plants so that the flavonoid content can be high. As stated by Pratiwi (2017), the higher the concentration of fertilizer, the production of flavonoids actually decreases because there is suppression of PAL activity which results in a bottle neck effect on flavonoid synthesis.

Flavonoids in plants usually function as pigments, such as anthocyanins. This compound serves to protect chlorophyll from ultraviolet exposure. Flavonoids are usually found in the upper epidermal tissue so that the chlorophyll contained in the parenchymal tissue below is safe from ultraviolet exposure. Ultraviolet radiation can damage the structure of chlorophyll, if chlorophyll is damaged then the photosynthesis process of plants will experience obstacles and ultimately plant growth can be disrupted (Pramushinta, 2019).

The content of vitamin A (beta-carotene) in red spinach leaves is one of the biochemical parameters of plants because it is known to be an antioxidant for plants, especially during drought stress conditions. In accordance with the statement of Prasiddha et al., (2016) that carotenoids such as beta-carotene have a role as antioxidants (defense compounds) that are able to bind singlet oxygen (O₂) molecules or control the formation of ROS in cells during drought stress conditions. Based on the results of Anova, giving POC *Lemna* sp. did not give significantly different results.

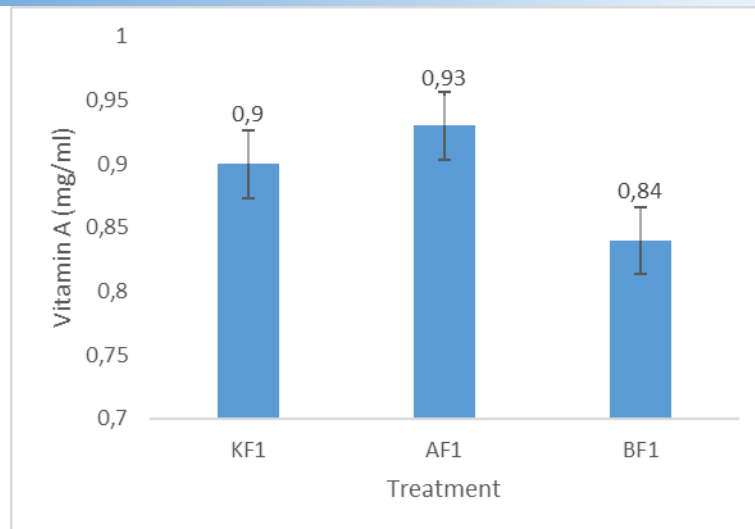


Fig. 3. Average Vitamin A Content (mg/ml) of Red Spinach Leaves (*A. tricolor* L.).

The average vitamin A in red spinach (*A. tricolor* L.) was highest in the AF1 treatment. Giving POC *Lemna* sp. can increase the beta-carotene content of red spinach plants. *Lemna* sp. liquid organic fertilizer, with the content of N, P, K is able to add nutrients to the soil which can later play a role in increasing the formation of beta-carotene in plants. According to the statement of Manurung et al., (2020) that the fulfillment of nutrients in plants will also increase the process of forming chlorophyll and plant carotenoids. Mualim in Rosmayati et al., (2020) also stated that the application of potassium fertilizer as much as 100 kg/ha can increase the beta-carotene content in plants. In addition, drought stress is also known to increase beta-carotene content which is related as a form of self-defense for red spinach plants.

Chlorophyll content in red spinach (*A. tricolor* L.) leaves is one of the parameters because it is known to be one of the indicators that affect the adaptability of plants to water deficit conditions. According to research by Kamanga et al., (2018) there is a decrease in chlorophyll content during drought conditions but there is also an increase in chlorophyll a, b and total chlorophyll in plants when water pressure is low (stressed) and actually decreases when water pressure is high.

Based on the results of Anova, the administration of POC *Lemna* sp. Did not give significantly different results to chlorophyll a and chlorophyll b.

