International Journal of Horticultural Science and Technology
Vol. 7, No. 2; June 2020, pp 139-151
Print ISSN: 2322-1461 Online ISSN: 2588-3143
DOI: 10.22059/ijhst.2020.286884.312
Web Page: https:// ijhst.ut.ac.ir, Email: ijhst@ut.ac.ir

Stimuli Effects of Different LEDs on Some Morphological and Biochemical Traits of Two Varieties of *Calendula officinalis*

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(Received: 6 August 2019, Accepted: 27 December 2019)

Abstract

In the production of flowers and ornamental plants, especially in the advanced greenhouse conditions, it is important to have a good light source and its accurate management. This study aimed to evaluate the effect of light quality on morphological and biochemical traits of two Marigold genotypes (Iranian-native and Gitana). This experiment was conducted in a completely randomized design with three replications. The treatments included five light qualities including red, blue, 70% red:30% blue (70%:30%), and white lights with an intensity of 500 µmol m^{-2} s^{-1} [photosynthetic photon flux density (PPFD)] and greenhouse natural light (with an average intensity of 650 PPFD). The results showed that light quality had significant effects on all studied traits at $p<0.01$. Genotypes had significant effects on the dry weight of the aerial parts, the number of open flower, and chlorophyll b concentration. The interaction effect of light and genotype was significant on the fresh and dry weight of the aerial parts. Between the two genotypes, the Gitana was significantly superior to Iranian-native genotype for the content of chl b. Among the light qualities, the highest number of flowers per plant, chlorophyll b and carotenoid concentrations were observed in plants that exposed to red light. Increase in all studied traits especially in plant height, total flavonoids and chlorophyll a, b, total, and carotenoid concentrations were observed in the plants that exposed to red, blue, and red/blue lights. In conclusion, growing both Marigold genotypes under red, blue and composition of red/blue light, improves quality and quantity of production of Marigold flower in the greenhouse condition.

Keywords: Blue light, Greenhouse condition, Marigold, Red light, Flavonoid

Abbreviations: LED, Light Emitting Diodes; **ROS**, reactive oxygen species; **TFC**, Total flavonoid content; **CAR,** Carotenoid; **Chl**, Chlorophyll; **FWAT**, Fresh weight of the aerial tissues; **DWAT**, Dry weight of the aerial tissues; **LL**, Leaf length; **LW**, leaf width; **NFFB**; Number of flower and flower bud; **NOF**, number of open flower; **BL,** Blue light; **RL,** Red light; **PL,** Purple light; **GL,** Green light; **YL,** Yellow light; **WLB,** White light bulb**; RL/BL,** Red/Blue light.

Introduction

Marigold or pot Marigold (*Calendula officinalis* L.) is an annual plant from the Asteraceae family that has spread extensively in many parts of the world. This plant is grown in Europe, Asia, and America continents (Muley et al., 2009; Safdar et al., 2010; Caliskan and Kurt; 2018). Marigold plant has a stem with 20-50 cm long and leaves with elliptical and tomentose shapes, which are green to light brown in color. This plant grows in western parts of Iran (at an

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altitude of 2500 meters above sea level) (Moghaddasi and Haddad Kashani, 2012; Moghtader et al., 2016). It has been reported that Marigold has an exactly long flowering period, which is resistance to cold conditions (Muley et al., 2009). Marigold is used as an ornamental plant with medicine and cosmetic purposes (Khalid and da Silva, 2012; Caliskan and Kurt, 2018). It has been reported that the mean economic purpose for the breeding and developing of this plant is to produce medicine, as active ingredients in its flowers and petals (Martin et al., 2005). Various studies have revealed that there are some compounds including carbohydrates, amino acids, lipids, carotenoids, terpenoids, flavonoids, volatile oil, quinines, coumarins, phytosterols, saponins and other constituents in leaves, flowers, and roots of Marigold (Naved et al., 2005; Ukiya et al., 2006; Kurkin and Sharova, 2007; Khalid and Teixeira da Silva, 2012; Hernandez-Saavedra et al., 2015; Ashwlayan et al., 2018). Marigold is used as a sedative traditional medicine as well as for wound therapy, ulcers, herpes, scars, skin damage, frostbite, and blood purification (Verma et al., 2018). This plant is mainly used for its various biological activities as antihypertensive, analgesic, anti-diabetic, anti-ulcer, anti-inflammatory, gastrointestinal diseases, gynecological problems, eye diseases, skin injuries (Ashwlayan et al., 2018), antiseptic, stimulant, diaphoretic, antispasmodic, and antipyretic agents (Jan et al., 2017).

Furthermore, plant growth and developmental stages are depended on genetic factors, environmental cues, and their interactions (Shahmoradi and Naderi, 2018; Rao et al., 2006). It has been reported that light is one of the main important environmental factors affecting plant growth (Zhang et al., 2017) and necessary driving force for its photosynthesis (Aliniaeifard et al., 2018). This factor can influence plant growth and development through its intensity, spectrum, and duration by producing the ATP and NADPH and by activating the enzymes involved in photosynthesis (Brotosudarmo et al., 2016; Johnson, 2016; Aliniaeifard et al., 2018). Furthermore, some researchers have shown that light signal perceives via photo morphogenetic pigments, which include the red/far-red light-absorbing phytochromes and blue/UV light-absorbing pigments (Cosgrove, 1993). Besides, light stimulates the production of various nutrients, antioxidants, and secondary metabolites in plants and help plants to cope with reactive oxygen species (Darko et al., 2014).

