ITALUS HORTUS
Vol. 30 (2023), Pages 12-24
doi: 10.26353/j.itahort/2023.3.1224
Original Research Paper

Evaluating spearmint's morphological and physiological responses under different artificial lighting systems

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Received: 17 October 2023; Accepted: 21 December 2023; Published: 31 December 2023

Abstract: Indoor agriculture is becoming more relevant as a result of artificial lighting, which makes it possible to increase productivity, improve quality and cultivate where natural light is insufficient. The present study was conducted by growing spearmint inside a glasshouse under light emitting diode (LED), high pressure sodium (HPS), and light emitting diode assisted infrared (LED+IR) light treatments, while the control plants were grown outside the glasshouse under natural sunlight. Morphological analyses revealed that LED supplemental light performed better in terms of plant height, number of stems per plant, and fresh weight of the produce than all other tested light treatments, whereas HPS favored increased internode spacing. The analytical determinations revealed the outperformance of LED in terms of total carotenoids, total anthocyanins, and total sugar accumulation in spearmint leaves. Plants grown under both LED and LED+IR accumulated less nitrate than plants grown under HPS. HPS treated spearmint plants also showed a reduction in the total carotenoids and total sugar levels. Moreover, no significant changes were observed in lipid peroxidation (as measured by the thiobarbituric acid reactive substances, TBARS assay) among all treatments. On the other hand, control plants showed the highest phenolic index relative to the other light treatments which provided a brief overview of the effects of the light spectrum of artificial lighting, such as LED, HPS, LED+IR, and natural sunlight on spearmint growth, oxidative stress, and secondary metabolite production.

Keywords: LED; HPS; TBARS; Infrared (IR); total chlorophyll; nitrates

1. Introduction

The rapid growth of greenhouse technology has improved resource usage efficiency, environmental management, and high-tech applications. This reduces negative environmental consequences and results in crops that are more plentiful, of better quality, and consistently produced throughout the year (Nguyen et al., 2022). Several primary parameters can be managed in modern greenhouses, such as the quantity, quality, duration, and direction of light. For plants to grow and develop properly during winter or in northern regions, additional light is required to produce high-quality vegetables (Paradiso and Proietti, 2022). The most widely used light over time in greenhouses is high pressure sodium (HPS) lamps, which emit light in the yellow-orange-red region (between 550 and 650 nm) and are responsible to generate high temperatures that can cause heat injuries in plants. Moreover, HPS lamps do not offer spectral distribution limiting their efficiency and usage. This provides a reason for growers to switch to LED technology (Kuijpers et al., 2021).

LED lights are available in different monochromatic wavelengths and recipes where different wavelengths of the light spectrum, in combination, prompt plants to respond morphologically and physiologically. Photoreceptors respond to changes in the spectral composition of light and assist plants in sensing light intensity, light quality, light direction, and photoperiod (Teixeira, 2020). Phytochrome photoreceptors are responsible for detecting red light in plants, stimulating dry mass accumulation, stem length, and wider leaf area in many species. Red LED light allows plants to grow and complete their life cycle; however, when small amounts of blue light are introduced, plant growth and development increase considerably (Taiz and Zeiger, 2013).

Red (R) and blue (B) LED lights are the most effective for photosynthesis, and red light affects the growth of photosynthetic machinery (Fan et al., 2013). Far red (FR) light increases blooming in long-day plants, whereas low red/far-red ratios control stem elongation and branching, leaf expansion, and reproduction (Demotes-Mainard et al., 2016). B-LED light affects the stomatal opening, plant height, and chlorophyll biosynthesis (Wang et al., 2020). Moreover, green LED light (G), functioning from the chloroplast scale to the whole-plant level, can drive long-term development and short-term adaptation to light conditions. G-LED light goes through the leaf mesophyll layers deeply and reaches the lower and inner canopy levels, advocating photosynthesis in the deepest chloroplasts and in the least exposed leaves, and provides signals to react to the environmental irradiance, thereby increasing crop productivity and yield (Schenkels et al., 2020). In addition, the use of LED recipes coupled with infrared to assist the temperature variations in glasshouse was found to be pivotal to obtaining taller plants compared to the non-illuminated ones (Fang et al., 2020).

Mentha spicata L., a member of the Lamiaceae family, was developed by crossing *M. longifolia* with *M. rotundifolia,* and is found in the temperate and sub-temperate regions (Ali et al., 2023). Additionally, the biological functions of carvacrol, menthol, carvone, methyl acetate, limonene, and menthone found in the essential oil composition of spearmint are well known, which may lead to the market for it as a functional food ingredient, marking spearmint as an economically important crop (Sommano et al. 2022). In the present study, spearmint was grown in an experimental glasshouse under different supplemental lighting conditions, such as HPS, LED, and LED+IR, while a set of control plants were grown outside of the glasshouse. Various morpho-physiological parameters were considered when evaluating the effects of different artificial lights compared. The plant height, fresh weight, number of stems per plant, water content, and internode spacing were measured. Various analytical determinations such as chlorophyll *a* and *b*, total carotenoids, phenolic index, total anthocyanins and TBARS, were performed. Nitrate concentration and total sugar levels were also determined.

