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# Evaluation of Growth, Yield and Bioactive Compounds of Ethiopian Kale (*Brassica carinata* A. Braun) Microgreens under Different LED Light Spectra and Substrates

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**Abstract:** Microgreens are innovative vegetable products whose production and consumption are gaining popularity globally thanks to their recognized nutraceutical properties. To date, the effects of lighting conditions and growing substrate on the performances of *Brassica carinata* microgreens (indigenous to Africa) remain underexplored. The present study aimed at providing insights into the influence of different lighting treatments provided by LEDs, namely monochromatic blue (B), red (R), cool white (W) and a combination of three color diodes (B + R + W), and substrates (cocopeat, sand and cocopeat–sand mix (*v*/*v*) (1:1)) on the growth, yield and bioactive compounds of *B. carinata* microgreens. Seeds were germinated in dark chambers and cultivated in growth chambers equipped with LED lighting systems for 14 days under a fixed light intensity of 160 ± 2.5 µmol $m^{-2}$ $s^{-1}$ and photoperiod of 12 h $d^{-1}$. The best performances were associated with the spectrum that combined B + R + W LEDs and with substrate resulting from the cocopeat–sand mix, including the highest yield (19.19 g $plant^{-1}$), plant height (9.94 cm), leaf area (68.11 $mm^2$) and canopy cover (55.9%). Enhanced carotenoid and flavonoid contents were obtained with B + R + W LEDs, while the B LED increased the total amount of chlorophyll (11,880 mg $kg^{-1}$). For plants grown under B + R + W LEDs in cocopeat, high nitrate levels were observed. Our results demonstrate that substrate and light environment interact to influence the growth, yield and concentration of bioactive compounds of *B. carinata* microgreens.

**Keywords:** African indigenous vegetables; healthy diets; light quality; functional foods; nutraceutical; phytochemical



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## 1. Introduction

Microgreens are gaining attention and recognition as a new class of food due to their unique characteristics such as flavor, tenderness, color [1,2] and nutrient density [3]. Microgreens are young plants harvested shortly after the first true leaves emerge, usually between 7 and 21 days after sowing. They are harvested by cutting the stem just above the medium, or over the roots when soilless cultivation is adopted [4]. The harvested shoots are eaten raw, either alone or in mixed salads, or used as a garnish for dishes [2]. The

superiority of microgreens over other plant stages of the same plant species is attributed to the germination process from dry seeds to growing plants which involves many metabolic activities and de novo synthesis of nutrients [5]. Microgreens are mainly grown in indoor hydroponic systems using different growing substrates and integrating supplemental lighting [2].

Ethiopian kale (*Brassica carinata* A. Braun) is one of the indigenous African leafy vegetables (ALVs) that are rich in nutrients and health-promoting secondary plant metabolites [6] with potential for use against non-communicable diseases (e.g., cancer). The leaves and seeds of *B. carinata* are rich in nutrients with high concentrations of glucosinolates, especially 2-propenyl glucosinolate (sinigrin), as well as phenolic compounds. *B. carinata* has been reported to reduce afb1-induced DNA damage [7]. *B. carinata* microgreens have been shown to contain flavonoids, phenols, tannins, saponins, alkaloids and terpenoids but not glycosides [8].

Growth substrate is critical in the production of microgreens as it is a major contributor to production costs [9]. Substrates will affect the growth, yield and environmental sustainability of microgreen production [10]. Locally available and inexpensive substrates that have good water-holding capacity and provide aeration are ideal for microgreen production. Those derived from renewable resources and/or those that can be recycled are to be preferred [11]. According to several authors, peat and peat-based mixes represent the most used growing substrates for the production of microgreens because of their good physicochemical properties, but coconut coir (also referred to as cocopeat) is common as well [10–13]. However, these substrates are quite expensive, and when they are not locally available, they require importation. The use of peat poses environmental concern due to its continuous extraction which contributes to the emission of carbon dioxide. On the other hand, cocopeat (derived from the coconut processing industry and its discarded fibers) is a renewable resource and could be used as an alternative to peat [11]. However, it can also be an expensive material and requires treatment for the removal of its concentrated salts before use, which increases costs. Accordingly, the exploration of alternative substrates or additives enabling a reduction in the amount of cocopeat needed may lead to the identification of sustainable, cheaper and renewable growing substrates for microgreens.