Most of the light sources that used to enhance plant growth and photosynthesis processes, have low energy use efficiency and are not result in the proper plant functioning (Kim et al., 2004). Therefore, present light source should be optimized and should be replaced with other efficient light sources. Light Emitting Diodes (LEDs), attracted so much attention because they have various advantages for plants (Lin et al., 2013). LEDs can affect plant morphology and physiology, as well as, can promote plant growth and development via manipulating their qualities (color and wavelength) (Aliniaeifard et al., 2018). Some studies reported that the combination of red and blue light spectra have important roles in leaf development and biomass accumulation (Stutte et al., 2009; Johkan et al., 2010; Shengxin et al., 2016; Aliniaeifard et al., 2018). Inoue and Kinoshita (2017) stated that blue light can keep stomata open through promoting entering potassium ion into guard cells and excreting the proton by activating the plasma membrane proton pumps. Further, by stomatal opening more CO_2 is provided for plants to drive its photosynthesis (Mott, 2009). Schwartz and Zeiger (1984) stated that since the opened stomata are controlled by light blue receptors, it is possible to increase the dry weight of the plants by increasing the blue light ratio in overall intercepted light spectrum.

Wang et al. (2009) have investigated the leaf morphology and shoot dry weight under different ratio of red and blue lights. They

revealed that the shoot dry weight increases by higher red/blue light ratios. This occurred due to increase in the leaf number and leaf area index. Zhang et al. (2018) in their study examined the eight spectral LED lights (white light as control, monochromatic red light, monochromatic blue light, monochromatic green light, monochromatic yellow light, monochromatic purple light and a combination of red and blue lights with ratios of 9/1 and 4/1) on the phenotypic and physiological characteristics of lettuce. They showed that the light treatments had significant effects on morphological and biochemical traits in lettuce so that the application of red/blue (4/1) light improved plant height, stem diameter, and fresh weight of aerial parts; while anthocyanin content significantly increased under green light treatment.

Despite the numerous studies concerning the effects of different LEDs on plants, there was no comprehensive study about the effects of LED lights on different varieties of pot Marigold. Therefore, the present study aimed to investigate the effects of various light qualities on some morphological, physiological, and biochemical traits of two varieties of pot Marigold.

Materials and Methods

Plant material

In the present study, the effects of five levels of LED lights on some morphological, physiological, and biochemical traits of two varieties of pot Marigold were investigated. To do so, an experiment was conducted in May-December 2017-2018 growing seasons in the research greenhouses of Khorasgan, Isfahan, Iran (geographical coordinates= 32:38N and 51:45E; 40% relative humidity, greenhouse temperature with an average of 28 $^{\circ}$C, and light intensity with an average of 650 PPFD).

Treatments and growth conditions

The research was conducted using a factorial experiment based on completely randomized design with five levels of light qualities, including 100% red, 100% blue, red/blue with 70/30 ratio, and white spectrum (400-700 nm, peak at 500 nm) in incubator, and natural greenhouse light with an average of 650 PPFD and two varieties of pot Marigold (Gitana and Isfahan Native Genotype) that were provided from the research greenhouses of Isfahan University of Technology.

At first, two varieties of the Marigold were planted in research greenhouses of the Islamic Azad University of Khorasgan in pots (with a depth of 12 cm and a diameter of 14 cm). The soil used in this study contained 20% vermicomposting-enriched soil (Gilda Company) and 80% of farm soil. Daily irrigation was performed to ensure the uniformity in seed germination. After germination and at seedling growth stage, the pots were transferred to an incubator (at 4 to 6-leaf stage) under aforementioned light qualities with 500 PPFD with 16 h light and 8 h dark cycle. In order to prevent any space limitation for growth of the seedlings, the size of the pots changed to 20×20 cm, and three seedlings were transferred to each pot. Plants were grown for three months under different light spectra. Differences in plant growth rate and their vegetative traits were recorded after one month growth under different light spectra. Following three months of plant growth, the final measurements including plant height, leaf length, leaf width, number of opened flowers, Chl α, Chl b, total chlorophyll concentrations, carotenoids, and total flavonoid content were performed.

Soil characteristics

To determine the soil physic-chemical characteristics, before the experiment, a sample of the soil used in the present study was sent to the soil laboratory to determine its physical and chemical properties. The results of soil analysis are shown in Table 1.

Table 1. Physical and chemical characteristics of soil sample

Soil texture	pH	O.C (% Mass of dry matter)	Humidity (% Mass)	Ec (dS.m $^{-1}$)	Available Nitrogen (% Mass of dry matter)	P (% Mass of dry matter)	K (% Mass of dry matter)	Zn (mg.kg $^{-1}$ soil)	Cu (mg.kg $^{-1}$ soil)	Co (mg.kg $^{-1}$ soil)
Loam-clay	7.81	20	30	3.18	2	2.14	1.19	124.97	21.3	12.4

Measurements

Plant height

The plant height of pot Marigold was recorded by measuring the distance of crown to end of the plant using a ruler.

Fresh and dry weights of the aerial tissues

To determine the fresh weight, the aerial parts of the Marigold plant were harvested and their fresh weight was measured by a Mettler Toledo scale. Then, the samples were put in special paper pockets and placed in an oven (Shimazco model) for 48 hours at 70 °C. After drying the samples, the dry weight of the aerial tissues was measured.

Leaf length and leaf width

To measure the dimensions of the leaves, the average distance from tip to the end of the leaf was recorded as leaf length and an average of the widest part of the leaves were measured as leaves width using a ruler.

Number of opened flower

The number of open flowers (NOF) per pots was obtained by counting the mean number of opened flowers (not flower bud) per pots on 25 July 2017 (102 days after transplanting).

Photosynthetic pigments

Chlorophyll a, b, and total concentrations were assayed based on the Arnon (1949) method. To do so, 0.1 g of fresh leaf tissue was weighed and was triturated in a mortar by acetone 80% until a scum was achieved. Then, the scum was isolated by a filter paper. The resulting scum was triturated by acetone 80% and was isolated. Next, the extract volume was reached to 10 mL by acetone 80%. Then, the extract was transferred to a cell and absorbed by a spectrophotometer (Model U-1800, Hitachi) at 645, 663 wavelengths. Furthermore, the concentration of carotenoids was measured based on the Lichtenthaler (1987) method. Accordingly, again, the above steps were performed to determine the content of carotenoids, and finally, the content of carotenoids was read at 470 nm wavelength.

