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Short Communication

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Rise of activity of antioxidant enzymes and secondary metabolites under treatment with LED lights in two genotypes of *Melissa officinalis*

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ABSTRACT- The innovation of light-emitting diodes (LEDs) has provided a new opportunity to grow plants in controlled environments. In this study, the plantlets of two genotypes of lemon balm were put for seven weeks in an incubator containing LED lamps with red, blue, red + blue (70:30 ratio), and white light spectrum with a light intensity of 300 µmol $m^{-2}s^{-1}$ and with a light cycle of 16 hours of light and 8 hours darkness and temperature of 25±2°C, and to compare with these conditions; some plantlets were also put in the greenhouse. The plantlets grown in incubators with red + blue LED lamps had higher activity of some antioxidant enzymes including catalase, guaiacol peroxidase, and ascorbate peroxidase, higher amounts of phenolic compounds and the valuable active substance of rosmarinic acid (the latter only in Ilam genotype), and phenylalanine ammonia lyase (PAL) enzyme activity than the plantlets grown in the greenhouse. However, in the Isfahan genotype significant difference in the content of rosmarinic acid was observed only between white LED light with other LED and greenhouse lights. Overall, according to the results of the current study, it can hope to use LED light sources to improve the growth and qualitative and quantitative properties of the medicinal plant, lemon balm, and also to create high-quality plants.

Light-emitting diodes (LEDs) have emerged as promising alternatives for light sources in controlled agricultural environments. In comparison to conventional horticultural lighting, LEDs offer distinct advantages, including prolonged lifetimes, compact dimensions, lower emission temperatures, and reduced energy consumption (Sabzalian et al., 2014, Ahmadi et al., 2020 and 2021).

Morphological and physiological alterations induced by these light sources have been extensively studied across various plant species, including peppers (Schuerger et al., 1997) and wheat (Goins et al., 1997). Given the high light intensity of LEDs, these sources can be deemed as inducing intense light stress. Each type of LED light, whether employed individually or combined with other sources, exerts distinct effects on plants, influencing primary and secondary metabolite (such as phenolic compounds and rosmarinic acid) production (Sabzalian et al., 2014, Iwai et al., 2010, Close & McArthur, 2002). Changes in antioxidant enzymes, gene expression, and metabolism at different wavelengths of LED lights have been reported in rice (Jung et al., 2013).

The aim of this research was to study the effects of four distinct types of LED lighting and greenhouse light treatments on antioxidant enzyme activity, phenolic compound content, and the presence of the active substance rosmarinic acid in two genotypes of the medicinal plant, lemon balm.

To conduct this research, the plant materials, lemon balm plants, were collected from the farms in Eyvan City, Ilam Province, and in Isfahan City, Isfahan Province in March 2016 and transferred to 24 pots that were placed in four growth cabinets (i.e., three pots of each genotype per cabinet). The lighting system of each cabinet included red (660 nm), blue (460 nm), white (760-380 nm), or red + blue (70:30 ratio) LED lamps with a light intensity of 300 µmol m-2s-1, with 16 hours of illumination and eight-hour dark cycle and at a temperature of 25±2°C. In the same way and at the same time, 6 pots were placed in the research greenhouse of Isfahan University of Technology in the same conditions under natural sunlight. All the pots were watered daily with water and once a week with 1/2 Hoagland solution (containing Macro nutrients: KNO_3 101.10 $g.l^{-1}$, $Ca(NO_3)_2$-$4H_2O$ 236.16 $g.l^{-1}$, $NH_4H_2PO_4$ 115.08 $g.l^{-1}$, $MgSO_4$-$7H_2O$ 246.49 $g.l^{-1}$, and Micro nutrients: KCI 1 .864 $g.l^{-1}$, H_3BO_3 0.773 $g.l^{-1}$, $MnSO_4$-

H_2O 0.169 $g.l^{-1}$, $ZnSO_4$-$7H_2O$ 0.288 $g.l^{-1}$, $CuSO_4$-$5H_2O$ 0.062 $g.l^{-1}$, H_2MoO_4(85% Mo03) 0.040 $g.l^{-1}$, NaFeDTPA(10% Fe) 30.0 $g.l^{-1}$). Seven weeks later, the plantlets were transferred to the laboratory for analysis.