Light is another major factor in plant growth and influences the development and production of phytochemical and bioactive compounds [14]. Light quality (its composition in the spectral regions), quantity (intensity), direction and duration (photoperiod) are vital components in microgreen production. In plants such as lettuce, high light intensity results in the production of high amounts of phenolics, anthocyanins and carotenoids, among others, which could be beneficial to human health [15]. Regarding the effects of light on microgreen growth, research results vary across studies and for different vegetable species. For example, it has been found [16] that growth and phytochemical accumulation in *Brassica juncea* and *Brassica napus* using different R and B ratios differed depending on the species. The chlorophyll, carotenoid and soluble protein contents depended on photoperiod [17] in other *Brassica* species. Artificial light sources such as light-emitting diodes (LEDs) have been used as a source of supplemental lighting in controlled environments such as indoor spaces and greenhouses in the production of microgreens [17]. B, R and W LEDs used alone or in combination have been used to produce high-quality microgreens with various nutritional benefits [17]. However, the influence of LED grow lights on *B. carinata* microgreens is still unknown. In addition, it is unclear how plants respond to LEDs in combination with substrates since most of the previous studies assessed either LEDs or substrates alone. Therefore, this study aimed to investigate the influence of different LED lights and growing substrates on the growth, yield and phytochemical content of *B. carinata* microgreens. The results obtained from this study provide a baseline towards an understanding of the influence of the interactions between the substrate and LEDs on quality traits and bioactive accumulation of *B. carinata* microgreens.

## 2. Materials and Methods

### *2.1. Experimental Materials and Design*

The experiment was conducted in a controlled environment in a locally fabricated walk-in growth chamber at Tokyo University of Agriculture between April and October 2023. The chamber was divided into four compartments using black opaque fabric to prevent light interference. Each compartment measured 100 cm by 100 cm. In each compartment, an LED fixture was placed 50 cm above the surface of the substrate. Ethiopian kale (*Brassica carinata*) seeds used in the study were sourced from a commercial vendor in Kenya. A phytosanitary certificate allowing entry of seeds to Japan was obtained from the Kenya Plant Health Inspectorate Service (KEPHIS). *B. carinata* was identified by a taxonomist at JKUAT GoK laboratories, and a voucher specimen (JMW/JKUAT/BOT/H001) is maintained at the JKUAT herbarium.

### *2.2. Growing Environment*

Seeds of *B. carinata* were sown and grown using three substrates under four LED light spectra in a factorial experiment. The light spectra used were B (with a peak at 450 nm), R (with a peak at 650 nm), W, and B + R + W (managed by having one light with three diodes; B, R and W combined in the ratio of 1:1:1) LEDs in each compartment. The three substrate types (cocopeat, sand and a mix of cocopeat and sand) and one LED light were placed in each compartment to give a split plot design with light being the main plot factor and substrate the subplot factor. There were three replicates for light spectra and twelve for the substrate. The lights had a fixed light intensity of 160 $\pm$ 2.5 µmol $m^{-2}$ $s^{-1}$, and a 12 h photoperiod was applied. The air temperature in the walk-in growth chamber was set and maintained at 26 $\pm$ 2 °C while relative humidity was maintained at approximately 60% during the experimental period. Temperature and relative humidity were monitored using a data logger (HOBO, OnSet Data Logging Solutions, Bourne, MA, USA). There were no nutrients supplied throughout the growing period. Irrigation was performed using capillary wick technology [18].

### *2.3. Growth Measurements*

Growth was assessed at the end of the experiment (14 days after sowing) in terms of height, leaf area and canopy cover. Ten plants were randomly selected from each subplot and harvested for height and leaf area measurements. The plants were harvested by cutting above the substrate. The individual height of each plant was measured using a ruler. Leaf area values were estimated using ImageJ v.1.5 software [19]. Leaves from the ten selected plants were spread on a clean white sheet of paper, and photographs were taken against a ruler as a reference. Additionally, a square paper of known area (2 $\times$ 2 mm) was included for verification of the measurements obtained. Canopy cover was estimated using Canopeo software (version 1.1.7) [20]. This was done by taking aerial photographs of all the above-ground plant materials. To achieve uniformity in all the photographs, a 30 cm distance from the camera to the treatment was maintained. The photographs were processed with Canopeo software, and canopy cover was calculated as a percentage of the total surface area.

### *2.4. Yield and Biomass Analysis*

Yield and dry biomass were obtained by weighing the whole harvested microgreen shoots 14 days after sowing (DAS). All above-ground parts including the leaves, stems and cotyledons were harvested by cutting them at the base, and fresh weight (yield) and dry biomass (after freeze drying at −41 °C for 24 h) were weighed using a weighing balance. The samples were further powdered and used for phytochemical analysis.

### *2.5. Phytochemical Analysis*

#### 2.5.1. Flavonoids

The estimation of total flavonoids in the sample was performed using the aluminum chloride method. Rutin was used as the standard [21]. The sample (0.1 mL) and standards were prepared in triplicates, vortexed and incubated for 5 min at room temperature. Afterward, 10% aluminum chloride was added, vortexed and incubated for 6 min at room temperature. The absorbance was measured against the blank at 510 nm using a spectrophotometer (Shimadzu model UV-1601 PC, Kyoto, Japan). The standard curve was plotted, and the total amount of flavonoids in the sample was expressed as mg of rutin equivalent (RE)/g of dry weight of the sample. Equation (1) was used to compute flavonoids (mg/100 g) from absorbance.

$$Flavonoids = 0.0001 * \frac{(A_s - A_b)}{0.0018 * W} * D \quad (1)$$

where $A_b$ = absorbance of the blank, $A_s$ = absorbance of the sample, $D$ = dilution factor (30), $W$ = weight of the sample (g), 0.0018 is the slope of the standard curve and 0.0001 is the factor for conversion to mg/100 g.