2. Materials and Methods

2.1. Experimental setup

The study was conducted in the glasshouse of the Faculty of Agricultural and Food Science, University of Milan, Italy in the months of June-July, 2022. Spearmint (*Mentha spicata* L.) was obtained from Ingegnoli nursery, Milan, Italy: identical rhizomes of 5 cm length were obtained from mother plants and used as planting material in order to reduce the plant variability and producing uniform seedlings for this trial. Seedlings were then transplanted in pots filled with a peat-based substrate and were divided into four groups, six pots each under different light treatments such as LED (Dutch lighting instruments: DLI DIODE-Series Top Lighting Fixture 400W), HPS (DLI JOULE-Series 6/750W Fixtures-HPS Agro Spectrum), LED supplemented with an infrared (LED+IR) (VORTICE Termologika Soleil System-1500W) in which the intensity was lowered to 30% of its nominal power load in order to avoid the possible heat damage to the spearmint plants.

The temperature and photoperiod were automatically managed in the glasshouse while the control was managed outside of the glasshouse under natural sunlight. Under each treatment, plants were fertilized twice during the experiment using slow released fertilizer (15% total N, 9%P_2O_5, 15% K_2O, 2%

MgO, 22.5% SO_3, 0.015 B, 0.002% Cu, 0.3% Fe, 0.1% Mn and 0.002% Zn), whereas no thinning was done throughout the experiment with an objective to record the plant height and fresh weight.

Greenhouse growth conditions were: mean temperature 24.3±0.03 °C, mean relative humidity 62.7±0.12%, and daily mean light intensity as 43.7±0.45 Wm^{-2}. All supplemental light treatments experienced the same environmental conditions inside the glasshouse. The growth condition for the control which was outdoor were: mean temperature 25.5±0.13 °C, mean relative humidity 48.6±0.35%, and daily mean light intensity as 269.94±6.77 Wm^{-2}. The difference can possibly be due to the shading effect in the greenhouse.

Spectral composition of the lamps is reported in Figure 1. In each supplementary light condition, photosynthetically active radiation (PAR) at plant level was around 55 µmol m^{-2} s^{-1}.

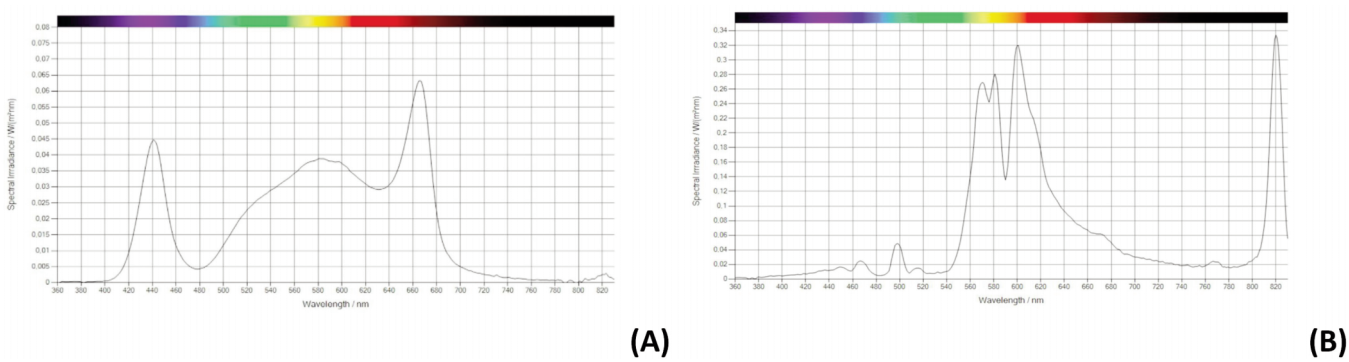

Figure 1. Spectral composition of the artificial light sources used in the experiment: (a) LED, (b) HPS.

The chosen IR fixtures was providing a very uniform heat distribution in order to cover all the crop area. Figure 2 shows the heatmap - at ambient temperature of 20 °C, relative humidity 48%, no airstreams).

