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Effect of Light Quality on the Morphological Structure of Rootless Duckweed (*Wolffia arrhiza*)

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Abstract

Duckweeds (Lemnoideae, Araceae) are the world's smallest and fastest-growing flowering plants, free-floating in freshwater, consisting of 5 genera (*Spirodela*, *Lemna*, *Landoltia*, *Wolffia* and *Wolffiella*) and 36 well-known species. It includes species ranging in size from 1 mm to 1.5 cm. *Wolffia* (watermeal, eggs of the water), which contains the smallest individuals of duckweed and is rootless, attracts attention with its high protein content. Therefore, more focus has been placed on this genus in recent years. *Wolffia* has a significant market potential as a plant-based protein source. In this study, the morphological structure (granule frond length and width) of *W. arrhiza* grown under different LED lights (white, red, blue and blue-red combination) in a climate chamber was examined. In the measurements made with a stereo microscope, it was observed that the light quality influenced the morphological structure of *W. arrhiza*. While *W. arrhiza* grown under blue LED was larger, red LED samples were smaller. The frond length and width were determined in blue > white > purple > red LED lights, respectively. The use of LED applications in vegetable cultivation is becoming widespread for high-efficiency and sustainable production. As a result of this study, it was determined that LED lights with different wavelengths were effective on the morphological structure of the plant. In targeted duckweed cultivation, it is important to select the appropriate light quality for the desired product (protein, starch, bioactive components, fresh vegetables, etc.).

Keywords: rootless duckweed, growth, light quality, LED, *Wolffia*

Işık Kalitesinin Köksüz Su Mercimeğinin (*Wolffia arrhiza*) Morfolojik Yapısı Üzerindeki Etkileri

Öz

Su mercimekleri (Araceae, Lemnoideae) 5 cins (*Spirodela*, *Lemna*, *Landoltia*, *Wolffia* ve *Wolffiella*) ve 36 tanınmış türden oluşan, tatlı sularda serbest yüzen, dünyanın en küçük ve en hızlı büyüyen çiçekli bitkilerdir. Boyutları 1 mm'den 1.5 cm'ye kadar değişen türler içerir. Su mercimeklerinin en küçük bireylerini içeren ve köksüz olan *Wolffia* (su unu, su yumurtaları), yüksek protein içeriği ile dikkat çekmektedir. Bu nedenle son yıllarda bu cinsin üzerine daha fazla odaklanılmıştır. Bitki bazlı protein kaynağı olarak *Wolffia* önemli bir pazar potansiyeline sahiptir. Bu çalışmada, iklimlendirme kabininde farklı LED ışıklar altında yetiştirilen (beyaz, kırmızı, mavi ve mavi-kırmızı kombinasyonu) *W. arrhiza*'nın morfolojik yapısı (granül yaprak uzunluğu ve genişliği) incelenmiştir. Stereo mikroskopta yapılan ölçümlerde ışık kalitesinin *W. arrhiza*'nın morfolojik yapısı üzerinde etkili olduğu gözlenmiştir. Mavi LED'de yetişen *W. arrhiza* daha iri iken kırmızı LED örnekleri daha küçük yapılıdır. Yaprak uzunluğu ve genişliği sırasıyla mavi> beyaz> mor> kırmızı LED ışıklarda tespit edilmiştir. Yüksek verimli ve sürdürülebilir üretim amacıyla LED uygulamalarının sebze yetiştiriciliğinde kullanımı yaygınlaşmaktadır. Bu çalışma sonucunda da farklı dalga boylarına sahip LED ışıkların bitkinin morfolojik yapısı üzerinde etkili olduğu tespit edilmiştir. Hedefe yönelik su mercimeği yetiştiriciliğinde, istenilen ürün için (protein, nişasta, biyoaktif bileşenler, taze sebze vb.) uygun ışık kalitesinin seçilmesi önemlidir.

Anahtar Kelimeler: köksüz su mercimeği, büyüme, ışık kalitesi, LED, *Wolffia*

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Introduction

The rapid increase in the global population, urbanization, climate change, and the reduction of arable land are making global food scarcity an increasingly critical issue (van Dijk et al., 2021). It is projected that by 2050, the world population will reach 9.7 billion, and global food demand will nearly double (Malila et al., 2024). Scientists agree that feeding this growing population will be one of the greatest challenges in the coming decades. Moreover, excessive livestock production is known to be unsustainable (Takács et al., 2025). The livestock sector uses 26% of land resources and 15% of groundwater, is responsible for 12% of water pollution, and contributes approximately 13% to global greenhouse gas emissions (Mehrabi et al., 2020; Wood & Tavan, 2022). Greenhouse gas emissions accelerate climate change, while the conversion of natural ecosystems into agricultural and pasturelands for feed production leads to ecological degradation and loss of biodiversity (Panja, 2021).