In the present test, it is noteworthy that acetone 80% was used as a Blank solution. At the end of the test chlorophyll a, b, and total and carotenoids concentrations for each sample were determined by using the following equations, respectively:

$$Chla = \frac{[(12.7 \times D663) - (2.69 \times D645)] \times V}{1000 \times W} \quad (1)$$

$$Chlb = \frac{[(22.9 \times D645) - (4.93 \times D663)] \times V}{1000 \times W} \quad (2)$$

$$Totalchl = \frac{[(20.2 \times D645) - (8.02 \times D663)] \times V}{1000 \times W} \quad (3)$$

$$Carotenoids = \frac{[(1000 \times D470) - (1.82 \times Chl.a) - (85.02 \times Chl.b)]}{198} \quad (4)$$

V: Final volume of extracts per mL, W: Tissue weight per gram, D: Optical absorption

Total flavonoid content

Total flavonoid content in the leaf of the Marigold plant was determined by Aluminum chloride complex-forming assay (Biju *et al*. 2014). Quercetin was used as a standard, and flavonoid content was determined as the quercetin equivalent. Based on the procedure, at first, the samples

were dried and were milled. Then, 2.5 g of each sample was weighted, and 10 mL of 80% methanol was added to them. The samples were stored at room temperature for 24 h in the absolute dark. The above extracts were then centrifuged at 10,000 RPM for 15 min, and the supernatant fluids dissociated to determine the flavonoid content. The calibration process was measured at 510 nm wavelength to draw the curve. Flavonoids were reported based on the mg of quercetin per g dry weight of the sample (mg QUE g^{-1}DW).

Statistical analysis

In the present study, the above traits were measured based on the average of three plants in each experimental unite (per pot). The research was conducted using a factorial experiment based on the completely randomized design. The data were analyzed by SAS software (version 9.4), and to compare the data mean, the LSD test was used at $p< 0.05$. The charts were drawn by using Microsoft Excel software.

Results

Plant height

The results of analysis of variance for the effects of light, genotype, and their interaction on plant height showed that it significantly affected by different light spectra at $p<0.01$, but genotype treatment and light × genotype interaction had not significant effects on plant height (Table 2).

The tallest plants (37 cm) were observed under blue light spectrum, which had a significant difference in comparison with white and greenhouse light treatments. On the other hand, the shortest plants (25.33 cm) were seen in plants exposed to greenhouse light. There was no significant difference between the plant height of greenhouse and white light-grown plants (26 cm). The height of plants under blue light was increased up to 11.55, 15.63, 42.31, and 46.07% when compared to plant height of red, red/blue, white, and greenhouse light treatments, respectively (Table 3).

Table 2. Analysis of variance for plant height, leaf length, leaf width, fresh weight of the aerial tissue, dry weight of the aerial tissues, and number of open flower traits in two Marigold genotypes exposed to different light qualities

Treatments	df	PH	LL	LW	FW	DW	NOF
Light	4	147.53 **	9.51 *	0.91 **	1567.74 **	93.21 **	116.62 **
Genotype	1	36.30 ^{ns}	0.43 ^{ns}	0.003 ^{ns}	109.94 ^{ns}	12.50 *	229.63 **
Light× Genotype	4	4.13 ^{ns}	3.92 ^{ns}	0.72 ^{ns}	287.50 *	17.42 **	37.72 ^{ns}
Error (E)	10	15.16	4.88	0.25	64.36	2.71	9.00
CV		12.68	28.12	21.61	18.18	22.67	34.67

ns: non-significant; * and **: significant at $p<0.05$ and $p< 0.01$, respectively.
PH, Plant height; LL, Leaf length; LW, leaf width; FWAT, Fresh weight of the aerial tissues; DWAT, Dry weight of the aerial tissues; NOF, number of open flower

Table 3. Effects of light spectra on plant height, leaf length, leaf width, and number of open flowers traits of Marigold plants exposed to different light spectra

Treatments	PH (cm)	LL (cm)	LW (cm)	NOF (Number)
Red	33.17 ^{a}	6.83 ^{c}	1.93 ^{b}	11.50 ^{a}
Blue	37.00 ^{a}	9.50 ^{a}	2.63 ^{a}	7.16 ^{bc}
Red/Blue	32.00 ^{a}	8.93 ^{ab}	2.80 ^{a}	9.83 ^{ab}
White	26.00 ^{b}	7.11 ^{bc}	2.20 ^{ab}	0.33 ^{d}
Greenhouse Light	25.33 ^{b}	6.91 ^{c}	1.98 ^{b}	4.67 ^{c}
LSD	5.89	1.96	0.61	2.79

Mean of each data fallowed by the non-similar letters have significantly difference at $p<0.0$

Leaf length and leaf width

Despite the significant effects of light quality on the leaf length at $p<0.05$, the mentioned trait was not affected by the genotype treatment and light × genotype interaction (Table 2). The longest leaves (9.50 cm), was recorded in the plants exposed to blue light, which had a significant difference compared to the leaf length of plants grown under other light spectra. The length of the leaf in the blue light-grown plants was 39.09, 6.38, 33.62, and 37.48% longer than the leaf length of red-, red/blue-, white-, and greenhouse light-grown plants, respectively (Table 3). Furthermore, there was a significant difference for the leaf width trait at $p<0.01$ (Table 2) in plants exposed to different light spectra, however, the genotype and interaction of light × genotype did not result in any significant effect on this trait (Table 2). The widest leaf was observed in plants grown under 70% red-30% blue (with an average of 2.80 cm), which was 45.08, 6.46, 27.27, and 41.41% wider leaves compared to the leaves of red-, blue-, white-, and greenhouse light-grown plants, respectively (Table 3).

