

## HOW MONOCHROMATIC AND COMPOSED LIGHT AFFECT THE KALE ‘SCARLET’ IN ITS INITIAL GROWTH STAGE

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### ABSTRACT

Interest in vegetables at their microgreen stage, especially those from the Brassicaceae family, has constantly grown due to their numerous health-promoting compounds. *Brassica oleracea* convar. *acephala* var. *sabellica* cv. Scarlet with purple leaf discolouration was used in the study. Four LED lighting treatments were applied: white light (control), monochromatic blue (430 nm), monochromatic red (660 nm) and purple, i.e., blue (30% in spectrum, 430 nm) mixed with red (70%, 620 nm and 660 nm in equal shares). Photosynthetic photon flux density (PPFD) was 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , photoperiod – 16 h light. The purple light promoted the cotyledon growth but decreased the soluble sugars content. The blue light significantly enhanced the anthocyanins synthesis and the radical scavenging activity (RSA). While under white light, the highest concentration of free amino acids and the lowest RSA were observed. As regards the phenolic compounds and photosynthetic pigments content, the reaction of kale to white light was similar to those observed under the purple and red light conditions. The experiment discussed here is of great practical importance and reveals the need for more in-depth research.

**Key words:** LED, pigments, phenolic compounds, radical scavenging activity

### INTRODUCTION

Microgreens are crops of vegetables or herbs consumed at initial growth stages. Some authors claim that ‘microgreen’ is a marketing term [Renna and Paradiso 2020]. However, no legal definition of this speciality crop has emerged. It seems that the term ‘microgreen’ is used flexibly depending on the species; it means a plant which is harvested between 7 and 21 days from sowing, has fully developed cotyledons, and the first true leaves have emerged [Di Gioia et al. 2017, Renna and Paradiso 2020]. According to recent studies, microgreens are rich in nutritional and bioactive compounds, which play a significant role in health-promoting diets [El Nakhel et al. 2021, Teng et al. 2021]. Brassicaceae microgreens are considered to be an excellent source of antioxidant bioactive

compounds for human health, such as ascorbic acid, polyphenols or carotenoids [De la Fuente et al. 2019, Fiutak and Michalczyk 2020]. Due to their small size, many microgreens are cultivated in indoor conditions on a micro-scale, which helps to control proper irradiation (light spectrum and intensity). Using an effective light spectrum to produce this speciality crop is a new and unresolved issue.

Light is commonly responsible for plants’ growth and biochemical composition. In the last few decades, LED light technology has expanded in horticulture research because it offers a narrow waveband, crucial for a specific effect in plant production [Bantis et al. 2018, Matysiak and Kowalski 2021]. It is worth emphasising that exposure to blue wavelengths stim-

ulates the production of more essential pro-health substances in many horticultural products [Kopsell et al. 2015, Samuolienė et al. 2017]. However, the reaction of plants to light in their initial growth stages may be quite different from that of mature plants [Brazaitytė et al. 2016, Turner et al. 2020]. In microgreens cultivation, most studies concern mixed light: blue is often used in combination with red, green or other wavelengths in various ratios, and the effect of pure light colour is analysed rarely [Gerovac et al. 2016, Viršilė et al. 2017].

In this paper, we present the results of our study on *Brassica oleracea* convar. *acephala* var. *sabellica* 'Scarlet'. We hypothesised that purple (red + blue) light would be the most effective in modifying the growth, pigments or phenolic compounds content in kale of this variety. However, the way monochromatic blue or red light affects morphogenesis and the levels of biochemical compounds was also investigated. Information in the literature about the effects of monochromatic light on kale with purple leaves discolouration in the microgreen phase is limited. Our experiment also focused on practical aspects. White light is the safest for the human eye (e.g., for staff supervising plants' growth), so it was used as the reference and compared with other light treatments. We chose kale microgreen short production (not longer than 7 days) in small boxes to limit personnel's participation in the production process. It ensured that the personnel would be protected against single-colour light.

