LED Light Sources Improved the Essential Oil Components and Antioxidant Activity of Two Genotypes of Lemon Balm (*Melissa Officinalis* L.)

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Abstract

Here we investigated the effect of different LED light sources on the essential oil components and antioxidant activity of *Melissa officinalis*. Two genotypes of lemon balm (Ilam and Isfahan) were subjected to four artificial light treatments including white, red, blue, red + blue LEDs and greenhouse light as natural lighting. The LED lights significantly increased shoot fresh and dry weights and Leaf number in the two genotypes as compared to greenhouse condition. The results showed that the content and composition of essential oil in two genotypes was variable under different light treatments and the total amount of compounds in Ilam genotype was higher than the other genotype. The results of analysis of the essential oil by GC/MS indicated that the highest amount of monoterpenes in the genotypes was related to citronellal under red + blue LED lamps (15.3 and 17.2% in Ilam and Isfahan genotypes, respectively) but blue, white, and greenhouse condition had the most effect on sesquiterpenes content in both genotypes. Furthermore, the observed variation between the two genotypes in the essentials oil composition was related to the relative percentage of the constituents and not to the appearance or lack of a specific component. Red + blue lighting also caused the highest radical scavenging activity % in both genotypes (80.77 and 82.09% for Ilam and Isfahan genotypes, respectively). Based on principal component analyses (PCA), three main groups were identified regarding genotypes and all light treatments. Overall, results indicated that the essentials oil composition of two genotypes of lemon balm was affected both qualitatively and quantitatively by different LED light sources; hence, LED lights might be used to improve monoterpenes, sesquiterpenes and antioxidant activity in the selected genotypes.

Highlights

Red+blue LEDs increased growth parameters, monoterpens and RSA% in both genotypes.

Ilam genotype had most total amount of compounds of essential oils than Isfahan.

Citronellal was highest monoterpenes in both genotypes.

1. Introduction

Melissa officinalis (lemon balm) is native to the north of Iran, western Asia, southern Europe, and northern Africa (Noorul Basar and Zaman 2013). This medicinal plant belongs to the Lamiaceae family that its scented leaves have been consumed as spice for salads and cold drinks, decoction, infusion, or directly used in food for decades. Several studies have shown antioxidant, hypoglycemic, hypolipidemic, anti-cancer, anti-depressant, sedative, and anti-inflammatory effects, in addition to known effects of this plant for digestive problems, rheumatism, or headache (Weidner et al. 2015; Birdane et al. 2007). Generally, the essential oil extracted from the aerial parts of lemon balm (0.02–0.8 %, with the main components being citral and citronellal) is one of the classes of compounds that are useful for food, nutrition, pharmacological and cosmetic applications, and antioxidant activities of the plant (Mazzanti et al. 2008).

Many secondary metabolites, such as flavonoids, essential oils, and phenolic acids are produced in response to environmental stresses (Weitzel and Peterson 2010). As an important environmental factor, light is an important source of energy for photosynthesis of the plant, as well as an important message for plant growth and development. Nowadays, new technologies such as light-emitting diodes (LEDs) lamps provide better growth of plants by providing an appropriate intensity and wavelength factors as an alternative source of sunlight (Sabzalian et al. 2014). LEDs also can stimulate the production of secondary metabolites and essential oils in plants (Ghaffari et al. 2019; Tohidi et al. 2019). Blue LED light caused the highest amount of essential oil in basil leaves (Amaki et al. 2011) and *Perovskia atripliciolta* (Gaffari et al. 2019). The highest thymol content was observed in *Thymus migricus* under blue LED light (Tohidi et al. 2019). Red LED light significantly increased lutein and glycosinolate in *Brassica oleracea* leaf (Lefsrud et al. 2008) and phenylacetaldehyde in *Petunia hybrid* flower (Colquhoun et al. 2013). Sabzalian and co-workers (2014) showed a four-fold increase in essential oil production of *Mentha longifolia* under red + blue LED light. The positive effect of blue, white, and green LED lights equally reported by Jung et al. (2013) on the antioxidant activity of rice leaves. Johkan and his colleagues (2010) also stated that the antioxidant activity of lettuce seedlings treated with blue LED was higher than red and fluorescent light. Additionally, the variability among plant genotypes influence the quantity and quality of secondary metabolites, which leads to large differences in secondary metabolite synthesis (Gahler et al. 2003). Several studies have shown the essential oil components and yield can vary with plant genotypes (Gholami-Zali and Ehsanzadeh 2018; Rajendra et al. 2016). However rare data have beem published about the variations in the essential oil composition between the lemon balm genotypes.

Today, the global strategy in the production of medicinal and aromatic plants is to improve the quantity, quality and health of their essential oils, and due to the emergence of adverse effects of chemicals used in agriculture, such as fertilizers and pesticides, the tendency to use approaches that are healthier and more environmentally friendly are on the rise. In this regard, the use of LED lamps thechnology while meeting plants' better growth and production, also help to better protect the environment. The goal of this study is to investigate the effects of four light wavelengths of LED lamps in two genotypes of lemon balm (Ilam and Isfahan), on growth, antioxidant activity and essential oil constituents.

2. Material And Methods

2.1. Plant material

The plant material of this study was *Melissa officinalis* plants that were collected from fields in Eyvan city of Ilam province (with geographical coordinates 33°53'N 46°11'E) and Isfahan city of Isfahan province (with geographical coordinates 32°38'41"N 51°40'03"E). Three rhizomes with 3–4 leaves were planted in pots (15 cm diameter) containing 3:1 loam-sandy soil (30 pots for two genotypes).

