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Research Article

Essential oil constituents and secondary metabolites of *Mentha viridis* under tissue culture technique using violet visible light emitting diodes (LEDs)

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Abstract

This study aimed to propagate the valuable medicinal plant *Mentha viridis* through *in vitro* culture of nodal segments measuring approximately 1-1.5 cm. Two different types of light-emitting diode (LED) systems were used to apply three different concentrations of two different cytokinins: 6-benzylaminopurine (BAP) and thidiazuron (TDZ) at 0, 1, or 2 mg/L. The LED systems were white as a control and violet, which is a 1:1 ratio of red and blue light. After a 30-day incubation period, the results revealed significant improvements in the survival rate and the number of shoots per explant across the various treatment groups. With MS medium supplemented with 2 mg/L TDZ and illuminated by white and violet LEDs, the highest values were obtained, yielding survival rates of 93.3% and 13.3 shoots per explant, respectively. Moreover, the treatment involving 2 mg/L TDZ under violet LEDs illumination exhibited superior outcomes in terms of leaf count per explant, callus formation, and callus size. Notably, no callus formation was observed in response to BAP treatments. All treatments resulted in a significant increase in antioxidant enzyme activity and the accumulation of various compounds, such as anthocyanin, ascorbic acid, phenols, flavonoids, peroxidase, and polyphenol oxidase, when compared to the control in a broader context, the levels of IAA, kinetin, and zeatin increased, while GA_3 and ABA decreased in response to the applied treatments, as compared to the control. Additionally, ten compounds were consistently found in all treatments by GC/MS analysis of the micro-propagated *Mentha*, with carvone accounting for the highest proportion (43.5%) and being the predominant component. Among all treatments,

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nodal segments that were exposed to violet LEDs and grown on MS medium supplemented with 1 mg/L TDZ had the highest carvone content.

Keywords: essential oil constituents; light emitting diode systems; medicinal herbs; *Mentha viridis*; natural products; secondary metabolites; tissue culture

Introduction

Medicinal plants remain the most important source of treatment for various ailments, and only some of the herbal products are obtained from cultivated biomass. It threatens biodiversity and the quality and safety of end products. Since most plants have not been grown or micropropagated under similar conditions, their properties differ, according to the study by Moraes *et al*. (2021). The use of synthetic drugs, the finding of unwanted side effects, and the high cost of traditional medicines was studied by Aleksic and Knezevic (2014). The use of herbal medicinal products and supplements has increased tremendously over the past three decades with not less than 80% of people worldwide relying on them for some part of primary healthcare (Ekor, 2014). About 40% of the compounds used in the pharmaceutical industry are directly or indirectly derived from plants (Stafford *et al*., 1986; Sidhu, 2010) due to the chemical synthesis of such compounds is either impossible or not viable economically (Oksman-Caldentey and Inzé, 2004). Therefore, many plants (particularly medicinal plants) are threatened with extinction due to over-exploitation (Edwards, 2004). High-demand plants face major challenges, including biodiversity loss due to over-exploitation and pollution affecting natural populations. These are strong factors in favor of the cultivation of rare, elite, high-yielding medicinal plants. Moreover, the cultivation of medicinal plants is the most effective way to bridge the gap between demand and supply. Breeding research is needed to develop medicinal plants and increase their production (Wang *et al*., 2020). *In vitro* micropropagation offers medicinal plant growth rates, environmental control, year-round availability, cloning identification, transgenic plant production, endangered plant conservation, Moreover, the cultivation of medicinal plants is the most effective way to bridge the gap between demand and supply. Breeding research is needed to develop medicinal plants and increase their production and genetic material preservation through crop reservation (Sidhu, 2010).

Plant tissue culture techniques are said to be a more suitable alternative to solve this alarming problem, opening new avenues in plant biotechnology (Dagla, 2012). 6-Benzyleaminopurine, or BAP, is a synthetic cytokinin that promotes plant growth and development by stimulating cell division. It is the plant growth regulator that has been successfully used in several tissue culture studies to induce adventitious shoot formation and promote apical and axillary shoot proliferation under in vitro conditions. The success of the tissue culture of several medicinal plants depends upon the use of BAP in their culture medium. As thidiazuron is resistant to degradation by cytokinin oxidase (Mok *et al*., 1987) it is quite stable in tissue culture. TDZ is more biologically active than BAP or zeatin, and lower concentrations are needed in tissue culture, especially during micropropagation. TDZ is as effective as or more effective in most of the species in which it has been tested, particularly woody species. In certain species, disadvantages have been reported to be associated with using TDZ. These include: (a) hyperhydricity (Debergh *et al*., 1992) of the regenerated shoots (Cousineau and Donnelly, 1991); (b) short and compact shoots (Fasolo *et al*., 2012); (c) difficulty in the elongation and rooting of the regenerated shoots. In some cases, TDZ is not always superior to benzyl aminopurine (BAP). For example, in peach, TDZ produced excessive callus and minimal shoot proliferation, whereas shoot multiplication was greatest with BAP (Zimmerman and Scorza, 1992).

Light quality affects the laboratory production of plants and, thus, plant breeding or genetic modification programs. The application of wide-spectrum LED lamps in micropropagation allows producers and researchers to mix wavelengths that effectively promote consistent and healthy plant growth (Miler *et al*.,

2019). LED lighting systems exhibit wave length specificity, durability, small size, long life, and relatively cool-emitting surfaces. Several types of plants grow well under LEDs, such as lettuce, peppers, cucumbers, wheat, Spanish seedlings, and potato seedlings. LED lights are an ideal tool to use for lighting designing explant production (Li *et al*., 2019). The absorption of the light spectrum is specific to plants. Red light plays an important role in controlling chloroplast, stem, petiole growth and reproductive system function, while blue light mainly regulates plant growth, leaf expansion, photomorphogenesis, stomatal opening, photosynthesis and pigment accumulation. The spectra of red and blue lights are similar to those required for plant photosynthesis, so most studies focus on evaluating the effects of monochrome or mixed red and blue LEDs (Xu *et al*., 2019).

M. spicata, also known as *M. viridis*, is a medicinal plant in the Lamiaceae family known for its ability to synthesize and secrete secondary metabolites, which are essential oils. In addition to their traditional uses, these aromatic molecules have found other fields of application (e.g., medicine, agriculture, food and beverages) are of great interest (Çakılcıoğlu and Türkoğlu, 2009; Selvi *et al*., 2022; Zhang *et al*., 2022). Importantly, toxicological studies of *M. spicata* demonstrate the safety of this species at various doses and multiple applications, justifying its use in traditional medicine as an herbal tea. Pharmacological biology explorations demonstrated that extracts and essential oils of *M. spicata* showed different pharmacological properties such as antibacterial, antiparasitic activity, insecticidal, anti-inflammatory, antidiabetic, antioxidant, diuretic, analgesic, antipyretic, antihemolytic, and protective activities. (El Menyiy *et al*., 2022). *M. spicata* is one of the most popular species in this *Mentha* genus and has received considerable attention as a gastrointestinal and sedative in folk medicine systems (Zengin *et al*., 2022). Plant tissue culture techniques involving plant materials like nodal segments, indirect organogenesis, multiplication using immature seeds, and regeneration of axillary cotyledons and buds are preferable (Bukar and Abba, 2022). This study aimed to establish a plant tissue culture protocol for *M. viridis* using nodal segments as explants by optimizing various factors, viz., plant growth regulators (two types of cytokinin: benzyl aminopurine; BAP; and thidiazuron; TDZ) at three different concentrations (0, 1 or 2 mg/L) for each, as well as light-emitting diode systems (LEDs) (white as a control and violet; combination of red and blue; 1:1) for secure micropropagation and production of secondary metabolites of the medicine plant in a short time.

Materials and Methods

Plant materials and chemicals used

The current study was conducted at the experimental station and tissue culture laboratory of the Vegetable and Floriculture Department, Faculty of Agriculture, Mansoura University and in the incubator of the physiology lab at the Botany Department, Faculty of Sciences, Mansoura University, during the seasons of 2021-2022. *M. viridis* (spearmint) nodal segments (about 1-1.5 cm) were obtained from the nursery plantation of Mansoura University selected for apparent uniformity of size and shape. The chemicals used were supplied by Sigma Chemical Company. 2.2. Time course of the experiment.

Time course of the experiment

Surface sterilization of *M. viridis* explant

Nodal segments of *M. viridis* were excised using a sharp scalpel and then washed for 30 minutes under running tap water with 4 drops of liquid soap to remove dust particles. The explants were surface sterilized by immersion in a 25% Clorox commercial bleach solution (6% sodium hypochlorite; NaOCL) for 6 minutes then finally rinsed three times with distilled sterilized water for 5 min each.

Culture media and conditions

Sterile explants were transferred into jars containing 25 ml of MS (Murashige and Skoog, 1962) basal medium containing 3% sucrose as a carbon source (Table 1) and supplemented with two types of cytokinins: 6-benzylaminopurine (BAP) and thidiazuron (TDZ) singly at three concentrations (0, 1, or 2 mg/L) for each cytokinin. The medium was solidified with 0.7% plant agar. The pH of the medium was adjusted to 5.75 before adding agar and autoclaved at 121 °C (1.1 kg / cm^2) for 25 minutes. The cultures were incubated in growth chambers at 25 ± 1 °C under a 16-hour light/8-hour dark treatment with two different light types (white LEDs as a control and a combination of red and blue LEDs (violet LEDs)) for 30 days, each treatment contained 20 explants.

Table 1. Components of MS basal medium (Murashige and Skoog, 1962)

Constituents	Concentration
Macroelements	
NH_4NO_3	1650 mg/l
KNO_3	1900 mg/l
$CaCl_2.2H_2O$	440 mg/l
$MgSO_4.7H_2O$	370 mg/l
KH_2PO_4	170 mg/l
Microelements	
H_3BO_3	6.2 mg/l
$MnSO_4.2H_2O$	16.9 mg/l
$ZnSO_4.7H_2O$	8.6 mg/l
KI	0.83 mg/l
$Na_2MoO_4.2H_2O$	0.25 mg/l
$CuSO_4.5H_2O$	0.025 mg/l
$CoCl_2.6H_2O$	0.025 mg/l
Na_2 EDTA. $2H_2O$	37.3 mg/l
Myo-inositol	80.0 mg/l
Glycine	2.0 mg/l
Nicotinic acid (B5)	0.5 mg/l
Pyridoxine – HCl(B6)	0.5 mg/l
Thiamine – HCl (B1)	0.1 mg/l

Data recorded and analytical methods

Sampling takes place after 30 days from incubation for determination of growth parameters (survival percentage %, shoots number/explant, shoot length (cm), leaves number/shoot, callus volume (cm^3), shoot and/or callus fresh and dry weights), antioxidant enzymes and compounds [peroxidase (POX), polyphenol oxidase (PPO), catalase (CAT), and phenols, flavonoids, anthocyanin, and ascorbic acid]. In addition, phytohormones were also determined (in the case of BAP application only, as TDZ did not propagate shoots). In addition, GC/MS analysis of essential oil constituents for micro propagated *Mentha* was also carried out. The following data were recorded:

1-Survival percentage = (Number of the grown explants/ All number of the cultured explants) × 100.

2-Shoots and leaves number per explant.

3-Shoots length (cm).

4-Callus formation percentage was calculated from the present formula;

Callus formation % = (Number of explants formed callus/ All number of the cultured explants) × 100.

