

## Article

# Effects of Light Intensity and Spectrum Mix on Biomass, Growth and Resource Use Efficiency in Microgreen Species

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**Abstract:** Light spectrum and intensity is one of the key factors in the production of microgreens in controlled-environment agriculture and is directly related to plant growth and biomass accumulation. Hence, the objective of this research study was to investigate the biomass, growth, and resource use efficiencies (RUEs) in 14 different species of microgreen grown in two light recipes with 209.5 (OSRAM LED) and 45  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (INSTAGREEN LED) with a 16/8 h light/dark photoperiod in a growth chamber. Under both LEDs, fresh biomass accumulation and the SPAD content were highest in sunflower. Nasturtium recorded the maximum hypocotyl length under both LEDs. The leaf area index (LAI) was significantly higher in mungbean under the INSTAGREEN LED compared to other microgreens, while the maximum LAI was measured in lentils under the OSRAM LED. This shows that the two different LEDs had species-specific effects. The RUE of the cheaper INSTAGREEN LED was more efficient in terms of light and energy use efficiency, while OSRAM LED was more efficient in terms of water and surface use efficiencies. Overall, the results showed that different species of microgreens exhibit different responses to fresh biomass accumulation and SPAD contents in the leaves, demonstrating the diversity of their growth responses. Across both LEDs (OSRAM LED and INSTAGREEN LED), the top performing microgreen in terms of biomass accumulation as well as SPAD contents in the leaves was sunflower. Consequently, a high chlorophyll content in sunflower led to a higher biomass production by enhancing photosynthesis.

**Keywords:** microgreens; light recipe; resource use efficiencies; photoperiod; LED



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## 1. Introduction

Microgreens are young, tender, and flavorful leafy greens of different species like vegetables, herbs, and legumes. Microgreens is available in a wide range of colors, textures, and flavors [1]. Depending on the variety grown, these plants are harvested between 7 and 21 days and are consumed fresh [2]. As compared to sprouts, their leaves are greener and larger in size [3]. In addition to adding texture and color to main dishes, they have a number of quality attributes that enhance their sensory appeal. In comparison with mature greens or seeds, microgreens contain a greater amount of antioxidants, phenolics, vitamins, and minerals, which makes them functional foods that not only provide nutritional value but also promote health and prevent disease [2,4]. The compounds found in microgreens are more concentrated than those found in mature plants or seeds, making them highly valued for their health benefits. Since consumers have recently become interested in functional foods, the demand for these products has grown rapidly [5]. A variety of crops are used to grow microgreens, including radish, cabbage, kale, lettuce, mustard, amaranth, etc., and there are almost 100 different species of microgreens that can be grown in controlled-environment agriculture (CEA) [6]. Among microgreens, *Amaranthaceae*, *Apiaceae*, *Asteraceae*, and *Brassicaceae* are the most commonly used species.

Environmental factors that affect plant growth are light (photoperiod, light intensity, and spectrum mix), relative humidity, temperature, electrical conductivity (EC), pH, nutrients, and moisture [7]. Out of these factors, light is a key factor which regulates the growth and quality of microgreens [8]. A phenomenon related to light called solar dimming affects solar light intensity and quality. Possible reasons for solar dimming include air pollution (aerosols) and clouds [9]. In conventional agriculture, these shifts can change growth patterns, reduce productivity, and disrupt crop quality [10]. CEA allows growers to maintain the light intensity, spectrum mix, and photoperiods regardless of external environmental conditions, reducing reliance on natural light and ensuring consistent growth. In addition to providing sufficient energy for photosynthesis, light acts as a signal to regulate photomorphogenesis [11] through the production of morphogenic pigments (carotenoids, chlorophyll a and b) and response by photoreceptors (phytochromes, cryptochromes, and phototropins), causing the light to transform the plant by triggering developmental and physiological changes [12,13]. A major contributor to the photosynthesis process is the spectrum mix [14]. Based on some previous studies, regarding plant growth and development, vegetative growth is primarily influenced by red and blue light [15,16]. In particular, phytochromes are highly sensitive to red (600–700 nm) as well as far-red light (700–800 nm), which possess distinct photosensory properties in determining leaf expansion, flowering time, and the elongation of the plant [17,18]. Blue light (400–500 nm) and UV light (315–400 nm) are primarily absorbed by phototropins and cryptochrome photoreceptors [19] to regulate hypocotyl elongation, blue light-induced stomatal opening, and photoperiodic flowering [20,21]. Apart from playing a significant role in photosynthetic carbon assimilation, the green light spectrum (500–600 nm) promotes the accumulation of biomass in the lower layers of leaves while also enhancing the efficiency of water use [22].

In order to meet the growing demand for fresh and nutritious microgreens year-round, optimizing microgreens' cultivation to increase yields, quality, and resource efficiency has become a major area of agricultural research. Resource use efficiency (RUE) is the proportion of inputs that are converted into biomass (productivity) based on the inputs provided, which include water, light, energy, surface and nutrient use efficiencies. WUE (water use efficiency) is defined as the amount of fresh biomass (g) produced per liter of water consumed. Graamans et al. [23] reported that CEA improved the WUE by 28–95% more than greenhouses, while Orsini et al. [24] reported that CEA improved the WUE by 12% more than greenhouses and 200% more than open fields for the same crop. LUE (light use efficiency) is the measure of plants to utilize light for growth, which can provide valuable insights into the performance of plants [25]. The energy consumption of CEA is substantially higher than that of conventional (traditional or field) production methods in greenhouses and open fields, primarily due to the use of electricity for lighting [26]. Therefore, improving the LUE is key to improving CEA's economic feasibility. Surface use efficiency refers to the amount of fresh biomass per 1 m<sup>2</sup> of area. In CEA, there is potential for reducing surface use by growing the plants in a vertical dimension [27] and the possibility of year-round production due to consistent environmental conditions [28]. Previous studies reported that CEA with LED lights have improved the efficiency of water, surface, and nutrients [29,30]. These improvements in resource use are due to the production of crops in multi-layered systems that increases the production per unit area, reduction in water, and nutrient loss due to reduced transpiration and recirculation of water and nutrient solutions [31,32]. While CEA farming has numerous advantages, it also has several challenges, such as the high cost of initial farm establishment and the high cost of energy associated with light, cooling, heating, and dehumidification [33]. While the intensity of light and duration plays a key role in microgreens' production, their importance in accelerating growth and development as well as facilitating photosynthesis process is well established. There is growing evidence that the spectral composition of light may have profound effects on plant morphology as well as nutritional properties. The use of light-emitting diodes (LEDs) in CEA offers unprecedented opportunities for tailoring

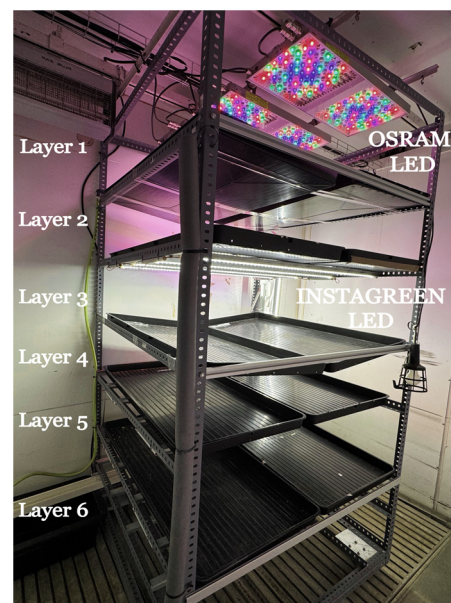
growth conditions and optimizing plant performance, as LEDs can provide light spectra tailored to plant-specific needs.

Therefore, the objective of this study is to investigate the biomass, growth, and resource use efficiency (RUE) of 14 microgreen species under two LEDs, i.e., the OSRAM LED and INSTAGREEN LED.

## 2. Materials and Methods

### 2.1. Description of Climate Chamber and Grow Rack for Trial

The experimental setup for the research trial comprised a climate chamber (Convion CMP5090: Winnipeg, MB, Canada). Instagreen.eu, a commercial microgreens producer company, provided a professional six-layered Instagreen growth cultivation system (Figure 1) from Barcelona, Spain, that was setup inside the climate chamber. This Instagreen grow system measures 217.5, 120, and 112 cm in height, width, and depth, respectively. Each growing layer consists of 2 growing trays, which makes a total of 12 trays for 6 growing layers. The dimensions of each tray were 110, 55, and 5 cm in length, width, and height, respectively; thus, the whole growing system has a total growing space of 7.26 m<sup>2</sup> with a capacity of 40 growing cups per each tray. The growing cup has a top dimension of 14.5 and 10 cm in length and width, respectively, while it has a narrower bottom dimension of 11.5 and 8.2 cm in length and width, respectively, and a cup height of 5 cm. Water for irrigation is pumped from a reservoir to the topmost layer using a water pump. The reservoir has a capacity of approximately 40 L of water. Each tray is positioned with a slight tilt in the cultivation system, allowing water to flow from the top layer to the next through perforations in the bottom-most section of each tray. The water then cascades over a slide into the top of the next layer before returning to the reservoir. The cultivation system was set to be watered twice a day for 15 min with an 12 h interval for the entire growth period.



