

## OPTIMIZING GROWTH CONDITIONS FOR WOLFFIA (*Wolffia globosa*) USING AN AB HYDROPONIC FERTILIZER CULTURING SYSTEM

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### Abstract

This research focuses on the optimization of growth conditions for *Wolffia*, the smallest flowering plant, utilizing an AB hydroponic fertilizer culturing system. The study addresses the increasing global demand for sustainable and nutritious food sources, positioning *Wolffia* as a promising solution due to its rapid growth and high protein content. The research methodology involves a systematic investigation into the effects of varying nutrient concentrations, light intensities, and pH levels within the AB hydroponic system. The results highlighted the significance of AB fertilizer concentrations, with 5 ml/l demonstrating superior growth rates and quality metrics. Surprisingly, lower light intensities (3,000-6,000 lux) yielded the highest growth rates and thallus size, challenging conventional expectations. pH levels exhibit a non-significant impact on growth, emphasizing the adaptability of *Wolffia* to a broader pH range. Controlled environmental conditions further validate the success of the optimized hydroponic system. Proximate analysis reveals *Wolffia*'s nutritional richness, supporting its potential as a functional food source. This research contributes valuable insights to

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hydroponics and sustainable agriculture, positioning *Wolffia* as a resilient and resource-efficient food source. The findings have implications for global food security and potential applications in space agriculture.

**Keywords:** *Wolffia*, AB hydroponic fertilizer, culturing system

## Introduction

The increasing global demand for sustainable and nutritious food sources has driven the exploration of unconventional alternatives. *Wolffia*, the smallest flowering plant, offers a promising solution due to its rapid growth, high protein content, which can range from 20% to 45% of its dry weight depending on the species and growing conditions (Sree et al., 2016) and potential applications in both food production and environmental remediation. However, to fully realize *Wolffia*'s potential as a viable food source, there is a critical need to standardize its growth conditions, particularly within hydroponic systems. Our research addresses this gap by optimizing the growth conditions of *Wolffia* using an AB hydroponic fertilizer culturing system, which has yet to be thoroughly explored in existing literature.

Hydroponic systems have emerged as efficient alternatives to traditional soil-based cultivation, providing controlled environments that can be precisely tailored to meet the specific needs of various plant species. The AB hydroponic fertilizer, known for its balanced nutrient composition, presents a unique opportunity to fine-tune the nutrient supply for *Wolffia*. AB fertilizer, commonly used in hydroponic systems, is composed of two main components: Solution A, which typically contains calcium nitrate and iron chelates, and Solution B, which includes potassium nitrate, magnesium sulfate, and a blend of micronutrients such as boron, manganese, zinc, copper, and molybdenum. These nutrients are essential for the growth and development of *Wolffia*, as they support various

physiological processes. Despite its potential, no standardized hydroponic protocol currently exists for *Wolffia*, limiting its scalability and broader application. Our study aims to bridge this gap by systematically investigating the effects of varying nutrient concentrations, light intensities, and pH levels within the AB hydroponic system, thereby laying the groundwork for the development of a standardized protocol.

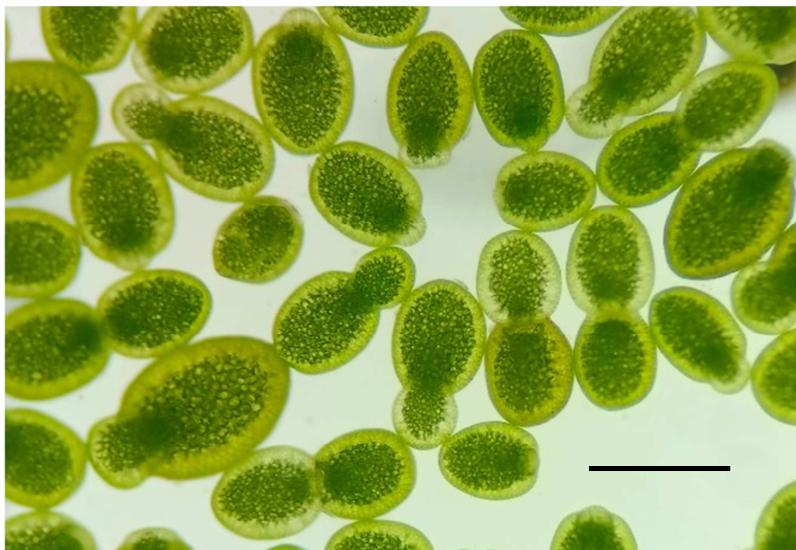
Previous studies have underscored *Wolffia*'s potential as a high-yield, low-resource-input crop with diverse applications. Nutritional studies, such as those by Kaplan et al. (2018) and On-Nom et al. (2023), have highlighted *Wolffia*'s rich protein content and its applicability in addressing malnutrition, particularly in resource-limited regions. Environmental applications have also been explored, with Pandey et al. (2020) and Delgado-González et al. (2021) demonstrating *Wolffia*'s effectiveness in phytoremediation and wastewater treatment. Additionally, the potential of *Wolffia* in space agriculture has been investigated, with Romano & Aronne (2021) and Romano et al. (2024) examining its adaptability to altered gravity conditions and its suitability for extraterrestrial cultivation.

