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Response of bioactive phytochemicals in *in vitro* shoot cultures of *Gardenia jasminoides*, Variegata to fluorescent light as a physical elicitor

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Abstract

An assessment was conducted on the impact of white, red, blue, and green fluorescent lighting on the fresh weight and production of bioactive phytochemicals in the shoot cultures of *Gardenia jasminoides*, Variegata. In both harvests, the shoot cultures exposed to blue fluorescent light had the maximum fresh weight (1.84 ± 0.16 and 2.413 ± 0.13 g, respectively). In contrast, the fresh weight measurements for both harvests (0.945± 0.055, 1.42± 0.2, respectively) were lowest under white fluorescent light. The shoot cultures that were exposed to green light for two weeks (first harvest) and four weeks (second harvest) showed the highest total flavonoid content (1.02 ± 0 mg / g dw) and total phenolic content (11.19 ± 0.43 mg / g dw), respectively. The shoot cultures exposed to blue light showed the highest level of antioxidant activity. Regarding the influence on the content of secondary metabolites, it is clear that only three phenolic compounds—chlorogenic acid, rosmarinic acid, and rutin—were found in variable amounts throughout various harvests and treatments. First, the extracts of shoots exposed to red light for two weeks (first harvest) had the highest concentration of chlorogenic acid (840±1.4 µg/g dw), followed by shoots exposed to green light for two weeks (first harvest) (835±1.6 µg/g dw). In the second harvest (four weeks), the white light yielded the highest rosmarinic acid concentration (3965± 3 µg / g d w), while the red light yielded the lowest quantity (3175± 1.5 µg / g d w). Regarding rutin, it was noted that the green light in the second harvest possessed the highest rutin concentration (1800± 3, 1515±3 µg/g d w, respectively), with the green light in the first harvest coming in second.

Keywords: Chlorogenic acid, fluorescent light, *Gardenia jasminoides* Variegata, rosmarinic acid, rutin.

1. Introduction

The initial energy source for photosynthesis and plant growth is light. Light triggers many signals and information related to morphogenesis and numerous other physiological processes [1]. Plant growth and development can be influenced by various aspects of light, including wavelengths, intensity, duration, and direction. In addition to providing energy for photosynthesis, light is a signal for the morphogenesis of plants [1]. Plant development, growth, yield, and quality are significantly influenced by photoperiod and light quality. The biosynthesis and accumulation of many secondary plant metabolites, which are essential for crop quality, are also significantly impacted by light [2]. Secondary metabolites (SMs), which are found in plants in fewer amounts than primary metabolites, include anthocyanins, carotenoids, phenolic acids, and flavanols. Although they are not necessary for life, they are very important to the plant's ability to survive [3].

Medicinal plants are known as the backbone of the global pharmaceutical industry. Different chemical groups are thought to be primarily responsible for the various therapeutic benefits of plants. The most well-known components of medicinal plants are flavonoids, alkaloids, phenolic compounds, and tannins. [4]. A variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides, and volatile oils, are plentiful in plants [5]. Environmental variables like water, light, temperature, soil characteristics, and chemical stress have an impact on the biosynthesis and accumulation of SMs in medicinal plants [6-7]. *Gardenia jasminoides* is a plant from the family *Rubiaceae*, which has its origins in the Near East. It grows in many temperate locations and has fragrant white blossoms. It has anti-atherosclerotic, antithrombotic, and anticancer properties in addition to its capacity to lower blood sugar, reduce inflammation, treat depression, increase antioxidant capacity, and improve sleep quality due to its high content from different secondary metabolites [8-14].

Plants in the *Lamiaceae* family possessed rosmarinic acid (RA) before its chemical structure was well understood [15]. RA is a polyphenolic secondary metabolite that is found in an array of plants and has a

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broad range of pharmacological properties. Among these are its capacities to stop low-density lipoprotein (LDL) from oxidizing and have an antiallergic impact. RA has been shown to have anti-viral, anti-bacterial, anti-mutagenic, antioxidant, astringent, and anti-inflammatory characteristics [15-18].

