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The influence of LED light on the development and antioxidant status of broccoli (*Brassica oleracea* var. *italica*) microgreens

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Abstract: The aim of the research was to examine the influence of different light treatments on the growth, phytochemicals and antioxidant potential of broccoli microgreens. Plants were grown in a growth chamber under LED (light-emitting diode) cold white, red and blue light and under fluorescent cold white light (control). The results showed that white and blue light treatments were the best for microgreen growth. Higher concentration of pigments was recorded in plants grown under LED light compared to those grown under FL (fluorescent lamp) light. The content of phenols and flavonoids had a positive and significant correlation with DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidative capacity (r = 0.66 and r = 0.90, respectively). The first two principal components account for 97.92 % of the total variation of all observed traits in this trial. Based on the PCA (principal component analysis) results, it can be concluded that the traits total phenols content, carotenoid content, chlorophyll *a* and *b* content make up the largest share of variability in the obtained results and that the red light conditions were the most unfavourable for the content of phytochemical compounds and antioxidant potential.

Keywords: broccoli; flavonoids; LED lights; phenols; photosynthetic pigments

Broccoli (*Brassica oleracea* var. *italica*) microgreens are edible young vegetable greens that are approximately 2.5–7.5 cm tall. Microgreens are very rich in antioxidants and often have higher quantities than mature plants (Choe et al. 2018). Thanks to the high amounts of antioxidants they contain, microgreens reduce the risk of heart disease (Huang et al. 2016), diabetes (Wadhawan et al. 2018), and certain cancers (Zhou et al. 2016). The growth of plants as well as the production of secondary metabolites

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can be regulated by using different LED (light-emitting diode) lights (Manivannan et al. 2015; Lobiuc et al. 2017; Ali et al. 2019).

Plants use light as the main source of energy for photosynthesis, which regulates numerous other processes related to plant growth and morphology (Devlin et al. 2007; Paradiso, Proietti 2022). Fluorescent lamps (FL) (usually cold white), which emit a wide spectrum of light and imitate outdoor conditions, were previously used for growing plants in the controlled conditions of growth chambers. In recent years, LED lamps have been increasingly used, which have several advantages over FL: they are more energy efficient, last longer, offer the possibility of spectrum and intensity optimisation, and have low heat emission (Singh et al. 2015). Through specific photoreceptors, plants react both to the intensity of light and to its spectral composition. Both morphogenesis processes and the synthesis and accumulation of secondary metabolites depend on the spectral quality of light (Brazaitytė et al. 2015; Dou et al. 2017; Jones 2018; Landi et al. 2020; Turner et al. 2020). Blue (450 nm) and red (650 nm) light have the greatest effect on plant growth and the intensity of photosynthesis due to the absorption peaks of chlorophyll molecules (Lefsrud et al. 2008), but also on primary and secondary metabolism (Hasan et al. 2017; Bartucca et al. 2020). Hypocotyl growth was strongly enhanced in red light and reduced in blue light compared to white light in *Arabidopsis*. This might be caused by both enhanced elongation growth and the extended reproductive phase in the condition of red light (Spaninks et al. 2020). Blue and red light activate cryptochromes and phytochromes, which stimulate the accumulation of phenols and flavonoids in different plant species during germination (Acharya et al. 2016; Nam et al. 2018) and in adult plants (Kim et al. 2014; Taulavuori et al. 2018; Wang et al. 2020).

The influence of light of different spectra depends on the plant species, so it must be optimised for each plant species and working conditions (Liang et al. 2021). In *Brassicaceae* plants, an increased percentage of blue light affects the accumulation of phenols, anthocyanins (Ying et al. 2021), macro and micronutrients (Brazaitytė et al. 2021), while in the case of other species (green basil, peas, borage), these processes are more favorably affected by red light (Bantis 2021). Compared to FL, red or blue LEDs clearly showed an increase in main and secondary chemicals, including sugars, starches, proteins, polyphenols, and vitamin C (Mohidul et al. 2017). While blue LED light increased the antioxidant capacity, total amount of isoflavones, and phenolic content of soybean seeds, red LED light increased the accumulation of anthocyanins in *Malus domestica* Borkh more than blue LED light (Lekkham et al. 2016). *Anoectochilus roxburghii* responded favorably to blue LED light, and the plants' biomass, chlorophyll content, and secondary metabolites (flavonoids and total polyphenols) were all noticeably higher compared to the other LED lights used in the experiment (Wang et al. 2018). Phenolic and flavonoid compounds are considered to be the most important antioxidants and play important roles in plants, such as protecting against herbivores and pathogens (Kumar et al. 2014). They are also important for human health and can protect consumers from some types of cancer and cardiovascular diseases (Pérez-López et al. 2018).

