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The Morphological Responses of *Calendula officinalis* L. "Radio" to the Foliar Application of Benzyladenine and Different Light Spectra

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Abstract: Pot marigold is a valuable medicinal plant with great decorative value. Three combinations of light (white (W)—170 µmol m^{-2} s^{-1}, white + blue (W+B)—230 µmol m^{-2} s^{-1}, white + red (W+R)—230 µmol m^{-2} s^{-1}) were used to analyse the influence of a diversified light spectrum on the morphological traits and flowering of *Calendula officinalis* L. "Radio". The effect of foliar treatment of the plants with 6-benzyladenine (BA) at concentrations of 100, 150 and 200 mg dm^{-3} at all the light spectrum combinations was analysed. BA had negative influence on the earliness of florescence and delayed it even by more than 10 days. W+B light intensified the delay, whereas red light partly reduced it. The BA treatment had the greatest influence on the biometric traits of the plants at the initial period of their development. W+B light significantly inhibited the growth of the plants. A high share of red light in the spectrum positively affected the Fv/Fm value, the relative chlorophyll content and the percentage of dry matter in the plants. When the amount of blue or red light in the spectrum increased, it was possible to obtain specific biometric traits of *Calendula* without the BA treatment.

Keywords: blue light; cytokinins; LED; pot marigold; red light

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1. Introduction

Calendula officinalis L. (pot marigold, common marigold, garden marigold, English marigold) is an annual ornamental plant of the Asteraceae family, *Calendula* genus. It is indigenous to central, eastern and southern Europe. It has been grown in European gardens and used in popular culture since the 12th century [1]. In Poland it has been cultivated for many years for ornamental and medical purposes.

Pot marigold is a valuable medicinal plant. The cultivars with intense orange flowers prevail in herbal cultivation. *C. officinalis* flowers are a source of biologically active substances, dominated by flavonoids and terpenoids, used in the pharmaceutical industry and cosmetic. They are currently used as pharmaceutical raw material in official phytotherapy [2–4].

Plant growth retardants enable the production of high quality, compact pot plants [5]. Their effectiveness depends on their concentration, the time and method of application, the plant species and cultivars, the target organ as well as physiological and environmental conditions [6,7]. Plant growth retardants are usually applied as foliar sprays and soil drenches [8]. They can delay cell division and the elongation of aerial parts of plants, restrict the biosynthesis of gibberellins, and thus reduce the length of internodes and vegetative growth [9]. Growth regulators from the cytokinin group regulate many fundamental physiological and developmental programmers in plants, including embryo

and seed development, germination, photomorphogenesis, plant growth and expansion, organ formation, vascular development, leaf senescence, plant immunity, and circadian rhythms [10,11]. Benzyladenine may either positively or negatively affect the qualitative traits of flowers, i.e., the peduncle length, the size and weight of flowers [12].

In the cultivation of ornamental plants, growth regulators are used in order to increase the habit formation capacity and improve the durability and quality of flowering. However, due to the protection of the natural environment and human health, there is a tendency to limit their use [13]. There are many methods that has the potential of replacing chemical growth regulators in the commercial production of ornamental plants and nowadays chemicals should be used as a last resort [14]. The results of various studies show that the growth and development of plants can be strongly modified by different colour of light during cultivation [15–17]. It is known that the increasing portion of blue LED light complementing red light may reduce stem extension and result in more compact plants [18]. The use of modern LED technology enables precise adjustment of the spectral composition to the needs of a given species, and allows control over the growth and development of plants [19]. The blue, red, and far-red lights are generally effective for controlling morphogenesis, because of associated with different photoreceptors. The photoreceptor responsible for absorbing the red (600–700 nm) and far red (700–800 nm) wavelengths is phytochrome. The blue light (400–500 nm) photoreceptor is the cryptochrome [20].

