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The Improvement in the Growth and Biosynthesis of Polyphenols in *Ocimum basilicum* L. Plants Through Simultaneous Modulation of Light Conditions and Soil Supplementation

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Abstract: The sweet basil *Ocimum basilicum* L. is the subject of numerous studies and is cultivated as a food and ornamental plant. Moreover, *O. basilicum* could be useful in the prevention of stroke ischemia, and its anticancer properties were recently shown. Caffeic acid derivatives, such as rosmarinic acid (RA), chicoric acid, salvianolic acids, and anthocyanins, provide the medicinal properties of basil. Therefore, investigations of the optimal growth conditions that can provide cost-effective cultivation of highly productive basil plants are relevant and important. The aim of the present work was to study the effects of a combination of soil composition and light conditions on the morphological and biochemical characteristics of *O. basilicum*. In totally artificial (indoor) environments, light-emitting diodes (LEDs) may provide a broad range of narrowband wavelengths with different intensities. This technology can lower operating costs. In addition to the spectral composition, light intensity (PPFD, μmol $m^{-2}s^{-1}$) is an important parameter for the optimal growth of plants. In the experiment, we used different spectra of LED lamps with intensities of 300 μmol $m^{-2}s^{-1}$: warm white, monochromatic (green and red), and a combination of blue and red. Plants were grown under various lighting conditions in soil supplemented with fertilizer, Z-ion, and Crystallon. The results showed that supplementation of soil with Crystallon had a greater effect on the growth of both above- and below-ground parts of *O. basilicum* plants. Interestingly, growing *O. basilicum* plants under R and RB light led to a 2-fold increase in the biosynthesis of both the key caffeic acid derivative RA and anthocyanin. However, given that under RB light, there is no positive effect of Crystallon on growth, the productivity of RA and anthocyanin reached a maximum when *O. basilicum* plants were grown under R light and Crystallon. Under these conditions, the productivity of anthocyanins and caffeic acid derivatives in *O. basilicum* was more than eight times greater than that in untreated *O. basilicum* plants.

Keywords: anthocyanins; artificial light; caffeic acid; light-emitting diode; sweet basil; rosmarinic acid; soil fertilizer

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1. Introduction

The sweet basil *Ocimum basilicum* L. is an annual herbaceous plant from the genus Lamiaceae that is native to India and Asia [1]. Many species of this genus are rich in essential oils, and sweet basil is considered the main essential oil crop throughout the world.

These species and, first, *O. basilicum*, are the objects of numerous studies and are cultivated as food and ornamental plants [1]. *O. basilicum* could be useful in the prevention of stroke ischemia, reperfusion-induced cerebral damage, and motor dysfunctions in mouse brains [2]. Moreover, the anticancer properties of *O. basilicum* extract were recently shown [2]. Therefore, investigations of the optimal growth conditions that can provide cost-effective cultivation of highly productive basil plants are relevant and important.

These medicinal properties of basil are provided by caffeic acid derivatives (CADs), the main polyphenols of *O. basilicum* [3–5]. The main phenolic compound, rosmarinic acid (RA), is found in the leaves and stems of *O. basilicum* plants [5]. Another CAD, chicoric acid, was detected in the stems of some varieties of basil in small amounts [6]. RA may be further converted into salvianolic acid; however, the biosynthetic pathway of this conversion has not yet been studied [7–9]. Anthocyanins constitute another class of *O. basilicum* polyphenol chemicals that have been linked to a number of health benefits [10]. Because of their anti-inflammatory, antioxidant, and photoprotective qualities, anthocyanins have the potential to treat a variety of illnesses, including cancer [10]. The physiological functions of plants depend heavily on anthocyanins. They exhibit antibacterial qualities, provide a photoprotective screen in plant tissues, and aid in the creation of visual attractors during pollination [11]. The anthocyanin content of certain plants has been thoroughly investigated in relation to light exposure of varying intensities or spectral compositions [12].

O. basilicum has a relatively short growth period and can be consumed as a micronutrient in approximately 1–2 weeks, and given its small size, it is beneficial for growth in greenhouses. A number of studies have shown that plants grown via traditional methods and in soilless systems have different levels of various nutrients. Thus, basil plants grown hydroponically have more significant antioxidant effects than do those grown in soil because of their higher contents of vitamin C, vitamin E, and lipoic and rosmarinic acids [13,14]. Cultivation in soil leads to lower values of plant development characteristics, but with the help of various fertilizers, the results can be significantly improved [15–17]. One of the most promising areas for increasing plant productivity is the use of ion exchange nutrient substrates and universal water-soluble complex fertilizers [15–17].

Another approach for optimizing production is the use of artificial lighting. Like any other type of plant, basil has its own needs for lighting conditions. In totally artificial (indoor) environments, light-emitting diodes (LEDs) may provide a broad range of narrowband wavelengths with different intensities. This technology can lower operating costs [18]. When the LED treatments were compared with high-pressure sodium (HPS), the energy cost per gram of new biomass increased by 95% to 98%. Supplementation with blue (B) and red (R) LEDs has been shown in earlier research to affect the morphology, physiology, and development of basil plants. The effectiveness of supplemental B- and R-LED narrowband wavelengths in comparison with that of conventional lighting systems such as HPS lamps in terms of yield, quality, and energy consumption for a range of high-value specialty crops cultivated in greenhouses requires further study [19]. In terms of biomass partitioning, the individual main stems, branches, and leaves of each plant in the LED treatment were relatively high. Greater height, main stem diameter, and phytochemical composition are the outcomes of LED treatments [19]. While RB is ideal for development and photosynthesis, white and red light cause RA more than twice as much as blue light does [20,21].

In addition to the spectral composition, light intensity (PPFD, μmol $m^{-2}s^{-1}$) is an important parameter for the optimal growth of plants. When *Arabidopsis* plants are grown hydroponically, increasing the intensity of red–blue light (3:1) from 100 to 250 μmol $m^{-2}s^{-1}$ leads to an increase in the values of fresh and dry biomass; however, with a further increase in intensity, the mass does not change [22]. Among the intensity options with intensities ranging from 160 to 310 μmol $m^{-2}s^{-1}$, a light intensity of 224 μmol $m^{-2}s^{-1}$ was optimal for growing basil plants, although higher intensities led to increased biomass accumulation [23]. Compared with low-intensity light, high-intensity light resulted in accelerated development and greater yields of various basil cultivars and increased their marketability by 3–5 days. However, green-leafed basil cultivars developed light avoidance responses when exposed to light levels greater than approximately 300 μmol $m^{-2}s^{-1}$ [23].

Therefore, the aim of the present work was to study the effects of a combination of soil composition and light conditions on the morphological and biochemical characteristics of *O. basilicum*. In the experiment, we used different light intensities of 300 μmol $m^{-2}s^{-1}$: warm white, monochromatic (green and red), and a combination of blue and red. Plants were grown under various lighting conditions in soil supplemented with fertilizer, Z-ion, and Crystallon. Z-ion consists of cations that are more easily absorbed by plants than they are in natural soil, and most of the cations are part of the crystal lattices of various minerals and are less accessible to plants. Another fertilizer used in this work was the water-soluble complex fertilizer Crystallon, which is used in any irrigation system and for foliar feeding. It has a high degree of purity and does not contain sodium, chlorine, or carbonates. As a result, the optimal combination of soil supplementation and light quality for cost-effective cultivation of *O. basilicum* was determined.