Chlorogenic acid is a significant and physiologically active dietary polyphenol that has a number of therapeutic uses, including the scavenging of free radicals, inhibition of bacterial growth, hepatoprotection, cardioprotection, anti-inflammatory, antipyretic, neuroprotective, anti-obesity, and anti-microbial effects [19-20].

The glycoside that combines the flavonoid quercetin and the disaccharide rutinose is called rutin. It has attracted notice due to its medicinal properties. Antioxidant, anti-carcinogenic, vaso-protective, neuroprotective, and cardioprotective are just a few of the numerous pharmacological properties it possesses [21-24]. Buckwheat is regarded as the third primary source of rutin among other plants [25]. Gardenia (*Gardenia jasminoides* Ellis) may come in at the fourth position for rutin accumulation, according to Gabr *et al.* [26].

In our earlier research on how light quality affects growth and secondary metabolites in tissue cultures of Gardenia jasminoides, Variegata, we found that chlorogenic acid was the predominant phenolic compound in both types of cultures, with significantly higher levels in the shoot cultures compared to the calli cultures. Additionally, exposure to white LED light enhanced the content of chlorogenic acid. [27].

Consequently, the purpose of this study is to assess how fluorescent light—which comes in white, red, blue, and green—affects the development and synthesis of secondary metabolites in shoot cultures of *Gardenia jasminoides*, Variegata.

Experimental:

Plant material:

In vitro cultivated *G. jasminoides* Variegata plantlets served as the source of the plant material. The plantlets underwent three subcultures on Murashige and Skoog (MS) medium, supplemented with 2 mg/l of benzyl adenine (BA) to promote shoot multiplication.

Impact of subjecting shoot cultures to varying fluorescent light qualities on the fresh weight of *Gardenia jasminoides* Variegata across two harvests:

To investigate how various fluorescent light qualities influence Gardenia shoot cultures, gardenia shoots were subcultured on MS medium enriched with 2 mg/l BA. Each set of ten jars was subjected to white, red, blue, and green fluorescent lights for four weeks, with five jars harvested every two weeks (representing the first and second harvests). The fresh weight was measured, and the samples were dried using a freeze-dryer. The extracted samples were then stored at -20 °C for future use.

Sample extraction:

The extraction process was carried out following the methodology outlined by Gabr *et al.* [27]. A total of 0.1 grams of freeze-dried samples was utilized, and the extraction was performed using 1.5 ml of 80% methanol for 24 hours. Following this, the extracts were subjected to sonication in an ultrasonic water bath (Grant, United Kingdom) for 20 minutes. The samples were then centrifuged for 5 minutes at 6000 rpm (Sigma, 2-16 PK, Germany). The supernatants were collected, and the remaining pellets underwent two additional extractions with 500 µl of the solvent. The resulting extracts were preserved at -20°C until needed for further analysis.

Total phenolic content:

Total phenols were quantified using the Folin–Ciocalteu micro-method [29-30]. A volume of 20 µl of the extract solution was combined with 1.16 ml of distilled water and 100 µl of Folin–Ciocalteu's reagent, followed by the addition of 300 µl of a 200 g/l Na2CO3 solution. The resulting mixture was placed in a water bath at 40 °C for 30 minutes, and its absorbance at 760 nm was recorded using a Jenway 6715 UV/Vis spectrophotometer. A control sample without the extract was also evaluated. Gallic acid served as the standard for constructing the calibration curve. The total phenolic content, expressed as gallic acid equivalent, was determined using the following equation:

$A = 0.98C + 9.0925 \times 10 -3$ ($R2= 0.9996$)

where A represents the absorbance of gallic acid and C denotes the concentration.

Total flavonoid content:

The total flavonoid content was assessed following the protocol outlined by *Ordon et al.*, [31]. An aliquot of 0.5 ml of a 20 g/l AlCl3 methanolic solution was mixed with 0.5 ml of the extract solution. After incubation for 1 hour at room temperature, the absorbance at 420 nm was recorded using a Jenway 6715 UV/Vis spectrophotometer. The presence of flavonoids is indicated by a yellow color. A control sample without the extract was also tested. The total flavonoid content, expressed as quercetin equivalent (QE), was calculated using the equation derived from the calibration curve:

$Y= 0.0255X$($R2= 0.9812$)

In this equation, X represents the absorbance, while Y denotes the concentration.

