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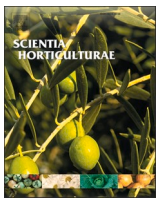

# Quality and physiological evaluation of tomato subjected to different supplemental lighting systems

Awais Ali [a,1,*], Viviana Cavallaro [a,1,*], Piero Santoro [b], Jacopo Mori [c], Antonio Ferrante [a], Giacomo Cocetta [a]

[a] Department of Agricultural and Environmental Sciences, Universita Degli Studi Di Milano, via Celoria, 2, Milano, MI 20133, Italy
[b] MEG Science., via Aleardo Aleardi 12, Milano, MI 20154, Italy
[c] ALMECO S.p.A., Via della Liberazione 15 – 20098 Civesio di S. Giuliano, Milanese, MI, Italy

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ABSTRACT

Light is a crucial mediator in plants of growth and development, secondary metabolism, and signaling of potentially beneficial phytochemicals. The present study investigated three indoor supplemental light treatments, HPS, LED, and LED+IR, applied during tomato cultivation, in which non-destructive and destructive analyses were performed at two time points to record the physiological responses and effects of these artificial lights on the growth and quality of the produce. An infrared thermometer and thermal cameras were used throughout the experiment to record temperature changes under each growing condition. LED+IR supplemented light showed a decrease in photosynthetic pigments, such as chlorophyll *a, b*, and carotenoids but induced an increased number of flowers, heavier tomato fruits, enhanced anthocyanins, phenolic index, lipid peroxidation, titratable acidity, and reduced nitrate accumulation. Moreover, higher generated temperatures under LED+IR helped tomato plants to reduce white fly infestation, but suppressed plant height. HPS light performed better than both LED and LED+IR in terms of total sugar accumulation, total carotenoids, water content, plant height, and leaf lipid peroxidation at harvest. Lycopene, ß-carotene, brix index were remarkable under HPS lighting but under the same conditions the number of whiteflies, however, was the highest in HPS among all the tested light treatments. In terms of fruit color analysis, maximum redness (a*), reduced hue angle, and chroma were observed under LED+IR while LED lighting in brightness (L*), and HPS in yellowness (b*) were prominent. However, LED in particular was insufficiently effective in acquiring any possible physiological and qualitative characteristics of tomato plants and fruits for the observed time span of the experiment. Hence, LED+IR has been shown to boost the accumulation of bioactive chemicals, improve fruit quality, promote more rapid and early flowering in tomato plants, and can serve as an efficient replacement for traditional indoor illumination.

## 1. Introduction

Climate change, reduction in fresh water supply, expansion of drylands, and a continuously growing population (expected to reach 9.6 billion by the end of 2050, according to FAO, 2016) have made farmers increasingly interested in controlled environmental cultivation. This approach can help to achieve higher food production and quality by regulating various factors that affect plant development (Benke and Tomkins, 2017; Marcelis et al., 2019). Greenhouse horticulture is an important agricultural indoor system that enables the efficient control of different parameters (such as light and temperature) and the effective use of vital resources (*e.g.*, water and fertilizers) to produce high-quality ornamental, vegetable, medicinal, and officinal plants. In 2019, the area occupied by greenhouses was estimated to be approximately 496,800 hectares in eight countries, namely China, Spain, South Korea, Japan, Turkey, Italy, Morocco, and France, with a market value of approximately 30 billion US dollars (Koukounaras, 2021; Krishna, 2022). Although the high initial cost of greenhouse technology is one of the biggest concerns for farmers, controlled environment farms have higher yields per unit area (Hemming et al., 2019).

The quantity, quality, duration, and direction of light are among the main factors that can be controlled in a greenhouse. During winter or in

* Corresponding authors.
*E-mail addresses:* awais.ali@unimi.it (A. Ali), viviana.cavallaro@unimi.it (V. Cavallaro).
[1] The first two authors contributed equally to the work.

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countries located in northern climates, supplemental light is needed to ensure proper plant growth and development, and to obtain high-quality vegetables (Ouzounis et al., 2015; Paradisoand Proietti, 2022). High pressure-sodium (HPS) lamps have been the most commonly used system in greenhouses since the 1930s, but they have limitations. HPS lamps do not allow for spectral distribution variety, emitting only in the yellow-orange-red region (between 550–650nm), and they generate high temperatures (over 200°C), which can cause heat injuries to the plants. Additionally, their commercial life is generally low (no more than one year) (Kuijpers et al., 2021). A possible new technology with potential advantages is a lighting system that uses solid-state light--emitting diodes (LEDs). LED lamps generate less heat than HPS lamps, reducing heat stress in plants and allow for the control of light spectral composition (*i.e.*, blue, green, red, and far-red wavelengths) with different benefits for plant quality and vegetable production (Olle and Viršile, 2013; Monostori et al., 2018). Although the cost of installing LED systems is initially higher than HPS, they reduce energy consumption by up to 70% and have a longer shelf life (2–3 times more) with less maintenance requirements (Mitchell et al., 2012; Singh et al., 2015; Kuijpers et al., 2021).

Temperature is also a crucial factor in greenhouse cultivation, especially in countries with cold or mild winters, where an adequate heating system is required to sustain plant growth and production. Various heating methods available, including the circulation of hot water in pipes, forced air, concrete floor heating, or systems able to heat directly at the hypogeal part of plants (Bartzanas et al., 2005; Perdigones et al., 2006; Reiss et al., 2007; Vasilevska et al., 2011; Nawalany and Sokołowski, 2019), however, all of these methods aim to maintain the correct temperature to support plant growth. Over the past two decades, the use of infrared radiation to heat greenhouses has been studied (Kavga et al., 2012; Nawalany and Sokołowski, 2019) which is inspired by the sun's action, where heat from a source (typically composed of a burner box and a booster fan) travels through the air, eventually warming the surface and surrounding space. Compared to other methods, infrared heating has two main advantages: it is highly directional and zone-specific, and causes fewer energy losses, resulting in a nearly 50% reduction in energy costs and efficient maintenance of optimal environmental conditions compared to traditional heating systems. This contributes to uniform plant growth and the production of high-quality final products while also suppressing pests and diseases (Kavga et al., 2015).

The tomato (*Solanum lycopersicum* L.) production industry is among the largest and most advanced industries in the world. In 2021, FAOSTAT reported a total production of 123 million tons of tomatoes distributed over 4.5 million hectares. In the coming years, significant growth in tomato production in greenhouses is expected because of the availability of better conditions, shorter maturity rates, and higher and more consistent productivity per unit area, which can be more easily achieved in a controlled environment than in open fields (Tao et al., 2016; Gatahi, 2020; Maureira et al., 2022). Tomatoes are among the most widely consumed vegetables in the human diet and serve as a source of minerals, vitamins, and antioxidant compounds (Ali et al., 2020; Lima et al., 2022). Therefore, it is therefore essential to identify innovative methods that can promote the production of this fruit while maintaining or enhancing the biosynthesis of important quality related compounds.

In the present study, tomato plants were grown in a greenhouse under three different light conditions: HPS, LED, and LED with thermal infrared supplementation. The primary aim was to determine how different lighting systems affected not only plant growth, biomass, and metabolism (both primary and secondary), but also the quality and yield of the fruits produced.

## 2. Material and methods

### 2.1. Experimental set-up

The study was conducted from February to July 2022 in the greenhouse of the Faculty of Agricultural and Food Science, University of Milan, Italy. Tomato seeds (*S. lycopersicum* L., var. Leader F1) were sown as one seed per pot in seed tray with peat-based soil. After a month, eighteen plantlets were transferred in vase (diameter 22cm, height 20cm) and were divided in three group, based on different light treatments such as LED, HPS, and LED supplemented with an infrared (IR) heat source. The light distributions of the luminaires can be seen in Fig. 1. 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 $Wm^{-2}$.