As an alternative to these problems, aquatic plants such as algae have gained attention as functional and nutritious food sources (Taş & Şengüllendi, 2022a). Duckweeds offer significant advantages over conventional animal-based proteins and crops in terms of sustainable production methods and high nutritional value. As seaweeds and microalgae are increasingly studied as alternative food sources, duckweeds have similarly attracted growing interest in recent years (Appenroth et al., 2017, 2018; Taş & Şengüllendi, 2023; Xu et al., 2012). Thanks to their balanced composition and time-cost-efficient production, duckweeds are considered promising food alternatives (Prada et al., 2024). Ongoing R&D efforts are overcoming challenges related to mass cultivation and harvesting of this plant, enabling its broader use in food, environmental, and industrial applications (Takács et al., 2025).

Duckweeds (Lemnoideae, Araceae) are small, fast-growing, morphologically simple, floating aquatic plants that inhabit freshwater environments. The family comprises 5 genera (*Spirodela*, *Lemna*, *Landoltia*, *Wolffia, Wolffiella*) and 36 recognized species (Appenroth et al., 2013). The genera *Wolffia* and *Wolffiella* include the world's smallest flowering monocotyledons. In particular, the rootless species *Wolffia*, with a diameter of only about 1 mm, represents one of the most morphologically reduced angiosperms (Bernard et al., 1990; Landolt, 1986). *Wolffia* primarily reproduces asexually via budding, and due to its rapid vegetative growth, it can form genetically uniform populations (Sree et al., 2015; Xu et al., 2011).

Duckweeds have been intensively studied as potential food sources for human consumption (Appenroth et al., 2017; Dhamaratana et al., 2025; Hu et al., 2022). The genus *Wolffia* is particularly notable for its high protein content, comprising about 50% of its dry weight, and its digestible essential amino acid profile. Additionally, it possesses high levels of phenolics and flavonoids, which contribute to its strong antioxidant capacity (Xu et al., 2011). These properties make *Wolffia* a promising nutritional and functional food component (On-Nom et al., 2023). It also has wide potential applications in nutraceuticals, pharmaceuticals, bioremediation, and bioenergy (Cui & Cheng, 2015; Petersen et al., 2022).

Duckweeds can be cultivated year-round in open or closed systems in tropical regions, and in greenhouses under suitable conditions in subtropical regions (Arslan Günal & Taş, 2022). Their 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). Studies particularly focusing on *W. arrhiza* have investigated the effects of different LED light spectra on growth and metabolism (Taş & Şengüllendi, 2022b; Şengüllendi, 2024). Through environmental manipulation, the nutritional composition of duckweeds can be optimized (Takács et al., 2025).

Light is a key environmental factor in plant development. Both the spectrum and intensity of light affect not only growth and development but also metabolite production (Zhong et al., 2022). However, the limited number of studies conducted on duckweed species have reported inconsistent results regarding the effects of LED light spectra on growth (Gallego et al., 2022; Petersen et al., 2022). Therefore, further research is needed to better understand the impact of different LED lights on *W. arrhiza*.

In this study, the effects of different LED light spectra (white, red, purple [red-blue], blue) on the morphological characteristics of the rootless duckweed *W. arrhiza* were evaluated under controlled conditions.

Materials and Methods

Plant Material

The rootless duckweed species *Wolffia arrhiza* was collected and described from a local wetland in the Yeşilırmak Delta in the Terme district of Samsun province (Taş & Topaldemir, 2021). Lacking typical plant organs such as roots, stems and leaves, *W. arrhiza* reproduces vegetatively by "budding" in the form of granular leaves (Schmitz & Kelm, 2017). The leaves are generally 1–1.5 times longer than they are wide, spherical to ellipsoid in shape; their upper surfaces are convex, and their greatest width is usually just below the water surface. In the literature, the leaf length of this species is reported as 0.5–1.5 mm and its width as 0.4–1.2 mm (Schmitz & Kelm, 2017; Bog et al., 2020). This species, which has a rather small structure, rarely flowers and produces fruit. However, it has the capacity to reproduce rapid vegetatively under suitable conditions (Figure 1).

