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Influence of colorful light-emitting diodes on growth, biochemistry, and production of volatile organic compounds *in vitro* of *Lippia filifolia* (Verbenaceae)

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ABSTRACT

Lippia filifolia Mart. & Schauer belongs to the Verbenaceae family and it is endemic from the rupestrian fields of the Espinhaço mountain range, located in Minas Gerais, Brazil. It is an aromatic species with medicinal potential due to the production of volatile compounds that constitute its essential oil. The objective of this work was to evaluate the effects of light quality using light-emitting diodes (LEDs) over the growth of L. *filifolia* grown *in vitro* after 45 days of culture, analyzing its volatile organic compounds (VOCs), biochemical, and biometric traits. This study had four treatments according to the wavelength of LED lamps: (i) white (control), (ii) blue, (iii) red, and (iv) a combination of red + blue (mix). The light quality influenced the growth, metabolism, and VOCs production of plantlets. The specimens showed higher height under red and white treatments and higher biomass accumulation, nodal segments, and shoot numbers under the mix treatment. Higher total carbohydrate content was also observed on the mix treatment, while the white LED provided higher chlorophylls and carotenoids contents. In addition, the lipid peroxidation was more pronounced in mix and white LEDs treatments, and it was also observed significant but not quite changes in VOCs profiles due to light quality. Eucalyptol was the compound found in a higher concentration among the VOCs of L. *filifolia* grown *in vitro* at all light quality treatments studied.

1. Introduction

Lippia filifolia Mart. & Schauer is an endemic plant from the rupestrian fields of Espinhaço mountain range, located in Minas Gerais, Brazil. However, its habitat has been devastated by increasing mining activities. With aromatic characteristics, this endangered species is a shrub with an average height of 1 m, densely glandular leaves, and yellow or orange-colored flowers. This species has aromatic and possibly medicinal characteristics related to the production of volatile organic compounds (VOCs) that composes its essential oils [1]. Thus, techniques that allow us to modulate the production of these compounds, such as plant tissue culture, are important for the pharmaceutical industry aiming the production of new medications and other essential oils-based products, such as larvicides, repellents, and so on [2].

Plant tissue culture technique is an *in vitro* cultivation mainly used for the multiplication of plantlets at an exponential pace. Therefore, it is an important tool for fast micropropagation and conservation of rare, endemic, or endangered plant species [1,3,4]. In addition, this technique has diverse applications, such as *in vitro* cultivation of plant tissues combined with the use of colored light-emitting diodes (LEDs) to modulate the production of secondary metabolites [5–8]. *In vitro* cultivation allows us to easily choose and set both light quality and intensity in which plantlets will grow, as well as temperature and

Abbreviations: LED, Lighting emitting diode; TSS, Total soluble sugars; TCA, Trichloroacetic acid; TBA, Thiobarbituric acid; MDA, Malondialdehyde; VOC, Volatile organic compound

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photoperiod, given plantlets are commonly cultivated in either growth chamber or room [9]. Several factors influence the growth and physiology of plantlets grown *in vitro*, but the luminosity stands out as the most influential one [10].

Light is the primary energy source for the photosynthetic process and it modulates the plant growth on the photomorphogenesis [11]. Thus, LEDs are a major alternative of artificial lighting source that presents many advantages over conventional fluorescent lighting systems, such as specific wavelength, great durability, and no heat emission [6,12,13]. Therefore, the objective of this work was to evaluate the effects of light quality using light-emitting diodes (LEDs) over the growth of L. *filifolia* grown *in vitro*, analyzing its volatile organic compounds (VOCs), biochemical, and biometric traits.

2. Materials and Methods

2.1. Plant Material and Cultivation

The experiment was conducted in the Laboratory of Plant Tissue Culture (Department of Biology), located at the Universidade Federal de Lavras. The initial plantlets that were used in this study were obtained from the *in vitro* collection of the Laboratory of Plant Physiology (Department of Botany), located at the Universidade Federal de Juiz de Fora (UFJF). The *in vitro* provided plants were originally collected in a region of the Espinhaço mountain range, Minas Gerais, Brazil (coordinates 18° 17′49.8 "S 43 ° 50'20.4" W, 1262 m of altitude), identified under CESJ 49008 voucher number, deposited in the herbarium Leopoldo Krieger/UFJF.

For the plantlets multiplication, it was followed the protocol described by Pereira Peixoto et al. [1] with modifications free of growth regulators. Nodal segments (~1 cm) were inoculated in MS culture medium [14], plus agar (7.0 g L^{-1}) and sucrose (30 g L^{-1}). The pH of the culture medium was adjusted to 5.7 before autoclaving at 121 °C; 1.1 atm for 21 min. The inoculation of the vegetal material occurred under aseptic conditions in a laminar airflow cabinet and test tubes were used, containing 10 mL of culture medium in which each explant was placed. The test tubes were closed with polystyrene caps and sealed with PVC film. Subsequently, the plantlets were kept for 45 days in a growth room under 25 ± 2 °C, 16 h of photoperiod, and 36 μmol photons m^{-2} s^{-1} of photosynthetic active radiation (PAR, measured using LI-COR Quantum Sensor Q41031 coupled to IRGA model LI-6400XT, LI-COR, US) supplied by LED lamps.

2.2. Experimental Design

The experiment was carried out in a completely randomized design, with four treatments and 100 plantlets per treatment. The treatments were composed by LED lamps with different monochromatic or combined wavelengths: (i) white (control, 400–700 nm), (ii) blue (peak at 450 nm); (iii) red (peak at 653 nm), and (iv) mix (blue + red; peaks at 448 and 664 nm, respectively). The light spectra were measured using a Red Tide USB 650 UV spectroradiometer (Ocean Optics™, US) (Fig. 1).

2.3. Biometric Analyses

The following biometric analyses of 21 plantlets of each treatment were performed using the ImageJ software: height (cm), nodal segments, and shoot numbers. Fresh biomass (g) data were obtained of plantlets weighed on a Shimadzu ATX224 analytical balance (Shimadzu, JP).

2.4. Biochemical Analyses

Fresh shoot parts (leaves and stems) of each plantlet were used in the biochemical analyses. All the vegetal samples were collected after a 45-day growth period, frozen in liquid nitrogen, and stored at −80 °C until the analysis stage. In addition, 12 replicates were considered for each analysis.

The extraction and quantification of chlorophylls and carotenoids were done according to the Lichtenthaler and Buschmann [15] method, with modifications. Approximately 200 mg of fresh biomass was immersed in 10 mL of acetone 80% (*v*/v) for 2 days in amber flasks under the temperature of 8 °C, in the absence of light. Afterward, readings were performed on an Epoch™ Microplate Spectrophotometer (Biotek, US) at the following wavelengths: 663.2 nm for chlorophyll *a*, 646.8 nm for chlorophyll *b,* and 470 nm for carotenoids. According to the concentration values of the three mentioned photosynthetic pigments, total chlorophylls content (chlorophyll *a* + chlorophyll *b*), chlorophyll *a*/chlorophyll *b* ratio, and total chlorophylls/carotenoids ratio were calculated.