Chemical growth regulators are often used to produce the desired plant habit. However, using LEDs in production makes it possible to obtain the proper plant habit using the appropriate spectrum of light, without using any chemicals. Light of a specific color can affect growth and morphogenesis of some plant species and also accelerate their flowering. This study was conducted to evaluate the light spectrum and benzyladenine effects on some morphologic (i.e., earliness of flowering, number and diameter of inflorescences plant height, number of lateral shoots) and physiologic (i.e., relative chlorophyll content and efficiency of photochemical performance of PSII) parameters of pot marigold. The main aim of the study was to determine the morphological changes—the growth and flowering parameters of *C. officinalis* "Radio" depending on the light spectrum and benzyladenine concentration.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The experiments were carried out in controlled-environment growth chambers. Pot marigold (*Calendula officinalis* L.) "Radio" seeds were sown into germination trays in a greenhouse at a temperature of 18–20 °C. When young plants had three-four true leaves, they were transplanted into individual pots and transferred to growth chambers. The plants were grown in 500 cm^3 pots filled with peat substrate for vegetable transplant production (TS-1, Klassmann-Deilman, Germany). Two weeks after planting Peters Professional Blossom Booster 10-30-20+MgO+TE fertiliser was applied at a concentration of 0.2% at seven-day intervals throughout the experiment. A 16-h photoperiod and a day/night temperature of 21/18 °C were maintained. The relative air humidity was 65–70%.

2.2. Treatment of Cytokines and Light

Seven days after being planted in pots the marigold plants were sprayed with aqueous solutions of cytokinin—6-benzyladenine—BA (100, 150 and 200 mg dm^{-3}), which was applied to the leaves only once for all light treatments. Untreated (control) plants were sprayed with distilled water.

Three combinations of light were used in the experiment: white light (W), white light with red light (W+R), white light with blue light (W+B). The red, blue and white light came from a high-power solid-state lighting module (LED) (SMD type, Seoul Semiconductor, South Korea). The spectra of the light sources are shown in Figure 1 and Table 1. The photosynthetic photon flux density (PPFD) from the top of the plants amounted to about 170 μmol m^{-2} s^{-1} ($\pm 14_{SD}$) for W light and additionally 60 μmol m^{-2} s^{-1} ($\pm 8_{SD}$) for blue

(B) and red (R) light. The PPFD for combinations W+R and W+ B light amounted to about 230 µmol m^{-2} s^{-1}. The daily light integral (DLI) amounted to about 9.8 mol m^{-2} d^{-1} for W light and to about 13.2 mol m^{-2} d^{-1} for W+R and W+B light. The method developed by [21] was used to calculate the phytochrome photostationary state (Φ). The PPFD was measured with a quantum sensor (PAR-10; Sanopan, Białystok, Poland). The spectral distribution of light treatments was measured with a spectroradiometer BLACK-Comet CXR, 280–900 nm (UV-VIS by StellarNet Inc.; Tampa, USA). The measurements were made 15 cm under the lamps, more or less at the height of the tops of the plants. As the plants grew, the lamps were gradually raised to a higher position.

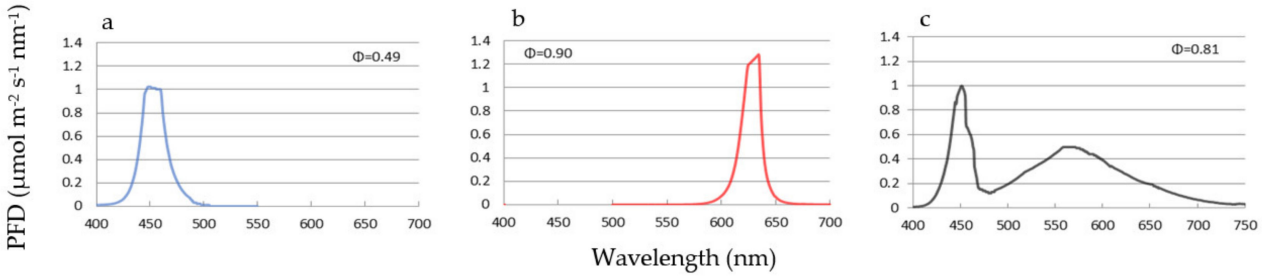

Figure 1. Spectral photon distribution of LEDs light sources: (**a**)—blue light; (**b**)—red light; (**c**)—white light.