Spectral composition of the lamps is reported in Fig. 2. In each condition, light intensity at plant level was around 55 μmol $m^{-2}$ $s^{-1}$. Plants status was checked daily, and *in vivo* analyses were conducted once a week for a total of 20 weeks starting from the transplants, between March and July 2022. Sampling for destructive analyses were done at two different time points: one on April 27 (T1), before the appearance of fruits, and a second one on July 27 (T2) at the end of the experiment (harvest).

The chosen IR fixtures provided an even heat distribution to cover the entire area of the crop. The heat generated by the infrared source has been managed with the addition of a dimer. For this study it was kept at minimum throughout the experiment. The heat map for the infrared source used can be seen in Fig. 3.

### 2.2. In vivo analyses

#### 2.2.1. Chlorophyll content, flavanols, anthocyanins, nitrogen flavanol index, and chlorophyll a fluorescence

*In vivo* levels of chlorophyll, flavanols, anthocyanins, and the Nitrogen-Flavanol Index (NFI), which is an indicator of the nitrogen nutritional status of the plants, were determined using the multipigment meter MPM-100 (ADC BioScientific Ltd.). Additionally, chlorophyll *a* fluorescence was measured using the Handy-PEA handportable fluorimeter (Hansatech Instruments). Prior to the measurements for fluorimeter, the leaves were dark-adapted using leaf clips (4mm in diameter) for 30–40 min and then exposed to saturating light (3000μmol $m^{-2}$ $s^{-1}$) for 1 s, provided by an array of three high-intensity light-emitting diodes. The JIP test calculation was used to derive the parameters that provide information on the structural and functional status of the photosynthetic apparatus, including the maximum quantum efficiency of photosystem II (Fv/Fm), the performance index (PI), the time intercourse to reach maximum fluorescence (Tfm) in milliseconds (ms), the area (a parameter proportional to the number of electrons transferred by the reaction centers to QA during photosynthesis), and the dissipation of heat per reaction center (DIo/RC). Please see the supplementary files (Fig S1 and Fig S2)

#### 2.2.2. Thermal images acquisition and thermal detections

Thermal images were taken between 11 a.m. and 2 p.m. (when the stomatal conductance remains most constant) using an infrared camera (FLIR C2) from approximately 120cm from the plants after the instrument was left in the growth chamber for two hours to calibrate to the thermal conditions of the environment (James and Sirault, 2012). The FLIR tools software as represented in Fig. 4, was used to evaluate the temperatures of the leaves, vase, and bench under different light treatments based on ten random points for each.

Additionally, the temperatures of the soil, leaves, pots, and bench under LED, HPS and LED+IR were evaluated simultaneously based on ten random points using an infrared thermometer.

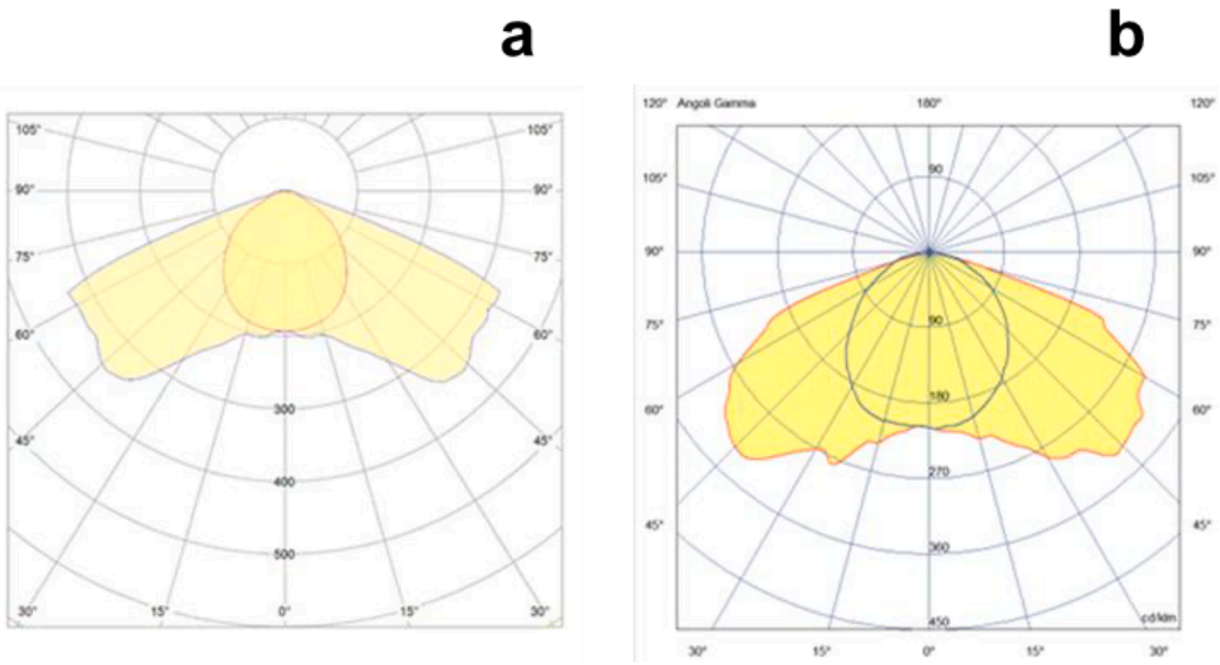


**Fig. 1.** Lighting distributions of the luminaires (a) LED and (b) HPS lighting.

### 2.3. Destructive analyses

#### 2.3.1. Chlorophyll (a+b) and total carotenoids concentration

To extract chlorophylls and carotenoids, leaf disk samples (5mm diameter, 30mg FW) from each condition were immersed in 5mL of 99.9% (v/v) methanol and left in a dark room at 4°C for 24 h. The levels of pigments were calculated using Lichtenthaler's formula based on absorbance readings taken at 665.2 and 652.4nm for chlorophylls and 470nm for total carotenoids. The results were expressed as μg of pigments per gram of fresh weight (FW), and the analysis was conducted in biological triplicate (Lichtenthaler, 1987). Results are expressed as μg of pigments $g^{-1}$ FW. Analysis was conducted in biological triplicate.

#### 2.3.2. Phenolic index and total anthocyanins concentration

Total phenols and anthocyanins were determined from leaf disk samples (5mm diameter, 30mg FW) for each condition. The leaf samples were kept in a tube containing 3mL of methanol acidified with hydrochloric acid (1% v/v) for 24 h at 4°C. Absorbance readings were measured at 320nm for total phenols and at 535nm for anthocyanins using a spectrophotometer. The phenolic index was expressed as Abs320 nm $g^{-1}$ FW, while the concentration of anthocyanins was expressed in mg cyanidin-3-glucoside equivalents per 100g of FW, using a molar extinction coefficient (ε) of 29,600L $M^{-1}$ $cm^{-1}$ (Klein and Hagen, 1961; Ke and Saltveit, 1989). Analysis was conducted in biological triplicate.

#### 2.3.3. Total sugars concentration

Around 1g of leaves was ground with 5mL of distilled water. The extract was centrifuged (ALC centrifuge-model PK130R) at 4000rpm for 15min and the supernatant was recovered and used for the colorimetric determination of nitrate and sugars. Total sugars were then determined from the extract using the anthrone method with slight modifications. Anthrone reagent was prepared and mixed with the extract before being heated and cooled. Readings were performed at 620nm and a glucose standard solution was used for calibration. Analysis was conducted in biological triplicate. The experiment aimed to determine the nitrate and total sugar content of the samples (Yemm and Willis, 1954). Calibration curve (0–4mM) was carried out using a glucose standard solution. Analysis was conducted in biological triplicate.

#### 2.3.4. Nitrate concentration

To extract fresh leaf tissue, distilled water was used at a ratio of 1g of tissue per 5mL of water as mentioned above in nitrates. The resulting homogenate was centrifuged at room temperature (RT) and the supernatant collected for colorimetric analysis. A portion of the extract was mixed with 5% salicylic acid in concentrated $H_2SO_4$, followed by the addition of 1.5N NaOH. The resulting mixture was allowed to cool before measuring absorbance at 410nm to calculate nitrate content using a $KNO_3$ standard calibration curve (0–10mM). Nitrate concentration was expressed as mg of $NO^{3-}$ $kg^{-1}$ of FW (Cataldo et al., 1975). Analysis was conducted in biological triplicate.