## MATERIAL AND METHODS

### Plant material and growth conditions

*Brassica oleracea* convar. *acephala* var. *sabellica* cv. Scarlet (W. Legutko Company, Poland) with a purple leaf discolouration, recommended for microgreen cultivation, was used in the experiment. Kale seeds (after decontamination in 70% ethanol and then rinsed out twice in distilled water) were transferred into plastic trays (20 × 15 × 8 cm), each padded with 6 sheets of autoclaved paper towel and moistened with 30 cm<sup>3</sup> of 10% Hoagland medium. Two grams of seeds were sown into each tray, sealed with a transparent lid and placed in a lighting chamber for 7 days (with a constant ambient temperature of 24°C). There were four growing repetitions for each light treatment: one rep-

etition was the one tray with sprouts germinated from 2 g of seeds; i.e., about 600 plants were grown in one repetition.

The experimental lighting chamber was equipped with white, red and blue diodes connected with a system controlling the spectral composition, light intensity, and photoperiod. The experimental lighting chamber was custom-made by PXM Marek Żupnik sp.k. company (Podłęże, Poland), using 24 OSRAM OSOLON diodes (it is not mass-produced). Four lighting treatments were used in the study: (1) white light as a control treatment – White; (2) monochromatic blue (430 nm) – Blue; (3) monochromatic red (660 nm) – Red and (4) 30% blue (430 nm) with 70% red (mixed 35% 620 nm with 35% 660 nm) – Purple. The spectral composition of the light used in each treatment is shown in Figure 1. The photosynthetic photon flux density (PPFD) reaching the plants was 100 μmol m<sup>-2</sup> s<sup>-1</sup>, and the photoperiod was 16 h day/ 8 h night. After 7 days, when the plants reached the complete development phase of the cotyledons, and the first true leaves emerged (microgreens phase assumed), growth and chemical analyses were conducted.

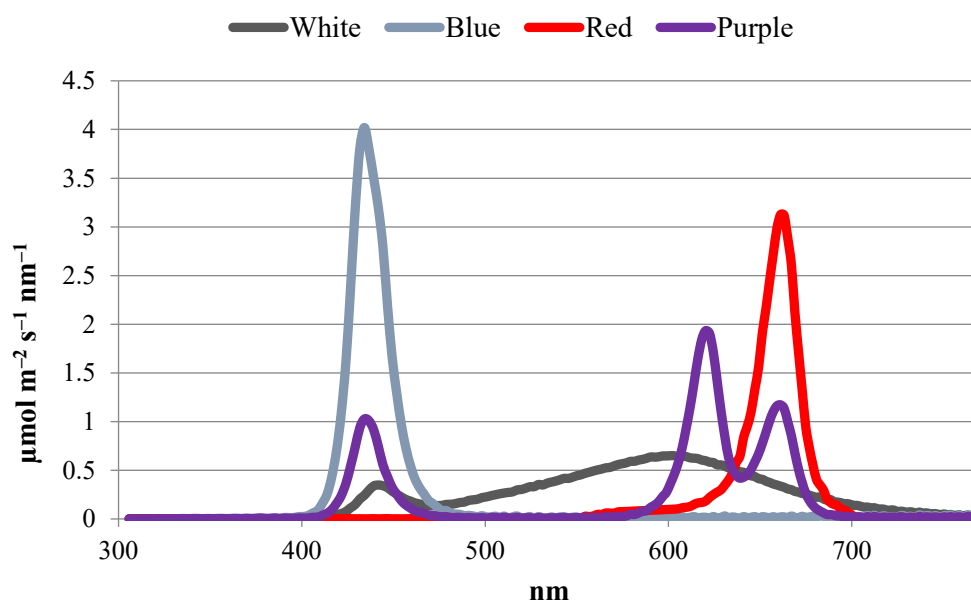
### Growth parameters and chemical analyses

Forty randomly chosen plants (n = 40) from each light treatment (ten from each repetition) were harvested to perform the following growth measurements: hypocotyl length and diameter; cotyledon length, width and thickness (length or width by using a ruler, and other parameters – with an electronic calliper, thickness – using dial thickness gauges from Mitutoyo, Japan).

