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White, blue and red LED lighting on growth, morphology and accumulation of flavonoid compounds in leafy greens

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Abstract

Growth, morphology and accumulation of flavonoid compounds in green- and purple-leaved sweet basil (*Ocimum basilicum* L.), lamb's lettuce (*Valerianella locusta* (L.) Laterr.) and garden rocket (*Eruca sativa* L.) grown in a greenhouse under natural day light supplemented with white, blue (440 nm) and red (660 nm) light-emitting diodes (LEDs), all at 130 µmol m^{-2} s^{-1}, were studied in winter time. Under white and red LED lights, fresh weights of above-ground parts of lamb's lettuce and garden rocket were the highest, whereas under blue light they were the lowest, in comparison to the control (natural light). For green- and purple-leaved basil no significant differences in biomass production were observed when different light spectra qualities were applied. Supplementation of daily light with blue light resulted in more compact growth of green-leaved basil, as compared to plants grown under natural light and those supplemented with white and red lights. A non-destructive method using an optical sensor was used for evaluation of flavonol, anthocyanin and chlorophyll indexes in plants. Blue light led to significantly increased flavonol index in both green- and purple-leaved basil, lamb's lettuce and garden rocket plants.

Key words: crop improvement, *Eruca sativa*, flavonols, *Ocimum basilicum*, *Valerianella locusta*.

Introduction

Leafy greens are considered an exceptional source of various bioactive compounds (Natesh et al., 2017) including health-promoting plant secondary metabolites, especially polyphenols, vitamins and essential oils. The yield and content of health-promoting compounds depend on the geographic location of a plantation as well as seasonal weather conditions. In order to provide high-quality and stable supply of herbs and leafy vegetables, controlled-environment horticulture is practiced as an alternative to field production (Kozai et al., 2015). Growing systems of plants in greenhouses during the light-limited winter months require the application of additional light sources to ensure plant growth as well as high nutritional value of food. Biosynthesis of polyphenols is enhanced by light, whereas flavonoid formation is light dependent, and its biosynthetic rate is related to light intensity (Darko et al., 2014). Leafy vegetables contain a lower concentration of polyphenols when cultivated in a greenhouse without supplementary lighting than when grown in the field (Romani et al., 2002), which may be explained by differing radiation intensities and spectra (greenhouse glass absorbs UV radiation).

Light-emitting diodes (LEDs), due to high photosynthetically active radiation (PAR) efficiency, have the possibility to adjust the light spectrum to the requirements of different plant species as well as have low energy cost, can provide appropriate growth, development and quality of plants cultivated in greenhouses (Morrow, 2008; Darko et al., 2014; Stutte, 2015). Recent scientific achievements indicate the possibility of plant growth regulation, development and accumulation of bioactive compounds in leafy vegetables and herbs by using LEDs (Lin et al., 2013; Olle, Viršile, 2013; Darko et al., 2014; Wojciechowska et al. 2015; Carvalho et al., 2016; Dou et al., 2017; Samuolienė et al., 2017). More and more scientific papers are being published every year on LED lighting applied in horticulture. A review of this literature shows that plant responses to LEDs are species and even cultivar dependent. Previous studies indicate that red LEDs generally stimulate biomass accumulation, stem elongation and increase leaf area (Johkan et al., 2010; Li et al., 2012; Son et al., 2012), while blue LEDs are more involved in chlorophyll production, the opening of stomata and photosynthesis (Muneer et al., 2014; Wang et al., 2015; 2016; Miao et al., 2016). Research has also shown that exposure to red and blue light increases the content of phenolic compounds, including flavonoids and anthocyanins, in various leafy greens and improve antioxidant activity of plants in contrast to white LED light; however, these effects are dependent on species

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/ cultivars, bioactive ingredients and light quality (Li, Kubota, 2009; Mizuno et al., 2011; Piovene et al., 2015; Naznin et al., 2016; Dou et al., 2017; Hasan et al., 2017). Leafy greens is an encompassing term that applies to a variety of plants, including lettuce, microgreens, herbs and specialty leafy vegetables.