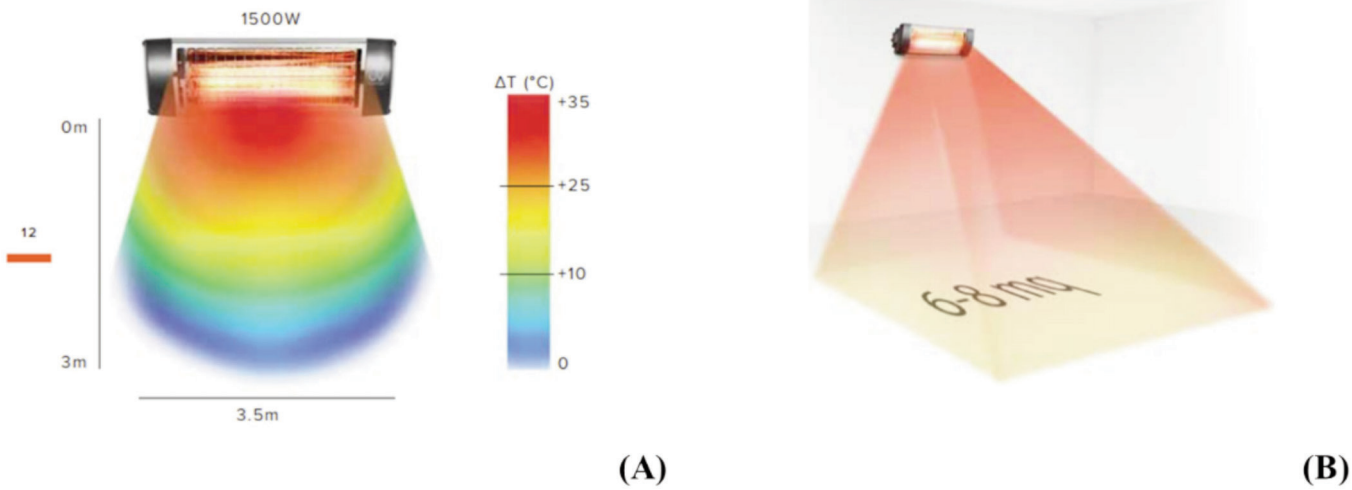

Figure 2. (a) Heatmap and (b) distribution of infrared used in the experiment.

2.2. Morphological Analyses

2.2.1 Plant height, fresh weight, water content, number of stems and internode distancing

Plant height was measured in centimetres (cm) by using a ruler from the base of the plant to the tip. Fresh weight was measured in grams (g) by using weighing balance by harvesting the total spearmint plant from each pot under each light treatment. Water content was measured as the percentage (%) of water loss during drying of samples in the oven. The number of stems were recorded manually while the internode distancing was measured in centimetres (cm) by using a ruler.

2.3. Analytical determinations

2.3.1. Chlorophyll (a + b) and total carotenoids

Total chlorophylls and carotenoids were extracted from the fresh matured leaf tissues (around 50 mg) in 5 mL of 99.9% methanol. The samples were kept in a dark room at 4 °C for 24 h. Absorbance readings were measured at 665.2 nm and 652.4 nm for chlorophyll pigments and 470 nm for total carotenoids. Chlorophyll and carotenoid concentrations were calculated using Lichtenthaler's formula (Lichtenthaler, 1987).

2.3.2. Phenolic index and total anthocyanins

For the extraction of the phenolic compounds, around 50 mg of matured leaves were placed in 5 mL of acidified methanol (1% HCl v/v) and extracted overnight in the dark. The phenolic index was calculated as the absorbance measured at 320 nm. The phenolic index was used as an indication of the total phenolics content. In this method, the total phenols were estimated by measuring absorbance at 320 nm using a UV-Vis spectrophotometer, as previously showed (Ke and saltveit, 1989). The total anthocyanins were measured from the same extracts. The concentration of anthocyanins was expressed as cyanidin-3-glucoside equivalents and determined spectrophotometrically at 535 nm using an extinction coefficient εM of 29,600 (Ferrante et al. 2004).

2.3.3. Nitrates and total sugars

The nitrate concentration was assessed based on Cataldo's method (Cataldo et al. 1975). Around 1 g of leaves was ground with 4 mL of distilled water. The extract was centrifuged (ALC centrifuge-model PK130R) at 4000 rpm for 15 min and the supernatant was recovered and used for the colorimetric determination of nitrate and sugars. Twenty µL of the sample was added to 80 µL of 5% salicylic acid in sulphuric acid (H_2SO_4) and to 3 mL of 1.5 N (NaOH) sodium hydroxide. The samples were cooled at room temperature and the spectrophotometric readings were done at 410 nm. The nitrate content was estimated based on a potassium nitrate (KNO_3) standard calibration curve (0-10 mM).

The extract used for total sugars was the same previously prepared for the nitrate determination. The total sugars concentration was assessed spectrophotometrically following the anthrone method (Yemm and Willis, 1954) with slight modifications. The anthrone reagent (10.3 mM) was prepared dissolving anthrone in ice-cold 95% H_2SO_4. In the next step, 0.5 mL of extract was placed on top of 2.5 mL of anthrone reagent and kept in ice for 5 min. the mix was vortexed vigorously and heated at 95 °C for 10 min and left to cool in ice. Readings were performed at 620 nm and total sugars concentration was calculated, based on a glucose calibration curve (0-4 mM).

2.3.4. Thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation was measured to estimate the possible oxidative damage of leaves subjected to different UV-B doses. This was assessed by using the thiobarbituric acid reactive substances (TBARS) method (Heath and Packer, 1968). Briefly, one gram of leaf tissue was ground in 5 mL of trichloroacetic acid (TCA) of 0.1% w/v and centrifuged (ALC centrifuge-model PK130R) at 4000 rpm for 10 min. One mL of the extract was mixed with 4 mL of 20% (w/v) TCA, 25 L of 0.5% thiobarbituric acid (TBA), and distilled water. After mixing, the extract was heated at 95 °C for 30 min in a Dubnoff bath (PID) and then cooled in ice. The absorbance at 600 nm was subtracted from the reading at 532 nm (as an index of non-specific turbidity) and the concentration of TBARS were expressed as malondialdehyde (MDA) equivalents (nmol g^{-1} F.W.), with the extinction coefficient εM = 155 mM-1 cm-1.