5-Callus fresh and dry weight (g).

6-Callus size (cm^3) was calculated by water displacement. As the callus was laid down in a beaker and the increase in the volume pointed to the callus volume.

Quantitative determination of some secondary metabolites

Flavonoid content was determined by the method accompanied by Kujala *et al*. (2000). Meanwhile, anthocyanins were extracted from oven-dried *M. viridis* tissue samples of leaves and measured using a Spekol Spectro Colorimeter (Mirecki and Teramura, 1984) and calculated as mg anthocyanins g-1 dry mass (Lange *et al*., 1971; Lindoo and Caldwell, 1978). In addition, total phenols were determined by the modified Folin–Ciocalteau reagent method according to Sadasivam and Manickam (2008). Ascorbic acid content was determined as described by Omaye *et al*. (1979).

Antioxidant enzyme activity: the enzyme extract was prepared according to Agarwal and Shaheen (1975). Peroxidase (POX) and polyphenol oxidase (PPO) activities were assayed by the method of Devi (2002). Catalase activity was assayed by the methods of Aebi (1983) and El-Bialy (2005).

The Arid Land Agricultural Research and Services Center, Faculty of Agriculture, Ain Shams University received fresh frozen samples that had been extracted in accordance with Shindy and Smith (1975) for phytohormone analysis (auxins; IAA; gibberellins; GA_3; cytokinin; CK (Kinetin and Zeatin); and abscisic acid, ABA).

Chemical composition of the essential oils

a. Extraction of essential oil: The essential oil content was determined in the air-dried samples of each treatment using a modified Clevenger apparatus according to the Egyptian pharmacopoeia (Egyptian pharmacopoeia, 1984). Essential oil content was determined and expressed as ml/100 g dry weight. The essential oils of each treatment were collected, dehydrated over anhydrous sodium sulfate, and kept in a refrigerator until GC-MS analyses.

b. Qualitative and quantitative analyses of the oils: Qualitative and quantitative analyses of the oils were performed using GC and GC-MS. The GC analysis of the oil was carried out on a GC HP-5890 II apparatus, equipped with a split-splittles injector, attached to an HP-5 column (25 m × 0.32 mm, 0.52 µm film thickness), and fitted to the FID. Carrier gas flow rate (H_2) was 1 ml/min, split ratio 1:30, injector temperature was 250 °C, detector temperature was 300 °C, and column temperature was linearly programmed from 40 °C to 240 °C (at a rate of 4 °C/min). The same analytical conditions were employed for GC-MS analysis, where an HPG 180 °C series II GCD system equipped with an HP-5MS column (30 m × 0.25 mm, 0.25 µm film thickness) was used. The transfer line was heated to 260 °C. Mass spectra were acquired in El mode (70 eV) in the m/z range of 40-400. Identification of the individual oil components was accomplished by comparison of retention times with standard substances and by matching mass spectral data with those held in the Wiley 275 library of mass spectra. Confirmation was performed using AMDIA software and literature (Adams, 2007) For the purpose of quantitative analysis, the area percent obtained by FID was used as a base.

Statistical analysis

The means of the data obtained were analyzed by the least significant difference (LSD) test at a probability of 0.05. ANOVA analysis was done with Costat (CoHort software, USA).

Results

Changes in variables in vitro culture parameters

Data in Table 2 and Figures 1-4 illustrate the variable growth parameters of *M. viridis* plantlets and calli *in vitro* cultured on different MS nutrient media for 30 days.

The survival percentage

Under white and violet LED illumination, all the treatments used caused a marked increase in the survival percentage of micropropagated *M. viridis*. The highest value for Mentha *invitro* was estimated in MS medium with 2 mg/L TDZ grown under white LED illumination. On the other hand, the control medium recorded the lowest survival percentage (55% and 60%) in white and violet LED illumination, respectively.

Table 2. Effect of two different visible light emitting diodes (white and violet) on variable invitro culture parameters of miropropagated *M. viridis* after 30 days from transplanting

Light Quality	Plant growth regulators		Callus formation %	Callus size (cm^3)	Shoot or callus F.WT (g/ explant)	Shoot or callus D.WT (g/ explant)
	Cytokinin type	Concentration (mg/L)				
White LEDs	Control	0	0%	NO	0.6427^{def}±0.040	0.375^{bcd}±0.0217
	BAP	1	0%	NO	0.912^{bc}±0.1323	0.5195^{bc}±0.0741
		2	0%	NO	0.978^{b}±0.147	0.5307^{b}±0.0828
	TDZ	1	66.67%	5.133^{b}±0.285	0.9868^{b}±0.1459	0.5216^{bc}±0.0808
		2	75%	7.0166^{a}±0.899	1.3003^{a}±0.1675	0.6957^{a}±0.0906
Violet LEDs	Control	0	0%	NO	0.4213^{f}±0.16758	0.169^{e}±0.0943
	BAP	1	0%	NO	0.5013^{ef}±0.161	$0.235d^{e}$±0.0913
		2	0%	NO	0.685^{cde}±0.154	0.355^{cd}±0.0866
	TDZ	1	86.667%	7.1166^{a}±0.950	0.828^{bcd}±0.1495	0.4772^{bc}±0.086
		2	93.333%	7.5^{a}±0.982	1.0168^{b}±0.1499	0.6898^{a}±0.0911
LSD 0.05			-	1.0909	0.2356	0.1505
Light Quality	**Plant growth regulators**		**Survival%**	**No. of shoots/ explant**	**Shoot length (cm)**	**No. of leaves/explant**
	Cytokinin type	**Concentration (mg/L)**				
White LEDs	Control	0	55%	1.8333^{e}±0.152	12.383^{a}±0.831	12^{c}±0.943
	BAP	1	73.33%	4.333^{de}±0.73	11.292^{a}±0.998	16^{c}±2.224
		2	81.25%	6.333^{cd}±1.025	7.033^{b}±1.089	22^{b}±2.8317
	TDZ	1	87.5%	9^{bc}±1.329	6.417^{bc}±1.086	26.333^{b}±3.648
		2	93.33%	11.333^{ab}±1.702	5.892^{bcd}±1.053	27.5^{b} ±3.809
Violet LEDs	Control	0	60%	2^{e}±1.637	11.367^{a}±1.094	15.333^{c}±3.601
	BAP	1	65%	4^{de}±1.531	7.392^{b}±1.053	25^{b}±3.619
		2	70%	6.333^{cd}±1.459	6.133^{bcd}±1.023	27.333^{b}±3.744
	TDZ	1	80.952%	12.333^{ab}±1.665	5.292^{cd}±1.006	33.333^{a}±4.050
		2	89.47%	13.333^{a}±1.914	4.808^{d}±0.994	34.667^{a}±4.313
LSD 0.05			-	3.332	1.378	5.2739

Number of shoots /explants

Under white and violet LED illumination, the number of shoots/explants of *in vitro*-cultured *Mentha* plantlets increased by the used treatments; these treatments were either non-significant in MS medium supplemented with 1 mg/L BAP or significant when applied to the other treatments. Hence, the minimum value was recorded in the control MS nutrient medium under white light illumination. The maximum value was estimated under violet LEDs in MS medium enriched with 2 mg/L TDZ.

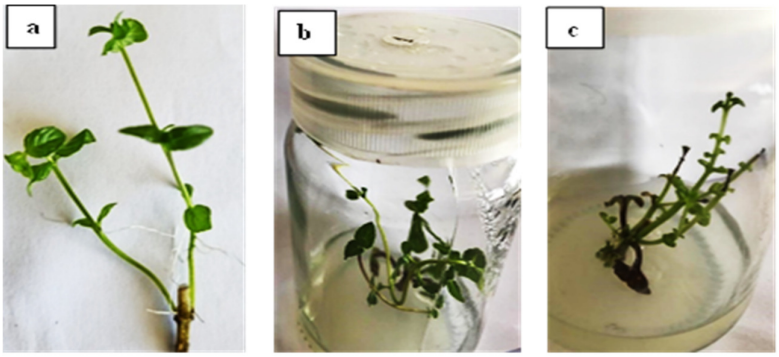

Figure 1. *Mentha viridis* plantlets grown under white LED for 30 days
a) into control medium (MS basal medium); b) into MS medium supplemented with 1 mg/L BAP; c) into MS medium supplemented with 2 mg/L BAP

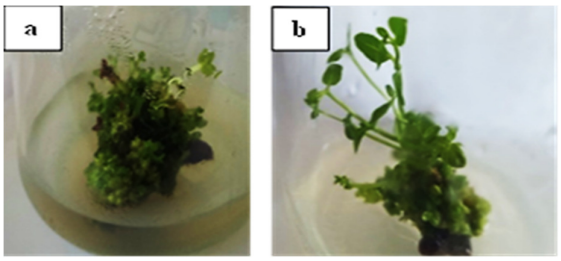

Figure 2. *Mentha viridis* plantlet and differentiated callus grown under white LEDs for 30 days
a) into MS medium supplemented with 1mg/L TDZ; b) into (Murashige and skoog medium) MS medium supplemented with 2 mg/L Thidiazrun (TDZ)

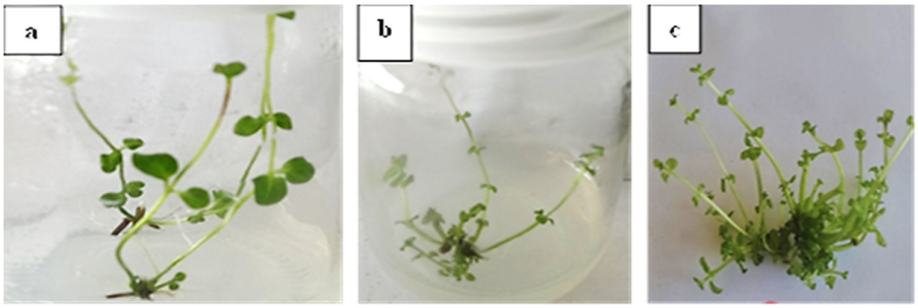

Figure 3. *Mentha viridis* plantlets grown under violet LED for 30 days
a) into (control) free hormone medium (MS basal medium). b) into MS medium supplemented with 1 mg/L BAP. c) into MS medium supplemented with 2 mg/L BAP.

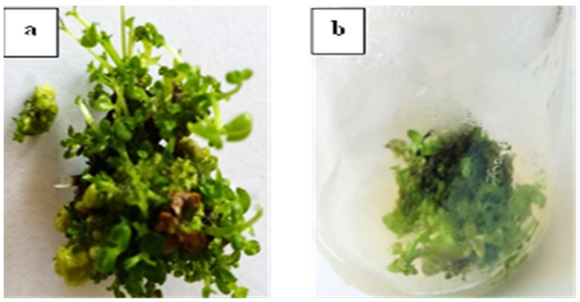

Figure 4. *Mentha viridis* plantlet and differentiated callus grown under violet light LEDs for 30 days
a) into MS medium supplemented with 1 mg/L TDZ. b) into (Murashige and Skoog medium) MS medium supplemented with 2 mg/L thidiazrun (TDZ

Shoot length and number of leaves per explant

In vitro cultured *Mentha* plantlets, the treatments used decreased shoot length compared to the control, either non-significant in MS medium supplemented with 1 mg/L BAP under white LED illumination or significant by the other treatments under white and violet LED illumination, compared to the control that records the highest shoot length (12.38 cm) under violet LED illumination. The number of leaves/explants invitro cultured *Mentha* plantlets increased by the treatments used under white and violet LED illumination, either non-significant in MS medium supplemented with 1 mg/L BAP under white LED illumination or significant by the other treatments under white and violet LED illumination, compared to the control that recorded the minimum values (12 leaves/explant) under white light illumination. The maximum number of leaves (33.333 and 34.667) were recorded under violet LED illumination in the case of MS medium enriched with 1 and 2 mg/L TDZ, respectively.