The aim of this research was to compare the effects of different LED lights (blue, red and cold white) compared to cold white FL light on morphological growth parameters and antioxidant status (phenol, flavonoid content and antioxidant potential) of broccoli microgreens.

MATERIAL AND METHODS

Material. The seeds of the selected broccoli variety Br-3 were sown in containers filled with sterile soil substrate (Florabalt® Seed 2, Floragard), pH 5.6, which contains nitrogen (180 mg/L), phosphorus (100 mg/L), potassium (230 mg/L) and 0.8 g/L of salt. After germination, the plants were transplanted into individual pots and their growth and development were monitored. The effect of different light quality [LED red (LED R), blue (LED B), cold white (LED CW) and fluorescent cold white (FL CW)] on growth, photosynthetic pigment contents and antioxidants of broccoli microgreens was investigated. Photosynthetic photon flux density (PPFD) measured at the top of the plants was 14.5 μmol/m^2s^1.

All plants were grown in a growth chamber at a temperature of 23 ± 2 °C and under a light regime of a long day (16 h day, 8 h night). Morphological growth parameters were measured [length of stem and roots, fresh weight/matter (FW) and dry weight/matter (DW) of stem and roots, number of leaves] after 2 and 4 weeks of growth in growth chamber conditions. The DW was expressed

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as a percentage in relation to the FW according to the formula:

$$DW\ (\%) = DW\ (g)/FW\ (g) \times 100 \quad (1)$$

Based on the data, the growth index (*Gi*) was calculated (Klimek-Szczykutowicz et al. 2022):

$$Gi = (G_1 - G_0)/G_0 \quad (2)$$

where: G_1 – morphological growth parameter after 4 weeks of growth; G_0 – morphological growth parameter after 2 weeks of growth.

The content of photosynthetic pigments, flavonoids, phenol, and antioxidant potential were measured after 2 weeks.

Determination of photosynthetic pigments. The isolation and determination of chlorophyll and carotenoid content were carried out using Brouers and Michel-Wolwertz's method (1983). Chlorophyll content (Chl *a* and *b*) and carotenoid content (TCC) were determined spectrophotometrically (JENWAY 6850, Cole Parmer, Great Britain). Absorbance was measured at three wavelengths: 470 nm (maximum absorption for carotenoids), 645 nm (maximum absorption for chlorophyll *b*) and 663 nm (maximum absorption for chlorophyll *a*). The total content of chlorophyll and carotenoids was calculated according to the formulas of Lichtenthaler (1987) and expressed in mg/g of fresh sample weight.

Sample preparation for the determination of total flavonoids, phenols and antioxidant potential. About 0.5 g of the sample was macerated with 5 mL of methanol. The extraction lasted 24 h in the dark. After that, the mixture was centrifuged for 5 min at 6 000 rpm (Eppendorf 5430 R, Eppendorfg CA, Hamburg, Germany) and the supernatants were stored as prepared extracts at –20 °C until the time of analysis. The extracts were used to determine the total content of flavonoids, phenols and antioxidant potential. The measurement was performed in triplicate.

Total flavonoid content (TFC). Total flavonoids were determined based on a slightly modified version of the method of Zhishen et al. (1999). Forty (40) µL $NaNO_2$ and 70 µL $AlCl_3$ were mixed with 100 µL methanol extract and made up to 1 000 µL with water. After 6 min, NaOH was added to the reaction mixture, and after mixing, the absorbance was measured at 510 nm. The results are expressed as mg rutin equivalents (RE) per gram of fresh sample weight (mg RE/g FW).

Total phenols content (TPC). Total phenols were determined based on Folin-Ciocalteu method (Singleton et al. 1999). The methanolic extracts were mixed with an aqueous solution of Folin-Ciocalteu reagent, after which Na_2CO_3 was added. The sample prepared in this way was first incubated for 25 min in a water bath at 45 °C, then for 2 h in the dark at 25 °C, after which the absorbance was measured at 765 nm. The results are expressed as mg of gallic acid equivalents (GAE) per g of fresh sample weight (mg GAE/g FW).

