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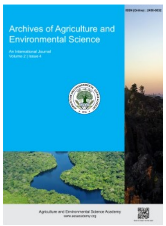

ORIGINAL RESEARCH ARTICLE

Comparative analysis of red and green lettuce microgreens under different artificial LED lighting conditions

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ABSTRACT

This study investigated how LED light exposure influences the growth and nutrient content of two lettuce microgreens over 10 days in a randomized setup. Lettuce seeds underwent surface sterilization, germination in prepared soil, and exposure to different LED light conditions. Morphological parameters and pigment analysis, including stem length, petiole length, leaf area, plant height, root length, fresh weight, dry matter percentage, were evaluated. Green lettuce outperformed red lettuce in the studied morphological parameters, including stem length (2.74±0.22 cm), plant height (4.54±0.21 cm), and fresh weight (3.79±0.32 g/100 plants) under different LED. White light promoted taller plants with higher fresh weight (4.45±0.43 g/100plants), dry matter (4.84±0.38%), and leaf area (0.76±0.06 cm^2) in both lettuce species. In contrast, red light reduced overall growth and development, as evidenced by a 54% decrease in leaf area, despite a 23.36% increase in plant height. Chlorophyll levels varied significantly among LED treatments, with white LED yielding the highest levels in both red and green lettuce. Highest chlorophyll *a* (146.37±6.27 μg/g FW), chlorophyll *b* (86.74±2.44 μg/g FW), total chlorophyll (233.11±8.69 μg/g FW) and relative chlorophyll (215.84±8.05 μg/cm^2) content was found in green lettuce under white light condition. Similarly, green lettuce grown under white LED had the highest total carotenoid, β-carotene, and lutein. The study concludes that optimizing white LED illumination has the potential to improve the nutritional value of lettuce microgreens by enhancing growth and pigment content, particularly in green varieties. These findings emphasize the crucial role of LED light color in optimizing the nutritional quality of microgreens.

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INTRODUCTION

Microgreens are a distinct type of specialty crop that includes young seedlings of vegetables, ornamental plants, and herbs. These seedlings are harvested when their cotyledons have fully developed, which can occur before or after the first true leaves emerge (Xiao *et al.*, 2012; Orlando *et al.*, 2022). They are typically consumed fresh after being harvested within 7-14 days of growth (Xiao *et al.*, 2015; Polash *et al.*, 2019). With up to 40 times more essential nutrients, microgreens boast significantly higher concentrations of bioactive compounds, antioxidants, and vitamins than their mature parts or seeds, despite their diminutive size, all without genetic engineering or bio-fortification (Xiao *et al.*, 2012; Polash *et al.*, 2019; Treadwell *et al.*, 2020). Among the many varieties of microgreens, lettuce microgreens, favored for their versatility, are nutrient-packed additions to salads and various healthy dishes, offering ample fiber, low calorie content, and a rich array of essential vitamins with beneficial phenolic compounds (Mampholo *et al.*, 2016; Sinaga *et al.*, 2023). Lettuce microgreens also contains beneficial substances such as flavonoids, β-carotene, and chlorophyll, all of which contribute significantly to the diet's antioxidant properties and nutritional value (Martínez-Ispizua *et al.*, 2022; Sinaga *et al.*, 2023).

Furthermore, the cultivation of microgreens, including lettuce varieties, has been revolutionized by advancements in lighting technology. Light plays a vital role in shaping the growth and development of plants (Seyedi *et al.*, 2024). Due to its higher luminous efficiency and lower power consumption as compared to conventional fluorescent lights, Light Emitting Diode (LED) lights are widely used in the cultivation of various plant species, particularly horticultural plants (Sena *et al.*, 2024). Both red (R) and blue (B) wavelengths play significant roles in plant growth and development, primarily by influencing photosynthetic pigment absorption and carbon assimilation, while also impacting plant architecture and development (Chen *et al.*, 2017; Alrajhi *et al.*, 2023). White LED lighting influences the fresh weight of lettuce plants (Lu *et al.*, 2019) and also promotes plant growth development as well as different necessary pigments (Sinaga *et al.*, 2023).