(mg QE g−1 fW).

The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical cation (ABTS•+) Radical – scavenging activity

For the ABTS assay, the procedure described by Re *et al.* [32] was utilized. The stock solutions included a 7 mmol/l ABTS solution and a 2.4 mmol/l potassium persulfate solution. The working solution was created by combining equal volumes of the two stock solutions and allowing them to react for 12 to 16 hours at room temperature in the dark. Subsequently, 1 ml of

the resulting ABTS solution was diluted with 60 ml of methanol to achieve an absorbance of 0.706 ± 0.001 units at 734 nm, as determined using a spectrophotometer. A fresh ABTS solution was prepared for each assay. Extract solutions from various treatments (1 ml each) were allowed to interact with 1 ml of ABTS solution for 7 minutes, after which the absorbance at 734 nm was recorded using a Jenway 6715 UV/Vis spectrophotometer. A control sample without any added extract was also examined. The scavenging activity was calculated using the following formula:

ABTS radical-scavenging activity (%) = [(ABTS control – ABTS sample) / ABTS control] x 100.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity:

According to Gabr *et al.* [33], the DPPH assay was performed with several modifications. A methanolic extract from various treatments (0.1 ml of each) was mixed with 1.9 ml of DPPH solution by vortexing for 30 seconds and allowed to react for 30 minutes, after which the absorbance at 515 nm was measured using a Jenway 6715 UV/Vis spectrophotometer. A control sample without any extract was also assessed. The scavenging activity was determined using the following formula:

DPPH radical-scavenging activity (%) = (A control - A sample / A control) × 100,

where A represents the absorbance at 515 nm.

Determination of phenolic acid content by High-Performance Liquid Chromatography (HPLC)

The methanol solution was evaporated to yield a dry residue. This extract was then dissolved in 1 ml of methanol and stored at 4°C in the dark. The phenolic compound content was analyzed using HPLC on a UNICAM CRYSTAL 200 Liquid Chromatograph (Column: Kromasil C18 5um250*4.66 mm). The mobile phase was comprised of methanol and water, both acidified with 0.3% orthophosphoric acid p.a. (w/v). The phenolic compounds were separated using a linear gradient from water to 50% methanol over 5 minutes, followed by isocratic elution at 50% methanol for 20 minutes. The flow rate was maintained at 1.4 ml/min. Substances were detected through absorption at λ = 280 nm, and their identification was performed by comparing retention times and absorption spectra with standards complex of phenols: chlorogenic acid, vanillic acid, ferulic acid, rosmarinic acid, and cinnamic acid. For flavonoid standards, Rutin (quercetin-3-rutinoside), apigenin-7-glucoside, and Kaempferol (kaempferol-3-rutinoside) were used. The content in the samples was expressed in µg/g of dry weight and calculated using the known concentration of the standard and the peak areas of both the standard and the sample.

Where Concentration of sample = [Area sample / Area of standard] * Concentration of standard.

Statistical analysis

All analyses utilized five replicates, and results were presented as Mean ± Standard Deviation (SD). A Two-way ANOVA was conducted with GraphPad Prism version 5.01 to assess the p-value and its significance.

Results and Discussion

Impact of subjecting shoot cultures to varying fluorescent light qualities on the fresh weight of *Gardenia jasminoides* Variegata across two harvests:

The initial energy source for photosynthesis and plant growth is light. By taking a glum on Fig. (1). It could be noticed that exposing the shoot cultures to the blue fluorescent light recorded the highest fresh weight in both harvests (1.84 ± 0.16, 2.413± 0.13 g, respectively). Comparing with the white fluorescent light which recorded the lowest fresh weight in both harvests (0.945± 0.055, 1.42± 0.2, respectively) Fig. (2). According to the two-way ANOVA there is a significant difference among different fluorescent light wavelengths and there is a significant difference among time (first and second harvest) but there is no significant difference among interaction between different fluorescent light wavelength and time.

