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The Physiology, Growth and Essential Oil Content of Lemon Balm (*Melissa officinalis* L.) in Response to Different LED Treatments

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ABSTRACT

Background: Light-emitting diodes (LEDs) are a promising technology that has enormous potential to improve irradiance efficiency and to replace traditionally used horticultural lighting. Compared with traditional light sources (*e.g.,* high-pressure sodium lamps and metal halide lamps) used in crop production, LEDs tends to have long life, generate less heat waste and providing the perfect spectrum for plant growth.

Methods: This work sought to investigate the effect of using blue LED lights on growth and development in Lemon balm (*Melissa officinalis* L.) under sole-source LED lighting spectra. Lighting spectra were provided by differing combinations of LEDs of 4 different peak wavelengths, (Blue 435, Blue 450, Red 663 nm and Green 540 nm) with ratios of (1.95:2.3:1 R: G: B 450 nm (white), 5: 1R: B 450 nm, 2.5:1 R: B 450 nm and 2.5:1 R: B435 nm) at a PPFD of 95 µmol m^{-2} s^{-1} (PAR).

Result: showed that the blue light with a wavelength peak at 435 nm in combination with red light at 663 nm at the ratio of (2.5:1 R: B) and white LEDs enhanced the physiology of lemon balm (*i.e.*, significantly increased assimilation rate, stomatal conductance and transpiration rate), consequently fresh and dry shoot weight increased and the shoot essential oil content.

Key words: Essential oil, Growth, LEDs, Lemon balm, Physiology.

INTRODUCTION

Lemon balm (*Melissa officinalis* L.), a member of the family Lamiaceae, is a medicinal aromatic plant. The aerial parts of lemon balm contain (0.02 - 0.8%) essential oils with the main components (citral and citronellal) being in one of the classes of compounds that are useful for food and nutrition due to the antioxidant activities of the plant (Mazzanti *et al.*, 2008). In general, the plant has economic importance in various countries due to its proven therapeutic and pharmaceutical properties (Weitzel and Petersen, 2010). In recent years, due to the new commercial interest in natural products, the increased demand for the production of this species has required the development of more intensive cultivation techniques that contribute to faster growth, higher economic production and improved and consistent quality. This has ignited interest in growing lemon balm under controlled conditions in plant factories which have complete or semi-complete control of growth conditions that facilitate the systematic production of crops of high value (Fujiwara *et al.*, 2011, Singh *et al.*, 2015). In plant factories artificial lighting is provided and the introduction of Light Emitting Diode (LED) lighting sources has considerable potential in reducing electrical energy consumption and making crop production more economically successful (Davis and Burns, 2016). Furthermore, LEDs with their narrow waveband spectra have the capacity to meet the wavelength and intensity dose requirements of different plant species (Davis and Burns, 2016). In the first application of LED lighting systems for plants, the combination of red and blue light were investigated as the most effective lighting source for producing many plant species, following the Macree experiments (Goto, 2012) and because chlorophyll a and b efficiently absorb wavelengths in the red (maximum absorption at 663 and 642 nm) and blue (maximum absorption at 430 and 453 nm) (Hopkins and Huner, 2004). However, there is less consensus about the optimal red: blue combination partly due to differences in stomatal responses to specific wavelengths between species (Ouzounis *et al.*, 2014). Many previous experiments have applied an increased fraction of red 660 nm to blue 450 or 459 nm and compared these with white LEDs which comprised three LEDs (red, green and blue).

Some studies showing the effect of using LED lighting on the Lamiaceae family have reported that a combination of 91% red and 9% blue increased the fresh and dry mass of Basil (*Ocimum basilicum* L.). Others reported the highest amount of total phenolic and anthocyanin content in green and purple Basil varieties were observed in leaves of plants