While these studies provide valuable insights, none have focused on optimizing *Wolffia*'s growth within a hydroponic system, particularly using the AB fertilizer. This study builds upon the existing body of research by introducing and evaluating a novel approach to *Wolffia* cultivation that could enhance its growth efficiency and nutritional profile. Specifically, this study seeks to investigate the effects of varying nutrient concentrations on the growth rate of *Wolffia* in an AB hydroponic system, assess the impact of different light intensities and pH levels on *Wolffia*'s growth performance, and develop a standardized hydroponic protocol for optimizing *Wolffia* cultivation.

## Materials and Methods

### 1. Experimental Site and Plant Selection

The experiments were conducted at Udon Thani Rajabhat University in Samprao District, Udon Thani Province, Thailand. Locally sourced *Wolffia globosa* (Fig. 1) from Nongbua Lamphu province was chosen for this study due to its availability and previous indications of robust growth in regional conditions. The species was identified and verified through morphological examination and comparison with standard taxonomic references. Cultivation took place in twelve rectangular basins, each measuring 90 cm in width and 120 cm in length, within a greenhouse environment (Fig. 2).



**Fig. 1** *W. globosa* collected from a natural pond from Nongbua Lamphu province. Bar = 0.5 mm



**Fig. 2** *W. globosa* cultured indoor in three different culturing systems and setup all culture systems in a greenhouse controlled by microcontroller connected with Wi-Fi and e-W Link software.

#### 2. Culturing System Design:

A completely randomized design (CRD) was employed for the indoor culturing of *W. globosa* to determine growth rate and quality. The variables under investigation included AB fertilizer concentrations (1, 3, and 5 ml/l). Each treatment was replicated thrice, and a control treatment was included for comparison.

#### 3. Environmental Condition and Monitoring:

Conditions such as light-dark period, light intensities (5,000-8,000 lux), pH levels (5-8) temperature (25-35 °C), moisture (70-92%), Dissolve oxygen (DO),  $\text{NO}_2$ , and  $\text{NO}_3^-$  were not directly controlled. However, monitoring occurred at seven-day intervals, aligning with data collection points.

#### 4. Growth Measurement:

The growth assessment of *W. globosa* involved tracking fresh weight and frond size over a 28-day cultivation period. Measurements were taken

weekly, and the plants were returned to their respective systems. Dry weight determination was carried out by overnight drying in a hot air oven at 60 °C.

#### 5. Data Analysis:

Data analysis involved descriptive statistics and one-way analysis of variance (ANOVA) with subsequent Duncan's multiple comparison tests. Statistically significant differences were determined at a probability level  $< 0.05$  ( $p < 0.05$ ).

#### 6. Proximate Analysis:

The same amount of 250 g for fresh and dry samples (dry samples derived from 5 kg of fresh samples by drying with an oven) of *W. globosa* subjected randomly to horizontal surface agitation were sent to the central laboratory Thailand Co., Ltd., for proximate analysis.

#### 7. Ethical Considerations:

Due to the small size of *Wolffia* plants, ethical guidelines and approvals were not applicable to this research.

## Results and Discussion

### 1. Effects of AB Fertilizer Concentration on Growth and Quality of *Wolffia globosa*

The growth metrics of *W. globosa* under varying concentrations of AB fertilizer revealed a clear dose-dependent response (Tables 1-3). The highest concentration of 5 mL/L consistently led to superior outcomes across all measured parameters, including fresh weight, production rate, and frond size. These results suggest that increased nutrient availability significantly enhances *W. globosa* growth and development.

