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Research Article
Effect of Light, Aeration and Nutrient Concentration on Wolffia (*Wolffia globosa* (Roxb.) Hartog & Plas) Growth

[1]Sumalee Chookhampaeng, [2]Sureewan Puntura and [3]Chowwalit Chookhampaeng

[1,2]Department of Biology, Faculty of Sciences, Mahasarakham University, Maha Sarakham 44150, Thailand
[3]Faculty of Education, Mahasarakham University, Maha Sarakham 44150, Thailand

Abstract

Background and Objective: Wolffia (*Wolffia globosa* (Roxb.) Hartog & Plas) is a nutritious plant that is used as food for humans and animals. In this study, the work aimed to determine the influence on the growth of *W. globosa* of light (non-shaded and shaded 50% of light intensity), aeration (aeration and without aeration) and Hoagland's solution concentration of 1/2, 1/4 and 1/6 compared with the control (full strength Hoagland's solution). **Materials and Methods:** Cultures of 100 g of *W. globosa* were grown in plastic boxes (32×40 cm) containing 3 L Hoagland's solution. Supplied during a 12 hrs photoperiod the light intensity was 10,000 lux. *W. globosa* was culture for 30 days. The fresh weight was recorded every 5 days until day 30 when the chlorophyll A and B contents were determined. **Results:** It was found that on days 15 and 20 the fresh weights of *W. globosa* cultured in 1/2 and 1/4 Hoagland's solution were the highest. *Wolffia globosa* cultured without shading had fresh weights higher than the 50% light intensity with statistical significance. In contrast, the 50% light intensity showed a higher total chlorophyll content than the non-shaded, with statistical significance at the 95% confidence level. *Wolffia globosa* without aeration had a higher fresh weight than that with aeration statistical but this did not affect the amount of chlorophyll. **Conclusion:** Therefore, appropriate conditions for *W. globosa* are a dilute nutrient solution and light at 50% light intensity without aeration.

Key words: Wolffia (*Wolffia globosa* (Roxb.) Hartog & Plas), light, aeration, nutrient concentration, beta-carotene, dietary fibre, photoperiod, hydroponics

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Corresponding Author: Chowwalit Chookhampaeng, Faculty of Education, Mahasarakham University, Maha Sarakham 44150, Thailand

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Wolffia or water meal, (*Wolffia globosa* (Roxb. Hartog & Plas)[1] is in the family *Lemnaceae*. It is classified as the smallest aquatic and flowering plant in the world[2]. It is a local vegetable used by people in the Northern and Northeastern Regions of Thailand. This plant is popular for consumption due to its high nutritional value as it contains useful protein-protein, with all nine essential amino acids in a ratio equivalent to that of egg protein, omega-3 fatty acids, dietary fiber[3,4] antioxidant, anti-inflammatory and cytotoxic activities, etc[5]. *Wolffia globosa* is an aquatic weed that is presumed to be used as alternative food and feed source[6]. If the amount of Wolffia can be increased, it could be a useful human food or animal feed, which will reduce food costs in the future[7]. This plant grows quickly in an environment with suitable conditions, such as nutrients, light and temperature and it will sprout new plants for a period of 16-48 hrs[8,9]. It is a protein source with a short culture time that can be harvested quickly to produce large quantities with low production costs. Wolffia needs nutrients for growth, currently, the technique of growing plants in a nutrient solution has become more advanced. Plants receive various nutrients from the nutrient solution prepared by the grower from a fertilizer or chemical solution added to water[10]. Hydroponic crops will help to effectively manage crop production systems and reduce nutrient deficiencies in crops[11]. In water culture, it was found that the growth rate was the best at a water pH of 5-5.5[12].