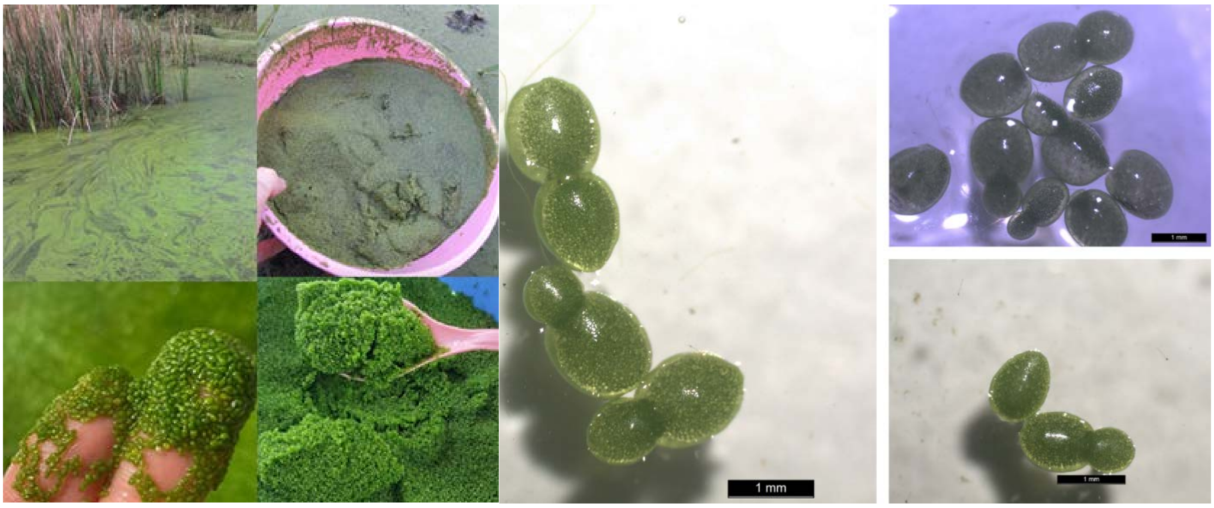

Figure 1. Morphological Appearance of *Wolffia arrhiza*; Granulated Leaves and Vegetative Reproduction (Original)

Growth Environment

W. arrhiza samples were cultured at Ordu University (OTU) Hydrobiology Laboratory. In the cultivation of plants, "N-medium", a synthetic medium recommended for duckweeds, was used (Appenroth, 2015). Four different stock solutions were prepared in the preparation of the medium:

Stok 1: 4.083 g L^{-1} KH_2PO_4

Stok 2: 47.23 g L^{-1} $Ca(NO_3)_2·4H_2O$

Stok 3: 161.8 g L^{-1} KNO_3, 61.8 mg L^{-1} H_3BO_3, 514.5 mg L^{-1} $MnCl_2·4H_2O$, 9.4 mg L^{-1} $Na_2MoO_4·2H_2O$, 49.30 g L^{-1} $MgSO_4·7H_2O$

Stok 4: 1.835 g L^{-1} FeNaEDTA

The prepared stock solutions were stored in the refrigerator at +4°C. 5 mL of each stock was taken for each 1 liter of N-medium preparation and mixed, the pH value was adjusted to 5.5 and sterilized in an autoclave at 121°C for 15 minutes. After sterilization, the media cooled to room temperature were used for *W. arrhiza* cultivation.

Trial Pattern and Light Applications

W. arrhiza cultivation was carried out under controlled conditions in a sterile climate cabinet (Grotech/GR08, Unitroniks® Vision350™) located in the Hydrobiology Laboratory of Ordu University (ODU). LED light sources with different spectral properties (white, red, violet [red-blue combination] and blue) were mounted under each of the four shelves in the climate cabinet. The light intensity provided by the LEDs was kept in the range of 70–80 μmol m^{-2} s^{-1}. The climate conditions applied in the experiment were fixed as 16 hours of light, 8 hours of dark photoperiod, 24±1°C temperature and 70% relative humidity. *W. arrhiza* samples were grown in plastic containers placed on each shelf and harvested regularly every week (Figure 2).

Morphological Measurements

W. arrhiza samples cultivated under different LED light spectra were washed with distilled water (dH_2O) after harvest and 20 individuals were randomly selected from each group and placed in petri dishes. Length and width measurements of the granule leaves of the samples were performed from the top view. For morphological measurements, a LEICA L2 stereo microscope with LAS V3.7 image analysis software (Leica Microsystems, Wetzlar, Germany) was used. The obtained data were used to determine morphological differences depending on light quality.