#### 2.3.5. Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) were measured to determine lipid peroxidation levels (Heath and Packer, 1968). One g of leaf tissue was homogenized with 5mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged (ALC centrifuge-model PK130R) at 4500rpm for 10min at RT. For the TBARS assay, 1mL of the supernatant was mixed with 4mL of 20% (w/v) TCA, 25 μL of 0.5% thiobarbituric acid (TBA), and incubated in a water bath at 95°C for 30min. After cooling the samples on ice, they were centrifuged at 4000rpm for 10min, and the optical density was determined at 532 and 600nm. The absorbance at 600nm was subtracted from the absorbance at 532nm (to eliminate non-specific turbidity), and the concentration of TBARS was calculated using the Lambert-Beer law with an extinction coefficient εM = 155 $mM^{-1}$ $cm^{-1}$. The results were expressed as malondialdehyde (MDA) equivalents (nmol $g^{-1}$). Analysis was conducted in biological triplicate.

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

### 2.4. Analyses on fruit yield and quality

#### 2.4.1. Fresh fruit weight, yield and color of the fruits

The quantity of fruit produced was determined by tallying and weighing fully ripened tomato fruits obtained from each experimental condition throughout the trial until July 27th 2022. Tomato yield (total production per treatment Kg/plant) has been monitored for a limited time-lapse (from May to July 2022) and for this reason, these data can be considered for making a comparison among plants grown under

