EFFECTS OF LED LIGHT SPECTRUM ON NUTRITIONAL COMPOSITION OF *Wolffia globosa* UNDER PLANT FACTORY CONDITIONS

Chairat Burana[1*], Viwat Maikaensarn[2], and Laddawan Kowitcharoen[3]

[1,2]Faculty of Innovative Agriculture and Management, Panyapiwat Institute of Management, Thailand
[3]School of Agriculture and Cooperatives Sukhothai Tammathirat Open University, Thailand
[*]**Corresponding Author, E-mail:** chairatbur@pim.ac.th

Abstract

This research aimed to develop an efficient, clean, safe, and precise production process for Wolffia (duckweed), an aquatic plant recognized as a highly nutritious food source. *Wolffia globosa* (*W. globosa*) contains high protein content ranging from 25–40% of dry weight and provides a complete profile of essential amino acids. Its protein content is higher than many plant-based raw materials currently used in commercial plant-based food products, indicating strong potential as a functional food ingredient. In this study, a controlled cultivation system was developed using light-emitting diodes (LEDs) with different ratios of red and blue light. The cultivation conditions were set at a 12-hour photoperiod, temperature of 25±2°C, and relative humidity (RH) of 70±2%. The results showed that the optimal growth of *W. globosa* was obtained under a color ratio (RGB) of 58.8, 41.0, and 18.0. Under these conditions, fresh biomass contained 2.6 g 100 g^{-1} DW, vitamin B_{12} at 1.24 µg 100 g^{-1} DW, and the lowest sodium content at 18 mg 100 g^{-1} DW. Moreover, the results confirmed that *W. globosa* grown in the experimental system contained no detectable nitrate residues, indicating the safety and suitability of the production method for food applications. Our results implied that, compared with other light treatments, white LED (Wave: 453.0 nm Value: 68.859 uW/cm²/nm Color Ratio (RGB) 58.8,41.0,18.0) illumination was more suitable for Wolffia cultivation under controlled environments conditions.

Keywords: Future Food, Plant Based Protein, LED, Longevity

Introduction

Wolffia globosa (W. globosa) is a tiny rootless duckweed with very rapid clonal propagation, making it attractive for high-yield protein production and resource-efficient cultivation (shallow water depth, fast turnover) (Appenroth *et al*.). It is increasingly discussed as a novel plant-based protein and functional ingredient, partly because some cultivated *W. globosa* lines (e.g., ‘Mankai’) have been reported to contain bioactive vitamin B_{12} a rare claim for plant foods linked to associated microbiota and/or cultivation conditions. (Ziegler *et al.,* 2015). Outdoor cultivation is commonly used because it is low-cost and scalable, but performance varies with temperature, light, rainfall dilution, contamination (algae), pests, and inconsistent nutrient supply. A recent study comparing nutrient media under outdoor conditions showed that medium choice substantially changed growth rate and productivity, demonstrating the importance of nutrient formulation and management in open systems (Sirison *et al*., 2025). Controlled tanks allow tighter control of nutrients, pH, aeration/mixing, water depth, and hygiene. Reviews on duckweed “bioreactors” and stacked/vertical concepts highlight that duckweeds are well-suited to multi-level shallow systems (very high area per floor area) if engineering constraints (mixing, oxygen, harvesting) are solved (Coughlan *et al*., 2022). While Plant Factory with Artificial Lighting (PFAL) research is more mature for leafy greens, duckweed-focused vertical designs are emerging because *W. globosa* can be grown in thin water layers and harvested frequently (Sree *et al*., 2015). The key barriers are water handling, microbial safety, automation, and consistent product quality rather than photosynthesis alone (Coughlan *et al.,* 2022.) Light is a critical environmental factor regulating plant growth and development. Both light spectrum and light intensity influence not only biomass accumulation but also the synthesis of primary and secondary metabolites (Zhong *et al*., 2022). Nevertheless, the limited number of studies investigating duckweed species have reported inconsistent findings regarding the effects of LED light spectra on growth performance (Gallego *et al*., 2022; Petersen *et al*., 2022). Therefore, further research is required to clarify the effects of different LED spectra on Wolffia. In this study, we evaluated the effects of three LED light spectra on growth and nutritional content of the rootless duckweed Wolffia under controlled-environment conditions.

Material and Methods

Plant materials

The rootless duckweed species *W. globosa* were obtained from a commercial grower in Phra Nakhon Si Ayutthaya Province, Thailand, and immediately transported to the laboratory at Panyapiwat Institute of Management, Nonthaburi, Thailand within 2 hours (Picture 1).

Picture 1: Appearance of *W. globosa* samples.