2. Results

2.1. Effects of Artificial Light and Soil Composition on the Growth of O. basilicum Plants

Manipulation with the artificial light spectrum and intensities [24] as well as variations in soil composition [25] allows the modulation of plant growth and the biosynthesis of polyphenols in plants. However, there is not much information about the effects of the simultaneous action of growth stimulators and light conditions on polyphenol accumulation and productivity in crop plants. The effects of artificial lighting variations and soil composition on the growth and morphology of *O. basilicum* plants were studied in the present work. The construction of the chambers, experimental design, and other conditions are described in Section 4. Compared with that of the untreated soil, the biomass accumulation of the aboveground parts of the 35-day-old *O. basilicum* plants grown under W light was greater than two and four times greater (Figure 1A). The same intensity (300 μmol $m^{-2}s^{-1}$) of R did not affect biomass accumulation. In contrast, compared with W light, G light caused a decrease in biomass accumulation in S and S + Z plants. Interestingly, the combination of R and B lights abolished the positive effect of Crystallon on the growth of basil plants. A similar effect of the combination of light variation and soil supplementation was shown for the accumulation of root biomass (Figure 1B). Summarizing this information, it can be assumed that the positive effect of soil supplemented with Crystallon remains under R and G light, whereas RB light abolished this effect.

To evaluate the physiological parameters, the height (cm), number of internodes and leaves, stem diameter (cm), and total area of the leaf (cm^2) were analyzed. Statistically significant differences in these measurements are shown in the heatmap (Figure 2). Under W light, supplementation of the soil with Crystallon led to dramatic increases in all the parameters, whereas the effect of Z-ion was not as strong. Compared with W light, red light had no effect on the analyzed parameters, whereas G light, in contrast, caused a decrease in height, the number of internodes and leaves, the stem diameter, and the total area of the leaves of S and S+Z plants. The combination of R and B lights abolished the positive effect of Crystallon on the growth of basil plants (Figure 2).

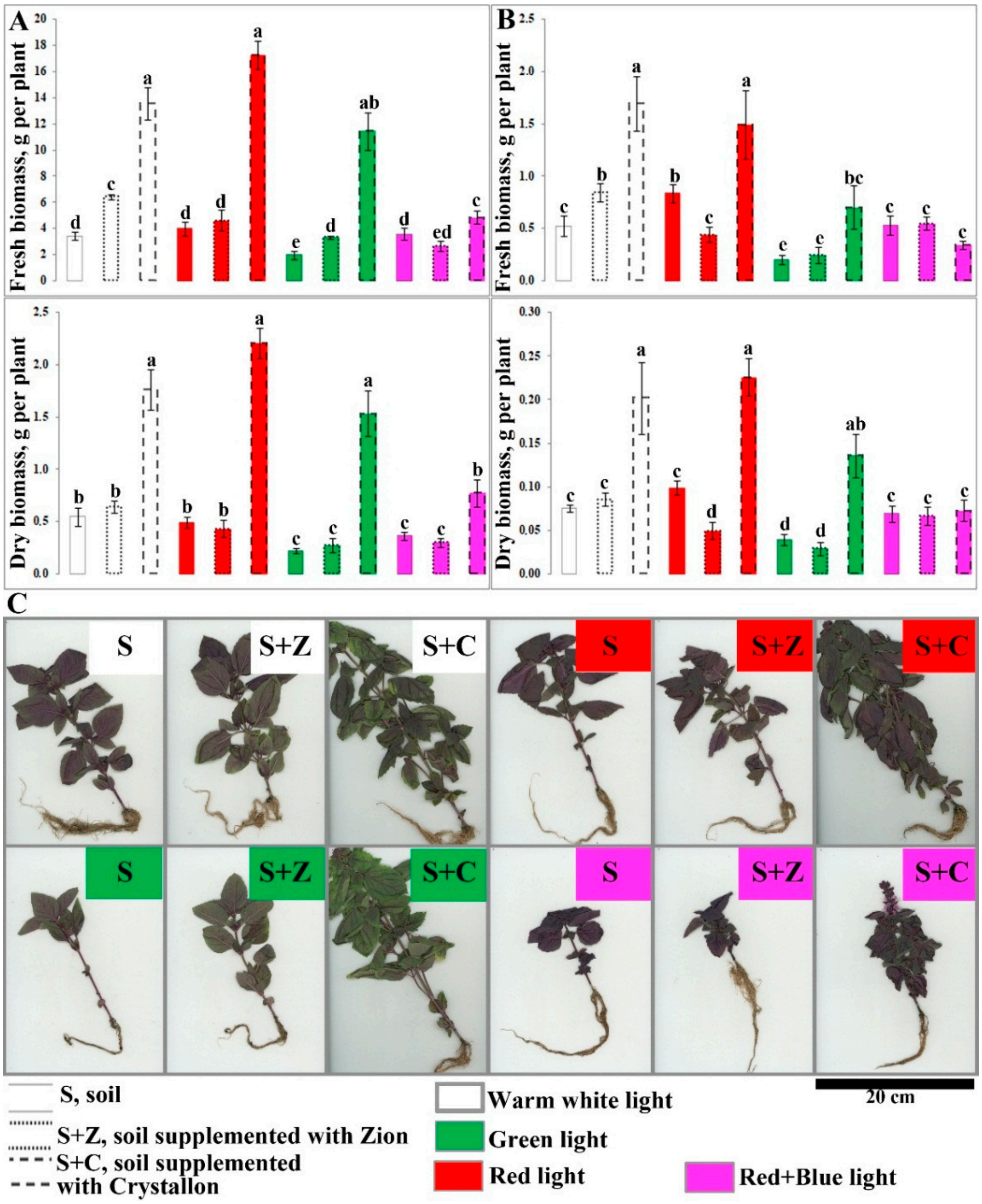

Figure 1. Impact of combinations of artificial lighting and soil composition on the growth and morphology of *O. basilicum* plants. (**A**) Biomass accumulation (g) of 40-day-old plants (aboveground part only); (**B**) biomass accumulation (g) of 40-day-old plants (roots); FW, fresh weight (left panel); DW, dry weight (right panel). (**C**) Morphology of 40-day-old plants. The plants were grown in soil (S), soil supplemented with Z-ion (S+Z), or soil supplemented with Crystallon (S+C). W, R, G, and RB represent different light variations with intensities of 300 μmol m^{-2}s^{-1}. Section 4 contains the specifics of the culture conditions. The mean ± standard error of the mean was used to represent data from three separate studies with 10 biological replicates. Statistical significance is shown by different letters above the error bars (ANOVA, $p < 0.05$).