All spectrophotometric determinations have been performed using the Evolution 300 UV-Vis spectrophotometer (Thermo Scientific).

2.4. Statistical Analyses

Data were subjected to a one-way analysis of variance (ANOVA) followed by Tukey's post-test with multiple comparisons test (n = 6, for plant height, no. of stems/plant and internode spacing while

(n=3 ± S.E.) for fresh weight, water content, chlorophyll (a + b), total carotenoids, phenolic index, total anthocyanins, nitrates, total sugars and Thiobarbituric acid reactive substances (TBARS*)*. Analyses were performed using GraphPad Prism version 6 for Windows (GraphPad Software; La Jolla, California, USA, www.graphpad.com).

3. Results

3.1. Morphological Analyses

3.1.1 Plant height, fresh weight, water content, number of stems and internode distancing

A significant increased fresh weight was recorded under supplemental LED compared to all other treatments, while the control exhibited the lowest fresh weight among all the treatments (Figure 3A). Moreover, a significant reduction in plant height was observed for the control compared to all supplemental light treatments (Figure 3B). A significant increase in number of stems was found in LED compared to LED+IR and the control, while this increase was not significant in plants grown under HPS supplemental lighting (Figure 3C). However, both the LED+IR and control groups showed a non-significant reduction in the number of stems. Moreover, an increased internode spacing was observed under HPS supplemental lighting compared to both the control and LED+IR (Figure 3D), both of which experienced a reduction in internode spacing among all the treatments. However, no significant internode length was observed in plants under LED light treatment. No significant differences were recorded in water content among all treatments (Figure 3E).