Cultivated condition and Light Applications

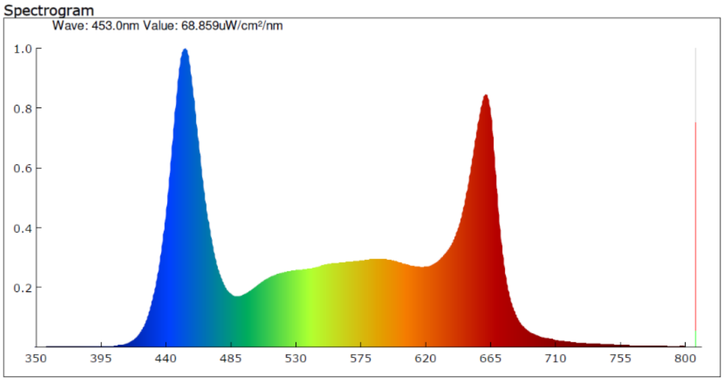
W. globosa cultivation was carried out under controlled conditions in a DRFT Duckweeds Tower (VD3-150, BANGSAI®) located in Laboratory of Faculty of Innovative Agriculture and Management. A tower including with 4 trays each layers different spectral properties LED lighting following as 1) White 2) White+Red Wave: 453.0 nm Value: 68.859 uW/cm²/nm Color Ratio (RGB) 58.8,41.0,18.0 3) Blue+White+Red Mix Wave: 453.0nm Value: 27.576uW/cm²/nm Color Ratio (RGB) 40.8,32.5,35.0 and 4) White+Red Wave: 660.0nm Value: 40.155uW/cm²/nm Color Ratio (RGB) 58.8,41.0,18.0.

The experiment was carried out in 100-L fiberglass tray (surface area: 0.8 m²; water depth: 19 cm), each serving as an independent cultivation unit. Treatments were arranged with four replicates per treatment. Fresh *Wolffia globosa* (200 g) was introduced into each tray, corresponding to approximately 50% initial surface coverage to promote optimal growth. This inoculation density was established based on preliminary experiments demonstrating that surface coverage exceeding 50% resulted in overcrowding within 5 days of cultivation. At the end of the experiment, biomass from each treatment was analyzed.

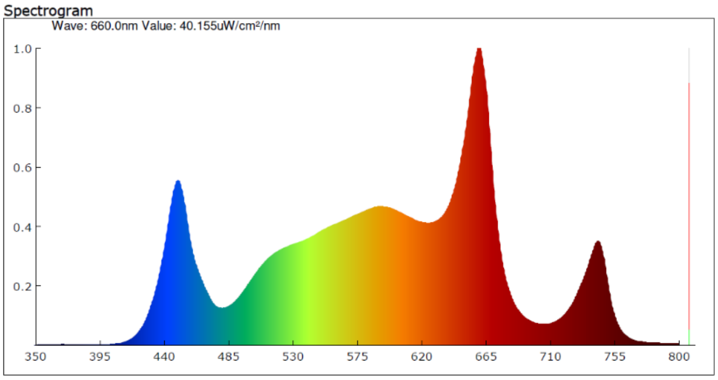
Prior to experimentation, stock cultures of *W. globosa* were acclimated in dechlorinated tap water for three days to stabilize growth conditions. Following acclimation, the cultures were transferred to AB medium (Commercial hydroponic fertilizer). Electrical Conductivity (EC) has been monitored throughout the experiment, resulting in an average EC level as 900 $\mu S\ cm^{-1}$.

The climate conditions applied in the experiment were fixed as 12 hours of light, 12 hours of dark photoperiod, 24±2°C temperature and 70±1% relative humidity. *W. globosa* samples were grown in plastic containers placed on each shelf and harvested regularly every week (Picture 2).

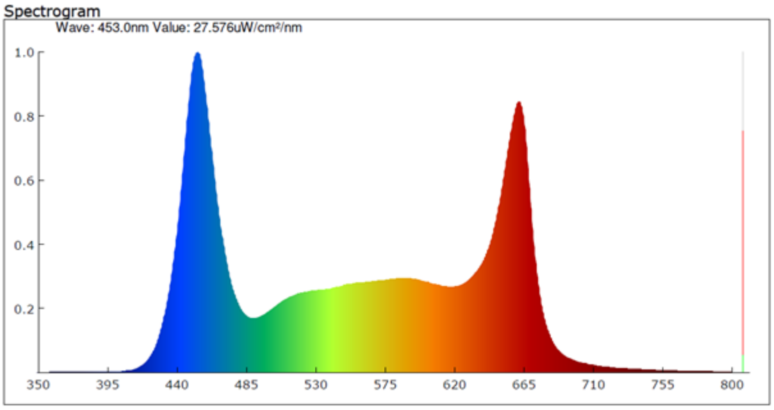
Picture 2: Growing *W. globosa* under different LED spectral power distribution.



