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Influence of Light Quality on Growth and Secondary Metabolite in Tissue Cultures of *Gardenia jasmonides*, Variegata

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Abstract

The influence of white, blue and red Light-Emitting Diodes (LED) on the growth and the secondary metabolites production in calli and shoots cultures of *Gardenia jasmonides*, Variegata was evaluated. The white light generally has a powerful effect on both fresh and dry weights of the two types of cultures. White light treatment recorded (11.88 and 0.63 g) for the fresh and dry weight (in case of calli) followed by the blue light which recorded (11.78 and 0.60 g), respectively. The extracts of shoots exposed to white LED light recorded the highest reducing efficiency (74.40 %). It could be justified as the shoots that exposed to the white light recorded the highest cholorogenic acid (CGA) compared to all the other treatments. Among different phenolic and flavonoids compounds which were tested in the calli and shoots cultures exposed to the different LED light quality, only the chlorogenic acid was detected in the different treatments in both cultures with varying quantities. The white LED light recorded the highest content of chlorogenic acid with both cultures, since it scored 52.43 µg / gdw with calli cultures versus 8783.53 µg / gdw with shoots cultures.

Key words: *Gardenia jasmonides*, Variegata ; light effect ; secondary metabolites; chlorogenic acid

1. Introduction

Plants may play an essential role in production of pharmaceutical compounds and other raw materials. The medicinal plants are recognized as the main stone in drug industries around the world. There are different chemical groups which can be considered as the main responsible for the different medicinal value plants. Phenolic compounds, flavonoids , tannins and alkaloids are the most famous compounds of the medicinal plants [1].

Gardenia is an ornamental and medicinal woody plant, belongs to the coffee family (Rubiaceae). There are more than hundred different species belongs to the genus Gardenia among them the specie jasmonide which include two sub- species (Ellis and variegata).The double-flowered form of gardenia is used for ornamental purposes, whilst the single-flowered one is considered as a medicinal plant, since the double – flowered one does not bear fruits which is considered as the medicinally used organ [2].Gardenia plays an important role in the traditional Chinese medicine [3]. It is full of phenolic compounds (i.e. cinnammic acid, chlorogenic acid) and anti-inflammatory flavonoids (i.e. rutin) which qualifies it for use as a pain treatment or for the treatment of inflammatory diseases [4].

Chlorogenic acid (CGA) is considered as an ester of the caffeic acid and (−)-quinic acid [5]. Chlorogenic acids (CGA) are recognized as cinnamic acid derivatives which have different biological effects mostly related to their anti- inflammatory activities.

In the last few years, chlorogenic acid has been shown to have a series of medicinal benefits, among them it minimize the relative risk of Alzheimer's disease , diabetes type 2 and cardiovascular disease [6–8], and it has different antibacterial and anti-inflammatory activities [9-10]. The major source of CGA in nature (5–12 g/100 g) is the Green (or raw)

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coffee [11], also buckwheat seedlings could be considered as a good source of chlorogenic acid [12] so, among the plant biodiversity it would be good to find new original sources for chlorogenic acid as well.

In order to face the increasing demand for plant metabolites used in the pharmaceutical industry, recent researches focused on the development of new biotechnological techniques from them the in vitro culture to elevate the bioactive metabolites in plant material is needed[13]

Biotechnology offers the different techniques to use cells, tissues, organs or whole organisms by growing them in vitro (under controlled environmental conditions) and manipulate with them to get the desired compounds. Light is the main physical factor in the controlled environmental conditions, which is directly affects the plant growth and development, also the production of plant secondary metabolites is greatly affected with light. Light is essential for photosynthesis process and also for the regulation of the plants development [14] . Utilizing different spectral composition could be considered as an efficient way to affect the plant growth and quality of plant production in controlled environments [15]. Recently, it was found that light emitted diodes (LED) lighting play an efficient role in in vitro plant growth [16] and plant productivity [17-19]. Since they are more preferred in the in vitro growing environments due to their small size, durability, low heat emission and energy consumption; all of which traits make them the most preferred for the in vitro plant propagation work [20].