Wolffia is highly nutritious and has interest from consumers but there have been only a few studies. For current production, most are harvested from natural water sources. Therefore, it may be contaminated and have an unpleasant smell, so it cannot be sold or consumed, while in some natural water sources, it cannot grow or produce only low yields. Hydroponics is used as a guideline for Wolffia production to replace natural sources so that it is clean and safe from contamination. However, no solution is perfect. It is necessary to adjust the selected production formula to suit the plants we want to grow by studying the appropriate culturing factors. The aim is to study the effect on the growth and increase production, for example, by altering the amount of light or aeration that may result in better plant growth and by studying the concentration of suitable nutrients as another option to reduce the cost of production. Hence, the objective of the current study was to determine the impact of the factors of light, aeration and nutrient concentration on the growth, chlorophyll A content and chlorophyll B content in Wolffia.

MATERIALS AND METHODS

Study area: Plants were cultured at the Department of Biology Nursery, Faculty of Sciences, Mahasarakham University, Thailand. The experiment was conducted in February, 2001.

Materials: Wolffia was obtained from commercial aquaculture farms, in Yang Talat District, Kalasin Province.

Methodology: To determine the effect of light on Wolffia growth, the experiment was divided into two levels: No shading and shading at 50% of light intensity. The study on the effect of aeration on Wolffia growth had an experiment that was divided into two levels: With aeration and without aeration. The plan was a Complete Randomized Design (CRD) trial in which 100 g of Wolffia seed were grown in a black plastic tank, size 32×40 cm. They were placed on a filter cloth for easy weighing and changing solutions. Seeds cultivated in Hoagland's solution[13] (control group) were compared with concentrations of 1/2, 1/4 and 1/6 that were adjusted to pH values of 5-6. Add 3 L of solution/plastic tank and change the solution every 5 days. Provide plants with fluorescent lighting for 12 hrs a day (10,000 lux light intensity). The growth was collected every 5 days to measure the fresh weight. When the plants were 30 days old, the amount of chlorophyll A and chlorophyll B were measured using Padilla *et al.*[14] methods. In which, 100 mg of plants were ground thoroughly. After which, 20 mL of acetone was extracted until the tissue turned clear to filter out the residue. The volume of the solution was adjusted with acetone to 30 mL. Then cover the chlorophyll solution container with aluminium foil to prevent the loss of chlorophyll from light exposure. The extracted chlorophyll solution was then measured for absorbance at wavelengths 645 and 663 nm with a spectrophotometer (Shimadzu UV-Vis 1201).

Statistical analysis: Data variance was analyzed (ANOVA) and the data were compared between the means by Duncan's New Multiple Range Test (DMRT)[15] at the 95% confidence level.

RESULTS

The study on the effect of light on fresh weight of Wolffia (Table 1) showed that the plants grown under non-shading had maximum fresh weight when cultured in 1/4 Hoagland's solution with a fresh weight of 833 g after 20 days of culture, while the plants cultured under shading of 50% of the light intensity resulted in the highest fresh weight when cultured in 1/2 Hoagland's solution with 651 g after 20 days of culture,

Table 1: Effect of light on fresh weight of Wolffia at 5-30 days of culture

Treatments		Fresh weight (g)					
Light	Concentration of solution	5 Days	10 Days	15 Days	20 Days	25 Days	30 Days
Non-shading	1	356±31^{c}	511±20^{c}	627±18^{b}	734±32^{ab}	725±29^{b}	712±26^{b}
	1/2	455±18^{b}	609±15^{b}	764±29^{a}	762±25^{a}	766±33^{b}	703±28^{b}
	1/4	482±13^{ab}	655±23^{a}	787±37^{a}	833±30^{a}	815±17^{a}	798±31^{a}
	1/6	491±16^{a}	573±12^{b}	721±16^{a}	716±28^{ab}	715±14^{b}	688±37^{b}
	Average	446	587	725	761	743	725
Shading (50% light intensity)	1	309±14^{d}	379±35^{d}	451±43^{c}	576±17^{c}	560±32^{d}	542±19^{d}
	1/2	422±25^{b}	481±36^{c}	564±29^{c}	651±19^{b}	614±29^{c}	602±33^{c}
	1/4	407±28^{bc}	499±19^{c}	689±15^{b}	648±26^{b}	620±18^{c}	594±21^{c}
	1/6	288±32^{d}	386±28^{d}	502±35^{c}	502±37^{c}	488±41^{e}	467±52^{e}
	Average	357	436	552	594	571	551