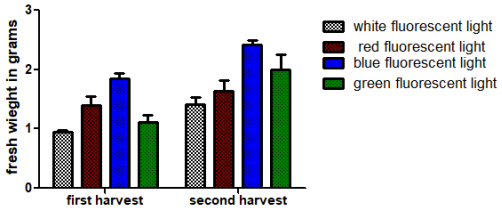

Fig (1): Effect of different fluorescent light quality on the fresh weight of *G. jasminoides* Variegata during two harvests.

A **B** **C** **D**

Fig (2): Effect of different fluorescent light quality on the fresh weight of *G. jasminoides* Variegata

A: shoot culture exposed to white fluorescent light for four weeks

B: shoot culture exposed to red fluorescent light for four weeks

C: shoot culture exposed to blue fluorescent light for four weeks

D: shoot culture exposed to green fluorescent light for four weeks

Plant growth is influenced by several processes, including cell division, elongation, directional growth, and branching. The growth-related processes are directly affected by the direction, intensity, and spectrum of light [34]. In our earlier research on how light quality affects growth and secondary metabolites in tissue cultures of *G. jasminoides*, Variegata, we found that both fresh and dried weights of calli and shoots were significantly influenced by white LED

light [27]. To further investigate the effects of different fluorescent lights on growth and secondary metabolites, we observed that blue light resulted in the highest fresh weight. These findings align with those of Srivastava *et al.* [35], who noted that blue light effectively promotes the growth of pea plants, with red light following as a close second. As noted by Ptushenko *et al.* [36], plants mainly absorb light in the red and blue spectrums. Moreover, Srivastava *et al.* [35] remarked that phototropism, which is the bending or growth of plants in response to light, is predominantly regulated by blue light. This type of light plays a significant role in seed germination, stimulates upward growth and development, and helps plants orient their leaves and stems toward the light source. Blue light also influences the opening of stomata, thereby affecting plant water balance and transpiration rates. It provides energy for the light-dependent processes of photosynthesis, which subsequently promote the elongation of leaves and stems and enhance overall plant growth.

Total phenolic content:
Impact of subjecting shoot cultures to varying fluorescent light qualities on total phenolic content of *Gardenia jasminoides* Variegata across two harvests:

During two harvests the phenolic acid accumulation was assessed in various treatments (Fig. 3). Data from Fig. (3) analysis revealed that exposing the shoot cultures to the green light for four weeks (second harvest) exhibited the highest total phenolic content (11.19± 0.43 mg / g dw) followed by exposing the shoot cultures to the green light for two weeks (first harvest) which exhibit (10.66± 0.014 mg / g dw) total phenolic content.

According to the two ANOVA test, among different fluorescent light wavelengths there is a significant difference and among time (first and second harvest) there is a significant difference but there is no significant difference among interaction between different fluorescent light wavelengths and time in analyzing the total phenolic content data.

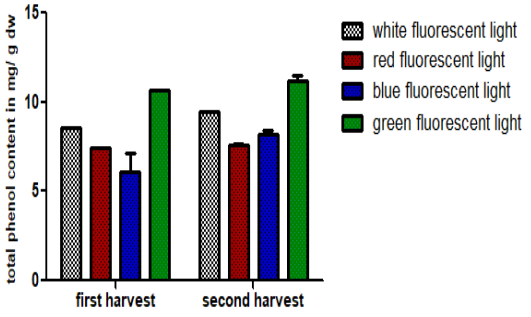

Fig (3): Effect of different fluorescent light quality on the total phenolic content of *G. jasminoides* Variegata during two harvests.

Total flavonoid content:
Impact of subjecting shoot cultures to varying fluorescent light qualities on total phenolic content of *Gardenia jasminoides* Variegata across two harvests:

During two harvests the flavonoid accumulation was assessed in various treatments (Fig. 6). Data from Fig. (4) analysis revealed that exposing the shoot cultures to the white, green light for two weeks (first harvest) exhibits the highest total flavonoid content (1.02± 0.0004, 1.02 ± 0 mg / g dw, respectively).