One potential reason for the superior performance at the 5 mL/L concentration is the balanced nutrient composition of the AB fertilizer, which may have provided optimal levels of macro and micronutrients necessary for

*W. globosa*'s rapid growth. Higher nutrient availability likely supports increased photosynthetic activity and protein synthesis, crucial for the plant's biomass accumulation.

However, it is important to consider whether an even higher concentration could yield further improvements or if there exists an upper limit where the beneficial effects plateau or even reverse. Although this study did not test concentrations higher than 5 mL/L, future research could explore this to determine the optimal nutrient range for *W. globosa* cultivation.

## 2. Influence of Light Intensity on Growth

Interestingly, the study found that *W. globosa* exhibited the highest growth rates and larger frond sizes under lower light intensities within the 3,000-6,000 lux range, which contrasts with typical expectations that higher light intensities would enhance growth. This unexpected result could be due to *W. globosa*'s adaptation to shaded aquatic environments in its natural habitat, where excessive light might lead to photo-oxidative stress or hinder nutrient uptake efficiency.

Comparing this finding with existing literature, it appears that while many aquatic plants thrive under moderate to high light conditions, *W. globosa* may have evolved mechanisms to optimize its growth under lower light intensities. On-Nom et al. (2023) demonstrated *W. globosa*'s versatility in different applications, but their study focused on its use in processed forms rather than on raw growth conditions, making direct comparisons challenging. Nevertheless, our findings suggest a unique light requirement for *W. globosa* that could inform cultivation practices, particularly in controlled environments with limited light availability.

## 3. pH Level Adaptability

The study also explored the effects of pH levels on *W. globosa* growth and found no significant differences in growth rates and frond size across

the pH range of 5-8. This adaptability to a broad pH spectrum is noteworthy, as it contrasts with many hydroponic crops that often exhibit optimal growth within a narrow pH range.

*W. globosa*'s ability to thrive across various pH levels may be attributed to its efficient regulation of ion exchange processes and cellular homeostasis, allowing it to maintain growth and nutrient uptake under different pH conditions. This resilience to pH fluctuations enhances its potential for diverse hydroponic systems, where pH control can be challenging.

#### 4. Environmental Conditions and Their Influence

The controlled environmental conditions during the cultivation period (Table 4) provided a stable framework for evaluating the effects of the experimental variables. The maintenance of suitable light-dark periods, light intensities, pH levels, temperature, and nutrient concentrations minimized external variability, ensuring that the observed differences in growth were attributable to the AB fertilizer concentrations and other controlled factors.

However, slight fluctuations in environmental parameters, such as temperature and dissolved oxygen (DO), were observed. While these did not appear to significantly impact the overall growth outcomes, they highlight the importance of continuous monitoring and potential adjustments in long-term cultivation practices.

#### 5. Proximate Analysis and Nutritional Composition

The proximate analysis of fresh and dry *W. globosa* of random samples (Table 5) revealed a nutrient-rich profile, including high protein content, essential amino acids, dietary fiber, and minerals such as calcium and iron. These findings align with Kaplan et al.'s (2018) emphasis on *Wolffia*'s potential to address malnutrition, particularly in resource-limited regions.

The nutritional composition of *W. globosa* supports its application as a functional food source, with potential for inclusion in diverse dietary products.

As explored by Monthakantirat et al. (2022) and Siriwat et al. (2023), the rich protein and antioxidant content of *Wolffia* could be harnessed in developing health-promoting foods, furthering its relevance in the context of global food security.

### 6. Implications for Sustainable Agriculture and Space Exploration

The optimized hydroponic system developed in this study provides valuable insights into the cultivation of *W. globosa*, with implications for sustainable agriculture. Given its rapid growth, minimal resource requirements, and nutritional richness, *Wolffia* presents a viable alternative for enhancing food security, particularly in regions with limited arable land.

Moreover, the adaptability of *W. globosa* to varying pH levels and lower light intensities may extend its potential for space agriculture. Romano et al. (2024) explored *Wolffia*'s growth under altered gravity conditions, suggesting its suitability for extraterrestrial cultivation. Our findings reinforce this potential, demonstrating that *Wolffia* can thrive under controlled environmental conditions, making it a strong candidate for inclusion in space missions focused on sustainable food production.