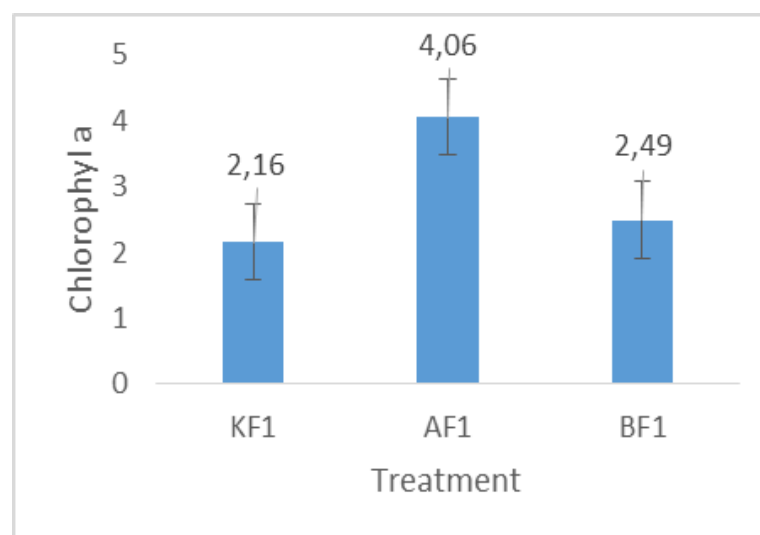


Fig. 4. Average Chlorophyll a (mg/l) Red Spinach Leaves (*A. tricolor* L.).

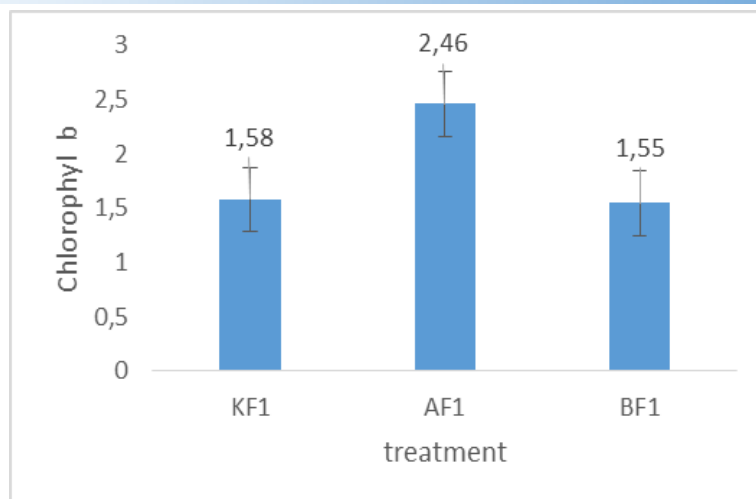


Fig. 5. Average Chlorophyll b (mg/l) Red Spinach Leaves (*A. tricolor* L.)

The highest average of chlorophyll a and b was in the A treatment. Nutrient content in POC *Lemna* sp. sufficient for the formation of chlorophyll because it contains the nutrients nitrogen and magnesium. As stated by Yama and Kartiko (2020), Sufficient nitrogen and magnesium elements in the formation of chlorophyll, providing adequate nutrition to plants causes optimal plants in the formation of chlorophyll. Especially the macro element nitrogen which is closely related to the formation of chlorophyll. In addition to nitrogen, the magnesium micro element group also plays a role in the formation of leaf chlorophyll as the nucleus of the chlorophyll molecule which is a Mg chelate in chloroplasts. Therefore, with the availability of these two elements, more leaf chlorophyll will be formed. In addition to macro elements, micro elements are also influential in the formation of chlorophyll, although the effect is indirect.

4. Conclusion

Based on the research that has been done, it can be concluded that the administration of POC *Lemna* sp. With a concentration of 5% can increase the growth of plant height (44.50 cm), stem diameter (3.13 mm), and flavonoids (7.86 mg QE/ 100 g).

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