For the carbohydrates extraction, 400 mg of fresh biomass were macerated using a potassium phosphate buffer solution (100 mM, pH 7.0), followed by a water bath for 30 min at 40 °C. The homogenate was centrifuged at 5000*g* for 10 min, collecting the supernatant after centrifugation. Then, specific protocols were followed and the contents of total soluble sugars (TSS) [16], reducing sugars [17], and sucrose [18] were measured.

To quantify the lipid peroxidation, the thiobarbituric acid (TBA) method was used, according to the methodology described by Buege and Aust [19], with modifications. Thus, 400 mg of fresh biomass was macerated in liquid nitrogen and polyvinylpolypyrrolidone (PVPP). After, the resultant was homogenized in 1.5 mL of trichloroacetic acid (TCA) 1% (*v*/v) and centrifuged at 12000 *g* for 15 min at 4 °C. Thereupon, a 250 μL aliquot was collected from the supernatant and pipetted into microtubes containing 250 μL of a reaction medium composed by TBA 0.5% (v/v) and TCA 20% (v/v). Then, the samples were kept in a water bath at 95 °C for 30 min. Subsequently, they were placed in an ice bath, aiming to stop the reaction. Lastly, the samples were read on a spectrophotometer at 535 nm (A_{535}) and 600 nm (A_{600}). The concentration of malondialdehyde (MDA)/TBA complex was calculated through the following equation: $[MDA] = (A_{535}–A_{600})/(\xi.b)$, where: ξ (extinction coefficient = 1.56×0^{-5} cm^{-1}); b (optical length = 1).

2.5. Volatile Organic Compounds Analysis

Approximately 300 mg of fresh plant material was placed in 2 mL microtubes, adding 500 μL of hexane. The microtubes were closed and taken to an ultrasound bath for 10 min, to improve the extraction of VOCs. After this step, a small portion of anhydrous Na_2SO_4 was added to each flask to remove the water. The extract was collected using a micropipette and cotton, and then it was stored in the in-vial analysis to be analyzed in a gas chromatograph coupled to a mass spectrometer (GCMS-QP2010 Plus; Shimadzu, JP).

A 30 m × 0.25 mm Rtx-5MS (Restek, US) column was used. The schedule for the oven temperature started at 70 °C, for 3 min, followed by an increase of 6 °C $minute^{-1}$ until it reaches 300 °C. The injector was operated in split mode (1:10), at a temperature of 240 °C and the interface and mass detector operated at 300 °C. Helium was used as the carrier gas, with a 1.53 mL $minute^{-1}$ flow. A standard mixture of linear hydrocarbons (C_9H_{20}; $C_{10}H_{22}$; … $C_{25}H_{52}$ and $C_{26}H_{54}$) was injected under the same conditions as the samples. The identification of the constituents was made by comparison of the obtained mass spectra with the NIST 9.0 database (correlation > 97%), confirmed by their retention index [20] and compared to the data found in the literature [21]. The concentration for each compound was calculated by normalizing the values found, according to the peak areas of interest (the six largest in each sample) and expressed as a percentage (%). In addition, three samples per treatment were analyzed.

2.6. Statistical Analyses

All statistical analyses were performed using the statistical software

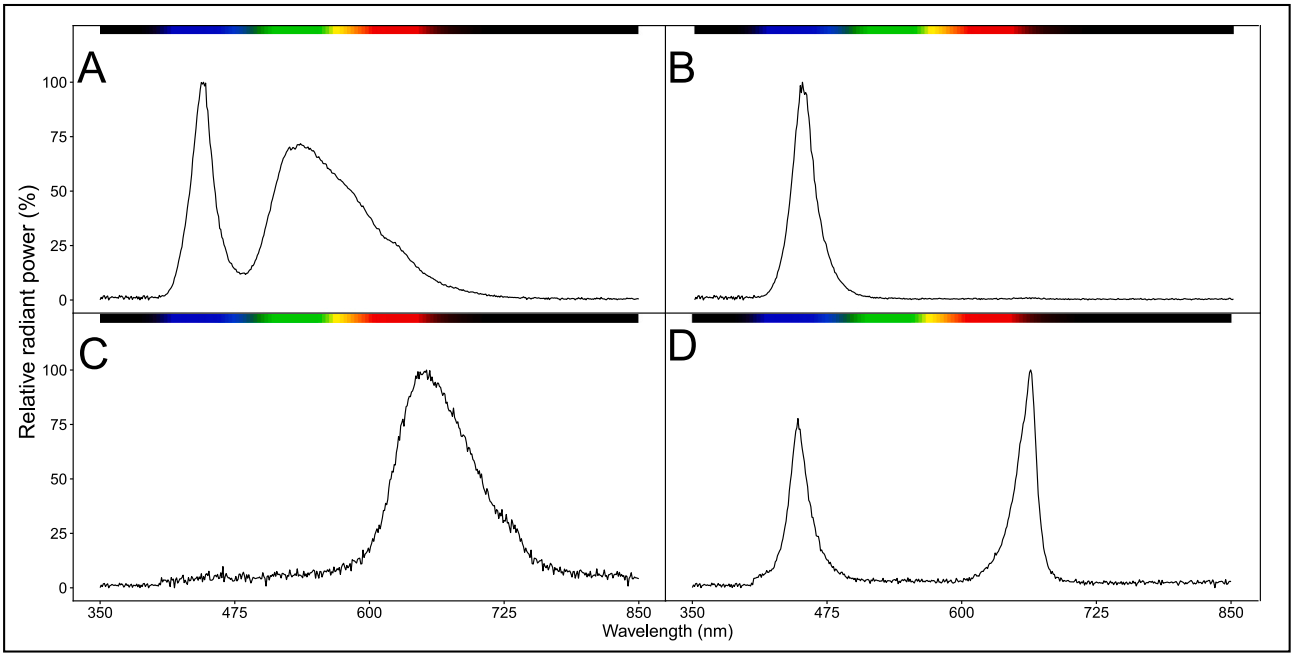

Fig. 1. Light spectra of the treatments: A) White LEDs, B) Blue LEDs, C) Red LEDs, and D) Mix (Blue + Red LEDs). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

R [22] and it was assumed a probability of 5% (p-value ≤ 0.05). The results were submitted to analysis of variance (ANOVA) and the normality of residuals was estimated by the Shapiro-Wilk test. Then, the results were compared using the Scott-Knott test. For biometric results, it was created a multiple correlation matrix (using *corrplot* package), applying Pearson's method. To analyze the results of VOCs, a principal component analysis (PCA) was performed (using the *factoextra* package). The plots were generated using the *ggplot2, cowplot,* and *ggspectra* packages.