**Table 1** Fresh weight (g/m<sup>2</sup>) of *W. globosa* cultivated in three different concentrations of AB fertilizer

AB Fertilizer Concentration (mL/L)	Days-culture				
	0	7	14	21	28
1 mL/L	25±0.1	53±0.22b	89±0.30c	129±0.33c	150±0.35c
3 mL/L	25±0.1	58±0.40ab	103±0.25b	149±0.45b	178±0.30b
5 mL/L	25±0.1	61±0.33a	122±0.22a	159±0.29a	219±0.30a
Control	25±0.1	50±0.56c	78±0.48d	112±0.40d	138±0.39d

<sup>a, b, c, d</sup> significant difference in mean of column at  $p < 0.05$

**Table 2** Production rate (g dry weight/m<sup>2</sup>/d) of *W. globosa* cultivated in three different concentrations of AB fertilizer

AB Fertilizer Concentration (mL/L)	Days-culture			
	7	14	21	28
1 mL/L	2.6±0.02b	4.45±0.03b	6.29±0.03b	7.35±0.05cd
3 mL/L	2.8±0.04ab	5.15±0.05ab	7.26±0.05ab	8.72±0.03b
5 mL/L	3.0±0.03a	6.1±0.02a	7.75±0.09a	10.73±0.03a
Control	2.46±0.06c	3.9±0.08c	5.46±0.04c	6.76±0.09d

<sup>a, b, c, d</sup> significant difference in mean of column at  $p < 0.05$

**Table 3** Frond size (mm<sup>2</sup>) of *W. globosa* cultivated in three different concentrations of AB fertilizer

AB Fertilizer Concentration (mL/L)	Days-culture				
	0	7	14	21	28
1 mL/L	0.40±0.01	0.48±0.04	0.50±0.05c	0.54±0.02b	0.49±0.04
3 mL/L	0.40±0.01	0.57±0.05	0.56±0.32b	0.55±0.03ab	0.54±0.02
5 mL/L	0.40±0.01	0.55±0.03	0.57±0.03a	0.56±0.03a	0.55±0.04
Control	0.40±0.01	0.51±0.05	0.50±0.04c	0.50±0.03c	0.48±0.04

<sup>a, b, c</sup> significant difference in mean of column at  $p < 0.05$

**Table 4** Environment condition during the cultivation (June 2023 - September 2023) in five culture systems

Parameters	value
Light-dark period	About 12:12 hrs
Light intensities	5,000-8,000 lux
pH levels	5-8
Temperature	25-35
Moisture	70-92% RH
Dissolve oxygen (DO)	3.2-12 mg/L
NO <sup>3-</sup> , NO <sub>2</sub>	0-12.5, <0.3 mg/L

**Table 5** Proximate analysis of Fresh and dry samples of *W. globosa* subjected to horizontal surface agitation

Nutrition information of <i>Wolffia globosa</i>				
Serving size: 1 sachet (250 g.)				
Amount per serving: 1	Fresh		Dry	
	(Percent Thai RDI)*		(Percent Thai RDI)*	
Total fat	0 g.	0%	0 g.	0%
Saturated fat	0 g.	0%	0 g.	0%
Cholesterol	0 mg	0%	0 mg	0%
Protein	2 g.		6 g.	
Total carbohydrate	2 g.	1%	19 g.	1%
Dietary fiber	2 g.	8%	8 g.	8%
Sugars	0 g.		0 g.	
Sodium	25 mg.	1%	85 mg.	1%
Vitamin A		10%		0%
Vitamin B1		0%		0%
Vitamin B2		0%		0%
Calcium		6%		70%
Iron		6%		150%

\*Percent Thai Recommended Daily Intakes for population over 6 years of age are based on a 2,000 kcal diet

## Conclusion

Our research provides valuable insights into the cultivation of *Wolffia globosa* using an optimized hydroponic system, particularly highlighting the optimal AB fertilizer concentration of 5 ml/L and the favorable light intensity range of 3,000-6,000 lux for maximizing growth. This study advances current knowledge by demonstrating *Wolffia's* adaptability to varying environmental conditions, offering a novel approach to its cultivation. The findings underscore

*Wolffia*'s potential as a resilient and resource-efficient food source, positioning it as a promising solution for addressing global challenges related to food security and sustainable agriculture.

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