Green- and purple-leaved basil, lamb's lettuce and garden rocket were used in this study. They are among the most important herbs and leafy vegetables that are widely cultivated in greenhouses worldwide. Leafy green vegetables and herbs were identified as potentially rich source of flavonols. Rocket crops, belonging to genera *Eruca*, accumulate kaempferol, wild rocket (*Diplotaxis tenuifolia* (L.) DC) accumulates quercetin, and lamb's lettuce (*V. locusta*) accumulates quercetin and luteolin (Bell, Wagstaff, 2014; Loedolff et al., 2017). Leaves of sweet basil (*O. basilicum*) show the presence of phenolic acids, such as caffeic and rosmarinic acids, flavonoids, such as orientin and vicenin, and essential oils (Gajula et al., 2009). Food derived flavonols are reported to exhibit biological activity, such as antioxidant, anti-inflammatory and anti-allergenic action (Panche et al., 2016). Studies on the effect of LED lighting on growth, development and accumulation of secondary metabolites in sweet basil, lamb's lettuce and garden rocket in greenhouses are very limited. Moreover, most studies on the influence of LED lighting on the accumulation of bioactive compounds in plants, and also biomass production were carried out in growth chambers under controlled conditions, without natural light. These conditions do not always produce the same results as in greenhouses, where leafy greens are usually produced for commercial purposes, especially when variable daylight effect is also involved.

The aim of the study was to determine whether application of supplementary white, blue and red LED lighting to low-level natural light conditions in a greenhouse affects polyphenolic compounds and chlorophyll as well as growth of green- and purple-leaved basil, lamb's lettuce and garden rocket plants.

Material and methods

Plant material and growth conditions. The experiment was conducted in a greenhouse chamber (5 × 4 m) fitted with the custom-made lighting equipment, containing OSRAM diodes and ebb-and-flow benches, belonging to the Research Institute of Horticulture in Skierniewice (lat. 51°57′ N), Poland. The lighting units for the greenhouse chamber were made to order (M-TECH). Fourteen-day-old seedlings of green- and purple-leaved sweet basil (*Ocimum basilicum* L. 'Sweet Genovese' and 'Red Rubin'), lamb's lettuce (*Valerianella locusta* (L.) Laterr.) and garden rocket (*Eruca sativa* L.) were planted in plastic 0.4 L single pots (four seedlings per pot). The substrate was a mixture of peat and perlite (3:1, v:v, pH 6.0). Plants were watered as needed to maintain adequate soil moisture. Fertigation was carried out once a week using a nutrient solution containing minerals in the following concentrations (in mg L^{-1}): N-NO_3 220, P 45, K 265, Ca 132, Mg 49, and microelements. The temperature during the day/night was 20/18°C, and relative humidity was 60–65%. The average daily light integral (DLI) inside the greenhouse during experimental period was 5.71 mol m^{-2} s^{-1}. The experiment was conducted in February and the first half of March 2017, and was terminated when commercial value of the plants was reached, at 30 days after planting in the case of lamb's lettuce and garden rocket, and at 45 days after planting in the case of green-leaved and purple-leaved basil.

Treatments and experimental set up. The greenhouse chamber was divided into four separate sections (four light treatments) and light-emitting diode (LED) arrays with different spectra, but the same photosynthetic photon flux density (PPFD) at plant level (130 µmol m^{-2} s^{-1}) was installed. LED lamps generated white, blue and red light spectra. One section was left without supplementary lighting (control). Blue monochromatic LED light with a max intensity of 450 nm and red monochromatic LED light with a max intensity of 660 nm were used. The white LEDs emitted broad spectrum light within the range of wavelengths between 400 and 700 nm with the peaks at 430 and 550 nm (5000 K). The light intensity was measured using a quantum photometer LI-189 (LI-COR Inc., USA). Ninety six plants of each of the species studied (24 pots of four plants) were exposed to each light treatment. Supplementary lighting was supplied to maintain a photoperiod of 16 h day^{-1}.