To measure the activity assay of each enzyme, one hundred-mg leaf sample was crushed in 1.5 ml of 50 mM phosphate buffer (pH 7.8) containing 0.1 mM EDTA. Then the activity of superoxide dismutase (SOD) (EC 1.15.1.1.), catalase (CAT) (EC 1.11.1.6), guaiacol peroxidase (GPX) (EC 1.11.1.7), ascorbate peroxidase (APX) (EC 1.11.1.11), glutathione reductase (GR) (EC 1.6.4.2) and glutathione-S-transferase (GST) (EC 2.5.1.18) was assayed by the method described by Beyer Jr and Fridovich, 1987, Aebi, 1984, Lin and Kao, 1999, Nakano and Asada, 1987, Foyer and Halliwell, 1976 and Carmagnol et al. 1981, respectively. To assay the total phenolic compounds the extract was centrifuged at 12000 *g* for 15 minutes. The optical absorbance of each reaction mixture containing 30 ml of extract of the tested plantlet, 120 ml of Na_2Co_3, and 150 ml of Folin Ciocalto reagent was read at a wavelength of 765 nm after being placed in the dark for 30 min (Singleton and Rossi, 1965).

The amount of rosmarinic acid in the samples was measured by the method of Öztürk et al. (2010). The standard solution of rosmarinic acid (Sigma) with a concentration of 1 mM was prepared by dissolving a certain amount of rosmarinic acid powder in ethanol. Then, standards with concentrations of 0, 0.01, 0.02, 0.03, and 0.04 M were prepared from this solution. To determine the amount of rosmarinic acid, 200 microliters of zirconium reagent (ZrOCl2.8H2O) was added to the ethanolic solutions of samples and standards, and after 5 minutes the absorbance of the samples was read at 362 nm.

Phenylalanine ammonia lyase **(**PAL) enzyme activity was measured according to the method of Morrison et al. (1994). For this, extraction was done first. Then after centrifuging of extract, 500 microliters of sample extract was added to 2.5 milliliters of phenylalanine (12 mM) dissolved in Tris-HCl buffer (0.1 M) with a pH equal to 8.5. Absorbance was read once immediately after the samples and controls were prepared and for the second time after the samples and controls were incubated for 60 minutes at 30°C.

Since the cultivation of plants was in the greenhouse and four incubators were in the laboratory, the experiment was considered in separate environments. Therefore, for the measured factors, the significance of variation was evaluated with the help of combined analysis of variance using the SAS statistical program (version 8; SAS Institute Inc., Cray, NC, USA) and MSTATC. The comparison of means was determined with the help of the least significant difference (LSD) test (P<0.05).

Catalase, guaiacol peroxidase, and Ascorbate peroxidase enzyme activities exhibited higher levels in both genotypes under the red + blue LED light treatment compared to the greenhouse light conditions (Fig. 1a, b, and d). Regarding the impact of genotype and light interactions, the analysis indicated that the superoxide dismutase enzyme activity remained unaffected, and all LED lights showed lower levels of activity of this enzyme in both genotypes compared to the greenhouse light conditions (Fig. 1c). Considering the glutathione reductase enzyme activity, red and red + blue LEDs in Ilam and Isfahan genotypes, respectively exhibited highest activity (Fig. 1e). Analyzing the glutathione-S-transferase (GST) enzyme activity, in both genotypes demonstrated the highest activity under blue LED light (Fig. 1f).