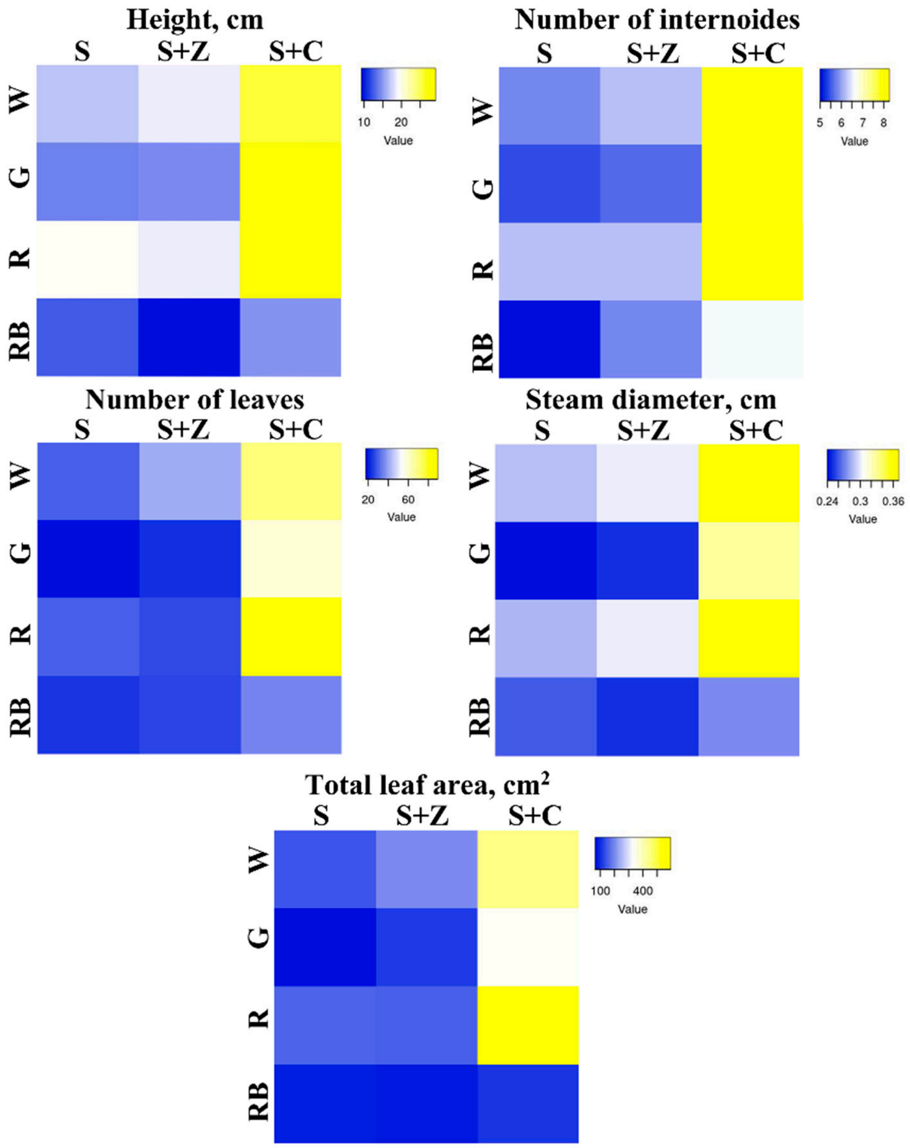

Figure 2. Morphological parameters of *O. basilicum* plants grown under different combinations of artificial lighting and soil composition. The heatmap shows statistically significant differences in the measurements of height (cm), number of internodes and leaves, stem diameter (cm), and total area of the leaf (cm^2). The plants were grown in soil (S), soil supplemented with Z-ion (S+Z), or soil supplemented with Crystallon (S+C). W, R, G, and RB represent different light variations with intensities of 300 μmol $m^{-2}s^{-1}$. The mean ± standard error of the mean was used to represent data from three separate studies with 10 biological replicates. Statistical significance is shown by different colors (ANOVA, $p < 0.05$).

2.2. *Identification of Caffeic Acid Derivatives in O. basilicum Plants*

A reversed-phase HPLC technique with UV–Vis and MS detection was used to investigate the polyphenol composition of *O. basilicum* plants. Thus, fifteen phenolic compounds have been identified in *O. basilicum* plants and can be divided into two groups. The chromatographic and mass-spectral information necessary for identification is summarized in Table 1.

The first group, caffeic acid derivatives, which include four substances, exhibit absorbance maxima at 325–340 nm (Figure 3). Compound **2** was recognized as rosmarinic acid because it fully coincided with the standard sample. With respect to the other caffeic acid conjugates, chicoric acid (compound **1**, also known as di-caffeoyltartaric acid) and two isomers of salvianolic acid F (compounds **3** and **4**) were identified to have mass spectrometric characteristics similar to those of the metabolites previously reported for *O. basilicum* [3].

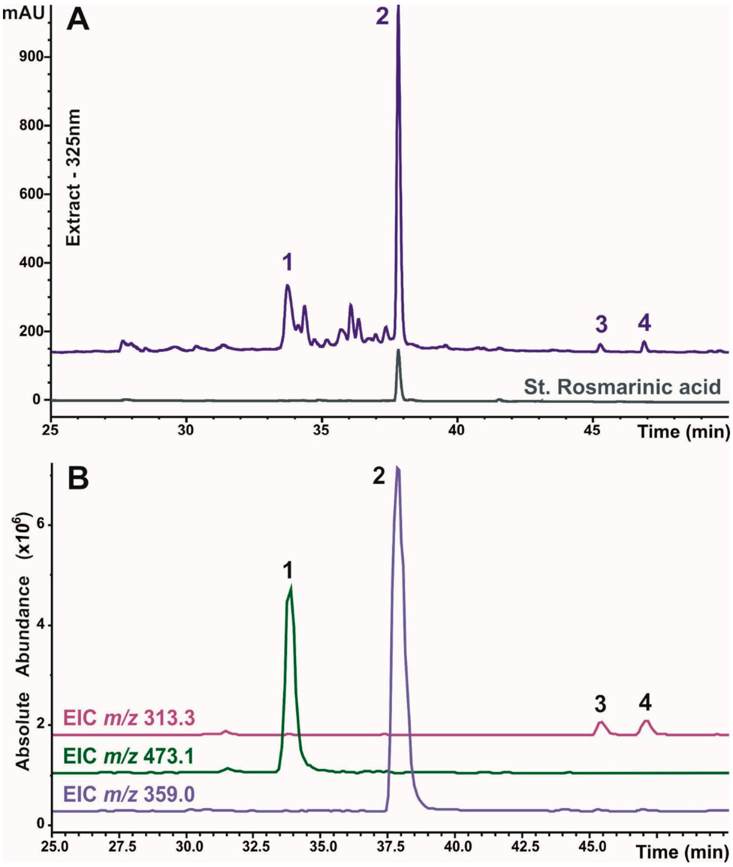

Figure 3. HPLC–UV–MS determination of caffeic acid derivatives identified in crude extracts of *O. basilicum*. The UV profile of a typical studied sample recorded at 325 nm is shown in comparison with that of a standard sample of rosmarinic acid (**A**). The extracted ion chromatograms (EICs) acquired in negative ion mode correspond to the deprotonated molecules of the individual compounds with a restricted window of ±0.5 m/z units and are shown with an overlay (**B**). The peak numbers correspond to the identified components and are listed in Table 1.

Table 1. List of phenolic compounds identified in the crude extracts of *O. basilicum* via HPLC–UV–ESI–MS (MS^2).

No	Rt, min	Compound Assignment	UV Max, nm	Molecular Formula	ESI–MS(MS^2) Data				Ref.
					Ion Composition	HRMS, *m/z* Values	Error, mDa	MS^2, Main Diagnostic Ions, *m/z*	
					Caffeic acid derivatives				
1	33.7	Chicoric acid	326	$C_{22}H_{18}O_{12}$	$[2M-H]^-$	947.1500	2.4	473, 311, 293	[3]
					$[M-H]^-$	473.0721	0.5	311, 293, 219, 179, 149	
2	37.7	Rosmarinic acid	230, 327	$C_{18}H_{16}O_8$	$[2M-H]^-$	719.1581	3.7	359	[3]
					$[M-H]^-$	359.0758	1.4	197, 179, 161	
3	45.3	Salvianolic acid F (isomer 1)	248, 339	$C_{17}H_{14}O_6$	$[M-H]^-$	313.0719	0.1	161	[3]
4	46.9	Salvianolic acid F (isomer 2)	252, 339	$C_{17}H_{14}O_6$	$[M+H]^+$	315.0884	2.1	nd	[3]
					$[M-H]^-$	313.0710	0.8	269, 161	
					Anthocyanins				