Table 1. Characteristics of white light source.

Light Colour	Wavelength (nm)	PFD * (µmol m^{-2} s^{-1})	% for W Light	% for W+R	% for W+B
UV	320–380	0.5	0.3	0.2	0.2
Violet	380–450	15.4	8.9	6.6	6.6
Blue	450-495	30.3	17.6	13.0	38.9
Green	495–570	53.5	31.1	23.0	23.0
Yellow	570–590	18.7	10.9	8.1	8.1
Orange	590–620	21.8	12.7	9.4	9.4
Red (R)	620–700	26.4	15.3	37.2	11.4
Far Red (FR)	700–780	5.6	3.3	2.4	2.4
sum	320–780	172.2	100	100	100
R:FR		4.7	-	15.5	-

* Photon flux density.

2.3. Plant Measurements and Experimental Design

The first biometric measurements were made when the first inflorescence developed. The next measurements were made when the second and third inflorescences developed and independently in the 8th, 10th, 12th and 14th week of growth in the growth chambers. The heights of the plants and the diameters of the anthodia were measured. The buds and lateral shoots were counted. The relative chlorophyll content in the fifth week of growth in chambers was measured with a Minolta SPAD apparatus on three randomly selected leaves of each plant. Chlorophyll fluorescence was measured with an OS1-FL modulated fluorometer (Optiscience) one hour after the termination of the lighting period. The dark adapted parameters were used to determine the maximum quantum yield of PS II (photosystem II): Fv/Fm = (Fm − Fo)/Fm (Fo—the dark adapted initial minimum fluorescence, Fm—the maximum fluorescence measured during the first saturation pulse after dark adaption) [22]. The dry mass was calculated by drying the material to a constant weight at 105 °C for 24 h [23].

The research was conducted as a two-factorial experiment in eight replicates as an independent design. One pot was treated as one replicate. The investigations were conducted in two series (replications after each other). The results are the means of two series. The data were analysed with ANOVA. Differences between the means were estimated with the

Newman-Keuls test at a significance level $\alpha = 0.05$. All the data were analysed statistically with the Statistica program (StatSoft, Poland).

2.4. Model Construction

The statistical analysis was based on discriminant analysis. It checked whether different concentrations of the BA phytohormone and different combinations of light wavelengths affected the morphological parameters of marigolds. The construction of the model was based on canonical variate analysis (CVA)—the canonical variation of Fisher's linear discriminant analysis (LDA).

The CVA enabled the construction of the CCA model (canonical-correlation analysis). Progressive stepwise analysis was applied to find which variables determined the morphological traits to the greatest extent. All variables were assessed. The ones whose p and F values for the variable under analysis discriminated the groups to the greatest extent were included in the model.

The cut-off significance level was determined with the Monte Carlo permutation test (9999 permutations). The Canoco for Windows software package and the Microsoft Excel spreadsheet were used for all comparisons, calculations and graphic elements. The following Canoco for Windows tools were used: Canoco for Windows 4.5, CanoDraw for Windows and WCanoIMP.

3. Results

3.1. The Earliness of Florescence

The research results showed that both the colour of light and BA significantly influenced the earliness of florescence of marigolds (Table 2). The plants which were not treated with BA and illuminated with R+W light were the first to bloom. BA, regardless of the concentration, delayed the emergence of the first inflorescence by about 10 days, as compared with the plants which were not treated with BA. This tendency continued throughout the experiment. The illumination of the plants with W+B light had similar effect. The difference in the emergence of successive inflorescences between the plants illuminated with W+R light and the other combinations tended to increase. The plants treated with BA at a concentration of 200 mg dm^{-3} and illuminated with W+B light were the last to start flowering, although the time of the emergence of subsequent flowers was shorter and shorter. The influence of BA was noticeably reduced by illumination with W+R light, whereas BA treatment and illumination with W+B light resulted in a synergistic effect.

