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Modification of the composition of thyme (*Thymus vulgaris*) essential oil based on the quality of the light

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ABSTRACT

Objective: To identify the changes in the concentration of the main components of thyme (*Thymus vulgaris*) essential oil in response to five different LED colors.

Design/Methodology/Approach: A completely randomized experimental design was used. The design included five treatments (white light; blue light; red light; 75% blue light and 25% red light; and 75% red light and 75% blue light) and 10 repetitions, at a 25 μmol m^{-2} s^{-1} luminous intensity, during a 16 h photoperiod. The thyme plants were sown in a pot with a substrate made up of 50% peat, 48% perlite, and 2% vermicompost. Each plant was an experimental unit. The plants were placed in light isolation chambers and subjected to the treatment for 35 days.

Results: The concentration of the main molecules in the essential oil recorded considerable changes between treatments: the concentration of thymol (its main component) increased in the white light treatments, as well as in the red light (75%) and blue light (75%) treatments. In addition, the composition of the essential oil resulting from these treatments is different to the composition reported in the references.

Study Limitations/Implications: The light intensity used in this experiment was lower than the light intensity required for plant growth; however, it was enough to produce changes in the secondary metabolism.

Findings/Conclusions: The changes in the quality of the light modify the composition of the thyme essential oil. Even at a low light intensity (25 μmol m^{-2} s^{-1}), the changes in the spectrum composition under which the plants grow influence the composition of the essential oil.

Keywords: *Thymus vulgaris*, LEDs, secondary metabolism, terpenoids.

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INTRODUCTION

Thyme (*Thymus vulgaris*) is a popular plant of the Lamiaceae family; it is native to the Mediterranean and its global distribution is the result of its multiple uses (Hosseinzadeh *et al.*, 2015). Thyme produces up to 1.2% (fresh weight) of essential oil, which is made up of thymol (40-50%), p-cymene (15-20%), y-terpinene (3-15%), carvacrol (3-4%), and δ-cadinene (1-3%) (Soković *et al.*, 2009; Nikolić *et al.*, 2014). Thyme essential oil has antioxidant (Kulisic *et al.*, 2005; Nikolić *et al.*, 2014), antibiotic (Basch *et al.*, 2004; Nikolić *et*

al., 2014), antiviral (Nolkemper *et al*., 2006), antitumor (Nikolić *et al*., 2014), and antitussive (Knols *et al*., 1994) properties. Marchese *et al*. (2016) and Martínez-Pabón and Ortega-Cuadros (2020) agree that these properties are the result of the high content of thymol in the essential oil. Consequently, many researches seek to change its composition, using different chemotypes of thyme and favoring thymol concentration.

Many researches have aimed to prove that changes in the quality of light can influence the growth, development, and secondary metabolism of plants. Currently, this type of researches uses light-emitting diodes (LED) to modify the light environment in which plants develop. LEDs have multiple advantages compared with the light sources used in the past, including: long life, low heat emission, light intensity adjustment, high energy conversion efficiency, and a specific wavelength (Gupta and Agarwal, 2017). As a result of the requirements of the plants, most of the experiments are carried out using at least 80 μmol m^{-2} s^{-1} light intensity.

Nishioka *et al*. (2008) used a red LED (150 μmol m^{-2} s^{-1}, 16 h photoperiod) to carry out a treatment with corn mint (*Mentha arvensis* L.), recording higher menthol, menthone, and limonene contents than the experiments carried out using blue and green LEDs. Nishimura *et al*. (2009) experimented with *Perilla frutescens* (L.) Britt. var. Acuta using red light, red-blue, and red-green wavelength treatments, and obtained plants with lower perillaldehyde and limonene concentrations. These two elements are the main components of that essential oil.