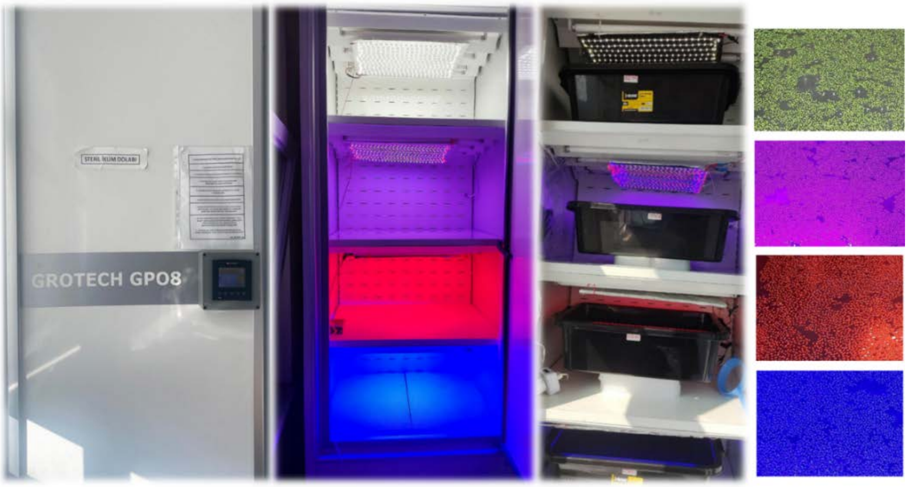

Figure 2. Growing *Wolffia Arrhiza* under Different Leds in Climate Cabinet

Statistical Analysis

After the research data was processed into an Excel file, data analysis was performed using Excel's statistical functions. The relationship between the length and width of rootless duckweed was evaluated by linear regression analysis, and the results were reported at a 5% significance level.

Result

In the width and length measurements made during the *W. arrhiza* cultivation process and on the harvest samples, it was observed that the leaf sizes of the plants grown under different LEDs were different. The width-length results of the granular leaf of *W. arrhiza* measured under a stereo microscope are given in Table 1.

Table 1. Width and Length Measurements of *W. arrhiza* Grown in Different Light Spectrums

	Width (mm)		Length (mm)	
Light	Min–max.	Mean±Std. dev.	Min–max.	Mean±Std. dev.
White LED	0.81–1.05	0.9±0.065	0.96–1.19	1.10±0.068
Red LED	0.69–0.88	0.77±0.051	0.83–1.10	0.97±0.082
Purple LED	0.79–0.95	0.88±0.039	0.93–1.92	1.05±0.202
Blue LED	0.93–1.32	1.15±0.105	1.12–1.59	1.36±0.137

According to the research results, different light spectrums are effective on the morphological structure of *W. arrhiza*. Leaf width and length were recorded in blue> white> purple> red LED lights, respectively (Figure 3). It was concluded that light quality (color or wavelength) changed the morphological structure of rootless duckweed (Figure 4). Length:width ratios of *W. arrhiza* grown in white, red, purple and blue LEDs were calculated as 1.22, 1.26, 1.19 and 1.18, respectively.