Callus formation percentage (%) and callus size (cm^2)

Our results showed that no callus formed in MS medium supplemented with 1 and 2 mg/L BAP or control MS medium either under white or violet LED illumination, and only MS medium enriched with 1 and 2 mg/L TDZ recorded callus formation. The maximum callus formation percentage (93.33%) was recorded under violet LED illumination in MS medium enriched with 2 mg/L TDZ. The maximum size of differentiated callus (cm^3) was 7.5 and 7.12 cm^3 in MS medium supplemented with 2 and 1 mg/L TDZ, respectively, under violet LEDs. The differences between the recorded data show significance in the case of MS medium enriched with 1 mg/L TDZ under white light illumination and non-significance in the case of the other treatments.

Shoot and callus fresh and dry weights

The treatments used in this study increased shoot and callus fresh weights under white and violet LED illumination, either non-significantly by treatment with 1 mg/L BAP under violet light illumination or significantly by the other treatments used. Thus, the maximum value of callus fresh weight was in the case of MS medium enriched with 2 mg/L TDZ (1.30 g) under white light illumination. Control medium recorded the least values (0.42 g) in the case of violet LEDs. The changes in callus dry weight were more or less similar to that of the determined fresh weight, so the maximum value of callus dry weight was in MS medium fortified with 2 mg/LTDZ (0.69 g) under white light. In the case of the control medium, it estimated the least dry weight (0.38 and 0.17 g) in the case of white and violet LEDs, respectively, but with a significant difference.

Changes in antioxidant enzymes: peroxidase (POX), polyphenol oxidase (PPO) and cata-lase (CAT) activity (µ mol/g f wt/min) of micropropagated M. viridis

Comparing to the control value, except for the non-significant increase in peroxidase (POX) activity of micropropagated *M. viridis* in the case of MS medium supplemented with 1 and 2 mg/L BAP, under white LED illumination, a significant increase in this enzyme activity was detected by the other treatments under both white and violet LED illumination. In this study, results showed that under violet LED illumination, the activity of polyphenol oxidase recorded the maximum values in MS medium supplemented with 1 and 2 mg/L TDZ without a significant difference between them. While the control medium recorded the lowest values under white and violet LED illumination, with a significant difference between them (Figure 5)

The obtained results in Figure 5 showed that, under white LED illumination, the used treatments caused an increase in CAT activity compared to the value of the control medium. As regards violet LED illumination, there were non-significant increments in CAT activity in MS medium supplemented with 1 and 2 mg/L BAP. Whereas, MS medium fortified with 1 and 2 mg/L TDZ significantly increased catalase activity and recorded the highest activity, while control medium recorded the least values under white LED illumination.

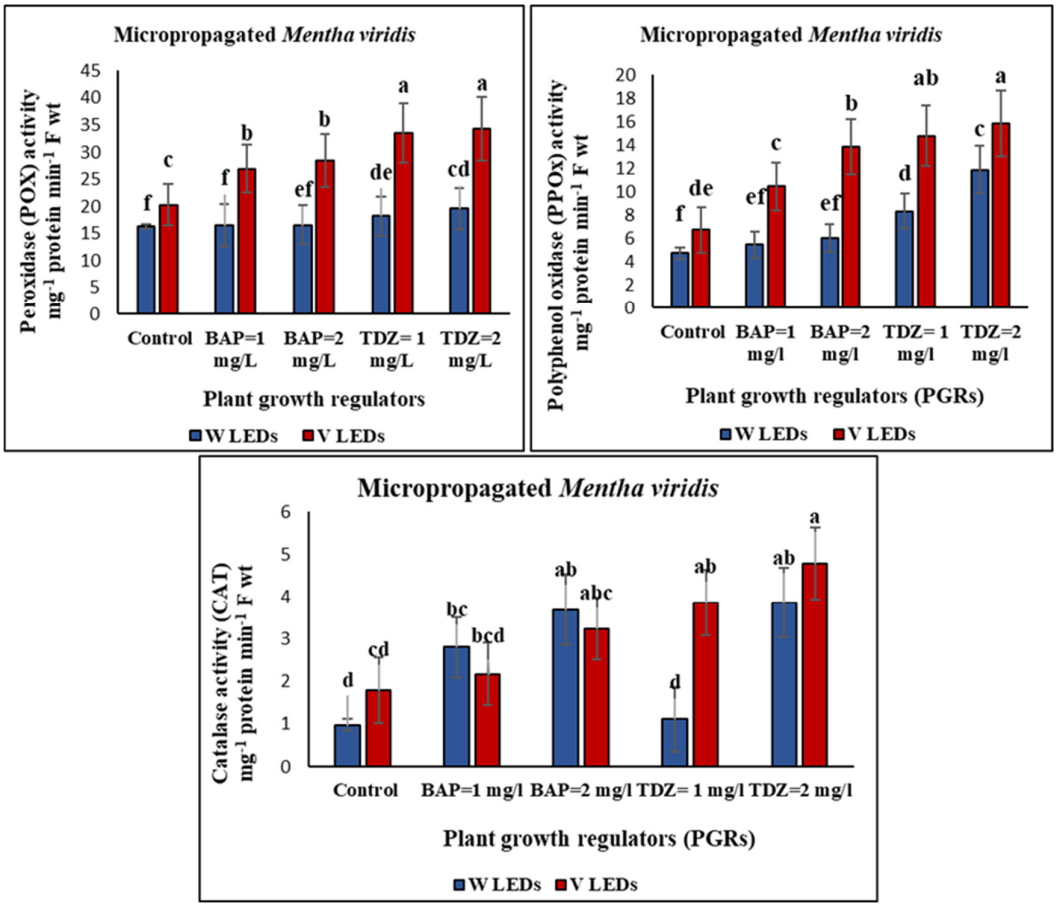

Figure 5. Effect of two different visible light emitting diodes (White and Violet) on antioxidant enzymes activity perpxidase (a), polyphrnol pxidase (b) and catalase (c) (unit mg^{-1} protein min^{-1} F wt) of micropropagated *M. viridis* after 30 days from transplanting

Values listed represent the mean ± standard error (SE). Different superscript letters refer to significant variation; with the least significant difference (LSD) at $p \leq 0.05$.

Changes in antioxidant compounds of micropropagated M. viridis

Phenols and flavonoids

In corresponding to the control value, phenol content in invitro cultured *M. viridis,* record an increment in MS medium supplemented with all the used treatments, generally. The control medium recorded the lowest values under white and violet LED illumination, without significant differences between them. Compared to the control values, MS medium supplemented with 1 and 2 mg/LTDZ recorded the maximum content of flavonoids under violet LED illumination. On the other hand, under white and violet LED illumination, the control medium recorded the least values (Figures 6a and b).

Anthocyanins and ascorbic acid

Under white and violet LED illumination and compared to the control values, all the treatments used generally significantly increased anthocyanin content in micropropagated *M. virdis*. So, the control medium recorded the lowest values of anthocyanin. Meanwhile, the highest value recorded was that in the case of MS medium fortified with 2 mg/L TDZ under violet LED illumination. In *M. virdis* under white and violet LED illumination, a general, significant increase in ascorbic acid was recorded in relation to the control values. In the case of MS medium, it is fortified with 1 and 2 mg/L TDZ. This vitamin content returned the maximum

values under violet LED illumination. On the other hand, white and violet LED illumination control medium recorded the least values of ascorbic acid (Figure 6c and d).

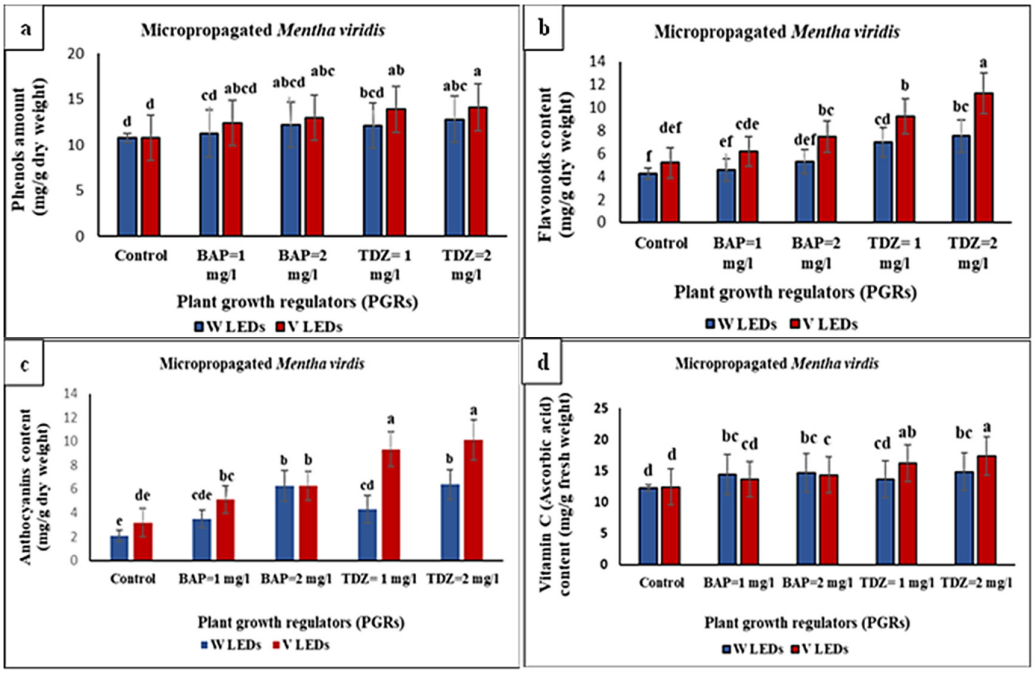

Figure 6. Effect of two different visible light emitting diodes (White and Violet on antioxidant compounds [phenols (a), flavonoids (b), anthocyanins (c) and ascorbic acid (d)] of micro propagated *M. viridis* after 30 days from transplanting

Values listed represent the mean ± standard error (SE). Different superscript letters refer to significant variation; with the least significant difference (LSD) at $p \leq 0.05$.

Changes in endogenous phytohormones of micropropagated M. viridis

Our results in Figure 7 indicated that GA_3 content recorded the highest values of micropropagated *M. viridis* cultured in control medium under white and violet LEDs because GA_3 content decreased by all the treatments, with significant differences between all values compared to the control values. This decline in GA_3 content was more pronounced in the case of MS medium fortified with 2 mg/L TDZ under violet and white LED illumination. A significant increment was quantified in the IAA content of micropropagated *M. viridis* by the used treatments, either under white or violet LEDs, as compared to the control value. The maximum value of IAA content was recorded in MS medium supplemented with 2 mg/L TDZ under violet LEDs. Naturally, control medium recorded the least values of IAA (46.56 and 58.79 g/100 g fresh weights, respectively) under white and violet LED illumination with a significant difference.