While numerous studies have investigated the effects of LED lighting on the growth and development of various crops, relatively limited research has focused specifically on red and green lettuce microgreens. Understanding how different LED lighting conditions with high intensity influence the pigments and bioactive compounds of these microgreens is essential for optimizing their production on a commercial scale. Therefore, the purpose of this comparative analysis is to assess the impact of various artificial LED lighting spectra, including blue, red, blue-red, and white, on the growth characteristics and pigment accumulation of red and green lettuce microgreens, thereby providing useful information for indoor growers and food enthusiasts as well as to improve growth, nutrition and advance sustainable food production.

MATERIALS AND METHODS

Experimental design

In December 2023, lettuce seeds were obtained from a local market and transported to the Crop Botany Department at Khulna Agricultural University (KAU) in Khulna, Bangladesh. Upon collection, the seeds underwent surface sterilization using a 2.5% NaOCl solution, followed by rinsing and overnight soaking in wet tissue. The growing medium was prepared using a blend of heavy soil (50%), vermicompost (30%), and compost (20%). Selected containers measuring 15×7.5 cm^2 were then filled with the growing medium, leaving approximately 1/2 to 1 inch of space at the top for planting. Subsequently, the containers were exposed to full sunlight for 3-4 days for surface sterilization. Finally, soaked seeds were evenly distributed on the surface, gently pressed into the soil, and lightly moistened with water to settle. The containers were kept in dark for 1 day to germinate evenly then subjected to different LED light environment-white, blue, red+blue, red. The light environment was created by using T-5 bulb (12W), made in China. The measured illuminance of the LED bulbs ranged from 1000 lux to 1200 lux. Utilizing conversion factors based on the spectral output of the LED bulbs, we calculated the approximate Photosynthetic Photon Flux Density (PPFD) for two different wavelengths. For 660 nm (red light), the PPFD was approximated to be around 1360 μmol/m^2/s to 1630 μmol/m^2/s. For 470 nm (blue light), the PPFD was calculated to be approximately 3790 μmol/m^2/s to 4530 μmol/m^2/s. These calculations were based on conversion factors derived from the relationship between lux and PPFD for each specific wavelength (Ashdown, 2015). Calculations were based on a standard 1 square meter area for simplicity. A fan was placed at the top of the LED tray to keep air flowing continuously. Each treatment was repeated four times in a random design. Seedlings were grown for ten days under consistent 10-hour light conditions, and then assessed for their morphological assessment and pigment content after harvest.

Morphological parameters

A meter scale was used to measure the seedling from the base of the stem to the tip of the leaf in order to determine its height. Using the scale, other morphological characteristics such as petiole length, stem and root length, and leaf area were also measured. After drying the sample in the sun for ten days to get a consistent weight, 100 seedlings from each treatment were weighed to estimate their dry weight. The following formulas were used to determine %DM:

$$\%\text{Moisture content} = \frac{\text{Fresh weight} - \text{Sun dry weight}}{\text{Fresh weight}} \times 100$$

% DM= 100 - % moisture contents

Pigment determination

Photosynthetic pigment levels were measured using a modified spectro-photometric approach based on Lichtenthaler (1987) method. Exactly 0.5 g of harvested microgreens were chopped and put into a small vial with 10 mL of 80% ethanol. In order to extract the pigments, the vials were placed in dark for 10 days. Using spectrophotometer with wavelengths of 480, 453, 495, 505, 645 and 663 nm (Shimadzu UV-1280, Kyoto, Japan). Finally, the amounts of chlorophyll a, b, carotenoids, lycopene, beta-carotene, and lutein were determined using the following equations:

Total Chlorophyll = Chlorophyll *a* + Chlorophyll *b*

Chlorophyll a = $(\lambda_{663}\times0.999 - \lambda_{645}\times0.0989) \times V/W$

Chlorophyll b = $(\lambda_{645}\times1.77 - \lambda_{663}\times0.328) \times V/W$

Total Carotenoids = $\{(\lambda_{663}\times0.114 - \lambda_{645}\times0.638) + \lambda_{480}\} \times V/W$

Lycopene = $(\lambda_{663} \times 0.0458 + \lambda_{645} \times 0.204 + \lambda_{505} \times 0.372 - \lambda_{453} \times 0.0806) \times V/W$

Beta-carotene = $(\lambda_{663} \times 0.216 - \lambda_{645} \times 1.22 - \lambda_{505} \times 0.304 + \lambda_{453} \times 0.452) \times V/W$

Lutein = $(\lambda_{480}\times11.51 - \lambda_{495}\times20.61) \times V/W$

Here, λ_{663} = Absorbance at 663nm wavelength; λ_{645} = Absorbance at 645nm wavelength; λ_{505} = Absorbance at 505nm wavelength; λ_{495} = Absorbance at 495nm wavelength; λ_{480} = Absorbance at 480nm wavelength; λ_{453} = Absorbance at 453nm wavelength.

Statistical analysis

The mean values of the studied parameters for the two lettuce varieties under different light conditions were subjected to two-way ANOVA analysis with Minitab 17.0 to determine the presence of significant differences between the groups. After obtaining significant F-ratios, the means were subjected to Tukey's test to determine whether there were any significant differences between the means.

RESULTS AND DISSCUSSION

Impact of LED light exposure on growth parameters

Appropriate light levels are crucial for optimal growth and development of plants, directly impacting the biosynthesis of secondary metabolites, and thus ensuring healthy plant growth (Seyedi *et al*., 2024). Table 1 presents numerical data on various growth parameters of red lettuce and green lettuce under different LED light conditions: White, Red, Blue, and Red + Blue from our study. Each cell in the table contains mean values, accompanied by standard errors, for parameters such as stem length (ranging from 0.98 to 3.98 cm), petiole length (ranging from 0.43 to 1.15 cm), leaf area (ranging from 0.33 to 0.83 cm^2), plant height (ranging from 2.45 to 5.55 cm), root length (ranging from 1.11 to 2.70 cm), fresh weight (ranging from 2.30 to 5.17 g/100plants), and dry matter percentage (ranging from 3.65% to 96.71%). Additionally, significant differences among values are denoted by letters (a, b, c, d) within the same column, indicating statistical significance. green lettuce consistently demonstrates superior performance compared to red lettuce across the studied morphological parameters (Table 1). Specifically, green lettuce exhibits significantly higher stem length (2.74±0.22 cm), plant height (4.54±0.21 cm) and fresh weight (3.79±0.32 cm) under various light conditions. Alrajhi *et al*. (2023) also found that in terms of growth and development, green lettuce had a significantly higher photosynthetic rate than red lettuce. Among the light conditions tested, white light emerges as the most favorable for overall growth, as it promotes taller plants with higher fresh weight (4.45±0.43 g/100plants), dry matter (4.84±0.38 %) as well as leaf area (0.76±0.06 cm^2) for both lettuce species (Table 1, Figure 1). On the contrary, the use of red light appears to result in diminished growth, evidenced by reduced leaf area (54%), despite an increase in plant height (23.36%). This suggests that red light may promote elongation of the plants, overall growth is compromised, especially evident in red lettuce, where several parameters exhibit minimal values (Figure 1 and Table 1). Sinaga *et al*. (2023) find boosted plant height (2.80 ± 0.51 cm) applying white light on green and led lettuce than blue and red light. The enhanced plant height and overall growth under white light compared to blue and red light may be due to its broader spectrum, offering a wider range of wavelengths for photosynthesis and growth. Sinaga *et al*. (2023) found that red light diminishes overall plant performance except the plant elongation which confirms our work. This could be due to red light predominantly activates the phytochrome system, stimulating elongation through the promotion of cell elongation processes (Darko *et al*., 2014; Dou *et al*., 2017; Trivellini *et al*., 2023). Again, blue light and the combination of blue and red light exhibit superior morphological parameters to red light. When applying a combination of red and blue light, showing similar morphological characteristics as white light except per cent dry matter. However, in our study, the order of LED light colors on plant morphological parameters was as follows: white > red + blue > blue > red (Table 1 and Figure 1).

