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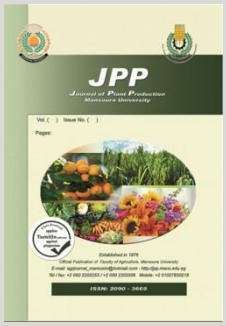
Influence of Light Type on the Propagation and Accumulation of Secondary Metabolites of *Ocimum basilicum l. Var. Genovese* under the Tissue Culture Technique

Heba. Y. El-Banna; M. M. Abd El-Baset and Noha A. Youssef *

Veget. and Flori. Depart, Fac. Agric., Mansoura Univ. Egypt.



ABSTRACT

This study aims to improve the production of secondary metabolites from (*Ocimum basilicum L. var. Genovese*) by using nodal segments and callus as explants. The research focused on Maximizing various factors, containing two cytokinins (benzylaminopurine (BAP) and thidiazuron (TDZ)) at three different concentrations (0, 1, or 2 mg/L) and plant growth regulators, as well as various light-emitting diode (LED) systems (white as a control, violet, and a 1:1 combination of red and blue). After 30 days of cultivation, the results showed that Murashige and Skoog control medium completely free of hormones gave superior results in terms of fresh and dry shoots weight (1.46 gm and 0.88 gm, respectively), while the Murashige and Skoog medium with 2 mg/L of (TDZ) and white and violet LED lighting, obtained highest values for fresh and dry callus weight (1.62 gm and 0.91 gm, respectively). Moreover, it is worth noting that callus formation was not observed in response to benzyl aminopurine treatments. In comparison with the control, it was found that all treatments used in general stimulated antioxidant compounds such as (phenolics, flavonoids, anthocyanins, and ascorbic acid), surprisingly, whether in the individual effects of white light or thidiazuron 2 mg/L alone or the interactions between them compared to the other treatments.

***Keywords*:** Italian basil, light type, tissue culture, cytokinin, Benzyl aminopurine, and thidiazuron

INTRODUCTION

Medicinal plants continue to be a crucial source of treatment for many health conditions, particularly considering the high cost of traditional synthetic medications and the discovery of unwanted side effects (Aleksic and Knezevic, 2014). In addition, plants provide only around 40% of the chemicals used in the pharmaceutical industry, directly or indirectly (Sidhu, 2011). This is because the chemical synthesis of these compounds is either impractical or not economically viable (Oksman-Caldentey and Inzé, 2004). Extracting pharmacologically significant plant-specialized metabolites in large, sustained, and continuous amounts from field-grown plant material may be challenging. However, in vitro cultures have been discovered as a potential biological system for the synthesis of these metabolites (Sasheva *et al*., 2013; Nazir *et al*., 2020). Compared to natural production methods, in vitro culture methods offer reliable production systems for metabolites that are not affected by environmental constraints, leading to higher biomass and greater concentrations of compounds (Khurshid *et al*., 2018; Nazir *et al*., 2020) Moreover, in this cultivation method, seedlings develop under carefully regulated conditions, including the culture medium and environmental factors (Weathers *et al*., 2010). As a result, the oil's chemical composition becomes more consistent and predictable. Additionally, the metabolism of the plant can be led to focus on producing chemical compounds that are of greater industrial value (Alvarez, 2014).

It is possible to produce secondary compounds in medicinal plants in vitro by changing the kinds and quantities of plant growth regulators in addition to the culture conditions (Palacio *et al*., 2012; Castro *et al*., 2016). The levels of growth regulators in the media and their proper proportions are crucial, as they significantly impact the plant development and productivity (Baque *et al*., 2010). Furthermore, improving the generation of bioactive chemicals through plant cell and tissue cultures requires an understanding of how the in vitro environment affects explant development and secondary metabolism (Georgiev *et al*., 2009). Lighting setups in plant tissue culture have often been updated or created using Light Emitting Diode (LED) technology in recent decades. As a result, there are several advancements in light quality accessible (Barceló-Muñoz *et al*., 2021). In vitro, LEDs have generated a lot of attention because of their narrow bandwidth and wavelength specificity, small mass and volume, long life, low energy consumption, and little heating (Shin *et al*., 2008; Alvarenga *et al*., 2015). Batista *et al*., (2018) they Discussed that plant metabolism is impacted by the various wavelengths. Light may serve as an external regulator in several morphogenic and physiological processes that modify the amount and structure of phytochemicals in plants, in addition to its role in photosynthetic activities.

One of the economically and botanically important aromatic medicinal and ornamental herbs is *Ocimum basilicum L.* var. "Genovese" which belongs to the family Lamiaceae (Enkhbileg *et al*., 2019) sweet basil cultivar native to Italy. Its large, strong leaves have a sweet, somewhat spicy flavor. Genovese basil leaves are bright green and somewhat crinkled, growing up to (7:6 cm) in length. Pesto, caprise salad, and other meals that call for big, fresh basil leaves are ideal. In truth, the applications of Genovese basil are like those of any other basil. Genovese basil plants may reach heights of (90-100cm). Using good growing media is essential to increase the growth, yield, quality, and profits of ornamental and medical crops (Bunt,

* **Corresponding author.**
E-mail address: noha.a.elwan@gmail.com
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1988). In addition, it has pharmaceutical chemical structure active ingredients including phenolics, flavonoids, terpenoids, anthocyanins, and alkaloids (Deineka *et al*., 2019; Açıkgöz, 2020). Moreover, it is rich in a variety of vital elements, including magnesium, potassium, iron, phosphorus, calcium, vitamin C, and vitamin A. Additionally, it has a high concentration of carotenoids such as β-carotene which the body converts to vitamin A (Sahu *et al*., 2022). Therefore, it is very important for the food industry (flavoring agent), cosmetic, and pharmaceutical industries (Da Silva *et al*., 2018; Sahu *et al*., 2022). Anxiety, the common cold, coughing, diabetes, migraines, headaches, fevers, and neuropathic issues have all been treated with it. Additionally, it is used to treat several neurological conditions, including cardiac issues, sinusitis, depression, cramping, insect stings, and gastrointestinal disorders (Bora *et al*., 2011; Sahu *et al*., 2022).In addition to, these features, sweet basil has been reported to have the potential as an antimicrobial, antioxidant (Koroch *et al*., 2017), antituberculosis (Siddiqui *et al*., 2012), anti-inflammatory (Aye *et al*., 2019), antiseptic (Koseki *et al*., 2002), antifungal (Piras *et al*., 2018) and antitumor (Tenore *et al*., 2017).