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grown under 70:30% R:B LEDs compared to 50:50% R:B (Hosseini *et al.*, 2018). Most recently, Lin *et al.* (2021) showed that Basil plants exposed to red+ green+ blue with a ratio of 4:1:1 at light intensity of 180 μmol/ m^{-2} s^{-1} had higher assimilation rate compared to 2:1:1 or 1:1:1. Moreover, Aldarkazali *et al.*, (2019) concluded that a combination of R/B compared to white LED and High Pressure Sodium (HPS) lights significantly increased stomatal conductance leading to increased assimilation rate and consequently increased leaf area, fresh and dry weight and essential oil yield. In addition to Basil, the 70:30% R:B LEDs increased assimilation rate, fresh weight and essential oil content in three Mint species (*Mentha piperita, Mentha spicata* and *Mentha longifolia*) compared to 100:100 R:B and 100% white (Sabzalian *et al.*, 2014). Work by Rihan *et al.* (2020) on sweet Basil led to the design a new light spectrum array to facilitate the spectrum absorbed by Basil pigments. This included the use of new wavelengths of blue LED at 435 nm. Moreover, the ratio between blue and red was rebalanced to match the absorbance spectrum of Basil leaf pigments. The use of a light spectrum that matches the plant absorbance significantly improved the physiological parameters and increased the growth and yield of Basil. This study was the first to confirm the positive impact of using 435 nm blue LEDs in comparison with the commercially widely used 450 nm blue LEDs.

To our knowledge very little is available in the published literature about the effect of the combination of R:B or B:R LEDs and white LEDs on the physiology, growth and essential oil in Lemon balm and the current study investigated aspects of this topic. Lemon balm was cultivated under controlled environmental conditions (temperature, humidity and light) in a plant factory system aimed at investigating whether the supply of a particular light regime that matches plant absorbance would improve the physiology, growth and quality of the crop.

MATERIALS AND METHODS

Lemon balm seeds were obtained from CN seeds (CN Seeds, Pymoor, Ely, Cambridgeshire, UK), sown in a rock wool plug in a tray and germinated in the greenhouse at Skardon Gardens, University of Plymouth. When seedlings had their first pair of true leaves, they were then transferred to the plant factory facility. The plant factory facility at the University of Plymouth is a converted insulated greenhouse where external light has been excluded and a multi tier hydroponic growing system consisting of plastic gullies for NFT (nutrient film technique) and is installed with interchangeable LED light units. The plant factory system hydroponic system units had a head space of 50 cm and were 80 cm wide. Each unit contained 63 plants.

The temperature and humidity were monitored using Gemini data loggers [Tinytag Plus (part No GP 1590)] and an instantaneous thermometer (Fisher Scientific) at 23±2°C. The dark/light period was set to 8/16 h. Four lighting treatments were designed and applied at an intensity of 95±2 μmol $m^{-2}s^{-1}$ using Sky LED lighting systems (LED Hydroponic LTD, UK) Light treatments were as follows:

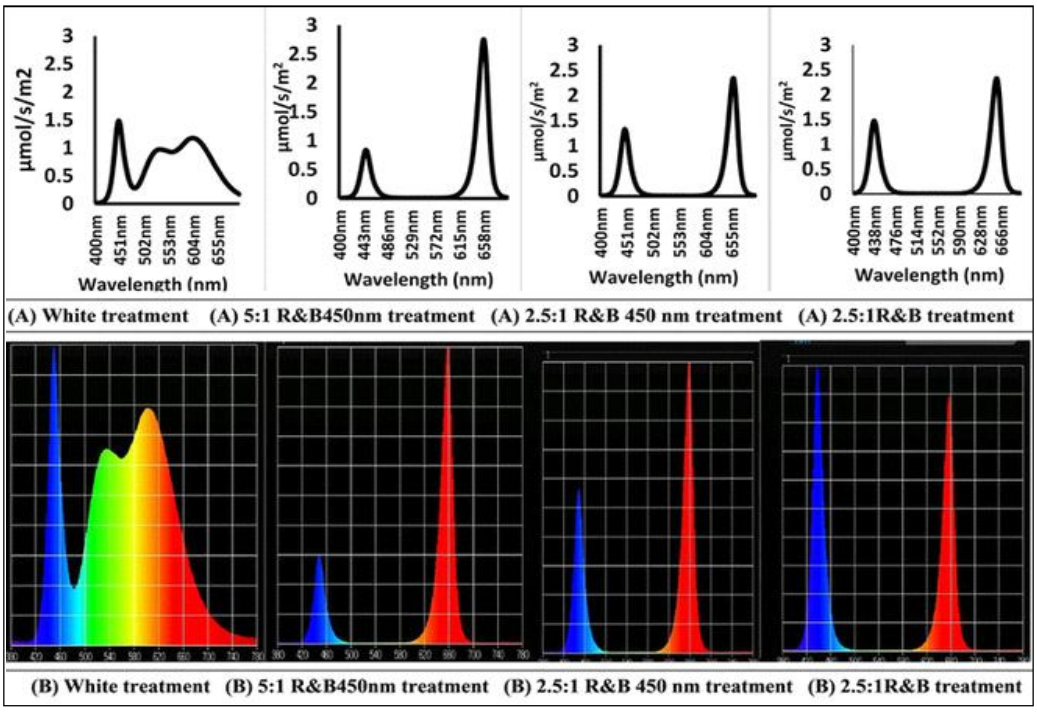


Fig 1: Spectra of the lighting treatments used as measured by an UPRtek spectrophotometer (A) the radiant density of the light spectrum intensity and (B) the relative light intensity.

White

A white LED light treatment with a combination of red (663 nm), green (540 nm) and blue (450 nm) with a ratio of (1.95:2.3:1 R: G: B).

RED and 450 Blue (5 to 1)

An LED light treatment with a combination of red (660 nm) and blue (450 nm) with a ratio of (5:1 R: B).

RED and 450 Blue (2.5 to 1)

An LED light treatment with a combination of red (660 nm) and blue (450 nm) with a ratio of (2.5:1 R: B).

RED and 435 Blue (2.5 to 1)

An LED light treatment with a combination of red (660 nm) and blue (435 nm) with a ratio of (2.5:1 R: B).

Light intensity from the LED lighting treatments was measured using a Skye PAR sensor (Fig 1A and B). (Photosynthetically Active Radiation) (Skye Instruments Ltd., Powys, UK).

Plants were grown for 2 months under the various light treatments and then physiological parameters (assimilation rate µmol m^{-2} s^{-1}, stomatal conductance mmol m^{-2} s^{-1} and transpiration rate mmol m^{-2} s^{-1}) were measured on three expanded leaves from five randomly chosen plants from each treatment using a portable LCi IRGA (LCi- highly portable Ambient Photosynthesis System) (ADC Bio Scientific, Herts,UK). Plant growth responses were also measured from another five plants randomly chosen from each treatment [plant height (cm), number of leaves, number of stem branches, fresh and dry weight (g)] and the leaf area of the four mostly recently fully expanded leaves was measured using the open source software, Image J (FIJI download, https://fiji.sc/), using code written specifically for this particular phenotyping setup (Ciaran Griffin, University of Plymouth 2020). This script scans and processes all new image files continuously using the steps: 1- calibrate the image dimensions; 2- segmentation of the image to separate the different leaves/plants; 3- extract the required data from the segmented images (Ealson and Bloom, 2014 and Kakorian *et al*., 2010). The plants were harvested and dried in an oven at 50°C for 72 hours and the essential oil determined using Soxhlet distillation. Finely ground whole plant samples (15 g) were transferred to a thimble then placed in a thimble chamber of the Soxhlet, Methanol (400 mL) was added and heated in the bottom flask and vaporized into the sample thimble. The distillate was returned into the bottom flask again and the distillation process was continued for a further 7 h. After that, any remaining liquid was evaporated off using a heated evaporator. The yield of oil extract (weight) was expressed as a percentage of the weight of plant sample (Testaya and Tefera, 2017).

$$\text{Yield of oil extraction} = \frac{\text{Weight of oil extracted}}{\text{Weight of sample used}} \times 100$$

Statistical analysis

All the data were subjected to analysis of variance (ANOVA) using Minitab software (version 19) and comparison of means were made using the least significant deference test (LSD) at 5% level of probability.