2.2. System of light and experimental condition

On March 11, 2016, 24 pots (12 of each genotype) were incubated in four growth cabinets (i.e. 3 pots for each genotype per each cabinet). Each cabinet's lighting system contained red (650 nm), blue (460 nm), white (380–760 nm) and red + blue (70:30 ratio) LED lamps with a light intensity of 300 $\mu mol.m^{-2}.s^{-1}$, with a 16h illumination and 8h darkness and 25 ± 2 ° C. On the same date, 6 pots (3 pots of each genotype) were kept in the research greenhouse of Isfahan University of Technology, under similar conditions under natural sunlight. All pots were irrigated daily with water and once a week with 1/2 Hoagland solution. After 7 weeks, on April 29, plants were transferred to the laboratory for sampling.

2.3. Assessment of growth parameters

First, the samples were washed with distilled water and their excess moisture was removed with filter paper. The fresh weight of plant shoots was determined in gram after cutting the roots from the crown. To calculate the dry weight, each sample, after being placed in paper bags, was dried in an oven at 65°C for 48 hours and then weighed in gram. The leaves were counted separately in each pot.

2.4. Extraction of essential oil

Seven weeks after exposure to LED lighting, 10 g of dry leaves of each light treatment was used for essential oil extraction. The dried and ground leaves were submitted to 150 ml water distillation using a clevenger apparatus. The sample of oils were extracted in triplicate. Essential oil was extracted for 6 h at 70–80°C temperature. The volatile fraction collected was kept in sealed glass vials and stored at 4°C before GC-MS analysis. The essential oil content (%) was calculated as follow:

Essential oil content (%) = Mass of isolated essential oil (g)/dried shoot (g) × 100

2.5. Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography-mass spectroscopy (GC–MS) analysis for the essential oil composition was carried out on a Hewlett–Packard 5890 gas chromatograph (Hewlett–Packard, EquipNet, Inc. 50 Hudson Road Canton, MA 02021 USA) coupled to a HP 5970 mass-selective detector (MSD) (Hewlett–Packard, EquipNet, Inc. 50 Hudson Road Canton, MA 02021 USA) using a fused silica ultra-performance cross-linked methyl silicone column (50 mm length, 0.2 mm inner diameter, film thickness 25 µm). Temperature programming was done from 100 to 250°C at a rate of $4°C.min^{-1}$. Helium was used as the carrier gas at 1 $ml.min^{-1}$ flow rate. Ionization energy at 70 eV and 250°C temperature were ordered for an ion source. Identification of each compound is based on the time of restoration and their recorded mass (Davies 1990; Li et al. 2009).

2.6. Determination of antioxidant activity

2.6.1. 2–2´-diphenyl-1-picrylhydrazyl radical scavenging activity

The radical scavenging activity (RSA) of the extracts was monitored using the stable free radical 2–2´-diphenyl-1-picrylhydrazyl (DPPH) following the method described by Kulisica et al. (2004). Extract solutions (1 ml) were mixed with 1 ml of a freshly prepared DPPH solution (0.1 mM in methanol) and 3 ml of 96% ethanol. The mixture was shaken vigorously and left to stand at room temperature for 30 min in dark (until stable absorbance values were obtained). The reduction of the DPPH radical was measured by monitoring the decrease of absorption at 517 nm. RSA of the extracts was calculated by the following formula:

% Radical scavenging activity = (OD control (optical density) - OD sample / OD control)×100.

Methanol (80%) and DPPH solution (0.1 mM, 5 ml) were used separately as a blank and control sample, respectively. The test also was done in triplicate.

3. Statistical Analysis

Since cultivation of plants were in greenhouse and 4 incubators in laboratory, considered as experiment in separate environment, so for the measured factors, significance of variation was assessed using the combined analysis of variance using SAS statistical program (ver. 8; SAS Institute Inc., Cray, NC, USA) and the least significant differences method was used for mean comparisons. Identification of interrelationships between genotypes of *Melissa* subjected to LED lighting with measured traits was done using principal component analysis (PCA).

4. Results And Discussion

4.1. Growth parameters

The effect of different light sources and genotypes on growth parameters was significant (Table 1). Table 2 showed that the highest shoot fresh and dry weight and Leaf number were observed under red+blue LED light source in plantlets of both genotypes. Increment in fresh and dry weight, leaf area, and chlorophyll content in lettuce plants in studies conducted by Kim et al. (2004), Johkan et al. (2010) and Yorio et al. (2001) showed that the highest amount of these indicators can be seen in the combination of red and blue light. The combination of red+blue light increased the fresh and dry weight by 3 times compared to the combination of blue and far-red light. It seemed that the combination of red and blue light increased plant growth by increasing the amount of pure photosynthesis because the red+blue light energy distribution corresponded to the absorption spectrum of chlorophyll (Wang et al. 2007). On the other hand, the intensity of photons from LED lamps is higher than other light sources, which is another mechanism of the positive effect of LED lights to improve plant growth indices (Yorio et al. 2001).

4.2. Essential oil content and constituents

The essential oil content of two genotypes of *M. officinalis* was not significantly affected by different LED light sources when compared to the greenhouse condition (in range of 0.27% to 0.32%, data not shown). However, the effect of different light sources and genotype on major compounds of lemon balm essential oil was significant (Table 1). A total of thirty-eight compounds were identified using GC-MS analysis (Table 3). A considerable difference was observed in the concentration of essential oil constituents in both genotypes of *M. officinalis* grown under various light sources (Table 3). In plantlets of Ilam genotype, ß-cubebene and citronellal had the lowest and highest amounts among all of the identified constituents under red and red+blue LEDs, respectively with 0.6 and 15.3%, but in the othergenotype, the lowest amount of essential oil content was belonging to bicyclo[2.2.1]heptan-2-one, ß-ionone, and eugenol in plantlets that were subjected to red, blue, and red+blue LEDs (0.8%). Among essential oil compounds, citronellal had the highest percent content (17.2%) under red+blue LED lights in the Isfahan genotype.