By employing nodal segments as explants, this work sought to create a dependable plant tissue culture methodology to produce secondary metabolites from *Ocimum basilicum* L. var. "Genovese." Plant growth regulators (two cytokinin types: benzylaminopurine (BAP) and thidiazuron (TDZ)) at three different concentrations (0, 1, or 2 mg/L) and various light-emitting diode (LED) systems (white as a control, violet, and a combination of red and blue in a 1:1 ratio) were among the factors that were optimized in this study.

MATERIALS AND METHODS

The study was carried out at the experimental station and in the tissue culture lab of Mansoura University's Faculty of Agriculture's Vegetable and Floriculture Department. throughout the years 2022–2023.

The main goal was to improve the propagation of Italian basil (*Ocimum basilicum L.* var. Genovese) Fig (1) family Lamiaceae by using tissue culture under two types of light and study the effect of BAP and TDZ on vegetative growth, chemical constituents of sweet basil plants.

Fig. 1. The plant material (*Ocimum basilicum* L., var. Genovese)

So, this work included two parts, the first was the including plant growth regulators BAP and TDZ, and the second was different light-emitting diode (LED) systems on chemical constituents.

Plant materials and chemicals used:

Healthy uniform seeds of *Ocimum basilicum* L., var. Genovese were obtained from a commercial Nursery in Beheira, Egypt were used as explant materials. For the chemicals used in this research, some of them such as plant growth regulators were supplied by Sigma Chemical Company. While others were supplied by different local companies.

Design of the experiment and statistical evaluation:

The design of the experiment was a factorial including two factors (first: types of cytokinin treatments, and second: different light types. However, the two-way completely randomized designs (CRD) were used to arrange the treatments of this test as outlined by Snedecor and Cochran (1967). It included ten treatments and three replications. Each of these included three jars. The statistical analysis was conducted using COSTAT v.6.3 software to perform an analysis of variance (ANOVA). To compare the means, the Least Significant Differences (LSD) method was applied, following the guidelines outlined by Steel and Torrie (1980), with a significance level of $P \leq 0.05$.

Surface sterilization of explants:

Seeds of *Ocimum basilicum* L., var. Genovese were immersed in 70% ethanol for 30 seconds and transferred to a solution of 30% (v/v) Clorox commercial bleach solution (6% Sodium hypochlorite; NaOCL) for 5-7 min then finally rinsed three times with distilled sterilized water for 5 min each.

Cultural media and conditions:

Sterile seeds were cultured onto wet cotton gars that have been sanitized. After 14 days, the epicotyls were removed from the sterilized seedlings that had sprouted and placed in glass jars that had been sterilized and contained 35 milliliters of MS (Murashige and Skoog, 1962). The carbon source in the baseline medium was 3% sucrose (Table 1), and two cytokinin types—benzylaminopurine (BAP) and thidiazuron (TDZ)—were added at concentrations of 0, 1, or 2 mg/L. The 0.7% plant agar was used to solidify the media. After bringing the medium's pH down to 5.75, agar was added, and it was autoclaved for 25 minutes at 121 ℃ and 1.1 kg/cm2.

Table 1. Constituents of Murashige and Skoog basal nutrient medium.

Basic ingredients	Concentration (mg/l)
NH_4NO_3	1650
KNO_3	1900
$CaCl_2 \cdot 2H_2O$	440
$MgSO_4 \cdot 7H_2O$	370
KH_2PO_4	170
H_3BO_3	6.2
$MnSO_4 \cdot 4H_2O$	16.9
$ZnSO_4 \cdot 7H_2O$	8.6
KI	0.83
$Na_2MoO_4 \cdot 2H_2O$	0.25
$CuSO_4 \cdot 5H_2O$	0.025
$CoCl_2 \cdot 6H_2O$	0.025
Na_2 EDTA ($2H_2O$)	37.3
Myo-inositol	80.0
Glycine	2.0
Nicotinic acid (B5)	0.5
Pyridoxine – HCl(B6)	0.5
Thiamine – HCl (B1)	0.1

Two distinct light-emitting diodes were applied to the cultures for 30 days: a combination of red and blue (1:1) LEDs and white LEDs as a control. Also,the cultures were kept in a growth chamber at 25 ± 1 °C, with 16 hours of light and 8 hours of darkness. There were twelve explants in each therapy.

Multiplication stage:

Trials were designed to study the effect of two types of cytokinins; benzyl amino purine (BAP) and thidiazuron (TDZ) as growth regulators with two different concentrations (1 & 2 mg/L) for each one in addition to control (free hormone MS medium) after incubation for 30 days under two different light emitting diodes illumination (white LEDs as control and violet LEDs) on proliferation, and some vegetative characteristics of *Ocimum basilicum* L. var. Genovese shoots. These experiments aimed to produce a high number of healthy proliferated shoots with a suitable length.

Data recorded:

After 30 days of incubation, the following data were recorded for vegetative parameters:

- Shoot fresh & dry weight (g)
-Callus fresh &dry weights (g).

Quantitative of some secondary metabolites:

Antioxidant enzymes and compounds [phenols, flavonoids, anthocyanin, ascorbic acid] in shoots and calluses.