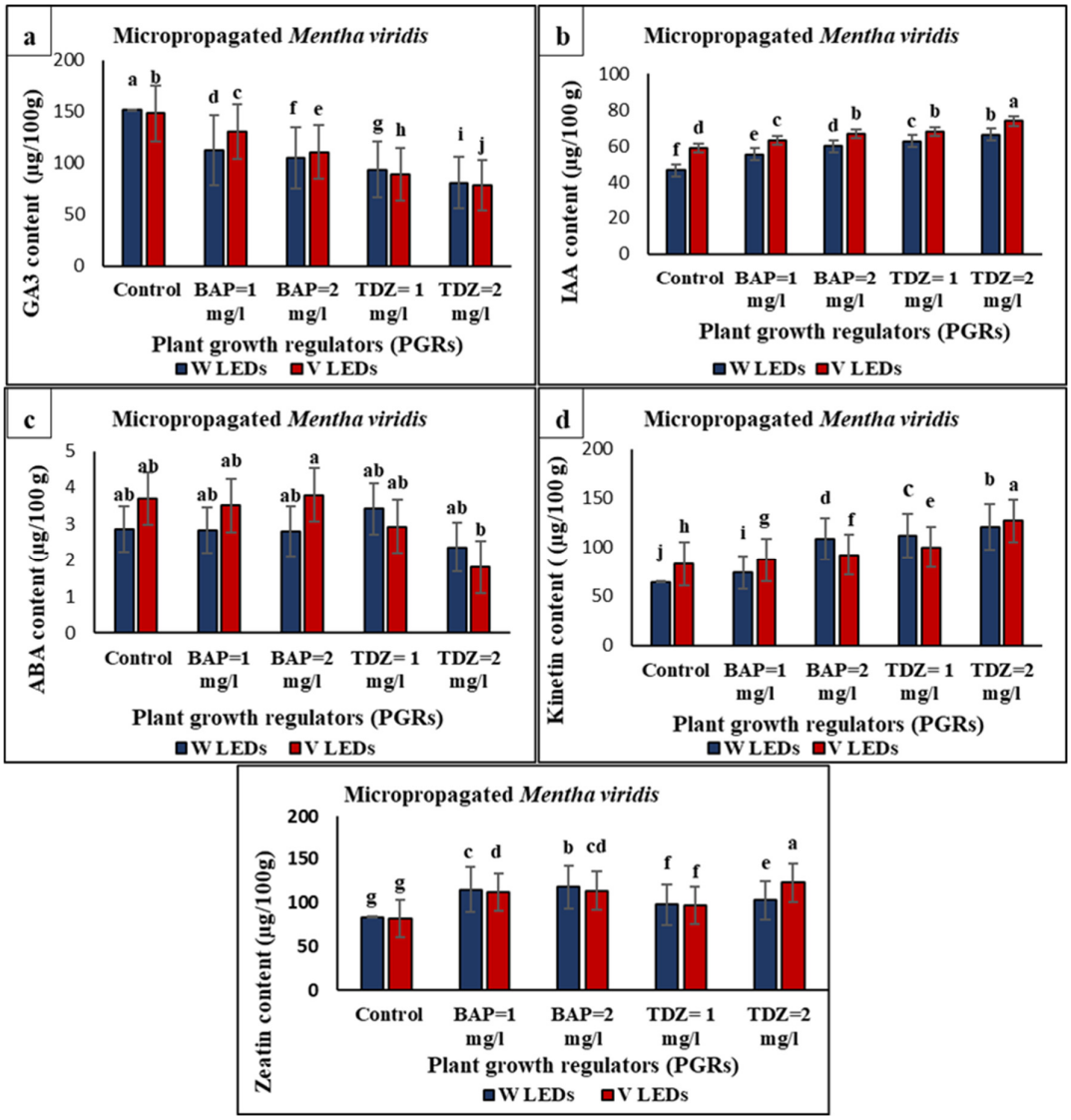

Figure 7. Effect of two different visible light emitting diodes (White and Violet) on phytohormones [GA_3 (a), IAA (b), ABA (c), Kinetin (d) and Zeatin (e)] (µg/100 g) of micropropagated *M. viridis* after 30 days from transplanting

Values listed represent the mean ± standard error (SE). Different superscript letters refer to significant variation; with the least significant difference (LSD) at $p \leq 0.05$.

Except for the non-significant increase that was recorded in MS medium supplemented with 1 mg/L TDZ under white LED illumination and in MS medium supplemented with 2 mg/L BAP under violet LED illumination, results reported a general non-significant decrease in ABA content of micropropagated *M. viridis* by the used treatments under white and violet LEDs as compared to control. Thus, the control medium estimated the highest value of ABA (3.70 g/100 g fresh weights) under violet LED illumination. The decline in ABA content was more pronounced in the case of MS medium fortified with 2 mg/L TDZ under violet LED illumination. The present findings revealed that the kinetin and zeatin content of micropropagated *M. virdis* increased with all the treatments used under white and violet LED illumination. Under violet LEDs, the maximum value of kinetin and zeatin content was recorded in MS medium supplemented with 2 mg/L TDZ.

Naturally, the control medium recorded the lowest values of kinetin and zeatin content under white and violet LED illumination.

Changes in essential oil constituents of micropropagated M. viridis under two different LEDS (white and violet)

The GC/MS analysis in Table 3 showed that ten compounds were identified in all treatments: a-pinene, d-limonene, 1,8-cineole, p-mentha-1(7), dien-2-ol, cis-carveol, trans-carveol, carvone, caryophyllene, germacrane d, and 2,4-di-tert-butylphenol. The main component was carvone, and compared to all other treatments, its highest value (43.53%) was recorded with nodal segments cultured with MS medium supplemented with 1 mg/L TDZ under violet LEDs. In consideration of light quality, it was a matter of importance to maintain that all treatments under violet light gave carvone higher than that obtained from white light at the same concentration of the cytokinin.

Table 3. Essential oil constituents' percentage of micropropagated *Mentha viridis* after incubation for 30 days from transplanting under two different visible light emitting diodes (LEDs) (white and violet)

Light quality	Plant growth regulators		Mean chemical constituents of *Mentha viridis* oils (%)														
	Cyt. type	Conc. (mg/L)	A-pinene	S-abinene	D-limonene	1,8-cineole	P-Mentha-1(7),8 dien-2-ol	l-Menthol	Cis-carveol	Trans carveol	Carvone	Caryophyllene	Dihydro Caryphyllene	Germacrane D	2-4-di-terr-butylphenol	Caryophyllene oxide	Benzoic acid
White LEDs	Control	0	2.85	-	6.31	8.10	1.71	4.50	8.75	1.95	25.16	4.46	4.34	4.33	14.61	2.63	4.10
	BAP	1	3.97	1.53	5.74	8.44	1.42	2.67	9.32	1.35	15.82	5.43	6.02	2.29	8.62	-	6.41
		2	2.84	-	3.22	3.37	2.56	-	6.39	2.19	22.45	3.49	-	2.23	7.53	1.87	6.54
	TDZ	1	1.68	0.84	2.68	2.93	0.90	2.66	5.80	2.34	10.31	4.20	3.39	2.29	4.21	0.84	3.15
		2	1.21	-	5.82	6.74	0.29	-	3.96	1.21	17.52	3.53	-	1.47	6.77	0.67	0.77
Violet LEDs	Control	0	2.06	0.93	2.73	8.05	1.32	1.86	6.24	1.31	33.39	3.57	5.82	4.60	6.20	2.25	3.26
	BAP	1	1.60	-	3.13	5.65	3.97	3.14	16.18	2.42	27.27	3.49	5.79	1.34	11.32	2.56	4.42
		2	1.96	0.92	5.96	8.57	1.62	4.79	8.06	1.32	28.70	4.79	3.45	1.69	2.13	-	-
	TDZ	1	2.70	0.10	6.50	10.69	0.25	4.49	5.43	1.09	43.53	1.78	-	1.27	2.06	1.28	-
		2	2.16	1.23	4.93	11.16	1.49	2.38	4.96	2.56	19.55	3.25	4.03	2.83	11.35	1.60	-

Other major constituents in the current study (i.e., cis-carveol, 1,8-cineole, and D-limonene) were influenced and detected in all treatments in different quantities. So, there is cis-carveol, which recorded the highest value (16.18%) under violet light in the case of MS medium supplemented with 1 mg/L BAP, while 8-cineole recorded the highest value (11.16%) under violet light in the case of MS medium supplemented with 2 mg/L TDZ, followed by the treatment of MS medium supplemented with 1 mg/L TDZ under violet light (10.69%). Also, D-limonene gave the highest value (6.50%) with the same treatment, which gave the highest value of carvone (1 mg/L TDZ under violet LED).

Discussion

Changes in in vitro culture parameters

The survival percentage, number of shoots and number of leaves per explant of *M. viridis* showed the maximum value in MS medium supplemented with 2 and 1 mg/L TDZ, respectively under violet LEDs. In support to using nodal segments of *M. viridis* as explant in the current study, earlier research on invitro propagation of medicinal plants recorded the proliferation efficiency of nodal explants was found significantly higher than that of other explants and BAP was most effective cytokinin for inducing multiple shoots in *in vitro* techniques (Raja and Arockiasamy, 2008). On the other hand, Rout and Das (1997) reported maximum (95%) proliferation of nodal explants of *M. viridis* in MS + 3.0 mg/l BAP. Rahman *et al*. (2013) reported when

BAP was applied singly, 50% explants responded with highest average number (3.40±0.24) of shoot buds per explant in 2.0 mg/L BAP. In accordance to the obtained results, Kadota and Nimii (2003) suggested that synthetic phenyl urea derivatives (CPPU and TDZ) produce more hyper hydric shoots than with adenine derivatives (BAP and KIN). However, increasing the level of BAP in the media to 3 mg/L caused a significant decline in the number of shoots per explant, indicating that the need to optimize the concentrations of cytokinin was critical in shoot multiplication (El-Shafey *et al*., 2019). In light of the present results, Omar (2017) found that different light sources altered potato plantlet development: red and green LEDs were the most and least prescribed for plantlet advancement, respectively. Meanwhile the present results opposite with the found of Al Khateeb *et al*. (2017) reported that BAP induced the best results in shoot multiplication from shoot apex of *Rumex cyprius* when compared with TDZ and KIN. Also, Rahman *et al*. (2013) indicated that the highest average number of micro shoots was achieved on MS medium with 2.0 mg/l BAP. Samantaray *et al*. (2012) studied the effect of BAP on proliferation of spearmint shoots and stated that the concentration of 2.5 mg/L BAP along with normal MS medium produced significantly maximum number of shoots per explants, maximum numbers of leaves and a smaller number of days required for shoot regeneration. A further increase in concentration of BAP showed some adverse effect and subsequently reduced the number of shoot development from callus.