**Figure 1.** Instagreen 6-layered cultivation system.

An RCBD experiment was implemented in the climate control chamber located at the University of Copenhagen's Taastrup Campus, Taastrup, Denmark, with fourteen microgreen species with three replicates (Table 1) separately in two layers of the cultivation system. Growing cups from three replicates were placed in separate blocks under both LEDs. Before sowing, some microgreen seeds such as peas, lentils, sunflower, borage, nasturtium, coriander, leek, and mungbean were soaked for 18–24 h. At the time of sowing, the substrate used in each layer was kept moist with water. Afterwards, seeds of 14 microgreen species (Table 1) were sown in growing cups on 30 January 2023, and some

weight was applied on top of each grow cup for 2–3 days to keep the growing environment humid for effective germination. Subsequently, growing cups were placed in the dark in the bottom grow trays, which were used for germinating the seeds before transferring them to the light treatment in the OSRAM (Osram, Beverley, MA, USA) and INSTAGREEN (Instagreen, Barcelona, Spain) LED layers. After germination, microgreens were transferred to the OSRAM LED (layer 1) and INSTAGREEN LED (layer 2), respectively, on 3 February 2023, except for coriander, nasturtium, and leek, which are slow-growing microgreens and take more time to germinate compared to other microgreens, so they were transferred to light on 6 February 2023. Then, after 3–4 days of exposure to light and a clear visible pair of cotyledons, the microgreens were harvested.

**Table 1.** Seeding density and growth habit of 14 microgreen species grown under OSRAM and INSTAGREEN LED.

| Family           | Crop           | Latin Name                                         | Seed Weight<br>(g m <sup>-2</sup> ) | Growth Habit                                                           |
|------------------|----------------|----------------------------------------------------|-------------------------------------|------------------------------------------------------------------------|
| (1) Brassicaceae | (a) Red Radish | <i>Raphanus raphanistrum</i> subsp. <i>Sativus</i> | 625                                 | Annual, fast-growing and cool-season crop.                             |
|                  | (b) Kale       | <i>Brassica oleracea</i> gr. <i>Acephala</i>       | 521                                 | Biennial, fast-growing crop.                                           |
|                  | (c) Kohlrabi   | <i>Brassica oleracea</i> gr. <i>Gongylodes</i>     | 521                                 | Biennial, fast-growing crop.                                           |
|                  | (d) Broccoli   | <i>Brassica oleracea</i> var. <i>Italica</i>       | 417                                 | Annual, fast-growing crop.                                             |
|                  | (e) Cress      | <i>Lepidium sativum</i>                            | 417                                 | Annual, fast-growing crop.                                             |
| (2) Leguminosae  | (a) Peas       | <i>Pisum sativum</i>                               | 2604                                | Hard, leafy, annual crop that grows in temperate and tropical regions. |
|                  | (b) Lentils    | <i>Lens culinaris</i>                              | 1875                                | Annual crop with fast vegetative and reproductive growth.              |
|                  | (c) Mungbean   | <i>Vigna radiata</i>                               | 1042                                | Herbaceous fast-growing annual crop.                                   |
| (3) Others       | (a) Amaranth   | <i>Amaranthus</i> spp.                             | 156                                 | Annual crop with an upright growing habit, producing spines or plumes. |
|                  | (b) Sunflower  | <i>Helianthus annuus</i>                           | 1875                                | Upright, tall, branched annual herb.                                   |
|                  | (c) Borage     | <i>Borago officinalis</i>                          | 625                                 | Annual fast-growing crop.                                              |
|                  | (d) Nasturtium | <i>Tropaeolum</i> spp.                             | 1562                                | Warm season, slow-growing annual crop.                                 |
|                  | (e) Coriander  | <i>Coriandrum sativum</i>                          | 937                                 | Glabrous, erect, and annual herb.                                      |
|                  | (f) Leek       | <i>Allium ampeloprasum</i>                         | 937                                 | Slow-growing perennial with strap-like leaves.                         |

## 2.2. Description of OSRAM and INSTAGREEN LED

The top layer of the cultivation system was equipped with LEDs called OSRAM provided by a company called OSRAM, Beverley, MA, USA. The distance between the OSRAM LED and the growing tray was 41 cm. There were three fixtures of the OSRAM LED, and each fixture had a maximum capacity of 150 watts power. This is a tunable LED system with six different channels of wavelength peaks with maximum emittance for UV, blue, green, red, far-red, and white light (385–730 nm) of 50, 250, 100, 250, 100, and 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively. In contrast, the second and third layers were fitted with four high-efficiency one-meter-long twenty-four-volt LED (24VHELED, Fullwat, Barcelona, Spain) lights evenly spaced, situated 32 cm from the tray surface, that had a color temperature of 4000 K and a maximum power draw of 12 watts. The ‘Paladin pro4 Bluetooth’ timer program was connected with the mobile app Save’n carry 2.0, which was used to schedule the INSTAGREEN LED and the irrigation system time, while the OSRAM LED was regulated with OSRAM Phytify software (Version 1.0). Both LED lights were programmed for a 16/8 h light/dark photoperiod over the entire growth cycle. The power consumption (Watts) of both light

recipes was measured with the help of an instrument, “Zimmer LMG610” (Frankfurt, Germany). The intensity and spectrum mix of both the OSRAM and INSTAGREEN LEDs can be found in Table 2, and the OSRAM LED can be individually adjusted for six different wavelengths to create a customized light recipe. For cost analysis, we did not use the upfront costs of the LEDs, instead, we calculated the operating costs of both LEDs (OSRAM and INSTAGREEN), where we used the energy use values of the OSRAM (0.1884 kWh) and INSTAGREEN LEDs (0.048 kWh) and multiplied them by cost (USD) per unit in the Danish market at the time of the experiment in January/February 2023.

**Table 2.** Description of OSRAM and INSTAGREEN LED power consumption (Watts) and energy use (kWh).

|                   | Unit                                 | Wavelength (nm) | OSRAM LED | INSTAGREEN LED |
|-------------------|--------------------------------------|-----------------|-----------|----------------|
| Blue              | $\mu\text{mol m}^{-2} \text{s}^{-1}$ | 400–500         | 25        | 7.91           |
| Green             | $\mu\text{mol m}^{-2} \text{s}^{-1}$ | 500–600         | 19        | 21.41          |
| Red               | $\mu\text{mol m}^{-2} \text{s}^{-1}$ | 600–700         | 157.5     | 15.68          |
| Far-red           | $\mu\text{mol m}^{-2} \text{s}^{-1}$ | 700–800         | 8         | 1.1            |
| PPFD              | $\mu\text{mol m}^{-2} \text{s}^{-1}$ | 400–700         | 209.5     | 45             |
| DLI               | $\text{mol m}^{-2}$                  | -               | 12.07     | 2.65           |
| Blue              | %                                    | 400–500         | 10        | 17             |
| Green             | %                                    | 500–600         | 19        | 46             |
| Red               | %                                    | 600–700         | 63        | 34             |
| Far-red           | %                                    | 700–800         | 8         | 3              |
| Blue/Red          | -                                    | -               | 0.16      | 0.5            |
| Blue/Green        | -                                    | -               | 0.53      | 0.37           |
| Green/Red         | -                                    | -               | 0.3       | 1.37           |
| Red/Far-red       | -                                    | -               | 7.87      | 14.25          |
| Far-red/PAR       | -                                    | -               | 0.04      | 0.02           |
| Power consumption | Watts                                | -               | 188.4     | 48             |
| Energy use        | kWh                                  | -               | 0.1884    | 0.048          |

### 2.3. Environmental Data Collection in Climate Chamber

For the effective maintenance of the environmental conditions inside the growth chamber, the air temperature ( $^{\circ}\text{C}$ ) and relative humidity (RH, %) of the top layers of the cultivation system were measured with the help of Tiny-Tag View-2 Loggers (Gemini Data Loggers, Chichester, UK). One logger was positioned at the top layer (OSRAM LED) while the second logger was placed in the INSTAGREEN layer, which enabled us to monitor the slight changes in the microclimate of the climate chamber. Moreover, the  $\text{CO}_2$  concentration levels were measured with a  $\text{CO}_2$  logger (HOBO MX, Onset, Bourne, MA, USA) inside the climate chamber. The electrical conductivity (EC) and pH of applied water were measured with the help of a pH and EC meter (Senmatic DGT-Voltmatic, Sønderød, Denmark). Table 3 shows the RH, air temperature,  $\text{CO}_2$  level, and water pH and EC in the growth chamber. Throughout the entire growth cycle, environmental parameters such as temperature,  $\text{CO}_2$  levels, and water pH and EC in the growth chamber were measured with the abovementioned instruments. During the entire growth cycle, the environmental parameters of the climate chamber and water pH and EC were within the range, such as  $\text{CO}_2$  levels at 473 ppm on average, temperature at  $21^{\circ}\text{C}$ , RH at 60%, water pH levels at 7–8, and water electrical conductivity (EC) at  $0.76\text{--}0.88 \text{ mS cm}^{-1}$ .