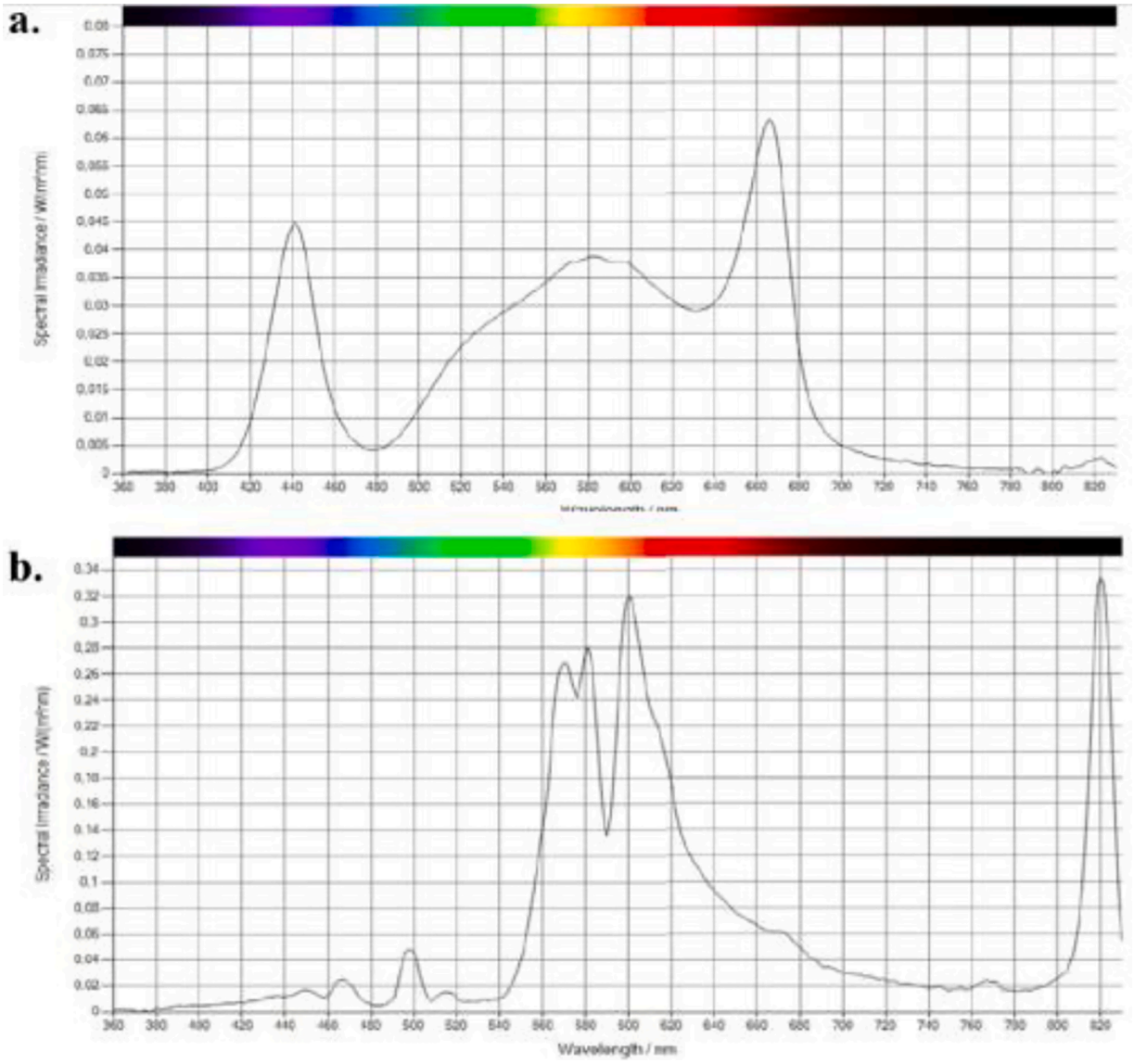


**Fig. 2.** Spectral compositions of the artificial light sources used in experiment. (a) LED (b) HPS.

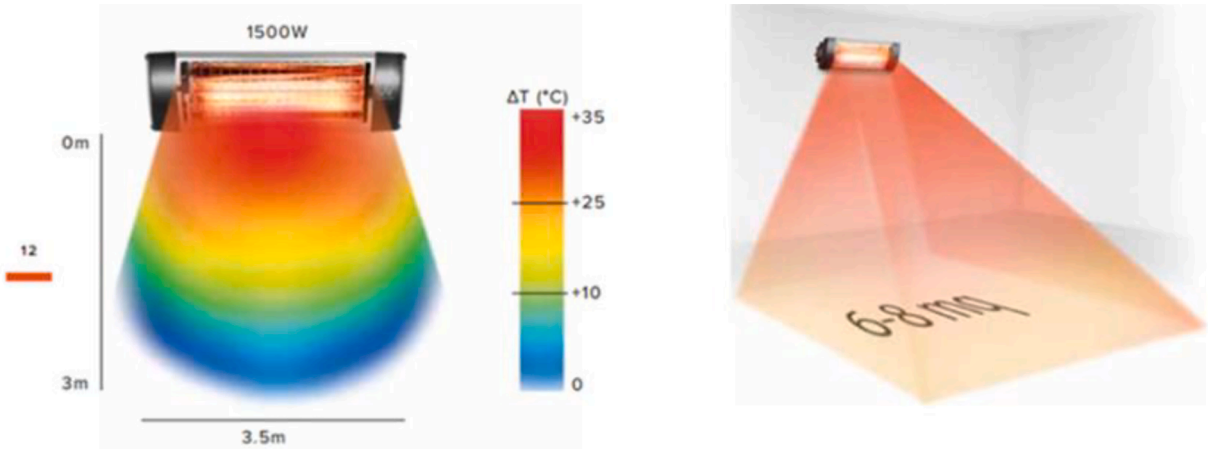


**Fig. 3.** The heatmap of infrared at ambient temperature of 20°C, relative humidity 48% with no airstreams.

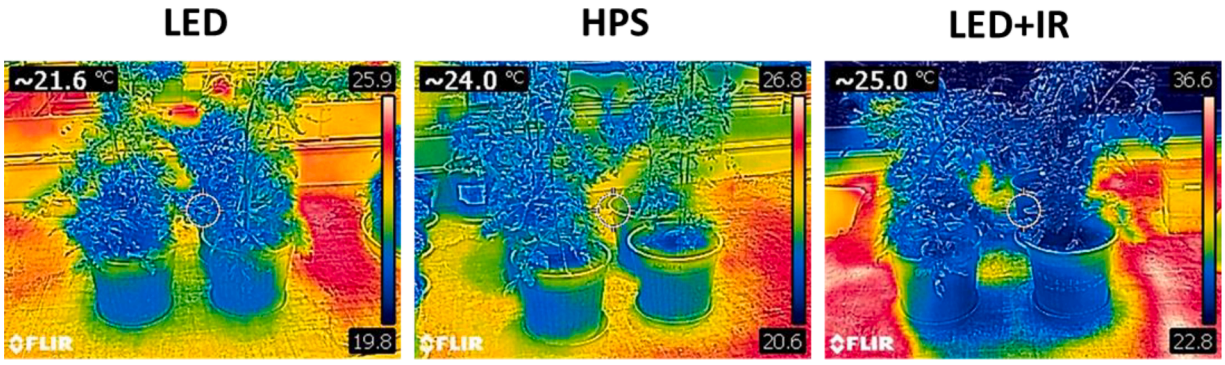


**Fig. 4.** FLIR tools software to evaluate the temperatures of the leaves, vase, and bench under different supplemental lighting conditions based on ten random points.

different lighting treatments, while they are not representative of the actual plant's productivity/yield. Additionally, the red color (a*), yellow color (b*), lightness (L*), hue angle ($H$=arctan(b*/a*)), and chroma [C*= sqrt ($a^2$+$b^2$)] values were assessed using a Minolta Chroma meter (CR-300 with an 8-mm aperture) on fifteen tomato fruits from each condition. The instrument was calibrated using a standard white tile.

#### 2.4.2. Lycopene and β-carotene determination

Three tomatoes from each group were individually weighed and homogenized together with a mixer. Then, 0.1 - 0.2 gs of the resulting mixture were measured and placed into plastic tubes that were covered with aluminum foil to prevent exposure to light. Lycopene and β-carotene were extracted from the samples using the method described by Sadler et al. (1990). Specifically, 8mL of hexane–acetone–ethanol (HEA, 2:1:1, v: v: v) were added to each sample, and the mixture was vortexed. After 10 min, 1mL of distilled water was added to the mixture. The solution was left to separate into polar and non-polar layers, and the absorbance of the hexane layer was measured at 444nm (β-carotene) and 503nm (lycopene) using a spectrophotometer.

#### 2.4.3. Titratable acidity and total soluble solids content

Juice extracted from four fruits was used to measure the titratable acidity (TA) and total soluble solids (TSS). The TA was determined by titrating 5g of juice with 0.1N sodium hydroxide until it reached a pH endpoint of 8.1, and the results were expressed as a percentage of citric acid. The titration was conducted using a Titrator Compact G10S (Mettler, Toledo, USA). On the other hand, TSS were estimated using a portable digital (model 53,011, Turoni, Italy) and expressed as °Brix.

### 2.5. Plant height and water content

For plant height, measurements were taken using a ruler from base of the stem to the tip of the plant, in order to determine total height of the tomato plants under each supplemental light treatment. The water content, expressed as a percentage (%), was estimated by allowing three plants from each condition to dry in an oven for five days at a temperature of 105°C. After this drying period, the dry weight (DW) of the plants was measured, and the water loss (WL%) was calculated as follow:

$$WL\% = 100 - \left(\frac{FW}{DW}\cdot 100\right)$$

Additionally, plant height was measured for three plants in each condition at the end of the experiment (T2).

### 2.6. Whitefly (Aleyrodidae) monitoring

Adhesive fly trap sheets were used to record the number of white fly infestation under different light treatments of HPS. LED and LED+IR. The total of 3 fly trap sheets per light treatment in the month of June 2022 for 3 consecutive weeks, were mounted above the plants and area of 10 square centimeters was drawn on front and back side of sheet to manually count the number of flies within the square box. The traps were changed weekly. Please see the supplementary image (Fig. S3).

### 2.7. Statistical analysis

Data are reported as mean ± standard error (S.E.) of the mean of the analyses (both destructive and non-destructive) that corresponded to the sampling time points. The statistical analysis was performed using GraphPad Prism version 8 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com). A two-way ANOVA was conducted, followed by a Tuckey post-test ($p < 0.05$), with the variables of treatment and time taken into consideration. The data that referred to the analyses conducted every week are reported in the supplementary material. For this, a one-way ANOVA was performed, followed by a Tuckey post-test ($p < 0.05$), considering the variable of different treatments at the same time.

## 3. Results

Experimental supplemented light treatments, such as HPS, LED, and LED+IR, in this experiment resulted in different flowering times in tomato plants, as shown in Table 1. LED+IR resulted in early and increased flowering; however, inflorescences experienced burns during the extreme temperature from mid-June, as shown in Fig. 5.

This characteristic burn was not observed in the other two light treatments. The lowest and late flowering was recorded under LED supplemented light, whereas the number and timing of flowers under HPS supplemented lighting were somehow intermediate between LED and LED+IR light treatments.

**Table 1**
Variations in flowering dates and observed notable effects in response to different light treatments.

| Crop | Experiment Duration | Light Treatments | Flowering dates | Notable effects |
|---|---|---|---|---|
| Tomato (*S. lycopersicum*, var. Leader F1) | February to July 2022 | LED | 25th April | Late flowering |
| | | HPS | 22nd April | Higher white fly infestation |
| | | LED+IR | 4th April | Thicker leaves, early flowering time but a few burnt flowers |

**Fig. 5.** Healthy (left) and burnt inflorescence (right) of tomato plants under LED+IR supplemental lighting.

### 3.1. Temperature monitoring by infrared and thermal camera

Using a thermal camera, a significant increase in temperature was recorded under the supplemented LED+IR treatment for bench and leaves compared to the other two treatments, while it was non-significant to HPS for pot temperature. However, non-significant temperatures were noticed between the supplemented LED and HPS light treatments. The order for the higher temperatures recorded by the thermal camera was LED+IR > HPS > LED as shown in Table 2.

Similar to the thermal camera, supplemental LED+IR showed significantly higher temperature values for bench, pot, leaf, and soil temperatures. Temperature readings were not significantly different between the HPS and LED for all recorded values.

### 3.2. Chlorophyll (a+b) and total carotenoid concentrations

Non-significant chlorophyll a and b were found for T2, where HPS light yielded slightly higher photosynthetic pigment accumulation than LED and LED+IR light treatments, as shown in Fig. 6a. However, significantly lower chlorophyll a and b were found during T1 for LED and HPS lights than for LED+IR. Moreover, unlike T2, LED+IR light produced more chlorophyll a and b at T1 than the other two supplemented light treatments.

Unlike chlorophylls a and b, carotenoids showed a decreasing trend from T1 to T2. Non-significant carotenoids accumulation was observed among all the light treatments at T1, with values moderately higher for the LED+IR supplemented light treatment. However, carotenoids production was significantly lower in the LED and LED+IR light treatments at T2 compared to not only the HPS light treatment but T1 readings, as shown in Fig. 6b.