3.2. Biometric Parameters

Both light and BA noticeably influenced the height of the plants. It is noteworthy that the addition of W+R light stimulated the growth of plants only in combination with BA at the concentrations tested in the experiment. By contrast, the correlation of BA with W or W+B light inhibited the growth of plants.

When BA was applied at concentrations of 100 and 150 mg dm^{-3}, it significantly influenced the branching of plants, especially at the beginning of the growing period. It is noteworthy how W+R light interacted with BA at these concentrations and affected the branching of plants. The number of lateral shoots in these combinations was about two times greater than in the other combinations. W+R light stimulated the branching of the plants throughout the experiment.

Neither the BA concentration nor the type of light had significant influence on the number of inflorescence buds. The comparison of interactions revealed the greatest number of buds at the beginning of flowering, when the first inflorescence developed in plants treated with BA at a concentration of 150 mg dm^{-3} and illuminated with W+R light. The most buds in the plants with three developed inflorescences was observed after illumination with W+R light, but without treatment with BA. At this stage of plant development BA had negative influence on the production of inflorescence buds.

Table 2. Morphological parameters of *Calendula officinalis* "Radio" in depending on light colour and benzyladenine concentration.

Light Colour	Benzyladenine (BA) Concentrations (mg dm^{-3})														
	0	**100**	**150**	**200**	**Mean for Light**	**0**	**100**	**150**	**200**	**Mean for Light**	**0**	**100**	**150**	**200**	**Mean for Light**
	first inflorescence					second inflorescence					third inflorescence				
	Earliness of flowering (days)														
W	44.2 bc	49.0 bc	49.0 bc	52.0 c	**48.5 b**	49.7 a	56.8 bc	75.1 cd	74.5 cd	**64.0 b**	68.0 bc	62.3 b	86.8 de	81.5 d	**74.7 b**
W+R	34.5 a	42.2 ab	48.7 bc	46.7 bc	**43.0 a**	47.1 a	54.1 bc	56.9 bc	56.4 bc	**52.5 a**	57.6 a	61.7 b	63.4 b	65.3 bc	**62.0 a**
W+B	47.3 bc	67.3 d	63.0 d	64.0 d	**60.4 c**	51.8 ab	71.4 cd	67.3 c	81.4 d	**68.0 b**	68.2 bc	80.3 d	74.2 c	90.0 e	**78.2 b**
Mean for BA	**42.0 a**	**52.8 b**	**53.6 b**	**54.2 b**		**49.5 a**	**60.8 b**	**71.2 c**	**70.8 c**		**64.6 a**	**68.1 ab**	**74.8 b**	**78.9 b**	
	Number of inflorescences buds														
W	1.4 ab	1.4 ab	1.3 b	0.6 b	**1.2 a**	1.0 ab	0.8 ab	1.0 ab	0.0 b	**0.7 b**	0.4 cd	0.0 d	0.7 bd	1.0 bd	**0.5 b**
W+R	0.9 b	1.2 b	2.2 a	1.2 b	**1.4 a**	1.0 ab	1.5 a	1.5 a	1.5 ab	**1.4 a**	3.0 a	0.4 cd	0.0 d	0.0 d	**0.9 ab**
W+B	1.0 b	1.3 b	1.2 b	0.5 b	**1.0 a**	0.0 b	0.5 ab	0.5 ab	1.0 ab	**0.5 b**	1.7 b	1.4 bc	1.0 bd	0.7 bd	**1.2 a**