**Table 3.** Mean  $\pm$  SE for  $\text{CO}_2$  levels (ppm), air temperature ( $^{\circ}\text{C}$ ), relative humidity (%), and water pH and water EC ( $\text{mS cm}^{-1}$ ) for the experimental period.

| $\text{CO}_2$ Level (ppm) | Air Temperature ( $^{\circ}\text{C}$ ) | Relative Humidity (%) | Water pH        | Water EC ( $\text{mS cm}^{-1}$ ) |
|---------------------------|----------------------------------------|-----------------------|-----------------|----------------------------------|
| $473.19 \pm 16.86$        | $21.1 \pm 0.04$                        | $60.7 \pm 1.90$       | $7.56 \pm 0.10$ | $0.78 \pm 0.01$                  |

#### 2.4. Microgreens' Biomass, Growth, and Resource Use Efficiency Measurements

At harvest, different parameters like the hypocotyl length, chlorophyll content, leaf area index (LAI), and fresh biomass of the crop were measured. The fresh biomass of each microgreen species per growing cup was measured with the help of a gravimetric scale. For the measurement of the hypocotyl length, SPAD content, and LAI, eight representative plants were taken from each growing cup. The hypocotyl length was measured using a ruler for representative plants, the SPAD content was measured with the help of a SPAD meter (SPAD-502; Konica Minolta Sensing Inc., Osaka, Japan) from the representative plants, and leaf area was measured using a mobile application called easy leaf area, as elaborated by Easlon and Bloom [34]. For LAI, representative leaves of 8 plants were taken, 2 cm<sup>2</sup> of red card paper was placed on the table, and the leaves were spread around the red card piece. Afterwards, an image was taken with the help of the easy leaf area app that gave the leaf area of those 8 representative plants. Then, the LAI was calculated. We also measured the resource use efficiency (RUE) of both LEDs according to the methods followed by Cowden et al. [35]. Resource use efficiencies were calculated as light use efficiency (LUEs), energy use efficiency (EUEs), total energy use efficiency (total EUEs), water use efficiency (WUEs) and surface use efficiency (SUEs): Below are the formulas used to calculate the resource use efficiencies of the grow light and the growing system.

$$\text{LUE} = \frac{\text{fresh biomass (g)}}{\text{mol PPFD}} \quad (1)$$

$$\text{EUE} = \frac{\text{fresh biomass (g)}}{\text{kWh of light}} \quad (2)$$

$$\text{Total EUE} = \frac{\text{fresh biomass (g)}}{\text{Total kWh of light}} \quad (3)$$

$$\text{WUE} = \frac{\text{fresh biomass (g)}}{\text{Water used (l)}} \quad (4)$$

$$\text{SUE} = \frac{\text{fresh biomass (gm}^{-2}\text{)}}{\text{No. of days}} \quad (5)$$

#### 2.5. Statistical Analysis

All the datasets collected during the experimental period were analyzed using R-studio software (R version 4.0.5). A one-way RCBD ANOVA was carried out on 14 microgreen species and 3 family groups in order to provide an overview of the effects of the OSRAM and INSTAGREEN LEDs on the fresh biomass, SPAD content, hypocotyl length, and LAI in 14 microgreen species and when categorized into 3 families. Fisher's LSD test was then used to compare all the means of the fresh biomass, SPAD content, hypocotyl length, and LAI at a significance level of 5% to see if any differences existed in terms of species and family. Standard errors for all the four traits (species and family level) and resource use efficiencies were calculated in an Excel spreadsheet.

### 3. Results

Table 4 shows the ANOVA table on the effects of the OSRAM and INSTAGREEN LEDs on all four traits, fresh biomass, SPAD, hypocotyl length, and the LAI, in 14 microgreen species grouped into three family categories. LEDs had highly significant ( $p < 0.001$ ) effects on all four traits, i.e., fresh biomass, SPAD, hypocotyl length, and LAI, in 14 microgreen species, whereas the LED effects differed when categorized as family groups. At the family-level comparisons, the INSTAGREEN LED had significant affects only on the LAI and SPAD, whereas the OSRAM LED had significant effects on the LAI and fresh biomass. Hence, the species-level comparison showed similar significant effects on all four growth parameters under both LEDs, whereas the comparison at the family level gave differing results under the OSRAM and INSTAGREEN LEDs.

**Table 4.** ANOVA table for 14 microgreen species and 3 family categories under OSRAM LED and INSTAGREEN LED.

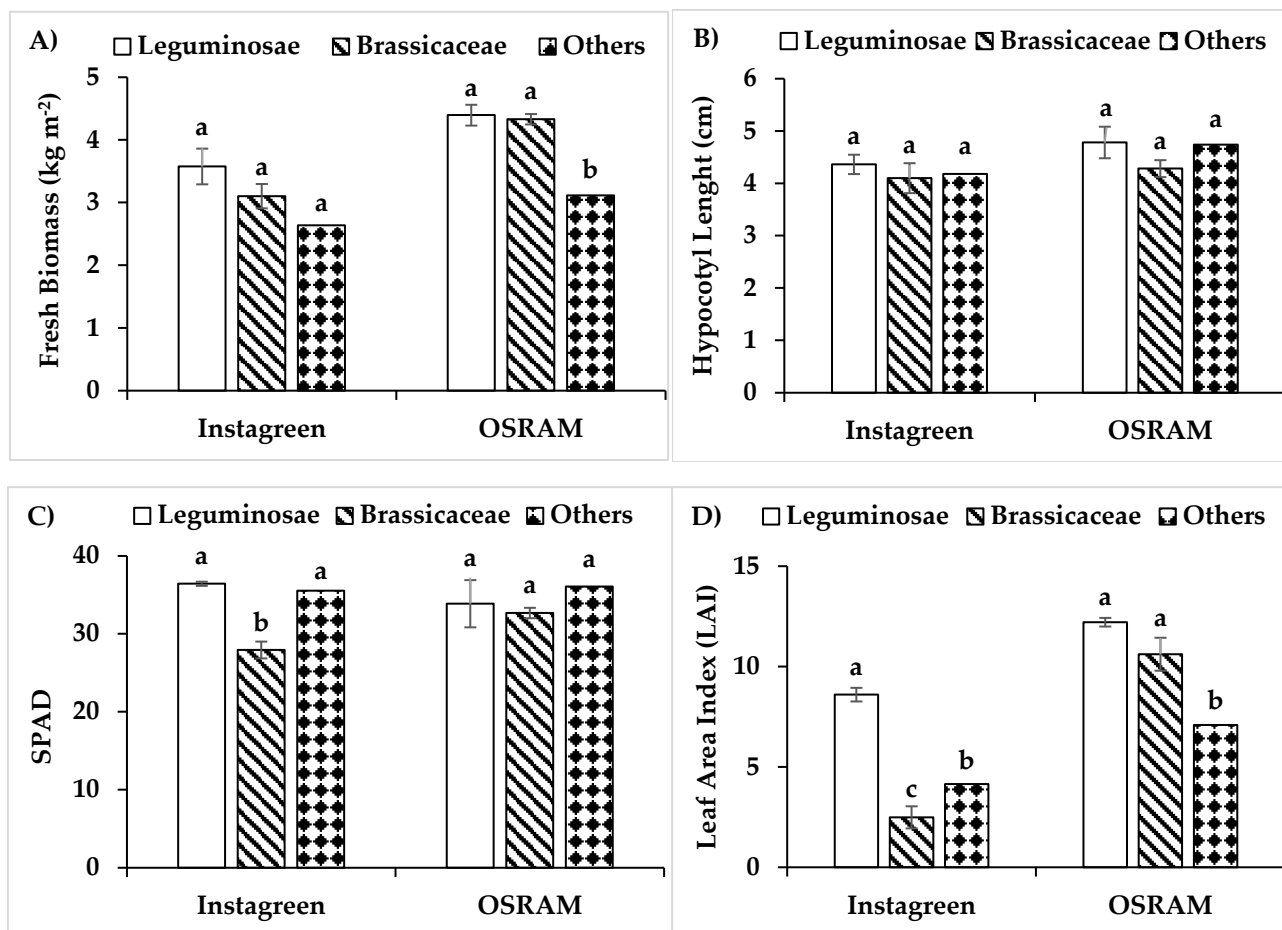
| Traits           | Source of Variation | Df | OSRAM LED       | INSTAGREEN LED  |
|------------------|---------------------|----|-----------------|-----------------|
|                  |                     |    | <i>p</i> -Value | <i>p</i> -Value |
| Fresh Biomass    | Species             | 13 | <0.001 ***      | <0.001 ***      |
|                  | Family              | 2  | 0.0359 *        | 0.151 ns        |
| SPAD             | Species             | 13 | <0.001 ***      | <0.001 ***      |
|                  | Family              | 2  | 0.133 ns        | 0.0432 *        |
| Hypocotyl Length | Species             | 13 | <0.001 ***      | <0.001 ***      |
|                  | Family              | 2  | 0.329 ns        | 0.751 ns        |
| LAI              | Species             | 13 | <0.001 ***      | <0.001 ***      |
|                  | Family              | 2  | <0.001 ***      | <0.001 ***      |

ns = not significant, \* =  $p < 0.05$ , \*\*\* =  $p < 0.001$ , Df = degrees of freedom.

