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The Effect of Various Wavelengths of LED Light on the Physiological and Morphological Parameters of Comfrey (*Symphytum officinale* L.)

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ABSTRACT

The aim of this study was to examine the effect of five different wavelengths of light on the comfrey plant (*Symphytum officinale*) (family Boraginaceae). The light source and wavelengths used in the study were UV-A (390-410 nm), blue (465-485 nm), red (620-630 nm) and cool white (CW) daylight (400-700 nm, 6500 K), LED (Light Emitting Diode). In the study, each of the 5 different light applications was applied for 45 days (T1: 100% blue; T2: 100% red; T3: 60% blue + 35% red + 5% UV-A; T4: 100% CW daylight; T5: 80% CW + 20% red). The experiments were carried out under conditions of 22°C temperature, 60% humidity, 16/8 hours light/dark and 180 $\mu mol.m^{-2}.s^{-1}$ Photosynthetically Active Radiation (PAR). After each application, measurements were taken of number of leaves, number of roots, height of plant, amount of chlorophyll in leaves, leaf colour and brightness. According to data obtained, the different wavelengths of the coloured light applied in the growing environment created a change in colour and brightness of the leaves, height of the plant, length of the roots, and number of leaves and roots.

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Introduction

Comfrey (*Symphytum officinale* L., family Boraginaceae) is a common and perennial plant that has been used as herbal medicine for years. It is reported that the leaves and roots of this plant can be used (Blumenthal et al., 1998) in the treatment of wound, joint disorders, sprain, pulled muscle and ligament, haemorrhoid, bone fractures, gastritis and ulcer and rheumatism pain (Blumenthal et al., 1998; Aceves-Avila et al., 2001; Hatfield, 2005; Grube et al., 2007; De Albuquerque et al., 2007; Frost et al., 2013). The plant has antimicrobial and antifungal properties (Heckman, 2004) and many components, primarily allantoin and hydroxycinamon acid derivatives, essential oils, and vitamin B12. However, it also contains pyrrolizidine alkaloids (PAs) which are hepatotoxic and this restricts long-term consumption (Bach et al., 1989; Oberlies et al., 2004).

To be able to obtain high productivity in plant production, the appropriate temperature, humidity percentage and CO_2 conditions must be provided, and there is a need for lighting and light energy that will accelerate photosynthesis. In addition to the main light source of sunlight, the use of artificial light sources provides significant advantages in increasing productivity. The provision of suitable amounts of blue, green and red-light waves in the visible light region (400-700 nm) and just outside this region (730 nm) is known to accelerate plant development.

While red light is of major importance for plant growth via inducing transformations in the photochromic system of plants, blue light is necessary for the effect on vegetative growth, including chlorophyll, opening of stomas, and photomorphogenesis (Furuya, 1993; Urbanicity et al., 2007). If red and blue light is given together, it promotes blooming and seed production in plants. If amount of light in greenhouse plant production is not sufficient for plant growth, synthetic light sources can provide lacking photosynthetic active radiation (PAR) energy. LED lamps that can emit a specific wavelength of light, are preferred for artificial illuminations (Caglayan and Ertekin, 2011).

Recent developments in artificial light source technology have opened new horizons for LED structures that are both sustainable and highly productive. Results of economic analyses comparing LED and traditional light sources have shown that LED could reduce the long-term costs of plant production because of the high energy effect, low maintenance cost and long life. Studies have been conducted, which have examined the responses given by different plant species to different wavelengths to be able to understand whether LED could really be an alternative to traditional light sources. However, there is a need for more detailed research to understand the effect on the plant physiology of different LED wavelengths (Caglayan and Ertekin, 2016).

In this research, effect of various wavelengths [UV-A (390-410 nm), blue (465-485 nm), red (620-630 nm) and cool white (CW) daylight (400-700 nm; 6500 K), LED (Light Emitting Diode)] of LED light on growth parameters (L^*, a^*, b^* values, amount of chlorophyll, leaf number, root number, plant length, root length) of the comfrey plant was investigated. The aim of the study was to find alternative light sources to increase plant yield in growth environments where natural and traditional light sources are insufficient.