Morphological measurements and quantification of flavonols, anthocyanins and chlorophyll indexes. At harvest time, 20 plants were randomly selected for morphological assessment within each treatment. Morphological characteristics, such as fresh shoot weight, plant height, number of leaves and leaf length, were determined. For the assessment of polyphenolic compounds and chlorophyll from the measurement of UV absorbance of the leaf epidermis by double excitation of chlorophyll fluorescence the optical sensor Force-A Dualex Scientific (Dynamax Inc., USA) was used in the study. This device allows performance of non-destructive measurements of chlorophyll, flavonol and anthocyanin contents in the leaves, which makes it particularly suitable for photophysiological research (Cerovic et al., 2012). For each light treatment, 20 young, fully expanded leaves were used for assessment of flavonol, chlorophyll and anthocyanin indexes.

Statistical analysis. Statistical analysis was performed using software *Statistica*, version 13.1 (StatSoft Inc., USA). Data were analysed using one-way analysis of variance (*ANOVA*), and the treatment means were compared using Tukey's HSD test at $\alpha = 0.05$.

Results

All supplementary light treatments (white, blue and red light-emitting diodes) stimulated biomass production of all the species used in the study compared to the plants illuminated with natural sunlight only (Table). At harvest time, shoots of purple-leaved basil, lamb's lettuce and garden rocket plants grown under supplemental 130 µmol m^{-2} s^{-1} white LED light weighed 2.1, 4.0 and 2.0 times more, respectively, than control plants grown without supplementary lighting. A similar positive effect of supplemental white LED light was observed for plant height, leaf number and leaf size. The weakest response to supplementary lighting was observed in green-leaved basil, which under white supplementary lighting had a fresh shoot weight only 11% higher than shoots of plants grown under natural sunlight (4.3 *vs* 3.2 g per plant). Plant height, leaf number and size of green-leaved basil under white LED light was similar to plants grown in control conditions.

Light quality affected growth and development of sweet basil and leafy vegetables; however, there were significant differences among species in response to monochromatic red and blue LED light (Table). Supplementary red LED light was more effective than blue LED light in increasing fresh weight of above-ground

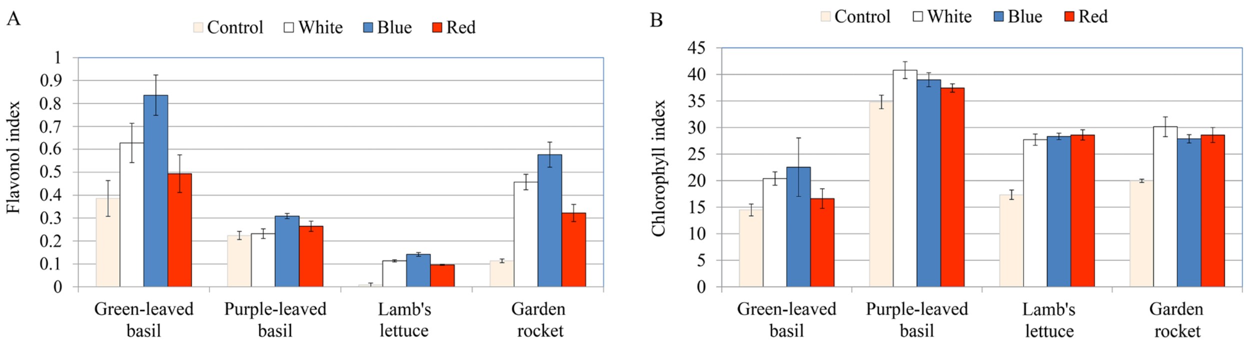
Table. The effect of supplementary white, blue and red LED lights on growth and morphological parameters of green- and purple-leaved basil, lamb's lettuce and garden rocket grown in greenhouse