Different alphabets in a column show different values and statistical significance at the 95% confidence level

Table 2: Effect of light on chlorophyll content of Wolffia after 30 days of culture

Treatments		Chlorophyll content (mg/100 g)		
Light	Concentration of solution	Chlorophyll A	Chlorophyll B	Total chlorophyll (A+B)
Non-shading	1	0.82±0.05^{b}	0.31±0.02^{b}	1.13±0.07^{b}
	1/2	0.78±0.07^{c}	0.33±0.01^{b}	1.11±0.08^{b}
	1/4	0.83±0.04^{b}	0.33±0.01^{b}	1.16±0.05^{b}
	1/6	0.81±0.06^{b}	0.32±0.01^{b}	1.13±0.07^{b}
	Average	0.81	0.32	1.13
Shading (50% light intensity)	1	0.85±0.05^{b}	0.35±0.01^{a}	1.20±0.06^{a}
	1/2	0.87±0.06^{b}	0.39±0.04^{a}	1.26±0.10^{a}
	1/4	0.92±0.08^{a}	0.37±0.02^{a}	1.29±0.10^{a}
	1/6	0.83±0.05^{b}	0.37±0.02^{a}	1.22±0.07^{a}
	Average	0.87	0.37	1.24

Different alphabets in a column show different values and statistical significance is at the 95% confidence level

Table 3: Effect of aeration on fresh weight of Wolffia at 5-30 days of culture

Treatments		Fresh weight (g)					
Aeration	Concentration of solution	5 Days	10 Days	15 Days	20 Days	25 Days	30 Days
With aeration	1	346±32^{d}	427±12^{c}	554±21^{d}	532±28^{c}	540±21^{c}	544±35^{b}
	1/2	395±16^{c}	482±29^{b}	651±30^{c}	706±36^{b}	685±25^{b}	644±32^{b}
	1/4	413±21^{bc}	497±37^{b}	667±36^{c}	699±39^{b}	689±28^{b}	657±43^{b}
	1/6	312±55^{d}	401±25^{c}	523±30^{d}	530±18^{c}	512±26^{c}	510±45^{b}
	Average	367	452	599	617	607	589
Without aeration	1	434±18^{b}	527±32^{b}	645±45^{c}	721±27^{b}	687±22^{b}	701±26^{a}
	1/2	474±24^{a}	641±29^{a}	779±25^{b}	812±19^{a}	744±22^{a}	712±27^{a}
	1/4	491±31^{a}	692±35^{a}	845±43^{a}	808±25^{a}	759±24^{a}	711±11^{a}
	1/6	376±21^{c}	493±38^{b}	679±32^{c}	710±33^{b}	681±36^{b}	651±41^{b}
	Average	444	588	737	763	718	694

Different alphabets in a column show different values and statistical significance is at the 95% confidence level

which were statistically different at the 95% confidence level. When considering both experiments, the highest fresh weight was obtained on day 20 with concentrations of the solution for the high fresh weight of Wolffia at 1/2 Hoagland's solution and 1/4 Hoagland's solution. A comparison between the shading and non-shading found that the Wolffia cultured under non-shading had a higher average fresh weight than when cultured under shading.

The study on the effect of the light on chlorophyll content (Table 2) found that the Wolffia cultured under non-shading resulted in chlorophyll A, B and total chlorophyll contents lower than when cultured under shading. The highest chlorophyll A and the total chlorophyll contents with 1/4 Hoagland's solution when cultured under shading were 0.92 and 1.29, respectively. When cultivated under non-shading with the same concentration, the chlorophyll A and total chlorophyll contents were 0.83 and 1.16, respectively, which were statistically different at the 95% confidence level. It was found that the chlorophyll B content was not statistically different at the 95% confidence level.