In this study, shoot length of *M. viridis* recorded the maximum value in case of control medium under violet LEDs illumination. The changes recorded in shoot length by the used treatments are in harmony with Poovaiah *et al*. (2006), the formation of shoots with a height greater than 2 cm in *in vitro* cultivation of *Mentha* is satisfactory, since they are suitable for the subsequent *in vitro* rooting step. The stems of potato plantlets developed invitro under pure red light exhibited shorter cells than those grown under blue light, according to histological examinations (Wilson *et al*., 1993). This could explain red and blue height performance in the present experiment. It seems that stem elongation can be promoted or inhibited by different synergistic interactions between blue/red light receptors and phytochrome according to species (Kim *et al*., 2004). In confirmation to the observed excellence of violet LEDs, in this investigation, red and blue LED lights, according to Folta and Maruhnich (2007), cause faster plantlet growth than white light. Poudel et al. (2008) studied growth and morphogenesis of the tested grape genotypes varied in response to the different LEDs and they stated that, plant height and inter-node length were significantly longer in plants cultured under red LEDs in all genotypes. Concerning of callus formation percentage (%) as well as the related determination, in the present study, no callus formed in MS medium supplemented with 1 and 2 mg/L BAP or control MS medium either under white or violet LEDs illumination and only MS medium enriched with 1 and 2 mg/L TDZ record callus formation with the maxi-mum recorded value with 2 mg/L TDZ under violet LEDs illumination. This is related to that of Lee and Lee (2003) who declared that TDZ effectively promotes callus formation, and induces the higher axillary shoot proliferation and shoot organogenesis of many recalcitrant orchid species at relative lower concentrations (from 0.45 to 4.52 μM). Also, Srivastava *et al*. (2017) stated that although induced callus was observed on both BAP- and TDZ-containing media, the frequency of callus induction on BAP-containing medium was considerably less than that on the TDZ-supplemented medium. Moreover, Alagarsamy *et al*. (2018) reported that the maximum callus induction rate (72.5%) was obtained from the leaf explants by supplementing B5 medium with 2 mg/L BAP, 1 mg/L TDZ. From other side of view, El-Shafey *et al*. (2019) indicated that the percentage of callus induction of *Rumex pictus*, when cotyledonary leaves were used as explants, reached the highest value (88.8%) at combination of 1 mg/ L 2,4-D and 0.8 mg /L BAP.

In accordance with the tabulated data in the present results, Ma *et al*. (2021) stated that the combination of red/blue LED lights only significantly increased dry weight by 161% among 25 plant characteristics analyzed, compared to the white LED light. Cytokinin supplementation of MS medium increased the biomass of *Dracocephalum forrestti* culture after 4 weeks of growth (Weremczuk-Jeżyna *et al*., 2018). Also, Nhut *et al*. (2015) reported that 60% red LED combined with 40% blue LED resulted in the highest values of micropropagated *Panax vietnamensis* fresh and dry weight, average plant height, leaf diameter and leaf length

higher than those recorded under fluorescent lamp. Gu *et al*. (2012) also suggested that plantlets of *Anthurium andraeanum* from leaf explants grown under red plus blue light had 22.7% greater total dry weight and more balanced root-to-shoot ratio than those grown under fluorescent white light. These results are in harmony with the present results using of complex of red blue (violt) LED could be an option for improving growth of *M. viridis* plantlets *in vitro*. The explant type, age and anatomical structure play an important role in the callus formation. The variety of callus formation of different explant types and age has been reported in many other plants (Zouzou *et al*., 2008).

Changes in antioxidant enzymes activity of micropropagated M. viridis

In this investigation, the maximum value of peroxidase, polyphenol oxidase activity and catalase activity were recorded by TDZ application; especially 2 mg/L TDZ under violet illumination. Differences in the antioxidant enzymes activity during invitro organogenesis in this investigation are in harmony by those stated that differences in the antioxidant enzymes activity in somatic embryogenesis in several plant species were reported (Gupta and Datta, 2004; Lipik *et al*., 2005). Furthermore, Xu *et al*. (2019) showed that the combination of red-blue-purple-green composite LEDs light could effectively improve the antioxidant capacity of *Cunninghamia lanceolata* tissue culture seed-lings and delay plant senescence and SOD, POD and CAT activities were significantly rise. In this concern, the non-significant increase of the determined peroxidase activity, in this study of micropropagated *M. viridis* in case of MS medium supplemented with 1 and 2 mg/L BAP compared to the least values that record-ed by the control medium is in harmony with the results of callus formation. This is confirmed by Srivastava *et al*. (2017) who noticed decreased POX activity of invitro cultured *Brassica oleracea* during differentiation. POX also plays a major role in an effective scavenging mechanism to remove H_2O_2 produced in plants, thereby lowering the oxidative stress and enhancing shoot growth and differentiation (Molassiotis *et al*., 2005). Similar results were also reported by Jana and Shekhawat (2012) in *Anethum graveolens*. In connection to the obtained results of PPO, phenolics released by injury act as signaling molecules and induce increases in PPO levels through feedback regulation. Under these conditions, PPO expression and PPO protein levels increase (Wang *et al*., 2008); these processes are correlated with the current results. From other side of view and about the response of PPO to white and violet LEDs illumination, Jung *et al*. (2021) stated that applying an appropriate LED spectral wave-length significantly increases antioxidant enzyme activity in plants, thereby enhancing the cell defensive system and providing protection from oxidative damage.

In this concern, increase in the activity of antioxidant enzymes, such as CAT, was found to be related to the delay in the onset of leaf senescence in banana (Chen *et al*., 2019). Also, Rajeswari and Paliwal (2008) stated that CAT plays an important role in shoot organogenesis in *Albizia adorratissima* and enhanced CAT activity is associated with increased adventitious shoot formation in plants, this is in harmony with the results of this investigation. According to Causin *et al*. (2006), blue light plays an important role in decreasing cellular oxidative dam-age by enhancing CAT activity in wheat plants. Gupta and Datta (2004) reported enhancement of SOD and CAT activities during adventitious shoot formation in *Gladiolus hybridus* grown under blue LED irradiation. Additionally, red light exposure decreased catalase and ascorbic acid peroxidase activities in some annual plants, but significantly increased their activities in some perennial plants (Li and Tang, 2010). In general, as recorded in catalase and peroxidase activity in the current study, Zhao *et al*. (2020) reported that the combination of red and blue light at a ratio of 1:1 enhanced the activity of antioxidant enzymes, including catalase, peroxidase, in *Carpesium triste* Maxim.

Changes in antioxidant compounds of micropropagated M. viridis

The determined antioxidant compounds (phenols, flavonoids, anthocyanins, ascorbic acid) content *in vitro* cultured *M. viridis*, record an increment in MS medium supplemented with all the used treatments,

comparing to the control values that record the least values; generally, with the maximum content by 2 mg/L TDZ under violet LEDs illumination. In this concern, *M. viridis* is a medicinal plant of the Lamiaceae family characterized by its potency to synthesize and secret secondary metabolites, essentially essential oils. Pharmacological properties of *M. viridis* extracts and essential oils were investigated for different health benefits such as antioxidant, anticancer, anti-parasitic, antimicrobial, and antidiabetic effects. *In vitro* and in vivo studies showed positives effects that could be certainly related to different bioactive compounds identified in *M. spicata* (El Menyiy *et al*., 2020). Eleven phenolic compounds were detected in 9 *Mentha* species including *M. spicata* (Park *et al*., 2019). The accumulation of phenolic compounds in plant tissues is a distinctive feature of environmental stress, and an increase in the biosynthesis of polyphenolic compounds helps plants cope with multiple bio-tic and abiotic stresses, such as drought, heavy metals, salinity, temperature, ultraviolet light (Tuladhar *et al*., 2022).In general, some studies report that the level of phenolics is higher in plants exposed to light than in samples from plants covered with nets and is highly influenced by the environmental conditions (Salazar-García *et al*., 2016). Light increases the total phenolic content by improving photosynthesis, as well as the malonyl-CoA pathway, which is associated with the synthesis of phenolic compounds (Qian *et al*., 2016). In this study a positive correlation was detected between phenols content in-vitro cultured *M. virdis* under white and violet LEDs illumination and the activity of peroxidase. In this respect, Liu *et al*. (2012) indicated that blue LED light should be recommended as a light source for enhancing the content of phenolic compounds of pea sprouts. Klimek-Szczykutowicz *et al*. (2022) concluded that red, blue and green LEDs light can be considered the best light quality variant for increasing the production of secondary metabolites and the antioxidant potential of *Nasturtium offcinale* microshoot cultures.

Ibrahim and Jaafar (2012) highlighted that the production of a wide range of flavonoids is possible by controlling the type and concentration of exogenic PGRs, as well as lighting conditions. The flavonoid biosynthesis in *in vitro* cultures has been found to be modulated significantly by exogenous auxins and cytokinins (Abbas *et al*., 2021). The use of LED light also caused an increased accumulation of many flavonoid compounds in *Cyathea delgadii* invitro culture, compared to fluorescent light (Mikuła *et al*., 2021). Liu *et al*. (2012) reported that the highest total flavonoid content was found in blue-LED grown sprouts. Consequently, from the aspect of the total phenolic and flavonoids, the blue LED light was determined to be the optimal culture condition for the production of pea sprouts. El-Shafey *et al*. (2019) revealed that increasing the concentration of BAP above 0.2 mg/L in combination with 1 mg/ L 2,4-D inhibited the accumulation of flavonoids. Although 2 mg/L 2,4-D induced lower callus growth with all combinations of BAP, it enhanced the accumulation of flavonoids, this hypothesis is in harmony with the results of this investigation.

Anthocyanins, a key component of secondary metabolites, were found to be increased by the LEDs. Antho-cyanins are water-soluble glycosides and acyl glycosides of anthocyanidins, a phenolic chemical class found in nature (Lian *et al*., 2019). Hasan *et al*. (2017) reported that blue light and plants subjected to multiple light cycles were found to have the most anthocyanins accumulation that induced by visible, ultraviolet, and various forms of LED lights in general. For *Gynura procumbens*, similar increases in phenolics, flavonoids, and anthocyanins were observed under various LEDs, with the biggest effect occurring when calli was exposed to blue light rather than red or white light (Lian *et al*., 2019). Lekkham *et al*. (2016) stated that anthocyanin concentration was increased in apples grown under red LED irradiation. Irradiation of grapes with both blue and red LEDs upregulated the expression of anthocyanin biosynthesis-related genes, such as MYB transcription factor genes. The results are confirmed by those of Kamal *et al*. (2020) who found that the highest ascorbic acid content was found with callus cultured on MS + 1.0 mg/l TDZ + 0.25 mg/l NAA + 5.0 mg/l $AgNO_3$, when used leaf explant in *Chinese cabbage*. Also, lettuce plants illuminated with single or combined blue and red lights showed higher vitamin C contents than plants grown in white light (Ohashi-Kaneko *et al*., 2007). Compatibility between the stimulation in secondary metabolites and the used treatments and light emitting diodes (LEDs) in this study is con-firmed with Shen *et al*. (2014) who found that in lettuce, continuous

illumination with red-blue light emitting diodes (LEDs) increases the vitamin C content in relation to the exposure time, in the same plants cultivated for 15 days under continuous red-blue LEDs exposure (Zha *et al*., 2019).