Light treatment	Fresh shoot weight g	Plant height cm	Leaf number	Leaf length cm
Green-leaved basil				
None (control)	3.2 a	7.0 b	4.0 a	7.1 a
White	4.3 b	7.1 b	4.2 ab	7.5 ab
Blue	4.4 b	5.1 a	4.5 b	7.6 ab
Red	3.8 ab	7.7 b	4.2 ab	8.4 b
Purple-leaved basil				
None (control)	0.8 a	10.7 a	5.1 a	3.2 a
White	1.7 b	14.4 b	7.0 b	4.1 b
Blue	2.1 b	13.5 b	7.6 b	4.4 bc
Red	2.1 b	14.5 b	7.3 b	4.8 c
Lamb's lettuce				
None (control)	0.5 a	6.5 a	7.5 a	4.9 a
White	2.0 c	9.1 b	9.6 b	7.3 c
Blue	1.7 b	8.9 b	9.4 b	6.4 b
Red	1.9 bc	9.0 b	8.6 ab	7.7 c
Garden rocket				
None (control)	1.1 a	11.1 a	5.4 a	9.6 a
White	2.8 c	14.0 b	6.0 b	10.8 b
Blue	2.1 b	12.6 ab	6.1 b	10.6 ab
Red	2.7 c	14.3 b	5.9 ab	12.1 c

Note. Different letters indicate significant differences at α = 0.05 (Tukey's HSD test).

parts of garden rocket plants. Fresh weight of garden rocket grown under red LED light was 29% higher than under blue light. There were no significant differences in biomass production in garden rocket between red and white LEDs. Lamb's lettuce grown under white LEDs had 18% higher fresh shoot weight than under blue LEDs. Similarly as in garden rocket, there were no significant differences in biomass production in lamb's lettuce grown under white and red LEDs. For green- and purple-leaved basil, no significant differences in biomass production were noted between all supplementary light treatments. Supplemental blue LED lighting resulted in more compact growth of green-leaved basil plants, with shorter stems, compared to other supplemental light treatments and to the control. Red LED light stimulated leaf elongation of lamb's lettuce and garden rocket plants compared to those grown under blue light. Purple-leaved basil under red LED light had longer leaves than under white light, but there were no significant differences between red and blue light treatments.

The Force-A Dualex Scientific sensor was found to be very useful for assessing flavonols and chlorophyll contents in leaves. Under control treatment, green-leaved basil had the highest flavonol indexes, followed by purple-leaved basil and garden rocket, whereas the lowest flavonol indexes were observed in lamb's lettuce (Fig.).



Means ± SE of 20 leaves

Figure. Flavonol (A) and chlorophyll (B) indices (relative units) for green- and purple-leaved basil, lamb's lettuce and garden rocket plants grown under supplementary white, blue and red LED lights in greenhouse

Flavonol indexes were enhanced with supplementary white LED lighting compared to the control in green-leaved basil (flavonol index 0.63 *vs* 0.39), lamb's lettuce (0.11 *vs* 0.02) and garden rocket (0.46 *vs* 0.11), but was unaffected in purple-leaved basil (0.23 *vs* 0.22). Red LED light was less effective than white LED light in stimulating flavonol biosynthesis in lamb's lettuce and garden rocket. Blue LED light was the most effective in stimulating flavonol biosynthesis. Plants grown under blue LED light had the highest flavonol index, and flavonol indexes were higher by 33% in the case of both green- and purple-leaved basil, 25% higher in lamb's lettuce and 26% higher in garden rocket, as compared with white LED light treatment.

Chlorophyll synthesis in leaves was stimulated by supplementary lighting in all species. White LED light enhanced the chlorophyll index compared to the control in green-leaved basil (20 *vs* 14), purple-leaved basil (41 *vs* 35), lamb's lettuce (28 *vs* 17) and garden rocket (30 *vs* 20). There were no significant differences between LED light treatments (white, blue and red) in chlorophyll contents for green leafy vegetables, except for lower chlorophyll indexes in green- and purple-leaved basil grown under red light. The purple pigmented leafy sweet basil was richer in anthocyanins than the green coloured ones (data not shown).

However, no significant differences in anthocyanin indexes among light treatments were observed in our experiments. The anthocyanin indexes in purple-leaved basil ranged from 0.40 for control and white light treatments to 0.42 for blue and red light treatments. Green-leaved basil, lamb's lettuce and garden rocket contained no detectable anthocyanins.