3. Results and Discussion

3.1. Biometric Analyses

The results showed that light quality influenced significantly all growth parameters evaluated (Figs. 2 and 3). The highest mean of plantlet height was observed in the control and red LED lamps treatments (Fig. 3A). However, the mix treatment induced the larger quantity of shoot number (Fig. 3B), nodal segments number (Fig. 3C), and fresh biomass accumulation (Fig. 3D). No root development was observed.

These results are similar to those described for *Lippia gracilis* grown *in vitro,* where plantlets under red and white LED lamps showed the highest means of height, beyond being observed a negative effect from blue light on the plantlets height [7]. In a study with *Lippia rotundifolia* also grown *in vitro*, the highest means of height were observed under the red light, followed by the white light and the treatments in which the quantity of red light was higher than the blue light [8]. Thus, the authors of this present article, suggest that white and red LEDs stimulate, *in vitro*, a higher height of plantlets from genus *Lippia*.

About the number of shoots and nodal segments, the light quality was not an influential factor on L. *gracilis* [7], on the contrary of our results, where the highest means were found in the mix treatment for both parameters (Fig. 3B and C). The greater fresh biomass

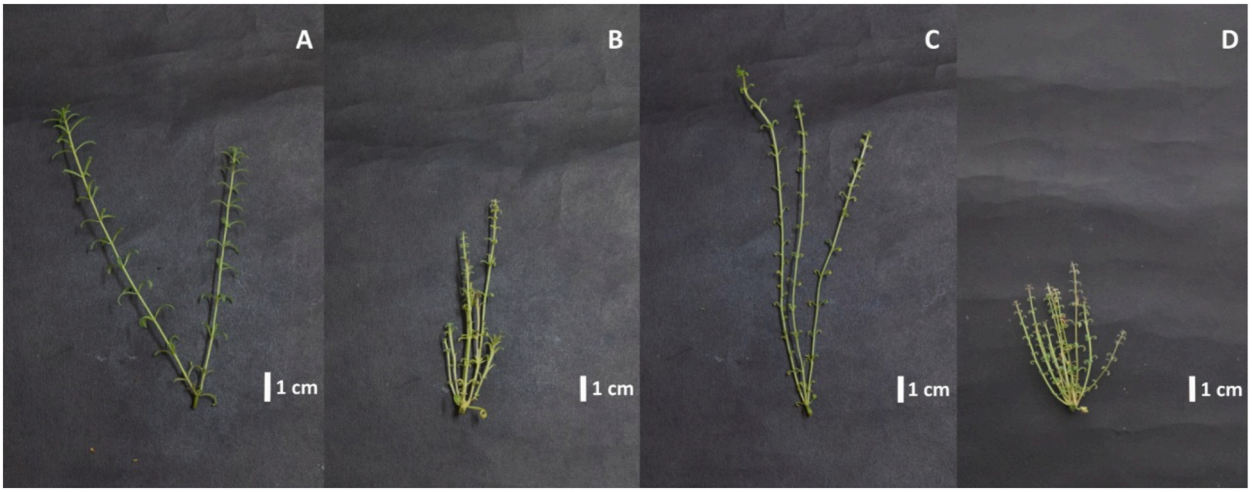

Fig. 2. Representative images of *Lippia filifolia* grown *in vitro* under: A) White LEDs, B) Blue LEDs, C) Red LEDs, and D) Mix (Blue + Red LEDs). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

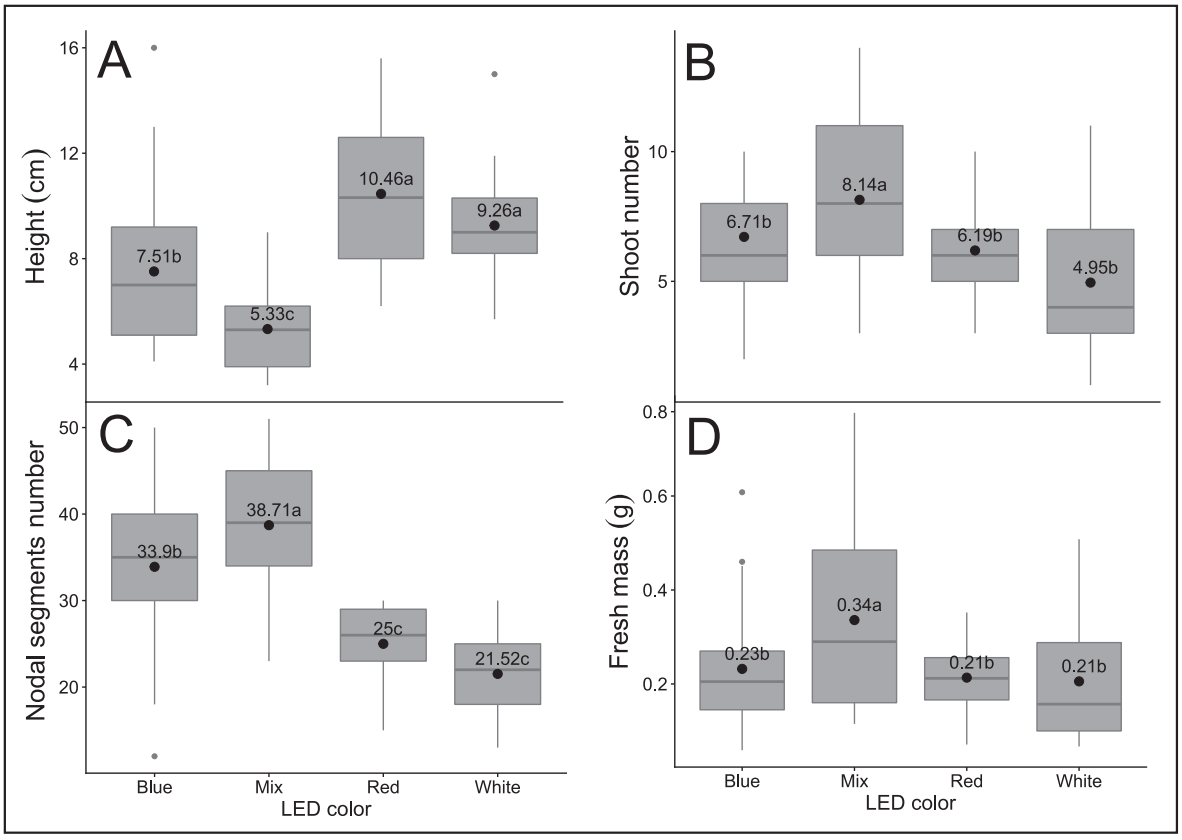

Fig. 3. Growth parameters of *Lippia filifolia* grown *in vitro* under different light conditions. Means followed by the same letters do not differ from each other according to the Scott-Knott test (p-value ≤ 0.05). The points inside the boxes represent the mean value and those outside represent outliers.