The effect of aeration on the fresh weight of Wolffia (Table 3) showed that the plants grown without aeration had

Table 4: Effect of aeration on chlorophyll content of Wolffia at 30 days of culture

Treatments		Chlorophyll content (mg/100 g)		
Aeration	Concentration of solution	Chlorophyll A	Chlorophyll B	Total chlorophyll (A+B)
With aeration	1	0.83±0.05[a]	0.35±0.06[a]	1.18±0.11[a]
	1/2	0.85±0.05[a]	0.36±0.03[a]	1.21±0.08[a]
	1/4	0.84±0.04[a]	0.38±0.04[a]	1.22±0.08[a]
	1/6	0.86±0.06[a]	0.36±0.03[a]	1.22±0.09[a]
	Average	0.85	0.36	1.21
Without aeration	1	0.87±0.07[a]	0.36±0.02[a]	1.23±0.09[a]
	1/2	0.85±0.03[a]	0.38±0.05[a]	1.23±0.08[a]
	1/4	0.88±0.06[a]	0.36±0.03[a]	1.24±0.09[a]
	1/6	0.84±0.05[a]	0.38±0.05[a]	1.22±0.10[a]
	Average	0.86	0.37	1.23

Different characters in a column show different values and statistical significance is at the 95% confidence level

a maximum fresh weight when cultured in 1/4 Hoagland's solution of 845 g after 15 days of culture, while the plants cultured without aeration had the highest fresh weight when cultured with aeration in 1/4 Hoagland's solution with 699 g after 20 days of culture. When considering both experiments, the highest fresh weight was on days 15-20 and the concentrations of solution for the highest fresh weights of Wolffia were 1/2 Hoagland's solution and 1/4 Hoagland's solution. From a comparison between aeration and without shading, it was found that Wolffia cultured without aeration had a higher average fresh weight than that cultured with aeration.

From the effect of aeration on chlorophyll content (Table 4), it was found that Wolffia cultured under aeration and non-aeration conditions gave results for the contents of A, B and total chlorophyll that were not statistically different at the 95% confidence level.

DISCUSSION

Wolffia reached its highest fresh weight on days 15 and 20, which was its period of maturity. After that, Wolffia will have slightly lower fresh weights. This may be due to a lower growth rate or some death. Ruekaewma *et al.*[16] found that Wolffia harvested at 21 days had the highest yield, after which the yield reduced. Therefore, the nutrient solution, if its concentration is too high, may inhibit the growth of plants because there may be some substances at too high concentrations, which thereby inhibit the growth of the plants. If the solution is too diluted, it will result in a lack of nutrients for the plant. At high concentrations, solutions of a nutrient may be excessive causing the growth to decline. Unfavourable conditions (high calcium content and alkaline pH) caused a decrease in the assimilation of minerals[17].

Wolffia cultured under non-shading conditions had a higher average fresh weight than those cultured under shading. Due to the light, the plant can grow to its fullest. This was consistent with the research of Chakrabarti *et al.*[18], who studied the intensity of the light and also played a major role in the production of *L. minor*. When light intensity decreased production also reduced drastically, an increasing trend was recorded as light intensity increased. The highest growth or maximum growth point is attained at a specific light intensity, growth declines if the light intensity is either increased or decreased from the maximum growth point[19]. It was found that the hydroponics fertilizer in combination with non-shading provided the maximum fresh weight and dry weight. Wolffia cultured without aeration had higher average fresh weights than when cultured with aeration. This may be due to aeration causing the solution to ripple constantly that affecting the distribution of the plants grown. In addition, aquatic plants under normal conditions are a source of oxygen for the environment[20]. The amount of oxygen present in the solution should be sufficient to meet the demand. Therefore, there is no need to add aeration as if it is too much, it may affect the metabolism and growth of the plants. This allowed the plants to have enough air so there was no need to add air to the solution.