Picture 3: Spectral power distribution of the white LED applied during cultivation.



Picture 4: Spectral power distribution of the white + red LED applied during cultivation.



Picture 5: Spectral power distribution of the white + red+blue LED applied during cultivation.

Protein content

The Dumas combustion method determined protein contents (In-house method T9258 based on AOAC (2023) 992.15). The nitrogen analysis, conducted by National Food Institute (Bangkok, Thailand) was used to measure nitrogen and crude protein (N× 6.25) through a standard nitrogen conversion factor (Mariotti et al., 2008).

Vitamin B_{12} content

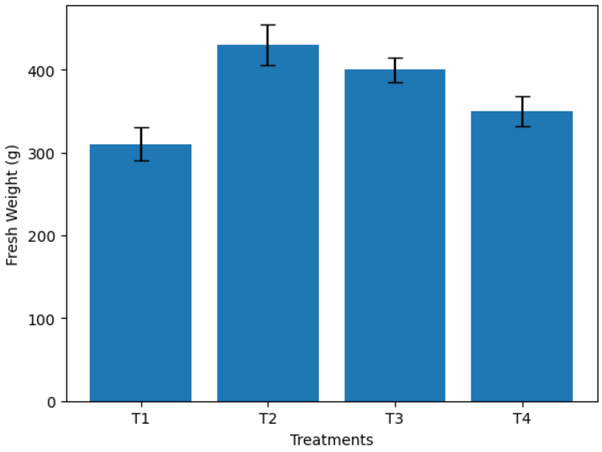
Vitamin B_{12} was determined by a microbiological assay method with *Lactobacillus leichmannii* as the test organism (In house method STM No. 01016 based on AOAC (2023) 952.20) conducted by National Food Institute (Bangkok, Thailand). Extraction of vitamin B_{12} was performed according to method AOAC 952.20, as described by Ball (2006).

Statistical Analysis

All experiments were performed in triplicate, and the results are presented as means ± standard deviation ($n = 3$). Statistical analysis was conducted using SPSS version 17 and Origin 2022 software (9.95), employing one-way analysis of variance (ANOVA) and Duncan's multiple range test ($p < 0.05$).

Results and Discussion

Variations in light spectral composition significantly regulate photomorphogenic processes and modulate metabolic responses in plants (Paradiso & Proietti, 2022). In this study, fresh weight was significantly influenced by the different treatments ($p < 0.05$). The highest fresh weight was observed in T2 (430 ± 15 g^a), which was higher than all other treatments significantly. Treatment T3 (400 ± 12 g^b) showed the second-highest value and was significantly higher than T4 and T1. Treatment T4 (350 ± 10 g^c) produced moderate fresh weight, significantly lower than T2 and T3 but higher than T1. The lowest fresh weight was recorded 310 ± 14 g^d in T1 (Picture 6).



Picture 6: Effects of different treatments on fresh weight (g). Values represent mean ± SD (n = 3). Different letters indicate significant differences among treatments according to one-way ANOVA followed by Tukey's HSD test at $p < 0.05$.

The significantly greater fresh weight observed under T2 suggests that this treatment provided optimal conditions for biomass accumulation. Enhanced fresh weight may be attributed to improved photosynthetic performance, efficient nutrient assimilation, and favorable physiological responses under this treatment (Zou *et al*., 2016; Zhou *et al*., 2019). Although T3 also promoted high biomass production, it was statistically lower than T2, indicating that while beneficial, it did not maximize growth to the same extent. The significantly lower fresh weight in T1 suggests suboptimal growth conditions, potentially limiting carbon assimilation or water balance. The progressive decline from T2 to T4 and T1 indicates that the treatment factor plays a critical role in biomass production. These findings highlight the importance of optimizing environmental or management factors to enhance fresh weight accumulation under controlled cultivation systems. Izzo *et al*. (2020) reported that blue (400-500 nm) and red (600-700 nm) wavelengths are key spectral regions governing plant developmental processes. Blue light regulates vegetative growth by stimulating chlorophyll biosynthesis and stomatal opening, while red light, mediated through phytochrome signaling, promotes flowering induction and reproductive development.