#### 2.5.2. Carotenoids

Total carotenoids were extracted using acetone and analyzed using column chromatography (Rodriguez- Amaya and Kimura, 2004; AOAC, 1996) and a UV spectrophotometer (Shimadzu model UV-1601 PC, Kyoto, Japan) [22]. Approximately 0.08 g of dried sample was weighed and ground in a mortar containing 10 mL of acetone, and extraction was repeated until the residue turned colorless. Then, 25 mL of the extract was evaporated to dryness using a rotary evaporator; the residue was dissolved in 10 mL of petroleum ether, and the solution was introduced into a chromatographic column. Absorbance was read at 450 nm in a UV-Vis spectrophotometer. Equation (2) was used to calculate carotenoids (mg/100 g) from absorbance.

$$Carotenoids = 0.001 * \frac{A}{2592 * W} \quad (2)$$

where $A$ = absorbance, $W$ = weight of the sample (g) and 2592 is the absorption coefficient of β-carotene in petroleum ether.

#### 2.5.3. Nitrates

The nitrate content in the test samples was determined by the calorimetric method using salicylic acid [22]. Samples of 0.3 g dry *B. carinata* were weighed and put in a test tube. Hot (90–95 °C) distilled water measuring 10 mL was added. The closed tubes were placed in a water bath at 80 °C and shaken for 30 min. The samples were then cooled and centrifuged at 4500 rpm. Chlorophyll in the sample was removed by adding 0.5 g $MgCO_3$ to the supernatant and centrifuging it again. The supernatant containing the nitrate extract was then treated with NaOH and a combination of salicylic acid and $H_2SO_4$. Nitrate standards were prepared using a sodium nitrate calibration curve. Absorbance was read at 410 nm in a UV-Vis spectrophotometer (Shimadzu model UV-1601 PC, Kyoto, Japan). The nitrate concentration was expressed on a dry weight basis (mg/100 g DW). Equation (3) was used to calculate nitrates (mg/100 g) from absorbance.

$$Nitrates = 0.1 * \frac{A_s - A_b}{0.0078 * W} * D \quad (3)$$

where $A_b$ = absorbance of the blank, $A_s$ = absorbance of the sample, $D$ = dilution factor (30), $W$ = weight of the sample (g), 0.0078 is the slope of the standard curve and 0.1 is the factor for conversion to mg/100 g.

2.5.4. Chlorophyll

Chlorophyll was extracted using acetone and analyzed using column chromatography (Rodriguez- Amaya and Kimura, 2004; AOAC, 1996) and a UV spectrophotometer (Shimadzu model UV-1601 PC, Kyoto, Japan) [23]. Approximately 0.08 g of a dry sample was weighed and ground in a mortar containing 10 mL acetone. The extraction was repeated until the residue turned colorless. An aliquot of 25 mL of the extract was evaporated to dryness using a rotary evaporator, and the residue was dissolved in 10 mL of petroleum ether. The solution was introduced into a chromatographic column, and absorbance was read at 645 nm and 663 nm in a UV-Vis spectrophotometer. Chlorophyll content was determined by computation from the absorbance using Equation (4).

$$\text{Total Chlorophyll (mg/100 g)} ChlA = 0.1 * (7.12 * A_{663} + 16.8 * A_{645}) * \frac{D}{W} \quad (4)$$

where $A$ = absorbance at indicated wavelength (645 or 663), $D$ = dilution factor (25), $W$ = weight of the sample (g).

*2.6. Statistical Analysis*

Statistical analysis was performed using GenStat software, version 12.1. Growth measurements (leaf area and plant height) were analyzed based on the individual values of the 10 sampled plants from each subplot, while canopy cover, yield and dry weight were analyzed at the subplot level. All data were subjected to two-way ANOVA, and significant differences among means were determined by Tukey's multiple comparison test at $p < 0.05$.

## 3. Results

*3.1. Effect of LED Light and Substrate on Height, Leaf Area and Canopy Cover*

The results from the ANOVA indicated that the interaction between substrates and LED light treatments did not have a significant effect on plant morphological parameters. However, height differed significantly in response to both different substrates and LED light treatments (Table 1). The microgreens grown using monochromatic R were significantly shorter compared to those grown using other LEDs. More specifically, microgreens grown under monochromatic R were 8% shorter compared to those under monochromatic B. Microgreens grown under B, W and B + R + W did not differ significantly in height. Microgreens grown in either sand alone or cocopeat–sand mix were significantly taller (F (3,108) = 3.92, $p < 0.001$) than those grown in cocopeat alone. Microgreens in cocopeat were shorter than those in sand and cocopeat–sand mix by 8%.