For fresh and dry weight analyses, two measurements in each of the four repetitions were conducted (n = 8). Single measurements consisted of randomly chosen 10 plants (whole, with roots) to calculate one plant's fresh or dry weight. Fresh material was dried at 105°C for dry matter measurements to determine their constant weight.

The remaining plants from the four growing repetitions were macerated in liquid nitrogen and refrigerated at –20°C. All chemical analyses were done in two laboratory replications (n = 8). A spectrophotometer HITACHI U2900 (Tokyo, Japan) was used for spectral analyses.

Photosynthetic pigments were extracted in 80% acetone (Stanlab; 0.1 g samples of plant material were



**Fig. 1.** Spectral characteristics of the light emitted by LED lamps measured using SpectraPen mini (Photon Systems Instruments, Drásov, Czech Republic) after dark calibration. Light treatments: White – white light as a control treatment; Blue – monochromatic blue (430 nm); Red – monochromatic red (660 nm) and Purple – 30% blue (430 nm) with 70% red (mixed 35% 620 nm with 35% 660 nm)

used; the final volume of the extract after centrifugation for 20 min at 5000 rpm was 10 mL); absorbance readings were carried out at wavelengths of 470, 646 and 663 nm. The procedure and calculations followed the method by Wellburn [1994].

To conduct the other chemical analyses (described below), methanol extracts from the frozen plant material were prepared: 0.1 g samples were extracted in 80% methanol; (Stanlab); the final volume after centrifugation for 20 min at 5000 rpm was 5 mL. The resultant extracts were used to carry out further chemical determinations.

Soluble sugars content was evaluated using the method described by Maness [2010]. The optical density of the methanol extracts after a reaction with an anthrone reagent (Sigma-Aldrich) and sulphuric acid (Chempur) was measured with a spectrophotometer at 578 nm. The content of soluble sugars was calculated based on the glucose (Sigma-Aldrich) calibration curve as a standard.

Free amino acids content was determined using ninhydrin (Sigma-Aldrich) according to the method of Chutipongtanate et al. [2012]. After the reaction with

ninhydrin, samples' absorbance measurements were carried out at 570 nm, and the calibration curve was prepared with glycine (Sigma-Aldrich) as a standard.

The total phenolics, phenylpropanoids, flavonols and anthocyanins contents were measured following the Fukumoto and Mazza [2000] method (aliquots of 0.25 mL of methanol extracts were added to 0.25 mL of 0.1% HCl in ethanol and 4.5 mL of 2% HCl). After incubation for 15 min at room temperature, the absorbance was measured at 280 nm, 320 nm, 360 nm and 520 nm. The total phenols, phenylpropanoids, flavonols and anthocyanins were calculated based on the calibration curves of chlorogenic acid (Sigma-Aldrich), caffeic acid (Sigma-Aldrich), quercetin (Sigma-Aldrich) and cyanidin (Sigma-Aldrich), respectively.