Mean for BA	**1.1 ab**	**1.3 a**	**1.6 a**	**0.8 b**		**0.7 a**	**0.9 a**	**1.0 a**	**0.8 a**		**1.7 a**	**0.6 b**	**0.6 b**	**0.6 b**	
	Diameter of inflorescence (cm)														
W	7.0 a	5.6 c	6.1 ac	6.4 ac	**6.3 a**	5.8 bc	5.4 bc	4.9 c	6.0 bc	**5.5 b**	5.7 b	5.6 b	4.4 c	6.3 a	**5.5 a**
W+R	5.7 c	5.9 c	6.0 bc	5.6 c	**5.8 b**	6.1 bc	7.8 a	5.5 bc	6.5 b	**6.5 a**	6.4 a	6.2 a	5.0 bc	5.6 b	**5.8 a**
W+B	6.9 ab	6.3 ac	5.8 c	6.0 bc	**6.3 a**	5.0 bc	6.2 bc	5.9 bc	5.9 bc	**5.8 b**	5.7 b	5.3 bc	5.8 b	6.2 a	**5.8 a**
Mean for BA	**6.5 a**	**5.9 b**	**6.0 b**	**6.0 b**		**5.6 b**	**6.5 a**	**5.4 b**	**6.1 ab**		**5.9 a**	**5.7 a**	**5.1 b**	**6.0 a**	
	Height (cm)														
W	26.4 bc	26.3 bc	23.5 cd	24.5 cd	**25.2 b**	30.0 c	27.0 cd	26.7 cd	26.3 cd	**27.5 b**	36.0 b	29.4 cd	33.0 c	30.3 cd	**32.2 b**
W+R	28.4 b	37.9 a	37.0 a	38.3 a	**35.4 a**	33.9 bc	41.4 a	39.0 ab	41.0 a	**38.8 a**	34.0 c	48.3 a	41.3 ab	43.0 ab	**41.7 a**
W+B	27.0 bc	20.5 e	20.7 e	20.9 e	**22.3 c**	30.7 c	23.0 d	22.8 d	32.7 bc	**27.3 b**	32.0 c	26.9 d	25.4 d	33.3 c	**29.4 b**
Mean for BA	**27.3 a**	**28.2 a**	**27.1 a**	**27.9 a**		**31.5 a**	**30.5 a**	**29.5 a**	**33.3 a**		**34.0 a**	**34.9 a**	**33.2 a**	**35.5 a**	
	Number of lateral shoots														
W	2.2 b	2.3 b	2.2 b	1.8 b	**2.1 b**	2.8 c	3.0 c	2.8 c	2.0 d	**2.7 b**	3.4 c	3.0 c	3.0 c	3.0 c	**3.1 b**
W+R	1.8 b	4.0 a	4.5 a	2.1 b	**3.1 a**	4.3 ab	5.5 a	4.7 ab	4.0 ab	**4.6 a**	5.7 a	6.4 a	5.1 ab	4.7 ab	**5.5 a**
W+B	1.9 b	2.0 b	1.9 b	1.2 b	**1.8 b**	2.3 cd	2.5 cd	2.5 cd	3.0 c	**2.6 b**	3.7 c	3.0 c	3.0 c	3.4 c	**3.3 b**
Mean for BA	**2.0 b**	**2.8 a**	**2.9 a**	**1.7 b**		**3.1 a**	**3.7 a**	**3.3 a**	**3.0 a**		**4.3 a**	**4.1 a**	**3.7 a**	**3.7 a**	

Datas followed by the same letter do not differ significantly at $\alpha = 0.05$ for each parameter. W—white light, W+R—white and red light, W+B—white and blue light.

The BA treatment had negative influence on the diameter of the first developing inflorescence regardless of the light colour. At later stages of florescence the factors under analysis did not have such strong influence on the anthodium diameter.

3.3. Relative Content of Chlorophyll, Fluorescence, Dry Mass

The BA treatment at concentrations of 150 and 200 mg dm^{-3} and illumination with W light and W+B light had negative effect on the relative content of chlorophyll in the leaves of *C. officinalis* "Radio" and the percentage of dry matter. No significant differences were found in fluorescence value under all the combinations. However, under R light Fv/Fm resulted in a higher value (but no significant) compared to W and W+B light. (Table 3).

Table 3. Some parameters characterising *Calendula officinalis* in depending of light colour and benzyladenine concentration.