It is very important to study the change in the amount of essential oil of medicinal plants because it is related to the change in their nutritional, medicinal and antioxidant properties. Scientists believed that chemical composition of essential oil and production of secondary metabolites were related to the physiology of plant, developmental stage, plant species, and various treatments such as various light sources (Yu et al. 2005; Fernandes et al. 2013; Mashkani et al. 2018). In the study by Batista et al. (2016) on three chemotypes of *Lippia alba*, they demonstrated that the differences in the composition of essential oil were more influenced by chemotype than light treatments (LED and fluorescent) applied. They and Viccini et al. (2014) stated that this difference between genotype and chemotypes could be depended on the content of plant DNA.

The content and composition of essential oils in two genotypes studied in the present work were variable under different light treatments and the total amounts of compounds in the Ilam genotype were higher than the other genotype. However the observed variation between two genotypes in the essentials oil composition was related to the relative percentage of the constituents and not to the appearance or lack of a specific component. Light quality and its intensity influenced on production of secondary metabolites and essential oils with changing in physiological and morphological properties of plants (Briskin et al. 2001). Light affects the number and morphology of leaves and essential oil storage structures such as trichrome, causing changes in the amount and chemical composition of essential oil in plants (Fernandes et al. 2013). In the present study, in plantlets of Ilam genotype, all LEDs lights had a greater effect on essential oil content than plants grown under greenhouse conditions but in Isfahan genotype, the effect of red+blue and red LEDs treatments were higher than plants grown under greenhouse conditions. Treatments of red and red+blue LEDs on Ilam and Isfahan genotypes have resulted in the greatest essential oil contents. Various studies have suggested the positive effect of LED light on increasing the essential oil content in *Mentha* species, green vegetables (such as parsley, onion, lettuce and…), and *Brassica oleracea* (Sabzalian et al. 2014; Žukauskas et al. 2011; Lefsrud et al. 2008).

4.2.1. Monoterpenes content under various light treatments in two genotypes of *M. officinalis*

The number of identified monoterpenes in the two genotypes of lemon balm was 21 compounds. The amount of these compounds was different under various light treatments. In Ilam genotype, the highest amount of monoterpenes was observed in incubators with white, red+blue, and red LEDs compared to Isfahan genotype,but in Isfahan genotype, these compounds were higher under blue LED and greenhouse condition than the other genotype. The red+blue LED light treatment in both genotypes produced the highest amount of monoterpene compounds compared to the greenhouse condition (Table 3).

In Ilam genotype, monoterpene compounds ranged from 0.9% for myrcene under red+blue LED to 15.3% for citronellal under the same light treatment (Table 3). Under LED light treatments, monoterpenes such as myrcene, alpha-phellandrene, para cymene, eucalyptol, γ-terpinene, linalool, cis-sabinene, trans-carveol, citronellal, citral, thymol, carveol, and carveol acetate were more affected than greenhouse condition. In this genotype, myrcene (0.9%) and para cymene (1%) had the lowest identified monoterpenes, regardless of light treatment. In this genotype, the red+blue LED treatment resulted in the highest amount of major essential oils namely citronellal (15.3%), trans-carveol (10.2%), linalool (8.9%), and citral (8.2%) compared to the other light.

The range of monoterpene composition in Isfahan genotype changed from 1% for thymol under the white LED light to citronellal with 17.2% under red+blue LED light (Table 3). Myrcene, limonene, eucalyptol (1.2%), and thymol (1%) had the lowest values among known monoterpenes, regardless of light treatments. Alpha-pinene, myrcene, γ-3-carene, limonen, eucalyptol, linalool, cis-sabinene, β-thujone, trans-carveole, citronellal, citral, thymol, and carveol had the most contents in plantlets grown in incubators with LED lamps compared to greenhouse condition. Among light treatments, red+blue LED light had the highest contribution to increasing the amounts of major essential oils including citronellal (17.2%), trans-carveole (11.1%), citral (9.1%), and linalool (8.9%).

The chemical composition of lemon balm essential oil (that is 0.02 - 0.3% of DW) had been investigated. The main combination was citronellal (2-40%) and citral (10-30%) with ß-caryophyllene, germacrene-D, ocimene and citronellol (Schultze et al. 1989; Adzet et al. 1992; Kreis and Mosandl 1994; Moradkhani et al. 2010). Citronellal, as one of the major components in the essential oil of lemon balm, is a monoterpene aldehyde (Chung et al. 2010). In the present study, citronellal percentage varied from 10.2-17.2%, that its highest amount (17.2%) was detected in plantlets of both genotypes grown in incubators containing red+blue LED. In many studies, citral after citronellal was the most commonly reported composition of lemon balm essential oil, but as was seen in the present study, the highest essential oil content after citronellal was citronellol with a varied range of 6.5–11.8%. In the present study, citral after citronellal, citronellol, and trans-carveol compounds has the highest amount of essential oil in two genotypes under different light treatments.

Light quality can change the composition of essential oils in medicinal plants (Amaki et al. 2011). In a study by Batista et al. (2016), different qualities of light caused a change in the pattern of essential oil in *Lippia alba*. These researchers showed changes in the number of monoterpenes such as eucalyptol and linalool in two *L. alba* chemotypes under LED and fluorescent lamps. Noguchi and Amaki (2016) and Nguyen and Saleh (2019) reported an increase in monoterpenes such as alpha-pinene, beta-pinene, limonene and carvone in mint plants under red LED light.