Determination of antioxidant potential – DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay. The total antioxidant potential was determined by the modified DPPH method (Molyneux 2004). The sample extracts (10 µL) were added to 1.990 mL of 0.01 mM methanol solution of DPPH and incubated in the dark for 30 min, after which the absorbance was measured at 517 nm. The results were expressed as mg Trolox equivalents per g of fresh sample weight (mg TXE/g FW).

Statistical analysis. All data were statistically processed using Statistica software version 8.0 (StatSoft Inc., 2007). Statistical processing of the data included analysis of variance of a one-way analysis of variance (ANOVA) and separation based on Fisher's least significant difference (LSD) test at the level of significance $P \leq 0.05$, correlation and principal component analysis (PCA). The graphic presentation of the results was done using the computer program Microsoft Office Excel.

RESULTS AND DISCUSSION

In the experiment, the influence of different lights on morphological parameters, content of pigments, phenols, flavonoids and antioxidant potential was examined. Significant morphological differences were observed between the observed light treatments. After 2 weeks, plants grown under LED R light had the longest stem length (8.08 cm), but the length was not followed by stem weight. The length of these plants was 67 % higher compared to the control plants grown under FL CW (4.84 cm), while the weight (0.021 g) was fourfold lower compared to the same plants (Table 1). Compared to the plants grown under LED B light (3.51 cm), the stem length of these plants was 130 % higher, while the weight

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Table 1. Morphological growth parameters after two weeks of growth in growth chamber conditions

Light treatment	Stem lenght (cm)	Stem FW (g)	Stem DW (%)	Root length (cm)	Root FW (g)	Root DW (%)
LED CW	5.46 ± 0.45^{b}	0.034 ± 0.016^{b}	10.03 ± 0.58^{a}	1.324 ± 0.079^{a}	0.024 ± 0.11^{a}	10.12 ± 0.21^{b}
LED B	3.51 ± 0.27^{c}	0.047 ± 0.007^{b}	8.06 ± 0.53^{b}	0.962 ± 0.023^{b}	0.037 ± 0.09^{c}	9.87 ± 0.25^{b}
LED R	8.08 ± 0.75^{a}	0.021 ± 0.005^{c}	4.06 ± 0.04^{c}	0.737 ± 0.011^{c}	0.016 ± 0.02^{d}	7.23 ± 0.13^{c}
FL CW	4.84 ± 0.12^{bc}	0.085 ± 0.004^{a}	9.85 ± 0.05^{a}	1.17 ± 0.023^{b}	0.069 ± 0.06^{b}	15.26 ± 0.17^{a}

Results are expressed as a mean value ± SE (standard error) (n = 3)

DW – dry weight; FW – fresh weight; LED – light-emitting diode; CW – cold white; B – blue; R – red; FL CW – fluorescent cold white

$^{a–d}$means with the same small letters within the same column are not significantly different (P < 0.05)

was twice lower (0.021g vs. 0.047 g). The plants were thin, pale and brittle, in contrast to the plants grown under LED B light, which were more vital although slightly shorter than the control plants. Similar results were observed on different crops such as *Rehmannia glutinosa*, potato and strawberry (Sivakumar et al. 2006; Manivannan et al. 2015; Rocha et al. 2015). Edesi et al. (2017) showed that the highest FW of potato shoots and roots occurred in a wide spectrum (white LED). Also, plants grown under LED R lights had the smallest root length (0.737 cm), 37 % less compared to FL CW plants (1.17 cm), 23 % compared to plants grown under B (0.962 cm) and even 44 % compared to plants under LED CW light (1.324 cm) (Table 1). According to Wei et al. (2020), red light induces endogenous gibberellins that are involved in cell elongation and root inhibition.