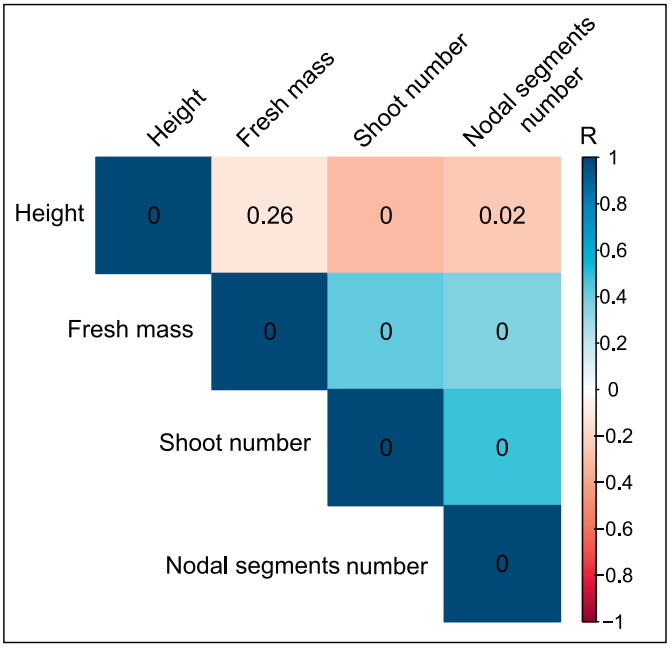

Fig. 4. Multiple correlation matrix of growth parameters of *Lippia filifolia* grown *in vitro* under different light conditions. The values inside the squares represent the p-value of each correlation.

accumulation was also observed in this same treatment (Fig. 3D), similarly to the results described by Batista et al. [5] with L. *alba* grown *in vitro* and it is reported for many plant species also grown *in vitro*, like *Pfaffia glomerata* [23] and *Solanum lycopersicum* [24]. Complementary, the multiple correlation matrix (Fig. 4) showed that the height was inversely proportional to the fresh biomass accumulation and the number of shoots and nodal segments. By joining this information to the results showed in Fig. 2, we observe that different light qualities have altered the L. *filifolia* growth pattern, characterizing a trade-off among investing in height or the remaining biometric characteristics evaluated.

Red light has a direct role in plant morphogenesis in our experiment, probably due to the induction of phytochrome transformation [25] and its essentiality for photosynthetic development [10]. Phytochromes, receptors of red and far-red light spectra, control plant development in various environmental conditions throughout the entire plant life cycle [25] and the effects of light quality in height are known [26,27]. Thus, we hypothesize that plantlets height was phytochrome-mediated. However, it is important to highlight that similar patterns for height were observed in plants grown *ex vitro* under blue light *via* phytochrome modulation in petunia, calibrachoa, geranium, and marigold [26]. Despite that, our height results are in accordance with those found for other *Lippia* plantlets [7,8], bell pepper seedlings [28], and other species, as revised by Huché-Thélier and colleagues [27]. Therefore, further studies are necessary for the elucidation of how red and blue lights affect *Lippia filifolia* growth pattern, using molecular, physiological, and morphological approaches.

3.2. Biochemical Analyses

Chlorophylls and carotenoids contents were higher in plantlets under white LED lamps (Fig. 5A-D), being the lowest values observed in plantlets under blue light. In the mix treatment, we observed the lowest concentrations of chlorophyll *a* (Fig. 5A) and carotenoids (Fig. 5C), besides intermediate values of chlorophyll *b* (Fig. 5B) and total chlorophylls content (Fig. 5D). By contrast, for L. *gracilis*, the white light had

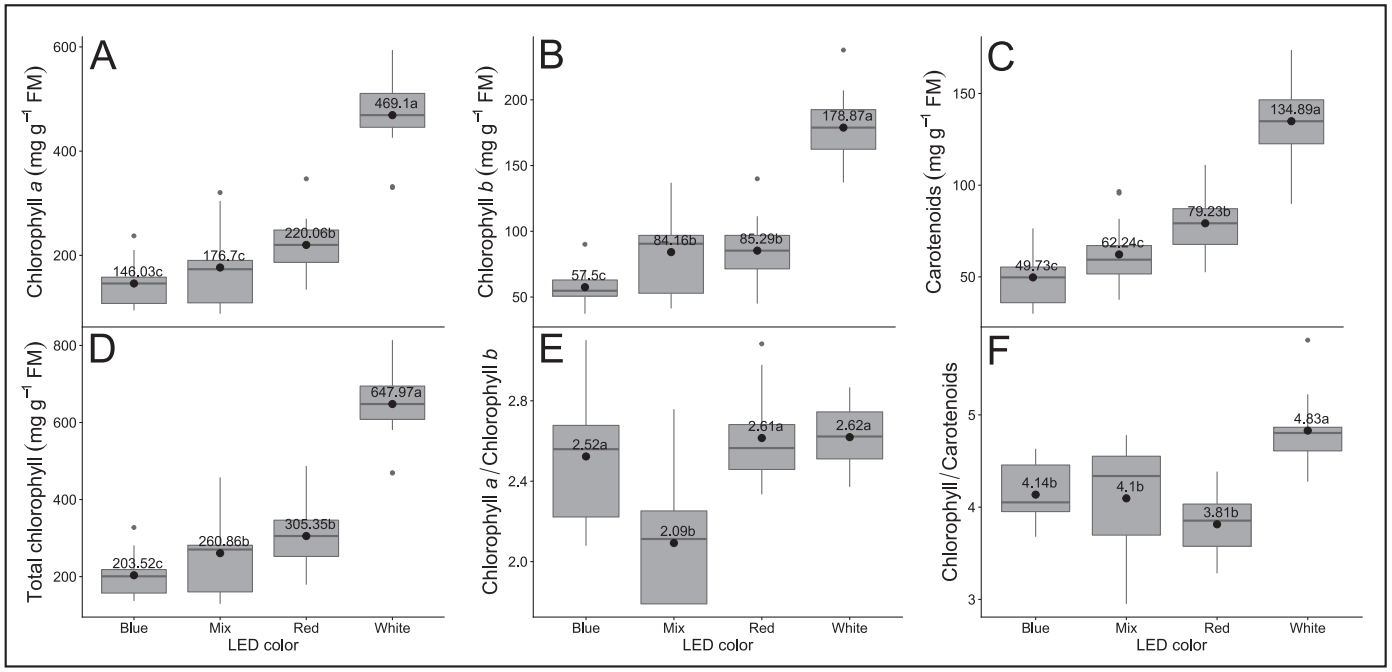

Fig. 5. Photosynthetic pigments contents of *Lippia filifolia* grown *in vitro* under different light conditions. Means followed by the same letters do not differ from each other according to the Scott-Knott test (p-value ≤ 0.05). The points inside the boxes represent the mean value and those outside represent outliers.

also promoted the greatest means of photosynthetic pigment contents, however with high and intermediate values for blue light, and the lowest to the red light [7]. Similarly, the blue light also promoted greater content of photosynthetic pigments than the red or the white lights in L. *rotundifolia* [8]. The mix of blue and red wavelengths are generally correlated to an increase in the photosynthetic process, being these the most absorbed by the photosynthetic pigments [29] and the most important in the chlorophylls and carotenoids synthesis [10]. However, the response of each species to the different blue and red lights proportions, are considered species-specific [6].