**Table 2**
Mean temperature values recorded during the entire experiment by Thermal camera and Infrared thermometer for Bench, Leaves, Pot and Soil under supplemented LED, HPS and LED+IR ($n$=20±S.E.). Different letters indicate significant differences among treatment after one-way ANOVA ($p < 0.05$).

| Temperature (°C) | THERMAL CAMERA | | | INFRARED THERMOMETER | | |
|---|---|---|---|---|---|---|
| | LED | HPS | LED+IR | LED | HPS | LED+IR |
| Bench | 25.7 ±0.6$^{b}$ | 26.4 ±0.7$^{b}$ | 31.3 ±1.3$^{a}$ | 24.9 ±0.4$^{b}$ | 25.7 ±0.4$^{b}$ | 31.5 ±0.8$^{a}$ |
| Leaves | 23.4 ±0.9$^{b}$ | 23.9 ±0.7$^{b}$ | 26.1 ±0.6$^{a}$ | 23.2 ±0.3$^{b}$ | 23.3 ±0.4$^{b}$ | 26.2 ±0.3$^{a}$ |
| Pot | 23.9 ±0.7$^{b}$ | 24.2 ±0.7$^{ab}$ | 26.5 ±0.7$^{a}$ | 22.4 ±0.4$^{b}$ | 22.8 ±0.4$^{b}$ | 25.7 ±0.7$^{a}$ |
| Soil | – | – | – | 22.4 ±0.3$^{b}$ | 22.9 ±0.3$^{b}$ | ±0.4$^{a}$ |

### 3.3. Phenolic index and total anthocyanin concentrations

As shown in Fig. 7a, no noticeable changes were observed in the production of phenols among all supplemented light treatments at either time point. However, a slight increase was observed in the LED+IR treatment at T2 compared to the LED and HPS supplemented treatments. Similar to the phenolic index, non-significant differences in anthocyanins prevailed throughout the experiment for all supplemented light treatments, as shown in Fig. 7b. Moreover, it has been observed that tomato plants under all light treatments for T2 have accumulated higher anthocyanins compared to T1, with HPS supplemented light responsible for the highest increment in anthocyanins followed by LED+IR and LED supplementation.

### 3.4. Total sugars, nitrates and thiobarbituric acid reactive substances (TBARS)

As shown in Fig. 8a, an increase in total sugars was recorded for T2 compared to T1 in tomato plants. At T2, HPS supplemented light resulted in significantly higher total sugars than the other two treatments at T2 and T1. LED supplemented light lagging was behind HPS in terms of total sugars accumulation, whereas LED+IR was observed to be the lowest total sugars producing light treatment. However, a slight non-significant increase in total sugar accumulation was recorded in LED+IR at T1.

Non-significantly increased nitrate, as shown in Fig. 8b, was produced at T1 by LED supplemented light compared to the HPS and LED+IR light treatments. However, a clear reduction in nitrate was recorded at T2 for all supplemented light treatments. HPS at T2 was responsible for a non-significant increase in nitrate content compared to LED and LED+IR supplementation, both of which showed significantly lower nitrate values in relation to T1 and HPS at T2.

A non-significant increase in lipid peroxidation was observed for LED+IR followed by HPS and LED supplemented light treatments at T1; however, no variation was recorded under LED+IR at either time point. In contrast, a significant reduction in lipid peroxidation was observed in HPS and LED supplemented plants, as shown in Fig. 8c.

### 3.6. Fresh fruit weight, yield and tomato fruits color

Significant increase in fresh tomato fruit weight was obtained under LED+IR supplemented light compared to the other two tested light treatments; however, the lowest berry weight was recorded under LED supplemented tomato plants compared to HPS, as shown in Fig. 9a. A significantly higher yield was recorded for LED+IR compared to LED, which showed the lowest yield considering the fixed time span of recording the tomato productivity under different supplemental light treatments. However, the yield was intermediate for HPS compared to both LED and LED+IR lighting, as shown in Fig. 9b.

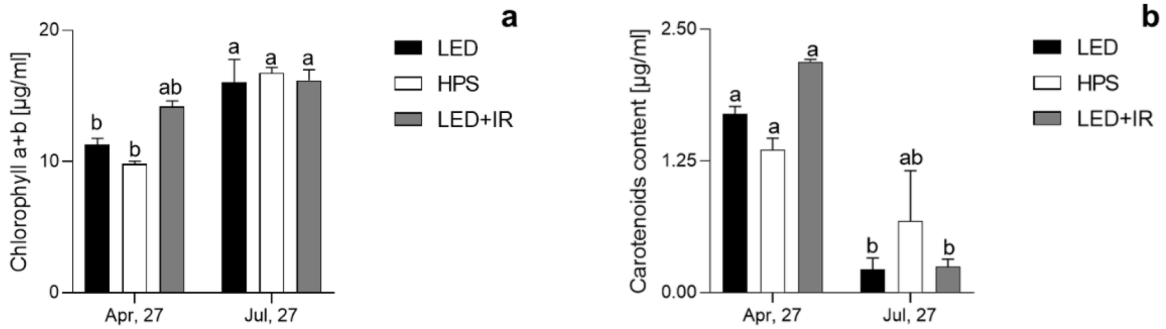


**Fig. 6.** (a) Chlorophyll *a* & *b* (b) Carotenoids contents of tomato plant treated with supplemental LED, HPS and LED+IR lighting. Values are mean ($n$=3±S.E.). Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p$<0.05).

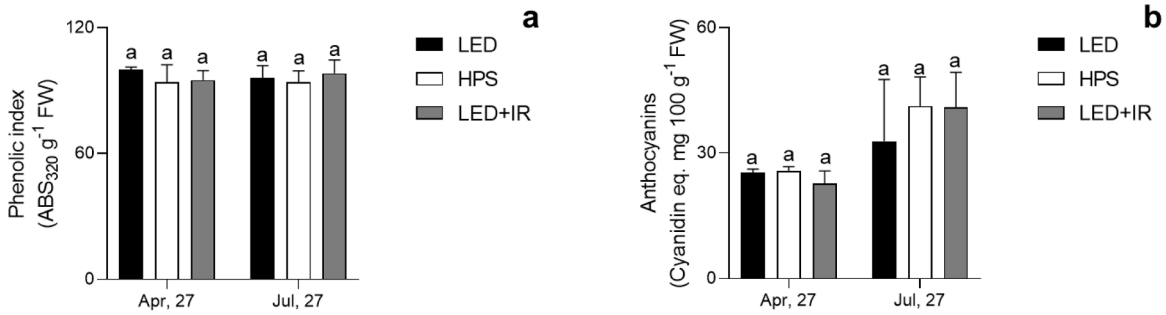


**Fig. 7.** (a) Phenolic Index (b) Anthocyanins contents of tomato plants treated with supplemental LED, HPS and LED+IR lighting. Values are mean ($n$=3±S.E.). Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p$<0.05).

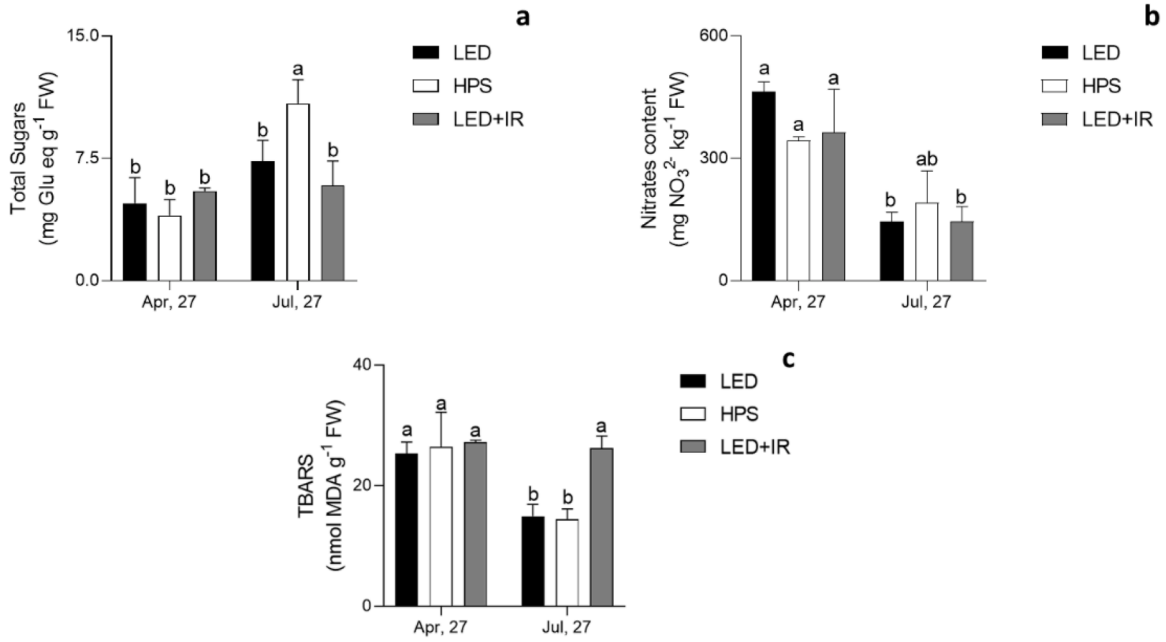


**Fig. 8.** (a) Total sugars (b) Nitrates (c) TBARS values of tomato plants treated with supplemental LED, HPS and LED+IR lighting. Values are mean ($n$=3±S.E.). Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p$<0.05).