After the initial growth, based on the calculated *Gi*, it was shown that LED R light was the least favourable for the plants, which showed the smallest increase in the length and weight of the stem (1.24 cm for length and 0.34 g for weight) and roots (0.56 cm for length and 1.24 g for weight). The increase in stem length was over 80 % lower compared to other light treatments, while the increase in stem weight was up to 95 % lower compared to LED CW light (7.33 g) (Figure 1). The highest increase in stem length (9.84 cm) and weight (7.33 g) was recorded in plants grown under LED CW. However, this light did not favour the growth of the length (0.91 cm) and weight (2.58 g) of the roots, which was almost half compared to the LED B light (1.72 cm for length and 4.52 g for weight) and a third less compared to the FL CW (1.25 cm for

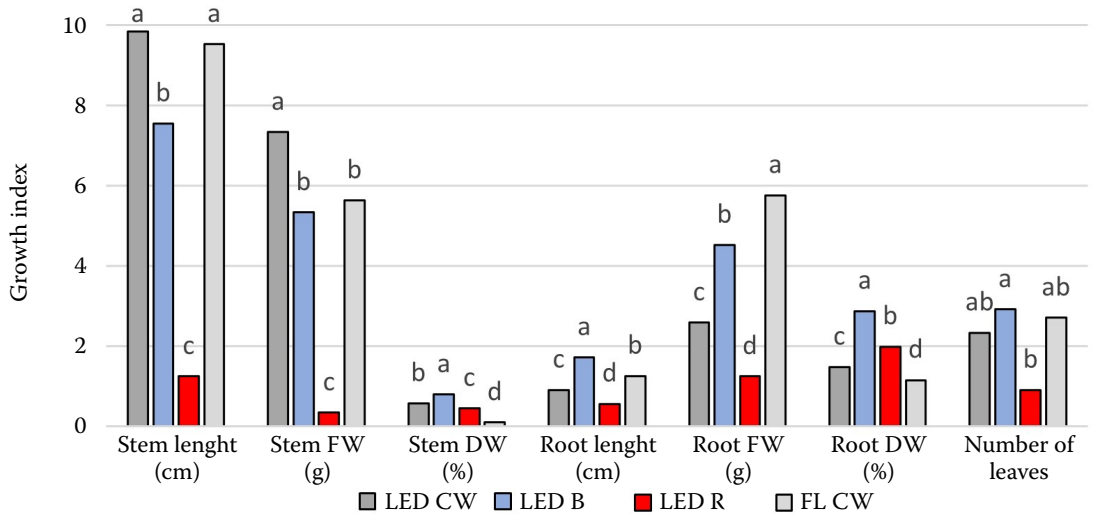

Figure 1. Growth index of morphological characteristics

FW – fresh weight; DW – dry weight; LED – light-emitting diode; CW – cold white; B – blue; R – red; FL CW – fluorescent cold white

$^{a–d}$values with the same small letters within the same column are not significantly different (P < 0.05)

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length and 5.76 g for weight) (Figure 1). Higher root growth under LED B compared to LED R and LED CW light was also observed in works with other crops (Manivannan et al. 2015).

After 4 weeks, plants had a uniform number of leaves under all light treatments, but during further growth, this number was significantly lower (3–4 fold) in plants under LED R light (0.90) compared to other treatments (Figure 1). Plants under LED B light produced more leaves (2.91), but not statistically significantly compared to LED CW light (2.33). Similar results were obtained in Manivannan et al. (2015) in the work with *R. glutinosa* and Borowski et al. (2014), who obtained a 2-fold higher leaf yield of lettuce under FL light than under LED light. Other authors have also found lettuce to show distinctly better growth under the white light of FL than under LED lamps (Hyeon-Hye et al. 2004).

The value of *Gi* for DW (%) was in the range from 0.090 to 0.793 in the case of the stem, while in the case of the root, it was in the range from 1.153 to 2.872. The highest increase in stem and root DW (%) was recorded in plants grown under LED B light (0.79 and 2.87) (Figure 1). A significant increase in DW (%) in the roots was also recorded in plants that grew under LED R light (1.99) compared to LED CW (1.48) and FL CW (1.15), 35 % and 72 %, respectively. The improvement of DW (%) by LED B and R was reported in *Oncidium* (Mengxi et al. 2011). FW and DW and seedling index of oriental melon seedlings were the highest under LED R and B 6 : 1 treatment (Cui et al. 2017)

A higher concentration of pigments was recorded in plants grown under LED light compared to those grown under FL CW light (Table 2). Chlorophyll content under FL CW light (1.04 mg/g FW) was 22 % lower compared to LED CW light (0.71 mg/g FW), while there was no statistically significant difference between this and LED B light (0.97 mg/g FW). The chlorophyll *b* content was the highest under the blue light in oriental melon (Cui et al. 2017). Borowski et al. (2014) obtained the same results regarding the content of pigments in lettuce, i.e. that LED light has a significantly better effect on the content of pigments. The lowest total chlorophyll content was measured in plants grown under LED R light (0.59 mg/g FW), 56 % less compared to FL CW and 41 % less compared to LED B light. These results are in contrast to those obtained in oilseed rape (Saleem et al. 2020), where red light increases and blue light decreases pigment content. Several reports have shown that the different reactions of plants to the same light treatments are determined by genetic diversity between plant species and among different cultivars within a species (Rocha et al. 2015; Edesi et al. 2017; Paradiso et al. 2018).