Light Colour	Benzyloadenine (BA) Concentrations (mg dm^{-3})				Mean for Light
	0	**100**	**150**	**200**	
			the relative content of chlorophyll		
W	18.3 b	16.5 c	12.3 d	12.9 d	15.0 b
W+R	22.0 a	21.8 a	18.9 b	15.5 c	19.6 a
W+B	14.5 cd	14.8 cd	12.3 d	12.2 d	13.4 b
Mean for BA	18.3 a	17.7 a	14.5 b	13.5 b	
			fluorescence Fv/Fm		
W	0.805 cd	0.815 ac	0.809 cd	0.814 bc	0.811 b
W+R	0.824 ab	0.826 a	0.823 ab	0.826 a	0.825 a
W+B	0.801 d	0.809 cd	0.813 bc	0.821 ab	0.811 b
Mean for BA	0.810 b	0.817 a	0.815 ab	0.820 ab	
			dry mass (g)		
W	0.69 b	0.60 bc	0.61 bc	0.55 c	0.61 b
W+R	0.86 a	0.75 b	0.89 a	0.74 b	0.81 a
W+B	0.65 b	8.7 0.59 c	0.49 d	0.57 c	0.57 b
Mean for BA	0.73 a	0.64 b	0.66 b	0.62 b	

Data followed by the same letter do not differ significantly at $\alpha = 0.05$ for each parameter. W—white light, W+R—white and red light, W+B—white and blue light.

3.4. The Morphological Parameters in the 8th, 10th, 12th and 14th Week of Pot Marigold Growth

The morphological traits of *C. officinalis* "Radio" were determined in the 8th, 10th, 12th and 14th week of its growth, regardless of the phase of development of individual plants. The height of the plants and the number of lateral shoots were stimulated by illumination with W+R light (Table 4, Figure 2). The lowest morphological parameters of the plants were observed in the 8th week of the experiment. The experiment showed that the BA treatment at a concentration of 200 mg dm^{-3} and the illumination of the plants with W+B light may inhibit their growth and development.

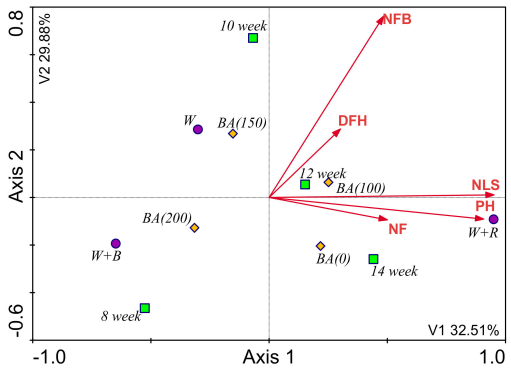

Figure 2. CCA analysis (n = 48)—relationships between the morphological parameters of marigolds

(PH, NLS, NF, DFH, NFB) and BA concentration levels and combinations of light spectrum in different weeks of growth.

Table 4. Statistical parameters for the CCA (canonical-correlation analysis) analysis.

	number of variables included: 5 number of variables rejected: 0 number of permutations: 9999		
	E %	*p*	*F*
NFB The number of flower buds	11.59	0.001	19.72
PH The plant height	9.78	0.001	16.69
NLS The number of lateral shoots	9.22	0.001	16.21
NF The number of inflorescences	8.74	0.001	11.42
DFH Diameter of flower heads	6.47	0.001	8.99

4. Discussion

4.1. Flowering

Light on its own or in combination with other factors significantly affects the development of many plant species [24].