ANALYTICAL METHODS

Extraction and estimation of antioxidant compound

Total phenols determination: as mg phenol equivalent/g dry weight by (Sadasivam and Manickam, 2008).

Quantitative estimation of flavonoids:

According to Rolim *et al*., (2005) and Kujala *et al.* (2000).

Quantitative estimation of anthocyanins:

using SpekolSpectro Colorimeter (Mirecki and Teramura, 1984). Anthocyanin contents were calculated as mg anthocyanins g^{-1} dry mass (Lange *et al*., 1971; Mancinelli *et al*.,1975; Lindoo and Caldwell, 1978).

Quantitative estimation of ascorbic acid:

Ascorbic acid content was determined as described by Omaye *et al.* (1979).

RESULTS AND DISCUSSION

Results

Changes *in vitro* culture variable parameters:

Table (2) shows that the variable growth parameters of *Ocimum basilicum* L. var. Genovese shoot callus *in vitro* cultured on different MS media for 30 days.

1.Effect of light types, cytokinin types at different concentrations, and their interactions on shoot fresh and dry weight.

Effect of light type on shoot fresh and dry weight.

Regarding the shoot fresh weight of *Ocimum basilicum* L. var. Genovese, data in Table, 2 indicate that using violet LEDs significantly counted the highest shoot fresh and dry weight, since it was (1.18 g and 0.63 g, respectively). When compared with the other type of light (white LEDs), it gave 0.99 gm and 0.49 gm, respectively.

Effect of cytokinin types on shoot fresh and dry weight.

It appears from the data presented in Table,2 reveals that the highest value of shoot fresh weight was obtained with the control (1.28 gm), followed by BAP 1.00 mg/L (1.15 gm). For shoot dry weight, also the control recorded the highest value of shoot dry weight (0.71 gm), but with a significant difference between it and BAP 1.00 mg/L (0.59 gm).

Effect of the relationship between cytokinin type and light types on the fresh and dried weight of shoots.

It appeared from data in the same Table,2 and Figures (2&3) that the maximum shoot fresh weight was obtained with control and in MS medium enhanced with BAP at 1mg/L under violet LEDs (1.46 gm and 1.28 gm, respectively) without significant difference. Also, the same previous treatments recorded the highest value of dry weight but with significant differences between them.

However, under white LEDs, the lowest values of shoot fresh weight were obtained by MS medium supplemented with 1 and 2 mg/L TDZ; the estimated value for both was 0.92 gm. Under white LEDs, the MS medium supplemented with BAP 2 mg/L produced the lowest shoot dry weight value (0.46 gm).

Table 2. Two distinct visible light emitting diodes (white and violet) and their effects on shoot fresh and dry weight (gm) parameters of micropropagated *Ocimum basilicum* var. Genovese after 30 days.

Treatments		Shoot fresh weight (gm)	Shoot dry weight (gm)
Light type			
White		0.99±0.03b	0.49±0.01b
Violet		1.18±0.05a	0.63±0.04a
	Growth regulators		
Control		1.28±0.09a	0.71±0.07a
BA 1.00 mg/L		1.15±0.06ab	0.59±0.05b
BA 2.00 mg/L		0.98±0.03c	0.47±0.02c
TDZ 1.00 mg/L		0.98±0.04c	0.51±0.04bc
TDZ 2.00 mg/L		1.02±0.05bc	0.52±0.03bc
	Interactions		
White light	Control	1.11±0.08bc	0.541±0.04c
	BA 1.00 mg/L	1.023±0.02c	0.48±0.02c
	BA 2.00 mg/L	0.95±0.06c	0.46±0.04c
	TDZ 1.00 mg/L	0.92±0.03c	0.47±0.03c
	TDZ 2.00 mg/L	0.92±0.04c	0.47±0.02c
Violet light	Control	1.46±0.11a	0.88±0.07a
	BA 1.00 mg/L	1.28±0.09ab	0.69±0.07b
	BA 2.00 mg/L	1.015±0.04c	0.48±0.02c
	TDZ 1.00 mg/L	1.05±0.04c	0.55±0.06bc
	TDZ 2.00 mg/L	1.12±0.07bc	0.57±0.06bc

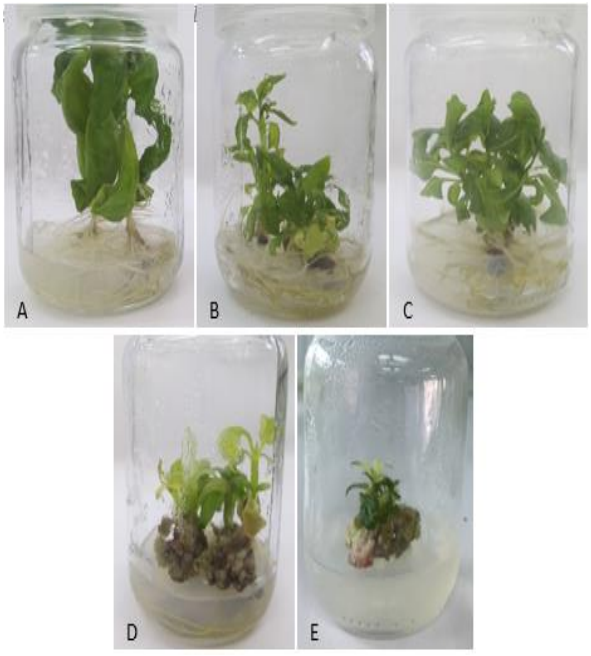

Figure 2. In vitro propagation of Ocimum basilicum L. var. Genovese seeds under white LEDs. A) MS-free content. B) MS + 1.00 mg/L BAP. C) MS + 2.00 mg/L BAP. D) MS + 1.00 mg/L TDZ. E) MS + 2.00 mg/LTDZ.