The mix treatment showed the lowest values of the chlorophyll *a*/ chlorophyll *b* ratio by stimulating more the chlorophyll *b* content when in comparison to the chlorophyll *a* (Fig. 5E). Besides, the control treatment stimulated more chlorophylls synthesis than carotenoids synthesis (Fig. 5F). Thus, the different LEDs lamps influenced the absolute and relative values of photosynthetic pigment contents in L. *filifolia*.

The light quality showed significant effects on the carbohydrate contents in L. *filifolia*. The highest means of TSS, reducing sugars and sucrose were observed in the mix and blue, mix, and mix and red treatments, respectively (Fig. 6). Similarly, studying *Solanum tuberosum* grown *in vitro* under LEDs lamps, Jiang et al. [30] observed the highest TSS content under the combination of red and blue lights. Correspondent results were described for *Phoenix dactylifera* also grown *in vitro*, where the highest TSS content was observed in the LEDs combination of red + blue [31]. Indeed, the regulation of gene expression related to starch and sucrose metabolic pathways were modulated by different wavelengths of LEDs in micropropagated *Vitis vinifera* [32].

Despite the mix treatment showed high levels of all carbohydrates analyzed, beyond the highest accumulation of fresh biomass, in this same treatment was observed a low content of chlorophylls and carotenoids when in comparison to the others. As described in scientific literature, plants grown *in vitro* are subjected to low photon flux density and, consequently, cannot fix CO_2 properly, resulting in low photosynthetic rates and deficient biosynthesis of carbohydrates, requiring an external source of sugar to grow *in vitro* [33]. However, a negative effect of adding sugars, such as sucrose, is known on photosynthetic capacity, based on the concept of balance between the processes of synthesis and consumption of carbohydrates [33,34]. Given that, we suggest that the highest contents of carbohydrates in those plantlets were obtained from the sucrose source provided by the culture medium and that the mix treatment contributed to stimulate this process.

The MDA content was also influenced by light quality. As evidenced in Fig. 7, the treatments using blue or red lights showed the lowest concentrations of MDA when in comparison to the control and mix treatments, which did not present differences from each other. MDA is a widely used marker of lipid peroxidation caused by oxidative stress due to different environmental stresses and several studies have investigated MDA of plants under different stressful conditions [35]. Some species grown *in vitro* under blue light showed significant activity of antioxidant enzymes in this condition, followed by red light [36], consequently decreasing the reactive oxygen species concentrations and, with this, avoiding the oxidative stress [37]. Indeed, our results agree with these data, since lower contents of MDA were observed in plantlets under red and blue LED lamps.

The blue light can benefit the *in vitro* growth, given that in the micropropagation plantlets are exposed to artificial stressful conditions, as high humidity into the tube, low carbon dioxide concentration, and decreased gas exchange rates [33]. In addition, these factors led to physiological and morphological disorders in plantlets. Thus, the use of monochromatic blue or red LEDs decreased considerably the MDA content, being important to avoid damages to the cell membranes of L. *filifolia* grown *in vitro*.

3.3. Analysis of Volatile Organic Compounds (VOCs)

According to the qualitative analysis of L. *filifolia* VOCs chemical composition, it was observed little differences related to the light quality (Fig. 8). In this present study, the eucalyptol was identified as the majority compound of L. *filifolia* VOCs, followed by α-pinene, thujene, γ-terpinene, caryophyllene, and limonene, all terpenoids. It was observed that the treatments using blue and red LED lamps promoted a

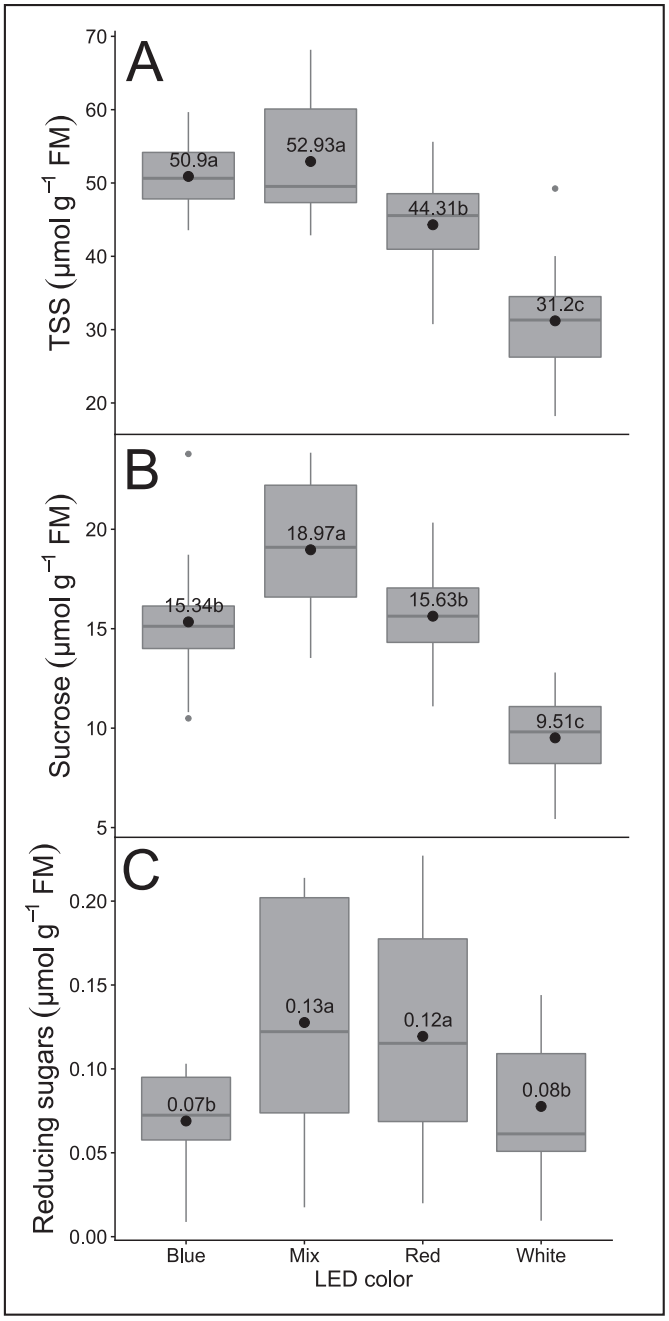

Fig. 6. Carbohydrates contents of *Lippia filifolia* grown *in vitro* under different light conditions. Means followed by the same letters do not differ from each other according to the Scott-Knott test (p-value ≤ 0.05). The points inside the boxes represent the mean value and those outside represent outliers. TSS = Total soluble sugars.