Fresh and dry weights of the aerial tissues

Analysis of variance showed that light treatment ($p<0.01$) and interaction of light × genotype ($p<0.05$) had significant effects on fresh weight of the aerial tissues. Nevertheless, there were no significant differences between fresh weight of the aerial tissues of genotypes (Table 2). The results of light × genotype interaction showed that the highest fresh weight of the aerial tissues observed in Gitana genotype grown under red light (61.49 g). On the other hand, the lowest fresh weight of the aerial tissues (Table 4) was recorded in Gitana genotype grown under greenhouse light (12.14 g), which had no significant difference with the fresh weight of the aerial tissues of Native genotype grown under white light (23.63 g).

Table 4. Effects of interaction between light quality and genotype on fresh and dry weights of the aerial tissues of Marigold plant

Treatments		FWAT (g)	DWAT (g)
LEDs	Genotypes		
Red	Native	55.25 ^{a}	8.04 ^{bc}
	Gitana	61.49 ^{a}	9.98 ^{ab}
Blue	Native	59.76 ^{a}	9.43 ^{abc}
	Gitana	50.28 ^{ab}	7.29 ^{bcd}
Red/Blue	Native	54.69 ^{a}	7.23 ^{bcd}
	Gitana	53.37 ^{a}	6.79 ^{cd}
White	Native	23.63 ^{cd}	3.64 ^{e}
	Gitana	33.70 ^{c}	4.56 ^{de}
Greenhouse light	Native	36.79 ^{bc}	11.17 ^{a}
	Gitana	12.14 ^{d}	4.43 ^{de}
LSD		14.60	2.99

Mean of each data fallowed by the non-similar letters have significantly difference at $p<0.05$
FWAT, Fresh weight of the aerial tissues; DWAT, Dry weight of the aerial tissues

Analyzing the effects of LEDs, genotypes, and light × Genotype interaction on the dry weight of the aerial tissues of Marigold showed that genotype, and light × genotype interaction significant influenced this trait. Highest dry weight of the aerial tissues was achieved in native genotype of Marigold grown under greenhouse light (with an average of 11.17 g), and the lowest dry weight of the aerial tissues was obtained in the native genotype of Marigold grown under white light (with an average of 3.64 g).

Number of open flowers

The results of the analysis of variance (Table 2) showed that the number of open flowers was only affected by the single effects of light and genotype treatments at $p<0.01$. The highest number of open flower was observed in red light-grown plants with an average number of 50.11 flowers per pot. The lowest number of open flowers was observed in white light-grown plants with an average number of 0.33 flowers per pot (Table 3). The native genotype (with an average number of 9.47) had more open flowers (+140.97%) than the Gitana genotype (with an average number of 3.93) (Fig.1).

Photosynthetic pigments

Analysis of variance showed that the effects of light quality was significant for the Chlorophyll (Chl) α, Chl b, total Chl and carotenoid concentrations at $p<0.01$, but no significant difference was observed for the genotype treatment and interaction of light × genotype for the Chl α, total Chl, and carotenoid concentrations. Moreover, there was a significant difference for Chl b at $p<0.05$ between the genotypes (Table 5).

The highest concentration of Chl α (1.22 mg g^{-1} FW) was observed in red light-grown plants, which was significantly different from greenhouse light-grown plants. On the other hand, the lowest concentration of Chl α was recorded for greenhouse light treatment (0.51 mg g^{-1} FW), which was significantly different from other treatments. Chl α concentration under red/blue treatment was increased by 3.28, 15.60, 27.27, and 147.06%, compared to its concentration in plants grown under red, blue, white, and greenhouse light conditions, respectively (Table 6).

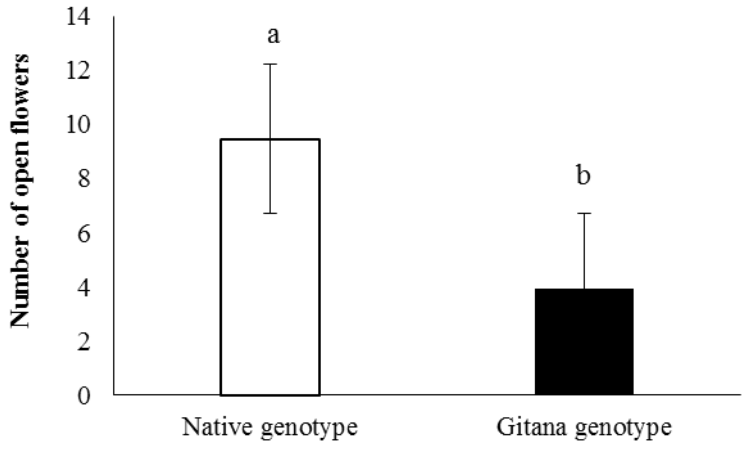

Fig. 1. Effects of Marigold genotype on number of open flower

Table 5. Analysis of variance for Chlorophyll (Chl) α, Chl b, total Chl and carotenoid concentrations and total flavonoid content in two Marigold genotypes exposed to different light qualities

Treatments	df	Chl α	Chl b	Total Chl	CAR	TFC
LEDs	4	0.54 **	0.44 **	0.89 **	0.001 **	10012.72 **
Genotype	1	0.10 ns	0.36 *	0.26 ns	0.0001 ns	1713.78 ns
Light× Genotype	4	0.08 ns	0.014 ns	016 ns	0.0007 ns	749.52 ns
Error (E)	10	0.03	0.005	0.06	0.001	402.81
CV		18.43	25.69	19.48	18.90	17.21

ns: non-significant; * and **: significant at $p<0.05$ and $p< 0.01$, respectively.