RESULTS AND DISCUSSIUON

Physiological response

There was a highly significant effect (P≤0.000) of the LED treatments on assimilation rate. In comparison to both combinations of (5:1R: B450 nm and 2.5: 1 R: B 450 nm), the (white and 2.5:1 R/B 435 nm) LED treatments gave the highest assimilation rate (Fig 2A). The white LED treatment increased assimilation rate by 33% and the 2.5:1 R:B 435 nm treatment increased assimilation rate by 41% compared to both of the of R:B 450 nm treatments used.

As with assimilation rate, stomatal conductance and transpiration rate highly significant differed (P≤0.000) between LED treatments (Fig 2B and C). White + (2.5:1 R/B 435 nm) LED treatments promoted the highest stomatal conductance and transpiration rate, compared to the different ratio of R: B 450 nm used.

Morphology response

Different LED combinations led to a significant difference in plant morphology (Table 1). Plants were tallest under 5:1 R/B 450 nm and followed by white and 2.5:1 R/B 450 nm and the smallest plants were under 2.5:1 R/B435 nm. The highest values for leaf and branch number were obtained from the 5:1 R/B 450 nm LEDs followed by the 2.5:1 R/B 435 nm treatment (Table 1). The greatest shoot fresh (P≤0.000) and dry weight (P= 0.003) and leaf area (P= 0.004) were obtained by plants under both white and the 2.5: 1R/B 435 nm (Fig 3) and the smallest shoot fresh and dry weight and leaf area were produced by plants under 2.5:1 R:B 450 nm which was no significantly differ from 5:1 R:B 450 nm. Both white and 2.5:1 R: B 435 nm LED increased the shoot fresh weight approximately by 33% compared to 2.5:1 R:B 450 nm. While, white LED was increased the plant shoot dry weight by 87% as compared to (5:1R/B 450 nm) and 2.5:1 R/B 450 nm), respectively, although 2.5:1 R:B 435 nm LED increased the plant shoot dry weight approximately by 21 and 79% as compared to 5:1 R/B 450 nm and 2.5:1 R/B 450 nm) respectively. As shown in (Fig 4) White LED increased plant leaf area by 32 and 45% as compared to both different ratio of R: B 450 nm and the biggest leaf area was produced by plants under 2.5: 1 R:B 435 nm which increased leaf area by 29 and 41% as compared to both different ratio of R:B 450 nm.

Essential oil content

There was a significant (P = 0.003) effect of LED treatment on plant essential oil content (Fig 5), the highest amounts of plant shoot essential oil content were obtained from plants under white, 2.5: 1 R:B 450 nm and 2.5 R:B 435 nm LEDs, increasing the essential oil content approximately by 20% compared to 5:1 R: B 450.

This study clearly confirmed that different LED array combinations of red, with a wavelength peak at 663 nm and blue light (with peaks of 450 and 435 nm) can differentially

affect the growth, physiology and essential oil content in lemon balm. All LED treatments were capable of growing acceptable crops of lemon balm, but despite identical PPFD (light energy supplied to the crops) growth and yield were influenced by specific combinations of LED wavelengths. In general, a combination of red and blue has been proved to be effective in driving assimilation rate (Hernández and Kubota, 2014, Hogewoning *et al.*, 2010, Wang *et al.*, 2016) with a higher proportion of red to blue light often promoting plant assimilation rate and growth (Wang *et al.*, 2018). However, there are discrepancies for different plants in response to the blue wavelength accompanying the red. Using a higher proportion of blue to red light (60% blue to 40% red compared with 20% blue to 80% red) had a positive impact on stomatal functioning in sweet basil (Jensen *et al.*, 2018). A blue deficient light treatment resulted in acclimation of light energy portioning in PSII and CO_2 assimilation rate in other plant species such as spinach (Matsuda *et al.*, 2008).