Table 1. The morphological characteristics of harvested (ten days after planting) red and green lettuce microgreens under various LED colors.

LED Light	Plant species (Microgreens)	Stem length (cm)	Petiole length (cm)	Leaf area (cm)	Plant height (Stem base to leaf tip) (cm)	Root length (cm)	Fresh weight (g/100plants)	Dry matter (%)
White	Red Lettuce	1.78±0.27^{bc}	0.88±0.05^{a-c}	0.83±0.10^{a}	3.75±0.25^{bc}	2.65±0.24^{a}	3.80±0.41^{ab}	5.11±0.55^{a}
	Green Lettuce	2.25±0.1^{b}	1.10±0.09^{a}	0.69±0.05^{ab}	4.28±0.26^{ab}	2.70±0.23^{a}	5.10±0.63^{a}	4.57±0.58^{ab}
Red+Blue	Red Lettuce	2.35±0.13^{b}	1.15±0.06^{a}	0.64±0.05^{a-c}	4.60±0.23^{ab}	2.70±0.21^{a}	3.70±0.23^{a-c}	4.54±0.50^{ab}
	Green Lettuce	2.58±0.18^{b}	0.80±0.07^{a-c}	0.60±0.02^{a-d}	4.53±0.19^{ab}	2.33±0.35^{ab}	4.58±0.17^{a}	3.88±0.10^{ab}
Blue	Red Lettuce	0.98±0.15^{c}	0.43±0.02^{d}	0.48±0.06^{b-d}	2.45±0.13^{c}	1.10±0.18^{b}	3.03±0.15^{bc}	3.65±0.19^{ab}
	Green Lettuce	2.18±0.35^{b}	0.58±0.03^{cd}	0.71±0.06^{ab}	3.80±0.28^{b}	1.95±0.50^{ab}	3.15±0.06^{bc}	96.71±0.04^{b}
Red	Red Lettuce	2.40±0.24^{b}	1.03±0.09^{ab}	0.38±0.05^{cd}	4.35±0.36^{ab}	1.11±0.12^{b}	2.3±0.06^{c}	4.15±0.19^{ab}
	Green Lettuce	3.98±0.24^{a}	0.73±0.13^{b-d}	0.33±0.05^{d}	5.55±0.43^{a}	2.08±0.21^{ab}	2.7±0.02^{bc}	5.17±0.17^{a}
White		2.01±0.16^{bc}	0.99±0.06^{a}	0.76±0.06^{a}	4.01±0.19^{b}	2.68±0.15^{a}	4.45±0.43^{a}	4.84±0.38^{a}
Red + Blue		2.46±0.11^{b}	0.98±0.08^{a}	0.62±0.028^{ab}	4.56±0.14^{ab}	2.51±0.20^{a}	4.14±0.21^{a}	4.21±0.27^{b}
Blue		1.58±0.29^{c}	0.50±0.03^{b}	0.59±0.06^{b}	3.13±0.29^{c}	1.53±0.29^{b}	2.49±0.18^{b}	3.47±0.11^{ab}
Red		3.19±0.33^{a}	0.88±0.09^{a}	0.35±0.04^{c}	4.95±0.35^{a}	1.59±0.21^{b}	3.06±0.12^{b}	4.66±0.23^{a}
Green Lettuce		2.74±0.22^{a}	0.80±0.06^{a}	0.581±0.054^{a}	4.54±0.21^{a}	2.26±0.17^{a}	3.79±0.32^{a}	4.23±0.23^{a}
Red Lettuce		1.88±0.18^{b}	0.87±0.08^{a}	0.58±0.045^{a}	3.79±0.24^{b}	1.89±0.22^{a}	3.28±0.19^{b}	4.36±0.22^{a}

Letters a, b, c, and d demonstrated that each letter was significantly different between various LEDs using the Tukey test.