**Table 1.** Effect of LED light and substrate on height, leaf area and canopy cover.

| Treatment | Height (cm) | Leaf Area ($cm^2$) | Canopy Cover (%) |
|---|---|---|---|
| **LED Lights** | | | |
| B | 9.9 (0.16) [a] | 57.62 (1.40) [c] | 50.68 (4.51) [a] |
| R | 9.2 (0.16) [b] | 57.36 (1.46) [c] | 44.45 (2.66) [b] |
| W | 9.7 (0.18) [a] | 63.43 (1.56) [b] | 56.39 (2.85) [a] |
| B + R + W | 9.8 (0.11) [a] | 68.11 (1.96) [a] | 55.15 (2.76) [a] |
| *P* | 0.011 | <0.001 | <0.001 |
| $LSD_{0.05}$ | 0.39 | 4.32 | 5.87 |
| F Value | F (3,108) = 3.92 | F (3,108) = 11.18 | F (3,33) = 13.12 |
| **Substrates** | | | |
| Sand | 9.8 (0.13) [a] | 60.0 (1.36) [b] | 56.0 (3.26) [a] |
| Cocopeat | 9.2 (0.12) [b] | 59.1 (1.44) [c] | 47.1 (2.07) [b] |

**Table 1.** *Cont.*

| Treatment | Height (cm) | Leaf Area ($cm^2$) | Canopy Cover (%) |
|---|---|---|---|
| Sand + Cocopeat | 9.9 (0.14) [a] | 65.6 (1.66) [a] | 51.9 (3.394) [ab] |
| *P* | <0.001 | 0.001 | 0.005 |
| $LSD_{0.05}$ | 0.34 | 3.74 | 5.08 |
| F Value | F (3,108) = 11.86 | F (3,108) = 7.28 | F (3,33) = 12.02 |

Mean separation by the Tukey test at the 5% significance level. Values in brackets are standard errors of means. Values without a letter in common in a column within a factor are significantly different ($p < 0.05$).

Both substrate and LED treatment had a significant effect on leaf area (Table 1). Microgreens grown under B + R + W had significantly higher leaf area (68.11 $mm^2$) compared to microgreens grown under W (63.43 $mm^2$) and both under monochromatic B and R (57.62 $mm^2$ and 57.36 $mm^2$). Leaf area in the cocopeat–sand mix was significantly higher by 22% (65.75 $mm^2$) compared to microgreens produced using cocopeat alone (59.12 $mm^2$).

Both the growing media and LED treatments had a significant effect on canopy cover. Canopy cover values under B + R + W treatment were significantly higher (55.15%) than those produced in monochromatic R (44.45%). On the other hand, microgreens in sand had a significantly higher canopy cover (55.95%) compared to those in cocopeat (47.11%).

### *3.2. Effect of LED Light and Substrate on Yield and Dry Weight*

The results from the ANOVA indicated that the interaction between substrates and LED light treatments was not significant. Similarly, no significant differences in yield were noted among LEDs. Regarding the effects of LEDs on dry weight, significant differences were noted between monochromatic R and all other LEDs. No differences were noted between B + R + W, W and monochromatic B LEDs (Table 2). Dry weight among the substrates ranged from about 1.0 g (cocopeat) to 1.3 g (sand). Regarding the yield, significant differences were found among the substrates but not the LED lights. The microgreen yield in sand and cocopeat–sand mix differed significantly from cocopeat alone ($p < 0.05$). Sand alone had a yield that was not significantly different from the cocopeat–sand mix.

**Table 2.** Effect of LED light and substrate on yield and dry weight of *Brassica carinata*.

| Treatment | Yield (g) | Dry Weight (g) |
|---|---|---|
| **LED Lights** | | |
| B | 17.9 (1.94) [a] | 1.2 (0.11) [ab] |
| R | 16.0 (0.86) [a] | 1.0 (0.06) [c] |
| W | 18.8 (2.36) [a] | 1.3 (0.17) [a] |
| B + R + W | 19.5 (2.22) [a] | 1.2 (0.10) [ab] |
| *P* | 0.339 | 0.053 |
| $LSD_{0.05}$ | 3.73 | 0.23 |
| F (3,33) | 1.28 | 5.38 |
| **Substrates** | | |
| Cocopeat | 15.2 (1.75) [b] | 1.0 (0.08) [a] |
| Sand | 19.2 (1.54) [a] | 1.3 (0.10) [b] |
| Cocopeat + sand | 19.8 (1.76) [a] | 1.2 (0.13) [ab] |
| *P* | 0.013 | 0.016 |
| $LSD_{0.05}$ | 3.23 | 0.20 |
| F (2,33) | 11.29 | 13.14 |

Mean separation by the Tukey test at the 5% significance level. Values in brackets are standard errors of means. Values without a letter in common in a column within a factor are significantly different ($p < 0.05$).