According to the findings of the present study, it was found that in the two genotypes of lemon balm, light treatments play an effective role in changing the number of monoterpenes. Since most of the essential oil components of lemon balm in this study were monoterpenes, it is concluded that the quality and property of essential oil will probably change significantly. Ghaffari et al. (2019) and Tohidi et al. (2019) reached the same results with the current study about increase in the number of monoterpenes under LED lamps. In the present study, red+blue LED light was the major contributor to the increase in monoterpenes,

but in studies by Ghaffari et al. (2019) and Tohidi et al. (2019), blue light played this role. Therefore, this difference reflects the different effects of LED light on the essential oil of different plants.

It is well known that the increase in the production of secondary metabolites is related to plant growth conditions such as temperature, light regime, stress, nutrition source, etc. (Selmar and Kirinwächter 2013). If the light energy that is absorbed by the photosynthetic apparatus exceeds compared to the energy required for CO_2 fixation, large amounts of this energy according to the energy dissipation mechanism proposed by Selmar and Kirinwächter (2013) ultimately led to the production of secondary metabolites. In the present study, since the intensity of light in LEDs (300 $\mu mol.m^{-2}.s^{-1}$) is continuously higher than the greenhouse conditions, these light sources, especially red+blue LED light, can be considered as severe light stress and increased monoterpenes according to the mechanism described above. Recently, researchers have found that terpenoids biosynthesis in addition to the mevalonic Acid (MVA) pathway is made from primary metabolites through another pathway called methylerythritol phosphate (MEP), which is present in plastids and chloroplasts (Lichtenthaler et al. 1999). Therefore, it can be stated that under the treatment of LED lights, especially under red+blue LED light, due to the matching of these wavelengths to the absorption peak of photosynthetic pigments, it resulted in increase of photosynthesis and production of photosynthetic intermediates, especially IPP (isopentenyl diphosphate). This process may have resulted in the activation of the MEP pathway more and more and produced high levels of these terpenoids.

4.2.2. Sesquiterpenes content under various light treatments in two genotypes of *M. officinalis*

According to the results shown in Table 3, the number of sesquiterpene compounds in lemon balm genotypes was also significantly affected by light treatments. In Ilam and Isfahan genotypes, the highest amount of these compounds were obtained under blue and white LED and greenhouse conditions, respectively. In Ilam genotype, LED light treatments increased the level of sesquiterpene such as ß-caryophyllene, α-humulene, germacrene-D, ß-ionone, and ß-cubebene but decreased the amount of caryophyllene epoxide, calamenene, and α-muurolene compared to greenhouse condition. In contrast, in Isfahan genotype, only α-humulene, ß-ionoe, and germacrene-D levels were higher in most LED treatments compared to the greenhouse condition. The reduction in the amount of ß-caryophyllene, caryophyllene epoxide, and calamenene treated with LED light was significantly higher than greenhouse condition. In plants of two genotypes, caryophyllene epoxide, germacrene-D and ß-caryophyllene had the highest sesquiterpene compounds, respectively, regardless of light treatments. As in the present study, in Jalal et al.'s study (2015), the main sesquiterpene of lemon balm was ß-caryophyllene epoxide (11%) and in the study of Kittler et al. (2018) ß-caryophyllene, germacrene-D and ß-caryophyllene epoxide constituted the main sesquiterpene compounds of lemon balm genotypes. However, in the study conducted by Chung et al. (2010), caryophyllene (0.8%) and farnesene (0.1%) were the most common sesquiterpene. So, it can be said that the essential oil compositions of species and genotypes of a plant are different under different conditions. ß-cariophyllene is a natural bicyclic sesquiterpene that has also been involved in the creation of a spicy taste of black pepper and many essential oils such as *Rosmarinus officinalis*, *Syzygium aromaticum*, and *Cannabis sativa*. This compound has received much attention because of its cyclobutane ring, that is scarce in nature (Taherpour et al. 2012).

Many studies have been shown that LED lights induced several metabolic changes in plants, including increased levels of β-farnecene, germacrene-D, and elmene in Mexican mint grown under blue light (Noguchi and Amaki 2016). Alvarenga et al. (2015) showed that high light intensities, such as 47 and 69 $\mu mol.m^{-2}.s^{-1}$, increased monoterpene compounds content in *Acillea millefolium* L., but these intensities reduced the number of sesquiterpenes. Sesquiterpenes such as ß-caryophyllene and ß-cubebebene have the highest values at 13 $\mu mol.m^{-2}.s^{-1}$ intensity. Blue LED light in *Mentha spicata* increased ß-caryophyllene compared to control condition. The concentration of beta-bourbonene as a sesquiterpene was also higher in mint cultivated under LED light compared to control (Nguyen and Saleh 2019.). Increment in trans-caryophyllene and α-humulene in two species of *Perovskia* has also been reported under the white LED light (Ghafari et al. 2019). Tohidi et al. (2019) study showed an increase in sesquiterpene levels of three species of thyme under red+blue and red LED lights.

4.3. Radical Scavenging Activity analysis

The results of the analysis of variance of free radical scavenging activity (RSA or antioxidant activity) showed that there was no significant difference between two genotypes in terms of this activity, while the effect of different lights on RSA was significant ($p \leq 0.01$) (Table 1). In both genotypes, the highest RSA% was obtained for plantts grown under red+blue LED light (80.77 and 82.09% for Ilam and Isfahan genotypes, respectively) and the lowest was for plants grown under greenhouse condition (34.29 and 25.67% for Ilam and Isfahan genotypes, respectively) (Fig. 1).