From the study of the effect of light on chlorophyll content, it was found that Wolffia cultured under shading (50% light intensity) resulted in the chlorophyll A, B and total chlorophyll contents being lower than when cultured under non-shading. Maybe too much light intensity inhibits the synthesis of chlorophyll. This was in line with the research of Ma *et al.*[21], who studied the suitable light levels for Anoectochilus plants and found that the chlorophyll concentration in the plants was significantly influenced by the different light intensities. Among all the treatments, the chlorophyll concentration was greatest in the low light

intensity treatment and then decreased consistently as the light intensified as it was lowest in the high light intensity treatment. In response to high light intensities, bryophytes experienced a decrease in chlorophyll. Both chlorophyll and DNA are easily damaged by high intensities of direct sunlight[22]. Therefore, introducing farmers to cultivating Wolffia should be cultured under non-shading and without aeration to achieve high productivity.

CONCLUSION

This study on the effect of light on the fresh weight of Wolffia grown showed that the growth rate may not be that high as it may be an adaptation to the cultural conditions. Wolffia reached its highest fresh weight on days 15 and 20, which is its period of maturity. After that, Wolffia will have a slightly lower fresh weight. The highest fresh weight of Wolffia was obtained when cultured in 1/2 Hoagland's solution and 1/4 Hoagland's solution. Wolffia cultured under non-shading had a higher average fresh weight than when cultured under shading. Wolffia cultured without aeration had a higher average fresh weight than when cultured under aeration. The study of the effect of light on chlorophyll content found that the Wolffia cultured under shading (50% light intensity) resulted in chlorophyll A, B and total chlorophyll contents lower than when cultured under non-shaded conditions. These studies have shown that if Wolffia is raised under suitable conditions, it will grow well and increase its yield.

SIGNIFICANCE STATEMENT

This study discovers Wolffia need suitable growth factors, especially concentration of the solution and light intensity. Farmers should attention to these factors to produce enough for consumers. In addition, cultured without aeration had a higher fresh weight than cultured with aeration making it easy and convenient and reducing production costs.

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REFERENCES

1. Ivan, K. and V. Katya, 2013. *Wolffia globosa* (Roxburgh) hartog et plas (Lemnaceae): A new species in Bulgarian flora. J. Biol. Sci. Opin., 1: 356-357.
2. Park, H., J.H. Park, Y. Lee, D.U. Woo and H.H. Jeon *et al*., 2021. Genome of the world's smallest flowering plant, *Wolffia australiana*, helps explain its specialized physiology and unique morphology. Commun. Biol., Vol. 4. 10.1038/s42003-021-02422-5.
3. Appenroth, K.J., K.S. Sree, M. Bog, J. Ecker and C. Seeliger *et al*., 2018. Nutritional value of the duckweed species of the genus *Wolffia* (Lemnaceae) as human food. Front. Chem., Vol. 6. 10.3389/fchem.2018.00483.
4. Sela, I., A.Y. Meir, A. Brandis, R. Krajmalnik-Brown and L. Zeibich *et al*., 2020. *Wolffia globosa-Mankai* plant-based protein contains bioactive vitamin B_{12} and is well absorbed in humans. Nutrients, Vol. 12. 10.3390/nu12103067.
5. Tipnee, S., A. Jutiviboonsuk and P. Wongtrakul, 2017. The bioactivity study of active compounds in *Wolffia globosa* extract for an alternative source of bioactive substances. Cosmetics, Vol. 4. 10.3390/cosmetics4040053.
6. Said, D.S., T. Chrismadha, N. Mayasari, D. Febrianti and A.R.M. Suri, 2022. Nutrition value and growth ability of aquatic weed *Wolffia globosa* as alternative feed sources for aquaculture system. IOP Conf. Ser.: Earth Environ. Sci., Vol. 950. 10.1088/1755-1315/950/1/012044.
7. Chareontesprasit, N. and W. Jiwyam, 2001. An evaluation of Wolffia meal (*Wolffia arrhiza*) in replacing soybean meal in some formulated rations of Nile Tilapia (*Oreochromis niloticus* L.). Pak. J. Biol. Sci., 4: 618-620.
8. Suppadit, T., S. Jaturasitha, N. Sunthorn and P. Poungsuk, 2012. Dietary *Wolffia arrhiza* meal as a substitute for soybean meal: Its effects on the productive performance and egg quality of laying Japanese quails. Trop. Anim. Health Prod., 44: 1479-1486.
9. Yan, Y., Y. Candreva, H. Shi, E. Ernst, R. Martienssen, J. Schwender and J. Shanklin, 2013. Survey of the total fatty acid and triacylglycerol composition and content of 30 duckweed species and cloning of a Δ6-desaturase responsible for the production of γ-linolenic and stearidonic acids in *Lemna gibba*. BMC Plant Biol., Vol. 13. 10.1186/1471-2229-13-201.
10. Pandey, A. and R.K. Verma, 2018. Nutritional composition, taxonomical and phytoremediation status of duckweed (*Wolffia*): Review. Ann. Plant Sci., 7: 1928-1931.
11. Sardare, M.D. and S.V. Admane, 2013. A review on plant without soil-hydroponics. Int. J. Res. Eng. Technol., 2: 299-304.
12. Sree, K.S., S. Sudakaran and K.J. Appenroth, 2015. How fast can angiosperms grow? Species and clonal diversity of growth rates in the genus *Wolffia* (Lemnaceae). Acta Physiologiae Plant, Vol. 37. 10.1007/s11738-015-1951-3.