A1	29.6	Cyanidine-3-(Cou-diHex)-5-Hex	225, 281, 520	$C_{42}H_{47}O_{23}$	M+ $[M-2H]^-$ $[M-2H+H_2O]^-$	919.2512 917.2335 935.2423	0.9 2.2 4	757, 595, 449, 287 755, 593, 447, 285 773, 755, 611, 447, 285	[26]
A2	31.3	Cyanidine-3-(Mal-Cou-diHex)-5-Hex	226, 283, 523	$C_{45}H_{49}O_{26}$	M+ $[M-2H]^-$ $[M-2H+H_2O]^-$	1005.2495 1003.2339 1021.2432	1.2 2.2 3.5	843, 681, 449, 287 959, 797 977, 815, 797	[26]
A3	33.5	Cyanidine-3-(Caf-hydroxybensoyl-diHex)-5-Hex	221, 287, 523	$C_{49}H_{51}O_{26}$	M+ $[M-2H]^-$	1055.2623 1053.2537	4.0 1.9	893, 449, 287 891	[27]
A4	34.1	Cyanidine-3-(Caf-Caf-diHex)-5-Hex	222, 286, 528	$C_{51}H_{53}O_{27}$	M+ $[M-2H]^-$ $[M-2H+H_2O]^-$	1097.2802 1095.2632 1113.2689	3.3 0.9 4	935, 773, 611, 449 933, 771, 609, 447 1095, 951, 933	[26]
A5	34.3	Cyanidine-3-(Caf-Cou-diHex)-5-Hex	228, 283, 528	$C_{51}H_{53}O_{26}$	M+ $[M-2H]^-$ $[M-2H+H_2O]^-$	1081.2836 1079.2643 1097.2753	1.6 3.1 2.7	919, 757, 595, 449 917, 755 1079, 935, 917, 465	[3,26]
A6	35.2	Cyanidine-3-(Caf-Cou-diHex)-5-Mal-Hex	225, 295, 529	$C_{54}H_{55}O_{29}$	M+ $[M-2H]^-$ $[M-2H+H_2O]^-$	1167.2850 1165.2706 1183.2763	2.6 2.8 2.1	1081, 1005, 919, 535, 449 1121 1139, 1121	[3,26]
A7	35.7	Cyanidine-3-(Mal-Caf-Cou-diHex)-5-Hex	226, 285, 528	$C_{54}H_{55}O_{29}$	M+ $[M-2H]^-$ $[M-2H+H_2O]^-$	1167.2840 1165.2666 1183.2773	1.6 1.2 1.1	1005, 449 1121 1139	[3,26]
A8	36.0	Cyanidine-3-(diCou-diHex)-5-Hex	229, 283, 529	$C_{51}H_{53}O_{25}$	M+ $[M^{-}2H]^{-}$ $[M-2H+H_2O]^-$	1065.2882 1063.2722 1081.2828	1.2 0.3 0.3	903, 757, 595, 449 901, 615, 447 919, 901, 465	[3]
A9	36.3	Cyanidine-3-(Caf-Fer-diHex)-5-Hex	226, 292, 530	$C_{52}H_{55}O_{26}$	M+ $[M-2H]^-$ $[M-2H+H_2O]^-$	1095.3008 1093.2787 1111.2905	3.2 4.4 3.1	933, 757, 595, 449 931, 755, 593, 447 949, 931	[26]
A10	36.8	Cyanidine-3-(diCou-diHex)-5-Mal-Hex	224, 288, 530	$C_{54}H_{55}O_{28}$	M+ $[M-2H]^-$ $[M-2H+H_2O]^-$	1151.2917 1149.2715 1167.2809	4.3 1.4 2.5	1065, 989, 903, 535, 449 1105, 943, 901 1123	[3,26]
A11	37.4	Cyanidine-3-(Mal-diCou-diHex)-5-Hex	228, 284, 529	$C_{54}H_{55}O_{28}$	M+ $[M-2H]^-$ $[M-2H+H_2O]^-$	1151.2915 1149.2702 1167.2829	4.1 2.7 0.6	989, 945, 843, 681, 449 1105, 943 1123	[3,26]

Abbreviations: Cou—*p*-coumaroyl, Hex—hexoside, Mal—malonyl, Caf—caffeoyl, Fer—feruloyl.

2.3. Content and Productivity of Caffeic Acid Derivatives in O. basilicum Plants

Basil plants growing in the control untreated soil under W light contained more than 40 mg/g DW of RA in their leaves. Chicoric and salvianolic acids are minor compounds. CA and SA were found at concentrations of 13 and 2 mg/g DW, respectively. R and RB light of the same intensity slightly increased the content of RA, whereas the content of CA increased in RB-treated plants, and the content of SA increased in R-treated plants. Interestingly, an increase in the biomass accumulation of plants growing in Z-ion- and Crystallon-supplemented soil was accompanied by a more than twofold reduction in the content of caffeic acid derivatives. However, the R and RB light treatments increased the CAD content up to the control (untreated soil, W light) level (Figure 4, left panel). Therefore, the simultaneous effects of light conditions and soil supplementation significantly affect the productivity of basil plants. We have shown that supplementation of soil with Crystallon and cultivation of basil plants under R light provided the maximum productivity of each CAD in terms of fresh biomass. Compared with that of the control conditions, the productivity of RA increased more than 6-fold. The productivity of basil plants growing in soil supplemented with Crystallon under G or RB light was close to that of the control (Figure 4, right panel).