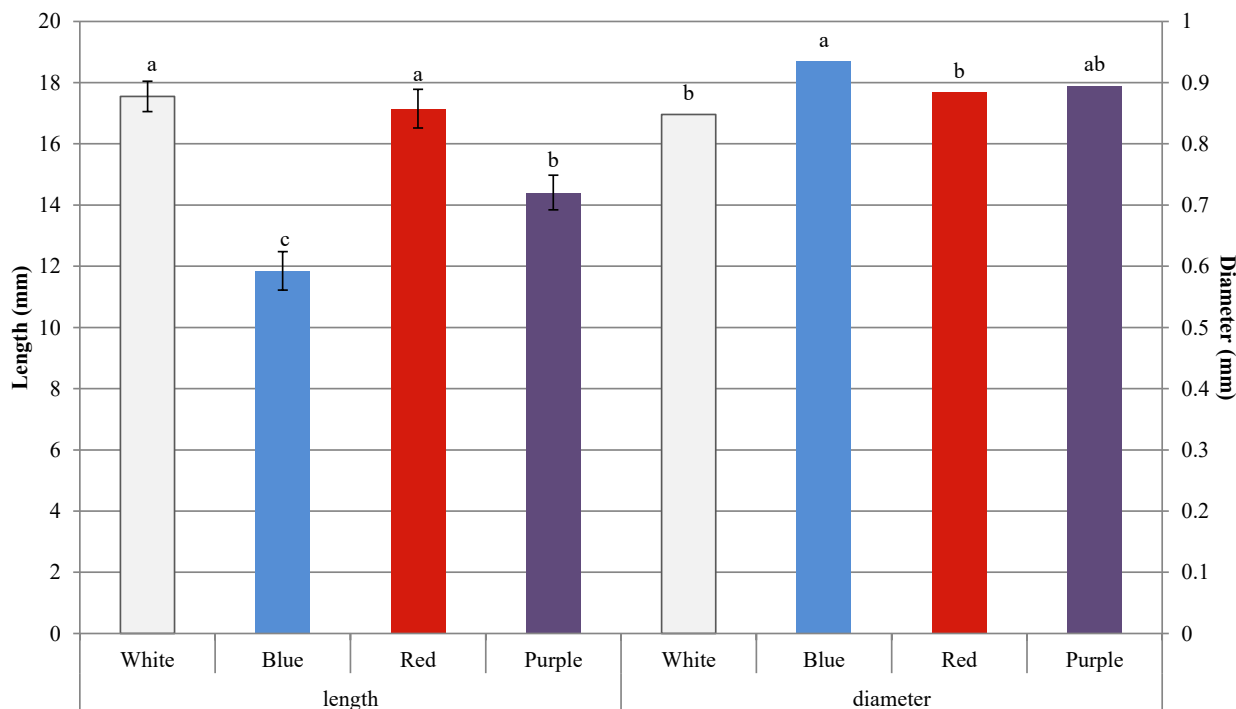
Radical scavenging activity (RSA) was found following Pekkarinen et al. [1999] with the use of 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich) free radical. The radical scavenging activity of the methanol extracts was measured at 516 nm after 5 min of incubation with a DPPH ethanolic solution (0.05 mL extract was added to 2.95 mL DPPH solution). The results were expressed as the percentage of DPPH neutralisation.

**Statistical analysis.** The results were subjected to ANOVA analysis (STATISTICA 13) using Fisher's LSD posthoc test for homogeneous groups determination and recognised as significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

The hypocotyl length of kale 'Scarlet' decreased significantly under the Blue spectrum compared to the other light treatments (Fig. 2). The difference between the Blue spectrum and the control (White) or Red light was about 32.5% and 31%, respectively. This observation can be explained by the fact that blue light through cryptochrome effectively degrades auxins and gibberellins in the stem, hence the weaker elongation growth [Folta et al. 2003]. However, Brazaitytė et al. [2021] obtained different results from our experiment with kale 'Red Russian' microgreens. They found that monochromatic blue and monochromatic red light similarly promoted the hypocotyl elongation compared to mixed (blue + red) light. In other research, solely