According to the two ANOVA tests, among different fluorescent light wavelengths there is a significant difference and among time (first and second harvest) there is a significant difference and there is also a significant difference among interaction between different fluorescent light wavelengths and time in analyzing the total flavonoid content data.

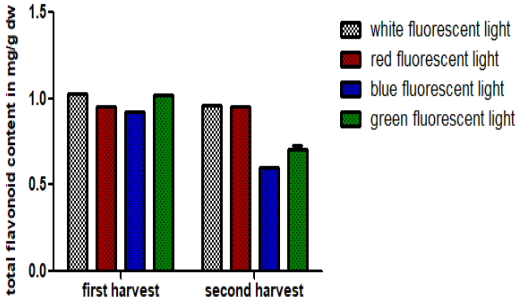

Fig (4): Effect of different fluorescent light quality on the total flavonoid content of *G. jasminoides* Variegata during two harvests

Our findings are consistent with those of Tariq *et al.* [37], who indicated that in the calli cultures of A. absinthium, total flavonoids, total phenolics, and antioxidant activity were more effectively supported by exposure to the green spectrum for three weeks at a photosynthetic photon flux density of 40–50 µmol m−2 s−1. Conversely, our results differ from those of Fazal *et al.* [38], who investigated the impact of monochromatic blue, green, yellow, and red light wavelengths on polyphenol production in *P. vulgaris* calli. They found that the highest accumulation of total phenolics (23.9 mg g-1 DW) and total flavonoids (1.65 mg g-1 DW) occurred under blue light. Similarly, Kapoor *et al.* [39] reported that calli cultures of *Rhodiola imbricata* achieved the highest levels of total flavonoids and total phenolics when grown under blue light.

The anti-oxidant activity:

As noted by Dorman *et al.*,[40], there is no one method that is suitable for determining the effectiveness of antioxidants because there are multiple ways that may yield wildly inconsistent results. It is necessary to employ several strategies based on various mechanisms. The ABTS and DPPH radical-scavenging activity tests were used in this instance.

Impact of subjecting shoot cultures to varying fluorescent light qualities on ABTS percentage of *Gardenia jasminoides* Variegata across two harvests:

Regarding data presented in Fig (5), it could be concluded that the highest percentage of ABTS (99.8 %) was recorded with the shoot cultures exposed to the white and blue light for two weeks (first harvest) followed by the shoots exposed to the red light for four weeks (second harvest) recording (99.55%).

Impact of subjecting shoot cultures to varying fluorescent light qualities on DPPH percentage of *Gardenia jasminoides* Variegata across two harvests:

By analyzing data presented in Fig (6), it could be observed that the highest percentage of DPPH (89.045 %) was recorded with the shoot cultures exposed to the blue light for four weeks (second harvest) followed by the shoots exposed to the blue light for two weeks which recorded (89.065 %). According to the two ANOVA test, among different fluorescent light wavelengths there is a significant difference and among time (first and second harvest) there is a significant difference and there is also a significant difference among interaction between different fluorescent light wavelengths and time and that was with the ABTS and DPPH tests.

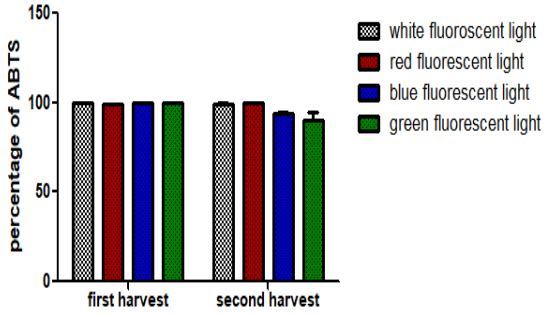

Fig (5): Effect of different fluorescent light quality on the antioxidant activity of *G. jasminoides* Variegata during two harvests using ABTS radical-scavenging activity assay