Changes in endogenous phytohormones of micropropagated M. viridis

In the current study, IAA, kinetin and zeatin content of micropropagated *M. virdis* increased by all the used treatments under white and violet LEDs illumination, with, the maximum value in MS medium supplemented with 2 mg/L TDZ under violet LEDs. On the contrary, GA_3 and ABA recorded the maximum value in the control MS medium and decreased by the other used treatments, in general. In this connection, endogenous phytohormones are organic substances synthesized in plant tissues that trigger physiological processes such as growth, differentiation and development (Isoda *et al*., 2020). They are chemical messengers, which play various roles at different stages, in different tissues or under different environmental conditions (Yu *et al*., 2020). Cio´c *et al*. (2022) indicated that LED light (Blue and Red: Blue) lowered total gibberellin content at the end stage of shoot propagation of *Gerbera jamesonii*. Lance *et al*. (2006) reported that culture conditions, light versus darkness and the quantity of cytokinin in the medium, affected the content of gibberellins found in the tissue. These culture conditions were also important in controlling growth rate of the callus and modified the ability of the tissue to respond to exogenous gibberellins. In harmony with the results of this study, Yu *et al*. (2020) declared that ABA contents were the greatest under the blue LED light and also, ABA has an inhibitory effect on plant growth, and the ABA content was consistent with the low plant height and fresh weight of the rice seedlings obtained under the blue LED light. Yu *et al*. (2020) found that IAA and GA_3 contents of the rice seedlings were the greatest under the red LED light. IAA promotes the longitudinal elongation of cells, while GA_3 and IAA can promote cell division of the formation layer; therefore, the rice seedlings under the red LED light treatment exhibited the greatest plant height and fresh weight. These results were consistent with the present results on micropropagated *M. viridis* as well as previous findings on the effects of blue and red LED light on the IAA content and ultimately affected the growth of cucumber leaves (Su *et al*., 2014).

Bairu *et al*. (2011) indicated that the exogenous application of cytokinin does have an effect on the endogenous cytokinin pool. The positive effects of applied cytokinin on increasing the endogenous cytokinin pool has been reported also by Ivanova *et al*. (2006). From other side of view, Cio´c *et al*. (2022) declared that zeatin (t-Z and c-Z) with two forms have stimulating effect on *Gerbera jamesonii* growth and was particularly visible in the plants grown under RB LED light, which showed the highest multiplication rate and high concentration of both zeatin forms at the end of the culture. Attibayeba *et al*. (2020) highlighted that the highest content of cyto-kinin was observed in Cichorium intybus L. explants exposed to either red light or long-day periods. In addition to endogenous hormones, phenolic compounds and antioxidants may also affect the success of invitro cultures (Karakas, 2020). Reactive oxygen species (ROS) interact with plant hormones and affect callus induction directly or indirectly (Mhamdi and Breusegem, 2018). These findings are in harmony with the obtained results in this study on micropropagated *M. viridis*.

Changes in essential oil constituents of micropropagated Mentha viridis under two different LEDS (white and violet)

In this study it is evident that there is wide range of variation in the oil content of propagated *M. viridis* in response to the used treatments. In support to the obtained analytical data of the essential oil constituents of micropropagated *M. viridis*, Bayan and Küsek (2018) showed that the gas chromatography/mass spectrometry (GC/MS) analysis of *M. spicata* showed the main component was carvone (56.94%). Ahmadi *et al*. (2011) reported that LED lights have a positive effect on the essential oil content. Light quality can change the composition of essential oils in medicinal plants (Amaki *et al*., 2011). Light affects the number and

morphology of leaves and essential oil storage structures such as trichrome, causing changes in the amount and chemical composition of essential oil in plants (Fernandes *et al*., 2013). Moreover, the current results are in harmony with the finding of Mkaddem *et al*. (2009) who revealed that *M. viridis* was rich in carvone (50.47%), 1,8-cineole (9.14%), and limonene (4.87%). It should be emphasized that the chemotype of plants is extremely important. Some of them were characterized by higher amounts of limonene and 1,8-cineole (eucalyptol), which corresponded to the results of Łyczko *et al*. (2020) who reported that a difference was found in other major constituents of peppermint which are expected to occur along with limonene and 1.8-cineole, such as carvone. These results are in harmony with the present results of the oil content detected by the GC/MS analysis of propagated *M. viridis* in response to the used treatments.

From other side of view, Alvarenga *et al*. (2018) reported that the production of volatile compounds in micro-propagated *M. viridis* was affected by quality and quantity of light conditions applied. Similar enhancing effects were obtained by Ahmadi *et al*. (2011) indicating that the essentials oil composition of two genotypes of lemon balm was affected both qualitatively and quantitatively by different LED light sources; hence, LED lights might be used to improve monoterpenes, sesquiterpenes, and antioxidant activity in the selected genotypes. They also reported that essential oil estimated by GC/MS indicated that the highest content of monoterpenes in the genotypes was under red+ blue LED lamps, this is in harmony with the current results of micropropagated *M. viridis*. Also, Tohidi *et al*. (2019) showed an increase in sesquiterpene levels of three species of thyme under red+ blue LED lights. While, Nguyen and Saleh (2019) reported an increase in monoterpenes such as alpha-pinene, beta-pinene, limonene and carvone in mint plants under red LED light. Moreover, the concentration of beta-bourbonene as a sesquiterpene was also higher in mint cultivated under LED light compared to the control condition (Nguyen and Saleh, 2019).

Conclusions

Plant tissue culture is an effective technology, it may open up new avenues for the economically viable commercial cultivation of even uncommon or exotic plants. It became evident from this study that combined red: blue LEDs; ''violet'' LEDs is more energy efficient than "white" or full spectrum LEDs because blue and red LEDs have the highest photon efficacy compared to other colors as they convert the highest amount of electricity in photons, so produce more growth from plants and anyhow, violet LEDs are more effective than white. Moreover, the addition of thidiazuron and benzyl aminopurine to the medium enhancing growth and the determined metabolites variably, but in general, the former is the most superior, although protocols for optimizing the use of medical plants have made excellent progress, more study is still required in these areas to optimize and preserve the use of medicinal plants.

Authors' Contributions

Conceptualization, H.E., S.A.H., L.A., M.A., A.M., and R.G.; methodology, H.E., R.G. and S.A.H.; software, H.E., S.A.H., and K.A.; validation, H.E., and S.A.H.; formal analysis, H.E., R.G.; investigation, S.A.H., R.G.; resources, H H.E., S.A.H., L.A., M.A., A.M., and R.G.; data curation, H.E., S.A.H., and R.G.; writing—original draft preparation, H.E., S.A.H., L.A., M.A., A.M., K.A. and R.G..; writing—review and editing, H.E., A.M., K.A., D.J., A.A. and R.G..; supervision, H.E., S.A.H., and S.A.H.; funding acquisition: A.A., D.J.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abbas GM, Bar FMA, Sallam A, Elgamal RM, Lahloub MFI, Gohar AA (2021). *In vitro* callus culture of *Cynara cardunculus* subsp. *scolymus*: a biosystem for production of caffeoylquinic acid derivatives sesquiterpene lactones and favonoids. Plant Biosystems 156(4):865-874. *https://doi.org/10.1080/11263504.2021.1947406*

Adams R (2007). Identification of essential oil components by gas chromatography mass spectroscopy. 4th Ed. Allured Publishing Crop. Carol stream IL 60188 USA. *https://doi.org/10.1016/j.jasms.2007.01.001*

Aebi HE (1983). Catalase. In: Bergmeyer HU, Bergmeyer J, Grable M (Eds). Methods of Enzymatic analysis. Vol III. Verlag Chemie Weinheim, pp 273-286. *https://doi.org/10.1016/b978-0-12-091302-2.50032-3*

Agarwal S, Shaheen R (2007). Stimulation of antioxidant system and lipid peroxidation by abiotic stresses in leaves of *Mormoordica charantia*. Brazilian Journal of Plant Physiology 19:149-161. *https://doi.org/10.1590/S1677-04202007000200007*

Ahmadi T, Shabani L, Sabzalian MR (2011). LED light sources improved the essential oil components and antioxidant activity of two genotypes of lemon balm (*Melissa offcinalis* L.). Botanical Studies 62:9. *https://doi.org/10.21203/rs.3.rs-254821/v1*

Al Khateeb W, Alu'datt M, Al Zghoul H, Kanaan R, El-Oqlah A, Lahham J (2017). Enhancement of phenolic compounds production in invitro grown *Rumex cyprius* Murb. Acta Physiologiae Plantarum 39:14. *https://doi.org/10.1007/s11738-016-2312-6*

Aleksic V, Knezevic P (2014). Antimicrobial and antioxidative activity of extracts and essential oils of *Myrtus communis* L. Microbiological Research 169:240-254. *https://doi.org/10.1016/j.micres.2013.10.003*

Alagarsamy K, Shamala LF, Wei S (2018). Influence of media supplements on inhibition of oxidative browning and bacterial endophytes of *Camellia sinensis* var. *sinensis*. Biotechnology 8:356-363. *https://doi.org/10.1007/s13205-018-1378-9*

Alvarenga JP, Pacheco FV, Bertolucci SV, Silva ST, de Oliveira T, Pinto JEBP (2018). *In vitro* culture of *Mentha viridis*: quality and intensity of light on growth and production of volatiles. Acta Horticulture 1224:175-182. *https://doi.org/10.17660/ActaHortic.2018.1224.23*

Amaki W, Yamazaki N, Ichimura M, Watanabe H (2011). Effects of light quality on the growth and essential oil content in sweet basil. Acta Horticulture 907:91-94. *https://doi.org/10.17660/ActaHortic.2011.907.9*

Attibayeba, Jean-Luc B, Genevieve OA (2020). Changes in endogenous cytokinins and *in vitro* photoperiodic flowering induction in *Cichorium intybus* L. Pakistan Journal of Nutrition 9(10):230-234. *https://doi.org/10.3923/pjn.2010.230.234*

Bairu M, Novak O, Doležal K, Staden JV (2011). Changes in endogenous cytokinin profiles in micropropagated *Harpagophytum procumbens* in relation to shoot-tip necrosis and cytokinin treatments. Plant Growth Regulation 63:105-114. *https://doi.org/10.1016/j.sajb.2010.02.011*

Bayan Y, Küsek M (2018). Chemical composition and antifungal and antibacterial activity of *Mentha spicata* L. volatile oil. Ciencia e Investigación Agraria 45(1):64-69. *https://doi.org/10.7764/rcia.v45i1.1897*

Bukar SM, Abba HM (2022). Macro- and micropropagation of *Moringa oleifera* Lam (Moringaceae): A mini review. Asian Journal of Plant Biology 4(1):20-25. *https://doi.org/10.54987/ajpb.v4i1.699*

Çakılcıoğlu U, Türkoğlu I (2009). Plants used for hemorrhoid treatment in Elazığ central district. Acta Horticulturae 826:89-96. *https://doi.org/10.17660/ActaHortic.2009.826.11*

Causin HF, Jauregui RN, Barneix AJ (2006). The effect of light spectral quality on leaf senescence and oxidative stress in wheat. Plant Science 171:24-33. *https://doi.org/10.1016/j.plantsci.2006.02.009*

Chen J, Li F, Li Y, Wang Y, Wang C, Yuan D, Jiang Y (2019). Exogenous procyanidin treatment delays senescence of harvested banana fruit by enhancing antioxidant responses and in vivo procyanidin content. Postharvest Biology and Technology 158:110999. *https://doi.org/10.1016/j.postharvbio.2019.110999*

Cio′c M, Dziurka M, Pawłowska B (2022). Changes in endogenous phytohormones of *Gerbera jamesonii* axillary shoots multiplied under different light emitting diodes light quality. Molecules 27:1804. *https://doi.org/10.3390/molecules27061804*

Cousineau JC, Donnelly DJ (1991). Adventitious shoot regeneration from leaf explants of tissue cultured and greenhouse-grown raspberry. Plant Cell Tissue and Organ Culture 27:249-255. *https://doi.org/10.1007/BF00157588*

Dagla HR (2012). Plant tissue culture. Historical developments and applied aspects. Resonance 17(8). *https://doi.org/10.1007/s12045-012-0086-8*

Debergh P, Aitken-Christie J, Cohen D (1992). Reconsideration of the term 'vitrification' as used in micropropagation. Plant Cell Tissue and Organ Culture 30:135-140. *https://doi.org/10.1007/BF00034307*

Devi P (2002). Principles and methods in plant molecular biology biochemistry and genetics. Agrobios India, pp 41.