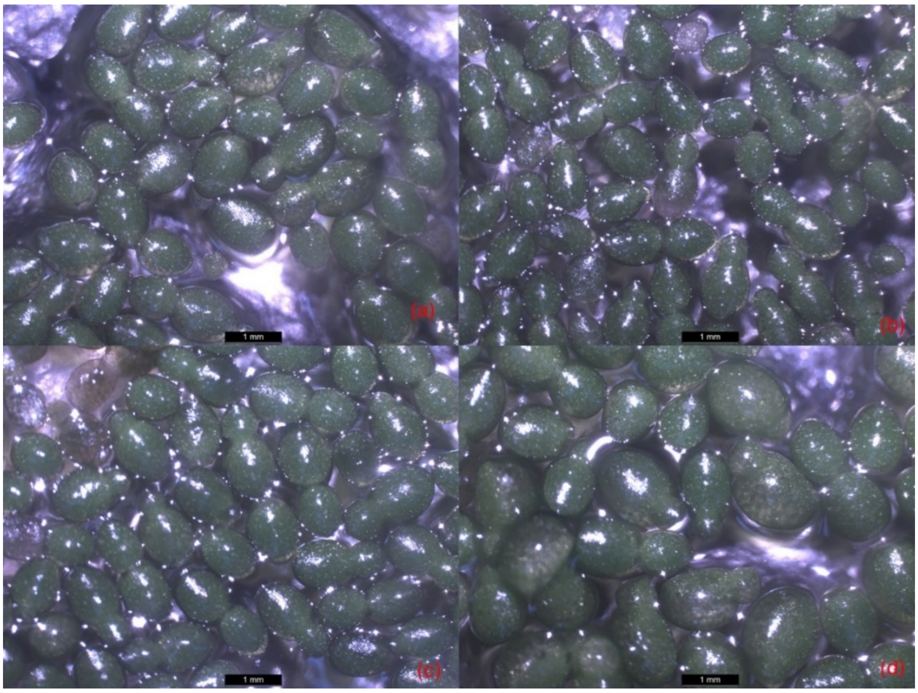

Figure 3. Morphological Appearances of *Wolffia arrhiza* Grown in Different LED Lights

(a: White LED, b: Red LED, c: Purple LED, d: Blue LED)

The relationship between width-length measurements of *W. arrhiza* samples grown under different LED lights was evaluated by linear regression analysis. The results of the statistical analyses are summarized in Table 2. In the analyses, leaf length was used as the dependent variable and leaf width was used as the independent variable.

Table 2. Linear Regression Analysis Results

LED	*n*	R^2	*Std. error*	*F-value*	*Sig. F-value*	*Intercept P-value*	*Width P-value*
White	20	0.502	0.049	18.155	0.0005	0.0143	0.0005
Red	20	0.267	0.072	6.547	0.020	0.1869	0.0197
Purple	20	0.070	0.200	1.363	0.258	0.0389	0.2582
Blue	20	0.440	0.105	14.141	0.001	0.1856	0.0014