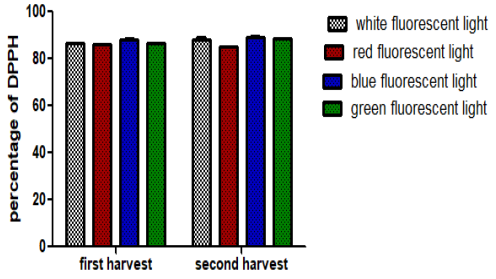

Fig (6): Effect of different fluorescent light quality on the antioxidant activity of *G. jasminoides* Variegata during two harvests using DPPH radical-scavenging activity assay

Our results indicate that the highest antioxidant activity was observed with exposing the shoot cultures to the blue light either for two or four weeks using the two methods for assessing the antioxidant. Although the phenolic acids detected in the different extracts was not detected with their highest value when exposing the shoot cultures to the blue light, there may be other phenolic acids not detected increasing the antioxidant activity with the blue light. Our results are matching what was reported by Nam *et al.* [41], who stated that sprouts of common buckwheat cultivated in blue light have the highest levels of antioxidant activity, total flavonoids, and total phenolics. While our results oppose what was found by Shiga *et al.*, [42]., who suggested that exposure to constant white light might effectively raise basil's RA concentration, leading to a high level of antioxidant activity.

Determination of phenolic acids content by High Performance Liquid Chromatography (HPLC)

For studying the effect of different fluorescent light quality on Gardenia shoot cultures. Samples from different treatments (white, red, blue, and green fluorescent light) were harvested after two and four weeks and extracted for further analysis. HPLC was used to assess how different fluorescent lights affected phenolic compounds. Considering the data in Table (1), it could be obviously declared that among the different phenolic compounds that were examined only the chlorogenic acid, rosmarinic acid, and rutin were detected with varying quantities across different treatments and different harvests. Firstly, the chlorogenic acid recorded its highest content in the extracts of shoots exposed to red light for two weeks, followed by the shoots exposed to green light for two weeks (840±1. 4, 835±1.6 µg/g d w, respectively). Also, it could be observed that the chlorogenic acid content decreased in the second harvest with the different treatments. As for the rosmarinic acid, it could be observed that the second harvest recorded higher rosmarinic acid content among the different treatments except the blue light. The highest rosmarinic acid content (3965±3 µg / g d w) was recorded with white light in the second harvest, followed by the red light in the second harvest, which recorded (3175± 1.5 µg / g d w). The lowest rosmarinic content was recorded with the blue light in both harvests. Finally, as for rutin which is considered a flavonoid, it could be observed that the highest rutin content was recorded with the green light in the second harvest followed by the green light in the first harvest (1800±3, 1515±3 µg/g d w, respectively). First of all, it must be clarified that using the LED illumination as an elicitor with the G. jasminoides, Variegata shoots, and calli cultures, the most prevalent phenolic compound in both cultures was chlorogenic acid, which is significantly more prevalent in shoot cultures than in calli cultures. Additionally, the amount of chlorogenic acid rose after being exposed to white LED light. [27].

As for chlorogenic acid, our results are on line with what was reported by Wu *et al.*,[43], who declared that red light significantly increased the accumulation of luteoloside, rutin, ferulic acid, and chlorogenic acid (CGA) at lower auxin concentrations, while CGA production under red light was significantly decreased at higher auxin concentrations. It suggests that auxin and light interact to control secondary metabolism. The fundamental mechanisms still need to be investigated. While our results oppose what was reported by Hashim *et al.*, [44], who reported that a high quantity of chlorogenic acid was found in *Berula erecta* cultivated in tissue culture under conditions of low temperature (12 °C) and blue light. They added that this plant species could be considered a good alternative for in vitro production of chlorogenic acid. These results also contradict what was found in our previous work [27], since the highest chlorogenic acid content (8783.53 µg / g dry weight) was recorded with the white LED light in the shoot cultures of *G. jasminoides*, Variegata.