Chl α, Chlorophyll α; Chl b, Chlorophyll b; Total Chl, Total chlorophyll; CAR, Carotenoids; TFC, Total flavonoid content

Table 6. Effects of light spectra on Chlorophyll (Chl) α, Chl b, total Chl and carotenoid concentrations and total flavonoid content of Marigold plant

Treatments	Chl α (mg.g^{-1} FW)	Chl b (mg.g^{-1} FW)	Total Chl (mg.g^{-1} FW)	CAR (mg.g^{-1} FW)	TFC (mg QUE.g^{-1} DW)
Red	1.22 ^{a}	0.37 ^{a}	1.59 ^{a}	0.199 ^{ab}	148.66 ^{a}
Blue	1.09 ^{a}	0.28 ^{a}	1.37 ^{a}	0.194 ^{ab}	151.25 ^{a}
Red/Blue	1.26^{a}	0.35^{a}	1.61^{a}	0.21^{a}	138.40^{a}
White	0.99^{a}	0.28^{a}	1.27^{a}	0.16^{b}	67.09^{b}
Greenhouse Light	0.51^{b}	0.15^{b}	067^{b}	0.11^{c}	77.61^{b}
LSD	0.30	0.11	0.400	0.03	49.97

Mean of each data fallowed by the non-similar letters have significantly difference at p<0.05
Chl α, Chlorophyll α; Chl b, Chlorophyll b; Total Chl, Total chlorophyll; CAR, Carotenoids; TFC, Total flavonoid content

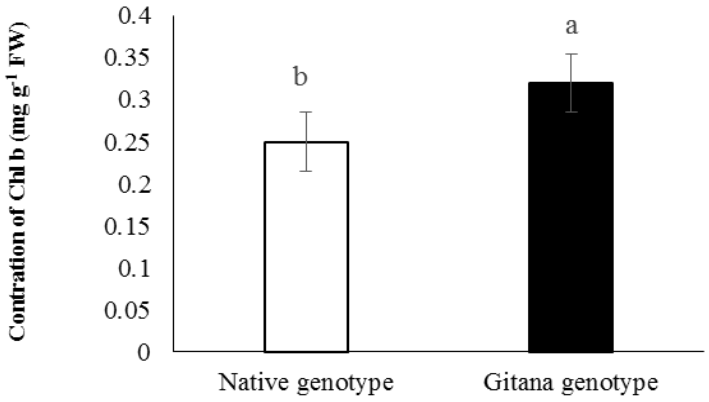

Fig. 2. Effects of Marigold genotype on concentration of Chlorophyll (Chl) b

Also, there was a significant difference between the concentration of Chl b of plants grown under different light spectra. The highest content of Chl b (0.37 mg g^{-1} FW) was achieved in plants grown under red light, without significant difference with its concentration in blue light- (0.28 mg g^{-1} FW), red/blue light- (0.35 mg.g^{-1} FW), and white light- (0.28 mg.g^{-1} FW) grown plants. On the other hand, the lowest concentration of Chl b (0.15 mg g^{-1} FW) was observed in plants grown under greenhouse light, which was significantly different from other treatments (Table 6). Concentration of Chl b in Gitana genotype (0.32 mg g^{-1} FW) was 28% more than its concentration in native genotype (Fig.2).

The highest total Chl concentration (1.59 mg g^{-1} FW) was obtained in plants grown under red light. On the other hand, the lowest total Chl concentration (0.67 mg g^{-1} FW) was achieved in plant grown under greenhouse light. There was a significant difference between the total Chl concentration in plants grown under greenhouse light compared to the other light treatments (Table 6).

The highest carotenoid concentration (0.21 mg g^{-1} FW) was obtained in the red/blue light-grown plants and the lowest carotenoid concentration (0.11 mg g^{-1} FW) was detected in the plants grown under greenhouse light. There was no significant difference between the carotenoid concentration of plants grown under red and blue lights, while there was a significant difference between the carotenoid concentration in plants grown under greenhouse light in comparison with the carotenoid concentration in other plants (Table 6).

Total flavonoid content

Total flavonoid content was influenced by the effect of light spectra at $p<0.01$. There was no significant difference between the genotypes and among the interactions of light and genotype (Table 5). The highest total flavonoid content was achieved in blue light-grown plants (151.25 mg QUE g^{-1} DW). On the other hand, the lowest total flavonoid content (67.09 mg QUE g^{-1} DW) was detected in white light-grown plants (Table 6), without significant difference with plants grown under white and greenhouse lights (77.61 mg QUE g^{-1} DW). Blue light caused 1.74, 9.28, 125.44, and 94.89% increase in total flavonoid content compared to its content in red-, red/blue-, white-, and greenhouse light-grown plants, respectively (Table 6).

Discussion

Light is one of the most effective and stimulatory factors for plant growth and development (Hogewoning et al., 2010; Curreyand Lopez, 2013). It is also an energy source for plant photosynthesis and a regulator factor for plant physiological activity (Son and Oh, 2013; Aliniaeifard et al., 2018). Nowadays, artificial lights are used in greenhouse and laboratory experiments to reduce the adverse effects of unfavorable radiation and to provide favorable growth conditions for plants. For this purpose, the development of LEDs has been suggested to optimize radiation use efficiency of the crops (Gruda and Tanny, 2014). It has been reported that responses of plants to the application of different light spectra, such as blue and red, are not the same in different environments (Randall and Lope, 2014). These photo-responses are of valuable significance in agricultural sciences, due to their effects on plant growth, development, and nutritional quality (Lin et al., 2013). Furthermore, it has been shown that light quality, intensity, and photoperiod influence morphological, physiological, metabolic traits and nutritional quality of plants (Ward et al., 2005; Perez-Balibrea et al., 2008; Liu et al., 2011; Zhang et al., 2018). In the present study, our results indicated that light quality had significant effects on morphological and growth traits in the Marigold plant. In a way that, the best growth performance was obtained under blue, red and red/blue treatments (Table 3). In agreement with our results, Fukuda et al. (2011) found that blue light promotes the stem elongation in the petunia plant. On the other hand, Steele (2004) revealed that plants under the red light treatment had more height compared to the other light treatments. Furthermore, some researchers believed that different lights, such as blue, have different effects on stomatal opening, photosynthesis process, leaf physiological responses, and thus, provides the CO_2 for the proper plant growth (Frechilla et al., 2000; Muneer et al., 2014; Miao et al., 2016). In the present study the biggest leaves were obtained under blue light, and in confirmation of the positive impacts of blue light on plants, similar studies revealed that blue light is essential light spectrum for leaf expansion, and for increase the leaf area (Li et al., 2010; Johkan et al., 2012). Therefore, it is possible that the morphological traits of the Marigold plant under blue light, such as plant height, leaf area, and leaf width, may depend on the effects of this light on physiological activity of the plants.