Edwards R (2004). No remedy in sight for herbal ransack. New Scientist 181:10-11.

Egyptian pharmacopoeia (1984). Text book of the Egyptian pharmacopoeia. Third Edition. Vol. (1). Cairo general organization for government printing office (with Memphis modification).

Ekor M (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in Pharmacology 4:177. *https://doi.org/10.3389/fphar.2013.00177*

El-Bialy DMA (2005). Effects of salinity and fertilization on growth and productivity of lettuce plants. MSc Thesis Faculty Science Mansoura University Egypt.

El Menyiy N, Mrabti HN, El Omari N, EI Bakili A, Bakrim S, Mekkaoui M, ... Bouyahya A (2022). Medicinal uses phytochemistry pharmacology and toxicology of *Mentha spicata*. Evidence-Based Complementary and Alternative Medicine 799:050832. *https://doi.org/10.1155/2022/7990508*

El-Shafey NM, Sayed M, Ahmed ES, Hammouda O, Khodary SA (2019). Effect of growth regulators on micropropagation callus induction and callus flavonoid content of *Rumex pictus* Forssk. Egypt. Journal of Botany 59(2):269-278. *https://doi.org/10.21608/ejbo.2019.4873.1202*

Fasolo F, Zimmerman RH, Fordham I (2012). Adventitious shoot formation on excised leaves of invitro grown shoots of apple cultivars. Plant Cell Tissue and Organ Culture 16:75-87. *https://doi.org/10.1007/BF00042180*

Fernandes VF, de Almeida LB, Feijó E, Silva D, de Oliveira RA, Mielke MS, Costa L (2013). Light intensity on growth leaf micromorphology and essential oil production of *Ocimum gratissimum*. Revista Brasileira de Farmacognosia 23(3):419-424. *https://doi.org/10.1590/S0102-695X2013005000041*

Folta KM, Maruhnich SA (2007). Green light: a signal to slow down or stop. Journal of Experimental Botany 58(12):3099-3111. *https://doi.org/10.1093/jxb/erm130*

Gu A, Liu W, Ma C, Cui J, Henny R, Chen J (2012). Regeneration of *Anthurium* and *Raeanum* from leaf explants and evaluation of micro cutting rooting and growth under different light qualities. HortScience 47:88-92. *https://doi.org/10.21273/HORTSCI.47.1.88*

Gupta SD, Datta S (2004). Antioxidant enzyme activities during invitro morphogenesis of gladiolus and the effect of application of antioxidant on plant regeneration. Biology of Plant 47:179-183. *https://doi.org/10.1023/B:BIOP.0000022248.62869.c7*

Hasan MM, Bashir T, Ghosh R, Lee SK, Bae H (2017). An overview of LEDs' effects on the production of bioactive compounds and crop quality. Molecules 22(9):1420. *https://doi.org/10.3390/molecules22091420*

Ibrahim MH, Jaafar HZ (2012). Primary secondary metabolites H_2O_2 malondialdehyde and photosynthetic responses of *Orthosiphon stimaneus* Benth. to different irradiance levels. Molecules 17:1159-1176. *https://doi.org/10.3390/molecules17021159*

Isoda R, Yoshinari A, Ishikawa Y, Sadoine M, Simon R, Frommer WB, Nakamura M (2020). Sensors for the quantification localization and analysis of the dynamics of plant hormones. Plant Journal 105:542-557. *https://doi.org/10.1111/tpj.15096*

Ivanova M, Novák O, Strnad M (2006). Endogenous cytokinins in shoots of *Aloe polyphylla* cultured *in vitro* in relation to hyperhydricity exogenous cytokinins and gelling agents. Plant Growth Regulation 50:219-230. *https://doi.org/10.1007/s10725-006-9139-x*

Jana S, Shekhawat GS (2012). *In vitro* regeneration of *Anethum graveolens* antioxidative enzymes during organogenesis and RAPD analysis for clonal fidelity. Biologia Plantarum 56:10. *https://doi.org/10.1007/s10535-012-0009-2*

Jung WS, Chung IM, Hwang MH, Kim SH, Yu CY, Ghimire BK (2021). Application of light-emitting diodes for improving the nutritional quality and bioactive compound levels of some crops and medicinal plants. Molecules 26:1477. *https://doi.org/10.3390/molecules26051477*

Kadota M, Niimi Y (2003). Effects of cytokinin types and their concentrations on shoot proliferation and hyperhydricity in *in vitro* pear cultivar shoots. Plant Cell Tissue and Organ Culture 72:261-265. *https://doi.org/10.1023/A:1022378511659*

Kamal OM, Shah SHA, Li Y (2020). Production of ascorbic acid total protein callus and root invitro of non-heading Chinese cabbage by tissue culture. Molecular Biology and Reproduction 47:6887-6897. *https://doi.org/10.1007/s11033-020-05745-4*

Karakas FP (2020). Efficient plant regeneration and callus induction from nodal and hypocotyl explants of goji berry (*Lycium barbarum* L.) and comparison of phenolic profiles in calli formed under different combinations of plant growth regulators. Plant Physiology and Biochemistry 146:384-391. *https://doi.org/10.1016/j.plaphy.2019.11.009*

Kim SJ, Hahn EJ, Heo JW, Pae KKY (2004). Effects of LEDs on net photosynthetic rate growth and leaf stomata of *Chrysanthemum* plantlets *in vitro*. Science Horticulture 101:143-151. *https://doi.org/10.1016/j.scienta.2003.10.003*

Klimek-Szczykutowicz M, Prokopiuk B, Dziurka K, Pawłowska B, Ekiert H, Szopa A (2022). The influence of different wavelengths of LED light on the production of glucosinolates and phenolic compounds and the antioxidant potential in invitro cultures of *Nasturtium officinale* (watercress). Plant Cell Tissue and Organ Culture (PCTOC), 149:1-10. *https://doi.org/10.1007/s11240-021-02148-6*

Kujala TS, Loponen JM, Klika KD, Pihlaja K (2000). Phenolics and betacyanins in red beetroot (*Beta vulgaris*) root: distribution and effect of cold storage on the content of total phenolics and three individual compounds. Journal of Agriculture and Food Chemistry 48(53):38-42. *https://doi.org/10.1021/jf000523q*

Lance B, Reid D, Thorpe T (2006). Endogenous gibberellins and growth of tobacco callus cultures. Physiologia Plantarum 36(3):287-292. *https://doi.org/10.1111/j.1399-3054.1976.tb04429.x*

Lange H, Shropshire W, Mohr H (1971). An analysis of phytochrome-mediated anthocyanin synthesis. Plant Physiology 109:1159-1166. *https://doi.org/10.1104/pp.47.5.649*

Lekkham P, Srilaong V, Pongprasert N, Kondo S (2016). Anthocyanin concentration and antioxidant activity in light-emitting diode (LED)-treated apples in a greenhouse environmental control system. Fruits 71:269-274. *Https://doi.org/10.1051/fruits/2016022*

Lee YI, Lee N (2003). Plant regeneration from protocorm-derived callus of *Cypripedium formosanum*. In Vitro Cellular and Developmental Biology-Plant 39:475-479. *https://doi.org/10.1079/IVP2003450*

Li H, Xu Z, Tang C (2010). Effect of light-emitting diodes on growth and morphogenesis of upland cotton (*Gossypium hirsutum* L.) plantlets invitro. Plant Cell Tissue and Organ Culture 103:155-163. *https://doi.org/10.1007/s11240-010-9763-z*

Liu H, Chen Y, Hu T, Zhang S, Zhang Y, Zhao T, Yu H, Kang Y (2012). The influence of light-emitting diodes on the phenolic compounds and antioxidant activities in pea sprouts. Journal of Functional Foods 25:459-465. *https://doi.org/10.1016/j.jff.2016.06.028*

Lindoo SJ, Caldwell MM (1978). Ultraviolet–B radiation–induced inhibition of leaf expansion and promotion of anthocyanin production. Lack of involvement of the low irradiance phytochrome synthesis. Plant Physiology 97:13-20. *https://doi.org/10.1104/pp.61.2.278*

Li Z, Chen Z, Yang Y (2019). Modulation of recombination zone position for quasi-two-dimensional blue perovskite light-emitting diodes with efficiency exceeding 5%. Nature Communications 10:1027. *https://doi.org/10.1038/s41467-019-09011-5*

Lian TT, Cha SY Moe MM, Kim YJ, Bang KS (2019). Effects of different colored leds on the enhancement of biologically active ingredients in callus cultures of *Gynura procumbens* (Lour.) Merr. Molecules 24(23):4336. *https://doi.org/10.3390/molecules24234336*

Libik M, Konieczny R, Pater B, S ′lesak I, Miszalski Z (2005). Differences in the activities of some antioxidant enzymes and in H_2O_2 content during rhizogenesis and somatic embryogenesis in callus cultures of the ice plant. Plant Cell Reproduction 23:834-841. *https://doi.org/10.1007/s00299-004-0886-8*

Łyczko J, Masztalerz K, Lipan L, Lech K, Carbonell-Barrachina A, Szumny A (2020). Chemical determinants of dried Thai basil (*O. basilicum* var. *thyrsiflora*) aroma quality. Industrial Crops and Products 0926-6690. *https://doi.org/10.1016/j.indcrop.2020.112769*

Ma YL, Sun P, Feng J, Yuan J, Wang Y, Shang YF, Niu XL, Yang SH, Wei ZJ (2021). Solvent effect on phenolics and antioxidant activity of Huangshan Gongju (*Dendranthema morifolium* (Ramat) Tzvel. cv. Gongju) extract. Food Chemistry and Toxicology 147:111875. *https://doi.org/10.1016/j.fct.2020.111875*

Mhamdi A, Breusegem FV (2018). Reactive oxygen species in plant development. Development 145(15):164376. *https://doi.org/10.1242/dev.164376*

Mikuła A, Tomaszewicz W, Dziurka M, Kaźmierczak A, Grzyb M, Sobczak M, Zdańkowski P, Rybczyński J (2021). The origin of the *Cyathea delgadii* Sternb. Somatic embryos are determined by the developmental state of donor tissue and mutual balance of selected metabolites. Cells 10(6):1388. *https://doi.org/10.3390/cells10061388*

Miler N, Kulus D, Wozny A, Rymarz D, Hajzer M, Wierzbowski K, Szeffs L (2019). Application of wide-spectrum light-emitting diodes in micropropagation of popular ornamental plant species: A study on plant quality and cost reduction. In Vitro Cellular Development and Plant Biology 55:99-108. *https://doi.org/10.1007/s11627-018-9939-5*

Mirecki RM, Teramura A (1984). Effects of ultraviolet-B irradiance on soybean v. The dependence of plant sensitivity on the photosynthetic photon flux density during and after leaf expansion. Plant Physiology 74:475-485. *https://doi.org/10.1104/pp.74.3.475*