### 3.1. Light Spectrum Mix and Intensity Effects on Biomass and Growth of Microgreens

Figure 2 shows the fresh biomass, hypocotyl length, SPAD content, and LAI of three family groups under the OSRAM and INSTAGREEN LEDs. Under the OSRAM LED, among the three family groups, the highest fresh biomass ( $4.39 \text{ kg m}^{-2}$ ) and LAI (12.21) were recorded in the leguminosae family, followed by brassicaceae ( $4.32 \text{ kg m}^{-2}$  and 10.61, respectively), and the least fresh biomass ( $3.11 \text{ kg m}^{-2}$ ) and LAI (7.08) were found in the others family group. The maximum amount of SPAD content was measured to be in the others family group (36.05), followed by leguminosae (33.86), while the smallest amount of SPAD content was found in the brassicaceae family (32.67). The hypocotyl length was recorded in the maximum amount in leguminosae (4.78 cm), followed by the others family group (4.74 cm), and the least amount was measured in the brassicaceae family (4.28 cm).

Species-level details of the fresh biomass, hypocotyl length, SPAD content, and LAI of 14 microgreen species under the OSRAM and INSTAGREEN LEDs can be seen in Table 5. Under the OSRAM LED, among the brassicaceae family, radish recorded the highest biomass ( $6.49 \text{ kg m}^{-2}$ ), followed by kohlrabi ( $4.76 \text{ kg m}^{-2}$ ), and the lowest was in kale ( $2.42 \text{ kg m}^{-2}$ ). The SPAD content was highest in radish (45.11), followed by broccoli (39.56), and the lowest was in cress (7.12). The hypocotyl length was highest in broccoli (6.83 cm), followed by radish (5.67 cm), while the lowest was recorded in kale (2.63 cm). The LAI was highest in kohlrabi (13.89), followed by kale (11.28), and the LAI was the lowest in cress (8.60). Among the leguminosae family, peas recorded the highest fresh biomass ( $5.22 \text{ kg m}^{-2}$ ), followed by lentils ( $4.14 \text{ kg m}^{-2}$ ), and the lowest was in mungbean ( $3.81 \text{ kg m}^{-2}$ ). Similarly, the SPAD content was highest in peas (41.70), followed by lentils (33.90), and the lowest was in mungbean (25.99). The maximum hypocotyl length was measured in lentils (6.07 cm), followed by mungbeans (5.32 cm), while the minimum was measured in peas (2.93 cm). The LAI followed a similar trend as hypocotyl length, where the LAI was the highest in lentils (14.04), followed by mungbean (12.07), and the LAI was the lowest in peas (10.50). Among the other family group, sunflower recorded the maximum fresh biomass, and SPAD content ( $7.22 \text{ kg m}^{-2}$  and 66.93, respectively), followed by borage ( $3.89 \text{ kg m}^{-2}$  and 42.22, respectively), while the least biomass was found in nasturtium and least SPAD content was measured in amaranth ( $0.90 \text{ kg m}^{-2}$  and 12.31, respectively). LAI was measured maximum in borage (11.12) followed by sunflower (7.99), whereas least LAI was recorded in nasturtium (2.44). However, the hypocotyl length was highest in nasturtium (7.32 cm), followed by leek (6.42 cm), while the lowest hypocotyl length was recorded in borage (2.27 cm).



**Figure 2.** Mean ( $n = 3$ ) of (A) fresh biomass ( $\text{kg m}^{-2}$ ), (B) hypocotyl length (cm), (C) SPAD content, and (D) leaf area index (LAI) for three family groups, i.e., leguminosae, brassicaceae, and others, under two light recipes separately, INSTAGREEN and OSRAM LED. Error bars show the mean standard error. At the 5% probability level, small letters indicate a significant difference among the means for OSRAM and INSTAGREEN LEDs separately, where different letters show significant differences among the means.

Under the INSTAGREEN LED, among the three family groups, the maximum SPAD content, hypocotyl length, and LAI (36.42, 4.36 cm, and 8.60, respectively) were recorded in the leguminosae family followed by the others family group (35.52, 4.17 cm, and 4.14, respectively), and the least SPAD content, hypocotyl length, and LAI (27.93, 4.1 cm, and 2.48, respectively) were found in the brassicaceae family. Fresh biomass was measured at the maximum amount in leguminosae ( $3.57 \text{ kg m}^{-2}$ ), followed by brassicaceae ( $3.10 \text{ kg m}^{-2}$ ), while the least amount of biomass was found in the others family group ( $2.63 \text{ kg m}^{-2}$ ) (Figure 2).

Under the INSTAGREEN LED, among the brassicaceae family, radish recorded the highest biomass ( $4.76 \text{ kg m}^{-2}$ ), followed by broccoli ( $3.68 \text{ kg m}^{-2}$ ), and least was recorded in kale ( $1.09 \text{ kg m}^{-2}$ ). The SPAD content was the highest in radish (40.64), followed by kale (40.05), and least amount was recorded in cress (7.26). The hypocotyl length was highest in broccoli (7.48 cm), followed by radish (4.79 cm), while the lowest was recorded in cress (2.50 cm). The LAI was highest in kohlrabi (4.26), followed by kale (3.17), and the LAI was the lowest in broccoli (1.02). Among the leguminosae family, peas recorded the highest fresh biomass ( $4.24 \text{ kg m}^{-2}$ ), followed by mungbean ( $3.36 \text{ kg m}^{-2}$ ), and least amount was recorded in lentils ( $3.12 \text{ kg m}^{-2}$ ). The SPAD content was highest in peas (43.92), followed by lentils (33.66), and the lowest was recorded in mungbean (31.68). The maximum hypocotyl length was measured in lentils (5.81 cm), followed by mungbeans

(4.87 cm), while the minimum was measured in peas (2.39 cm). Mungbean recorded the highest LAI (9.65), followed by peas (7.94), and the lowest LAI was measured in lentils (6.86). Among the other family group, sunflower recorded the maximum fresh biomass and SPAD content (5.99 kg m<sup>-2</sup> and 73.51, respectively), followed by borage (3.16 kg m<sup>-2</sup> and 52.67, respectively), while the least biomass and SPAD content were measured in amaranth (0.60 kg m<sup>-2</sup> and 4.72, respectively). Sunflower recorded the highest LAI (8.54), followed by borage (4.34), and the lowest LAI was measured in coriander (1.49). However, hypocotyl length was highest in nasturtium (7.95 cm), followed by coriander (4.29 cm), while the shortest hypocotyl length was recorded in amaranth (2.07 cm) (Table 5).

**Table 5.** Fresh biomass, LAI, and SPAD values of 14 microgreen species (means  $\pm$  SE) under OSRAM LED and INSTAGREEN LEDs. Lowercase letters show significant differences among the species means ( $p < 0.05$ ). Uppercase letters show significant differences between the same species under the two LEDs at  $p < 0.05$ .