The TPC was slightly higher in plants grown under FL CW light (3.03 mg GAE/g FW) compared to plants under LED B light (2.95 mg GAE/g FW) (Table 2), and 30 % higher compared to LED CW light (2.13 mg GAE/g FW). The lowest TPC was recorded in plants that grew under LED R light (0.47 mg GAE/g FW). The TFC was higher in plants grown under LED CW light (7.96 mg RE/g FW) compared to LED B (5.44 mg RE/g FW) and LED R lights (3.14 mg RE/g FW) by 31 % and 60 %, respectively. The highest TFC was recorded in plants grown under control FL CW light. Total antioxidant potential was higher in plants grown under LED CW light than under LED B and R lights, and a 15 % higher antioxidant potential was measured compared to FL CW light. (Table 2).

Table 2. The total content of phytochemical compounds in broccoli extract

Light treatment	TPC (mg GAE/g FW)	TFC (mg RE/gFW)	DPPH (mg TXE/g FW)	Chl *a* (mg/g FW)	Chl *b* (mg/g FW)	Chl *a* and *b* (mg/g FW)	TCC (mg/g FW)
LED CW	2.13 ± 0.23^{b}	7.96 ± 0.54^{b}	1.40 ± 0.34^{a}	0.56 ± 0.24^{b}	0.14 ± 0.04^{b}	0.71 ± 0.13^{c}	0.17 ± 0.06^{c}
LED B	2.95 ± 0.47^{a}	5.44 ± 0.46^{c}	0.99 ± 0.23^{c}	0.73 ± 0.32^{a}	0.23 ± 0.08^{a}	0.97 ± 0.24^{b}	0.27 ± 0.09^{b}
LED R	0.47 ± 0.14^{c}	3.14 ± 0.21^{d}	0.63 ± 0.09^{d}	0.42 ± 0.12^{c}	0.14 ± 0.03^{b}	0.59 ± 0.22^{d}	0.13 ± 0.03^{d}
FL CW	3.03 ± 0.19^{a}	8.96 ± 0.14^{a}	1.19 ± 0.19^{b}	0.80 ± 0.16^{a}	0.23 ± 0.02^{a}	1.04 ± 0.12^{a}	0.29 ± 0.07^{a}

Results are expressed as a mean value ± SE (standard error) (n = 3)

TPC – total phenolic content; GAE – gallic acid equivalents; FW – fresh weight; TFC – total flavonoid content; RE - rutin equivalents; DPPH – 2,2-diphenyl-1-picrylhydrazyl; TXE – trolox equivalents; Chl *a*, *b* – chlorophyll *a*, *b*; Chl *a* and *b* – total chlorophyll content; TCC – total carotenoid content; LED – light-emitting diode; CW – cold white; B – blue; R – red; FL CW – fluorescent cold white

$^{a–d}$means with the same small letters within the same column are not significantly different ($P < 0.05$)

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Table 3. Correlation coefficients among phytochemical compounds and total antioxidant potencial of broccoli leaf extract

Traits	TPC	TFC	Chl *a* and *b*	TCC	DPPH
TPC	1.00	–	–	–	–
TFC	0.73*	1.00	–	–	–
Chl *a* and *b*	0.91*	0.58*	1.00	–	–
TCC	0.93*	0.59*	0.99*	1.00	–
DPPH	0.66*	0.90*	0.37	0.41	1.00

TPC – total phenolic content; TFC – total flavonoid content; Chl *a* and *b* – total chlorophyll content; TCC – total carotenoid content; DPPH – total antioxidative potencial
*significance at $P < 0.05$

TPC and TFC had a positive and significant correlation with DPPH antioxidative capacity ($r = 0.66$ and $r = 0.90$, respectively) (Table 3). Similar results were obtained in earlier studies on other cultures (Lachowicz et al. 2018; Gordanić et al. 2022). Kumar et al. (2014) found a positive correlation between TPC and antioxidant activity of *Lantana camara* leaf extract, but not between TFC and DPPH. Correlation between TCC and DPPH was positive but not significant, which is not in agreement with the study of Lachowicz et al. (2018) but is in agreement with Gordanić et al. (2022). These results indicate that phenols and flavonoids contribute the most to the antioxidant potential of broccoli leaf extract.