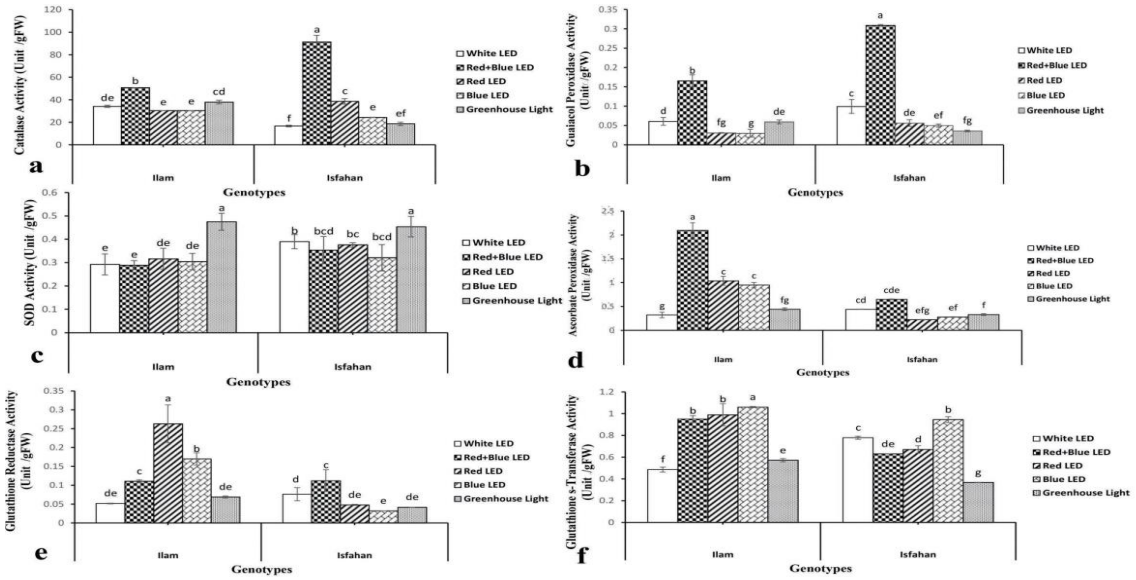

Fig. 1. Activity of catalase (a), guaiacol peroxidase (b), superoxide dismutase(c), ascorbate peroxidase (d), glutathione reductase (e) and glutathione-S-transferase (f) enzymes in two genotypes of lemon balm under the influence of different types of LED lighting and greenhouse light (non-identical letters indicate a significant difference in means at the 0.05 probability level of the LSD test)

Red + blue LED light treatment compared to greenhouse light produced the highest content of phenolic compounds and PAL activity among all light treatments in both genotypes (Table 1). However, no significant

difference was observed in phenolic compounds between red + blue, white, and blue LED light treatments in Ilam genotype (Table 1). In the Ilam genotype, different LED lights had a different effect on the content of rosmarinic acid. The highest content of this substance was observed under red + blue and red LED lights. However, the situation was different in the Isfahan genotype, so different LED light treatments except white LED had no significant effect on the content of rosmarinic acid in treated plantlets (Table 1).

It has been shown in this study that the light-emitting diode lights, chiefly mixed red (and blue (70:30 ratio) LEDs, had the most positive effect on increasing the activity of the antioxidant enzymes such as GPX, CAT, and APX in Ilam and Isfahan genotypes. Blue LED lamp increased GST enzyme activity in two tested lemon balm genotypes. However, it has already been reported that the lowest activity of APX and SOD enzymes was observed in peppermint (Mentha piperita L.) plants grown in a combination of red and blue light (Heydarizadeh et al., 2013).

Another study (Shohael et al., 2006) showed that APX activity was higher in Eleutherococcus senticosus plant embryos under fluorescent light, and blue LED in comparison with red and blue plus far-red light treatment. GR activity increased in red, blue, and blue plus far-red light treatments. SOD and CAT activities were more than others in the red LED light treatment. High SOD activity reflects less production of superoxide radicals or a high ability of SOD to scavenge this radical. In the aforementioned study, the activity of APX enzyme was inhibited in embryos treated with red LED. APX is a member of the ascorbate-glutathione cycle, which plays an essential role in removing hydrogen peroxide from plant cells (Shohael et al., 2006).