Mkaddem M, Bouajila J, Ennajar M, Lebrihi A, MarhieuI F, Romdhane M (2009). Chemical composition and antimicrobial and antioxidant activities of *Mentha longifolia* L. *viridis* essential oils. Food Microbiology and Safety 74(7). Journal of Food Science 74(7):358-363. *https://doi.org/10.1111/j.1750-3841.2009.01272.x*

Molassiotis AN, Diamantidis GC, Therios IN, Tsirakoglou V, Dimassi KN (2005). Oxidative stress antioxidant activity and Fe (III)-chelate reductase activity of five *Prunus* rootstocks explants in response to Fe deficiency. Plant Growth Regulation 46:69-78. *https://doi.org/10.1007/s10725-005-6396-z*

Moraes RM, Cerdeira AL, Lourenço MV (2021). Using micropropagation to develop medicinal plants into crops. Molecules 26:1752. *https://doi.org/10.3390/molecules26061752*

Mok MC, Mok DWS, Turner JE, Mujer CV (1987). Biological and biochemical effects of cytokinin-active *Phenylurea* derivatives in tissue culture systems. HortScience 22(6):1-4. *https://doi.org/10.21273/hortsci.22.6.1194*

Murashige T, Skoog F (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum 15:473-497. *https://doi.org/10.1111/j.1399-3054.1962.tb08052.x*

Nguyen TL, Saleh MA (2019). Effect of exposure to light emitting diode (LED) lights on essential oil composition of sweet mint plants. Journal of Environmental Science Health 54(5):435-440. *https://doi.org/10.1080/10934529.2018.1562810*

Nhut DT, Huy N, Tai N, Nam N, Luan V, Hien V, Tung H, Vinh B, Luan T (2015). Light-emitting diodes and their potential in callus growth plantlet development and saponin accumulation during somatic embryogenesis of *Panax vietnamensis* Ha et Grushv. Biotechnology Biotechnological Equipment 29:299-308. *https://doi.org/10.1080/13102818.2014.1000210*

Ohashi-Kaneko K, Takase M, Kon N, Fujiwara K, Kurata K (2007). Effect of light quality on growth and vegetable quality in leaf lettuce spinach and komatsuna. Environmental Control of Biology 45:189-198. *https://doi.org/10.2525/ecb.45.189*

Oksman-Caldentey KM, Inzé D (2004). Plant cell factories in the postgenomic era: new ways to produce designer secondary metabolites. Trends in Plant Science 9:433-440. *https://doi.org/10.1016/j.tplants.2004.07.006*

Omaye ST, Turnbull JD, Sauberlich HE (1979). Selected methods for the determination of ascorbic acid in animal cells tissues and fluids. Methods in Enzymology 62:3-11. *https://doi.org/10.1016/0076-6879(79)62181-x*

Omar GF (2017). Growth responses of potato plantlets cultured invitro under different colors light emitting Diodes (LEDs). HortScience Journal of Suez Canal University 6(1):65-71. *https://doi.org/10.21608/hjsc.2017.6397*

Park YJ, Baek SA, Choi Y, Kim JK, Park SU (2019). Metabolic profiling of nine *Mentha* species and prediction of their antioxidant properties using chemometrics. Molecules 24:458. *https://doi.org/10.3390/molecules24020258*

Poudel PR, Kataoka I, Mochioka R (2008). Effect of red-and blue-light-emitting diodes on growth and morphogenesis of grapes. Plant Cell Tissue and Organ Culture 92:147-153. *https://doi.org/10.1007/s11240-007-9317-1*

Poovaiah CR, Weller SC, Jenks MA (2006). *In vitro* adventitious shoot regeneration of native spearmint using internodal explants. HortScience 41(2):414-417. *https://doi.org/10.1079/IVP2006783*

Qian H, Liu T, Deng M, Miao H, Cai C, Shen W, Wang Q (2016). Effects of light quality on main health-promoting compounds and antioxidant capacity of Chinese kale sprouts. Food Chemistry 196:1232-1238. *https://doi.org/10.1016/j.foodchem.2015.10.055*

Rahman MM, Ankhi UR, Biswas A (2013). Micropropagation of *Mentha viridis* L.: An aromatic medicinal plant. International Journal of Pharmacy and Life Sciences 4(9):0976-7126.

Raja HD, Arockiasamy DI (2008). *In vitro* propagation of *Mentha viridis* L. from nodal and shoot tip explants. Plant Tissue Culture and Biotechnology 18(1):1-6. *https://doi.org/10.3329/ptcb.v18i1.3243*

Rajeswari V, Paliwal K (2008). *In vitro* adventitious shoot organogenesis and plant regeneration from seedling explants of *Albizia odoratissima* L.f. (Benth.). Invitro Cellular and Developmental Biology – Plant 44:78-83. *https://doi.org/10.1007/s11627-008-9120-7*

Rout GR, Das P (1997). *In vitro* organogenesis in ginger (*Zingiber officinale* Rosc.), Journal of Herbs Spices & Medicinal Plants 4:41-51. *https://doi.org/10.1300/J044v04n04_05*

Salazar-García S, Medina-Carrillo RE, Álvarez-Bravo A (2016). Influencia del riego y radiación solar sobreelcontenido de fitoquímicosen la piel de frutos de aguacate 'Hass'. Revista Mexicana Ciencias Agrícolas 72565. *https://doi.org/10.29312/remexca.v0i13.483*

Sadasivam S, Manickam A (2008). Biochemical methods. 3rd ed. New Age international Limited New Delhi.

Samantaray A, Sial P, Kar M (2012). Micropropagation and biochemical analysis of spearmint (*Mentha spicata*). Indian Journal of Innovations and Developments 1(7):489-493.

Selvi S, Polat R, Çakılcıoğlu U, Celep F, Dirmenc T (2022). An ethnobotanical review on medicinal plants of the Lamiaceae family in Turkey. Turkish Journal of Botany 46(4):283-332. *https://doi.org/10.55730/1300-008X.2712*

Shen YZ, Guo SS, Ali WD, Tang YK (2014). Effects of illuminants and illumination time on lettuce growth yield and nutritional quality in a controlled environment. Life Sciences in Space Research 2:38-42. *https://doi.org/10.1016/j.lssr.2014.06.001*

Shindy WW, Smith O (1975). Identification of plant hormones from cotton ovules. Plant Physiology 55:550-554. *https://doi.org/10.1104/pp.55.3.550*

Sidhu Y (2010). *In vitro* micropropagation of medicinal plants by tissue culture. The Plymouth Student Scientist 4(1):432-449. *http://hdl.handle.net/10026.1/13944*

Srivastava S, Krishna R, Sinha RP, Singh M (2017). TDZ-induced plant regeneration in *Brassica oleracea* L. var. *botrytis*: effect of antioxidative enzyme activity and genetic stability in regenerated plantlets. Invitro Cell and Developmental Biology—Plant 53:598-605. *https://doi.org/10.1007/s11627-017-9861-2*

Stafford A, Morris P, Fowler MW (1986). Fowler plant cell biotechnology: A perspective. Enzyme and Microbial Technology 8:578-587. *https://doi.org/10.1016/0141-0229(86)90114-6*

Su L, Bassa C, Audran C, Mila I, Cheniclet C, Chevalier C, Bouzayen M, Roustan J, Chervin C (2014). The Auxin Sl-IAA17 transcriptional repressor controls fruit size via the regulation of endoreduplication-related cell expansion. Plant and Cell Physiology 55(11):1969-1976. *https://doi.org/10.1080/15592324.2015.1071001*

Tohidi B, Rahimmalek M, Arzani A, Sabzalian MR (2019). Thymol carvacrol and antioxidant accumulation in *Thymus* species in response to different light spectra emitted by light-emitting diodes. Food Chemistry 307125521. *https://doi.org/10.1016/j.foodchem.2019.125521*

Tuladhar P, Sasidharan S, Saudagar P (2011). Role of phenols and polyphenols in plant defense response to biotic and abiotic stresses. Biocontrol agents and secondary metabolites. Applications and Immunization for Plant Growth and Protection 419-441. *https://doi.org/10.1016/B978-0-12-822919-4.00017-X*

Wang J, Xu WM, Zhu YZ, Wang DY, Wang JZ (2008). Effects of storage temperature on browning of fresh-cut burdock (*Arctium lappa* L.). Jiangsu Journal of Agricultural Sciences 24(4):492-496. (in Chinese). *https://doi.org/10.1016/j.scienta.2003.09.002*

Wang Z, Chen X, Lu Y, Chen F, and Zhang W (2020). Clinical characteristics and therapeutic procedure for four cases with 2019 novel coronavirus pneumonia receiving combined Chinese and western medicine treatment. Bioscience Trends 14(1):64-68. *https://doi.org/10.5582/bst.2020.01030*

Weremczuk Jeżyna I, Kuźma Ł, Kiss AK, Grzegorczyk Karolak I (2018). Effect of cytokinins on shoots proliferation and rosmarinic and salvianolic acid B production in shoot culture of *Dracocephalum forrestii* W. W. Smith. Acta Physiologiae Plantarum 40189. *https://doi.org/10.1007/s11738-018-2763-z*

Wilson DA, Weigel RC, Wheeler RM, Sager JC (1993). Light spectral quality effects on the growth of potato (*Solanum tuberosum* L.) nodal cuttings *in vitro*. In Vitro Cellular and Developmental Biology– Plant 29(1):5-8. *https://doi.org/10.1007/BF02632231*

Xu Y, Liang Y, Yang M (2019). Effects of composite LED light on root growth and antioxidant capacity of *Cunninghamia lanceolata* tissue culture seedlings. Science Reproduction 9:9766. *https://doi.org/10.1038/s41598-019-46139-2*

Yu Z, Duan X, Luo L, Dai S, Ding Z, Xia G (2020). How plant hormones mediate salt stress responses. Trends in Plant Science 25:1117-1130. *https://doi.org/10.1016/j.tplants.2020.06.008*

Zengin G, Ak G, Ceylan R, Uysal S, Llorent-Martínez E, Di Simone SC, Rapino M, Acquaviva A, Libero ML, Chiavaroli A (2022). Novel perceptions on chemical profile and biopharmaceutical properties of Mentha spicata extracts: adding missing pieces to the scientific puzzle. Plants 11:233. *https://doi.org/10.3390/plants11020233*

Zhang L, Chen Y, Li Z, Li X, Fan G (2022). Bioactive properties of the aromatic molecules of spearmint (*Mentha spicata* L.) essential oil: a review. Royal Society of Chemistry 13:3110-3132. *https://doi.org/10.1039/D1FO04080D*

Zha LY, Zhang YB, Liu WK (2019). Dynamic responses of ascorbate pool and metabolism in lettuce to long-term continuous light provided by red and blue LEDs. Environmental and Experimental Botany 163:15-23. *https://doi.org/10.1016/j.envexpbot.2019.04.003*

Zhao J, Thi LT, Park YG, Jeong BR (2020). Light quality affects growth and physiology of *Carpesium triste* Maxim. cultured *in vitro*. Agriculture 10:258. *https://doi.org/10.3390/agriculture10070258*

Zimmerman TW, Scorza R (1992). Shoot growth and proliferation of peach under varying environmental regimes. HortSciene 27:696. *https://doi.org/10.21273/HORTSCI.27.6.696a*

Zouzou M, Hilaire KT, Mongomaké K, Georges AN, Justin KY (2008). Effect of genotype explants growth regulators and sugars on callus induction in cotton (*Gossypium hirsutum* L.). Australian Journal of Crop Science 2(1):1-9.

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