Synthesis of secondary metabolites in plants is an important part of the defense response to stress. Oxidative stress and reactive oxygen species (ROS) cause extensive damage to plants. Antioxidant systems that show antioxidant capacity in plants including flavonoids, ascorbate, carotenoids, phenolic compounds, and essential oils, protect plants against photo-oxidative damage (Shohael et al. 2006). Li and Kubota (2009) demonstrated the beneficial role of LEDs in improving antioxidant activity in baby leaf lettuce. In addition, Samuolienė et al. (2012) reported that LED supplementation with HPS lamps altered the antioxidant and nutritional properties of lettuce due to increased metabolic system activity to protect plantlets against moderate photo-oxidative stress induced by the use of LEDs. In the present study, it was observed that the red+blue LED light treatment produced the highest amount of major monoterpene components such as citronellal, trans-carveol, citronellol, linalool, and citral toghether with the highest RSA% in two genotypes. Therefore, it can be stated that the antioxidant capacity of these two genotypes under light treatments is directly related to the essential oil components, especially monoterpenes. Confirming this result, researches has shown that DPPH's scavenging activity is directly related to the levels of phenolic and antioxidant compounds of plants such as essential oils (Wojciechowska et al. 2015; Tohidi et al. 2017 and 2019).

4.4 Principal component analysis

Principal component analysis (PCA) was used to identify the relationship between growth parameters and essential oil components, antioxidant activity, and different wavelengths applied in two genotypes of lemon balm (Fig. 2). The results of PCA analysis identified three main groups and these results also indicated that the first and second groups accounted for 78.39% of the total variation. The first PC (PC1) and the second PC (PC2) revealed 64.61% and 13.77% of the total variation, respectively. The plants grown under LED light sources were completely separated based on the growth parameters, essential oil

constituents and antioxidant activity, from the plant grown in the greenhouse condition. In terms of caryophyllene, only plants of Isfahan genotype that were grown in incubators containing red and blue LEDs were similar to greenhouse light. In the other group, Ilam genotype that was under red+blue and red LEDs, and Isfahan genotype grown under red+blue, red and blue LEDs, there was the highest levels of main monoterpene compounds such as citronellal, trans-carveol, linalool, and citral and highest antioxidant activity plus shoot fresh and dry weights. The third group, Ilam genotype grown under white and blue LEDs and Isfahan genotypes with white LEDs also had the same and the highest amount of monoterpene compounds such as citronellol and γ-3-caren and sesquiterpene compounds such as caryophyllene epoxide and germacrene-D. Results showed that two genotypes of lemon balm were differentially affected by LEDs and greenhouse light, successfully distinguished by the main constituents of essential oil and antioxidant activity.

5. Conclusion

In the present study, the effect of different sources of LED light and greenhouse light on two genotypes of lemon balm was measured in terms of growth factors, amount and composition of essential oil content, and antioxidant activity. Different light sources had a significant effect on the measured characteristics of the two genotypes, which could also alter their medicinal and food properties. In the two genotypes, the positive effect of LEDs compared to greenhouse light on the measured properties was quite significant. In both genotypes, red + blue LED produced the highest amount of shoot fresh and dry weight, Leaf number, and essential oil monoterpenes, such as citronellal, trans-carveol, linalool, and citral, and the highest amount of antioxidant activity. It can be concluded that this light treatment with increasing antioxidant properties could have the best effect on improving the productional, nutritional and pharmaceutical characteristics of lemon balm.

Abbreviations

LED, Light-emittinf diode; RSA, Radical Scavenging Activity; DPPH, 2-2´-diphenyl-1-picrylhydrazyl; MEP, Methylerythritol phosphate; IPP, Isopentenyl diphosphate; PCA, Principal Component Analysis

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Agree.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceived and designed the experiments: Leila Shabani, Mohammad R. Sabzalian. Performed the experiments: Tayebeh Ahmadi. Analyzed the data: Tayebeh Ahmadi, Leila Shabani, Mohammad R. Sabzalian. Wrote the paper: Tayebeh Ahmadi, Leila Shabani. Edited the manuscript: Leila Shabani, Mohammad R. Sabzalian.

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Tables

Table 1. Analysis of variance (mean of squares) of genotype, light and light their interactions on growth factors and major compounds of essential oil of *Melissa officinalis*.

Germacrene-D	Caryophyllen epoxide	ß-Caryophyllene	Citral	Citronellol	Citronellal	Trans-Carveol	Linalool	γ-3-carene	Leaf number	Shoot dry weight (g)	Shoot fresh weight (g)
9.072**	9.072**	9.072**	9.072**	17.412**	28.792**	15.9**	11.787**	7.144**	75676.36**	14.37**	596.7**
0.012	0.012	0.012	0.012	0.008	0.12	0.12	0.12	0.009	884.54	0.65	6.69
0.192**	0.192**	0.192**	0.192**	5.808**	12.675**	7.203*	0.300**	7.203**	25317.07*	5.61**	487.14**
1.137**	1.137**	1.137**	1.137**	1.698**	1.057**	0.798**	1.860**	2.665**	22707.42**	0.71**	50.97**
0.008	0.008	0.008	0.008	0.012	0.008	0.008	0.008	0.017	876.4	0.32	2.83

Table 2. Interaction of genotype and light on growth indices in two lemon balm genotypes under different levels of light (different letters indicate a significant difference at the probability level of 0.05).

	Shoot fresh weight		Shoot dry weight		Leaf number	
light genotypes	Ilam	Isfahan	Ilam	Isfahan	Ilam	Isfahan
White LED	29.65±2.55^{b}	25.46±0.04^{c}	4.07±0.2^{c}	3.74±0.1^{e}	387±17^{c}	317±17^{d}
Red+Blue LED	44.32±1.1^{a}	45.11±0.66^{a}	7.17±0.4^{a}	7.74±0.2^{a}	646±20^{a}	688±15^{a}
Red LED	32.6±0.5^{b}	42.26±2.54^{a}	5.53±0.1^{c}	6.4±0.3^{b}	556±50^{b}	544±6^{b}
Blue LED	30±3.97^{b}	22.25±0.12^{d}	4.70±0.1^{d}	3.39±0.04^{f}	253±22^{e}	330±49^{d}
Greenhouse	22.82±1.04^{d}	18.4±0.63^{e}	3.54±0.4^{e}	3.54±0.1^{f}	469±12^{b}	213±40^{e}

Table 3. Essential oil composition in two genotypes of *Melissa officinalis* L. under various light treatments.