Raju et al. (2013) and Muneer et al. (2014) stated that the structure and physiology of plants are regulated by light signals, and the initial responses of plants for photosynthesis, growth, and yield depend on the lighting condition. In this regard, Lefsrud (2008) and Ramakrishna et al. (2011) indicated that light quality have great effects on the contents of pigments. The highest concentration of Chl α and total Chl were achieved under red/blue treatment, and the highest concentration of Chl b and carotenoids were obtained in plants grown under blue light. In accordance with the obtained results of the

present study, Wang et al. (2009) showed that the blue and red light spectra had positive effects on pigments, and as result the growth and yield of the plants will be also improved. Zhang et al. (2018) examined the effects of different LED sources including white light (control), purple, blue, red, green, yellow and red/blue light in 9:1 and 4:1 ratios on the growth, quality and nitrogen metabolism of lettuce and showed the purple, blue, red, green, yellow and red/blue light increase the mentioned traits in lettuce plants. Also, they found that the activities of the nitrogen metabolism-related enzymes were increased under purple, blue, red, and red/blue lights. Therefore, it can be possible that improvement of growth parameters in the present study has been linked to the nitrogen metabolism and enzymatic activity, but more researches in this context should be done. Furthermore, it has been stated that plants commonly show higher photosynthetic properties under blue light than the red and other lights (Savvides et al., 2012; Aliniaeifard et al., 2018). Accordingly, increase in the concentration of Chl α and other photosynthetic pigments under blue light treatments (Table 6) can be related to the above reason.

Flavonoids are phenolic compounds that can affect the color and flavor of the plants (Hichri et al., 2011), and it has been proven that different light sources can affect the content of flavonoids (Zhang et al., 2018). Zhang et al. (2018) showed that the content of total flavonoid in lettuce increases by application of blue light. In the present study, despite of no significant effects of cultivars on total flavonoid content, light spectra caused significant differences on this index so that the highest total flavonoid content was obtained under blue, red, and red/blue treatments and the lowest total flavonoid content was detected under greenhouse light. Lobiuc et al. (2017) showed that light quality has different effects on diverse plant species. They showed that red and blue LEDs caused rapid growth and increased phenolic content in basil, but total phenol content varied between different basil varieties. These results are in consistent with the obtained results of the present study about the effects of blue light on total flavonoid content. Based on our results, despite the positive effects of LEDs on the total flavonoid content, there was no significant difference between two cultivars on this regard.

Lee et al. (2014) examined the effects of light spectra on buckwheat and reported that blue and red lights elevate flavonoid content in buckwheat sprouts. In another study, Takemiya et al. (2005) showed that blue light photoreceptors realize the specific wavelengths and regulate photomorphogenic responses. On the other hand, cryptochrome, as photoreceptors for blue light, induces expression of the genes that are involved in flavonoid biosynthesis, e.g. chalcone synthase gene (Wade et al., 2001). Therefore, according to the above studies, it can be probable that there is a special connection between the perception of blue light and the biosynthesis of flavonoids. However, the effects of light spectrum on the mechanism of the biosynthesis of flavonoids are not well known yet (Nam et al., 2017), which can be investigated by future studies.

Conclusion

In conclusion, application of different light spectra, in LED forms, was effective in increasing the growth and yield of the pot Marigold. Therefore, due to the fact that there was no significant difference between different light spectra, it is recommended to use artificial lighting using LEDs, especially blue, red, and a combination of them, for improving growth and yield of pot Marigold plants.

Conflict of interest

The authors indicate no conflict of interest for this work.