(100%) blue light enhanced the elongation growth of plants in their initial growth phase [Kong et al.2019]. In contrast, Nanya et al. [2012] showed that the stem length of tomato seedlings grown under 100% blue light was significantly shorter than that under 100% red light. Also, in the research of Ying et al. [2020], the hypocotyl length of kale 'Red Russian' microgreen decreased with an increased proportion of blue light in the spectrum. More detailed studies are needed to investigate and account for the discrepancies between the growth of hypocotyls after exposure to blue light (for example, analysis of auxin degraded by blue light or photoreceptors content and action). In the present study, the hypocotyl diameter of kale (Fig. 2) under Blue light was increased by about 10% compared to the control, but no significant difference between the Blue and Purple (red with blue) light treatments was found. A beneficial role of Purple light in enhancing the growth of kale cotyledons was also found in our study (Fig. 3). This spectrum significantly increased the cotyledon thickness compared to the Red and

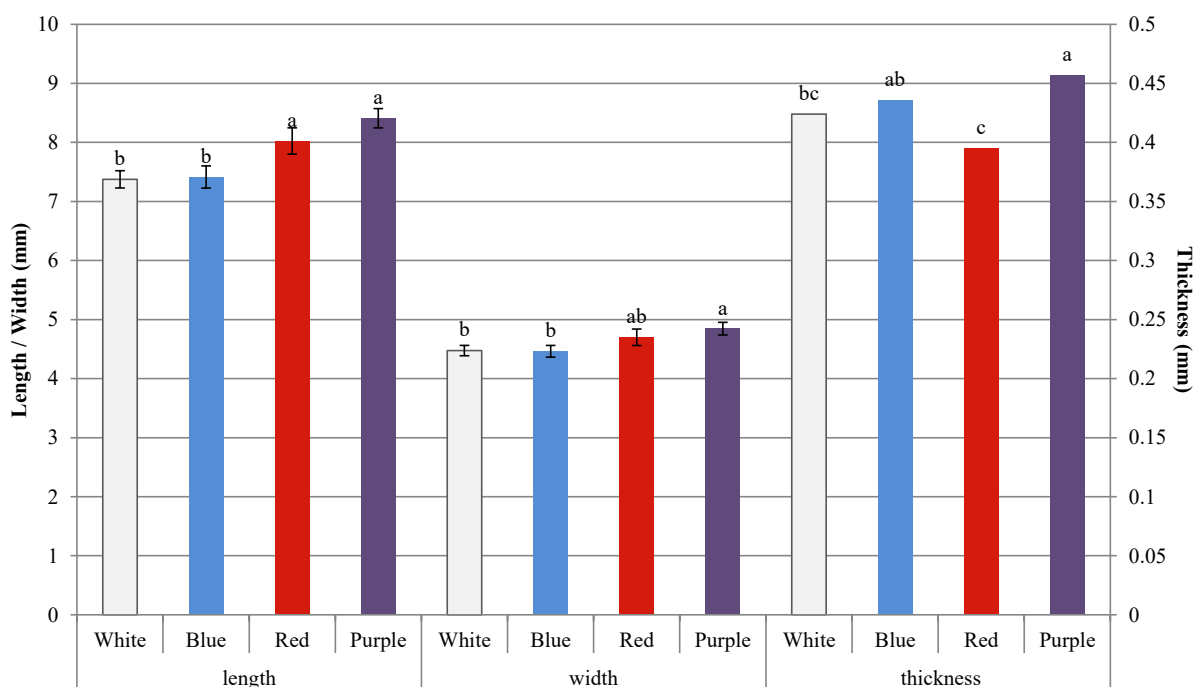


**Fig. 2.** Morphological characteristics of the hypocotyl of kale seedlings grown under various LED light spectra. Data were subjected to one-way ANOVA ( $n = 40$ ). Means for individual parameters followed by different letters are significantly different at  $p < 0.05$  according to Fisher's LSD test

White ones. Concerning the Blue and White spectrum, Purple and Red light stimulated cotyledon growth in length and width. Ying et al. [2020] observed that the hypocotyl length and cotyledon area of kale decreased linearly with an increasing blue light percentage in the spectrum, and they recommended 95% red light mixed with 5% blue for indoor production of kale microgreens. Similarly, 100% blue light reduced the cotyledon length and width compared to 100% red light in the experiment of Kong et al. [2019]. Our re-

sults show that 30% blue light combined with 70% red light (Purple light) positively impacted the cotyledon growth compared to 100% Blue light, but this spectrum (Blue), indeed, reduced the hypocotyl length.