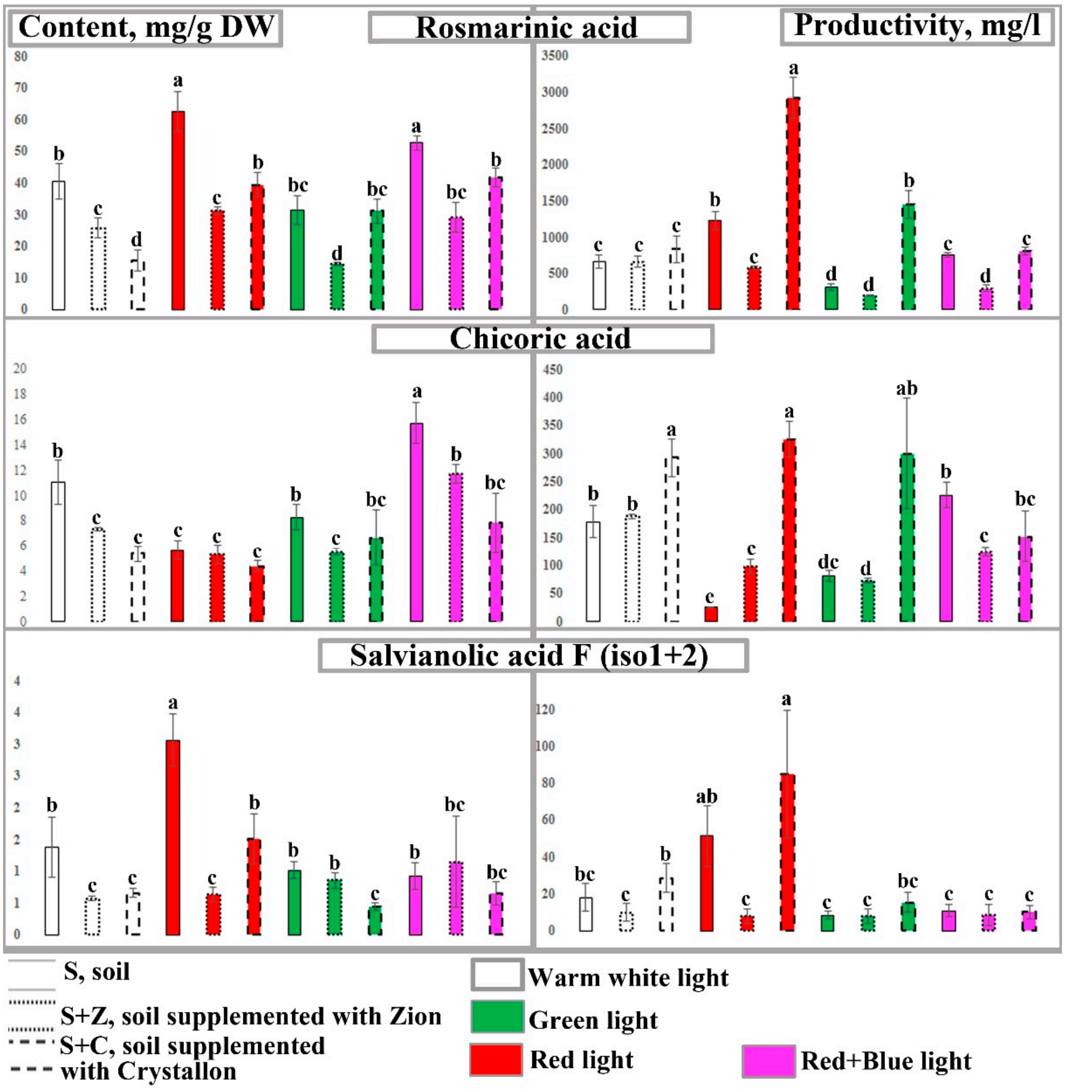

Figure 4. Analysis of caffeic acid derivatives in the leaves of *O. basilicum* plants growing under different combinations of artificial lighting and soil composition. Content (left diagrams, mg/g dry weight) and productivity (right diagrams, mg/L soil) of rosmarinic acid, chicoric acid, and salvianolic acid F (isomers 1 and 2) in 40-day-old *O. basilicum* plants. The plants were grown in soil (S), soil supplemented with Z-ion (S+Z), or soil supplemented with Crystallon (S+C). W, R, G, and RB represent different light variations with intensities of 300 μmol $m^{-2}s^{-1}$. The mean ± standard error of the mean was used to represent data from three separate studies with 10 biological replicates. Statistical significance is shown by different letters above the error bars (ANOVA, $p < 0.05$).

Additionally, we analyzed the CAD content in the roots of treated basil plants. Analysis of caffeic acid derivatives in the roots of *O. basilicum* plants growing under different combinations of artificial lighting and soil compositions revealed that the contents (mg/g dry weight) of rosmarinic acid, chicoric acid, and salvianolic acid F (isomers 1 and 2) in the roots of 40-day-old *O. basilicum* plants were relatively low. In addition to light treatment, soil supplementation did not significantly affect CAD biosynthesis, except for the RB variance. The RA content in the roots of the plants growing under RB light was 1.5 times greater than that in the roots of the plants growing under control W light. However, the sum of the CAD (mg/g DW of roots) did not significantly increase in the plants growing under RB light conditions (Figure 5).

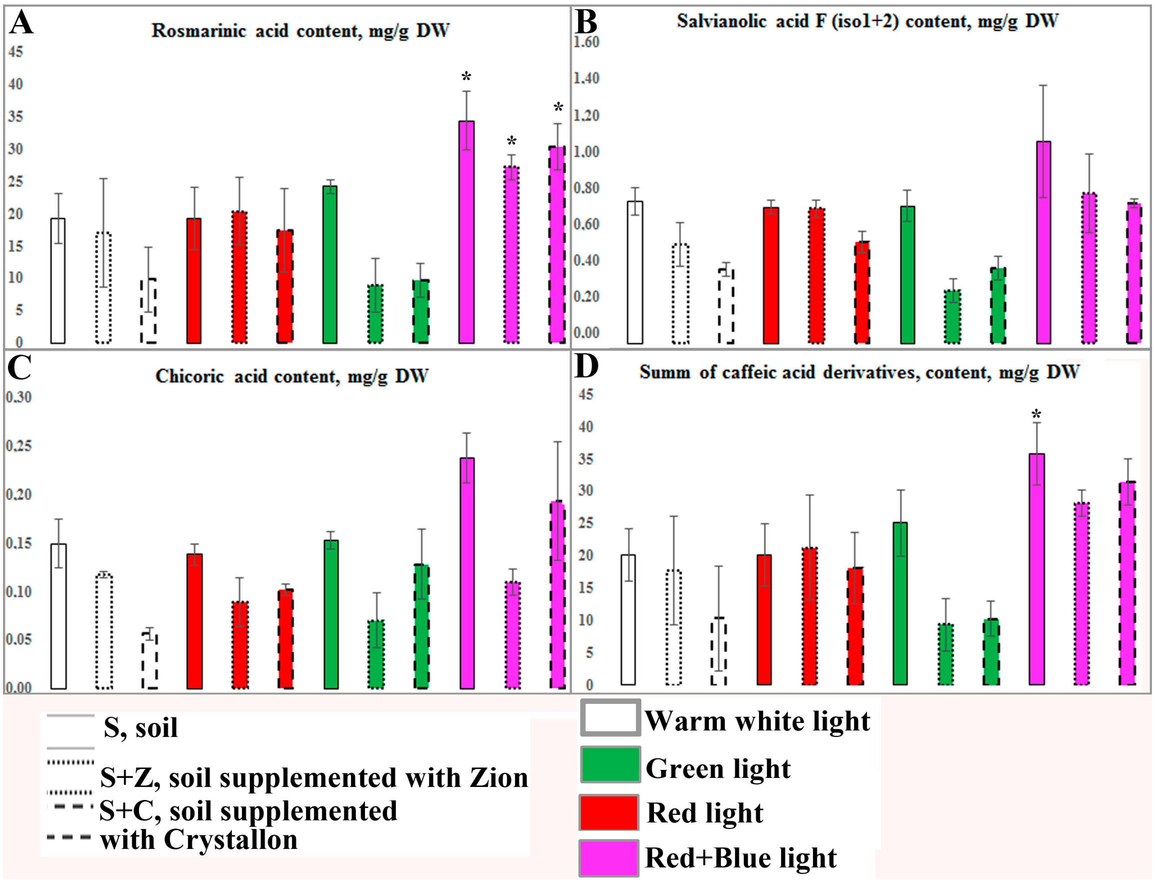

Figure 5. Analysis of caffeic acid derivatives in the roots of *O. basilicum* plants growing under different combinations of artificial lighting and soil composition. Contents (mg/g dry weight) of rosmarinic acid (**A**), salvianolic acid F ((**B**), isomers 1 and 2), chicoric acid (**C**), and sum of CAD (**D**) in the roots of 40-day-old *O. basilicum* plants. The plants were grown in soil (S), soil supplemented with Z-ion (S+Z), or soil supplemented with Crystallon (S+C). W, R, G, and RB represent different light variations with intensities of 300 µmol $m^{-2}s^{-1}$. The mean ± standard error of the mean was used to represent data from three separate studies with 10 biological replicates. Statistical significance is shown by asterix above the error bars (ANOVA, $p < 0.05$).

Analysis of the overall productivity of the CAD in the *O. basilicum* plants growing under different combinations of artificial lighting variation and soil composition revealed that the combination of R light and Crystallon supplementation resulted in the best productivity, as did the fresh and dry biomasses of the basil plants (Figure 6). The productivity (mg/L soil) of the sum of the caffeic acid derivatives in the leaves of the plants growing under these conditions (Figure 6A) was more than three times greater than that in the leaves of the plants growing under the control conditions (untreated soil and W light). The overall production of CAD in the dried leaves and roots (Figure 6B) of *O. basilicum* plants was more than three times greater than that in the plants growing under control conditions (untreated soil and W light).