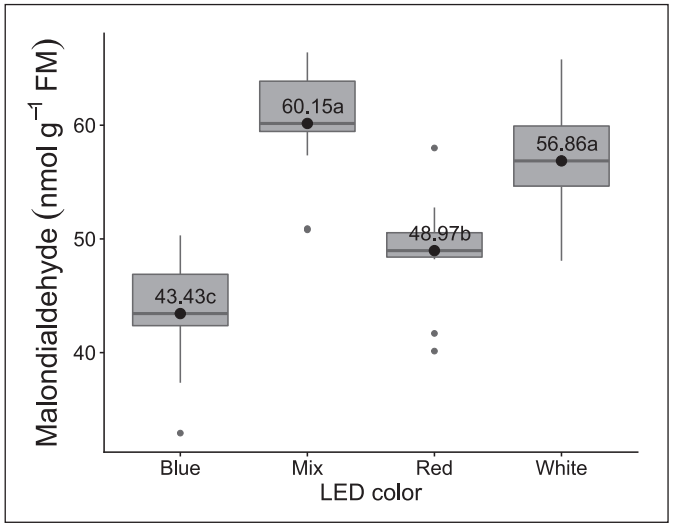

Fig. 7. Malondialdehyde content of *Lippia filifolia* grown *in vitro* under different light conditions. Means followed by the same letters do not differ from each other according to the Scott-Knott test (p-value ≤ 0.05). The points inside the boxes represent the mean value and those outside represent outliers.

higher concentration of limonene in comparison to the control treatment, and the plantlets grown under red LED lamps showed a higher concentration of γ-terpinene in comparison to the mix treatment. For some species from *Lippia* genus, it is known that light quality also promotes differences in the VOCs profile. For L. *rotundifolia*, myrcene was identified as the majority compound and its production was favored by red LEDs [8]. For L. *gracilis,* however, the majority compound was carvacrol and it presented the higher accumulation when the plantlets were grown under blue LEDs [7]. In addition, the luminous quality also affected the VOCs production in L. *alba* [5].

The PCA showed in an integrated manner that clusters were formed according to each treatment VOCs, explaining 73,4% of data total variance (Fig. 9). Given that, white and blue LED lamps were the treatments that differed from each other in an opposite behavior. Intermediary to these treatments are the mix and red LEDs treatments, being the red LED's closer to the control and the mix treatment, closer to the blue LEDs. It was also showed that the treatments using blue and mix LED lamps promoted higher synthesis of limonene, thujene, and caryophyllene compounds, while the treatments using white and red LEDs promoted higher production of eucalyptol and γ-terpinene. The α-pinene production was favored by control and blue LED treatments. All of these results are in accordance with the univariate ones (Fig. 8). With this, it was possible to observe that the differences in the profiles of the VOCs were few, but enough significant to be possible to divide the specimens into groups according to the treatments used, as previously related to other *Lippia* species [5,7,8].

Singulani et al. [38], analyzing the VOCs chemical composition of many species from the *Lippia* genus, reported that the camphor was the majority compound found in L. *filifolia*, whereas the eucalyptol appeared in very low concentration (3%) in the samples. These authors analyzed leaves collected *ex vitro*, differently from this present study, where we used plantlets grown *in vitro*. With this, it is possible to hypothesize that exists different chemotypes of L. *filifolia*, or the *in vitro* grown affects the VOCs profile.

Our study showed that L. *filifolia* growth and metabolism were influenced by the light quality *in vitro*. The combination of red + blue LEDs promoted, in general terms, higher biomass accumulation, nodal segments, and shoot numbers, as well as induced the higher content of carbohydrates in plantlets, thus being indicated to *in vitro* grown of L. *filifolia,* aiming higher regeneration and propagation rates. The luminous quality did not change the majority compound concentration (eucalyptol) in the plantlets, enabling an alternative, fast, and practical production of this compound.

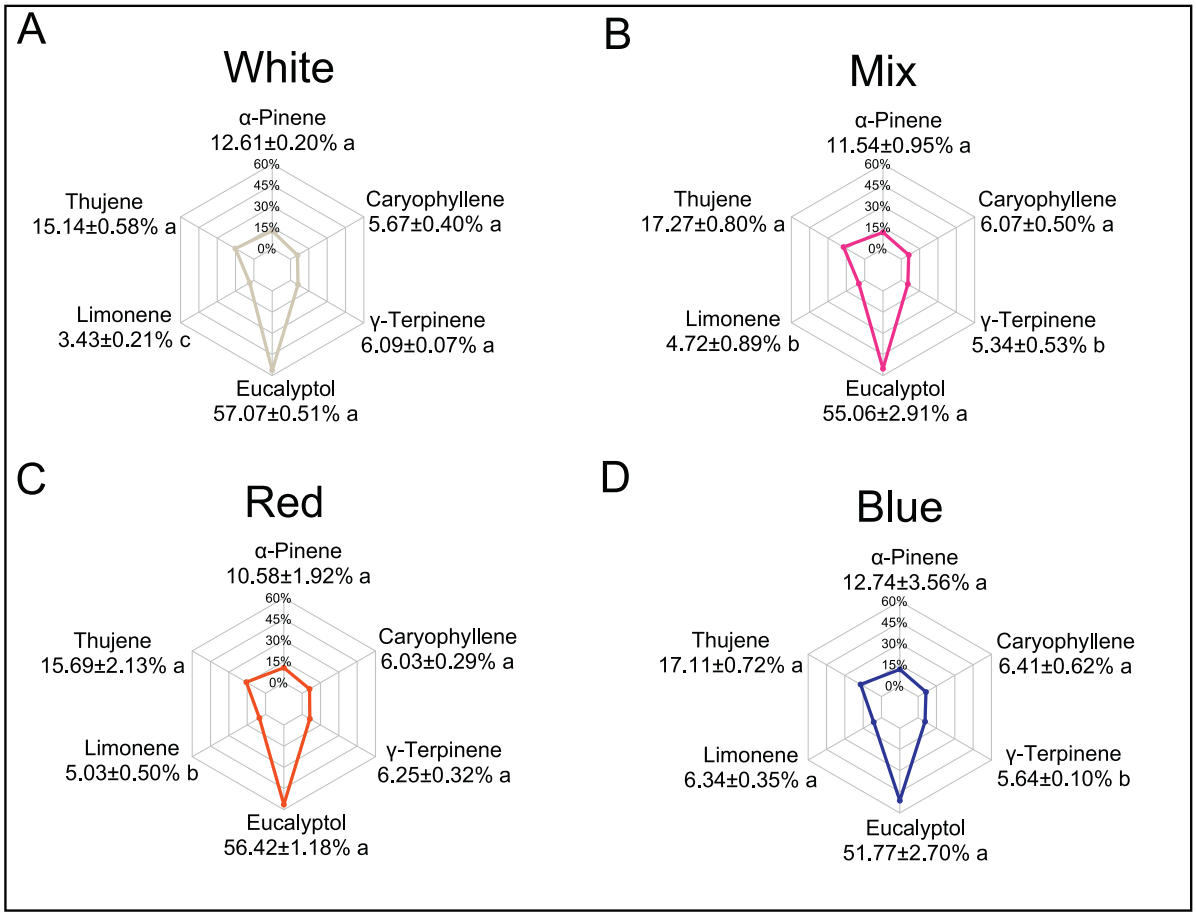

Fig. 8. Six major volatile organic compounds of *Lippia filifolia* grown *in vitro* under different light conditions. Means ± standard deviations followed by the same letters do not differ from each other according to the Scott-Knott test (p-value ≤ 0.05).