The L*a*b* color space indicates lightness, red and yellow colors of the tomato qualitative analysis. No significant differences with minute variations have been recorded for L* among all the three supplemented light treatments as shown in Table 3. Increased significant differences however, in the a* was recorded for LED+IR against HPS which showed the lower redness values among the three supplemented light treatments. Redness for tomatoes grown under LED supplemented light was non-significant among both HPS and LED+IR supplemented light treatments. b* exhibited somehow similar trend as L* in terms of non-significant differences and slight variations. Non-significant higher chroma (C*) values were measured between LED and HPS against the lower significant values under LED+IR supplemented light. Hue (h*) depicted the exact similar trend as of chroma.

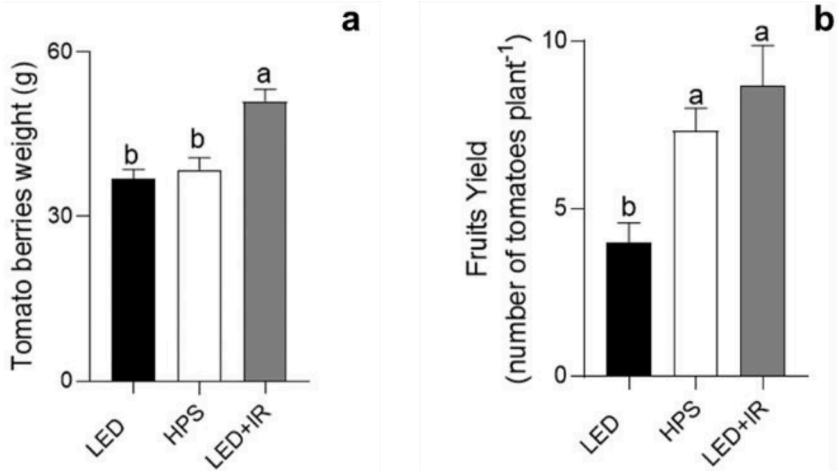


**Fig. 9.** (a) FW and (b) yield of tomato fruits grown under supplemental LED, HPS and LED+IR lighting. Values are mean ($n$=10±S.E.) for FW and ($n$=3±S.E.) for yield. Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p$<0.05).

**Table 3**
Effect of supplemental LED, HPS and LED+IR on tomato fruit color ($n$=15±S.E.). Different letters indicate significant differences among treatment after one-way ANOVA ($p < 0.05$).

| | Fruit Color Analyses | | | | |
|---|---|---|---|---|---|
| Light Treatments | (L*) | (a*) | (b*) | Chroma (C*) | Hue (h°) |
| LED | 42.81 ±0.65$^{a}$ | 31.63 ±0.27$^{ab}$ | 34.78 ±0.74$^{a}$ | 46.80 ±0.56$^{a}$ | 47.31 ±0.64$^{a}$ |
| HPS | 42.55 ±0.40$^{a}$ | 31.47 ±0.28$^{b}$ | 33.71 ±0.68$^{a}$ | 46.16 ±0.45$^{a}$ | 47.07 ±0.60$^{a}$ |
| LED+IR | 43.97 ±0.45$^{a}$ | 32.67 ±0.36$^{a}$ | 35.04 ±1.74$^{a}$ | 42.22 ±1.60$^{b}$ | 42.56 ±1.74$^{b}$ |

**Table 4**
Supplemented LED, HPS and LED+IR treatments on Tomato fruit quality ($n$=4 ±S.E.). Different letters indicate significant differences among treatment after one-way ANOVA ($p < 0.05$).

| | Fruit Quality Analyses | | | | |
|---|---|---|---|---|---|
| Light Treatments | Lycopene | ß-Carotene | pH | Titratable Acidity (TA) | Soluble solids content (Brix°) |
| LED | 65.12 ±3.69$^{a}$ | 60.40 ±2.28$^{a}$ | 4.29 ±0.04$^{a}$ | 0.36 ±0.008$^{b}$ | 3.63 ±0.18$^{a}$ |
| HPS | 86.22 ±3.28$^{b}$ | 84.90 ±3.21$^{b}$ | 4.24 ±0.01$^{ab}$ | 0.41 ±0.007$^{c}$ | 4.13 ±0.08$^{b}$ |
| LED+IR | 72.99 ±4.56$^{ab}$ | 63.20 ±4.54$^{a}$ | 4.21 ±0.01$^{b}$ | 0.46 ±0.001$^{a}$ | 4.80 ±0.05$^{c}$ |

### 3.5. Tomato fruit quality

Lycopene content was significantly enhanced under HPS supplemented light compared to the lowest lycopene production under LED as shown in Table 4. However, LED+IR supplemented tomatoes showed non-significant intermediate lycopene production in this experiment. A similar trend was been observed for the ß-carotene, in which significantly higher accumulation of ß-carotene was recorded in HPS compared to LED and LED+IR supplemented treatments. Non-significant intermediate pH values, however, were noticed in tomato fruits of HPS supplemented plants compared to the significantly higher pH values in LED with respect to LED+IR supplemented tomato fruits.

Significant titratable acidity values were found among all the treatments, with LED being the lowest recorded against the successive increased values of HPS and LED+IR supplemented light treatments respectively. However, the Brix index significantly increased under HPS compared to significant lowest value of LED and intermediate LED+IR supplemented light treatments.

### 3.7. Plant height and water content

Non-significant differences have been observed in terms of plant height in this experiment. However, plants under HPS lights were taller compared to other two light treatments as shown in Fig. 10a.

Non-significant differences were noticed for water content at both timepoints in this study. There had been minor fluctuations for the water content percentage among the treatments (Fig. 10b) but the trend was somehow similar at both timepoints in which a slight increase for HPS compared to other two supplemental light treatments have been recorded.

### 3.8. Whitefly monitoring

Significantly reduced white fly infestation was recorded under LED+IR supplemented light treatment compared to HPS and LED. However, plants under HPS were non-significantly more infested compared to the LED supplemented light treatment as shown in Fig. 11.

## 4. Discussion

Tomato farming is a significant source of income and a primary dietary requirement worldwide. Therefore, it is important to develop strategic measures to reduce production constraints that lower overall yields and quality of produce. Indoor tomato production is carefully researched, monitored, and improved throughout the time to meet the rising tomato consumption demands of an expanding population. Artificial lighting, such as LEDs and HPS, is a key element for aiding these production goals.