Similar to the results obtained by the present study, Heydarizadeh et al. (2013) showed that there is more variation between the studied genotypes of the peppermint (Mentha piperita) plant in terms of the activity of the two enzymes catalase and glutathione reductase in comparison with other enzymes in response to the light environment. The current study also showed that the effects of LED lights on the tested antioxidant enzymes were different in two genotypes of the lemon balm plant, which shows the effect and role of plant genotypes on this issue.

In the lemon balm plantlets studied in the present study, it was seen that the combination of red and blue LED light raised the content of phenolic compounds, rosmarinic acid and PAL enzyme activity. It has already been reported that the quality of light in terms of color and wavelength can affect the morphological structure and the amount of plant metabolites (Li et al., 2013; Marchant et al., 2022; Klimek-Szczykutowicz et al., 2022). It has been also shown that blue and red lights complementarily initiate mechanisms to motivate the plant defense responses and raise the biosynthesis of phenolic compounds (Taulavuori et al., 2016).

It has already been reported that the high-intensity effect of LEDs causes the aggregation of secondary metabolites by stimulating photoreceptors or by stimulating or inhibiting some regulatory genes (Pennisi et al., 2019; Bian et al., 2018). The two central receptors in plants that act together to regulate photomorphogenic responses are cryptochromes as blue light receptors and phytochromes as red light receptors (Son and Oh, 2015). It has been shown that adding blue light to red light or raising the intensity of blue light increased red leaf lettuce's total phenolic amounts (Son and Oh, 2015). It is possible that blue light activates the expression of the chalcone synthase and phenylalanine ammonialyase genes and important elements in the production of secondary metabolites (Son and Oh, 2015). Iwai et al., (2010) showed that artificial light irradiation increased the content of caffeic acid, rosmarinic acid, and luteolin-7-O-glucoside. It was assumed that this significant difference in the accumulation of these three compounds might be due to PPFD (Photosynthetic Photon Flux Density). The average PPFD in a greenhouse was 289 µmol m-2s-1, while it was 360 µmol m-2s-1 in artificial light sources. Since the biosynthesis of polyphenols is related to blue light receptors, i.e., phototropins and cryptochromes, it has been shown that the rise of the polyphenols is closely related to the induction of cryptochrome by light (Kang et al., 2008). In this regard, in the present study, the positive effect of LED light radiation, especially red + blue LED light, on increasing the phenolic compound content was observed in the lemon balm plantlets of both genotypes (Table 1).

Table 1. Interaction of genotype and light on phenolic content, rosmarinic acid, and PAL enzyme activity in two lemon balm genotypes under different levels of light (different letters indicate a significant difference at the probability level of 0.05).

	Phenolic content (mg/gFW)		Rosmarinic acid (mmol/gFW)		PAL activity (mmol cinnamic acid/gFW)	
genotypes / light	Ilam	Isfahan	Ilam	Isfahan	Ilam	Isfahan
White LED	28.43±1.14^{abc}	27.08±1.59^{bc}	1.22±0.2^{c}	0.72±0.08^{e}	25±1^{b}	19.6±1^{d}
Red+Blue LED	31.92±0.00^{a}	31.31±0.27^{a}	1.95±0.00^{a}	1.07^{cd}	28.26±1.1^{a}	22.4^{c}
Red LED	26.6±2.9^{bc}	17.22±3.45^{d}	1.87±0.06^{a}	0.97±0.007^{d}	14.67±0.8^{e}	10.32±1^{f}
Blue LED	27.6±2.8^{abc}	20.94±0.23^{d}	1.04±0.1^{cd}	0.97±0050^{d}	11.73±1^{f}	11±0.00^{d}
Greenhouse	25.96±1.8^{c}	19.89±0.00^{d}	1.64±0.14^{b}	0.92±0.00^{d}	8.00±1^{g}	14.00±1^{e}