Material and Method

Establishment of the LED Lighting System and the Plant Experiment

Shoots were grown from rhizomes of comfrey in pots (two weeks). When the plants were 10 cm in height, they were transferred to the experiment environment (plant growth cabinet) and the growth procedures were applied. The plant growth cabinet (Nuve, TK252) has a capacity of 252 litres and its climate can be controlled. The experiments were conducted as a randomized complete design method with three replications (five plants for each application). The light source used in the study was Light Emitting Diode (LED) at wavelengths of UVA (390-410 nm), blue (465-485 nm), red (620-630 nm) and CW daylight (400-700 nm; 6500 K). Each of the five different light applications was applied for 45 days (T_1: 100% blue; T_2: 100% red; T_3: 60% blue + 35% red + 5% UV-A; T_4: 100% CW daylight; T_5: 80% CW + 20% red). The study was carried out in conditions of 22°C temperature, 60% humidity, 16/8 hours light/dark and 180 $\mu mol.m^{-2}.s^{-1}$ Photosynthetically Active Radiation (PAR). The light sources used in the study were CW daylight, blue, red (CREE XLamp XP-C) and ultraviolet (UVA) (EDISON Opto- Edixeon S) LEDs (Figures 1a, b, c, d). A LED group was formed from each colour of the same wavelength LEDs connected in parallel or serially in a pattern. The organization of the LED groups was shown in Figure 1 and the technical properties of the LEDs were shown in Table 1.

To select the wavelength for each LED group and to adjust the amount of light, separate Pulse Width Modulation (PWM), 186 W, 30 Vdc, 6.2 A LED driver (Mean Well Inc, 2019) were used. The performance of the drivers varied between 70% and 95% depending on the output strengths and quality of the electronic circuit design. At the beginning of each experiment, the PAR value was adjusted by changing the PWM square wave signal from the control entry of the driver providing the energy to each LED group as shown in Figure 2. The mean sign of the square of the wave frequency $f_{(t)}$, the lowest general value y_{min}, the highest general value y_{max} and the duty cycle D was: (Equation 1)

When $f_{(t)}$ square wave was

$$\bar{y} = \frac{1}{T}\int_0^T f(t)\mathrm{dt} \quad (1)$$

$f_{(t)}$, values of $D.\ T<t<T$ can be obtained for y_{min} and $0<t<D.T$ for y_{max}. The following formulae was obtained (Equation 2, 3 and 4);

$$\bar{y} = \frac{1}{T}\left(\int_0^{\mathrm{DT}} y_{\max}\mathrm{dt} + \int_{\mathrm{DT}}^{T} y_{\min}\mathrm{dt}\right) \quad (2)$$

$$\bar{y} = \frac{D.T.y_{max}\,dt + T(1-D)y_{\min}}{D.y_{\max} + (1-D)y_{\min}} \quad (3)$$

$$\bar{y} = D.y_{max} + (1-D)y_{min} \quad (4)$$

The equations were used as $y_{min} = 0$ and $\bar{y} = \mathrm{D.y_{max}}$. The mean value obtained depends on the duty cycle (Huang et al., 2011).

The ability to adjust the severity levels of LED light between 0 and 100% and in very small gradations, is provided with control of the pulse widths produced with PWM (Figure 3). This procedure can be applied by the control card sending numerical signs to the driver in a sensitive manner and without vibration in the light. The Arduino Uno control card was used for PWM control. The light control of the LED groups is shown in block form in Figure 4.

Quality Analyses

The amounts of chlorophyll (from the leaf with a chlorophyll meter (SPAD 502) measured three times and average values were calculated), L, a, b values, stem and root lengths, the number of leaves and roots of the plant were measured (Zhu et al., 2012). In determining colour changes in leaves, leaf samples were measured using a chroma meter (Minolta CR-200) as CIE L, *a*, *b*. L value. When the value of L is 0, this indicates black colour, with no reverberation and when L= 100, this indicates white colour with full reverberation (Figure 5). Positive "a", negative "a", positive "b" and negative “b” values represent red, green, yellow, and blue, respectively. At the point of zero-cut (*a*=0 and *b*=0), it becomes colourless or grey (Figure 6). Chroma (C*) and hue angle (h*) values were calculated using the equation 5 and 6 below (McGuire, 1992).