As shown in Table 1, Blue light reduced the content of chlorophyll a in comparison to the White and Purple light treatments. The results indicate that the interaction of red and blue light enhances the synthesis of assimilatory pigments. However, there were no statistical differences between the contents of chloro-



**Fig. 3.** Morphological characteristics of the cotyledon of kale seedlings grown under various LED light spectra. Data were subjected to one-way ANOVA ( $n = 40$ ). Means for individual parameters followed by different letters are significantly different at  $p < 0.05$  according to Fisher's LSD test

**Table 1.** Effect of various light spectra on photosynthetic pigments content (mg/100 g FW) in kale seedlings

Light	Chl a	Chl b	Car	Chl a : b	Chl : Car
White	49.6 ±4.42 a	17.8 ±1.56	15.6 ±1.40 a	2.8 ±0.05 c	4.3 ±0.05 b
Blue	36.5 ±2.61 b	14.3 ±1.09	11.4 ±0.77 b	2.6 ±0.08 d	4.4 ±0.06 b
Red	45.4 ±3.44 ab	13.7 ±0.94	13.4 ±1.03 ab	3.3 ±0.05 a	4.4 ±0.04 b
Purple	49.9 ±6.62 a	17.0 ±2.40	14.3 ±1.80 ab	2.9 ±0.04 b	4.7 ±0.06 a

Chl a – chlorophyll a, Chl b – chlorophyll a, Car – carotenoids, FW – fresh weight. Data (mean ±SE) were subjected to one-way ANOVA ( $n = 8$ ). Means within a column followed by different letters are significantly different at  $p < 0.05$  according to Fisher's LSD test

phyll a or b in the plants treated with Red or Blue light separately. The effect of distinct wavelengths of light on pigment composition was also observed by Lefsrud et al. [2008]. The researchers noticed greater chlorophyll a and b accumulation in plants treated with red (640 nm) light compared to blue (440 nm). In contrast, increased accumulation of  $\beta$ -carotene was observed by Lefsrud et al. [2008] under blue rather than red light. The highest ratio of chlorophyll a : b was found in the case of red light, whereas the lowest ratio – was in the blue light treatment (the difference was about 20%). The contents of chlorophylls and carotenoids in tissues are often correlated, and an increase in the ratio of carotenoids to chlorophylls content may indicate stress or tissue ageing [Niroula et al. 2019]. Therefore, the ratio of both groups of pigments can be an interesting parameter describing the physiological state of leaves. In our experiment, Purple light increased the ratio of chlorophylls to carotenoids, unlike in the other

treatments, which may suggest that the leaves function well in this light. It must be admitted that the contents of the assimilation pigments shown in the present study are not significant. In a review paper, Turner et al. [2020] showed that microgreens of most species studied, including kale, contain less chlorophylls and carotenoids than mature plants.

No significant differences in the fresh weight of kale were observed (Tab. 2). Solely Blue irradiation increased the dry matter of kale microgreens compared to the other light treatments. In other research, kale microgreens' shoot fresh weight and dry weight was similar under 100% red, 100% blue, and the mixed (25% blue + 75% red) light [Brazaitytė et al. 2021].

The soluble sugars content significantly decreased when Purple light was used; in the case of the other light treatments, the sugars concentration was higher than in Purple light. Also, soluble sugars content was similar in plants treated with White, Red and Blue light.