Early flowering, thicker leaves, and few burnt flowers were observed under high temperature of LED+IR compared to the late and normal flowering of HPS and LED supplemental lighting. Tomato plants suffer significant damage during numerous phases of development, including seed germination, vegetative and reproductive growth, and fruit setting, when temperature conditions are higher than 35°C (Wahid et al., 2007). Moreover, Pan et al. (2017) stated that the floral morphology of tomatoes is severely harmed by prolonged exposure to high temperatures, such as stigma exertion which resulted in prevention of self-pollination. In a similar study, Pham et al. (2020) observed deformed tomato flowers

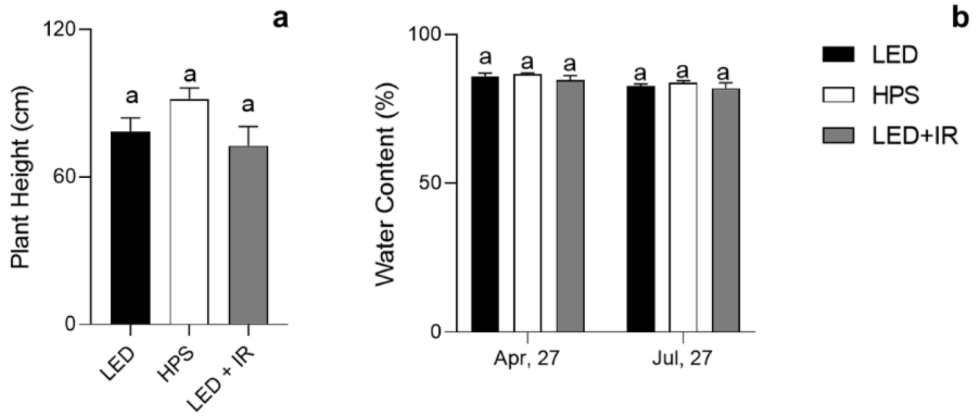


**Fig. 10.** (a) Plant height and (b) water contents of tomato plants treated with supplemental LED, HPS and LED+IR lighting. Values are mean ($n$=3±S.E.) for plant height and water content. Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p$<0.05).

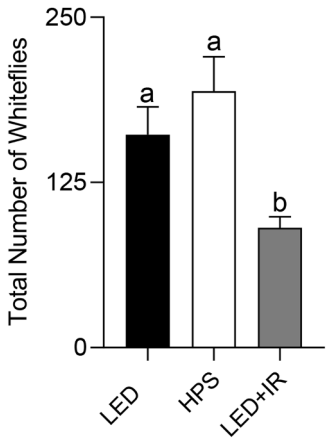


**Fig. 11.** Number of whiteflies under supplemental LED, HPS and LED+IR lighting. Values are mean ($n$=9±S.E.). Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p$<0.05).

and 50% damaged pollen viability under prolonged heat exposure at 35°C. Various changes in the production of chlorophyll a and b were noticed for the two studied timepoints in this experiment which indicated that throughout the course of tomato growth, plants experienced varied changes under supplemented light treatments. Increased chlorophyll content was observed for both LED and LED+IR treatments compared to HPS at T1 which indicated that LED assisted plants in the accumulation of photosynthetic pigments in a precise manner compared to HPS. However, this trend can be explained by a previous study in which the chlorophyll content is found to be proportional to the blue LED light by Hogewoning et al. (2010). Both LED and LED+IR supplemented light treatments in this experiment contained sufficiently higher proportions of blue light compared to HPS, therefore resulting in higher photosynthetic pigment accumulations. However, this trend switched opposite in the later timepoint T2 which mostly comprised of the reproductive stages of tomato, such as flowering and fruiting. The high temperature of LED+IR might have altered the membrane permeability and reduced the membrane capacity to retain solutes and water as explained by Camejo et al. (2005). This decreased the chloroplast's capacity to absorb light by reducing the light-harvesting chlorophyll proteins to help tomato plants to survive and thrive under high temperature but with a reduced accumulation of photosynthetic pigments. This finding is consistent with the findings reported by Shin et al. (2020), who noted a drop in the amounts of chlorophyll a and b in leaves exposed to high temperatures. Authors postulated that the rise in chlorophyllase activity, which also affected the overall membrane effectiveness of leaves, is responsible for the drop in photosynthetic pigments.

Likewise, a buildup of carotenoids has been observed in both LED and LED+IR light treatments for T1 which was otherwise for the later, showed that LED assisted tomato plants in accumulating these pigments which helped them to absorb excess light energy during vegetative phase and perform better growth and development. Moreover, previously when lettuce was exposed to 70% $R$+30% B LED, Ammozgar et al. (2017) found a higher accumulation of carotenoids in the plant. The production of more of these pigments aids plants in better light absorption, the control of reactive oxygen species, and better shoot development. To increase the accumulation of chlorophyll a, total chlorophyll, carotenoids, and chl a/b in tomato leaves, Wang et al. (2022) found that adding LED light with a 7R:2B ratio for three hours in the morning was more beneficial. However, a decrease in carotenoids under LED and LED+IR light treatments at the later stages of this experiment might be due to the increased fruit production under these treatments, where plants utilized most of the energy for fruit formation rather than using it in the production of carotenoids as a protective pigment against higher temperature induced by LED+IR. It has been established that abiotic stresses, such as high and low temperatures and high luminosity, influence the accumulation of carotenoids and these molecules are associated with thermotolerance in tomato (Scarano et al., 2020).

Even if non-significant, an increase in phenolic index at both time points suggested that LED in particular could be responsible for the higher accumulation of phenols in tomato leaves. The differences in the production were not affected much by the stages of development such as vegetative as well as reproductive throughout the experiment. It is well known that light composition affects the expression of genes that modulate the synthesis of secondary metabolites, including phenolic compounds, although such effects may depend on specific wavelength and/or plant species (Baenas et al., 2021). Anthocyanins on the other hand, showed a drastic increase at the later timepoint compared to the earlier, in which HPS light was quite prominent in producing an increased anthocyanins compared to the LED light while LED+IR was somehow parallel to HPS in producing the anthocyanins. According to a recent study, blue LEDs are the most effective light spectrum for promoting the expression of the genes PAL (phenylalanine ammonia-lyase), CHS (chalcone synthase), and DFR (dihydroflavonol-4-reductase), which are necessary for the synthesis of anthocyanins and phenols (Giliberto et al., 2005). These previous studies are in line with the antioxidant activity in LED+IR but increase antioxidant production in HPS might be due to the fact that tomato plants under this light treatment were highly infested by white flies. According to Krishna et al. (2019), the antioxidant potential of tomatoes comes from a mixture of

biomolecules such as ascorbic acid, vitamin E, lycopene, phenols and flavanols. As these compounds are typically used by plants to protect themselves against ultraviolet radiation, diseases, parasites, and predators, as well as to produce their distinctive hues, we can conclude that tomato plants yielded higher phenols and anthocyanins under HPS and LED+IR to cope with the whitefly's infestation in HPS treated tomato plants and higher temperature under LED+IR light treatment.

Sugar levels in plant cells, as well as their transport, utilization, and storage, are tightly controlled and highly influenced by physiological activity in the cells as well as plant organs, environmental factors, circadian rhythms, and plant developmental phases (Lemoine et al., 2013). Results from this study depicted variations in total sugars accumulation in tomato plants not only among the treatments but the timepoints of sampling as well. Sampling carried out at T1 showed a non-significant increase of total sugars in LED and LED+IR treatment which according to the findings from Li et al. (2017), suggested that tomato plants increased the buildup of sucrose in the leaves by increasing the activity of the enzymes such as sucrose-phosphate synthase (SPS) and sucrose synthase (SS) when exposed to 3R1B LED treatment. The T2 sampling for total sugars revealed an increase in HPS compared to both LED and LED+IR treatment. Sugars are known to play a key role in defense against biotic and abiotic stresses (Chen et al., 2010). Although the reason behind this drastic increase of total sugars under HPS is not clearly known, yet it can be assumed that an increased whitefly infestation might be the cause of increase total sugars accumulations as an activation for defense mechanism of tomato plants. Alsina et al. (2022) has also stated that HPS lights bring higher proportions of other lights compared to various LED combinations. This increased proportion of red light in HPS composition favors it in higher accumulation of total sugars. Similar results were recorded by Erdberga et al. (2020) who concluded that higher proportion of red facilitates in higher sugar accumulation in tomato leaves.