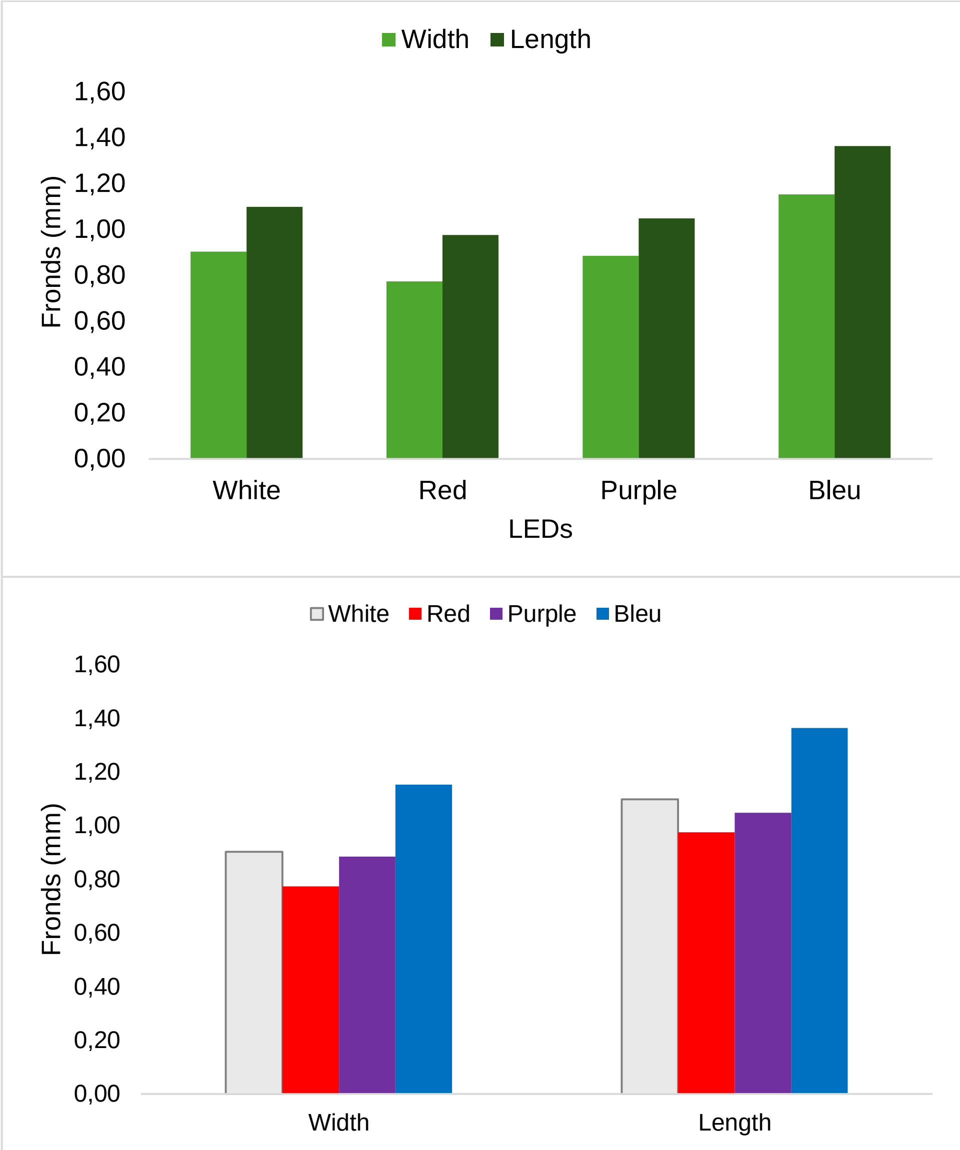

Figure 4. Changes in *Wolffia arrhiza* Width and Length Measurements on Different LEDs

When the white LED light results were evaluated; a strong linear relationship was determined with multiple R value of 0.709. The R^2 value explains half of the data (50.21%). The F-value (18.15) and the significance F-value (0.00047) show that it is statistically significant. These results confirm that the relationship between "width" and "length" creates a significant difference. The value of the intercept was found as 0.4274. The slope coefficient (0.7424) shows that the relationship between "width" and "length" is strong and that for each increase in width, the length increases by 0.7424 units (Figure 5). The P-value is very low ($p<0.05$), which shows that the effect of the width variable on length is statistically strong (Table 2). As a result, these regression analysis data show that the linear relationship between width (independent variable) and length (dependent variable) is significant.