Therefore, this research is conducted to evaluate the effect of white, red, blue Light-Emitting Diodes (LED) light on the growth and the production of secondary metabolites in calli and shoots cultures of Gardenia jasmonides, Variegata.

2. Experimental

Plant material:

In vitro growing Gardenia jasmonides Variegata plantlets were used as a source of plant material in this study. The plantlets were subcultured for three times on MS medium [21] supplemented with 2 mg/l BA for shoots multiplication needed in this study.

Calli cultures:

For callus induction, leaves of the in vitro growing gardenia plantlets were cut to segments of about 0.5cm long and placed on MS medium supplemented with 0.5 mg /l BA + 0.5 mg /l picloram for one subculture in dark. The inducted callus was transferred to MS medium supplemented with 4 mg/l TDZ for calli production needed in this study.

Effect of exposing the calli and shoots to the different light LED quality on the fresh and dry weights:

For studying the effect of different LED light quality on Gardenia calli cultures, about 0.5 g of calli were singled out on petri dishes containing MS medium supplemented with 4 mg/l TDZ. Ten petri dishes were placed under blue, red and white LED light for one month. While, for studying the effect of different LED light quality on gardenia shoots cultures, a single shoot of about 0.5 g was subcultured on MS medium supplemented with 2 mg/l BA. Ten jars were placed under blue, red and white LED light for one month. All the LED lamps are LED T8 True light (18 watt and 220 volt). Light intensity was measured with lux-meter it recorded 4130 lux with white light, 657 lux with red light and 283 lux with blue light. After one month, the explants were harvested and the fresh weights were recorded. The samples were dried using freeze dryer and dry weights were recorded.

Sample extraction:

100 mg of grounded dried samples each of calli and shoots in each treatment were extracted with 1.5 ml 80 % methanol for 24 hrs. The extracts were then sonicated in an ultrasonic water bath (Grant, United Kingdom) for 20 min. Samples were centrifuged for 5 min at 6000 rpm (Sigma, 2- 16 PK, and Germany). The supernatants were collected and the pellets were re-extracted twice with 500 µl of the solvent. The extracts were stored at - 20 ◦ C until further use.

DPPH Radical scavenging activity:

The DPPH assay was used with some modifications according to Gabr et al., (2017) [22]. The stock reagent solution (1 × 10-3 mol L-1) was prepared by dissolving 22 mg of DPPH in 50 ml of methanol and stored at - 20◦ C until use. The working solution (6× 10 – 5 mol L-1) was prepared by mixing 6 ml of stock solution with 100 ml of methanol to obtain an absorbance value of 0.8 + or – 0.02 at 515 nm, as measured using a spectrophotometer. Extract of the different samples (0.1 ml of each) were vortexed for 30 s with 1.9 ml of DPPH solution and left to react for 30 min, after which the absorbance at 515 nm was recorded. A control with no added

extract was also analyzed. Scavenging activity was calculated as follow:

Radical-scavenging (DPPH) activity (%) = [(Acontrol - Asample) / A control] × 100

Where A is the absorbance at 515 nm.

Determination of phenols and flavonoids content by HPLC:

The extraction was performed according to Gabr et al., [22]. The methanol solution of the extracted samples were evaporated and concentrated to a dry residue which was dissolved in 1 ml of methanol and kept at 4◦C in darkness. The content of flavonoids and total phenols were determined by HPLC on a UNICAM CRYSTAL 200 Liquid Chromatograph. The mobile phase consisted of methanol and water (both acidi¬fied with 0.3% orthophosphoric acid p.a. - w/v). Flavonoids were eluted with linear gradient from water to 50% methanol in 5 min, following by isocratic elution with 50% methanol for 20 min. The flow-rate was 1.4 ml/min. Substances were detected by absorption at λ = 288 nm and their identification were carried out by the comparison of retention times and absorption spectra with standards complex of flavonoids standards: Rutin (quercetin-3-rutinosid), apeginin-7-glucoside and Kaempferol (kaempferol-3-rutinoside). For phenolic acids: chlorogenic acid, vanillic, ferulic, rosmarinic and cinnamic. All Standards' used were manufactured by Sigma Aldrish. Samples content were expressed as µg/g dry weight and derived using a known concentration of standard and sample peak areas.