### *3.3. Effect of LED Light and Substrate on Phytochemical Content*

*Carotenoids:* There were significant differences for carotenoids among LED lights ($F(3,24) = 1270.56250$, $p < 0.001$), substrates ($F(2,24) = 50.24509$, $p < 0.001$) and their interactions ($F(6,24) = 1814.12864$, $p < 0.001$). Microgreens under B + R + W light in cocopeat had the highest carotenoid content (644.4 mg $kg^{-1}$ DW). Under monochromatic B and R, more

carotenoids were found in sand compared to cocopeat and in the cocopeat–sand mix. Under W and B + R + W, cocopeat had higher carotenoids relative to those in sand alone and the cocopeat–sand mix (Figure 1A).

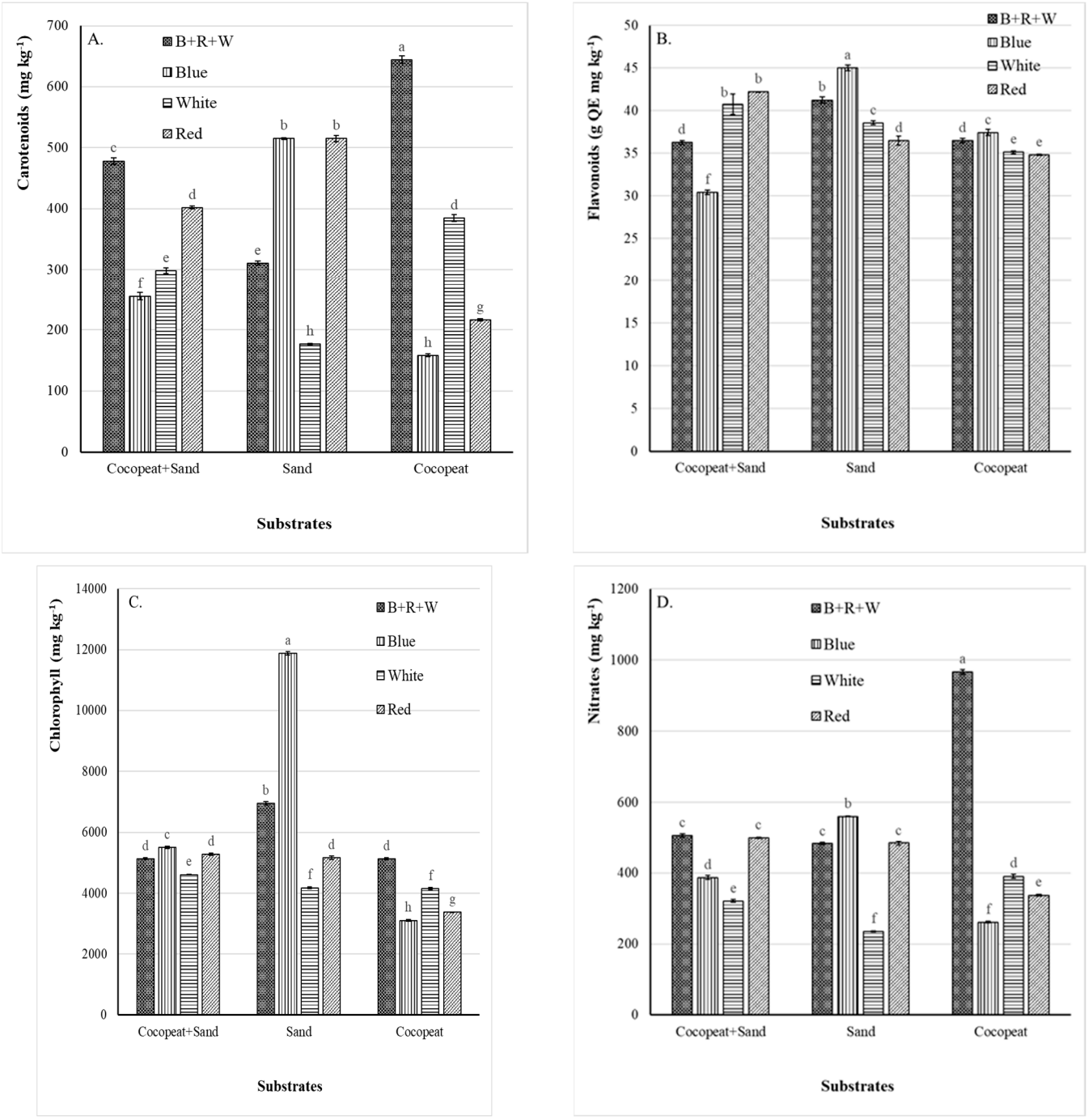


**Figure 1.** Effect of LED light on phytochemicals ((**A**) carotenoids, (**B**) flavonoids, (**C**) chlorophyll and (**D**) nitrates) under different substrates (cocopeat + sand, sand and cocopeat). Bars represent standard errors of means. Different letters indicate significant differences at $p < 0.05$.