13. de Bever, A., P.A. Ndakidemi and C.P. Laubscher, 2012. Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on growth and development of *Beta vulgaris* subsp. cycla 'Fordhook Giant' grown in hydroponic cultures. Int. J. Phys. Sci., 7: 5281-5287.
14. Padilla, F.M., R. de Souza, M.T. Peña-Fleitas, M. Gallardo, C. Gimenez and R.B. Thompson, 2018. Different responses of various chlorophyll meters to increasing nitrogen supply in sweet pepper. Front. Plant Sci., Vol. 9. 10.3389/fpls.2018. 01752.
15. Midway, S., M. Robertson, S. Flinn and M. Kaller, 2020. Comparing multiple comparisons: Practical guidance for choosing the best multiple comparisons test. PeerJ, Vol. 8. 10.7717/peerj.10387.
16. Ruekaewma, N., S. Piyatiratitivorakul and S. Powtongsook, 2015. Culture system for *Wolffia globosa* L. (Lemnaceae) for hygiene human food. Songklanakarin. J. Sci. Technol., 37: 575-580.
17. Giel, P. and K. Bojarczuk, 2011. Effects of high concentrations of calcium salts in the substrate and its pH on the growth of selected rhododendron cultivars. Acta Societatis Botanicorum Poloniae, 80: 105-114.
18. Chakrabarti, R., W.D. Clark, J.G. Sharma, R.K. Goswami, A.K. Shrivastav and D.R. Tocher, 2018. Mass production of *Lemna minor* and its amino acid and fatty acid profiles. Front. Chem., Vol. 6. 10.3389/fchem.2018.00479.
19. Magahud, J.C. and S.L.P. Dalumpines, 2021. Growth of duckweeds (*Lemna minor* L.) as affected by light intensity, nutrient solution concentration, and light x nutrient interaction. Philipp. Sci. Lett., 14: 119-129.
20. Knight, S., J. Hauxwell and E.A. Haber, 2014. Distribution and abundance of aquatic plants-human impacts. Ref. Module Earth Syst. Environ. Sci., 10.1016/B978-0-12-409548-9.09102-8.
21. Ma, Z., S. Li, M. Zhang, S. Jiang and Y. Xiao, 2010. Light intensity affects growth, photosynthetic capability, and total flavonoid accumulation of *Anoectochilus* Plants. HortsScience, 45: 863-867.
22. Glime, J.M., 2017. Chapter 9-3: Light: Effects of High Intensity. In: Bryophyte Ecology Volume 1: Physiological Ecology, Glime, J.M. (Ed.), Michigan Technological University, Houghton, MI, pp: 2-30.