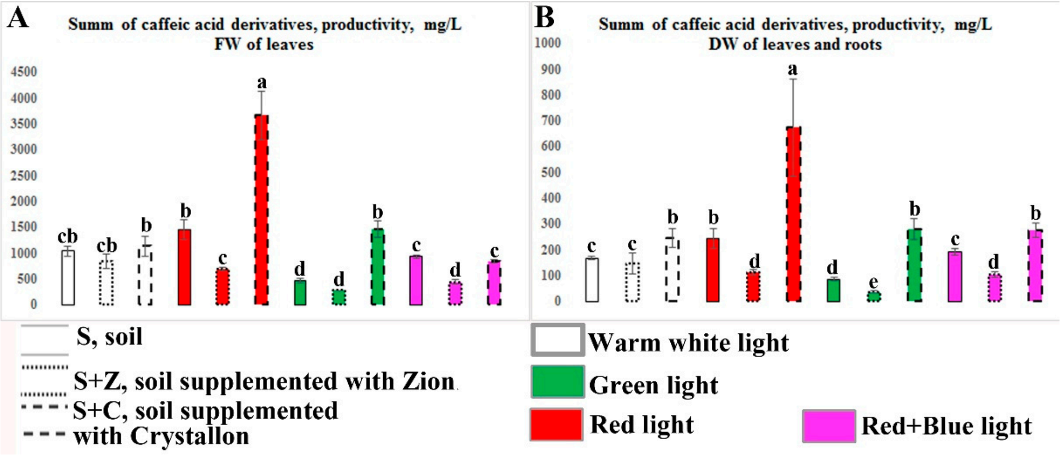

Figure 6. Analysis of the productivity of caffeic acid derivatives in *O. basilicum* plants growing under different combinations of artificial lighting and soil composition. Productivity (mg/L soil) of the sum of the caffeic acid derivatives in the leaves ((**A**), fresh weight) and leaves and roots ((**B**), dry weight) of 40-day-old *O. basilicum* plants. The plants were grown in soil (S), soil supplemented with Z-ion (S+Z), or soil supplemented with Crystallon (S+C). W, R, G, and RB represent different light variations with intensities of 300 μmol $m^{-2}s^{-1}$. The mean ± standard error of the mean was used to represent data from three separate studies with 10 biological replicates. Statistical significance is shown by different letters above the error bars (ANOVA, $p < 0.05$).

2.4. Content and Productivity of Anthocyanins in O. basilicum Plants

Anthocyanins are suggested to be among the main protective compounds in plants against light-induced damage [28]. Thus, the quantity and quality of light significantly affect the regulation of anthocyanin production [29]. The content and productivity of anthocyanins were analyzed in *O. basilicum* plants grown under different combinations of light and soil supplementation. The anthocyanin profile recorded at 530 nm (characteristic of pink-colored derivatives) revealed the presence of eleven compounds (Figure 7). The structural determination of each individual metabolite included multistage MS fragmentation (MS^3) and comparison with previously reported information [3,26,27]. Notably, all the identified anthocyanins were cyanidin derivatives. The aglycon fragments (at *m*/*z* 287 for positive mode and 285 for negative mode) were well observed in all the MS^2 and/or MS^3 spectra and corresponded to cyanidin. Moreover, most defined anthocyanins are derived from cyanidin triglycoside (cyanidin-3-sophoroside-5-glucoside, as known from published data) by acylation with malonic and various hydroxycinnamic acids and are described as the main anthocyanins of basil [3,26,27]. Thus, the anthocyanin composition of samples was as follows: one monoacylated derivative, **A1**; five diacylated derivatives, **A2**, **A4**, **A5**, **A8,** and **A9**; and four triacylated derivatives, **A6**, **A7**, **A10,** and **A11** (Table 1). Compound **A3** (with a molecular formula of $C_{49}H_{51}O_{26}$) was found in *O. basilicum* for the first time and was presumably assigned as cyanidine-3-(caffeoyl-hydroxybensoyl-sophoroside)-5-glucoside because of the similarity of its mass spectrometric behavior with previously published data for sweet potato [30, 31].

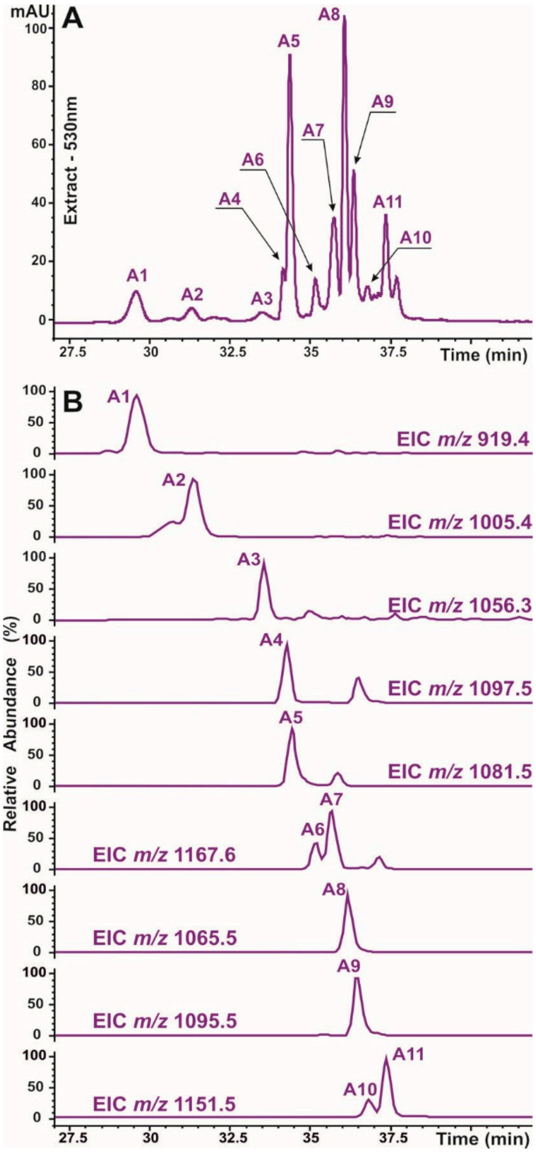

Figure 7. HPLC-UV/Vis-MS determination of anthocyanin compounds identified in crude extracts of *O. basilicum*. Vis profile of a typical studied sample recorded at 520 nm (**A**). Extracted ion chromatograms (EICs) of the studied anthocyanins acquired in positive ion mode with a restricted window of ±0.5 m/z are displayed in each trace (**B**). The peak numbers correspond to the identified components and are listed in Table 1.

Analysis of the total anthocyanin content revealed that the control warm white light (W) with an intensity of 300 µmol $m^{-2}s^{-1}$ resulted in anthocyanin accumulation of up to 100 µM/g DW (Figure 8). The supplementation of the soil with Z-ion did not affect anthocyanin accumulation, whereas the supplementation of the soil with Crystallon led to a greater than twofold decrease in anthocyanin accumulation compared with that of the plants growing in the untreated soil. The cultivation of basil plants in soil supplemented with Crystallon under R and RB light led to an almost twofold increase in anthocyanin biosynthesis, whereas G light had no effect. Moreover, only RB light had a significant effect on anthocyanin accumulation in plants growing in the control soil and soil supplemented with Z-ion (Figure 8A).

Considering the positive effect of Crystallon on the growth of basil plants, we obtained the maximum productivity of anthocyanins (µM/L soil, fresh weight) in plants growing in soil supplemented with Crystallon under R light. G light or RB light conditions had no considerable effect on the productivity of anthocyanins in basil plants (Figure 8B).

Thus, the combination of R light and supplementation of soil with Crystallon provided more than 4500 µM/L soil, which is three times greater than the productivity of basil plants growing in the control untreated soil under W light.