Isfahan genotype					Ilam genotype			
Greenhouse	Blue	Red	Red+ Blue	White	Grenhouse	Blue	Red	Red+ Blue
0.7±0.1^{c}	-	1±0.1^{b}	1.2±0.1^{a}	1±0.1^{b}	1±0.1^{b}	1.3±0.1^{a}	1.5±0.1^{a}	-
2.4±0.1^{b}	2.6±0.1^{b}	1.7±0.1^{d}	2±0.1^{c}	1.7±0.1^{d}	3.1±0.3^{a}	2.2±0.2^{c}	1.9±0.1^{c}	2±0.1^{c}
1.3±0.1^{a}	1.1±0.2^{a}	0.9±0.1^{a}	1.1±0.1^{a}	0.8±0.3^{a}	0.9±0.1^{a}	1.4±0.3^{a}	1.1±0.1^{a}	1.2±0.2^{a}
1.3±0.1^{c}	1.8±0.1^{a}	1.5±0.1^{b}	1.2±0.1^{c}	1.3±0.1^{b}	1.9±0.1^{a}	1.4±0.1^{b}	1.1±0.1^{c}	0.9±0.1^{c}
5.8±0.2^{f}	**6.1±0.1^{e}**	**5.4±0.1^{g}**	**4.5±0.1^{i}**	**7.4±0.1^{c}**	**8.5±0.1^{a}**	**8±0.1^{b}**	**6±0.1^{e}**	**4.9±0.1^{h}**
2.1±0.1^{b}	1.7±0.3^{c}	1.5±0.1^{c}	2±0.2^{b}	1.3±0.2^{d}	1.9±0.1^{b}	2.1±0.1^{b}	2.6±0.3^{a}	2±0.1^{b}
2.1±0.1^{a}	1.7±0.2^{b}	1.5±0.2^{b}	2±0.1^{a}	1.3±0.3^{c}	1.2±0.2^{c}	1.6±0.1^{b}	2±0.1^{a}	1.5±0.2^{b}
2.3±0.1^{b}	2.6±0.1^{a}	2.1±0.1^{b}	1.2±0.1^{d}	1.4±0.1^{d}	3±0.3^{a}	2.3±0.1^{b}	1.9±0.1^{c}	2.2±0.2^{b}
1.9±0.3^{b}	2±0.1^{a}	1.6±0.2^{c}	1.2±0.4^{d}	2.2±0.1^{a}	1.3±0.4^{d}	1.7±0.2^{c}	2.1±0.1^{a}	1.3±0.2^{d}
0.7±0.1^{e}	1.1±0.2^{c}	1.3±0.1^{b}	1±0.3^{d}	1±0.2^{d}	1.7±0.1^{a}	1.4±0.2^{b}	1.3±0.1^{b}	1.2±0.3^{c}
2.5±0.1^{a}	1.9±0.3^{d}	2.2±0.2^{b}	2.5±0.1^{a}	1.4±0.4^{e}	2±0.2^{c}	1.5±0.4^{e}	2.4±0.1^{a}	1.8±0.3^{d}
4.7±0.4^{h}	**7.3±0.3^{c}**	**7.4±0.3^{c}**	**8±0.4^{b}**	**6±0.2^{f}**	**4.6±0.3^{h}**	**5.3±0.2^{g}**	**7.1±0.3^{d}**	**8.9±0.2^{a}**
2.2±0.1^{a}	1.7±0.2^{c}	2.3±0.1^{a}	2±0.2^{a}	1.9±0.2^{b}	1.8±0.3^{c}	1.6±0.3^{c}	2±0.1^{a}	1.5±0.4^{c}
2.2±0.1^{a}	1.4±0.2^{b}	1.1±0.1^{c}	0.9±0.2^{d}	0.8±0.1^{d}	1.5±0.2^{c}	1.1±0.1^{c}	0.8±0.1^{d}	1.2±0.1^{c}
1.8±0.2^{d}	2.3±0.3^{c}	1.9±0.1^{d}	1.3±0.2^{f}	1.3±0.3^{f}	3±0.2^{a}	1.8±0.2^{d}	2.2±0.2^{c}	1.7±0.1^{e}
1.4±0.3^{b}	1±0.1^{d}	1.2±0.1^{c}	0.8±0.1^{e}	1.8±0.1^{a}	1.8±0.2^{a}	2±0.1^{a}	1.6±0.1^{b}	1.3±0.1^{c}
1.1±0.1^{b}	0.9±0.1^{c}	0.8±0.2^{c}	1.1±0.2^{b}	1.6±0.2^{a}	1.1±0.2^{b}	1.4±0.2^{a}	1.2±0.1^{b}	1±0.3^{c}
2.7±0.2^{a}	2.1±0.2^{b}	1.6±0.1^{d}	2±0.3^{b}	2.3±0.3^{b}	2.6±0.2^{a}	1.5±0.1^{d}	2.1±0.2^{b}	1.4±0.2^{d}