The research showed that the applied spectrum of light significantly influenced the flowering of *C. officinalis* "Radio". The plants grown under W+R light (over 50% of red light in the spectrum) bloomed 5 days earlier than the plants grown under W light and as much as 13 days earlier than the plants grown under W+B light. Previous studies reported that blue light may stimulate the flowering of plants [25–27], especially long-day plants (LDP) [28]. However, in our experiment when the amount of B light was three times greater than the amount of R light, it significantly delayed the emergence of the first inflorescences. At a later phase, the subsequent inflorescences of the plants grown under W light and the ones grown under W+B light developed at a similar time. Interestingly, W+B light influenced the flowering date, but it did not have such significant influence on the number of buds and inflorescences' diameter. In the study by [20] the flowering of *Petunia* (LDP) was significantly accelerated only in the combination without B light, where the R:FR ratio was 1. Some previous studies have shown that a small amount of blue light (5–25%) within the total spectrum can stimulate plant growth and flowering [29,30]. Probably a high amount of B light in the spectrum can inhibit flowering due to reduced plant growth. Additionally, phytochromes may be the dominating photoreceptors regulating the flowering of at least some long-day plants [20]. It is not without significance that the influence of blue light on plants is stronger when they grow only under artificial light in growth chamber compared to plants grown in a greenhouse with natural and artificial light [31].

When discussing the influence of red light on flowering, it is impossible to skip the red to far red (R:FR) ratio. In our experiment the R:FR ratio was high and FR light did not significantly influence on flowering. Probably in our studies flowering was induced by a high amount of R light and low-intensity FR radiation has much less influenced on flowering [32,33]. The light combinations in our experiment did not significantly affect other parameters of the anthodia, such as diameter and buds' number, especially at later stages of the plants' growth.

4.2. Growth

W+B light significantly inhibited the elongation growth of the plants, especially during the first 35–50 days after planting. When the first inflorescences appeared on the plants in the W+B combination, their height was about 40% lower than in the W+R combination and 12% lower than the height of the plants illuminated with W light, although the plants in the W+B combination began flowering much later. This result indicates that the quantity of B light controls stem elongation. This effect has been observed in various plant species [30,34,35]. Blue light inhibits cell growth and blue light photoreceptors might regulate and change the expression through which stem elongation is inhibited [36,37]. Blue light may substitute plant growth regulators and produce desirably compact ornamentals because they inhibit extension growth [15,38]. By contrast, plants illuminated with a high proportion of the red light spectrum were the tallest and had many branches. Some authors [39,40] made similar observations in their studies. The latter team of researchers grew cultivated flower buds earlier under R light compared to white light. However, the cultivation only under red light had negative influence on the shape of the leaves and the appearance of the plants. The absorption of a large amount of red light by plant photoreceptors may cause the production of meta-topolins. These cytokinins are plant growth regulators stimulating cell division and plant branching [41].

It is noteworthy that the W and W+R light had the same amount of blue light. Nathe-less the plants in the W light combination had a similar height, number of lateral shoots and other traits to those in the W+B light combination. It is likely that apart from the amount of blue light, the R:B ratio is also important. It was about 1:1 for the W light combination and 3:1 for the W+R light combination. According to [42] the effect of red light on sunflower compactness might be partially due to the lack of blue light. However, some authors [43] observed that the increasing dose of red light with a constant amount of blue light had no effect on the length of shoots of tomato seedlings. Our study showed that there was no continuous linear relationship between the amount of blue light and the morphological traits of plants. It is likely that above a certain dose and at a specific R:B ratio blue light does not affect the morphological traits of plants so intensely, especially at later stages of their growth.

4.3. BA

Various studies reported that growth regulators delayed or accelerated flowering. The effect is mainly influenced by the species, type and concentration of the growth regulator. In one research was observed that *Calendula* plants treated with highest concentration of cycocel had the longest time of flowering and the lowest number of inflorescences [44]. In our studies BA delayed the flowering of plants and the development of subsequent inflorescences. This phenomenon was partly reduced by W+R light and intensified by W+B light. Similar results were achieved by other authors [45]. In their study BA treatment promoted the initiation of floral buds and flowering in petunia under R light.