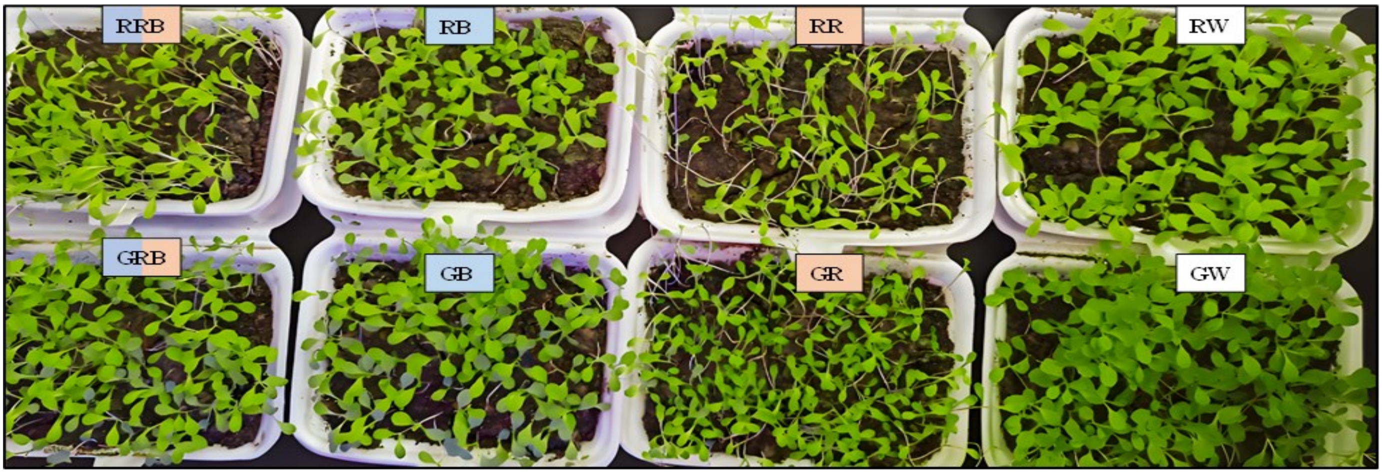

Figure 1. *Ten days old red lettuce and green lettuce microgreens under different LED light colors. Where, RRB = red lettuce under red and blue light environment, GRB = green lettuce under red and blue light environment, RB = red lettuce under blue light environment, GB = green lettuce under blue light environment, RR = red lettuce under red light environment, GR = green lettuce under red light environment, RW = red lettuce under white light environment, GW = green lettuce under white light environment.*