| Family       | Crop       | Fresh Biomass (kg m <sup>-2</sup> ) |                         | Hypocotyl Length, cm    |                        | SPAD                     |                          | LAI                    |                          |
|--------------|------------|-------------------------------------|-------------------------|-------------------------|------------------------|--------------------------|--------------------------|------------------------|--------------------------|
|              |            | Instagreen                          | OSRAM                   | Instagreen              | OSRAM                  | Instagreen               | OSRAM                    | Instagreen             | OSRAM                    |
| Leguminosae  | Peas       | 4.24 $\pm$ 0.16 bc (A)              | 5.22 $\pm$ 0.46 b (A)   | 2.39 $\pm$ 0.14 e (A)   | 2.93 $\pm$ 0.29 de (A) | 43.92 $\pm$ 0.57 bc (A)  | 41.70 $\pm$ 0.44 bc (A)  | 7.94 $\pm$ 0.44 a (A)  | 10.50 $\pm$ 1.27 a-c (A) |
|              | Mungbean   | 3.36 $\pm$ 0.26 b-e (A)             | 3.81 $\pm$ 0.19 de (A)  | 4.87 $\pm$ 0.18 bc (A)  | 5.32 $\pm$ 0.92 bc (A) | 31.68 $\pm$ 1.07 de (A)  | 25.99 $\pm$ 0.78 f (B)   | 9.65 $\pm$ 0.21 a (A)  | 12.07 $\pm$ 1.43 ab (A)  |
|              | Lentils    | 3.12 $\pm$ 0.70 c-e (A)             | 4.14 $\pm$ 0.08 cd (A)  | 5.81 $\pm$ 0.34 b (A)   | 6.07 $\pm$ 0.37 ab (A) | 33.66 $\pm$ 1.08 c-e (A) | 33.90 $\pm$ 2.02 de (A)  | 6.86 $\pm$ 2.28 bc (B) | 14.04 $\pm$ 1.33 a (A)   |
| Brassicaceae | Kohlrabi   | 3.20 $\pm$ 0.33 c-e (B)             | 4.76 $\pm$ 0.21 bc (A)  | 3.53 $\pm$ 0.31 c-e (A) | 3.4 $\pm$ 0.22 de (A)  | 29.25 $\pm$ 0.69 e (A)   | 34.80 $\pm$ 2.34 de (A)  | 4.26 $\pm$ 0.39 b (B)  | 13.89 $\pm$ 1.12 a (A)   |
|              | Radish     | 4.76 $\pm$ 0.09 ab (B)              | 6.49 $\pm$ 0.24 a (A)   | 4.79 $\pm$ 0.15 bc (A)  | 5.67 $\pm$ 0.44 bc (A) | 40.64 $\pm$ 1.97 cd (A)  | 45.11 $\pm$ 1.53 b (A)   | -                      | -                        |
|              | Broccoli   | 3.68 $\pm$ 0.55 b-d (A)             | 4.05 $\pm$ 0.05 c-e (A) | 7.48 $\pm$ 0.17 a (A)   | 6.83 $\pm$ 0.22 ab (A) | 35.78 $\pm$ 1.03 c-e (A) | 39.56 $\pm$ 3.15 b-d (A) | 1.02 $\pm$ 0.09 c (B)  | 8.68 $\pm$ 1.55 bc (A)   |
|              | Cress      | 2.75 $\pm$ 0.15 c-f (B)             | 3.90 $\pm$ 0.08 c-e (A) | 2.50 $\pm$ 0.04 e (A)   | 2.85 $\pm$ 0.27 de (A) | 7.26 $\pm$ 1.55 f (A)    | 7.12 $\pm$ 1.38 g (A)    | 1.84 $\pm$ 0.04 bc (B) | 8.60 $\pm$ 1.30 bc (A)   |
|              | Kale       | 1.09 $\pm$ 0.54 gh (A)              | 2.42 $\pm$ 0.08 fg (A)  | 3.26 $\pm$ 0.36 e (A)   | 2.63 $\pm$ 0.08 e (A)  | 40.05 $\pm$ 0.69 e (A)   | 36.77 $\pm$ 2.08 c-e (A) | 3.17 $\pm$ 0.25 bc (A) | 11.28 $\pm$ 2.79 ab (A)  |
|              |            |                                     |                         |                         |                        |                          |                          |                        |                          |
| Others       | Coriander  | 2.60 $\pm$ 0.46 d-g (A)             | 3.11 $\pm$ 0.22 ef (A)  | 4.29 $\pm$ 0.31 b-d (A) | 4.38 $\pm$ 0.27 cd (A) | 8.76 $\pm$ 1.22 f (B)    | 27.11 $\pm$ 4.45 f (A)   | 1.49 $\pm$ 0.21 c (B)  | 6.78 $\pm$ 0.51 cd (A)   |
|              | Amaranth   | 0.60 $\pm$ 0.02 h (B)               | 1.40 $\pm$ 0.10 hi (A)  | 2.07 $\pm$ 0.10 e (B)   | 3.62 $\pm$ 0.15 de (A) | 4.72 $\pm$ 0.41 f (A)    | 12.31 $\pm$ 2.18 g (A)   | -                      | -                        |
|              | Nasturtium | 1.28 $\pm$ 0.21 f-h (A)             | 0.90 $\pm$ 0.20 i (A)   | 7.95 $\pm$ 0.88 a (A)   | 7.32 $\pm$ 1.10 a (A)  | 37.96 $\pm$ 0.55 c-e (A) | 31.67 $\pm$ 0.46 ef (B)  | 2.19 $\pm$ 0.24 bc (A) | 2.44 $\pm$ 0.50 d (A)    |
|              | Sunflower  | 5.99 $\pm$ 1.37 a (A)               | 7.22 $\pm$ 0.67 a (A)   | 3.56 $\pm$ 0.78 c-e (B) | 4.40 $\pm$ 0.84 cd (A) | 73.51 $\pm$ 3.86 a (A)   | 66.93 $\pm$ 1.14 a (A)   | 8.54 $\pm$ 1.13 a (A)  | 7.99 $\pm$ 0.94 bc (A)   |
|              | Leek       | 2.16 $\pm$ 0.33 e-g (A)             | 2.13 $\pm$ 0.49 gh (A)  | 4.17 $\pm$ 0.53 cd (B)  | 6.42 $\pm$ 0.25 ab (A) | -                        | -                        | -                      | -                        |
|              | Borage     | 3.16 $\pm$ 0.08 c-e (A)             | 3.89 $\pm$ 0.51 c-e (A) | 3.00 $\pm$ 0.20 de (A)  | 2.27 $\pm$ 0.47 e (A)  | 52.67 $\pm$ 1.21 b (A)   | 42.22 $\pm$ 1.91 bc (A)  | 4.34 $\pm$ 0.66 b (A)  | 11.12 $\pm$ 2.24 a-c (A) |
|              |            |                                     |                         |                         |                        |                          |                          |                        |                          |

Among the three family groups, the brassicaceae family recorded an increase in fresh biomass by 39%, hypocotyl length by 4%, and the LAI by 327% under the OSRAM LED compared to the INSTAGREEN LED. The leguminosae family measured a fresh biomass increase of 23%, hypocotyl length increased by 10%, and the LAI increased by 42% under the OSRAM LED compared to the INSTAGREEN LED. Similarly, in the others family group, the OSRAM LED increased the fresh biomass by 8%, hypocotyl length by 13%, and the LAI by 71% compared to the INSTAGREEN LED. The SPAD content in the brassicaceae and others family groups was higher by 17% and 1.5%, respectively, when grown in the OSRAM LED compared to the INSTAGREEN LED, whereas in the leguminosae family, the INSTAGREEN LED recorded 8% more SPAD content compared to the OSRAM LED (Figure 2).

Comparing two light recipes at the species level, fresh biomass accumulation was consistently higher under the OSRAM LED compared to the INSTAGREEN LED. Amaranth recorded 133% more fresh biomass accumulation in the OSRAM LED than the INSTAGREEN LED, followed by kale with 122% higher. However, nasturtium and leek recorded 42% and 1% more biomass accumulation under the INSTAGREEN LED compared to the OSRAM LED. When exposed to the OSRAM LED, Amaranth recorded a maximum increase of 75% in hypocotyl length, followed by leek and sunflower, with increases of 54 and 23%, respectively, while the smallest increase was found in coriander by 2% compared to the

INSTAGREEN LED. On the other hand, under the INSTAGREEN LED, borage recorded 32% longer hypocotyl length compared to the OSRAM LED, followed by 24% longer in kale, and the smallest increase was found in kohlrabi by 4%. A maximum increase of 209% in the SPAD content was measured in coriander, followed by amaranth with 160%, and the smallest increase of 1% was found in lentils under the OSRAM LED compared to the INSTAGREEN LED. On the other hand, some microgreens resulted in higher SPAD contents in the INSTAGREEN LED than the OSRAM LED, where borage and mungbean recorded maximum increases of 25% and 22%, respectively, and the smallest increase was recorded in cress by 2%. Under the OSRAM LED, the maximum increase in the LAI was measured in cress by 367%, followed by coriander by 355% compared to the INSTAGREEN LED, and the smallest increase in the LAI was recorded in nasturtium by 11% under the OSRAM LED than the INSTAGREEN LED (Table 5). Therefore, in terms of family comparison, each family performed better for all the morphological traits under the OSRAM LED compared to the INSTAGREEN LED, except for the SPAD content in leguminosae, whereas the species-level comparison showed a lot of differences under the two LEDs, which showed species-dependent effects on the four morphological traits under investigation.

### 3.2. Resource Use Efficiencies of OSRAM and INSTAGREEN LEDs

Table 6 shows the mean + SE values of resource use efficiencies averaged across species for both light recipes, i.e., INSTAGREEN and OSRAM LEDs, which highlights the efficiency of each light spectrum. In our study, the LUE of the INSTAGREEN LED was 3.5 times more efficient compared to the OSRAM LED. In terms of energy use, the INSTAGREEN LED was 1.7 times more efficient than the OSRAM LED. However, the EUE included only the energy consumption for lights in both lighting systems, but we calculated the total energy use efficiency (total EUE) of the systems that, in addition to light, also includes the energy consumption for maintaining temperature, aeration, and running the water pump inside the growth chamber. For the total EUE, the OSRAM LED was 2.2 times more efficient than the INSTAGREEN LED. Under the OSRAM LED, the light EUE of the system was 27 times more efficient than the total EUE, while the light EUE was 103 times more efficient than the total EUE under the INSTAGREEN LED. A similar pattern was observed with the water and surface use efficiency (WUE and SUE), where the OSRAM LED was 1.2 times more efficient for the WUE and 1.5 times more efficient for the SUE compared to the INSTAGREEN LED; this means that we need less amount of water in the OSRAM LED than in the INSTAGREEN LED to produce the same quantity of fresh biomass. The cost of energy use calculated for the OSRAM and INSTAGREEN LEDs was USD 189 and USD 48, respectively, for one year. This shows that the operating cost of the OSRAM LED was almost four times higher compared to the INSTAGREEN LED. Hence, cheaper INSTAGREEN LEDs were more efficient in terms of light and energy consumption, while expensive OSRAM LEDs were more efficient in terms of water and surface use.