7±0.1^{g}	**8.7±0.3^{d}**	**9.4±0.1^{c}**	**11.1±0.3^{a}**	**7.5±0.3^{f}**	**5.7±0.4^{i}**	**6.8±0.2^{h}**	**8.5±0.3^{e}**	**10.2±0.3^{b}**
10.5±0.5^{i}	**13.7±0.7^{d}**	**14.4±0.4^{c}**	**17.2±0.3^{a}**	**12.7±0.3^{f}**	**10.2±0.4^{j}**	**11.3±0.4^{h}**	**13.4±0.3^{e}**	**15.3±0.2^{b}**
2.3±0.1^{a}	1.8±0.1^{b}	2.1±0.1^{a}	1.7±0.1^{b}	2±0.1^{a}	2.2±0.1^{a}	1.7±0.1^{b}	1.1±0.1^{d}	1.5±0.1^{c}
11±0.1^{c}	**8.8±0.3^{g}**	**8.1±0.3^{h}**	**6.5±0.4^{j}**	**10.3±0.5^{d}**	**11.8±0.2^{a}**	**11.2±0.3^{b}**	**9.1±0.4^{f}**	**7.3±0.4^{i}**
2.1±0.1^{a}	1.7±0.3^{b}	2±0.1^{a}	1.3±0.1^{e}	1.3±0.1^{e}	1.9±0.1^{b}	1.5±0.1^{c}	1.4±0.1^{d}	1.8±0.1^{b}
5.3±0.1^{i}	**6.8±0.3^{d}**	**6.9±0.3^{d}**	**9.1±0.5^{a}**	**5.9±0.1^{f}**	**5.7±0.1^{g}**	**5.5±0.1^{h}**	**7.4±0.3^{c}**	**8.2±0.3^{b}**
1.2±0.1^{b}	1±0.1^{d}	1.7±0.2^{a}	1.1±0.1^{b}	-	1.2±0.1^{b}	1.1±0.1^{c}	1±0.1^{d}	0.9±0.1^{e}
1.7±0.1^{g}	1.2±0.1^{h}	1.7±0.1^{g}	1±0.1^{i}	2.6±0.1^{c}	2.4±0.1^{d}	3±0.1^{a}	2.3±0.1^{e}	2±0.1^{f}
2.3±0.2^{b}	2.1±0.1^{d}	2.6±0.1^{a}	1.9±0.1^{e}	2.6±0.1^{a}	1.7±0.1^{a}	2.2±0.2^{c}	1.9±0.2^{e}	1.3±0.1^{f}
2.3±0.1^{b}	1.6±0.1^{d}	1.9±0.1^{c}	2.1±0.3^{b}	1.6±0.1^{d}	1.3±0.1^{e}	2.5±0.2^{a}	2.2±0.2^{b}	1.8±0.1^{c}
2.3±0.1^{a}	**1.6±0.1^{e}**	**1.9±0.1^{c}**	**2.1±0.1^{b}**	**1.6±0.1^{e}**	**1.4±0.1^{f}**	**1.8±0.1^{d}**	**1.1±0.1^{h}**	**0.9±0.1^{j}**
2.3±0.1^{a}	**1.1±0.1^{f}**	**1.7±0.1^{d}**	**1.2±0.2^{f}**	**2.1±0.1^{b}**	**1.2±0.2^{f}**	**1.5±0.1^{e}**	**1.7±0.1^{d}**	**1.9±0.1^{c}**
1.8±0.1^{a}	1.2±0.2^{d}	1.5±0.2^{b}	1.3±0.1^{d}	1.4±0.1^{c}	1.5±0.2^{b}	1.2±0.2^{d}	1±0.1^{e}	1.5±0.2^{b}
0.9±0.1^{a}	1.3±0.1^{b}	1±0.1^{d}	1.2±0.1^{c}	1.5±0.1^{a}	0.8±0.1^{f}	1.1±0.2^{c}	0.9±0.1^{e}	1.3±0.1^{b}
1.3±0.1^{d}	**1.5±0.1^{c}**	**1.2±0.1^{d}**	**1±0.1^{e}**	**2.2±0.1^{a}**	**1.2±0.1^{d}**	**1.7±0.1^{c}**	**2±0.1^{b}**	**1.5±0.2^{c}**
1±0.2^{c}	0.8±0.1^{d}	1.1±0.1^{c}	1.3±0.1^{a}	1.4±0.2^{b}	0.7±0.1^{d}	1±0.1^{c}	1.3±0.2^{b}	1.8±0.2^{a}
1.4±0.2^{a}	1±0.1^{c}	0.9±0.1^{d}	1.1±0.1^{c}	0.8±0.1^{d}	1.2±0.1^{b}	1.5±0.1^{a}	1.2±0.1^{b}	1±0.1^{c}