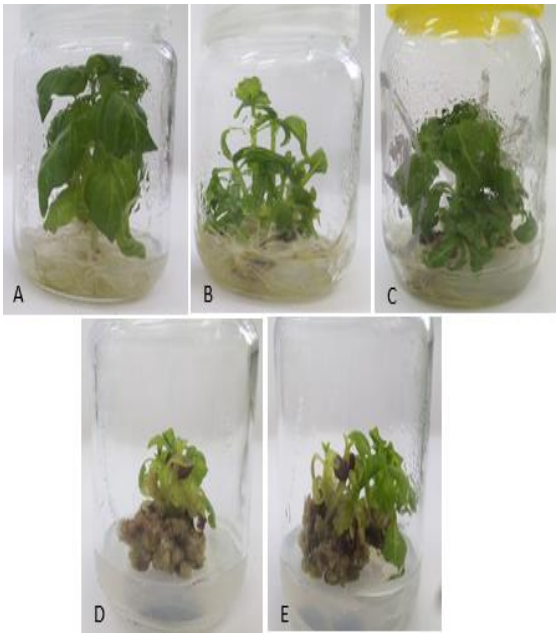

Figure 3. In vitro propagation of Ocimum basilicum L. var. Genovese seeds under violet LEDs A) MS-free media. B) MS + 1.00 mg/L BAP. C) MS + 2.00 mg/L BAP. D) MS + 1.00 mg/L TDZ. E) MS + 2.00 mg/LTDZ

2.Effect of light types, cytokinin types at different concentrations, and their interactions on Callus fresh weight.

Effect of light type on Callus fresh and dry weight:

According to callus fresh and dry weight data in Table (3) there was a significant difference detected between using the two types of light, Hence, the maximum callus fresh and dry weight formed (1.37 and 0.67 g) respectively, was under violet LEDs, followed by (0.99 and 0.50 gm) respectively under white LEDs with significant difference between them.

Effect of cytokinin types on Callus fresh and dry weight:

Table,3 shows that a noteworthy distinction between the two concentrations of TDZ as the highest significant callus fresh and dry weight values of (1.42 and 0.77 gm.), respectively was recorded at a concentration of 2mg/L. However, the lowest Callus fresh and dry weight of 0.95 and 0.40 gm was recorded at a concentration of 1mg/L with a significant difference between them.

Effect of the relationship between cytokinin type and light kinds on the fresh and dry weights of callus.

Regarding callus, fresh weight was clear that there is a significant difference between the two types of lighting at the same concentration of TDZ. Hence, the fresh weight of formed *Ocimum basilicum* L. var. Genovese callus under violet LEDs in MS medium fortified with 2 and 1 mg/L TDZ was 1.62 gm, and 1.12 gm, respectively. While MS medium fortified with 2 and 1 mg/L TDZ under white LEDs recorded 1.21 gm and 0.79 gm, respectively. Therefore, the highest dry weight of the callus of *Ocimum basilicum* L. var. Genovese callus (0.91 gm) was observed in MS medium complemented with 2 mg/L TDZ under violet LEDs, followed by (0.64 gm) in the same MS medium under white LEDs with a significant difference between them. The MS medium complemented with 1 mg/L TDZ under white LEDs produced the lowest dry weight of calli (0.37 gm) (Table 3).

Table 3. Effect of two distinct cytokinin types and visible light emitting diodes (LEDs) on varying *in vitro* culture parameters of micropropagated *Ocimum basilicum* L. var. Genovese callus after 30 days.

Treatments		Callus fresh weight	Callus dry weight
Light type			
White		0.99±0.08b	0.50±0.06b
Violet		1.37±0.11a	0.67±0.09a
Growth regulators			
TDZ 1.00 mg/L		0.95±0.07b	0.40±0.02b
TDZ 2.00 mg/L		1.42±0.09a	0.77±0.06a
INTERACTIONS			
White light	TDZ 1.00 mg/L	0.79±0.05c	0.37±0.024c
	TDZ 2.00 mg/L	1.21±0.05b	0.64±0.05b
Violet light	TDZ 1.00 mg/L	1.12±0.08b	0.44±0.04c
	TDZ 2.00 mg/L	1.62±0.07a	0.91±0.04a

Phenols and flavonoids

3.Effect of light types, cytokinin types at different concentrations, and their interactions on the content of total phenolic in shoot and callus.

Effect of light type on total phenolic in shoot and callus.

It appeared from the data There was a notable variation in Table (4) detected between using the two types of light, Hence, the maximum content obtained from shoots for total phenolic was 11.77 under violet LEDs, followed by (10.74) under white LEDs.

Also, the data in Table (5) produced the same trend for callus as using violet LEDs significantly counted the highest content of total phenolic, since it was 17.92 when compared with the other type of light (white LEDs), as it gave 14.98.

Effect of cytokinin types on content of total phenolic in shoot and callus.

From the results cited in Table (4), It is clear that the total phenolic content of Ocimum shoots varies greatly according to the treatments that are provided. In this respect, the MS medium enhanced with 2 mg/L TDZ had the maximum significant value of total phenolic content, 13.38. While a significant decrease with the minimum value (8.79) was recorded by basal (control) medium.

For total phenolic from callus data Table (5) stated that there was a significant difference between the two concentrations of TDZ as the highest significant total phenolic content value of 17.10 was recorded at a concentration of 2mg/L. However, the lowest total Phenolic content value of 15.79 was recorded at a concentration of 1.00 mg/L.

Effect of the interaction between light types and cytokinin type on the content of total phenolic in shoot and callus.