**Table 6.** Resource use efficiencies of OSRAM and INSTAGREEN LEDs averaged across the species. LUE, EUE, total EUE, WUE, and SUE denote light, energy, total energy, water, and surface use efficiencies (values after ‘±’ show the standard error of each RUE).

| Layer      | LUE                | EUE            | Total EUE   | WUE                            | SUE                   |
|------------|--------------------|----------------|-------------|--------------------------------|-----------------------|
| Unit       | FW (g)/mol<br>PPFD | FW (g)/kWh     | FW (g)/kWh  | FW (g)/H <sub>2</sub> O<br>(L) | FW (g)/m <sup>2</sup> |
| INSTAGREEN | 109.34 ± 5.19      | 369.01 ± 17.51 | 3.58 ± 0.17 | 106.33 ± 5.32                  | 256.97 ± 12.86        |
| OSRAM      | 31.07 ± 0.66       | 216.22 ± 5.15  | 8.01 ± 0.19 | 131.81 ± 2.95                  | 374.93 ± 8.01         |

## 4. Discussion

### 4.1. Light Spectrum Mix and Intensity Effects on Microgreens' Biomass and Growth

In CEA, in the absence of sunlight, plant growth is mainly affected by photoperiod, light intensity, and quality [36,37]. In our study, the OSRAM LED and INSTAGREEN LED had an intensity of 209.5 (DLI of 12.07 mol m<sup>-2</sup>) and 45 µmol m<sup>-2</sup> s<sup>-1</sup> (DLI of 2.65 mol m<sup>-2</sup>), respectively. It is essential to recognize how light affects the fresh biomass of microgreens, which mainly varies due to the specific light spectra being used and the species of microgreens. Our results showed that fresh biomass was higher in the majority of microgreens, except nasturtium and leek, under the OSRAM LED compared to the INSTAGREEN LED. Table 7 demonstrates eleven different studies from the literature on the effects of different light spectrum mixes and light intensities on microgreens' biomass and growth. Cowden et al. [35] used quite similar light recipes (high red and high far-red with an L.I of 245 and 207 µmol m<sup>-2</sup> s<sup>-1</sup>) for growing mustard, radish, and kohlrabi microgreens, where a similar higher fresh biomass was recorded with higher PPFD compared to the INSTAGREEN LED (24VHELED light with L.I of 45 µmol m<sup>-2</sup> s<sup>-1</sup>). Similarly, a considerable 34% increase in the fresh biomass of brassicaeae microgreens was observed with increasing intensity of light from as low as 105 to as high as 315 µmol m<sup>-2</sup> s<sup>-1</sup> [38], in alignment with our study, with lower biomass at lower PPFD. In our experiment, the OSRAM LED had the higher percentage of red light (63%), 19% green light, and 10% blue light, while the INSTAGREEN LED had 34% red light, 46% green light, and 17% blue light, which showed that the higher proportion of red light (63%) in combination with blue (10%) and green (19%) light increased the amount of fresh biomass in microgreens, which is in line with the findings of Kopsell et al. [39], where broccoli microgreen produced maximum fresh biomass with the light treatment of 5% blue, 10% green, and 85% red light. Similarly, the higher red light proportion in the spectrum produces more fresh biomass in amaranth [40] and cucumber [41] at 200–220 µmol m<sup>-2</sup> s<sup>-1</sup> of light intensity. However, two microgreens, nasturtium and leek, from our experiment resulted in higher biomass under the INSTAGREEN LED (R34:G46:B17), and Klimek-Szczykutowicz et al. [42] found increased fresh biomass of watercress in the light spectrum of 35% red, 15% blue, and 50% green light. This increase in the biomass accumulation of plants is driven by the process of photosynthesis [43].

**Table 7.** Studies on the effect of different light recipes on microgreens' growth and biomass accumulation.

| No. | Crop                                      | Light Spectrum                                                                          | Light Intensity (µmol m <sup>2</sup> s <sup>-1</sup> ) | DLI                       | Photoperiod (h) | Effects                                                                                                                                                                                                                                                                         | References |
|-----|-------------------------------------------|-----------------------------------------------------------------------------------------|--------------------------------------------------------|---------------------------|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| 1   | (1) Kohlrabi<br>(2) Mizuna<br>(3) Mustard | (1) R74:G18:B8<br>(2) R87:B13<br>(3) R84:FR7:B9                                         | (1) 105<br>(2) 210<br>(3) 315                          | (1) 6<br>(2) 12<br>(3) 18 | 16              | Fresh biomass of mustard and chlorophyll content of kohlrabi increased with the increase in light intensity, whereas hypocotyl length decreased for all microgreens with the increase in light intensity. At high light intensity, leaf area of kohlrabi and mustard decreased. | [38]       |
| 2   | Broccoli                                  | (1) Fluorescent light<br>(2) R95:B5<br>(3) R80:B20<br>(4) R70:G10:B20<br>(5) R85:G10:B5 | 250                                                    | -                         | 16              | Light treatment R80:B20 and R85:G10:B5 yielded significantly higher fresh and dry biomass of broccoli, while R80:B20 light recorded significantly higher chlorophyll content in broccoli than other light recipes.                                                              | [40]       |

Table 7. Cont.

| No. | Crop                                                   | Light Spectrum                                                                          | Light Intensity ( $\mu\text{mol m}^2 \text{s}^{-1}$ ) | DLI                  | Photoperiod (h)       | Effects                                                                                                                                                                                                                                                                                                           | References |
|-----|--------------------------------------------------------|-----------------------------------------------------------------------------------------|-------------------------------------------------------|----------------------|-----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| 3   | Rapeseed                                               | (1) R100:B0<br>(2) R0:B100<br>(3) R75:B25<br>(4) R50:B50<br>(5) R25:B75                 | 550                                                   | -                    | -                     | Blue light (R0:B100) increased chlorophyll content, leaf mass per area, leaf thickness, and photosynthetic capacity. Red (100%) and the higher percentage of red light increased the net photosynthetic rate.                                                                                                     | [44]       |
| 4   | Pea                                                    | (1) Blue 112 lux<br>(2) Red 128 lux<br>(3) White 135 lux                                | -                                                     | -                    | -                     | Under red light, stem length and leaf area significantly increased, while fresh biomass and stem diameter increased under blue light more than white light.                                                                                                                                                       | [45]       |
| 5   | Watercress                                             | (1) R100:B0<br>(2) R90:B10<br>(3) R80:B20<br>(4) R70:B30<br>(5) R60:B40<br>(6) R1:B1:G1 | 198                                                   | -                    | -                     | A R70:B30 light ratio produced the highest stem length, fresh shoot weight, dry shoot weight, and SPAD values, while a R1:B1:G1 light ratio produced the lowest results. This pattern was similarly reflected in the photosynthesis rate and stomatal conductance.                                                | [46]       |
| 6   | (1) Arugula<br>(2) Mustard<br>(3) Cabbage<br>(4) Kale  | (1) Pure Red (R)<br>(2) Pure Blue (B)<br>(3) Unpure Blue (BU)<br>(4) Unpure Blue (BF)   | (1) 50<br>(2) 100                                     | -                    | 24                    | Compared to R, B promoted hypocotyl length, petiole length in arugula, cabbage, and kale, but not mustard. Arugula and mustard stem extension rates and hypocotyl length were increased with BF compared to B for some species at 100, but not for R. BF compared to B inhibited elongation growth but R did not. | [47]       |
| 7   | (1) Argula<br>(2) Broccoli<br>(3) Mizuna<br>(4) Radish | R50.3:G21.1:B17.6                                                                       | 270                                                   | (1) 15.6<br>(2) 23.3 | (1) 16<br>(2) 24 (CL) | Under continuous light (CL), seedlings of all four crops were significantly heavier and more densely packed than 16 h photoperiods, regardless of light quality. In addition, ratios of carotenoid/chlorophyll and chlorophyll a/b were higher in all plants except mizuna.                                       | [48]       |

Table 7. Cont.