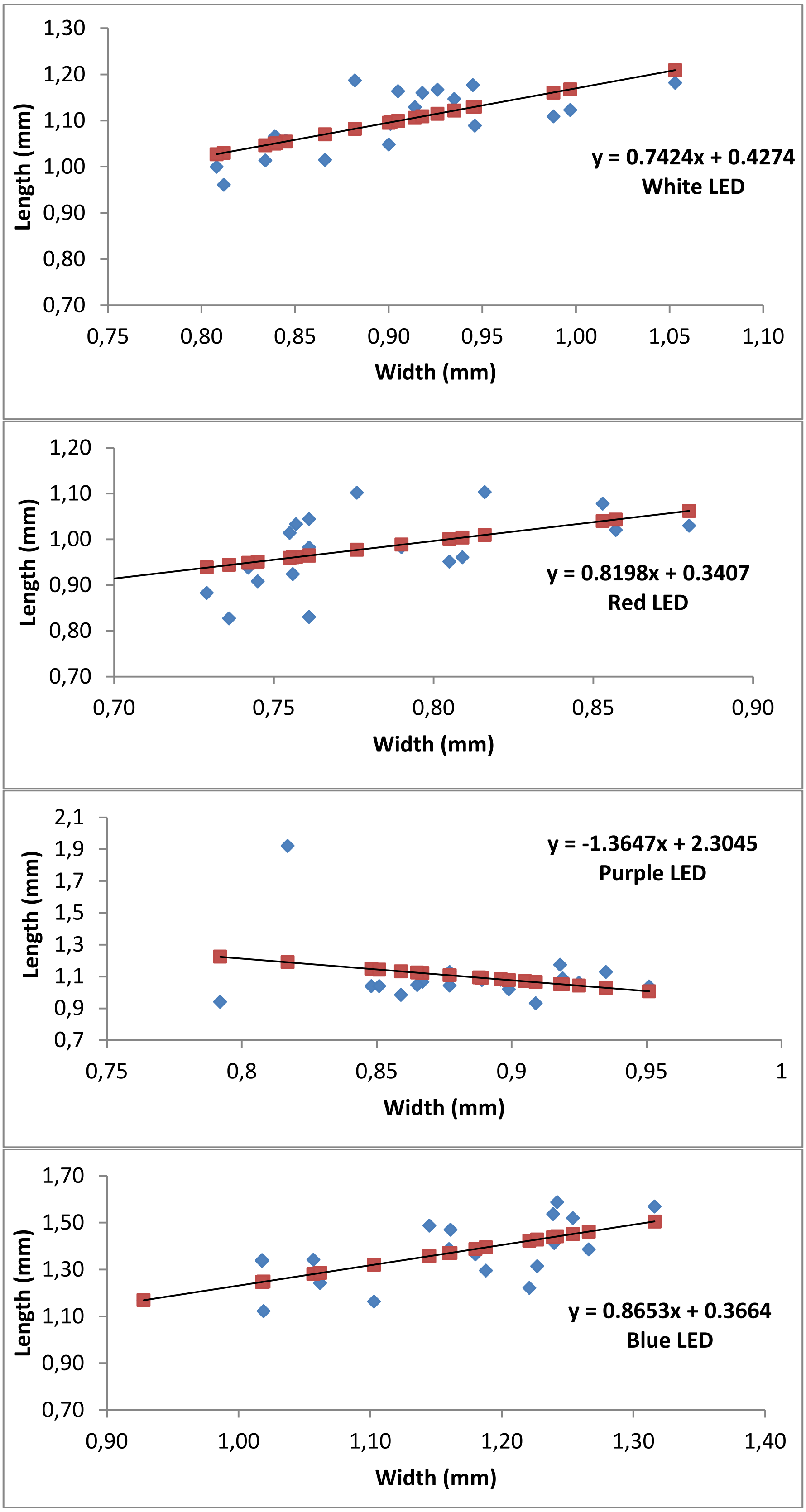

Figure 5. Regression Plot of Width–Length Measurement of *W. arrhiza* Grown in Blue LED Light

In the regression analysis of the red LED samples, the multiple R value showed that there was a moderate relationship between the dependent and independent variables. The R^2 value explained 26.67% of the data, so the explanatory power was slightly low. The F-value (6.5465) showed that the model was generally consistent, and since the significance F-value was less than 0.05, the model was statistically significant. The p-value (0.0197) at the 95% confidence interval showed that the effect of the width variable on the length was statistically significant. As seen in Figure 5, the effect of the slope and width variable on the height was statistically significant.

According to the purple LED results obtained with the red-blue combination, the multiple R (0.2653) value showed that the linear relationship between the independent variable and the dependent variable was weak. The R^2 value explained only 7.04% of the data, so it had low explanatory power. The F-value (1.3632) was low, and the significance F-value (0.2582) was greater than 0.05. These results mean that the model is not generally significant. In the width-length measurements we made in different LED lights, the smallest individuals were observed in the red LED light. Since the p-value of the intersection (0.0389) is less than 0.05, the intersection point is statistically significant (Figure 5).

In the regression analysis between the width-length measurements of *W. arrhiza* under the effect of blue LED light, the multiple R value (0.6633) showed a moderate positive linear relationship. The R^2 value (0.440) explained 44% of the data, meaning it has a moderate explanatory power. The F-value (14.1414) showed that the general fit of the data was good, and the significance F-value (0.0014) showed that the data was statistically significant. In short, the effect of the width variable on the length is significant. Since the p-value for the intersection is greater than 0.05, the intersection point is not statistically significant (Table 2). There is a statistically significant effect of leaf width on length as the p-value for slope (0.0014) is less than 0.05 (Figure 5).

Discussion

Light sources with different spectra can significantly affect photomorphogenesis processes and metabolic responses in plants (Paradiso & Proietti, 2022). In this study, morphological differences depending on light quality were detected in *W. arrhiza* samples grown under white, red, purple and blue LED light in controlled climate conditions (Table 1). According to the measurements, granule leaf length varied between 0.83–1.92 mm and width between 0.69–1.32 mm. These values are generally consistent with the leaf length and width ranges reported in the literature for *W. arrhiza* species common in Europe (Bog et al., 2020; Landolt, 1986; 1994; 2000).

Leaf length/width ratios determined according to LED light types were calculated as blue: 1.18, purple: 1.19, white: 1.22 and red: 1.26, respectively. The average ratio (1.21) coincides with the range of 1–1.33 reported in the literature. When Figure 3 is examined, it is seen that different spectra significantly affect *W. arrhiza* leaf morphology. The smallest granular leaves developed under red LED light, while the largest ones under blue LED light. The order in terms of leaf size is red < purple < white < blue LED.

These findings show that especially blue (400–500 nm) and red (600–700 nm) wavelengths play a critical role in plant development. It is known that blue light supports vegetative growth by increasing chlorophyll synthesis, while red light promotes flowering and fruit formation (Izzo et al., 2020). The fact that *W. arrhiza* forms smaller structures under red light may be related to the rare flowering of this species. In contrast, larger leaves observed under blue light may be associated with increased photosynthetic activity. It has been reported in various studies that blue light increases growth, chlorophyll levels and photosynthesis rate (Carvalho & Folta, 2014). In addition, it is supported by the literature that *W. arrhiza* has higher chlorophyll content under blue LED light. However, it should be noted that the effects of abiotic factors on different duckweed species may vary (Paolacci et al., 2018; Stewart et al., 2020).