Noguchi and Amaki (2016) conducted an experiment using Mexican mint (*Plectranthus amboinicus*) treated with red light (100 μmol m^{-2} s^{-1},16 h photoperiod). This treatment promoted a higher concentration of α-pinene, β-pinene, and limonene compounds, recording a lower retention time in the column, during the gas chromatography. Meanwhile, green light promoted the concentration of borneol and bornyl acetate compounds, recording an intermediate retention time in the column. Finally, blue light promoted an accumulation of β-farnesene, germancrene D, and elemene compounds, recording the maximum retention time in the column. The authors pointed out that Mexican mint is very sensitive to changes in the quality of the light. Therefore, they concluded that blue light promotes the biosynthetic metabolic pathway of the sesquiterpenoids.

These examples prove that modifying the light environment in which plants grow can promote phytochemical modifications. This situation opens the door to further researches aimed to improve the quality of essential oils of anthropocentric importance, using technologies such as high- and low-intensity LEDs. Consequently, the objective of this research is to describe the changes in the composition of the essential oil of thyme plants, treated with different qualities of low-intensity LEDs.

MATERIALS AND METHODS

The experiment was established from April to July 2021, in a greenhouse of the Posgrado en Horticultura of the Autonomous University of Chapingo. The thyme (*Thymus vulgaris*) seedlings used in the experiments were grown from seeds (Vita®).

The seeds were sown in a substrate made up of 70% peat and 30% perlite; they were grown under greenhouse conditions and watered with running water. Fourteen days

after germination, the seedlings were planted in 4-inch-wide pots, with a 50% peat, 48% perlite, and 2% vermicompost substrate. Fertilization was carried out once a week, using a dose of 1,000 mg L^{-1} of the Ultrasol® multipurpose fertilizer (NO_3^-: 9%, NH_4^+: 9%, P_2O_5: 18%, K_2O: 18%, MgO: 1%, EDTA-Fe: 0.04%, EDTA-Mn: 0.02%, EDTA-Zn: 0.02%, B: 0.01%, EDTA-Cu: 0.01%, Mo: 0.01%). Watering was carried out every two days, using running water. The plants were kept under natural light conditions for 28 days and were subjected to two trims (at 10 and 24 days), in order to promote branching and homogenization of the aerial part of the plant. Subsequently, the plants were subjected to completely controlled light conditions, remaining under these conditions until the experiment was completed.

Each thyme plant was an experimental unit. Five treatments with ten repetitions each were established. The experimental design was completely random. The treatments were: monochromatic blue (B) light; monochromatic red (R) light; a combination of 75% blue light and 25% red light (75B:25R); and 75% red light and 75% blue light (75R:25B); and white light (W).

RGB 5050 (Weluvfit®) LED lightning tapes were used as source of blue and red lights and their combinations. They had 30 modules per meter (5 m) and were installed on 15×40 cm wooden plates. As source of white light, a 3528 (Tunix®) LED lightning tape was used, with 60 modules per meter. The height of the plates was adjusted to influence the plants. The 25 μmol m^{-2} s^{-1} light intensity was measured with a QMSW-SS Apogee® radiometer. The photoperiod lasted for 16 hours, from 6:00 am to 10:00 pm. The treatments were established in 80 cm long×40 cm wide×80 cm tall boxes, with a white interior. Air circulation was provided with a 4-inch fan (12 v), placed lengthways in the back side of the chamber. The fan was switched on every hour: 10 minutes, from 6:00 to 11:00 am and 06:00 and 10:00 pm and 15 minutes, from 12:00 to 05:00 pm. The temperature inside the greenhouse was kept under 28 °C, using fans controlled by a temperature sensor. Inside the boxes, the plants were rotated on a daily basis and the treatments were changed to another box, every four days, in order to reduce the experimental error.

The aerial part of the plants was harvested 35 days after the beginning of the LED treatments. In order to extract the essential oil, the thyme was crushed and 7.4 ml of ethyl acetate (Sigma®) were added per gram of plant material. The mixture was allowed to rest for 30 m and, afterwards, it was filtered.