As regards phenol content from in-vitro cultured Ocimum shoots in white LEDs and violet LEDs illumination, it appeared from Table (4) that all treatments under violet LEDs gave the highest value of total phenolic content when compared with the same treatments under white LEDs. In this concern, the maximum significant value of phenols was in Ocimum micro propagated in MS medium supplemented with 2.00 mg/LTDZ (13.95) under violet LEDs, the next positive value in MS medium fortified with 2.00 mg/L TDZ recorded a value of 12.81 under white LEDs followed by (12.78) in MS medium fortified with 1.00 mg/L TDZ under violet LEDs without any obvious distinction between them. Under white LEDs, however, the control medium reported the lowest significant value (8.20).

Regarding the content of total phenolic from callus Table (5) demonstrated that the maximum content of Total Phenolic (18.47) was in MS medium supplemented with 2.00 mg/L TDZ under violet LEDs with a notable difference between them, and then (17.37) in MS medium supplemented with 1.00 mg/L TDZ under the same light. The lowest content of Total Phenolic (14.22) was obtained in MS medium supplemented with 1.00 mg/L TDZ under white LEDs.

4.Effect of light types, cytokinin types at different concentrations, and their interactions on the content of total flavonoids in shoot and callus.

Effect of light type on the content of total flavonoids in shoot and callus.

As for the effect of light type on the content of total flavonoids, data in Table (4) indicated that using violet LEDs significantly counted the highest content of total flavonoids from shoots, since it was 6.16 when compared with the other type of light (white LEDs), as it gave 4.96.

For callus, Table (5) revealed the highest significant total flavonoid value of 9.21 was observed under violet LEDs, indicating a substantial difference between the two forms of light. While the lowest total flavonoids of 7.43 were recorded under white LEDs.

Effect of cytokinin types on total flavonoids in shoot and callus.

The two forms of cytokinin differ significantly from one another, as Table (4) illustrates. It could be noted that the highest significant value content of total flavonoids was obtained with TDZ at 2.00 mg/L since it recorded 7.16., followed by 6.35 in MS medium complemented with 1.00 mg/L TDZ with significant differences between them. It also appeared that the control recorded extremely the lowest content of 4.09.

Also, the callus data in Table (5) indicated that there was a noteworthy distinction between the two concentrations of TDZ as the highest significant content of total flavonoid values of 8.74 recorded at a concentration of 2.00 mg/L. while the lowest content of total flavonoids of 7.90 was recorded at a concentration of 1mg/L.

Effect of the interaction between light types and cytokinin type on the content of total flavonoids in shoot and callus.

Concerning the flavonoid amount of Ocimum from shoots found by the applied procedures in Table (4). Thus, when illuminated with violet LEDs, the highest significant value was 7.70 in the MS medium complemented with 2.00 mg/LTDZ, while the MS medium supplemented with 1.00 mg/L TDZ estimated the next positive value of 7.05 of flavonoids under the same light. The control medium recorded the least value of flavonoids (3.56) under white LEDs.

Regarding the content of total flavonoids from callus, Table (5) presented that the maximum content of total flavonoids (9.66) was in MS medium complemented with 2.00 mg/L TDZ under violet LEDs. The lowest content of total flavonoids (7.09) was obtained in the MS medium complemented with 1.00 mg/L TDZ under white LEDs.

Anthocyanins and ascorbic acid

5. Effect of light types, cytokinin types at different concentrations, and their interactions on the content of total anthocyanins in shoot and callus.

Effect of light type on the content of total anthocyanins in shoot and callus.

Tables (4 and 5) demonstrate that a notable difference was detected between using the two types of light, Hence, the maximum content of total anthocyanins from shoots and callus were 6.30 and 9.96, respectively under violet LEDs, followed by 5.09 for shoots and 8.03 for callus under white LEDs.

Effect of cytokinin types on total anthocyanins in shoot and callus.

As shown in Table (4) the two forms of cytokinin differ significantly from one another. It could be noticed that the highest significant value content of total anthocyanins from shoots was obtained with TDZ at 2.00 mg/L since it recorded 7.58, followed by 6.64 in MS medium enhanced with 1.00 mg/L TDZ with significant differences between them. It also appeared that the control recorded the lowest content of 3.37.

For callus, Table,5 shows that the two TDZ concentrations differed significantly. as the highest significant content of total anthocyanins values of 9.52 recorded at a concentration of 2.00 mg/L. However, the lowest content of Total anthocyanins of 8.48 was recorded at a concentration of 1.00 mg/L.

Effect of the interaction between light types and cytokinin type on the content of total anthocyanins in shoot and callus.

Concerning the anthocyanins amount from shoots in Table (4) The maximum value (8.69) in MS medium containing 2 mg/L TDZ under violet LEDs and white LEDs, respectively, and the control medium estimated the minimum value of anthocyanins (2.95) under white LEDs.

Regarding the content of total anthocyanins from callus Table (5) presented that the concentrations of TDZ under violet LEDs noted the highest values of total anthocyanins when compared with the concentrations of TDZ under white LEDs. The maximum content of total anthocyanins (10.41) was in MS medium supplemented with 2.00 mg/L TDZ under violet LEDs. The lowest content of total anthocyanins (7.44) was obtained in MS medium fortified with 1.00 mg/L TDZ under white LEDs.

6. Effect of light types, cytokinin types at different concentrations, and their interactions on the content of total ascorbic acid in shoot and callus.

Effect of light type regarding the content of total ascorbic acid in shoot and callus.

As for the effect of light type on the content of total ascorbic acid, data in Tables (4 and 5) indicate that using violet LEDs significantly counted the highest content of total ascorbic acid in shoots and callus, since it was 14.88 and 19.30, respectively when compared with the other type of light (white LEDs), as it gave 13.48 and 16.32, respectively.

Effect of cytokinin types on total ascorbic acid in shoot and callus.