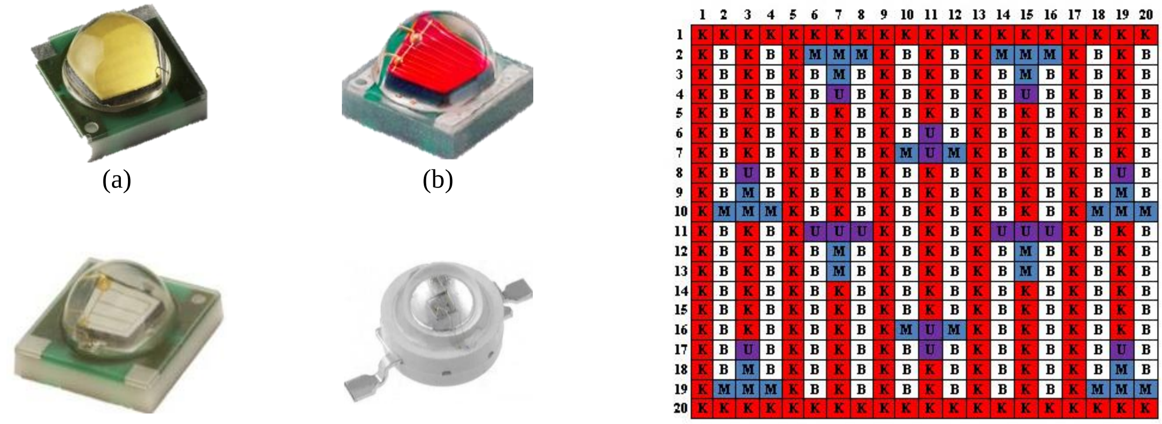
$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (5)$$

$$h° = \tan^{-1} (a^*/b^*)] \quad (6)$$

The angle formed by the X axis crossing the intersection point of the hue angle, *a** and *b** values (McGuire, 1992). A hue angle of 0°, 90°, 180° and 270° represent red, yellow, green, and blue, respectively. The chroma value represents the vitality-opacity of the leaves with a low chroma value in dull colours and a high chroma value in bright colours (Anonymous, 1996). After measurements of the amount of chlorophyll and colour, measurements were taken of the number of leaves and roots, the height of the plant and the length of the roots.

Statistical Analysis

The experiment was conducted as a randomized complete design method with three replicates. The obtained data was analysed using analysis of variance and the Duncan grouping method was performed to determine whether there was a relationship between the different light applications. MSTAT-C software was used for analysis of the data (Freed, 1988).



K: Red M: Blue B: Daylight U: UV-A

(c) d) (e)