| No. | Crop                                          | Light Spectrum                                                                                         | Light Intensity ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) | DLI  | Photoperiod (h) | Effects                                                                                                                                                                                                                                              | References |
|-----|-----------------------------------------------|--------------------------------------------------------------------------------------------------------|----------------------------------------------------------|------|-----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| 8   | Radish                                        | (1) R100<br>(2) R95:B5<br>(3) R90:B10<br>(4) R88:B10:FR2<br>(5) R83<br>(638 nm):R5<br>(669 nm):B10:FR2 | 200                                                      | -    | 16              | Under R100, radish elongated, but the hypocotyl formation was weak. Lower accumulation of photosynthetic pigment resulted in lower leaf dry weight and hypocotyl ratios. Hypocotyl thickening was observed with blue light supplementation (455 nm). | [49]       |
| 9   | Broccoli                                      | R1:G1:B1                                                                                               | (1) 30<br>(2) 50<br>(3) 70<br>(4) 90                     | -    | 12              | Under light intensity of 50, PPFD produced the highest fresh as well as dry biomass but the lowest phytochemical contents. As light intensity increased, chlorophyll content also increased.                                                         | [50]       |
| 10  | (1) Red pak choi<br>(2) Tatsoi<br>(3) Mustard | -                                                                                                      | (1) LED 150<br>(2) LED 250<br>(3) HPS 150                | 3.46 | 16              | Shorter hypocotyls were produced by the LED with light intensities of 150 and 250 PPFD, while mustard and tatsoi had small leaf area under LED 150 and LED 250 while higher leaf area in pak choi under LED 250.                                     | [51]       |
| 11  | (1) Mustard<br>(2) Kale                       | (1) R100:B0<br>(2) R90:B10<br>(3) R75:B25<br>(4) R50:B50<br>(5) R25:B75<br>(6) R0:B100                 | 250                                                      | -    | 18              | Under LED B100, fresh biomass and hypocotyl length of kale were highest, while R100 produced higher leaf area, whereas for mustard, fresh biomass and leaf area were highest under B100 while R100 produced the highest hypocotyl.                   | [52]       |

Chen and Blankenship [53] observed that chlorophyll (primary photosynthetic pigments) readily absorbs blue and red light spectrum during the photosynthetic process. The addition of red and blue light spectra enhances the fresh biomass of microgreens [54] by increasing photosynthesis, which increases the chlorophyll content that traps the incident red light by stomatal opening [55]. In this study, seven microgreens had higher chlorophyll contents under the OSRAM LED, which had a higher red light proportion than the INSTAGREEN LED, but this increase in chlorophyll is species-specific, i.e., influenced by a particular light intensity. In plants, processes like photosynthesis, chlorophyll formation, and stomata opening are regulated by blue light, mainly by means of cryptochrome and phototropin [56]. In addition to increasing photosynthetic activity and chlorophyll formation in the leaves, the addition of blue to red light increases the net photosynthetic rate and the accumulation of dry matter in plants [44]. Blue light is particularly effective at enhancing carotenoid synthesis by activating specific photoreceptors. Blue light also increases the proportion of total chlorophyll content and stomatal conductance [57]. In amaranth, at an intensity of  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ , blue light significantly increased the accumulation of carotenoids and total chlorophyll content than white light at the same light intensity [41]. Green light has a wavelength between the red and blue spectrum but

it has an increased tendency of penetrating the leaf compared to red or blue light, thus increasing the carbon fixation and photosynthetic activity [22]. Our results also show that few microgreen species, such as peas, mungbean, kale, etc., obtained a higher chlorophyll content under the INSTAGREEN LED than the OSRAM LED. This is similar to the results of Gerovac et al. [38], where by the intensity of light increasing from low  $105 \mu\text{mol m}^{-2} \text{s}^{-1}$  to high  $315 \mu\text{mol m}^{-2} \text{s}^{-1}$ , he found a decrease in the relative chlorophyll content of kohlrabi.

In our study, the highest hypocotyl length was recorded in nasturtium under the INSTAGREEN and OSRAM LEDs. However, nine microgreens had shown higher hypocotyl length under OSRAM LED than INSTAGREEN LED light. Under certain ratios of red to far-red, phytochrome photoreceptors are triggered to activate the elongation of hypocotyl as a type of shade-avoidance response [58]. There are two types of phytochromes, one for red (Pr) and the other for far-red (Pfr) ranges of light, which are interconvertible based on the ratio of wavelengths in the red and far-red spectrum of light when compared to the wavelengths in the blue range. Shade avoidance response occurs when far-red light increases in the light spectrum. Particularly, phytochromes, as well as cryptochromes (photoreceptors absorbing blue light and UV light, respectively), regulate the transcription factors HY5 and HYH that induce photomorphogenesis, as well as the transcription factor COP1, which suppresses photomorphogenesis [59,60]. Wu et al. [45] compared pea microgreens grown under white LED light to the combination of traditional red with blue light and he measured a 30% increase in stem length with the combination of two LED lights. Basil resulted in an increase in stem length under a high light intensity of  $141 \mu\text{mol m}^{-2} \text{s}^{-1}$  than  $107 \mu\text{mol m}^{-2} \text{s}^{-1}$  [61]. In watercress, an R7:B3 LED light measured the highest increase in the stem length as compared to the same wavelength but a different ratio [46]. In another research finding, blue light ( $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) increased cabbage, kale, and arugula hypocotyl length more than red light ( $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) [47]. Compared to continuous light (24 h), LED light with a 16 h photoperiod increased the broccoli and radish hypocotyl length at  $270 \mu\text{mol m}^{-2} \text{s}^{-1}$  of intensity [48]. Although previous studies reported that blue LED combined with red light has an inhibiting effect on the hypocotyl elongation of many microgreens [49,50], with the supplementation of green LED light with dichromatic blue and red light, this reduces the inhibition effect and increases the hypocotyl length of microgreens [51,62,63]. Few microgreens, i.e., kohlrabi, broccoli, kale, borage, and nasturtium, in our study resulted in a lower hypocotyl length under the OSRAM LED than the INSTAGREEN LED, similar to the findings of Gao et al. [50] where a low-intensity light ( $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) improved the hypocotyl length of broccoli. In another study, three microgreens, kohlrabi, mizuna, and mustard, obtained a lower hypocotyl length with a higher light intensity ( $315 \mu\text{mol m}^{-2} \text{s}^{-1}$  instead of  $105 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) [38]. This inhibition in the elongation of hypocotyl with the increase in light intensity is mainly due to the reduced levels of gibberellic acids [64]. This shows that different microgreen species had different patterns of hypocotyl elongation under two light recipes, as observed in one of the studies by Kong et al. [65].

Leaf area index (LAI) refers to the leaf area of a plant per unit of surface [66]. The results of our study show that under the OSRAM LED, kohlrabi and lentils had the highest LAI, while borage and mungbean had the highest LAI under the INSTAGREEN LED. However, the majority of the microgreen species had a higher LAI under a higher light intensity (OSRAM LED) than a lower light intensity (INSTAGREEN LED). Kyriacou et al. [67] observed that changing the wavelength of the light, especially red and blue spectral wavelengths, can be effective in controlling the leaf area. Hence, there is the possibility of the selective use of blue LED light with red LED light intercepted by the photomorphogenetic pigments for the better growth and elongation of leaves [68,69]. Microgreens grown under red light exhibited a significant increase in leaf area of 33% compared to microgreens grown under white light [45]. Similarly, a red monochromatic light source ( $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) increased the leaf area of microgreen kale [52]. However, the addition of green light was in the light spectrum of blue and red (R70:B10:G20), and there were increases in the leaf area of brassica microgreens as compared to other dichromatic light treatments at a light intensity of  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$  [70]. Pea, amaranth, and radish grown under the red/blue light ratio of 9 recorded a greater leaf area than a red/blue light ratio of 5 and 2 [71]. Out

of all the microgreens, borage at a lower light intensity measured the highest LAI than the higher light intensity (OSRAM LED), and Vaštakaite and Viršile, [51] observed similar results, where at a light intensity of  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ , HPS lamps had a greater leaf area in brassicaceae microgreens as compared to the LED light.

#### 4.2. Light Recipes Effect on Microgreens Resource Use Efficiencies

In this experiment, overall the OSRAM LED measured lower LUE (31.07 g FW/mol PPFD) as compared to the INSTAGREEN LED with higher LUE (109.34 g FW/mol PPFD), which means that microgreens grown under the INSTAGREEN LED were more efficient to convert incident light to biomass than the OSRAM LED. However, under the INSTAGREEN LED, sunflower had a significantly higher LUE (192.77 g FW/mol PPFD), while amaranth had the lowest LUE (19.50 g FW/mol PPFD); this indicated the higher efficiency of sunflower to convert incident light to biomass at a low light intensity. Table 8 shows the literature from five different studies on the effects of light spectrum mix and intensity on microgreens' resource use efficiencies. One of the factors for the higher LUE of sunflower under both lights is correlated to the chlorophyll content of sunflower, that was also higher, as shown by our results. This higher amount of chlorophyll resulted in the capturing of incident light, thus generating higher photosynthetic activity which increases the  $\text{CO}_2$  capture by leaves and hence results in more biomass accumulation [35,72]. Light EUE had similar results in our study where, overall, the INSTAGREEN LED (369.01 g FW/kWh) was more efficient compared to the high-intensity light EUE of the OSRAM LED (216.22 g FW/kWh). Similarly, at the species level, sunflower was most efficient under both light recipes in utilizing the light EUE. In our experiment, the OSRAM and INSTAGREEN LEDs had red/blue ratios of 6 and 2, respectively. It has been reported in a previous study that a red/blue light ratio of 3 was most efficient; in terms of the light EUE of lettuce, a red/blue ratio of 2 was most effective in the light EUE of basil and chicory, while a red/blue ratio of 4 was most efficient for rocket [73], highlighting the species-specific efficiency for particular light recipes. Similarly, a red/blue ratio of 3 was most effective in the production of lettuce in the context of light EUE [74]. Light EUE only included the energy consumption of LED lights; however, there were other operations like ventilation and the maintenance of the relative humidity and temperature of the growth chamber that needed more energy consumption. For this reason, we have calculated the total EUE of the growth chamber. Total EUE had a quite different pattern to light EUE; overall, the OSRAM LED's total EUE (8.01 g FW/kWh) was more effective than that of the INSTAGREEN LED (3.58 g FW/kWh). However, at the species level, sunflower was the most efficient crop under both lights in terms of total EUE than other microgreens. Reed et al. [35] used the same production system for his study, and our results are in line with his findings, where low-light-intensity LEDs (24VHELED) have lower total EUE compared to other light recipes.