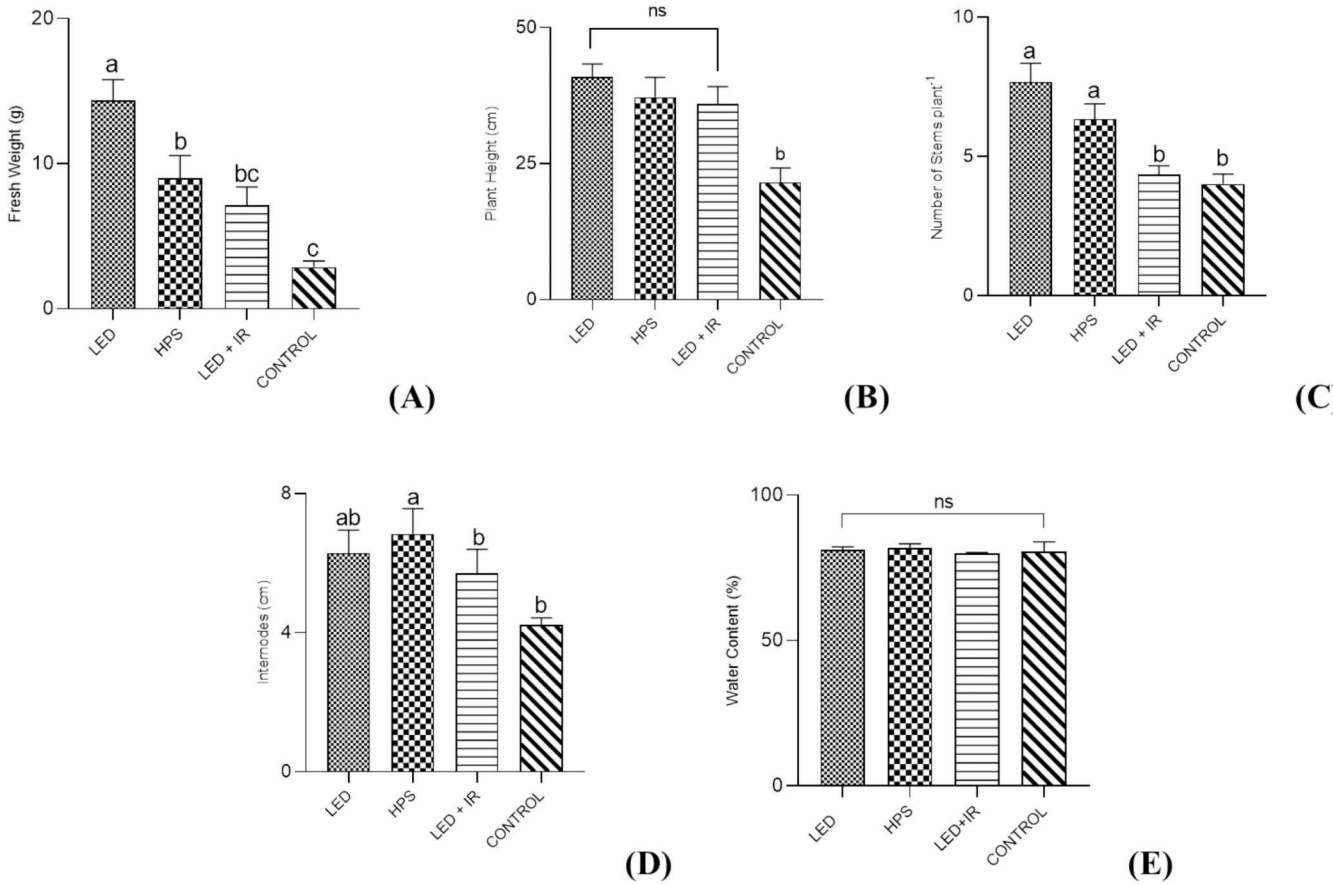

Figure 3. Fresh weight (A) plant height (B) number of stems/plant water content (C) internode length (D) and water content (E) in spearmint plants treated with supplemental LED, HPS and LED+IR lighting and control. Values are means ± S.E. (n=6 for plant height, no. of stems/plant and internode spacing; n=3 for fresh weight and water content). Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p<0.05$).

3.2. Analytical determinations

3.2.1. Chlorophyll (a + b) and total carotenoids

No significant differences with minor variations in total chlorophyll accumulation were observed among all the supplemental light treatments (Figure 4A). In terms of total carotenoids, control plants accumulated significantly higher carotenoids compared to HPS lighting however, it was non-significant compared to LED+IR and the LED treatment (Figure 4B).

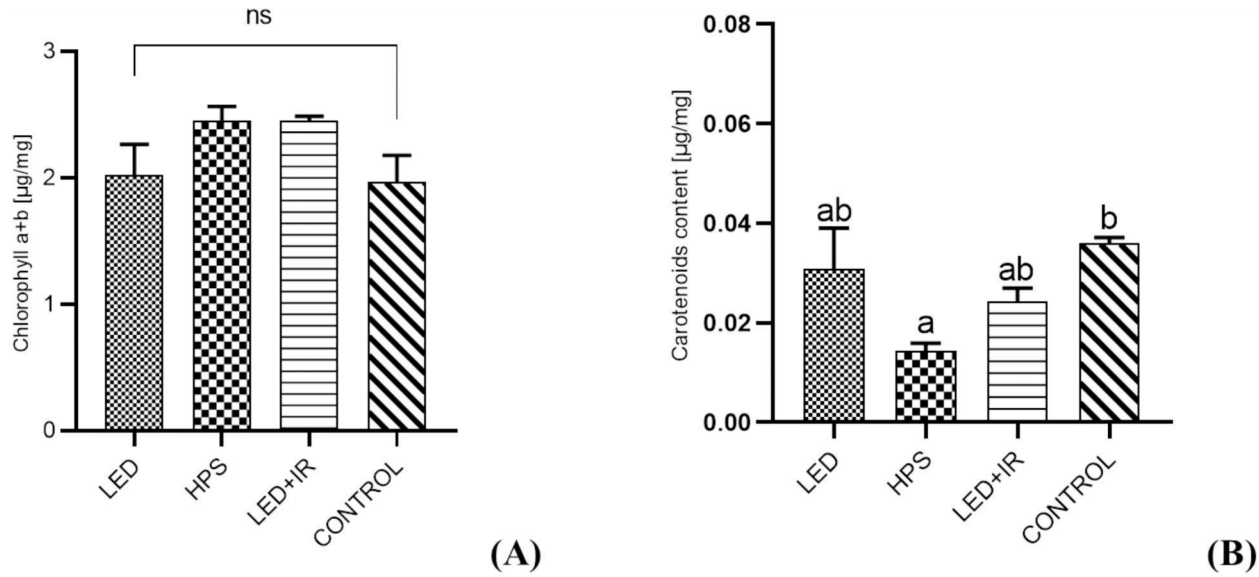

Figure 4. Chlorophyll a + b (A) and carotenoids contents (B) in spearmint plants treated with supplemental LED, HPS and LED+IR lighting and control. Values are means (n=3) ± S.E. Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p<0.05$).

3.2.2. Phenolic index and total anthocyanins

A significantly increased phenolic index was recorded in the control compared to LED+IR plants (Figure 5A), while no differences between treatments was found for anthocyanins (Figure 5B).