*Flavonoids:* Flavonoids similarly showed significant differences among LED lights ($F(3,24) = 100.7731207$, $p < 0.001$), substrates ($F(2,24) = 98.2264237$, $p < 0.001$) and interactions ($F(6,24) = 105.0911162$, $p < 0.001$). Monochromatic B and B + R + W had higher flavonoid contents in sand than in cocopeat alone as well as in the cocopeat–sand mix. Under

monochromatic B in sand, flavonoids were 16.8% higher than in cocopeat and 32.4% higher than in the cocopeat–sand mix. For B + R + W in sand, flavonoids were 11.5% higher than in cocopeat and 12.0% higher than in the cocopeat–sand mix. Monochromatic R had higher flavonoid contents in sand alone than in cocopeat alone by 4.6% but lower flavonoid contents in sand alone than in the cocopeat–sand mix by 15.7%. Similarly, under W, sand had 9.8% more flavonoids than cocopeat alone but less flavonoids by 6.3% than in the cocopeat–sand mix (Figure 1B).

*Total Chlorophyll:* Total chlorophyll content differed significantly among LED light ($F(3,24) = 2690.467$, $p < 0.001$) and substrates ($F(2,24) = 6647.472$, $p < 0.001$). In addition, the interaction between substrate and lights was significant ($F(6,24) = 2957.422$, $p < 0.001$). Except for W, total chlorophyll content under monochromatic B, R and B + R + W was higher in sand compared to cocopeat. The highest total chlorophyll content (11,880 mg $kg^{-1}$) was observed under monochromatic B in sand while the lowest (3100 mg $kg^{-1}$) was under monochromatic B in cocopeat, a reduction of 73.9%. The chlorophyll content under B + R + W was higher in sand by 26.1% compared to B + R + W in cocopeat substrate, while for monochromatic R it was 34.5% higher in sand than in cocopeat (Figure 1C).

*Nitrates:* There were significant differences for nitrates among LED lights ($F(3,24) = 1696.0669$, $p < 0.001$), substrates ($F(2,24) = 110.4731$, $p < 0.001$) and interactions ($F(6,24) = 983.5374$, $p < 0.001$). Microgreens under B + R + W in cocopeat had extremely higher nitrates (966.2 mg $kg^{-1}$ DW) compared to other treatments. Except under W and B + R + W, nitrate contents were higher in sand than in cocopeat. Under monochromatic B, nitrate content in sand was higher by 53.4% compared to cocopeat, while for monochromatic R it was 30.3% higher compared to cocopeat (Figure 1D).

## 4. Discussion

### *4.1. Effect of LED Light and Substrate on Height, Leaf Area and Canopy Cover*

In recent years, several scientific reports addressed the role of light in stimulating specific plant photoreceptors, allowing plants to be manipulated to produce desirable phytochemicals and nutrients. Lighting systems for indoor farming can therefore be designed to maximize growth, control morphology and optimize yield [24]. This study established that *B. carinata* grown under monochromatic B were significantly taller compared to those grown using a monochromatic R source. Such a result is surprising since it is commonly acknowledged that monochromatic B decreases hypocotyl elongation. For example, the stem length of baby lettuce decreased by 33% when a supplemental B treatment was provided [25]. Furthermore, lettuce grown using an increased ratio of red radiation had an increased shoot height and shoot/root ratio compared to that grown using a blue light source [26]. Inconsistencies in results on the effect of different spectral regions across plant species and phenological stages have been acknowledged as a gray area requiring further research [27]. Monochromatic B and B in combination with far-red light were found to increase mustard (*Brassica juncea*) and arugula (*Eruca sativa*) microgreen elongation (as defined as plant height) [28]. The results presented herein suggest that sand alone or the cocopeat–sand mix had better growth than cocopeat, indicating that these substrates provided a better growing environment. This could be due to the physiochemical properties such as low water retention capacity allowing good aeration as compared to cocopeat which could have retained excessive moisture potentially leading to anoxia conditions. Similarly, ref. [29] reported that using cocopeat-based mixes with other coarser materials such as burnt rice hull improved the growth of *Celosia cristata.*

The present research also found that B + R + W and white light resulted in better yield performances than monochromatic red or blue. This was previously associated with synergistic effects of the different spectral regions. Red light combined with varying ratios of blue has been reported to enhance the growth characteristics of lettuce, spinach, kale, basil and sweet pepper compared to red light alone [27]. Similarly, leaf area among other growth parameters of lettuce increased with an increase in the proportion of red light in combination with blue [30]. For leaf area and canopy cover, B + R + W LED in the ratio

of 1:1:1 and cocopeat–sand mix enhanced the leaf growth of *B. carinata* microgreens. In this study, a cocopeat-based substrate (cocopeat–sand mix) showed increased leaf area of *B. carinata* microgreens. Similar results showed that cocopeat-based substrate increased plant growth, yield, nutritional, biochemical composition and antioxidant activity of various microgreen species [31]. These positive effects were attributed to enhanced nutrient acquisition, water retention and root development.