In the current study, the physiological measurements in lemon balm indicated that net assimilation rate and stomatal conductance were more productive under the white and 2.5:1 R: B 435 nm, markedly increasing all physiological parameters measured compared to other LEDs. This is supported by a recent study on another of the Lamiaceae (sweet basil) where the combination of blue (435 nm) to red (663 nm) light promoted the highest assimilation rate and stomatal conductance subsequently leading to 20% enhanced growth of sweet basil compared to blue light at 450 nm (Rihan *et al.*, 2020). The favourable response to white light was interesting and in other studies significant increases in assimilation rate under white LED has been related to the inclusion of green wavebands. It has found that green LED might enhance photosynthesis more efficiently than red light when supplied in a white background LEDs (Terashima *et al.*, 2009).

Alongside physiological parameters, the lemon balm shoot fresh and dry weights and leaf area were comparatively greater in plants grown under the combination of 2.5: 1 R/B 435 (Fig 3A, B and 4). This indicates that the increased assimilation rate and leaf area under white and the 2.5:1 R/B 435 nm resulted in increased fresh and dry biomass compared to other LEDs. However, lemon balm leaf and branch number showed no significant differences among all LED treatments (Table 1) indicating that plant development was not affected by the LED spectra. In a similar study LEDs treatments significantly increased the shoot fresh and dry weight and also increased the Leaf number in two genotypes (Ilam and Isfahan) compared to greenhouse condition and compared to other LEDs treatments (white 380 to 760 nm, red and blue) the combination of (70:30 R 650 nm:B 460 nm) with a light intensity of 300 µmol. m^{-2} with a 16 h illumination and 8 h darkness and 25±2°C produced the tallest lemon balm in

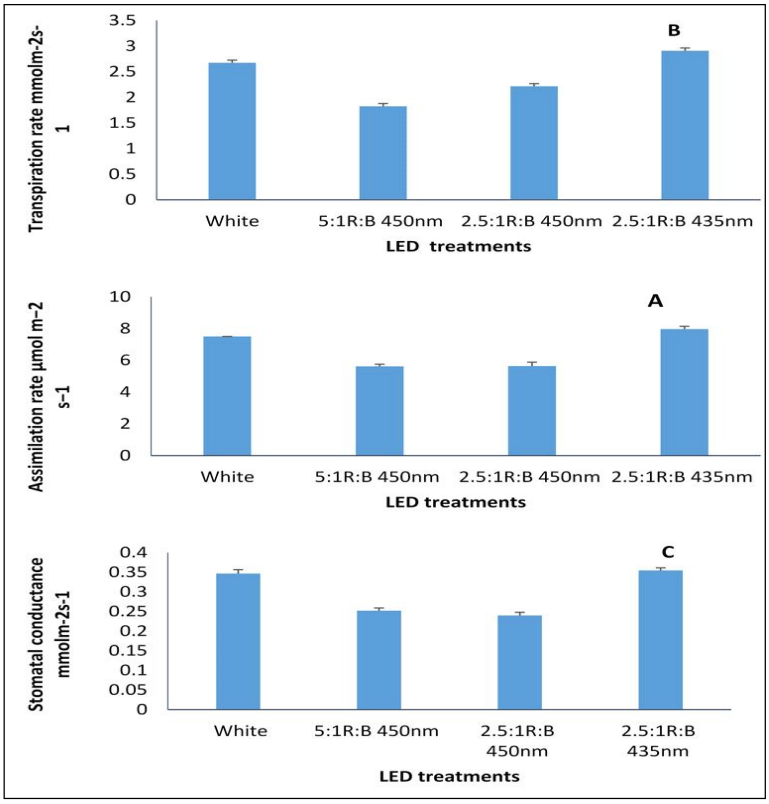


Fig 2: Lemon balm physiological response to LED treatments, A. Assimilation rate (L.S.D=1.36). B, Stomatal conductance (L.S.D=0.019), C. transpiration rate (0.13) at different combination of LSD treatments.