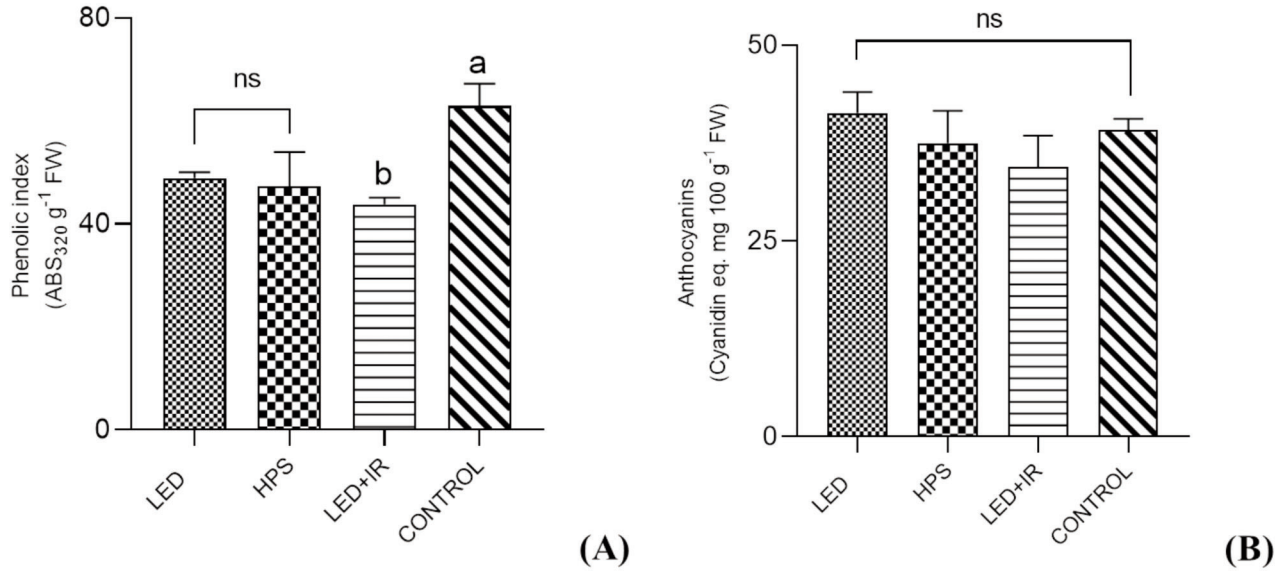

Figure 5. Phenolic index (A) and anthocyanins content (B) in spearmint plants treated with supplemental LED, HPS and LED+IR lighting and control. Values are means (n=3) ± S.E. Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p<0.05$).

3.2.3. Nitrates and total sugars

HPS supplemental lighting was responsible for the significantly increased nitrate accumulation in spearmint compared to LED+IR light treatment (Figure 6A). These increases in nitrate concentrations, however, were non-significant between LED and control plants, whereas the latter produced more nitrates than LED+IR plants (Figure 6A). Unlike nitrates, total sugars were significantly higher in plants exposed to LED light compared to the other treatments (Figure 6B).

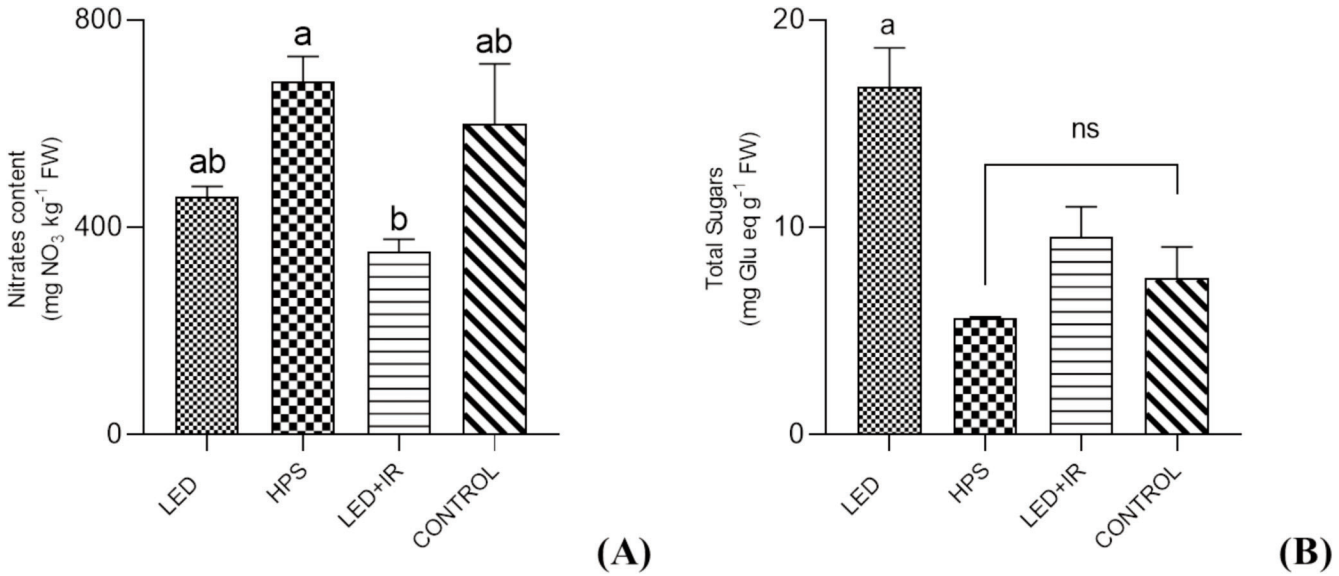

Figure 6. Nitrates (A) and total sugar (B) in spearmint plants treated with supplemental LED, HPS and LED+IR lighting and control. Values are means (n=3) ± S.E. Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p<0.05$).

3.2.4. Thiobarbituric acid reactive substances (TBARS)

No significant differences were found in TBARS values between all treatments (Figure 7).