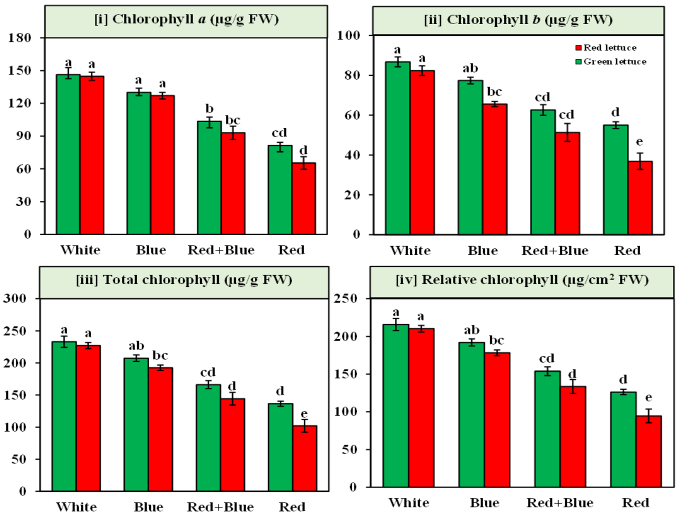

Figure 2. *Bar graph showing comparative LED light effect on chlorophyll related parameters of green and red lettuce microgreens. The studied parameters are- [i] chlorophyll a (µg/g FW), [ii] chlorophyll b (µg/g FW), [iii] total chlorophyll (µg/gm FW), [iv] relative chlorophyll (µg/cm^2 FW). The data is presented as means of 4 replicates ± SEM, with a sample size of n = 4. Two-way ANOVA with Tukey post hoc test was used for statistical analysis. Significant differences (P<0.05) were indicated by different lowercase letters.*

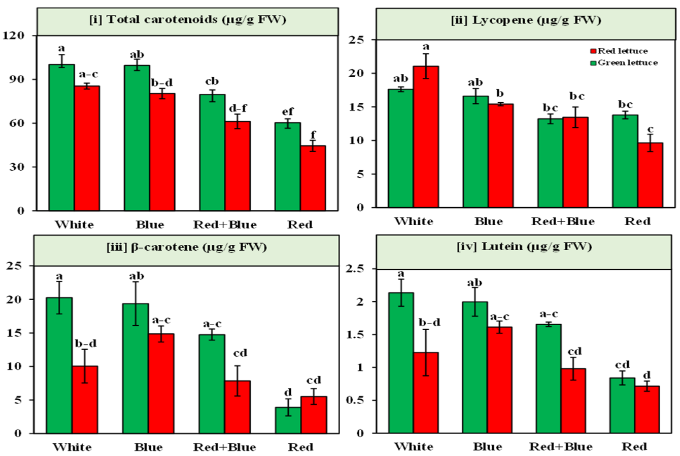

Figure 3. *Bar graph showing comparative LED light effect on carotenoids related parameters of green and red lettuce microgreens. The studied parameters are- [i] total carotenoids (µg/g FW), [ii] lycopene (µg/g FW), [iii] β-carotene (µg/gm FW), [iv] lutein (µg/m^2 FW). The data is presented as means of 4 replicates ± SEM, with a sample size of n = 4. Two-way ANOVA with Tukey post hoc test was used for statistical analysis. Significant differences (P<0.05) were indicated by different lowercase letters.*

Impact of LED light exposure on chlorophyll content

Chlorophyll, a photosynthetic pigment, promotes increased metabolite production in plants, thereby increasing photosynthesis activity (Saleem & Muhammaf, 2019). Previous investigations have revealed varying chlorophyll concentrations produced by different LEDs (Sinaga *et al.*, 2023). The LEDs utilized in this study resulted in variable chlorophyll concentrations for both lettuce species. Considering both light and species, both red and green lettuce exhibited higher chlorophyll *a* content under white light and lower under red light. However, there were no significant differences in chlorophyll *a* production between white and blue light. Chlorophyll *a* content in green lettuce ranged from 81.42±4.26 µg/g FW under red LED to 146.37±6.27 µg/g FW under white LED, while for red lettuce, concentrations ranged from 65.37±4.17 µg/g FW under red LED to 144.79±6.02 µg/g FW under white LED (Figure 2i). The combination of red and blue light yielded the second-highest chlorophyll *a* content for both species (Figure 2i). Significant differences were observed among LED treatments for both green and red lettuce ($p < 0.05$), with higher chlorophyll *b* levels generally observed under white LED compared to other treatments. The highest chlorophyll *b* content in green lettuce (86.74±2.44 µg/g FW) and red lettuce (82.36±3.06 µg/g FW) was obtained under white LED (Figure 2ii). Among the lettuce varieties, blue and red+blue LED treatment led to intermediate chlorophyll *b* levels, while red LED resulted in lower concentrations, with significant differences noted (Figure 2ii). Again, both total chlorophyll and relative chlorophyll showed similar results for different LED light exposures, with green lettuce under white light exhibiting the highest values, 233.11±8.69 µg/g FW for total chlorophyll and 215.84±8.05 µg/cm² for relative chlorophyll (Figure 2iii & 2iv). When only considering the effect of light irrespective of plant species, white LED had the most significant influence, yielding the highest chlorophyll content observed, followed by blue light, red+blue, and red light (Figure 4). Sinaga *et al.* (2023) found that different LED lights resulted in varying chlorophyll concentrations, with the white LED having the most significant impact, leading to the highest chlorophyll levels.