Discussion

Supplementary LED lighting at 130 µmol m^{-2} s^{-1} and a 16 h photoperiod had a positive impact on the biomass production of all leafy green species grown under low-level natural light conditions in a greenhouse during winter. Lamb's lettuce was distinguished for its highest and green-leaved basil for its lowest LED lighting efficiency based on their biomass production. Unlike monochromatic LEDs, the light from white LEDs covers a narrow spectrum band in the range of 460 nm (blue light) and wider emission of the longer wavelength part of the spectrum, which mimics natural sunlight. The spectrum of light emitted by white LEDs corresponds to plant requirements for appropriate growth and development and has the advantage of increasing light use efficiency compared to monochromatic LED lights (Pimputkar et al., 2009; Cope, Bugbee, 2013).

Our study confirmed that white LED light at 130 µmol m^{-2} s^{-1} with a 16 h photoperiod created favourable conditions for biomass production of all tested leafy greens. Fresh weights of plants grown under supplementary white LED light were the highest or at least the same as under red LED light for all the tested species. Our study also showed that monochromatic blue light in combination with natural sunlight inhibited biomass production of lamb's lettuce compared to white LED light, and production of garden rocket plants compared to white as well as red light. Plant response to the light quality seems to be complex, and opposite results have often been published. Positive effects of monochromatic blue light were demonstrated on biomass production and photosynthesis of several green leafy vegetables including green-leaf and red-leaf lettuce, spinach and sweet basil (Yorio et al., 2001; Johkan et al., 2010; Amaki et al., 2011; Muneer et al., 2014; Miao et al., 2016). On the other hand, higher shoot and root biomass of Chinese cabbage under red than under blue light was reported (Li et al., 2012).

In our study, green-leaved basil under blue LED light showed a compact growth pattern in contrast to plants grown under red and white LED lights. A similar effect was also reported for other plants, such as lettuce plants grown under a high share of blue light (Li, Kubota, 2009; Johkan et al., 2010). Light quality also affected leaf development, but the response was dependent upon genotype. In our study, purple-leaved basil grown under red LED light had 16% longer leaves than under white light. Lamb's lettuce under red light had 14% longer leaves compared to blue light. Garden rocket leaves exposed to red light were 14% and 12% longer than under blue and white LEDs lights, respectively. The above results indicate the significant role of red and blue light in morphology development.

Our study showed that plants exposed to supplementary LED lighting had greater leaf chlorophyll content than plants grown in natural sunlight. Plants grown under deep shade show a reduced ratio of red to far-red that triggers morphological alteration, such as inhibiting hypocotyl elongation and chlorophyll development (Solymosi, Schoefs, 2010). When plants are exposed to light conditions, chloroplast development and chlorophyll synthesis are induced, which might explain the increased chlorophyll accumulation in leafy greens grown under supplementary lighting. Our results also indicate that supplementary monochromatic blue and red LED light as well as white LED light stimulate chlorophyll accumulation in sweet basil, lamb's lettuce and garden rocket leaves to a similar extent. The exception was green- and purple-leaved basil, in which red light was less favourable than white light for chlorophyll accumulation. Previous studies indicated that blue light stimulates and red light inhibits chlorophyll synthesis in Chinese cabbage and grape (Poudel et al., 2008); however, the opposite trend has been shown in *Nicotiana tabacum* (Yiang et al., 2017).

Biosynthesis of flavonols is upregulated by high sunlight irradiance and plays an important photo-oxidative role in plant protection when exposed to excess UV-radiation (Siipola et al., 2015). It was shown that high light intensity (272 µmol m^{-2} s^{-1}) increased contents of resveratrol, quercetin, catechin and kaempferol in wild rocket microgreens (Loedolff et al., 2017) and can explain increased flavonol contents in garden rocket as well as lamb's lettuce and green-leaved basil exposed to white LED light in our study. Our results showed that purple-leaved basil contained much less flavonols than green-leaved basil. Analysis of the anthocyanin indexes made using the Force-A Dualex Scientific sensor also showed their presence in purple-leaved basil and absence in green-leaved basil plants.