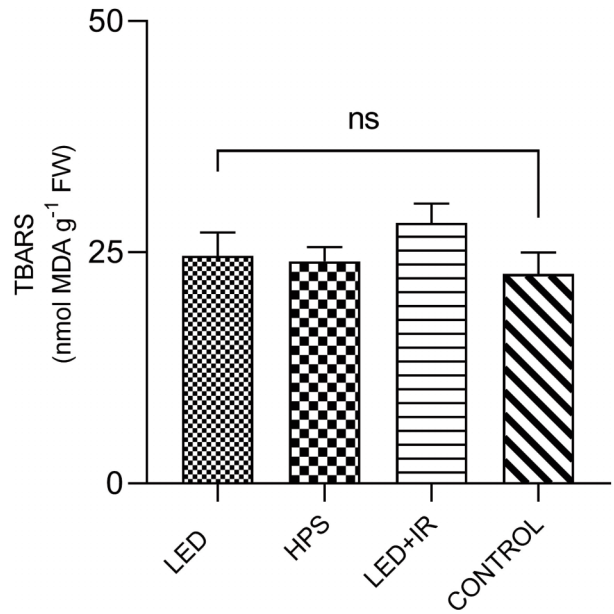

Figure 7. Thiobarbituric acid reactive substances (TBARS) values of spearmint plants treated with supplemental LED, HPS and LED+IR lighting and control. Values are means (n=3) ± S.E. Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p<0.05$).

4. Discussion

Light is an essential environmental factor for plants and plays an important role in the regulation of growth, morphology, and metabolism (Samuolienė et al., 2021). In contrast to natural light, all supplemental lighting in this experiment improved plant height, fresh weight, water content, number of stems per plant, and internode length in spearmint plants. Supplemental light is known to increase the chlorophyll content in leaves and possibly alter photosynthesis which can be the reason for the increase in elongation and in fresh and dry weight in the plant (Rahman et al., 2021). The fresh weight of lettuce has been positively influenced by monochromatic R-LED light and makeup of the R and B spectra which is proven to fit well with the absorption spectra of chlorophyll and carotenoid pigments possibly aiding in spearmint photosynthesis and improved morphology compared to the control (Lin et al., 2021). Moreover, Christaens et al. (2019) suggested that adding B to the R+FR LED spectrum is responsible for photomorphogenesis in crops otherwise, blue is added to the R-LED instead of the R+FR recipe. Contrary to our findings, Hernández and Kubota (2015) found that shoot dry weight, fresh weight, and leaf number in the HPS treatment were 28%, 28% to 32% and 9% to 12% higher than in the LED treatments. According to them, these discrepancies could be due to the greater canopy air temperature identified in the HPS treatment, which was induced by the stronger infrared radiation of the fixture.

Non-significant increases in chlorophyll a and b were observed under supplemental LED+IR followed by HPS, control, and LED lighting. All treatments which showed higher contents in chlorophyll a and b supposedly had higher temperatures compared to the LED supplemental lighting, a finding which is contradictory to those reported by Shin et al. (2020), who observed a drop in the chlorophyll a and b in the leaves of tomato plants exposed to high temperatures due to chlorophyllase activity. Moreover, increased temperature was reported to result in early maturity, leaf expansion, stem elongation, and thickening (Ohtaka et al., 2020). In this study, although stem elongation, thickness, and early maturity were not measured, it can be assumed that the plants under these treatments were in early generation cycling compared to the cold property of LED lighting, and hence possessed thicker and elongated leaves (not recorded) which may be responsible for the increased chlorophyll a and b in spearmint leaves. Carotenoids are important secondary metabolites that help plants absorb excess energy for photosynthesis and assist in photoprotection by non-photochemical quenching (Carvalho et al., 2011). Carotenoids were previously studied by Ammozgar et al. (2017), who suggested that, when plants are exposed to R, B, or in combination with LEDs, there is a potential increase in carotenoid pigments. Extensive research has been carried out to investigate the effects of LEDs on carotenoid accumulation and manipulation (Carvalho and Folta, 2014). It is also important to remember that carotenoid content is typically not altered by light intensity, which was previously proved by Camejo et al. (2020) and Grzegorzewska et al. (2023). Ali et al. (2023) also reported an increment in total carotenoids when lettuce was exposed to continuous LED light with an average PPFD of 150 μmol s^{-1} m^{-2}, photoperiod of 16 h and growing cycle of 30 days. Moreover, the HPS lighting in this study failed to accumulate a considerable amount of carotenoids, possibly because the spectrum of HPS has little or no wavelength in the blue region of spectrum which plays a key role in the production and accumulation of these accessory light pigments.