The extracts were taken to 10 mL with ethyl acetate; subsequently, they were dried with anhydrous sodium sulfate and filtered. In order to analyze the oils, an Agilent Technologies 5973Network Mass Selective Detector (with a HP 5973 coupled mass detector) and a column with an internal diameter of HP5/30 m×0.225 mm were used. The temperature of the oven was initially kept at 60 °C for 1 minute; afterwards, it was linearly increased to 260 °C, at a 7 °C min^{-1} speed. This final temperature was kept for 1 minute. The helium constant flux was 1 mL min^{-1} and the gas had a 99.999% purity. The temperature of the splitless injector was 260 °C. The ionization energy was 70 eV. The temperatures of the ion source and the quadruple were 250 and 150 °C, respectively. The interphase temperature was 280 °C. The injection volume was 1 μL. The compounds were identified comparing the mass spectra of the peaks of interest and the NIST 2011 library.

The statistical analysis was carried out with an analysis of variance, while the following variables were subjected to a Tukey's mean comparison test ($p \leq 0.05$): concentration of the five main molecules of the essential oil of each treatment and concentration of each main molecule in the different treatments. The SAS® 9.0 statistical software was used.

RESULTS AND DISCUSSION

The main compounds of the essential oil obtained in this research were: thymol (38-52%), β-terpinene (14-23%), sulphureous acid, dodecyl 2-propyl ester (6-20%), perillene (4-7%), and thymol acetate (3-8%). Except for thymol, the content and concentration of the other molecules contrast with the data reported in the references, because several authors agree that the thyme essential oil is made up of: thymol (40-50%), p-cimene (15-20%), γ-terpinene (3-15%), carvacrol (3-4%), β-cariophyllene (2-4%), and δ-cadinene (1-3%) (Soković *et al.*, 2009; Nikolić *et al.*, 2014). The practically inexistent match between those results and the results of this study can be the consequence of the different light intensity used in the researches. The plants of those studies were grown outdoor or in greenhouses (high light intensity), while the plants of this research were subjected to a low light intensity (25 μmol m^{-2} s^{-1}). These differences strongly influence the composition of the essential oil (Noguchi and Amaki, 2016; Milenković *et al.*, 2021).

Thymol recorded the highest concentration in all the treatments. In addition, all the following molecules were identified in all the treatments: the β-terpinene and perillene cyclical monoterpenoids (Figure 1), which are products of the same biosynthetic pathway than thymol (Tholl, 2015). Only the red light treatment and the two dichromatic treatments reported the presence of γ-terpinene di-epoxide (a precursor of thymol) (Thompson *et al.*, 2003).

The effect of the quality of the light on the concentration of the identified molecules is remarkable (Table 1). The thymol concentration was higher in the white light and 75R:25B treatments and lower in the rest of the treatments. These results contrast with the findings of Tohini *et al.* (2020), who reported a higher increase of thymol concentration in thyme plants subjected to a blue light treatment. Ahmadi *et al.* (2021) carried out an experiment with Melissa officinalis (300 μmol m^{-2} s^{-1}, 16 h photoperiod) and determined that the quality of the light interacts with the genotype in the case of the thymol synthesis. In one of the studied genotypes, the maximum amount of thymol was recorded with the blue light treatment, while the highest accumulation was recorded by another treatment with the use of white light. The authors concluded that red light promoted the accumulation of monoterpenes in both genotypes, while blue light promoted the accumulation of sesquiterpenes. Meanwhile, the effects of white light were intermediate for both colors. Such differences can be the result of the unequal light intensity used in both experiments. Tohini *et al.* (2020) used a 300 μmol m^{-2} s^{-1} intensity, while the intensity used in this study was 12 times lower. For their part, Rios-Estepa *et al.* (2008) reported that changes in light intensity can influence some of the steps of the biosynthetic path of the different molecules that make up the essential oil.

Consequently, further research is need to evaluate the effects of light intensity, through different spectrum compositions, on the secondary metabolism of different species.