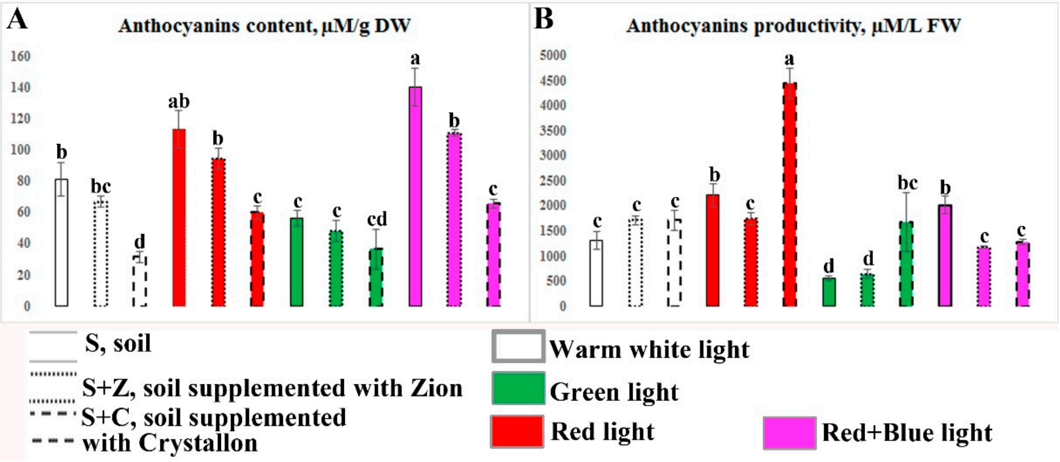

Figure 8. Content and productivity of anthocyanins in *O. basilicum* plants growing under different combinations of artificial lighting and soil composition. Total anthocyanin content ((**A**), µM/g DW) and productivity ((**B**), µM/l soil, fresh weight) in 40-day-old *O. basilicum* plants. The plants were grown in soil (S), soil supplemented with Z-ion (S+Z), or soil supplemented with Crystallon (S+C). W, R, G, and RB represent different light variations with intensities of 300 µmol $m^{-2}s^{-1}$. The mean ± standard error of the mean was used to represent data from three separate studies with 10 biological replicates. Statistical significance is shown by different letters above the error bars (ANOVA, $p < 0.05$).

3. Discussion

The aim of the present work was to study the simultaneous effects of a combination of soil composition and light conditions on the morphological and biochemical characteristics of *O. basilicum*. Despite the large number of publications devoted to the cost-effective cultivation of basil, works that have investigated the simultaneous effects of soil supplementation and light conditions on productivity and biosynthesis have not been reported. In the present work, for the first time, we investigated the effects of the combination of soil supplementation and monochrome lighting in comparison with standard white and often-used RB light on the productivity and accumulation of phytochemicals in basil plants.

Soil composition is an important factor for the cost-effective cultivation of crop plants. Ion exchange fertilizers are mixtures of anion exchangers, ion exchangers, and plant nutrient ions. Since ion exchangers have a high exchange capacity, the nutrient content in substrates can exceed that in the best natural soils [32]. The cations most easily absorbed by plants are in an ion exchange state in the form of the mobile ions K^+, Ca^{2+}, and Mg^{2+} [33]. Small proportions of ion exchange substrates can have positive effects on plant growth [34] and root mass gain [33]. Another pivotal parameter for the optimal growth of plants is light conditions [35]. The development of optimal light conditions for cost-effective growth, including the examination of biochemical and physiological parameters, is the primary scientific direction in this field [36]. Moreover, most recent works are aimed at studying the effects of supplementation with red and blue light or variations in the red/blue combination. A decrease in the intensity of radiation in the blue part of the spectrum led to a decrease in the fresh mass of basil plants, and the best ratio for the proper development of plants was a ratio of red to blue light of 0.7. Notably, the ratio of red to blue light in the radiation spectrum has different effects on the growth of basil plants and the content of phenols, depending on the plant variety [37]. In green holy basil, 3R:1B was favorable for biomass formation and photosynthetic responses, whereas 1R:3B promoted the accumulation of antioxidants. Furthermore, 1R:3B offered the best growing conditions for red holy basil cultivation, encouraging increases in plant biomass and physiological and antioxidant capacities [38]. In contrast, a relatively high B proportion suppressed stem elongation and leaf expansion and reduced shoot biomass in all the tested species except red mustard [39]. For basil cultivars, the inclusion of G wavelengths decreased shoot biomass compared with that of plants grown under R and B light combinations with similar B proportions [39].

In the case of LED light, it can be assumed that red light is more effective for CAD biosynthesis than blue or green light. A relatively high BP increased the phytochemical concentration but decreased the total amount of phytochemicals present per plant. The effects on phytochemical accumulation were species-specific for the inclusion of G wavelengths [39]. However, the different combinations of red, blue, and green light with intensities of 200 μmol $m^{-2}s^{-1}$ did not significantly affect the biosynthesis of CAD [39]. Increasing the intensity of the different combinations of red, blue, and green light to 300 μmol $m^{-2}s^{-1}$ had no significant effect on CAD biosynthesis in sweet basil plants [40-43]. The supplementation of white light with R and B (B2:R3:W5) led to a twofold increase in the levels of three major polyphenols in the leaves of sweet basil [43]. In the same experiments with fluorescent lamps, red light with an intensity of 100 μmol $m^{-2}s^{-1}$ caused more than twice as much RA as blue light did [22].

The results of the present study showed that supplementation of soil with Crystallon had a greater effect on the growth of both the above- and underground parts of *O. basilicum* plants. Compared with the control treatment, the addition of Z-ion to the soil led to a twofold increase in biomass accumulation. However, the effect of Crystallon on the growth of *O. basilicum* plants was significantly greater, with a fourfold increase compared with that in the control soil. Compared with 300 μmol $m^{-2}s^{-1}$ warm white light, monochromatic light with an intensity of 300 μmol $m^{-2}s^{-1}$ did not affect growth. However, 300 μmol $m^{-2}s^{-1}$ RB (50:50) significantly affected the effects of soil supplementation on the growth parameters. In addition to growth characteristics, we examined caffeic acid derivatives and anthocyanin contents in *O. basilicum* plants grown under different combinations of artificial lighting and soil compositions. We have shown that, along with a significant improvement in growth characteristics, supplementation of soil with Crystallon leads to a significant decrease in the content of caffeic acid derivatives and anthocyanin, more than 2-fold. Interestingly, growing *O. basilicum* plants under R and RB light led to a 2-fold increase in the biosynthesis of both RA and anthocyanin. However, given that under RB light, there is no positive effect of Crystallon on growth, the productivity of RA and anthocyanin reached a maximum when *O. basilicum* plants were grown under R light with Crystallon supplementation.

4. Materials and Methods

4.1. Plant Materials, Growth Conditions, and Experimental Design

The experiment was conducted using seeds of the O. basilicum variety "Rosy" (Netherlands). Following germination, identically sized seedlings were placed in 500 cm^3 plastic containers filled with soil (9 × 9 × 10 cm). As previously mentioned [27], ready-made soil for garden plants called "Universal" (Terra Master LLC, Novosibirsk, Russia) was used for planting. A photoperiod of 16/8 (light/darkness, hours) and an air humidity of 70% were maintained for 35 days while the plants were grown in soil at an average temperature of 21 °C. A standard TM618N-4 digital timer (SINOTIMER, China) was used to control the photoperiod. Every two days, 150 mL of water was given to the *O. basilicum* plants in each container. The experiment was conducted in triplicate over the course of 35 days. The study was conducted at the greenhouse of the Institute of Automation and Control Processes of the Far Eastern Branch of the Russian Academy of Sciences (IACP FEB RAS) from 2023 to 2024.

The plants were grown in three different types of soil (Table 2). The control soil without any supplementation was designated "S". Two variants of soil supplemented with growth stimulators were used as experimental conditions. The soil mixed (up to 5%) with Z-ion (Ecokhimprom, Republic of Belarus) was designated "S+Z". Z-ion contents were N1:P1:K1, mg/kg: 4960:4730:11,280, pH 6.9. The third variant was soil supplemented with complex fertilizer Crystallon (Fertica, Russia), designated "S+C". The contents of the microelements in the Crystallon samples were as follows: N:P:K—18:18:18, Mg—3, S—5, Fe—0.07, Mn—0.04, B—0.025, Cu—0.01, Mo—0.004, and Zn—0.025. Complex fertilizer Crystallon was applied in dissolved form during irrigation (0.002 g/mL).