Statistical analysis:

All analysis was performed in ten replicates and data reported as mean ± standard deviation (SD). Results were processed by Excel.

3. Results and Discussion

Effect of exposing the calli and shoots to the different light LED quality on the fresh and dry weights

It is well known that Light is very important for Plants either in photosynthesis process or also for the regulation of their development.

Table (1): Effect of exposing the calli and shoots cultures to different light LED quality on the fresh and dry weights, after one month of culturing.

Treatments	Explant	Mean of fresh weight in grams	Mean of dry weight in grams
Red LED	Calli	8.7276±1.6839	0.4890 ±0.1358
Blue LED	Calli	11.7815±2.5893	0.6037 ±0.1504
White LED	Calli	11.8885±0.7626	0.6315 ±0.1138
Red LED	Shoots	0.8265±0.7264	0.0828 ±0.05716
Blue LED	Shoots	0.7156±0.5083	0.0831 ±0.0473
White LED	Shoots	1.8753±1.4841	0.2686 ±0.1417

Data represent mean ±S.D.

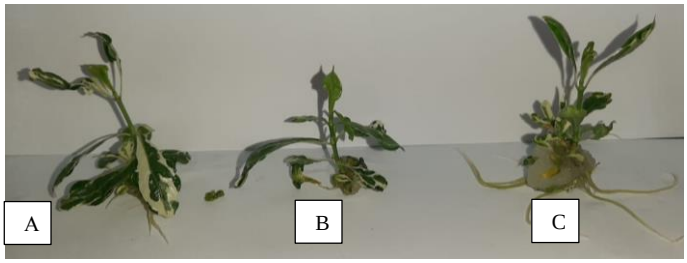

Fig. (1) Effect of different LED light quality on shoot cultures , after one month of culturing

A: Shoot in Red LED light

B: Shoot in blue LED light

C: Shoot in white LED light

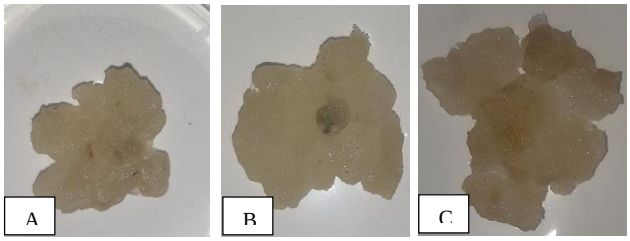

Fig. (2) Effect of different LED light quality on calli cultures , after one month of culturing

A: Calli in Red LED light

B: Calli in blue LED light

C: Calli in white LED light

Data presented in table (1) show the effect of exposing the calli and shoots cultures to different light LED quality (red, blue and white) on fresh and dry weight after one month of culturing. Data

declared that the white light generally has a powerful effect on both fresh and dry weights of the two types (calli and shoots) of cultures. White light treatment recorded (11.88 and 0.63 g) for the fresh and dry weight (in case of calli cultures) followed by the blue light which recorded (11.78 and 0.60 g) respectively. While, this treatment (white) recorded (1.8 7 and 0.26 g) with the shoots cultures. On the other hand, blue and red light treatments had a relatively weak or little effect on weights either fresh or dry in case of shoots cultures (figs 1 and 2).