Figure 1. a) CW daylight, b) Blue, c) Red, d) UVA and e) LED placement

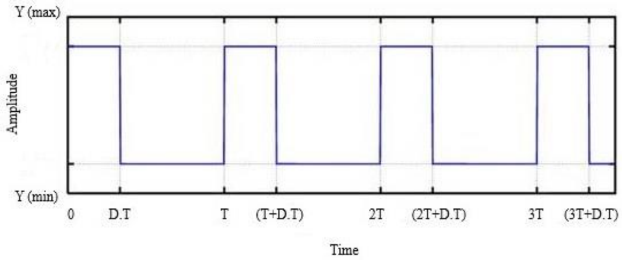


Figure 2. PWM wave ymin, ymax and D parameters

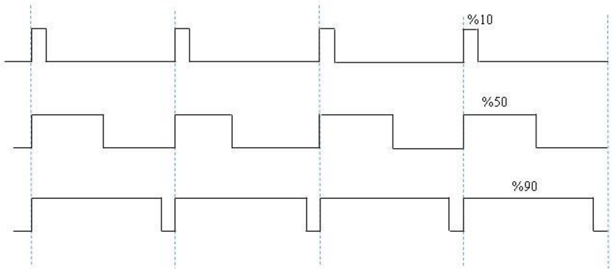


Figure 3. PWM ratios determining the light level

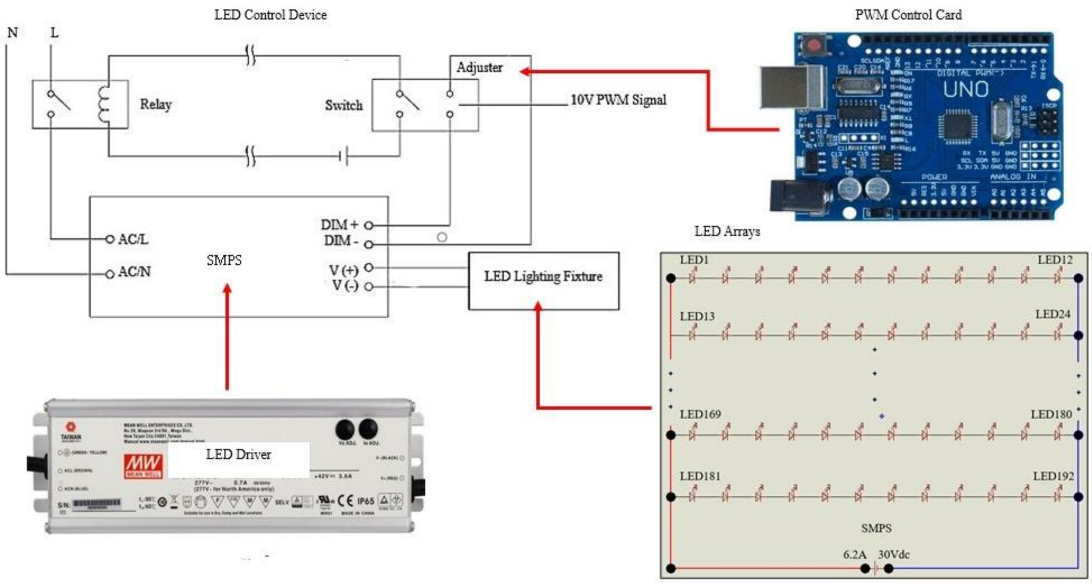


Figure 4. Block schematic form of the light level control

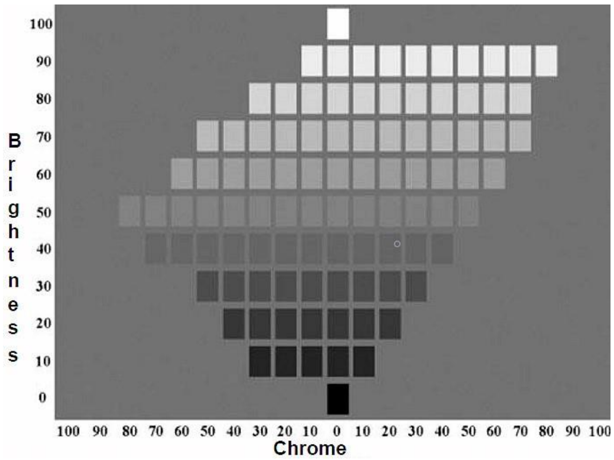


Figure 5. Brightness-chrome diagram

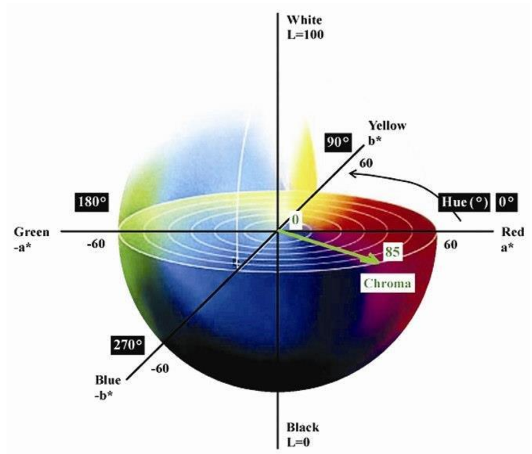


Figure 6. CIE *L*a*b** colour space diagram

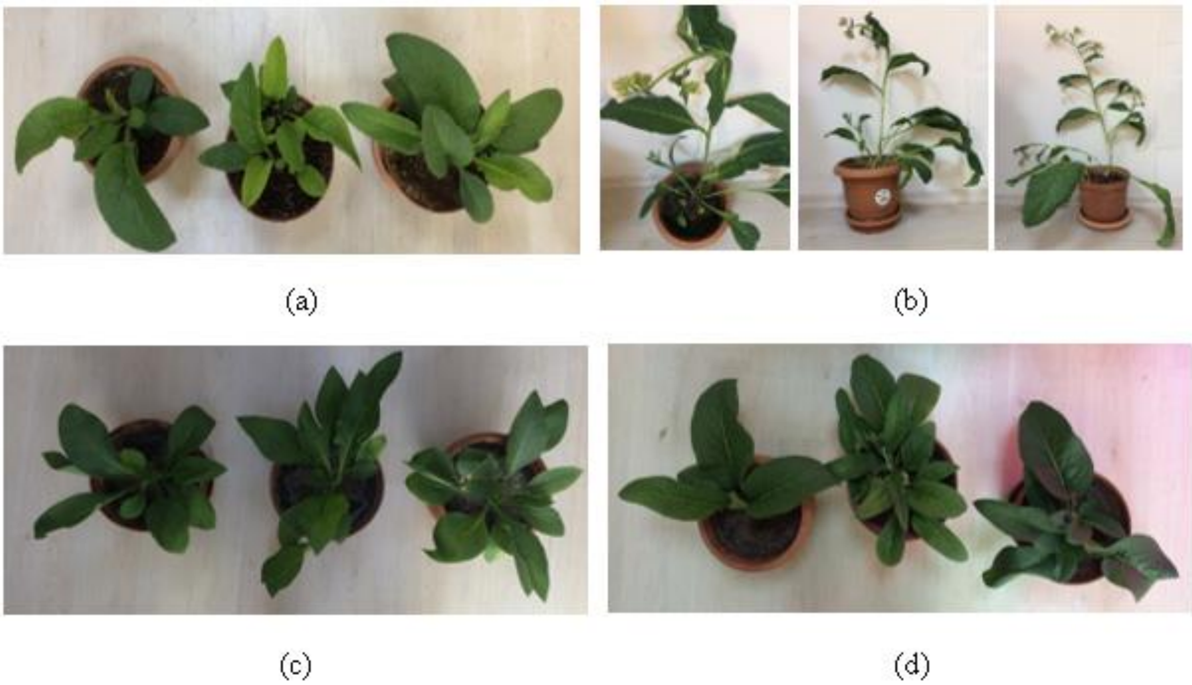


Figure 7. Appearance of the plants at the end of the experiment, a) CW, b) Red, c) Blue and d) Red + Blue + UVA

Result and Discussion

In this study, a LED lighting system has been designed and applied to provide the PAR energy necessary in plant growth cabinet applications. In LED lamp, the LED series giving cool white, red, blue and ultraviolet lights are efficient in increasing the photosynthesis rate. The Arduino Uno control card was used for selection of LED series and light level control.

Red and blue lights have the greatest impact on plant growth, because they are the major energy sources for photosynthetic CO2 assimilation in plants. Past studies examined the action spectra for photosynthesis of higher plants. It is well known that action spectra have action maxima in the B and R ranges. Combined R+B LED lights were proven to be an effective lighting source for producing many plant species in controlled environments. The paper used the LED light source of adjustable parameter as crop growth light source, with red, blue LED combination collocation design, LED red light could inhibit photosynthetic product output from leaf to increase leaf starch accumulation; LED blue light regulated the physiological process of chlorophyll formation, chloroplast development, stomata opening, rhythm of photosynthetic and so on, meet the needs of crop growth photosynthesis for light conditions. Throughout the 45-day period of the experiment, 5 different wavelengths (T_1, T_2, T_3, T_4, T_5) were applied in the growing environment of the comfrey plants. After each application, measurements were taken of the number of leaves, the number of roots, root length, height of the plant, amount of chlorophyll in the leaves, leaf colour and brightness (Table 2). According to the data obtained, the different wavelengths of the coloured light applied in the growing environment created a change in the colour and brightness of the leaves (Figure 7). Growth, yield and quality of plants associated with both genetic and environmental factors. Light, plant nutrition elements, quality of water and temperature are necessary and important for plant growth (Yağcıoğlu, 1996).