Plants effectively absorb photons in the blue and red regions of the light spectrum, and photosynthetic efficiency is generally higher in these regions. Therefore, the combination of these two types of light in appropriate proportions is recommended as an ideal lighting strategy for many plant species (Xu et al., 2019). The wavelength-specific effects of LEDs are an important tool for evaluating physiological responses in closed system plant production (Dutta Gupta & Jatothu, 2013; Yeh & Chung, 2009).

In the current literature, no study has been found that directly examined the effect of light spectra on the leaf length/width ratio of *W. arrhiza*. Xu et al. (2019) reported that the combination of blue and red light provided the highest photosynthetic efficiency in their study on *Spirodela polyrhiza*. Petersen et al. (2022) investigated the effects of different spectral ratios and light intensities on growth and pigment contents in *Lemna minor* and *Wolffiella hyalina* species; however, no significant differences were observed. In this study, white light background illumination was used, and blue/red lights were not tested individually.

In another study on *W. arrhiza*, elemental analysis and crude protein content of samples grown under different LED lights were evaluated (Taş & Şengüllendi, 2022b; 2023). The findings showed that the highest crude protein rate (41.6%) was obtained under red LED light; this rate remained at 10% in natural environment samples. In elemental analysis, the highest carbon and hydrogen content were recorded under blue LED, the highest nitrogen under red LED, and the highest sulphur content under purple LED light.

Similar results have been reported in LED lighting studies on different plants such as lettuce, tomato and broccoli. It has been observed that blue and red-light combinations increase the aboveground biomass of lettuce; blue light influences stomatal aperture, plant height and chlorophyll biosynthesis (Zhang et al., 2018; 2021; Zheng et al., 2019). It has been reported that blue LED increases yield and turgor pressure in tomato leaves; and provides enrichment of phytochemical components in broccoli sprouts (Kopsell & Sams, 2013; Xu et al., 2012). All these findings reveal that the light spectrum is a determining factor in plant morphogenesis.

Paradiso and Proietti (2022) drew attention to the effects of LED technologies on growth and morphology in vegetables and ornamental plants; they emphasized that smart lighting systems have great potential in greenhouse farming. However, it was stated that the optimum light spectrum and intensity for each plant species are still not clearly defined and interactions with environmental factors should be investigated in more detail.

Consequently, providing sufficient light with appropriate spectral components is critical for optimum growth in indoor *W. arrhiz*a cultivation. Efficient LED systems integrated with renewable energy sources can support sustainable agricultural practices by reducing production costs (Redmond et al., 2025).

Conclusion

Artificial lighting plays a critical role in plant development, and it is known that LED light applications have significant effects on the physiological, morphological and structural characteristics of plants. However, it should be kept in mind that light quality may affect growth and development processes differently depending on the plant species. As revealed in this and similar studies, light spectra used in duckweed (*W. arrhiza*) cultivation are determinants of plant growth rate, biomass, quality characteristics, nutrient content, phytochemical components and aesthetic appearance. In this context, selection of appropriate light quality according to production targets (e.g. protein, starch or bioactive components) is of strategic importance in terms of increasing the nutraceutical and pharmaceutical potential of plants such as duckweed.

As widely emphasized in the literature, blue light is an effective spectrum that promotes plant growth. In this study, it was determined that blue LED light supports larger granule leaf formation compared to white, red and purple (red-blue) combinations. *W. arrhiza*, which has no root system, is quite small in structure but has a high reproductive capacity, stands out as an efficient source in terms of time and cost in food production thanks to its balanced nutritional composition and rapid production potential. As a plant-based protein source, *Wolffia* is considered to have significant potential in pharmaceutical applications thanks to its bioactive components as well as being evaluated in nutraceutical and functional food production. The availability of this innovative "super food" in the Turkish market will be an important step in terms of sustainable food resources.

On the other hand, the use of LED technology in agricultural production offers significant advantages in terms of both energy and product efficiency; this indicates a widespread application area in the future. Thus, it will be possible to develop high value-added plant products with effective, sustainable, economical and environmentally friendly production methods. In future studies, detailed investigation of light duration and intensity as well as different light spectrums will contribute to the development of more efficient and target-oriented strategies in plant cultivation.

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Author Contribution

The authors collaborated throughout all stages of the study. All authors contributed equally to the article.

Ethics Statement

There are no ethical issues with the publication of this article.

Conflict of Interest

The authors state that there is no conflict of interest.

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