The plants were grown in chambers with light sources designed and manufactured at the IACP FEB RAS as described previously [27]. The matrices of the light sources were composed of three-watt (CHANZON, China) LEDs of different colors. In this study, the following varieties of light were used (Table 2): warm white (W) as a control; monochrome light sources, red (R, 100%) and green (G, 100%); and binary light sources, red/blue (RB, 50:50%). All light variants were used with an intensity of 300 μmol $m^{-2}s^{-1}$. The dimensions of the chamber were 100 × 50 × 50 cm; other parameters were described previously [27]. The temperature regime was maintained by an exhaust fan FFB1212SH 12025 (power: 14.8 W, speed: 3700 rpm, air volume: 140.16 cfm, Uni-T, Dongguan, China). The spectra were measured via a PG200N spectrophotometer (UPRtek, Taiwan). A UT61A digital multimeter (Uni-T, Dongguan, China) controlled the driver supply currents.

Table 2. Experimental design.

Soil Variants	Different Spectra of LED Lamps (of 300 μmol $m^{-2}s^{-1}$)			
	W (Warm White)	**R (Red)**	**G (Green)**	**RB (Red/Blue)**
Soil without any supplementation (S)	W, S	R, S	G, S	RB, S
Soil supplemented with Z-ion (S+Z)	W, S+Z	R, S+Z	G, S+Z	RB, S+Z
Soil supplemented with Crystallon (S+C)	W, S+C	R, S+C	G, S+C	RB, S+C

4.2. Measurements of Morphometric Characteristics

For analysis, adult plants were used on the 35th day of growth before the formation of buds. Measurements of the morphometric characteristics (area, length, width of the leaves) of the 35-day-old *O. basilicum* plants were carried out via an Epson Perfection V850 Pro scanner (Epson, Suwa, Japan) with WinFolia Pro 2020 software (Regent Instruments, Québec, Australia). The weights of the aerial parts of the plants and roots were measured via Ohaus EX225/AD automatic scales (Ohaus Corporation, Parsippany-Troy Hills, NJ, USA).

The dry weights were calculated using the following formula:

$$C = \frac{Wd}{Wf} \times 100$$

4.3. Anthocyanin and Caffeic Acid Derivative Analysis

Chemicals

An analytical standard of cyanidine chloride was obtained from Sigma–Aldrich (St. Louis, MO, USA). We used a standard sample of research-grade rosmarinic acid, which was previously obtained [44]. All extraction solutions and eluents were prepared with ultrapure water (Millipore, Bedford, MA, USA). All solvents were of analytical grade.

Sample preparation for analytical chromatography

The sample preparation procedure was carried out as described in our earlier publication [24]. To summarize, 80% v/v methanol was used for sonicated extraction of dried and powdered plant tissue. The resulting supernatant was then filtered through a 0.45-μm membrane (Millipore, Bedford, MA, USA), and a 1 μL aliquot was used for analysis.

Analytical chromatography and mass spectrometry

First, one typical plant sample was chosen to study and identify the phenolic metabolites. UV–Vis and mass–spectral data were collected and examined; the MS fragmentation of each individual compound was studied. A time-of-flight mass spectrometer was used to obtain high-resolution mass measurements (with a mass error of less than 4.5 mDa), and chemical formulas were determined for the identified substances. The identification of defined compounds was based on the analysis of their retention duration and UV–Vis and MS spectrometric data and the comparison of the same parameters with published data [3,26,27] and reference samples.

An Agilent 1260 Infinity analytical HPLC instrument (Agilent Technologies, Santa Clara, CA, USA) equipped with a PDA detector was used for metabolite profiling. The separation of the determined compounds was carried out on a Zorbax C18 column (150 mm, 2.1 mm i.d., 3.5 µm, Agilent Technologies, Santa Clara, CA,USA) at a column temperature of 40 °C. The gradient elution with a flow rate of 0.2 mL/min was realized with two eluents, A (0.1% aqueous formic acid) and B (acetonitrile with 0.1% formic acid addition). The following linear gradient was used: 0–10 min—0% B; 45 min—40% B; and 55 min—100% B. UV–Vis spectral measurements were recorded in the range of 200–800 nm via a PDA detector, and chromatograms for quantification were acquired at wavelengths of 325 and 530 nm. An ion trap mass spectrometer Bruker HCT ultra PTM Discovery System (Bruker Daltonik GmbH, Bremen, Germany) interfaced with an HPLC system was used for the MS experiments. The MS instrument was operated in electrospray ionization (ESI) mode and in both positive and negative ionization modes. The following settings were used: mass range, 100–1.400 Da; drying gas (N_2) flow rate, 8.0 L/min; nebulizer gas (N_2) pressure, 25 psi; and drying gas temperature, 325 °C. The auto-MS^n mode (smart fragmentation) was used for the MS^2 and MS^3 experiments. A Shimadzu LCMS-IT-TOF instrument (Shimadzu, Japan) including a tandem ion trap/time-of-flight mass spectrometer was utilized for the high-resolution MS studies. The following operating conditions were used: the drying gas (N_2) pressure was 100 kPa, the nebulizer gas flow rate was 1.5 L/min, the ion source potential was changed from −3.8 to 4.5 kV, and the interface temperature was 200 °C. The external standard method was employed for quantification of the detected compounds. An analytical standard of cyanidine chloride was chosen for quantifying the discovered anthocyanins, and a rosmarinic acid methanol solution was used for evaluating caffeic acid derivatives.

Since the plants were grown in separate containers and not by continuous sowing, the productivity was calculated per liter of soil; in one container, there was 0.5 L of soil. Productivity was calculated as follows:

$$\text{Productivity } (\mu\text{M/L}) = \text{Content} \times \text{FW},$$

where Content is the content of substances in a plant (µM/g FW) and FW is the fresh weight of the aboveground part of the *O. basilicum* plants per liter of soil (g/L).

4.4. Statistical Analysis

An Excel add-on called XFolia 2020 was used to handle the statistical data (morphometric parameters). The STATISTICA 12.6 software package (StatSoft, Inc., Tulsa, OK, USA) was used for statistical analysis. Every value is presented as the mean ± standard error (SE). Student's *t*-test was employed for the statistical assessment to compare two independent groups. Analysis of variance (ANOVA) and a multiple comparison process were used to compare multiple datasets. A post hoc test called Fisher's protected least significant difference (PLSD) was employed. At $p < 0.05$, the threshold for statistical significance was established. The correlations between the two variables were determined via Pearson correlation analysis.

5. Conclusions

Research on lighting settings that can increase the sustainability and profitability of PFALs has become increasingly important in recent years [1]. In the present work, in addition to growth characteristics, we examined the content of caffeic acid derivatives and anthocyanin in the leaves of *O. basilicum* plants growing under different combinations of artificial lighting and different soil compositions. We have shown that, along with a significant improvement in growth characteristics, supplementation of soil with Crystallon leads to a significant decrease in the content of caffeic acid derivatives and anthocyanin, more than 2-fold. Interestingly, growing *O. basilicum* plants under R and RB light led to a 2-fold increase in the biosynthesis of both the key caffeic acid derivative RA and anthocyanin. However, given that under RB light, there is no positive effect of Crystallon on growth, the productivity of RA and anthocyanin reached a maximum when *O. basilicum* plants were grown under R light and Crystallon. Thus, on the basis of the data obtained, a conclusion can be drawn. When growing on a large scale, two important factors must be taken into account. First, improving growth through soil supplementation can lead to plant depletion of bioactive phytochemicals such as CAD and anthocyanins. Second, this problem can be solved by modulating the lighting parameters.

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