Table (1): Impact of subjecting shoot cultures to varying fluorescent light qualities on phenolic compounds content (µg / g dry weight) of *Gardenia jasminoides* Variegata across two harvests:

Treatment	Harvest	Phenolic compound in µg / g d w		
		Chlorogenic acid	Rosmarinic acid	Rutin
White fluorescent	First harvest	693±1.4	1385±2	1450±2
Red fluorescent		840± 1.4	1430±3	230±1
Blue fluorescent		393.5±0.5	295±1	0±0
Green fluorescent		835±1.6	430±2	1515±3
White	Second harvest	452.4 ±0.6	3965±3	1290±5
Red fluorescent		388.4± 0.8	3175± 1.5	0± 0
Blue fluorescent		0±0	75.4±0.8	312±1
Green fluorescent		115±1	634±1.4	1800±3

Data represent mean± standard deviation.

Regarding the rosmarinic acid, our findings are consistent with those of Shiga *et al.* [42], who said that, within 14 days, RA accumulation was induced by both red and white light irradiation up to a level of 6 mg g^1 fresh weight, while only 3 mg g~1 fresh weight was induced by blue light irradiation in the sweet basil. This suggests that the red wavelength at 600–700 nm in both white and red irradiation promoted RA accumulation. Furthermore, RA accumulation was higher in the top leaves compared to the lower leaves and was influenced by the irradiation time. These findings suggest that exposure to constant white light can effectively raise basil's RA concentration, which in turn produces significant antioxidant activity. Additionally, our findings match with Pedroso *et al*.,[45] research, which stated that, despite the plant's height, leaf count, and fresh and dry biomass production all declining after 20 days of exposure, red light promotes the highest concentration of rosmarinic acid. Red and UV light stimulates the enzyme phenylalanine ammonia lyase (PAL), which catalyzes the deamination of phenylalanine into cinnamic acid, increasing the synthesis of phenolic compounds [46]. Compared to our findings, there was more fluctuation in the levels of RA identified in extracts from shoot cultures of Aronia melanocarpa, A. arbutifolia, and A. ×prunifolia, with the highest concentrations found in extracts from shoots cultured under blue light. [47].

As for rutin content, our results match what was found by De Souza *et al.*, [48], who declared that, In *Physalis angulata* (Linn.), the blue and green lights encouraged rutin accumulation to a larger extent. Our results contradict what was stated by Pedroso *et al.* [49], who declared that the red light enhanced plant development and increased dry weight and leaf number in in vitro-cultivated seeds of H. marrubioides, while white light and blue light were found to provide the maximum amount of rutin (0.308 mg/g of DW and 0.298 mg/g of DW, respectively). Additionally, it opposes what was found with Gabr *et al.*, [50] who stated that common buckwheat shoots grew best in hormone-free media with activated charcoal and blue light exposure, yielding extracts of rutin and quercetin of 4.3 mg and 7.0 mg/g, respectively, as opposed to 3.7 mg of rutin and traces of quercetin in the seeds of common buckwheat. Also, Nam *et al.*,[41] reported that, in typical buckwheat sprouts, blue light markedly increased the amounts of C-glycosylflavones, such as orientin, vitexin and their isomers, and rutin and an isomer of rutin.

Conclusion:

From our previous results on the effect of using different LED light quality on the growth and secondary metabolites of *G. jasminoides* Variegata calli and shoot cultures, it was declared that the independent phenolic acid found in both cultures was chlorogenic acid, and that the amount of this acid was higher in the shoot cultures than in the calli cultures. Exposure to white light also raises the level of chlorogenic acid. Here when using different fluorescent light quality to examine its impact on the

growth and secondary metabolites of *G. jasminoides* Variegata shoot cultures rosmarinic acid and rutin were detected with varying quantities along with the chlorogenic acid which may be explained as indicated by several researchers, there are numerous studies on various species that examine how light affects *in vitro* proliferation in order to determine the characteristics of light that can improve the effectiveness of the micropropagation process and the content of secondary metabolites. But the outcomes are frequently contradictory. Numerous writers attribute these outcomes to the varying reactions of plant species, cultivars, or even types of transplants to light, as well as the development of plant stages, medium composition, and vessel ventilation. Nonetheless, a significant contributor to variability could also be related to the challenge of implementing consistent intensities throughout the shelves and/or selecting the appropriate spectral composition for every type of light [51-52].

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