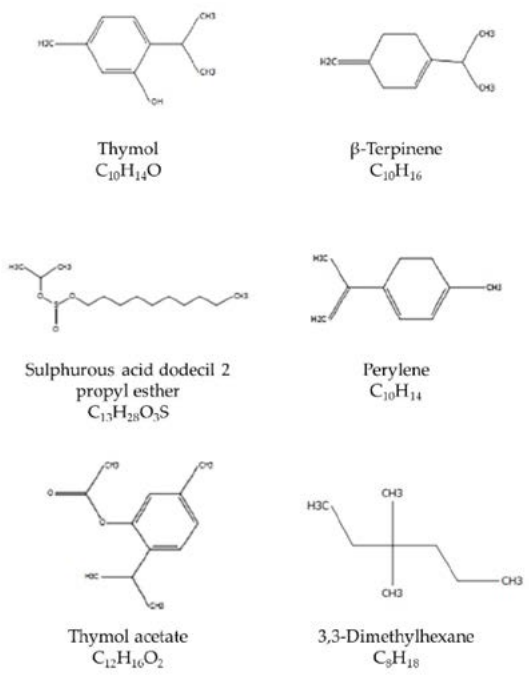

Figure 1. Structures of the molecules with higher concentration in the essential oil of thyme plants, grown under LEDs with different colors.

Table 1. Relative concentration of the main molecules found in the essential oil of thyme, grown with different LED colors.

<	R.T.* (min.)	Relative content (%)				
Molecule		**Blanco**	**Rojo**	**75R:25A**	**75A:25R**	**Azul**
Thymol	11.61	51.08 a**	43.98 b	52.26 a	38.56 b	41.42 b
β-Terpinene	7.01	21.55 a	23.13 a	21.13 a	16.44 b	14.42 b
Sulphurous acid dodecil 2 propyl esther,	29.42	14.72 b	13.9 b	6.4 c	17.75 a	18.24 a
Perylene	6.36	5.07	5.03	4.74	6.81	6.52
Thymol acetate	12.81	3.57 b	7.87 a	4.93 b	4.37 b	5.01 b
4-acetoxy-3-methoxystyrene	11.81	1.81	1.52	1.67	1.43	1.44
Hexadecane	29.18	-	-	-	-	14.28
3, 3-Dimethyilhexane	29.21	-	-	6.64 b	13.89 a	-
γ-Terpinene diepoxide	7.84	-	0.94 b	0.83 b	1.45 a	-

*RT (T.R): Retention time. **Values followed by similar letters in each row are not statistically different ($p \leq 0.05$). Relative content was calculated based on the areas of the curves obtained from the gas chromatography.

Most of the molecules found in the experiment recorded a low retention time in the column and only three of them reached a high value (>15 min). The blue and 75B:25R treatments recorded a higher ($p \leq 0.05$) relative concentration of sulfurous acid and dodecyl 2-propyl ester (Table 1). Hexadecane was only found in the blue monochromatic treatment; meanwhile, the highest 3, 3-dimethylhexane values were recorded when the blue light

ratio was higher. However, it was not identified in the monochromatic blue treatment. The results suggest that, under a monochromatic or a high ratio of blue light, the plants accumulate molecules of high retention time in the column. This effect has already been reported by Noguchi and Amaki (2016), who carried out experiments with *Plectranthus amboinicus*. In addition, these authors reported that red light promotes the synthesis of compounds with a low retention time in the column. These data match the findings of this research, but only in the case of β-terpinene and perillene. This response is related to the gene expression codified by different enzymes that take part in the biosynthetic path of terpenoids, which are positively regulated by blue and red lights (Fu *et al.*, 2015).

This study proves that modifying the composition of thyme essential oil is possible, if the plants are subjected to different spectra compositions, even at a low light intensity and for a relatively short period.

CONCLUSIONS

The changes in the quality of the light modify the composition of the thyme essential oil. Even at a low light intensity (25 μmol m^{-2} s^{-1}), the changes in the spectrum composition under which the plants grow influence the composition of the essential oil.

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