While the closest hue angle values to green were determined in applications 1, 4 and 5, the brightest leaf colour was determined in application 4. The highest amount of chlorophyll was determined in application 2 with 100% red light. Although the green colour of a leaf and the amount of chlorophyll was expected to be directly proportional, this was not the case in this study. When the green colour of the leaves was evaluated, statistical differences were determined between the applications, however numerically there was not much difference. When evaluated on the basis of the amount of chlorophyll, application 2 can be said to have been effective on the green colour of the leaves. There are indirect or direct effects of light intensity and light type time on plant growth together with photosynthesis ratio and dry matter production increasing (Matsuda et al., 2004; Spalding and Folta, 2005).

Chlorophyll molecules absorb blue (460-480 nm) and red (650-700 nm) radiations better and these wavelengths are more effective on plant growth (Yeh and Chung, 2009). Tripathi and Brown (1995) emphasized the effect of red LED light on chlorophyll synthesis. In a study carried out by Li et al. (2012), blue (440 and 476 nm) and red LED were used together on Chinese cabbage and it was concluded that this combination caused a higher rate of chlorophyll. Ucar et al. (2016) obtained maximum chlorophyll amounts with 50% blue light + 50% red light in stevia plant. When the brightness of colour was evaluated as represented by the L value, the best value in respect of leaf brightness was determined in application 4 with CW daylight. The best values in respect of plant height, number of leaves and root length were determined in applications 2 and 3, and the highest root count was found in application 5.

Table 1. Technical properties of the LEDs used in the study

Technical property	LED			
	CW	Red	Blue	UVA
Colour heat (K)	6500	-	-	-
wavelength (nm)	400-700	620-630	465-485	390-410
Light flow (lm), (mW)	100 lm	45.7 lm	23.5 lm	528 mW
Colour conversion index (CRI)	70	-	-	-
Operating current (mA)	350	350	350	700
Operating force (V)	3.2- 3.9	2.2–2.5	3.3–3.9	3.4
Power (W)	1	1	1	3
Heat resistance (°C W^{-1})	12	10	12	15
Angle of light radiation (°)	115	125	125	120
Package form	Surface Mount Device (SMD)			

Table 2. The effect of lighting applications in wavelength on morphological and physiological properties of the comfrey plant

Applications	L	Hue angle	Chroma value	K	PL	LN	RL	RN
T_1	42.07^{c}	156.7^{a}	24.13^{b}	21.37^{d}	36.33^{b}	11.00^{b}	27.67^{c}	3.3^{b}
T_2	38.70^{d}	152.7^{b}	14.73^{c}	53.63^{a}	33.00^{c}	15.33^{a}	35.33^{a}	3.0^{b}
T_3	37.63^{d}	149.0^{c}	15.77^{c}	31.97^{b}	83.67^{a}	14.00^{a}	$34.0a^{b}$	3.3^{b}
T_4	48.90^{a}	157.7^{a}	32.50^{a}	10.70^{e}	$35.33b^{c}$	11.67^{b}	15.0^{d}	3.7^{b}
T_5	44.73^{b}	156.7^{a}	23.17^{b}	25.93^{c}	32.33^{c}	11.00^{b}	31.0^{b}	5.3^{a}

The quality of light has an important effect on lengthening of the stem and leaves of plants (Yağcıoğlu, 2005). A wide range of light can be produced in LED light sources from ultraviolet to infrared. This presents extremely good possibilities for researchers and in recent years there has been a great increase in studies of plant production with light obtained in the visible and ultraviolet regions (Miyashita et al., 1995; Jao and Fang, 2003; Lawrance et al., 2005; Shimizu et al., 2005; Kondo et al., 2008). It has been concluded that significant changes can be made in the physiological and morphological growth and development of plants with different light combinations in particular and light quality. Brown (1995) observed that applying the combination of infrared (735 nm) and red (660 nm) light together (total 300 µmol m^{-2} s^{-1}) to the pepper plant (*Capsicum annum* L.) resulted in a greater increase in stem biomass and plant height than red light applied alone.

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