Nitrates are found in all plant tissues because they are essential for growth, development, and environmental adaption. Reduction in nitrates were seen between the two time points with the T2 showing reduced nitrates assimilations in the leaves for all the three tested light treatments while LED and LED+IR during T1 showed an increased nitrates accumulation compared to HPS which was otherwise in later timepoint. Wojciechowska et al. (2016) observed that the length of vegetation had a bigger impact on nitrate reductase activity (NR) than did the light treatments. Moreover, it was established by Anjana and Iqbal (2007) that nitrate content in the petioles and leaves of plants tends to decrease as the crop approaches maturity which in turn might be due to the translocation of nitrates in the fruit formation. However, Santamaria et al. (1999) listed plant organs in decreasing order of nitrate content as follows: petiole > leaf > stem > root > inflorescence > tuber > bulb > fruit > seed.

Malondialdehyde (MDA) is a by-product of lipid peroxidation, and it is released when the cell membranes are damaged. It was seen that LED+IR posed an increased lipid peroxidation due to an increased temperature, compared to the LED and HPS light treatments, which is known to eventually trigger the production of reactive oxygen species leading to higher oxidative stress. Previous study carried out by Natalini et al. (2014) stated that higher temperatures accelerated the loss of membrane integrity and hence increased the electrolyte leakage and TBARS values in fresh cut tomatoes. Larkindale et al. (2002) observed that higher temperature effects the photosynthetic machinery in Arabidopsis and hence resulted in higher TBARS values. On the other hand, HPS light treatment for this research resulted in lower TBARS values at T2 which suggested an increased accumulation of photosynthetic pigments such as chlorophyll a and b along with the accumulation of accessory light pigment carotenoids which might possibly protected tomato plants from cell membrane damage. Moreover, the relative lower temperature under HPS supplemented lighting compared to LED+IR possibly led to less oxidative damages to cell membranes.

FW of tomato berries significantly increased by LED+IR treatment compared to LED and HPS supplemented light treatments whereas the yield was also significantly higher under LED+IR compared to LED lighting. The dry weight in terms of water content was not largely affected by light treatments. These results are in line with those of Micken et al. (2018), who found that red-blue LED exposure to species of lentil, basil and mint increased yields and dry weight. This was true for the LED+IR lighting but not for LED, which may be because LED experienced late flowering. As a result, we assume that the growth cycle of tomato plants under LED was slightly delayed which resulted in lesser yield than LED+IR until the specified date of harvesting (T2). Studies have shown that the red and blue components of the spectrum correlate up well with the absorption spectra of both chlorophyll and carotenoids pigments hence might have resulted in an increased photosynthesis which directly affected biomass and yield (Lin et al., 2021). Moreover, an increased fruit number and weight under LED+IR has seen to accommodate less sugars for this experiment, a finding which is in line with the findings of Gautier et al. (2008) who stated that increased fruit load and high temperature both reduced the aggregation of sugars and acids within tomato fruits.

The color quality attributes (L* a* b*) of tomato fruits have shown distinct color changes in which the higher values for a* were recorded under supplemented LED+IR light, marking the fruits under this treatment more red and possibly more matured. The yellowness of the fruits as indicated by higher b* was found mostly in LED treated tomato plants, while for brightness as indicated by L*, the values showed non-significant trend in series as LED>HPS>LED+IR. According to Pek et al. (2010), the de novo synthesis of carotenoids, primarily lycopene and ß-carotene, gives red ripe tomatoes their distinctive fruit color such as a* represents the major carotenoid lycopene, which gives them their red color, and b* represents ß-carotene, which gives them their yellow hue (Pek et al., 2010; Sacks and Francis, 2001). Although higher lycopene, ß-carotene as well as carotenoids accumulation were found under HPS light treatment, yet inconsistency prevailed for redness and yellowness for tomato fruits under HPS treated tomatoes. Skin color of tomato as hue angle revealed the lower values for LED+IR supplementation while the maximum values in other treatments indicates the fruit maturity levels among all the tested light treatments. Presence of chlorophyll a and b can be an indicator for the differentiation of tomato fruits due to the distinguish photosynthesis process (Seifert et al., 2014). Lower total chlorophyll accumulation in the tomato leaves under LED+IR resulted in reduced chlorophyll florescence therefore resulted in decrease values of color measurements in terms of hue angle for LED+IR. Kim et al. (2020) also found inconsistent results on the effects of FR radiation on fruit pigmentation and color in tomatoes.

Moreover, lycopene and ß-carotene concentrations in greenhouse tomatoes were unaffected by the addition of B and FR (Dzakovich et al., 2017). The presence of malic and citric acids, the two main organic acids present in the majority of mature fruits, has a direct effect on titratable acidity and/or pH, which are key components of fruit sensory quality (Etienne et al., 2013). A pH gradient-driven symport of sugar and acid leads to the buildup of sugar and acid in fruit tissues. Since hydrogen ions are necessary for the symport of both sugar and acid into cells, their accumulation results in an accumulation of both in the tissues of fruit (Ho, 1988). Considering the lower pH values and higher temperature under both HPS and LED+IR, the above information has a potential to explain the higher accumulation of TSS (Brix) and titratable acidity in HPS and LED+IR supplemented light treatments which in fact are also the prime parameters to consider for post-harvest storage against microbiological stability. According to studies on various tomato genotypes, heat stress significantly decreased the amounts of soluble sugar, starch, and lycopene relative to ambient temperature while significantly increase the amounts of ascorbic acid, total soluble solids, and titratable acidity (Vijayakumar et al., 2021) which, in our experiment, can be further explained by the b* of the peel color. The production of ß-carotene, which was found to be higher under HPS and LED+IR, supports the yellowness of the peel and consequently led to

higher TSS and TA. The formation of ß-carotene was minimal under supplementary LED, which consequently resulted in decreased TSS and TA in tomato fruits, despite having a second higher b* values, a finding previously supported by Andelini et al. (2023).

## 5. Conclusion

According to these findings, different indoor supplemental lights had an impact on surrounding temperatures of the tomato plants, which in turn had an effect on the overall quality of the produce. Under LED+IR supplemental light, physiological studies showed a decrease in photosynthetic pigments like chlorophyll *a, b* and carotenoids, which resulted in less heighted plants with an increased inflorescence and heavier tomatoes. Increased lipid peroxidation under LED+IR was due to the higher temperature, which ultimately assisted tomato plants in reducing whitefly infestation. Additionally, this light treatment showed enhanced anthocyanins, phenolic index and reduced nitrate accumulations which marked it as the most advantageous light treatment of this experiment. However, in terms of total sugar accumulations, total carotenoids, lycopene, ß-carotene, Brix index, water content and plant height, supplemented HPS light performed better than both LED and LED+IR supplemented lights. Despite having the most severe whitefly infestation among all lights investigated, the tomato plants growing under this light had the lowest levels of lipid peroxidation. Tomato fruit color analyses found differences in tomato maturity, with LED+IR lighting showing the most redness and a reduced hue angle, followed by LED and HPS supplemental lighting. A greater number of tomatoes were also produced with the LED+IR light treatment for a specific time period, despite the burnt inflorescence, which necessitated a detailed investigation of the infrared exposure time and distance from the plant in addition to the growing month cycle. Additionally, the growing cycle must be lengthened and repeated tomato harvests must be carried out to precisely determine the amount of tomato plants under each tested light treatment.

## CRediT authorship contribution statement

**Awais Ali:** Investigation, Formal analysis, Writing – original draft, Data curation. **Viviana Cavallaro:** Investigation, Formal analysis, Writing – original draft, Data curation. **Piero Santoro:** Resources, Writing – review & editing. **Jacopo Mori:** Resources, Writing – review & editing. **Antonio Ferrante:** Conceptualization, Methodology, Project administration, Writing – review & editing. **Giacomo Cocetta:** Conceptualization, Methodology, Project administration, Writing – review & editing.

## Declaration of Competing Interest

The author Piero Santoro is employed by the company MEG Science. The author Jacopo Mori is employed by company ALMECO. All other authors declare no competing interests.

## Data availability

No data was used for the research described in the article.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.112469.

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