From the results cited in Table (4), It is clear that the total ascorbic acid concentration of Ocimum shoots varies greatly according to the treatments that are applied. In this respect, the highest significant value of total ascorbic acid content was 15.86 in MS medium fortified with 2 mg/L TDZ, then 14.89 in MS medium with 1.00 mg/L TDZ. While a significant decrease with the minimum value (12.25) was recorded by basal (control) medium.

It appeared from the data there was a notable distinction between the two in Table (5). concentrations of TDZ as the highest significant content of total ascorbic acid in callus was a value of 18.62 recorded at a concentration of

2.00 mg/L. while the lowest content of total ascorbic acid of 17.00 was recorded at a concentration of 1.00 mg/L.

Effect of the interaction between light types and cytokinin type on the content of total ascorbic acid in shoot and callus.

As regards ascorbic acid (vitamin C) of Ocimum shoots in Table (4) under violet and white LEDs lighting. Concerning violet LEDs illumination, the amount of ascorbic acid (vitamin C) was the maximum (16.96) regarding MS medium supplemented with 2.00 mg/L TDZ, furthermore, MS medium contained 1.00 mg/L TDZ estimated at 15.74 with significant differences. The control medium recorded the minimum value of ascorbic acid content with a value of (11.82) under white LEDs illumination.

For callus, regarding the content of total ascorbic acid Table (5) presented that the maximum content of total ascorbic acid (19.83) was in MS medium fortified with 2 mg/L TDZ under violet LEDs followed by TDZ at 1.00 mg/L (18.78) under the same light with significant differences. The lowest content of total ascorbic acid (15.22) was obtained in MS medium supplemented with 1mg/L TDZ under white LEDs.

Table 4. Effect of various cytokinin types and visible light emitting diodes (LEDs) on antioxidant chemicals of micropropagated *Ocimum basilicum L.* var. Genovese shoots after 30 days.

Treatments		Total Phenolic (mg/g dry wt)	Total Flavonoids (mg/g dry wt)	Total Anthocyanins (mg/g dry wt)	Total Ascorbic acid (mg/g fresh wt)
Light type					
White		10.74± 0.45b	4.96± 0.29b	5.09± 0.33b	13.48± 0.27b
Violet		11.77± 0.43a	6.16± 0.31a	6.30± 0.45a	14.88± 0.39a
Growth regulators					
Control		8.79± 0.28e	4.09± 0.26e	3.37± 0.22e	12.25± 0.21e
BA 1.00 mg/L		10.19± 0.39d	4.72± 0.27d	5.23± 0.18d	13.59± 0.31d
BA 2.00 mg/L		11.49± 0.17c	5.51± 0.30c	5.69± 0.19c	14.31± 0.23c
TDZ 1.00 mg/L		12.41± 0.27b	6.35± 0.33b	6.64± 0.34b	14.89± 0.38b
TDZ 2.00 mg/L		13.38± 0.27a	7.16± 0.25a	7.58± 0.51a	15.86± 0.49a
Interactions					
White light	Control	8.20±0.07g	3.56 ± 0.13h	2.95± 0.09i	11.82±0.17g
	BA 1.00 mg/L	9.48± 0.36f	4.13± 0.13g	4.91± 0.21g	12.93 ± 0.11f
	BA 2.00 mg/L	11.15± 0.11 de	4.85± 0.14f	5.28± 0.12fg	13.83± 0.10e
	TDZ 1.00 mg/L	12.04±0.1c	5.66± 0.16e	5.89± 0.17de	14.05± 0.06 de
	TDZ 2.00 mg/L	12.81± 0.07b	6.62± 0.04c	6.46± 0.06c	14.76 ± 0.09c
Violet light	Control	9.39± 0.17f	4.62± 0.18f	3.79± 0.25 h	12.67± 0.01f
	BA 1.00 mg/L	10.89± 0.36e	5.27± 0.13e	5.55± 0.09ef	14.27± 0.08d
	BA 2.00 mg/L	11.83± 0.13 cd	6.15±0.11d	6.09± 0.08cd	14.79± 0.14c
	TDZ 1.00 mg/L	12.78± 0.46b	7.05± 0.21b	7.39± 0.05b	15.74±0.03b
	TDZ 2.00 mg/L	13.95± 0.17a	7.70± 0.09a	8.69± 0.18a	16.96± 0.16a

Table 5. Effect of various cytokinin types and visible light emitting diodes (LEDs) on antioxidant chemicals of micropropagated *Ocimum basilicum* callus after 30 days.

Treatments		Total Phenolic (mg/g dry wt)	Total Flavonoids (mg/g dry wt)	Total Anthocyanins (mg/g dry wt)	Total Ascorbic acid (mg/g fresh wt)
Light type					
White		14.98±0.34b	7.43±0.18b	8.03±0.29b	16.32±0.50b
Violet		17.92±0.29a	9.21±0.20a	9.96±0.20a	19.30±0.28a
Growth regulators					
TDZ 1.00 mg/L		15.79±0.72b	7.90±0.39b	8.48±0.47b	17.00±0.81b
TDZ 2.00 mg/L		17.10±0.61a	8.74±0.42a	9.52±0.41a	18.62±0.55a
Interactions					
White light	TDZ 1.00 mg/L	14.22±0.10d	7.09±0.04d	7.44±0.11d	15.22±0.03d
	TDZ 2.00 mg/L	15.74±0.04c	7.82±0.11c	8.63±0.22c	17.41±0.24c
Violet light	TDZ 1.00 mg/L	17.37±0.36b	8.76±0.03b	9.52±0.07b	18.78±0.35b
	TDZ 2.00 mg/L	18.47±0.03a	9.66±0.09a	10.41±0.06a	19.83±0.11a