**Table 8.** Studies on the effect of different light recipes on resource use efficiencies (RUEs) of microgreens.

| No. | Crop                                      | Light Spectrum                                                                             | Light Intensity ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) | DLI                                                          | Photoperiod (h) | Effects                                                                                                                                                                                                                                                                                                                     | Reference |
|-----|-------------------------------------------|--------------------------------------------------------------------------------------------|----------------------------------------------------------|--------------------------------------------------------------|-----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| 1   | (1) Kohlrabi<br>(2) Mustard<br>(3) Radish | (1) R70:G19:B11<br>(2) R65:G0:B35<br>(3) R47:G27:B26<br>(4) R58:G22:B20<br>(5) R37:G46:B17 | (1) 245<br>(2) 252<br>(3) 250<br>(4) 207<br>(5) 45       | (1) 15.37<br>(2) 15.81<br>(3) 15.64<br>(4) 15.49<br>(5) 2.65 | 16              | Cheaper 24VHELED light (R37:G46:B17) was more efficient for light and energy use efficiency (LUE and EUE). However, higher far-red (R58:G22:B20) light recipe was more efficient in terms of water and surface use efficiency (WUE and SUE) as well as efficient for whole systems energy use, regardless of fertilization. | [35]      |

Table 8. Cont.

| No. | Crop                                                        | Light Spectrum                                                  | Light Intensity ( $\mu\text{mol m}^2 \text{s}^{-1}$ ) | DLI | Photoperiod (h) | Effects                                                                                                                                                                                                                                                                                                                   | Reference |
|-----|-------------------------------------------------------------|-----------------------------------------------------------------|-------------------------------------------------------|-----|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| 2   | (1) Lettuce<br>(2) Chicory<br>(3) Rocket<br>(4) Sweet basil | (1) R/B:0.5<br>(2) R/B:1<br>(3) R/B:2<br>(4) R/B:3<br>(5) R/B:4 | 215                                                   | -   | 16              | In terms of energy, the most efficient red/blue LED light ratio was 0.5 with lower biomass yield. However, red/blue ratio of 3 was most efficient in terms of energy for lettuce, a red/blue ratio of 2 was most effective in energy use of basil and chicory, while a red/blue ratio of 4 was most efficient for rocket. | [73]      |
| 3   | Lettuce                                                     | (1) R/B:0.5<br>(2) R/B:1<br>(3) R/B:2<br>(4) R/B:3<br>(5) R/B:4 | 215                                                   | -   | 16              | The use of LED lights improved the efficiency of light energy by 2.8-fold than fluorescent lighting. RB3 light recipe was most efficient treatment for WUE, light EUE, and SUE of lettuce.                                                                                                                                | [74]      |
| 4   | Basil                                                       | (1) R/B:0.5<br>(2) R/B:1<br>(3) R/B:2<br>(4) R/B:3<br>(5) R/B:4 | 215                                                   | -   | 16              | Results show that with the increase in red component in light recipe ( $\text{RB} \geq 2$ ) RUE, i.e., light EUE, WUE and SUE were most effective in growing the basil.                                                                                                                                                   | [75]      |
| 5   | Soyabean                                                    | (1) R/B:1<br>(2) R/B:3.2                                        | (1) 546<br>(2) 570                                    | -   | 13              | A vertical farming system produces approximately eight times more protein per square meter of crop than an open field. Open field requires 82 times more area, 46 times more water, and 1778 times less energy for the production of sufficient food to meet the protein requirement of person for one year.              | [76]      |

WUE is one of the important factors to consider in indoor farming systems. In our experiment, the overall WUE of the OSRAM LED (131.81 g FW/L H<sub>2</sub>O) was 1.2 times that of the INSTAGREEN LED (106.33 g FW/L H<sub>2</sub>O), and this result translates on a species level as well, where sunflower was most efficient in utilizing the WUE. Possibly, this increase in WUE is due to the increased red wavelength in the light spectrum that reduces the quantum efficiency of photosystem II, which in turn reduces transpiration, resulting in an increase in WUE [74]. This reduction in the transpiration rate is also associated with the stomatal conductance regulated by the temperature [77,78]. Comparing the WUE of broccoli grown in a vertical farming system than in an open field, it needs 158–236 times less water to produce the same amount of biomass [79]. In a previous study, lettuce yield was increased by 10-fold in hydroponics systems as compared to conventional production system with the water use of 250 L kg<sup>-1</sup> year<sup>-1</sup> in the conventional system and 25 L kg<sup>-1</sup> year<sup>-1</sup> (10 times less water requirement), while 82 times less energy was consumed in the conventional system than the hydroponic system [32]. SUE had a similar trend as of WUE, with the OSRAM LED being more efficient than the INSTAGREEN LED; also, on a species level, sunflower had the highest SUE in both light regimes. The addition of far-red light to red light had

significantly increased the SUE in mustard, kale, and kohlrabi [42]. In another study, a red/blue ratio 3 resulted in the highest SUE in basil microgreen [75]. Similarly, a high SUE was found in lettuce when grown in a red/blue light ratio of 3 [74]. In a comparative study of open field cultivation with a vertical farm, Raghini concluded that open field requires 82 times more area, 46 times more water, and 1778 times less energy for the production of sufficient food to meet the protein requirement of a person for one year [76]. In our study, the operating costs of the INSTAGREEN LED were four times less than the OSRAM LED. Similar findings were observed in Cowden et al.'s [35] study, where low-intensity 24VHELED light was more cost-efficient than other high-light-intensity treatments.

This study demonstrates the effects of spectrum mix and light intensity on the biomass, growth, and RUE of 14 microgreens species that are limited to controlled environmental and lighting conditions, as well as on microgreens, which are short-growing crops. The findings of controlled lighting and environmental conditions can be applicable to optimize production of other high-value microgreen species under controlled environment than uncontrolled environmental conditions.

## 5. Conclusions

This study assessed the variability in the growth and biomass yield of 14 different microgreen species, when they were grown in two distinct light recipes, i.e., the OSRAM and INSTAGREEN LEDs and RUE of two LEDs. According to our findings, light recipes (spectrum mix and intensity) are a major factor in determining the growth and overall amount of biomass produced by microgreens. Particularly, sunflower recorded the highest biomass and SPAD values under both lighting conditions, emphasizing the importance of chlorophyll content for enhancing photosynthetic efficiency. Comparatively, the majority of microgreen species resulted in higher yields under the OSRAM LED compared to the INSTAGREEN LED, except nasturtium and leek, which shows the species-specific sensitivity of microgreens with the two light recipes. Furthermore, the resource use efficiency, including the light, energy, water, and surface use efficiencies, varied significantly between the light recipes, which indicated the necessity of tailored light spectra as well as light intensity to optimize growth and sustainability. The cheaper INSTAGREEN LED was quite efficient for light and energy consumption. Although the initial cost for setting up the system for the OSRAM LED is quite expensive compared to the cheaper INSTAGREEN LED, these lights were effective for water and surface use efficiency. Hence, moderate light intensities can be beneficial between maximizing biomass and improving light and energy use efficiency. This highlights the potential for energy-saving strategies in CEA by choosing cost-effective LED lights. Under global climate change, where stressors like drought and temperature fluctuations may impair nutrient uptake or growth of crops, CEA enables a controlled, consistent, and adaptive approach to plant production by maintaining light intensity, spectra, and other environmental parameters such as temperature, relative humidity, and increases in resilience by enhancing root development and growth rate. This resilience is key for future-proofing agriculture and ensuring reliable crop production under global change. Furthermore, these findings indicate that specific light spectra can be utilized to enhance biomass, growth, and resource use efficiency in controlled-environment agriculture by enhancing light-mediated growth responses in microgreens. It is imperative that future research focuses on refining light recipes with the use of different efficient LEDs (OLEDs, etc.), which may offer unique benefits in terms of energy efficiency and spectral customization. Additionally, the effect of the varying light intensities and spectrum mixes on microgreens' physiology, nutrients, phytochemicals, and post-harvest shelf life should be studied in order to further increase the production and sustainability of microgreen cultivation in CEA.

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