Secondary metabolites, such as phenolic compounds and anthocyanins, are used by plants to protect themselves against a wide range of biotic and abiotic stresses, in addition to helping them to produce distinct hues (Sgherri et al., 2017). Previous studies have confirmed the role of LEDs in triggering the accumulation of phenols and anthocyanins in different crops (Soufi et al., 2023; Bian et al., 2018); however, the present study is in line with the anthocyanin accumulation in spearmints under LED lighting, whereas the phenolic index lagged behind the control. This increase in the phenolic index in the control might be due to the fluctuating environmental conditions outside the glasshouse, which were perceived by spearmint plants as stress and hence resulted in an increased phenolic index accumulation. Furthermore, anthocyanins in the control were also notable after LED lighting which also suggests that

external conditions were somewhat challenging for spearmint during the summer cycle. Artificial lights such as HPS and LED+IR supposedly increased temperatures which allowed the plant to grow morphologically in terms of the number of stems, plant height, and fresh weight, compared to the control plants, but lagged behind in the production of these secondary metabolites. Moreover, LEDs, such as B and R spectra have been previously shown to increase the content of flavonoids and phenolic compounds in cucumber and *R. hongnoensis* plants (Oh et al., 2021; Palma et al., 2022). According to Landi et al. (2020) the increase in the amount of most phenolics and anthocyanins under LED treatments could be mediated by an increase in phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and dihydroflavonol-4-reductase (DFR) through cryptochrome (a blue/UV-A light photoreceptor) in the biosynthetic pathways of flavonoid/phenolic metabolites. This may be the reason for the increased phenolic index and anthocyanin accumulation under supplemental LED lighting in addition to the control spearmint. In this study, lower nitrates as well as high total sugars were found in the plants of both LED and LED+IR treatments which confirmed that LED is beneficial in lowering the nitrates and elevating the sugar levels in spearmint. Similar findings were previously reported in leafy vegetables such as tatsoi (Simanavičius and Viršilė, 2018), basil (Piovene et al., 2015), spinach (Ohashi-kaneko, 2007), rocket (Signore et al., 2020), and lettuce (Bian et al., 2020). Soluble sugars serve various functions in plant cells, including critical components of energy and biosynthetic processes. However, they can also function as complimentary osmolytes, preserving osmotic balance, and as protective macromolecules (Gurrieri et al. 2020). Likewise, in the present study, Soufi et al. (2023) also reported an increased total sugar in lettuce when subjected to R/B and monochromatic R-LED spectra, and proposed that different light conditions influence sugar levels by modulating the activity of enzymes involved in sucrose metabolism. Additionally, subjection to different combinations of R and B LEDs in lettuce, such as 70/30%, 80/20%, and 100% red light combinations were found to be effective in increasing the sugar level (Chen et al., 2021), which further confirmed the results of increased sugar accumulation under supplemental LED and LED+IR. Increased membrane damage may result from an increased canopy temperature of the crops, which might result in elevated malondialdehyde (MDA), a byproduct of lipid peroxidation in membranes. Higher temperatures are known to accelerate membrane integrity, which results in increased electrolyte leakage, apart from the production of reactive oxygen species (ROS); hence, it causes a rise in TBARS values in tomato leaves (Natalini et al., 2014). Although non-significant in the present study, a marked increase in the TBARS values was recorded under LED+IR which confirmed previous findings. In addition to higher TBARS values under LED+IR, decreased TBARS values under supplemental LED and control might possibly be due to increased accumulation of phenols and anthocyanins, which prevented the production of ROS, helped maintain intact membrane integrity, and eventually resulted in lower TBARS values.

However, conducting a one-year experiment on supplementary lighting in a greenhouse introduces several limitations that may compromise the comprehensive understanding of the treatment effects. The inherent seasonal variability in greenhouse conditions, may not be fully captured within this timeframe. Additionally, the short growth cycles of the chosen crops might not align with the one-year observation period, limiting the study's applicability to plants with more extended life cycles. Also, there is a need to further evaluate the economical and environmental aspects of these artificial lighting systems both in glasshouses or growth chambers considering the increasing energy costs, which led the researchers to opt alternatives in designing researches using the pulsed LED mode and the moving LED lights, both vertically and horizontally to the plants. The present study proved that LED outperformed LED+IR treatment. This was possibly due to the heat generated under LED+IR supplemental lighting which was otherwise for LED which serves as the cool lighting option in agricultural operations. Moreover, the distance from the source, age of the plants and considerably the choice of the plant species should also be given importance while conducting lighting experiments. Hence a more prolonged and comprehensive investigation is essential to overcome these limitations and provide a more nuanced understanding of the benefits and challenges associated with supplementary lighting in greenhouses.

5. Conclusions

This study showed that different supplemental lighting has the potential to help plants promote various morphological and physiological indices. Although the LED was responsible for an increased plant height, fresh weight and number of stems per plant, HPS lighting was found second to LED in terms of all these parameters compared to the control and LED+IR lighting; however, HPS led to internode distancing among all the treatments. Natural sunlight was responsible for the increased phenolic index in control plants in this experiment, which can be triggered by various outdoor fluctuating parameters. The plants under LED, however, gained more anthocyanins, and increased total sugars. Nitrate concentrations were considerably lower under both LED treatments than their counterparts. None of these artificial lights influenced lipid peroxidation in a considerate manner, yet high temperature under LED+IR showed a slightly higher TBARS value among all the treatments.

Funding: This research received no external funding.

Conflicts of Interest: Author Piero Santoro is employed by the company MEG Science while Jacopo Mori is employed by ALMECO. The remaining authors declare no conflict of interest.

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