Discussion

Variations in the parameters of in vitro culturing

In this study, the fresh and dry weights of *Ocimum basilicum* shoots recorded the highest value when the control medium was illuminated with violet LEDs. In support of Ma *et al*. (2021), they discovered that among 25 plant characteristics examined, red and blue LED lights together greatly increased dry weight by 161% when compared to white LED lights in *Dendranthema morifolium* (Ramat). Nhut *et al.* (2015) they discovered that a combination of 40% blue LED and 60% red LED generated the highest dry and fresh weights of micropropagated *Panax vietnamensis*, as well as greater average plant height compared to fluorescent lamps. Also, Gu *et al*. (2012) they indicated that *Anthurium andraeanum* compared to plantlets produced under fluorescent white light, those developed from leaf explants under red and blue light exhibited a better-balanced root-to-shoot ratio and a 22.7% increase in total dry weight. However, the advantage is probably because the red + blue spectral energy distribution matches the one of chlorophyll absorption (Goins *et al.*, 1997), which raises the net photosynthetic rate and biomass accumulation. Abu-Ziada *et al.* (2022) found that in MS medium containing 2.00 mg/L TDZ, the highest fresh weight of callus for *Moringa olifera* was 4.779 g but under white light, this was followed by 3.448 g in the same medium under violet light. Phippen and Simon (2000) discovered that to achieve optimal callus and shoot induction across various basil cultivars, a higher concentration of TDZ (16.8 µM or 4 mg/L) is necessary. Meanwhile, the current findings are opposite to those found by Mansour *et al.* (2024)

who reported that the fresh weight of callus per explant (CFW/exp) in Balady basil ranged from 159.49 to 1275.00 mg, achieved with 20 and 10 µM BAP, respectively.

Additionally, this study found that when exposed to callus size violet light increased in MS medium containing 2.00 mg/L TDZ. These findings can be explained by the fact that the weight of the new callus increases as cell growth and division increase. Furthermore, the fresh weight of the callus is physiologically influenced by the water and carbohydrate content of the culture medium (Bajji *et al.*, 2000), and the rate of division and proliferation of these cells before the formation of a callus greatly influences the quantity of new weight generated (Phua *et al.*, 2018). However, these results may be due to an increase in cell division and expansion are the causes of an increase in the fresh weight of the callus. Additionally, the water and carbohydrates in the culture medium have a physiological impact on the callus's fresh weight (Indah and Ermavitalini, 2013), and the amount of fresh weight generated highly depends on how fast these cells divide, proliferate, and then produce a callus (Andaryani, 2010). In this connection, Islam *et al.* (2005) provided an illustration of the formation of a callus from explants by breaking the procedure down into three stages: induction, cell division, and differentiation phase. Also, the length of these stages is determined by the physiological state of the cells in the explant as well as culture parameters such as the right ratio of plant growth regulators. The existence of hormones found naturally in explants other than those supplied to the medium may result in different callus induction. In addition to their weights, calli vary in shape, including color, they may be yellow, white, or green (Mahood *et al.*, 2018).

Changes in antioxidant compounds of micropropagated *Ocimum basilicum*

When compared to the control treatment values, which show the lowest values, the amount of antioxidant chemicals (ascorbic acid, flavonoids, phenols, and anthocyanins) *in vitro Ocimum basilicum* shows an increase in MS medium supplemented with all the used treatments; typically, the maximum content by 2.00 mg/L of TDZ under violet LED illumination. Callus cultures of various plant species have been subjected to light exposure to boost metabolite biosynthesis (Fazal *et al*., 2016; Nadeem *et al*., 2018). Our results in harmony with those obtained by Dörr *et al.* (2020) stated that artificial light positively influences the levels of phenolic compounds, identifying the optimal concentration of these substances in *Ocimum basilicum*. Additionally, in the callus culture of *Cnidium officinale*, the use of mixed (red-blue) LED light significantly increased the production of flavonoids and phenolics (Adil *et al.*, 2019). Consequently, adjusting the ratios of blue and red LEDs can enhance growth and phenolic content in both green and red basil microgreens, providing a practical method for producing high-quality foods (Lobiuc *et al.*, 2017). Also, Abu-Ziada *et al.* (2022) they found that the MS medium supplemented with 2 mg/L TDZ had the highest significant value of phenols under violet light (142.31%), followed by MS media supplemented with 1 mg/L TDZ under the same illumination conditions (96.29%). Under white light, the same previous media recorded values of 59.38% and 7.05%, respectively on *Moringa olifera*. Phenolic compound accumulation is a feature of environmental stress in plant tissues., and plants may withstand a variety of biotic and abiotic stresses, including salinity, heavy metals, drought, temperature, and ultraviolet light by increasing the production of polyphenolic compounds (Cheynier *et al.*, 2013, Tuladhar *et al.*, 2021). By promoting photosynthesis and activating the malonyl-CoA pathway, which is essential for creating phenolic compounds, light raises the overall phenolic content (Qian *et al.*, 2016). A plant cultivated in vitro can enhance its two-way antioxidant capability: it may elevate the synthesis of phenolic substances in response to challenging circumstances (Matkowski, 2008), or the synthesis of these substances can be promoted through elicitation mechanisms, independent of stress (Alvarez, 2014; Trettel *et al*., 2018).

Ibrahim and Jaafar (2012) highlighted that a range of flavonoids may be produced by altering the kind and exogenic PGR concentration, in addition to the lighting. Additionally, using LED light instead of fluorescent light caused a higher accumulation of many flavonoid compounds in *Cyathea delgadii in vitro* culture (Mikuła *et al*., 2021). Also, De Queiroz *et al.* (2015) they highlighted that in studies focused on producing valuable compounds through callus culture, both the induction of callus and its ongoing proliferation are largely influenced by plant growth regulators (PGRs). They observed that different growth conditions can increase the amounts of bioactive secondary metabolites in the callus, particularly the kinds and concentrations of PGRs used. A notable overall increase in flavonoid synthesis in *Moringa olifera* was seen at around 11.292% on MS medium with 2 mg/L TDZ under violet light. The minimum is around 14.59 in the control medium when exposed to white light (Abu-Ziada *et al*., 2022). El-Banna *et al.* (2024) they stated that the control medium recorded the lowest flavonoid content in *Mentha viridis* under white and violet LEDs illumination, while the MS medium supplemented with 1 and 2 mg/L TDZ recorded the highest content under violet LEDs illumination.