1.3±0.1[a]	0.9±0.1[c]	1.2±0.1[a]	0.8±0.1[c]	1.1±0.1[b]	1±0.1[b]	0.8±0.1[c]	-	0.9±0.1[c]
1.2±0.1[b]	1±0.1[c]	1.4±0.1[a]	1.2±0.1[b]	1.2±0.1[b]	1.3±0.2[a]	1.1±0.1[c]	1±0.1[c]	0.8±0.1[d]
1.2±0.1[b]	1±0.1[c]	1.4±0.1[a]	1±0.1[c]	1.4±0.1[a]	1±0.1[c]	1.3±0.1[a]	0.6±0.1[d]	1.4±0.1[a]
78.5	78.8	80.2	80.6	76	76.8	76.7	80.7	81.9
13.4	10.5	10.3	11.4	13.6	10.3	12.2	10.8	11.1
7.7	7.4	9.2	8	8.1	10.2	10.5	8.5	6.3
99.6	96.5	99.7	100	97.7	97.3	99.4	100	99.3
38	37	38	38	37	38	38	37	37

Figures

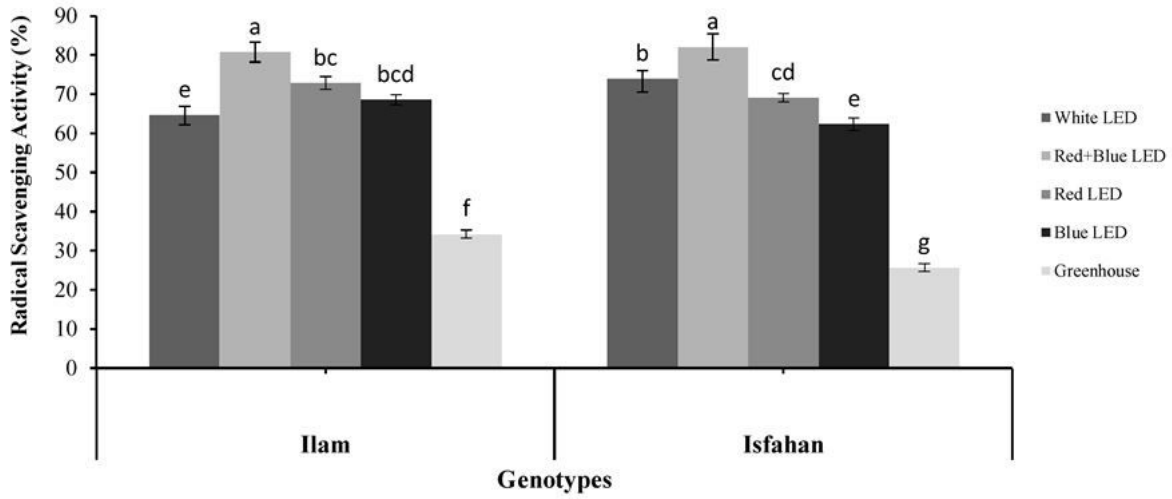

Figure 1

Interaction of genotype*light on free radical scavenging activity in leaves of two genotypes of Melissa officinalis affected by different levels of light (non-identical letters indicate significant difference at 0.01 probability level of LSD test).

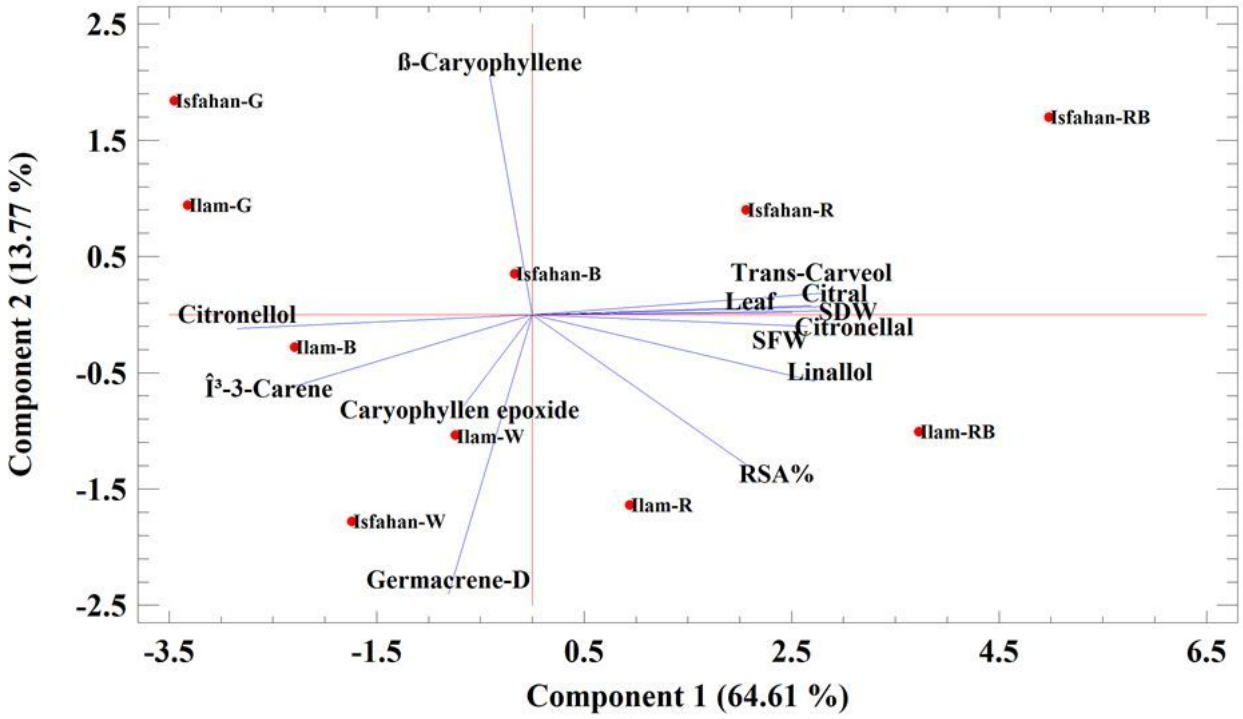

Figure 2

Principal component analysis (PCA) for essential oil components, antioxidant activity and different wavelengths applied in two genotypes of lemon balm. Ilam-W: Genotype Ilam-White LED; Ilam-RB: Genotype Ilam-Red+Blue LED; Ilam-R: Genotype Ilam-Red LED; Ilam-B: Genotype Ilam-Blue LED; Ilam-G: Genotype Ilam-Greenhouse; Isfahan-W: Genotype Isfahan-White LED; Isfahan-RB: Genotype Isfahan-Red+Blue LED; Isfahan-R: Genotype Isfahan-Red LED; Isfahan-B: Genotype Isfahan-Blue LED; Isfahan-G: Genotype Isfahan-Greenhouse; RSA%: Radical Scavenging Activity (%); SFW: Shoot Fresh Weight; SDW: Shoot Dry Weight; Leaf: Leaf Number.