Baidya *et al.* (2021) tells that blue LED light exposure enhances

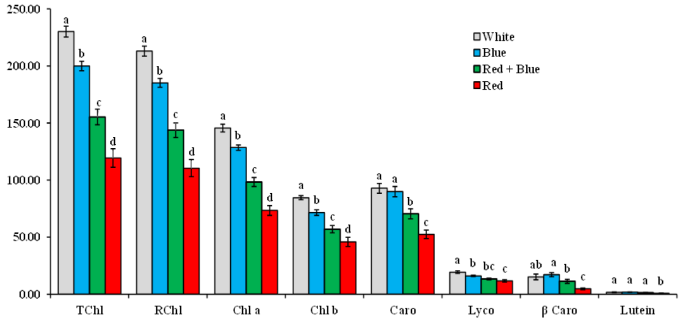

Figure 4. *Bar graph showing comparative LED light effect on pigments of green and red lettuce microgreens considering only light condition. The studied parameters are- TChl (total chlorophyll; µg/gm FW), RChl (relative chlorophyll; µg/cm² FW), Chl a (chlorophyll a; µg/g FW), Chl b (chlorophyll b; µg/g FW), Caro (total carotenoids; µg/g FW), Lyco (lycopene; µg/g FW), β Caro (β-carotene; µg/g FW), lutein (µg/m² FW). The data is presented as means of 4 replicates ± SEM, with a sample size of n = 4. Two-way ANOVA with Tukey post hoc test was used for statistical analysis. Significant differences (P<0.05) were indicated by different lowercase letters.*

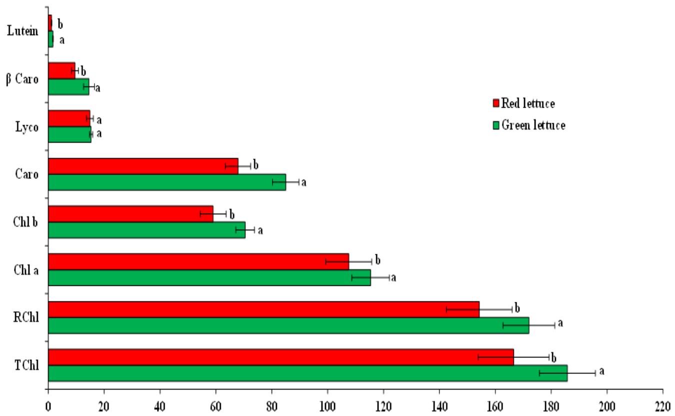

Figure 5. *Bar graph showing comparative study on several pigments of green and red lettuce microgreens considering only plant species. The studied parameters are- TChl (total chlorophyll; µg/gm FW), RChl (relative chlorophyll; µg/cm² FW), Chl a (chlorophyll a; µg/g FW), Chl b (chlorophyll b; µg/g FW), Caro (total carotenoids; µg/g FW), Lyco (lycopene; µg/g FW), β Caro (β-carotene; µg/g FW), lutein (µg/m² FW). The data is presented as means of 4 replicates ± SEM, with a sample size of n = 4. Two-way ANOVA with Tukey post hoc test was used for statistical analysis. Significant differences (P<0.05) were indicated by different lowercase letters.*

chlorophyll content and promotes nutritionally rich biomass production in *Chlorella ellipsoidea*. In our study we found limited growth in two lettuce varietirs when exposure to blue LED light which is quite different from the Baidya *et al.* (2021) study. This variation may be due to variations in experimental conditions such as light intensity, duration, or other environmental factors. When considering individual species, green lettuce surpassed red lettuce for all studied chlorophyll related parameters (Figure 5).