The LEDs were found to increase anthocyanins, a crucial component of secondary metabolites. Water-soluble glycosides and acyl glycosides of anthocyanidins, a class of phenolic chemicals present in nature, are called anthocyanins. (Lian *et al*., 2019). Nazir *et al.* (2019) found that adding TDZ significantly increased the accumulation of hydroxycinnamic acid derivatives and anthocyanins, leading to a deep purple coloration of the callus in *Ocimum basilicum*. Also, El-Banna *et al.* (2024) found that the control medium had the lowest anthocyanin levels in *Mentha viridis*, whereas the MS medium complemented with 2.00 mg/L TDZ had the greatest anthocyanin value when illuminated by violet LEDs. According to research, anthocyanins can prevent photo-inhibition in photosynthetic tissues by absorbing blue-green light and lowering the amount of light that reaches the chloroplasts (Merzlyak *et al.*, 2008). The rise in anthocyanin production may be triggered by mild stress during micropropagation. Anthocyanins are frequently synthesized in vegetative tissues when exposed to stressors such as high light intensity, low temperatures, nutrient deficiencies, or pathogen infections (Chalker-Scott, 1999). This induction shows that anthocyanins act as a safeguard. But this may be due to their antioxidant qualities, their visual characteristics, or a combination of both factors. This result agrees with the reports of El-Banna *et al.* (2024) observed that *Mentha viridis* was shown to have a general, significant increase in ascorbic acid levels compared to the control values. Additionally, the MS medium used in the experiment was supplemented with

TDZ at 1.00 and 2.00 mg/L. Under violet LED light, this vitamin content produced the highest results. On the other hand, ascorbic acid levels were lowest in the control media with violet and white LED light.

Antioxidants like glutathione and ascorbic acid, which are present in the chloroplasts of the moringa plant and other cellular compartments, are essential for plants' defense against oxidative stress (Badawy *et al.*, 2022; Sofy *et al.*, 2021). According to Lee *et al.* (2008), Blue and red light working together may increase the synthesis of flavonoids, anthocyanins, and total polyphenols for all the antioxidant syntheses that were examined. Compared to blue LED therapy, red LED therapy has an increased impact on anthocyanin levels and increases the antioxidant activity in crops like tomatoes, Chinese cabbage, peas, and Chinese kale while they are being stored (Kim *et al.*, 2013).

CONCLUSIONS

Blue and red light working together may increase the synthesis of flavonoids, anthocyanins, and total polyphenols for all the antioxidant components that were examined. Compared to blue LED therapy, red LED therapy has an increased impact on anthocyanin levels and increases the antioxidant activity in crops like tomatoes, Chinese cabbage, peas, and Chinese kale while they are being stored (Kim *et al.*, 2013).

El-Banna *et al.* (2024) they observed that *Mentha viridis* was shown to have a general, significant increase in ascorbic acid levels parallel to the control rates. Additionally, the MS medium used in the experiment was supplemented with TDZ at 1.00 and 2.00 mg/L. Under violet LED light, this vitamin content produced the highest results. On the other hand, ascorbic acid levels were lowest in the control media with violet and white LED light. whether in the individual effects of white light or thidiazuron 2.00 mg/L alone or the interaction between them compared to the other treatments.

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تأثير نوع الضوء على اكثار وتراكم المركبات الثانوية لنبات الريحان الايطالى تحت تقنية زراعة الأنسجة

هبه يوسف البنا ,مهند محمد عبد الباسط و نهى عادل يوسف

قسم الخضر و الزينة – كلية الزراعة – جامعة المنصورة

الملخص

تهدف هذه الدراسة إلى تحسين إنتاج المركبات الثانوية من نبات الريحان الايطالي والذي يتبع العائلة الشفوية باستخدام السيقان و الكالس. حيث تم إستخدام نوعين من السيتوكينين: بنزيل أمينوبيورين(BAP) والثيديازورون(TDZ) بشكل فردى بثلاثة تركيزات مختلفة 0 ، 1 أو 2 ملليجم / لتر بالإضافة إلى أنظمة اضاءه منها مصابيح الليد,(LED) الضوء الأبيض كعنصر ضابط والبنفسجي ؛ مزيج من الأحمر والأزرق (1:1) اوضحت النتائج التي تم الحصول عليها ان بيئة موراشيج وسكوج الكنترول الخالية تماما من الهرمونات اعطت نتائج متفوقة من حيث وزن السيقان الطازجة والجافة(1.46جرام و 0.88 جرام علي التوالي) بينما بيئة موراشيج وسكوج المدعمة بـ 2 ملليجرام / لتر من (TDZ)و إضاءة الليد البيضاء و البنفسجية، اعطت أعلى قيم لوزن الكالس الطازج والجاف(1.62 جرام و 0.91 جرام علي التوالي) . والجدير بالذكر أنه لم يتم ملاحظة تكوين الكالس استجابة لمعاملات بنزيل أمينوبيورين. وبالمقارنة مع معاملة الكنترول وجد ان جميع المعاملات المستخدمة عمومًا ساعدت في تحفيز تكوين المركبات المضادة للأكسدة مثل (الفينولات والفلافونويد والأنثوسيانين وحمض الأسكوربيك) بشكل ملحوظ, سواء في التأثيرات الفردية للضوء الأبيض او الثيديازورون 2.00 ملليجم / لتر كل بمفرده او التداخل ماينهما بالمقارنه بالمعاملات الأخرى.