### *4.2. Effect of LED Light and Substrate on Yield and Biomass*

Yield is an important parameter in microgreen production because microgreens are sold on a fresh weight basis [32]. One of the limiting factors in microgreen production continues to be low yield due to various elements [33]. Microgreen yield can be affected by seed quality [34], growing media [35], and light quality and intensity [36], among other factors. In our study, both substrate and light quality significantly affected the yield and dry matter accumulation for *B. carinata.* Notably, the yield of microgreens varied across the different light spectra used, being highest under W. The results obtained are similar to those reported in the literature where fresh weight, which was used as a measure of yield, responded differently in plants grown using different light spectra. On the other hand, in the experiments presented herein, the increase in yield also depended on the substrate used. For *B. carinata* microgreens, a higher yield was recorded in sand alone or in the cocopeat–sand mix. In previous research comparing different substrates, the yield of sunflower microgreens was significantly affected by the type of substrate used [12]. Dry mass yield is a good indicator of crop productivity and photosynthetic efficiency [37] in microgreens. In our study, the highest dry matter accumulation was in microgreens grown using W. Conversely, microgreens grown in cocopeat using R had the lowest dry matter accumulation. Therefore, a significant effect resulting from substrate was noticed in our trial indicating the importance of substrate and lighting on the yield of *B. carinata* microgreens. Other studies on dry matter assessment of microgreens seem to indicate interspecies variability. For example, ref. [38] found differences in dry mass accumulation within W and R for broccoli, cabbage and radish microgreens.

### *4.3. Effect of LED Light and Substrate on Phytochemical Content*

#### 4.3.1. Carotenoids

Microgreens grown using B + R + W and in cocopeat had higher amounts of carotenoids. This is consistent with previous observations on the effect of light treatments on carotenoid accumulation in plants, where the R + B combination increased carotenoid accumulation in lettuce, spinach and pepper [27], while in kale and basil, carotenoid accumulation was increased under monochromatic B. Earlier studies also demonstrated that R/B combinations positively influenced carotenoid accumulation in lettuce [26]. Conversely, however, enzymatic activities involved in the metabolic pathways of carotenoid pigments were largely increased under monochromatic B, resulting in higher carotenoid accumulation in Chinese cabbage [25]. For Brassica sprouts, carotenoid transcription of biosynthesis genes, namely PSY, βLCY and βOHASE1, was enhanced by a higher B percentage compared to R [39], therefore increasing the carotenoid accumulation in the sprouts. Similar results were associated with a combined spectrum (resulting from the integration of blue, red and amber diodes) that enhanced the transcription of a gene involved in carotenoid biosynthesis (PSY), leading to higher carotenoid accumulation in various *Brassica* plants [40]. In the present study, the results are consistent, as the treatment B + R + W often presented higher amounts of carotenoids. Such findings corroborate the concept that combined light spectra are superior to monochromatic B or R light supply. On the sand substrate, carotenoids were higher under monochromatic R and monochromatic B. We hypothesize that these two spectra may have boosted photosynthesis, and therefore leaf transpiration, a scenario that could have led to drought stress ultimately inducing carotenoid biosynthesis and accumulation. Further studies on water retention in sand (compared to other substrates) and how it influences carotenoid accumulation are needed to provide a conclusive explanation.

#### 4.3.2. Flavonoids

Flavonoids are important plant compounds that are produced as a result of stress to prevent DNA damage [41]. Light quality triggers different transcriptional genes that are used for the biosynthesis of flavonoids and could cause differences in the levels of flavonoid accumulation in plants [42]. In the current study, both monochromatic B and B + R + W enhanced the accumulation of flavonoid content in *B. carinata* microgreens grown on sand and cocopeat substrates, just as monochromatic R and W did in those grown on the cocopeat–sand mix. An earlier study indicates that monochromatic B highly influenced the accumulation of flavonoids by modulating the phenylpropanoid pathway, a pathway in which most plant secondary metabolites are synthesized [43]. The adoption of R/B combinations at low intensities was formerly found to increase the accumulation of flavonoids in lettuce [44]. This could have resulted from the influence of different R/B ratios on the phenylalanine ammonia lyase (PAL), chalcone synthase (CHS) and other enzymes involved in the flavonoid biosynthesis, ultimately leading to the accumulation of flavonoids [45]. For *Scrophularia kakudensis*, ref. [46] reported that flavonoid accumulation was higher in monochromatic B and R than in W. Furthermore, these effects of light were also influenced by the substrate used (although different from those adopted in this study). While monochromatic B enhanced flavonoid accumulation in cocopeat and sand, R and W enhanced the same phytochemical in cocopeat–sand mix. These subtle differences point toward a substrate–light interaction, as also previously hypothesized [47].