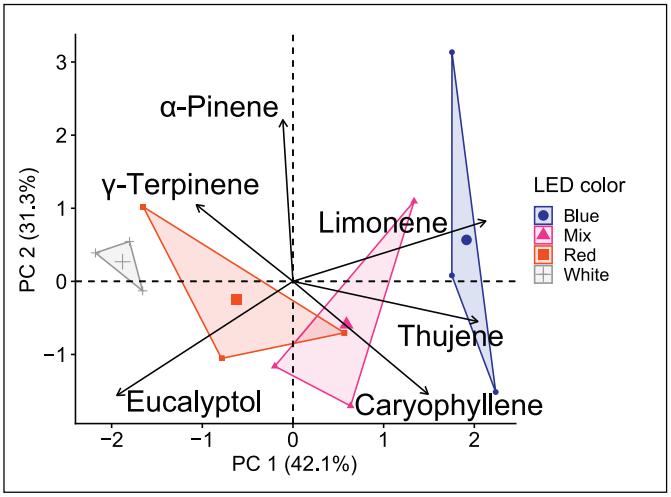

Fig. 9. Biplot containing scores and loadings of the PCA performed using data of six major volatile organic compounds of *Lippia filifolia* grown *in vitro* under different light conditions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] P.H. Pereira Peixoto, F.R. Goncalves Salimena, M.D.O. Santos, L.D.S. Garcia, P.M. De Oliveira Pierre, L.F. Viccini, W.C. Otoni, *In vitro* propagation of endangered *Lippia filifolia* mart. and Schauer ex Schauer, Vitr. Cell. Dev. Biol. 42 (2006) 558–561, https://doi.org/10.1079/IVP2006814.

[2] J.S. Raut, S.M. Karuppayil, A status review on the medicinal properties of essential oils, Ind. Crop. Prod. 62 (2014) 250–264, https://doi.org/10.1016/j.indcrop.2014.05.055.

[3] C.F. de Resende, R.E. Bianchetti, A.M. de Oliveira, V.F. Braga, P.H. Pereira Peixoto, In vitro propagation and acclimatization of *Lippia rotundifolia*, an endemic species of Brazilian campos rupestres, Rev. Ciênc. Agron. 46 (2015) 582–589, https://doi.org/10.5935/1806-6690.20150041.

[4] N.A. Hlatshwayo, S.O. Amoo, J.O. Olowoyo, K. Doležal, Efficient micropropagation protocol for the conservation of the endangered *Aloe peglerae*, an ornamental and medicinal species, Plants. 9 (2020) 506, https://doi.org/10.3390/plants9040506.

[5] D.S. Batista, K.M. de Castro, A.R. da Silva, M.L. Teixeira, T.A. Sales, L.I. Soares, M. das Graças Cardoso, M. de Oliveira Santos, L.F. Viccini, W.C. Otoni, Light quality affects *in vitro* growth and essential oil profile in *Lippia alba* (Verbenaceae), Vitr. Cell. Dev. Biol. – Plant. 52 (2016) 276–282, https://doi.org/10.1007/s11627-016-9761-x.

[6] D.S. Batista, S.H.S. Felipe, T.D. Silva, K.M. de Castro, T.C. Mamedes-Rodrigues, N.A. Miranda, A.M. Ríos-Ríos, D.V. Faria, E.A. Fortini, K. Chagas, G. Torres-Silva, A. Xavier, A.D. Arencibia, W.C. Otoni, Light quality in plant tissue culture: does it matter? Vitr. Cell. Dev. Biol. – Plant. 54 (2018) 195–215, https://doi.org/10.1007/s11627-018-9902-5.

[7] L.E.S. Lazzarini, S.K.V. Bertolucci, F.V. Pacheco, J. dos Santos, S.T. Silva, A.A. de