Some previous researchers reported that the application of cytokinin cause decreasing the plant height of *Calendula* [46]. Our study showed that W+R light and BA stimulated the growth of the "Radio" cultivar, whereas W+B light combined with BA inhibited it. The results reported that plants without BA under all light combinations had a similar height at first stage of florescence. After applying BA, regardless of the dose, pot marigolds grown under W light were higher than these ones grown under W+B light, but significantly lower than plants grown under W+R light. This results show the interaction of cytokinins and light and the enhancement of the effect of a particular light colour on stimulation (R light) or inhibition (B light) of growth. According to [47] exogenous cytokinin influences on plants morphogenesis similar as blue light, but both factors could independently decrease the rate of extension growth [48], but the effect of exogenous cytokinins on hypocotyl elongation take place only in the dark [47]. Previous studies also showed that the action of exogenous cytokinins and light on the inhibition of hypocotyl elongation is independent [49].

4.4. Dry Matter

The percentage of dry weight decreased as cytokinin concentration increased. Studies have shown the tendency of BA to promote minimum dry weight. Some authors [50] observed a smaller weight of *Zantedeschia* "Black Magic" flowers after GA_3+BA treatment. In some plants grown in vitro the highest BA concentration resulted in the lowest dry weight [51,52]. Dry weight increased under W+R light. The low percentage of dry matter in the plants grown under W and W+B light may also have been caused by the higher proportion of blue than red light in the spectrum. Many researchers found that dry mass decreased steadily as B light increased [29,53,54].

4.5. Chlorophyll

Cytokinins considerably influence the chlorophyll content in leaves [55]. In previous research was observed that the chlorophyll content in leaves increases after BA treatment [50]. Some authors found that only high concentrations of BA decreased the chlorophyll content significantly [56,57]. Our study showed that the BA concentration of 100 mg dm^{-3} had no effect on the chlorophyll content in the leaves of *C. officinalis* "Radio". Higher concentrations of BA inhibited the synthesis of chlorophyll.

4.6. Fv/Fm

The BA treatments did not significantly influence on Fv/Fm value. However, it can be seen, especially for W+B light, a gradual increase in the Fv/Fm value as the BA dose increases. Research results show that cytokinins positively affect photosynthesis and regulate this process at various levels [55]. However, very high doses of cytokinins may reduce the Fv/Fm value [57]. In general, the Fv/Fm values ranging from 0.8008 to 0.8262 are commensurable to optimal values for most field-grown plant species [58].

It is known that red light induces photosynthesis and promotes plant growth more effectively than other colours [59,60]. In our study a greater amount of red light in the spectrum (W+R) positively affected the relative chlorophyll content, the dry matter content and the Fv/Fm value.

4.7. Daily Light Integral (DLI)

Increasing DLI decreased time to flower, increased biomass accumulation, hastened development in many floriculture crops [61]. In our research we did not notice the influence of DLI on the studied parameters. Probably because there was difference in the DLI between W light (9.8 mol m^{-2} d^{-1}) and W+B or R light (13.2 mol m^{-2} d^{-1}) or DLI was sufficient even in the case of W light. Previous study reported that plant developmental rate increases as DLI increases until some threshold beyond which a further increase has little or no effect on developmental rate [62]. Important is also that in this research the plants were grown under the same photoperiod. On the other hand, the influence of other factors was probably so strong that it could lessen the effect of DLI on the growth and flowering of pot marigolds.

5. Conclusions

On average BA delayed the flowering of *C. officinalis* "Radio" by 10.8–12.2 days and the development of subsequent inflorescences. This phenomenon was partly reduced by W+R light and intensified by W+B light.

The development of inflorescence buds at the beginning of flowering was stimulated by BA at a concentration of 150 mg dm^{-3} only when the plants were illuminated with W+R light.

W+R light and BA stimulated the growth of the "Radio" cultivar, whereas W+B light in combination with BA inhibited it.

BA and W+R light positively affected the branching of the plants at the beginning of the growing period.

BA at concentrations of 150 and 200 mg dm^{-3} inhibited the synthesis of chlorophyll in leaves.

A greater amount of red light in the spectrum (W+R) positively affected the relative chlorophyll content, dry weight content and the Fv/Fm value.

It is possible to obtain plants with specific characteristics without BA treatment if there is an adequate spectrum of light.

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