#### 4.3.3. Chlorophyll

Besides its role as photosynthetic pigment, total chlorophyll content is also one of the key indicators of quality in vegetables, as the green color indicates freshness, which leads to product acceptability or rejection by consumers. In microgreens, vivid and intense colors are particularly appreciated and tend to influence consumer preference [48]. Chlorophylls represent part of the light-harvesting complex and therefore play a significant role in photosynthesis. As reported in the literature, significant genotypic variations were observed for chlorophyll content in microgreens, with their level also being highly dependent on the lighting conditions [2,49]. In the present study, monochromatic B increased chlorophyll biosynthesis and accumulation in plant tissues. The role of B in boosting chlorophyll accumulation was evidenced in previous studies thanks to both increased photosynthetic efficiency and a concentration factor (e.g., as a consequence of lower leaf extension as compared with spectra with a higher R fraction) [49,50]. Blue light improves the expression of genes such as MgCH, GluTR and FeCH, involved in chlorophyll biosynthesis, while red light may lead to a reduction in 5-aminolevulinic acid, a tetrapyrrole precursor required for chlorophyll synthesis [51]. Furthermore, when a monochromatic R, a monochromatic B and a combination of R and B ratio (with R/B = 6) were alternatively applied to Chinese cabbage, a lower chlorophyll content was associated with monochromatic R, as a result of reductions in the synthesis of chlorophyll precursors including ALA, Proto IX, Mg-Proto IX and protochlorophyllide [51]. In an analysis of the effect of the tested substrates, higher chlorophyll content was observed in *B. carinata* grown using sand compared to those grown using cocopeat, which could have contributed to the higher yield observed for the same treatments. The use of sand for microgreen production is not common. Elsewhere, the use of sand as a substrate is reported as an additive to another substrate [35]. The effects of sand as a microgreen substrate may thus require some further investigation, e.g., by using different mixture combinations.

#### 4.3.4. Nitrates

Nitrates are among the main compounds that may negatively affect food safety. Vegetables can accumulate nitrates which are associated with harmful effects on human health, with toxic effects of methemoglobinemia and the possibility of causing an endogenous formation of carcinogenic N-nitroso compounds. Accumulation of nitrates in vegetables may vary depending on the species, the substrate used for production or the stage of

plant growth at harvest. Several studies reported that microgreens recorded lower levels of nitrates compared to their mature counterparts [52,53]; therefore, microgreens are commonly considered safe to consume within a healthy diet. As reported earlier, lighting conditions can influence the accumulation of nitrates in vegetables, thus affecting their quality [52]. Regarding the substrates, the result contrasts with what was reported by two studies that evaluated microgreens grown on different substrates and found significantly lower concentrations of nitrates in microgreens grown using cocopeat substrate [2,10]. In our case, cocopeat showed a higher nitrate content compared to the other substrates. This could possibly be because of the differences in the lighting sources during cultivation. Notably, no such results have been reported for microgreens, and this assumption could be further investigated.

### *4.4. Interactive Effects of Light and Substrate on Phytochemicals*

The current study reports some significant interactions between lighting treatments and substrate composition. For example, the interaction between cocopeat and B + R + W and the interaction between sand and B enhanced the production of all phytochemicals investigated here. Further, the cocopeat–sand mix and R exhibit a strong interaction except in the accumulation of carotenoids. This suggests that the effect of light was dependent on the substrate. No such results have been previously reported for microgreens. Possibly, the cause of these interactive effects may be associated with either reflective or absorptive attributes of the substrates. This could be better studied, e.g., by measuring the light intensity in a sealed box with light turned on and only one substrate at a time. The incident radiation could be absorbed or reflected depending on the substrate, leading to differences in lighting conditions experienced by the microgreens. Sand for instance is known to have the capacity to cause light scattering [54], while cocopeat due to its color and texture would be expected to absorb light. The light absorption and reflection are further affected by moisture content, which varies across different substrates. It will be good to test this assumption to understand the mechanisms involved in the noted interactive effects.

## 5. Conclusions

This study aimed to investigate the influence of LED light quality and different substrates on the growth, yield and accumulation of selected bioactive compounds of *Brassica carinata* microgreens. Our results demonstrate that substrate and light environment interact to influence the growth, yield and concentration of bioactive compounds of *B. carinata* microgreens, enabling improved cultivation strategies. A combination of various light spectra (B + R + W) offers a better chance of obtaining higher yields and better-quality *B. carinata* microgreens. A combination of cocopeat with sand is a viable alternative to cocopeat considering the additional benefits of lower costs and ubiquitous availability of sand. Further studies are needed to elucidate media-related physical and biochemical dynamics that could potentially influence how different lighting systems lead to the varied accumulation of phytochemicals. Since *B. carinata* microgreens have not been extensively studied (compared to other species), such exploratory studies should first focus on the most commonly studied microgreen taxa. Such an understanding would help to describe the specific influence of the interactions between substrates and LED ratios on the quality traits (nutritional value, color, texture, taste, etc.) of microgreens.

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