both genotypes shoot fresh, shoot dry weight and leaf number (Tayebeh *et al.*, 2021). In their work the white LED was not significant as the combination of red and blue on the both of lemon balm genotypes that might be due to the inclusion the different light wavelengths such as far-red in the white LED with a higher light intensity which were different from the white LED used in current experiment. These results agree with those reported by Naznin *et al.*, (2019) who found that the combination of red 661 nm to blue 449 nm with a ratio of 91% R: 9% blue at the light intensity of 200±5 µmol $m^{-2}s^{-1}$ had a positive influence on basil (*Ocimum basilicum*) plant height, leaf number, fresh weight and dry weight compared to 83% R : 17% B, 95% R: 5% B and 100% R (Naznin *et al.*, 2019). In addition, The elongation of the main and lateral shoots in maxican mint were significantly increased under red 660 nm LEDs at a light intensity of 100 µmol m^{-2} s^{-1} compared to monochromatic blue 470 nm and green 525 nm and the a combined white, blue and green. Whilst the mint fresh weight was significantly increased under blue compared to other

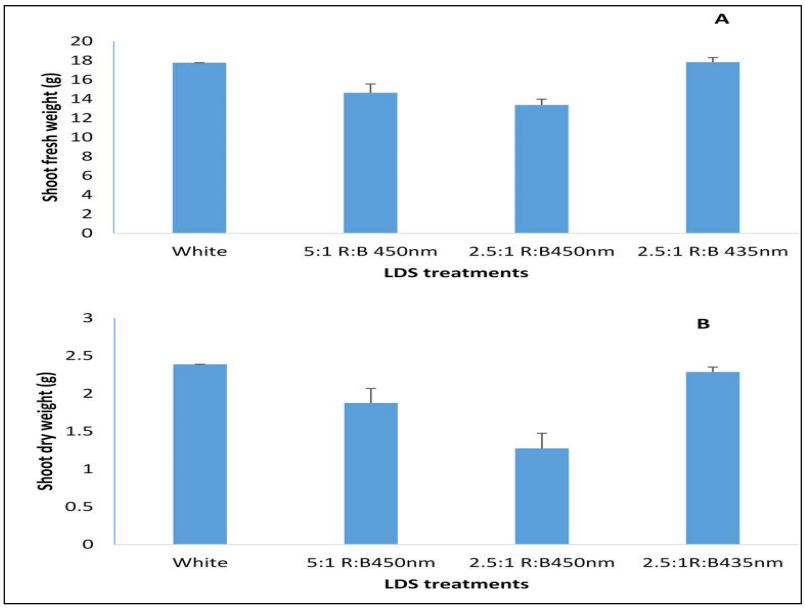


Fig 3: Lemon balm A. shoot fresh weight (L.S.D=1.240) B. shoot dry weight (L.S.D=0.47) under different combination of LED treatments.

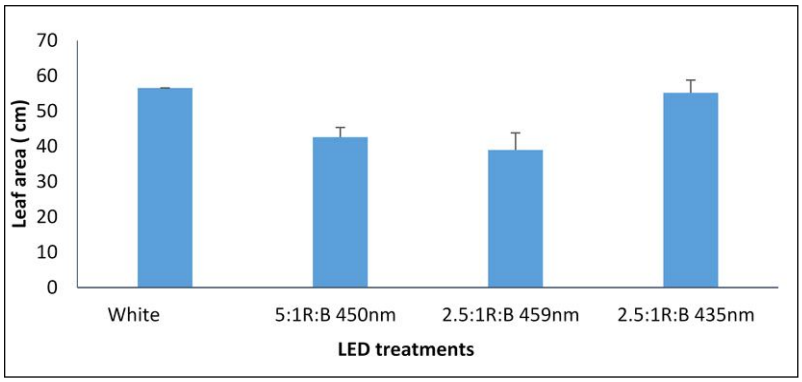


Fig 4: Lemon balm leaf area under different combination of LED treatments (L.S.D= 8.5).

Table 1: Means of lemon balm plant height, leaf number and branch number under different LED treatments.

Treatments	Plant height cm	SE	Leaf No./plant	SE	Branch No./plant	SE
White	35 b	0.707106781	72.4	11.96912695	6	0.707106781
5:1 R: B 450 nm	42 a	2.774887385	82.8	10.0518655	7.6	12.30934604
2.5:1 R:B 450 nm	33.2 b	0.969535971	54.8	8.479386771	5.6	0.678232998
2.5:1 R: B 435 nm	23.4 c	1.913112647	73.2	12.69409311	8.8	1.392838828
L.S.D at 5%	4.42		Not significant		Not significant	

• Means labelled with different letters in column are significantly different at 5% probability.