Previous studies have shown that blue light was the most effective among monochromatic lights in increasing the concentration of total phenolics, flavonoids and antioxidant capacity, even though blue LEDs had been used only as a supplement to natural light (Ouzounis et al., 2015). Bantis et al. (2016) showed that total phenolic content in sweet basil leaves was higher when plants were exposed to light with a relatively high portion of blue. The concentrations of flavonoids and total phenolics in the perennial herb dropwort (*Oenanthe stolonifera* Wall. ex DC) exposed to blue light were significantly higher than in the plants exposed to green light (Jeon et al., 2017). Other results showed that monochromatic blue as well as red light increased total phenolic compounds, flavonoids and anthocyanins contents in green perilla (*Perilla frutescens* L. Britton) and Chinese foxglove (*Rehmannia glutinosa* Libos.) plants in contrast to white light; moreover, blue light was much more efficient to synthesise bioactive ingredients than red light (Lee et al., 2014; Manivannan et al., 2015).

Our results are in line with the above finding, as the highest flavonols content in green-leaved basil, lamb's lettuce and garden rocket plants were shown under supplementary blue LED light. However, anthocyanin content in purple-leaved basil in our study was not dependent on the light quality.

Conclusions

1. White and monochromatic red LED lights applied as supplement to natural lighting in a greenhouse during winter are more effective than blue light to promote biomass production in lamb's lettuce (*Valerianella locusta* L.) and garden rocket (*Eruca sativa* L.) plants, while green-leaved sweet basil (*Ocimum basilicum* L.) 'Sweet Genovese' and purple-leaved basil 'Red Rubin' are less responsive to light quality.

2. 'Sweet Genovese' and 'Red Rubin' sweet basil as well as lamb's lettuce and garden rocket plants grown in a greenhouse during a low-level natural light period under blue LED light had significantly higher flavonol indexes compared to plants grown under white and red LED lights.

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Apšvietimo balta, mėlyna ir raudona LED šviesa įtaka lapinių žalumynų augimui, morfologijai ir flavonoidų kaupimuisi

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Santrauka

Tirta žalialapio ir raudonlapio saldžiojo baziliko (*Ocimum basilicum* L.), salotinės sultenės (*Valerianella locusta* (L.) Laterr.) ir sėjamosios gražgarstės (*Eruca sativa* L.), žiemos metu auginamų šiltnamyje natūralioje dienos šviesoje, papildžius 130 μmol m^{-2} s^{-1} baltais bei mėlynais (440 nm) ir raudonais (660 nm) šviesą skleidžiančiais diodais (LEDs), augimas, morfologija ir flavonoidų kaupimasis. Paveikus balta ir raudona LED šviesa didžiausią antžeminės dalies žalią masę užaugino salotinės sultenės ir sėjamosios gražgarstės, tačiau veikiant mėlyna šviesa abiejų rūšių augalų žalios masės derlius buvo mažiausias, palyginus su kontroliniu variantu (natūralia šviesa). Taikant skirtingus šviesos spektrus reikšmingų žalialapių ir raudonlapių saldžiųjų bazilikų biomasės derliaus skirtumų nebuvo nustatyta. Dėl dienos šviesos papildymo mėlyna šviesa žalialapių bazilikų augalai buvo kompaktiškesni, palyginus su augalais, augintais natūralioje šviesoje ir kai natūrali šviesa buvo papildyta balta bei raudona šviesa. Siekiant įvertinti flavonolių, antocianinų ir chlorofilo kiekį augaluose buvo taikytas nedestruktyvus metodas, naudojant optinį jutiklį. Dėl apšvietimo mėlyna spalva flavonolių indeksas esmingai padidėjo visuose tirtuose augaluose: žalialapiuose ir raudonlapiuose bazilikuose, salotinėse sultenėse ir sėjamosiose gražgarstėse.

Reikšminiai žodžiai: derliaus gerinimas, *Eruca sativa*, flavonoliai, *Ocimum basilicum*, *Valerianella locusta*.