References

1. Aliniaeifard S, Seif M, Arab M, Zare Mehrjerdi M, Li T, Lastochkina O. 2018. Growth and photosynthetic performance of *calendula officinalis* under monochromatic red light. International Journal of Horticultural Science and Technology 5(1): 123-132.
2. Arnon D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenol-oxidase in *Beta vulgaris*. Plant Physiology 2(1): 1-15.
3. Ashwlayan V.D, Kumar A, Verma M, Garg V.K, Gupta S.K. 2018. Therapeutic potential of *Calendula officinalis*. Pharmacy and Pharmacology International Journal 6(2): 149-155.
4. Biju J, Sulaiman C.T, Satheesh G, Reddy V.R.K. 2014. Total phenolics and flavonoids in selected medicinal plants from Kerala. International Journal of Pharmacy and Pharmaceutical Sciences 6: 406-408.
5. Brotosudarmo T.H.P, Prihastyanti M.N.U, Gardiner A.T, Carey A, Cogdell R.G. 2016. The light reactions of photosynthesis as a paradigm for solar fuel production. Energy Procedia 47: 283-289.
6. Caliskan O, Kurt D. 2018. Flower yields of pot Marigold (*Calendula officinalis* L.) plants as affected by flowering durations and number of harvests. Journal of Medicinal Plants Studies 6(6): 159-161.
7. Cosgrove D.J. 1993. Photomodulation of growth. *In:* R.E. Kendrick, G.H.M. Kronenberg (Eds.) *Photomorphogenesis in plants*, 2nd edition. Dordrecht, Kluwer Academic Publishers. Netherlands.
8. Currey C.J, Lopez R.G. 2013. Cuttings of Impatiens, Pelargonium, and Petunia propagated under light-emitting diodes and high-pressure sodium lamps have comparable growth, morphology, gas exchange, and post-transplant performance. Horticultural Science 48: 428-434.
9. Darko E, Heydarizadeh P, Schoefs B, Sabzalian M.R. 2014. Photosynthesis under artificial light: the shift in primary and secondary metabolism. Philosophical Transactions of the Royal Society B 369: 213-243.
10. Frechilla S, Talbott L.D, Bogomolni R.A, Zeiger E. 2000. Reversal of blue light-stimulated stomatal opening by green light. Plant Physiology 41: 71-176.
11. Fukuda N, Ishii Y, Ezura H, Jorunn E.O. 2011. Effects of light quality under red and blue light emitting diodes on growth and expression of FBP28 in petunia. Acta horticulturae 907: 361-366.
12. Gruda N, Tanny J. 2014. Protected crops. *In*: G.R. Dixon, D.E. Aldous (Eds) *Horticulture plants for people and places*. Prod Hort SSB Media Verlag, Heidelberg. New York.
13. Hernandez-Saavedra D, Perez-Ramirez I. F, Ramos-Gomez M, Mendoza-Diaz S, Loarca-Pina G, Reynoso-Camacho R. 2015. Phytochemical characterization and effect of *Calendula officinalis*, *Hypericum perforatum*, and *Salvia officinalis* infusions on obesity-associated cardiovascular risk. Medicinal Chemistry Research 25(1): 163-172.
14. Hichri I, Barrieu F, Bogs J, Kappel Ch, Delrot S, Lauvergeat V. 2011. Recent advances in the transcriptional regulation of the flavonoid biosynthetic pathway. Journal of Experimental Botany 62(8): 2465-2483.
15. Hogewoning S.W, Trouwborst G, Maljaars H, Poorter H, Van Ieperen W, Harbinson J. 2010. Blue light dose responses of leaf photosynthesis, morphology, and chemical composition of Cucumissativus grown under different combinations of red and blue light. Journal of Experimental Botany 61: 3107-3117.
16. Inoue S.I, Kinoshita T. 2017. Blue Light Regulation of Stomatal Opening and the Plasma Membrane H^+-ATPase. Plant Physiology 174(2): 531-538.
17. Jan N, Andrabi K. I, John R. 2017. *Calendula officinalis*- An important medicinal plant with potential biological properties. Proceedings of the Indian National Science Academy 83(4): 769-787.
18. Johkan M, Shoji K, Goto F, Hashida S.N, Yoshihara T. 2010. Blue light-emitting diode light irradiation of seedlings improves seed quality and growth after transplanting in red leaf lettuce. Horticultural Science 45: 1809-1814.
19. Johnson M.P. 2016. Photosynthesis. Essays in Biochemistry 60(3): 255-273.
20. Khalid A.K, Silva J.A.T. (2012). Biology of *Calendula officinalis* Linn. focus on pharmacology biological activities and agronomic practices. Medicinal and Aromatic Plant Science and Biotechnology 6(1): 12-27.
21. Kim S.J, Hahn E.J, Heo J.W, Paek K.Y. 2004. Effects of LEDs on net photosynthetic rate, growth and leaf stomata of chrysanthemum plantlets in vitro. Scientia Horticulturae 101: 143-151.

22. Kurkin V.A, Sharova O.V. 2007. Flavonoids from *Calendula officinalis* flowers. Chemistry of Natural Compounds 43: 216-217.

23. Lee S.W, Seo J.M, Lee M.K, Chun J.H, Antonisamy P, Arasu MV, Suzuki T, Al-Dhabi NA, Kim S.J. 2014. Influence of different LED lamps on the production of phenolic compounds in common and Tartary buckwheat sprouts. Industrial Crops and Products 54: 320-326.

24. Lefsrud M.G, Kopsell D.A, Sams C.E. 2008. Irradiance from distinct wavelength lightemitting diodes affect secondary metabolites in kale. Horticultural Science 43: 2243-2244.

25. Li H.M, Xu Z.G, Tang C.M. 2010. Effect of light emitting diodes on growth and morphogenesis of upland cotton (*Gossypium hirsutum* L.) plantlets in vitro. Plant Cell, Tissue and Organ Culture 103: 155-163.

26. Lichtenthaler H. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods of Enzymology 148: 350-382.

27. Lin K.H, Huang M.Y, Huang W.D, Hsu M.H, Yang Z.W, Yang C.M. 2013. The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactucasativa* L. var. capitata). Scientia Horticulturae 150: 86-91.

28. Liu M, Xu Z, Yang Y, Feng Y. 2011. Effects of different spectral lights on Oncidium PLBs induction, proliferation, and plant regeneration. Plant Cell, Tissue and Organ Culture 106(1):1-10

29. Lobiuc A.V, Vasilache M, Oroian T, Stoleru M, Burducea, Pintilie O.M. Zamfirache M. 2017. Blue and Red LED illumination improves growth and bioactive compounds contents in acyanic and cyanic *Ocimum basilicum* L. Microgreens. Molecules 22(12): 2111-2125.

30. Martin F, Mastebroek D. Gorp K.V. 2005. A grower's manual for *Calendula officinalis* L. ADAS Bridget Research Centre.

31. Miao Y.X, Wang X.Z, Gao L.H, Chen Q.Y, Qu M. 2016. Blue light is more essential than red light for maintaining the activities of photosystem ii and i and photosynthetic electron transport capacity in cucumber leaves. Journal of Integrative Agriculture 15(1): 87-100.

32. Moghaddasi M.Sh, Haddad Kashani H. 2012. Pot Marigold (*Calendula officinalis*) medicinal usage and cultivation. Scientific Research and Essays 7(14): 1468-1472.

33. Moghtader M, Salari H, Mozafari H, Farahmand A. 2016. Evaluation the qualitative and quantitative essential oil of *Calendula officinalis* and its antibacterial effects. Iranian Journal of Cellular and Molecular Researches 29(3): 331-339.

34. Mott K.A. 2009. Opinion: Stomatal responses to light and CO_2 depend on the mesophyll. Plant, Cell and Environment 32: 1479-1486.

35. Muley B.P, Khadabadi S.S, Banarase N.B. 2009. Phytochemical constituents and pharmacological activities of *Calendula officinalis* Linn (Asteraceae): A review. Tropical Journal of Pharmaceutical Research 8(5): 455-465.