Impact of LED light exposure on carotenoids

Carotenoids, found in diverse organisms, exhibit colors from yellow to purple, encompassing carotenes (e.g., β-carotene, lycopene) as hydrocarbons, and xanthophylls (e.g., lutein) (Britton *et al*., 2004; Maoka, 2020). Carotenoids play a dual role in photosynthesis by transferring light energy to chlorophylls through singlet-singlet excitation transfer and by quenching reactive oxygen species such as singlet oxygen through triplet-triplet transfer (Maoka, 2020). Previous studies found statistically significant interactions between the effects of light quality and intensity on total carotenoid content (Orlando *et al*., 2022). In our study all the carotenoids related parameters also exhibited similar trend like chlorophyll. Green lettuce under white LED possessing highest total carotenoids (100.26±6.78 µg/g FW) (Figure 3i), β-carotene (20.25±2.43 µg/g FW) (Figure 3iii) and lutein (2.13±0.21 µg/g FW) (Figure 3iv). On the other hand, red lettuce under white LED showing highest lycopene content (21.06±1.85 µg/g FW) than green lettuce (Figure 3ii). Previous study reported that blue-red light environment enhanced total carotenoids content than sole application of blue, red and white light (Seyedi *et al*., 2024). Another study also found the similar result that microgreens exhibited a preference for blue-red light environments, favoring the accumulation of carotenoids compared to environments with red or blue LEDs as the sole light source (Kyriacou *et al*., 2019). The difference of the two study from our result may vary due to research methodology, light intensity, the time exposure as well as the cultivars studied. Lee *et al*. (2023) found that microgreens exposed to white LEDs exhibited elevated levels of various carotenoids, such as lutein, 13-cis-β-carotene, α-carotene, β-carotene, and 9-cis-β-carotene, compared to those exposed to red or blue LEDs. In our study, regardless of plant species, the sequence of LED light color on plant carotenoid-related parameters was as follows: white > red + blue > blue > red (Figure 4). Again, green lettuce surpasses the other lettuce variety across all parameters studied (Figure 5).

Conclusion

The study found that, optimal LED light exposure significantly influenced the growth parameters, chlorophyll content, and carotenoid levels in lettuce microgreens. White LED light emerged as the most favorable for promoting robust growth and enhancing pigment accumulation, particularly chlorophyll and carotenoids. Conversely, red light inhibited growth, underscoring the importance of light quality in microgreen cultivation. Overall, green lettuce microgreens exhibited superior performance compared to red lettuce microgreens across various parameters, highlighting its potential for nutrient-rich microgreen production.

DECLARATIONS

Author contribution statement

Conceptualization: M.J.S. and S.I.; Methodology: M.J.S.; Formal analysis and investigation: M.J.S., S.S.T., R.H.M.R. and S.I.; Resources: M.J.S.; Data curation: M.J.S., and S.S.T.; Writing-original draft preparation: M.J.S., S.S.T., R.H.M.R., and S.I.; Writing—review and editing: S.I.; Visualization: S.I.; Supervision: S.I.; All authors have read and agreed to the published version of the manuscript.

Conflicts of interest: The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Ethics approval: This study did not involve any animal or human participant and thus ethical approval was not applicable.

Consent for publication: All co-authors gave their consent to publish.

Data availability: The data that support the findings of this study are available on request from the corresponding author.

Supplementary data: Not available.

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