According to the correlation matrix for phytochemical and antioxidative properties for several parameters such as TPC-TFC, TPC-Chl *a* and *b*, TPC-TCC, TFC-Chl *a* and *b*, TFC-TCC, Chl *a* and *b*-TCC, high positive correlations were observed ($P < 0.05$) (Table 3). In our study, the strongest correlation was observed between Chl *a* and *b* and TCC ($r = 0.99$), while the weakest correlations were observed between Chl *a* and *b* and TFC ($r = 0.58$) and TCC and TFC ($r = 0.59$). This can be concluded based on the distribution of vectors for these traits shown on the biplot (Figure 2). The first two principal components account for 97.92 % of the total variation of all observed traits in this trial. The first principal component (PC1) includes 77.52 %, while the second principal component (PC2) includes 20.40 % of the total variability. PC1 is negatively correlated with all five traits, while PC2 explains the DPPH trait the most. The trait DPPH stood out with the longest vectors in the direction of the PC2 axis, and the trait TPC is closest to the direction of the PC1 axis (Figure 2). Based on the presented results, it can be concluded that the properties of TPC, TCC, Chl *a* and *b*, and TFC make up the largest share of variability in the obtained results.

The LED R light stood out on the opposite side of PC1 in relation to the observed traits, which indicates that the LED R light conditions were the most unfavourable for the content of phytochemical compounds and antioxidant potential (Figure 2). TFC and DPPH values under LED CW light conditions were above average. The values of TPC, TCC and Chl *a* and *b* under LED B light conditions were

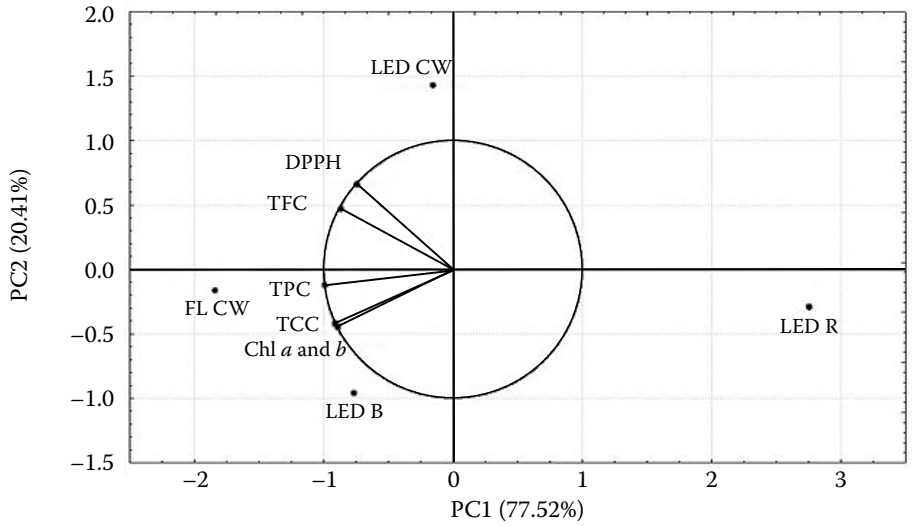

Figure 2. Principal component analysis (PCA) classification of light treatment based on the chemical parameters
TPC – total phenolic content; TFC – total flavonoid content; Chl *a* and *b* – total chlorophyll content; TCC – total carotenoid content; DPPH – 2,2-diphenyl-1-picrylhydrazyl; LED – light-emitting diode; CW – cold white; B – blue; R – red; FL CW – fluorescent cold white; PC1 – the first principal component; PC2 – the second principal component

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above average, while the values for the TFC and DPPH features were below average under the conditions of this lighting. The values of all observed traits under FL CW lighting conditions are above average.

CONCLUSION

The obtained results suggest that LED lighting can promote the growth of broccoli microgreens and improve morphological and phytochemical parameters. The LED CW, which emits a wide spectrum of light, and LED B are the best light treatments for broccoli. These treatments positively affected both morphological parameters and the content of phytochemical compounds. This may suggest that combinations of LED B and LED R light should be included in future research.

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