Carvalho, J.E.B.P. Pinto, Quality and intensity of light affect *Lippia gracilis* Schauer plant growth and volatile compounds *in vitro*, Plant Cell Tissue Organ Cult. 135 (2018) 367–379, https://doi.org/10.1007/s11240-018-1470-1.
[8] B.S. de Hsie, A.I.S. Bueno, S.K.V. Bertolucci, A.A. de Carvalho, S.H.B. da Cunha, E.R. Martins, J.E.B.P. Pinto, Study of the influence of wavelengths and intensities of LEDs on the growth, photosynthetic pigment, and volatile compounds production of *Lippia rotundifolia* Cham *in vitro*, J. Photochem. Photobiol. B Biol. 198 (2019) 111577, , https://doi.org/10.1016/j.jphotobiol.2019.111577.
[9] P.L. Sáez, L.A. Bravo, M.I. Latsague, M.J. Toneatti, M. Sánchez-Olate, D.G. Ríos, Light energy management in micropropagated plants of *Castanea sativa*, effects of photoinhibition, Plant Sci. 201–202 (2013) 12–24, https://doi.org/10.1016/j.plantsci.2012.11.008.
[10] S. Dutta Gupta, B. Jatothu, Fundamentals and applications of light-emitting diodes (LEDs) in *in vitro* plant growth and morphogenesis, Plant Biotechnol. Rep. 7 (2013) 211–220, https://doi.org/10.1007/s11816-013-0277-0.
[11] M. de Wit, R. Pierik, Photomorphogenesis and Photoreceptors, Springer, Dordrecht, 2016, pp. 171–186, https://doi.org/10.1007/978-94-017-7291-4_6.
[12] J.J. Bello-Bello, J.A. Pérez-Sato, C.A. Cruz-Cruz, E. Martínez-Estrada, Light-emitting diodes: progress in plant micropropagation, Chlorophyll, InTech, 2017, , https://doi.org/10.5772/67913.
[13] M.R. Shukla, A.S. Singh, K. Piunno, P.K. Saxena, A.M.P. Jones, Application of 3D printing to prototype and develop novel plant tissue culture systems, Plant Methods 13 (2017) 6, https://doi.org/10.1186/s13007-017-0156-8.
[14] T. Murashige, F. Skoog, A revised medium for rapid growth and bio assays with tobacco tissue cultures, Physiol. Plant. 15 (1962) 473–497, https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.
[15] H.K. Lichtenthaler, C. Buschmann, Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy, Curr. Protoc. Food Anal. Chem. 1 (2001) F4.3.1–F4.3.8, https://doi.org/10.1002/0471142913.faf0403s01.
[16] Z. Dische, Methods in Carbohydrate Chemistry, By RL Whistler, ML Wolfrom, Acad. Press Inc., New York. vol. 1 (1962) 507.
[17] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. 31 (1959) 426–428, https://doi.org/10.1021/ac60147a030.
[18] E. van Handel, Direct microdetermination of sucrose, Anal. Biochem. 22 (1968) 280–283, https://doi.org/10.1016/0003-2697(68)90317-5.
[19] J.A. Buege, S.D. Aust, Microsomal lipid peroxidation, Methods Enzymol. 52 (1978) 302–310, https://doi.org/10.1016/S0076-6879(78)52032-6.
[20] H. van Den Dool, P. Dec. Kratz, A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography, J. Chromatogr. A 11 (1963) 463–471, https://doi.org/10.1016/s0021-9673(01)80947-x.
[21] R. Adams, Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy, Carol Stream. 16 (2005) 65–120.
[22] R Development Core Team, R: A Language and Environment for Statistical Computing, http://www.r-project.org, (2009).
[23] T.D. Silva, D.S. Batista, E.A. Fortini, K.M. de Castro, S.H.S. Felipe, A.M. Fernandes, R.M. de J. Sousa, K. Chagas, J.V.S. da Silva, L.N. de F. Correia, L.M. Farias, J.P.V. Leite, D.I. Rocha, W.C. Otoni, Blue and red light affects morphogenesis and 20-hydroxyecdisone content of *in vitro Pfaffia glomerata* accessions, J. Photochem. Photobiol. B Biol. 203 (2020) 111761, , https://doi.org/10.1016/j.jphotobiol.2019.111761.
[24] M.T. Naznin, M. Lefsrud, M.O.K. Azad, C.H. Park, Effect of different combinations of red and blue LED light on growth characteristics and pigment content of *in vitro* tomato plantlets, Agriculture. 9 (2019) 196, https://doi.org/10.3390/agriculture9090196.
[25] M. Legris, Y.Ç. Ince, C. Fankhauser, Molecular mechanisms underlying phytochrome-controlled morphogenesis in plants, Nat. Commun. 10 (2019) 1–15, https://doi.org/10.1038/s41467-019-13045-0.
[26] Y. Kong, M. Stasiak, M.A. Dixon, Y. Zheng, Blue light associated with low phytochrome activity can promote elongation growth as shade-avoidance response: a comparison with red light in four bedding plant species, Environ. Exp. Bot. 155 (2018) 345–359, https://doi.org/10.1016/j.envexpbot.2018.07.021.
[27] L. Huché-Thélier, L. Crespel, J. Le Gourrierec, P. Morel, S. Sakr, N. Leduc, Light signaling and plant responses to blue and UV radiations-perspectives for applications in horticulture, Environ. Exp. Bot. 121 (2016) 22–38, https://doi.org/10.1016/j.envexpbot.2015.06.009.
[28] N.B. Claypool, J.H. Lieth, Physiological responses of pepper seedlings to various ratios of blue, green, and red light using LED lamps, Sci. Hortic. (Amsterdam) 268 (2020) 109371, , https://doi.org/10.1016/j.scienta.2020.109371.
[29] H. Lambers, R.S. Oliveira, H. Lambers, R.S. Oliveira, Photosynthesis, respiration, and long-distance transport: photosynthesis, Plant Physiol. Ecol, Springer International Publishing, 2019, pp. 11–114, , https://doi.org/10.1007/978-3-030-29639-1_2.
[30] L. Jiang, Z. Wang, G. Jin, D. Lu, X. Li, Responses of Favorita potato plantlets cultured *in Vitro* under fluorescent and light-emitting diode (LED) light sources, Am. J. Potato Res. 96 (2019) 396–402, https://doi.org/10.1007/s12230-019-09725-8.
[31] A.M.W. Al-Mayahi, Effect of red and blue light emitting diodes "CRB-LED" on *in vitro* organogenesis of date palm (*Phoenix dactylifera* L.) cv. Alshakr, World J. Microbiol. Biotechnol. 32 (2016) 1–8, https://doi.org/10.1007/s11274-016-2120-6.
[32] C.-X. Li, Z.-G. Xu, R.-Q. Dong, S.-X. Chang, L.-Z. Wang, M. Khalil-Ur-Rehman, J.-M. Tao, An RNA-Seq analysis of grape plantlets grown *in vitro* reveals different responses to blue, green, red LED light, and white fluorescent light, Front. Plant Sci. 8 (2017) 78, https://doi.org/10.3389/fpls.2017.00078.
[33] Q.T. Nguyen, Y. Xiao, T. Kozai, Photoautotrophic micropropagation, Plant Fact, Elsevier, 2020, pp. 333–346, , https://doi.org/10.1016/b978-0-12-816691-8.00023-6.
[34] L. Chen, Y. Lu, Y. Hu, X. Xue, RNA-Seq reveals that sucrose-free medium improves the growth of potato (*Solanum tuberosum* L.) plantlets cultured *in vitro*, Plant Cell Tissue Organ Cult. 140 (2020) 505–521, https://doi.org/10.1007/s11240-019-01743-y.
[35] C. Soares, M.E.A. Carvalho, R.A. Azevedo, F. Fidalgo, Plants facing oxidative challenges—A little help from the antioxidant networks, Environ. Exp. Bot. 161 (2019) 4–25, https://doi.org/10.1016/j.envexpbot.2018.12.009.
[36] A. Manivannan, P. Soundararajan, Y.G. Park, H. Wei, S.H. Kim, B.R. Jeong, Blue and red light-emitting diodes improve the growth and physiology of *in vitro*-grown carnations 'green beauty' and 'purple beauty, Hortic. Environ. Biotechnol. 58 (2017) 12–20, https://doi.org/10.1007/s13580-017-0051-2.
[37] L. Mengxi, X. Zhigang, Y. Yang, F. Yijie, Effects of different spectral lights on oncidium PLBs induction, proliferation, and plant regeneration, Plant Cell Tissue Organ Cult. 106 (2011) 1–10, https://doi.org/10.1007/s11240-010-9887-1.
[38] J. de L. Singulani, P.S. Silva, N.R.B. Raposo, E.P. de Siqueira Filho, C.L. Zani, T.M.A. Alves, L.F. Viccini, Chemical composition and antioxidant activity of *Lippia* species, J. Med. Plant Res. 6 (2012) 4416–4422, https://doi.org/10.5897/JMPR12.533.