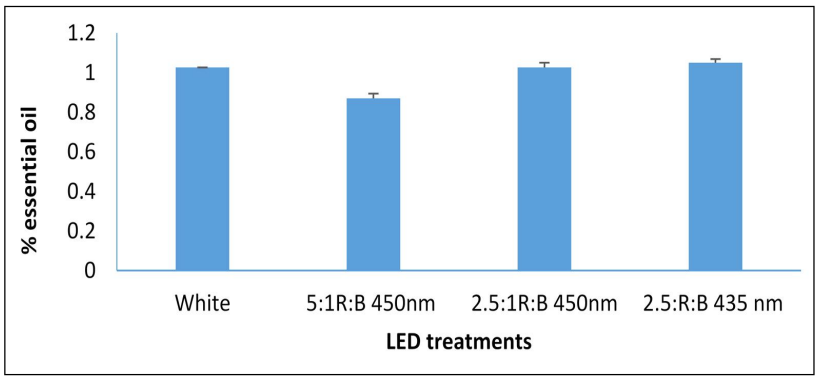


Fig 5: Lemon balm essential oil content under different combination of LED treatments (L.S.D=0.068).

LEDs used (Noguchi and Amaki, 2016). It has been well documented that R LED resulted in plant elongation and biomass reduction (Lin *et al.*, 2013) and B LED is important for expansion and biomass production (Johkan *et al.*, 2012).

In the current study, as shown in (Fig 5) the lemon balm shoot essential; oil content had significantly increased under white and the combination of 2.6:1 R: B 450 nm and 2.5:1 R: B 435 nm treatments increasing the essential oil content by about 20% compared to 5:1 R: B 450 nm treatment. This result indicates that for the oil composition blue light in the range from 435 to 450 nm is as important as red light. This impact on the level of essential oil in other Lamiaceae family species has also been reported elsewhere (Ichimura *et al.*, 2009). Their work with sweet basil revealed that the essential oil content of basil grown under blue light was between 1.4 to 4.4 fold higher than those grown without blue light.

The essential oil in maxican mint (*Plectranthus amboinicus* (Lour) was also higher under blue light compared to monochromatic green and red LEDs at the light intensity of 100 µmol m^{-2} s^{-1} (Noguchi and Amaki, 2016). Moreover, the addition of 30% of blue light to 70% red light increased the essential oil of three cultivated of mint species *Mentha piperita, Mentha spicata longifolia,* compared to 100:100 white (Sabzalian *et al.*, 2014). However, compared to the glasshouse condition the amount of essential oil content of two genotypes (Ilam and Isfahan) of *M. officinalis* was not significantly affected by different LEDs light sources (white, blue, red, blue +blue LEDs) with light intensity of 300 µmol. m^{-2} with a 16 h illumination and 8 h darkness and 25± 2°C but the composition of essential oil was variable under different LED treatments and the total amount of components in Ilam genotype was higher than Isfahan genotype (Tayebeh *et al.*, 2021). Previously well documented that Blue light has been enhanced the biosynthesis of the chemical composition of several plant species (Hoffmann *et al.*, 2015, Ichimura *et al.*, 2009).

In general, the results from the current study are supportive of the positive finding by Rihan *et al.* (2020) that using 435 nm blue light spectrum is better in comparison with the commercially widely used 450 nm LED spectrum when supplied in combination with 663 nm red light).

CONCLUSION

Increasing general understanding of phenological, physiological and biochemical changes that occur under different combinations of wavelength ranges may allow growers to better optimize selective light delivery for different varieties of lemon balm and further optimize light recipes. The results from this study concluded that white and the combination of red 663 nm in combination of blue 435 nm at the ratio of (2.5:1 R: B) significantly enhanced the assimilation rate, growth and shoot essential oil content in lemon balm.

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Conflict of interest

All authors declare that they have no conflict of interest.

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