**Table 2.** Effect of various light spectra on plant fresh or dry weight and some biochemical components of kale seedlings (FW – fresh weight)

Light	Fresh weight (mg/plant)	Dry weight (%)	Soluble sugars (mg/100 g FW)	Amino acids (mg/100 g FW)
White	42.5 ±1.54	6.68 ±0.21 b	1497.7 ±130.16 a	1316.5 ±97.68 a
Blue	45.0 ±0.70	7.92 ±0.22 a	1297.7 ±92.98 a	768.7 ±68.28 b
Red	42.2 ±3.71	7.23 ±0.17 b	1500.0 ±100.87 a	982.7 ±103.95 b
Purple	46.5 ±1.64	6.97 ±0.14 b	982.9 ±78.13 b	832.0 ±100.29 b

Data (mean ±SE) were subjected to one-way ANOVA (n = 8). Means within a column followed by different letters are significantly different at  $p < 0.05$  according to Fisher's LSD test

**Table 3.** Effect of various light spectra on some phenolic compounds and radical scavenging activity (RSA) of kale seedlings

Light	Total phenols (mg/100 g FW)	Phenyl-propanoids (mg/100 g FW)	Flavonols (mg/100 g FW)	Anthocyanins (mg/100 g FW)	RSA (%)
White	384.5 ±18.9 ab	132.6 ±7.78 ab	121.1 ±6.7 ab	46.1 ±3.43 b	2.30 ±0.19c
Blue	426.6 ±28.06 a	157.1 ±11.53 a	142.8 ±11.3 a	67.0 ±6.44 a	5.50 ±0.11a
Red	339.5 ±21.50 b	127.0 ±8.10 b	113.8 ±6.79 b	39.0 ±2.08 b	4.64 ±0.24b
Purple	332.4 ±35.20 b	121.0 ±12.71 b	108.4 ±11.0 b	40.3 ±3.70 b	4.66 ±0.37b

Data (mean ±SE) were subjected to one-way ANOVA (n = 8). Means within a column followed by different letters are significantly different at  $p < 0.05$  according to Fisher's LSD test

The free amino acids content in kale microgreens was the highest in control, whereas no significant differences were found between the other light treatments. The plants accumulated about 1.8-fold fewer amino acids than the control under the Blue light spectrum. In the research of Li et al. [2019], adding blue light as a supplementary in greenhouse cultivation of Chinese kale increased the content of soluble sugars and free amino acids in the mature crop. Our research did not show the effect of blue light on the accumulation of these compounds. A different phase of plant development may cause inconsistent results. Very young plants do not yet have an adequately developed photosynthetic apparatus; hence the lower production of soluble sugars than in mature plant organs can be observed.

The total phenols concentration in the kale grown under Blue irradiation was higher than that under Red and Purple ones. However, no significant difference was noticed between the Blue and the control treatments (Tab. 3). A similar situation was observed in the case of the phenylpropanoids and flavonols concentrations. Blue light significantly increased the content of anthocyanins in kale as related to the other treatments; the highest difference (about 2-fold) was observed concerning Red irradiation. Moreover, the Blue spectrum significantly enhanced the radical scavenging activity of kale tissues. Similarly, some authors have shown that blue LED light effectively increases the amounts of anthocyanin and total phenolics in Chinese kale sprouts [Alrifai et al. 2019]. Also, an increased percentage of blue light in kale plant irradiation has been found to stimulate the accumulation of phenolics and anthocyanins [Ying et al. 2021].

## SUMMARY

The study on *Brassica oleracea* convar. *acephala* var. *Sabellica* 'Scarlet' in the microgreen phase allowed us to verify the hypothesis that Purple (red + blue) light would be the most effective in promoting the quality of plants. The effect of Purple was similar to White's in synthesising assimilatory pigments and phenolic compounds, but worse in soluble sugars and amino acids. Although the seedlings were shorter and had longer cotyledons under Purple, the differences in fresh and dry weight between plants of these two light treatments (Purple and White) are insignificant. Monochromatic

Blue affected the most anthocyanin content and enhanced the radical scavenging activity. Considering future research, we should connect the light safe for the human eye and the light effective in increasing the quality of 'Scarlet' kale microgreen in closed production.

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