Protein content increased progressively from the control (T1) to T4. The lowest value was observed in control (0.80 ± 0.05 g 100 g^{-1} DW), while the highest protein concentration was recorded in white+red+blue LED treatment (T4) (2.60 ± 0.12 g 100 g^{-1} DW), followed by white + T3; red LED treatment (2.00 ± 0.10 g 100 g^{-1} DW) and T2; white LED treatment (1.50 ± 0.08 g 100 g^{-1} DW), respectively (Table 1.). The progressive increase in protein content from T1 to T4 suggests that the applied treatments enhanced nitrogen assimilation and protein biosynthesis in Wolffia. The significantly higher protein accumulation in T4 indicates that this treatment provided optimal environmental or nutritional conditions for amino acid synthesis and metabolic activity. Enhanced light quality or nutrient availability (depending on experimental design) may have stimulated photosynthetic efficiency, thereby increasing carbon skeleton availability for protein synthesis. Under steady-state cultivation conditions, *W. globosa* exposed to blue light exhibited significantly higher protein and chlorophyll contents compared with plants grown under red light (Landolt and Kandeler, 1987). The results are consistent with previous studies on duckweed species, which report that environmental optimization significantly enhances crude protein accumulation due to improved nitrogen uptake and assimilation efficiency (Gou *et al*., 2020).

Table 1: Protein, Vitamin B_{12}, and Sodium contents of *W. globosa* under different treatments.

Treatments	**Protein** (g $100g^{-1}$ DW)	**Vitamin B_{12}** (µg 100 g^{-1} DW)	**Sodium** (g $100g^{-1}$ DW)
T1 (Control)	0.80 ± 0.05^{d}	$<0.10^{c}$	99.0 ± 3.2^{a}
T2	1.50 ± 0.08^{c}	0.39 ± 0.04^{b}	14.0 ± 1.1^{b}
T3	2.00 ± 0.10^{b}	1.06 ± 0.07^{a}	18.0 ± 1.4^{b}
T4	2.60 ± 0.12^{a}	1.24 ± 0.09^{a}	18.0 ± 1.2^{b}

Statistical analysis indicated significant differences among all treatments ($p < 0.05$), as shown by different superscript letters.

Vitamin B_{12} content was significant affected by treatment. The control (T1) showed a negligible level (<0.10 µg 100 g^{-1} DW). T2 significantly increased vitamin B_{12} to 0.39 ± 0.04 µg 100 g^{-1} DW, while T3 and T4 showed the highest values (1.06 ± 0.07 and 1.24 ± 0.09 µg 100 g^{-1} DW, respectively). The substantial increase in vitamin B_{12} in T3 and T4 is particularly noteworthy. Since vitamin B_{12} in duckweed is often associated with microbial symbiosis or cultivation conditions, the higher levels may indicate enhanced microbial activity or favorable metabolic regulation under these treatments. The negligible level in the control suggests that standard conditions were insufficient to promote B_{12} biosynthesis or accumulation. This finding highlights the potential of optimized cultivation strategies to produce *Wolffia* as a functional food with elevated micronutrient value, especially for plant-based diets where vitamin B_{12} deficiency is a concern. Yu *et al*.(2015) revealed that *P. freudenreichii* presents high growth rate and vitamin B12 production in tofu wastewater under blue light conditions.

In contrast to protein and vitamin B_{12}, sodium content was highest in the control treatment (99.0 ± 3.2 g 100 g^{-1} DW), which was significantly higher than all other treatments. T2, T3, and T4 showed markedly reduced sodium levels (14.0–18.0 g 100 g^{-1} DW), with no significant differences among these treatments. Wolffia phytochemical profiles and nutritional content may vary depending on the cultivation environment, species, and other environmental factors (Ziegler *et al*., 2015; On-Nom *et al*., 2023). Moreover, different light spectrums also are effective on the morphological structure of *W. arrhiza.* The significantly lower sodium levels in T2–T4 compared to the control suggest that the treatments improved ion regulation or altered nutrient solution composition. Excess sodium accumulation can negatively affect nutritional quality and consumer health; therefore, the reduced sodium content observed under optimized treatments enhances the suitability of Wolffia as a healthy food ingredient. The high sodium concentration in the control may be attributed to uncontrolled ionic balance or stress conditions, which promoted sodium uptake and accumulation.

Conclusion

The combined blue + white + red LED spectrum (under a color ratio (RGB) of 58.8, 41.0, and 18.0) significantly enhanced the nutritional quality of *W. globosa* cultivated under controlled conditions. This light treatment increased protein content and vitamin B_{12} concentration, while simultaneously reducing sodium levels in the biomass. The improvement in protein accumulation may be associated with enhanced photosynthetic efficiency and nitrogen assimilation under the synergistic effects of blue and red wavelengths, while white light likely contributed to balanced photomorphogenic responses. The reduction in sodium content further improves the nutritional profile, making the product more suitable for health-conscious consumers.

Overall, the blue + white + red LED combination appears to be an optimal lighting strategy for producing high-value *W. globosa* with superior nutritional characteristics. Therefore, this light spectrum is recommended for commercial cultivation in PFAL system**,** particularly for functional food and nutraceutical markets.

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