Again in the present study, the highest level of phenylalanine ammonia lyase (PAL) enzyme activity was observed in both plant genotypes in the red + blue LED light treatment. It has already been reported that the levels of this enzyme are affected by age, light, phytochrome, wounding, infection, and growth modifiers (Camm and Towers, 1973). In the pointed study, the possibility that PAL is involved in the

control of phenolic metabolism has been critically examined. The involvement of phytochrome in affecting PAL activity has also been shown in the terminal buds of peas (*Pisum sativum*, Attridge & Smith, 1967). It has been illustrated that short-term exposure to red light followed by the return of darkness increased PAL activity. This activity depends on the logarithm of the red light irradiance (Attridge & Smith, 1967). On the other hand, exposure to far-red light in peas and mustard seedlings has been shown to inhibit response to red light (Attridge & Smith, 1967, Durst & Mohr, 1966). In addition to showing the sensitivity of red light to far-red light, many systems have been shown to be sensitive to blue light (Camm and Towers, 1973). For example, it has been reported that PAL is formed in *Helianthus tuberosus* plants when they are incubated in white, red (660-680 nm), and blue (427 nm) lights (Durst & Duranton, 1970). It has been also reported that the Piédallu 17 variety of this plant responds only to blue light and at least 2 photoreceptors are implicated in this process (Camm and Towers, 1973). Schopfer and Mohr (1972) reported that the effect of red and far-red light on the synthesis of PAL can be ascribed to the action of phytochrome Pfr. This was shown true not only for short-term irradiation but also for the action of continuous far-red light (Schopfer and Mohr, 1972). Therefore, the role of phytochromes in increasing the activity of PAL is one of the mechanisms that show the role of light in increasing the activity of this enzyme.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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مقاله علمی- پژوهشی

افزایش فعالیت آنزیم های آنتی اکسیدانی و متابولیت هـای ثانویـه تحت تیمار با لامپ‌های LED در دو ژنوتیپ *Melissa officinalis L.*

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کیفیت نور
نور LED

چکیده - نوآوری دیودهای ساطع‌کننده نور (LED) فرصت جدیدی را برای رشد گیاهان در محیط های کنترل شده فراهم کرده است. در مطالعه حاضر، گیاهچه-های دو ژنوتیپ بادرنجبویه به مدت ۷ هفته در انکوباتور حاوی لامپ های LED قرمز، آبی، قرمز + آبی (نسبت ۷۰:۳۰) و سفید با شدت نور ۳۰۰ میکرومول بر مترمربع بر ثانیه و با چرخه نور ۱۶ ساعت روشنایی و ۸ ساعت تاریکی و دمای ۲±۲۵ درجه سانتیگراد قرار داده شد و برای مقایسه با این شرایط، تعدادی گیاهچه از هر دو ژنوتیپ در گلخانه قرار داده شد. گیاهچه های قرار داده شده در انکوباتور با لامپ های LED قرمز + آبی دارای فعالیت بالاتر آنزیم‌های آنتی‌اکسیدان (کاتالاز، گایاکول پراکسیداز و آسکوربات پراکسیداز)، فعالیت بیشتر آنزیم فنیل آلانین آمونیالیاز (PAL)، مقادیر بیشتر ترکیبات فنلی، و ماده موثره ارزشمند رزمارینیک اسید (در ژنوتیپ ایلام) نسبت به گیاهچه های کاشته شده در گلخانه بودند. هرچند در ژنوتیپ اصفهان تفاوت معنی‌دار در میزان رزمارینیک اسید فقط بین نور سفید LED با سایر نورهای LED و گلخانه‌ای مشاهده شد. در مجموع، با توجه به نتایج مطالعه حاضر می توان به استفاده از منابع نور LED برای بهبود رشد و خواص کمی و کیفی گیاه دارویی بادرنجبویه (Melissa officinalis) و همچنین ایجاد گیاهان باکیفیت بالای این گونه امیدوار بود.