36. Muneer S, Kim E.J, Park J.S, Lee J.H. 2014. Influence of green, red and blue light emitting diodes on multiprotein complex proteins and photosynthetic activity under different light intensities in lettuce leaves (*Lactuca sativa* L.). International Journal of Molecular Sciences 15: 4657-4670.

37. Nam T.G, Kim D, Eom S.H. 2017. Effects of light sources on major flavonoids and antioxidant activity in common buckwheat sprouts. Food Science Biotechnoly 27(1): 169-176.

38. Naved T, Ansari S.H, Mukhtar H.M, Ali M. 2005. New triterpenic esters of oleanene-series from the flowers of *Calendula officinalis* L. Journal of Medicinal Chemistry 44: 1088-1091.

39. Perez-Balibrea S, Moreno D.A, García-Viguera C. 2008. Influence of light on health-promoting phytochemicals of broccoli sprouts. Journal of the Science of Food and Agriculture 88(5):904–910.

40. Raju S, Shah S, Gajbhiye N. 2013. Effect of light intensity on photosynthesis and accumulation of sennosides in plant parts of senna (*Cassia angustifolia* Vahl.). Indian Journal of Plant Physiology 3: 285-289.

41. Ramakrishna A, Dayananda C, Giridhar P, Rajasekaran T, Ravishankar G.A. 2011. Photoperiod influences endogenous indoleamines in cultured green alga *Dunaliella bardawil*. Indian Journal of Experimental Biology 49: 234-240.

42. Randall W.C, Lopez R.G. 2014. Comparison of supplemental lighting from high-pressure sodium lamps and light emitting-diodes during bedding plant seedling production. Horticultural Science 49(5): 589-595.

43. Rao M.K.V, Raghavendra A.S, Janardhan P.K. 2006. Physiology and molecular biology of stress tolerance in plants. Published by Springer, Dordrecht, Netherlands.

44. Safdar W, Majeed H, Naveed I, Kayani W. Kh, Ahmed H, Hussain S, Kamal A. 2010. Pharmacognostical study of the medicinal plant *Calendula officinalis* L. International Journal of Cell and Molecular Biology 1(2): 108-116.

45. Savvides A, Fanourakis D, van Ieperen W. 2012. Co-ordination of hydraulic and stomatal conductances across light qualities in cucumber leaves. Journal of Experimental Botany 63(3): 1135- 1143.

46. Schwartz A, Zeiger E. 1984. Metabolic energy for stomatal opening: Roles of photophosphorylation and oxidative phosphorylation. Planta 161: 129-136.

47. Shahmoradi H, Naderi D. 2018. Improving effects of salicylic acid on morphological, physiological and biochemical responses of salt-imposed winter jasmine. International Journal of Horticultural Science and Technology 5(9): 219-230.

48. Shengxin C, Chunxia L, Xuyang Y, Song C, Xuelei J, Xiaoying L, Zhigang X, Rongzhan G. 2016. Morphological, Photosynthetic, and Physiological Responses of Rapeseed Leaf to Different Combinations of Red and Blue Lights at the Rosette Stage. Front Plant Science 7: 1144-1156.

49. Shiga T, Shoji K, Shimada H, Hashida S.N, Goto F, Yoshihara T. 2009. Effect of light quality on rosmarinic acid content and antioxidantactivity of sweet basil, *Ocimum basilicum* L. Plant Biotechnology 26: 255-259.

50. Son K.H, Oh M. M. 2013. Leaf Shape, Growth, and Antioxidant Phenolic Compounds of Two Lettuce Cultivars Grown under Various Combinations of Blue and Red Light-emitting Diodes. HortScience 48(8): 988-995.

51. Steele R. 2004. Understanding and measuring the shelf-life of food. Woodhead Publishing.

52. Stutte G.W, Edney S, Skerritt T. 2009. Photoregulation of bioprotectant content of red leaf lettuce with light-emitting diodes. Horticultural Science 44: 79-82.

53. Sun N, Wei M, Yan L, Wang XF, Yang FJ, Shi QH (2016) Effects of light quality on carbon and nitrogen metabolism and enzyme activities in tomato seedlings. Acta Horticulturae 43(1):80-88.

54. Ukiya M, Akihisa T, Yasukava K, Tokuda H, Suzuki T and Kimura Y. 2006. Anti-inflammatory, anti-tumorpromoting and cytotoxic activities of constituents of Marigold (*Calendula officinalis*) flowers. Journal of Natural Products 69: 1692-1696.

55. Verma P.K, Raina R, Agarwal S, Kour H. 2018. Phytochemical ingredients and pharmacological potential of *Calendula officinalis* Linn. Pharmaceutical and Biomedical Research 4(2): 1-17.

56. Wade H.K, Bibikova T.N, Valentine W.J, Jenkins G.I. 2001. Interactions within a network of phytochrome, cryptochrome and UV-B phototransduction pathways regulate chalcone synthase gene expression in Arabidopsis leaf tissue. Plant Journal 25: 675-685.

57. Wang H, Gu M, Cui J, Shi K, Zhou Y, Yu J. 2009. Effects of light quality on CO_2 assimilation, chlorophyll-fluorescence quenching, expression of Calvin cycle genes and carbohydrate accumulation in *Cucumis sativus*. Journal of Photochemistry and Photobiology B: Biology 96(1): 30-37.

58. Ward J.M, Cufr C.A, Denzel M.A, Neff M.M. 2005. The Dof transcription factor OBP3 modulates phytochrome and cryptochrome signaling in Arabidopsis. Plant Cell 17(2):475-485.

59. Zhang T, Shi Y, Piao F, Sun Zh. 2018. Effects of different LED sources on the growth and nitrogen metabolism of lettuce. Plant Cell, Tissue and Organ Culture 134(2): 231-240.

60. Zhang T, Shi Y, Wang Y, Liu Y, Zhao W, Piao F, Sun Zh. 2017. The effect of different spectral LED lights on the phenotypic and physiological characteristics of lettuce (*Lactuca sativa*) at picking stage. Journal of Biochemistry and Biotechnology 1(1): 14-19.