Plant growth is considered a result of some processes like cell division and elongation, directional growth and branching. Light spectrum, intensity and direction affect directly these processes**[17].** The fluorescent lamps (FLs) are considered the common light sources used for in vitro culture. Which emit wide-spectrum light (350 to 750 nm), which in return may contains low-quality wavelengths unnecessary for promoting growth **[23].** Due to the light emitting diodes (LEDs) several advantages they could be considered as an alternative light source for *in vitro* culturing. The LED lights can causes higher growth and development in plants **[16]** since they eliminate light wavelengths that are inactive for photosynthesis. In spite of, blue and red light are absorbed by photosynthetic pigments more effectively than green **[18 and 24].** However, there are several studies declared that the white light could be equivalent to or more effective than red-blue (RB) LED light in plant growth promoting **[24].**

Regarding the effect of red, blue and white lights on crop growth, there are varying results. For example, it was declared that the growth under white light, produced by dysprosium and fluorescent lamps, was equivalent to that under red : blue (1:1) LED light at the same light intensity in tomato and chrysanthemum. **[25 and 23]**. As well as, **Lin *et al*. [26]** reported that the fresh and dry weights of plants grown under red: blue LED light were not significantly different from those grown under white fluorescent light in lettuce. While in petunia , **Phansurin *et al.* [27]** suggested that white LED light effectively supports petunia plant growth than red : blue LED light due to its ability to transmit through leaves and subsequently increases the photosynthesis at the canopy level. Contrary to this, what was reported with **Lee *et al.* [28]** who reported that, the blue light treatment increases fresh weight and dry matter production in buckwheat. Also, the fresh weight and the dry weight of *Anoectochilus roxburghii* which was exposed to the blue light treatment were significantly greater than in the control.

DPPH Radical scavenging activity:

DPPH is considered as a powerful method for assessment the antioxidant efficiency of plants crude extracts. Using an antioxidant compound and various plant extracts as hydrogen donors in the conversion process of the free radical 2,2-diphenyl-1-picrylhydrazyl to the stable diamagnetic molecule diphenyl picryl hydrazine by reduction which will be accompanied with a change in color from purple to yellow in a short time is the main base of this test**[29]**.

Table (2): Effect of exposing the calli and shoots cultures to different light LED quality on free radical scavenging capacity of DPPH (%), after one month of culturing.

Treatment	Explant	Scavenging activity %
Red LED	Calli	54.89333 ± 22.78017
Blue LED	Calli	73.4575 ± 13.51197
White LED	Calli	66.9675 ± 21.23941
Red LED	Shoots	68.005 ± 15.02168
Blue LED	Shoots	65.7525 ± 17.4874
White LED	Shoots	74.405 ± 3.389912

Data represent mean ±S.D.

Data presented in table (2) indicated that the extracts of calli exposed to blue LED light are more efficient in reduce the stable, purple-colored free radical DPPH into the yellow-colored DPPH (73.45 %) more than the extracts from calli exposed to white or red LED light which had low reducing efficiency (66.96 and 54.89 %) respectively. Whilst, the extracts of shoots exposed to white LED light recorded the highest reducing efficiency (74.40 %) comparing with those exposed to red or blue LED light which had low reducing efficiency (68.00 and 65.75) respectively . As for calli **,** our results may be explained as that the blue light have very high frequencies which in turn mean that it carries a large amount of energy which can cause damages to the cellular functions of the plant. To protect the plant tissues from the incoming energy as well as clean up

any “free radicals” produced in the cell, plants may produce different compounds which in turn increase the DPPH radical scavenging activity **[30].** In this respect, **Manivannan *et al.* [31**] reported that the red and blue light-emitting diode (LED) both elevate the antioxidant capacities in Chinese foxglove, and blue light was more efficient than red one. While , **Adil *et al,* [32]**recorded that The least DPPH percentage was observed in calli of *C. officinale* Makino grown in complete dark condition The percent antioxidant activity for calli of *C. officinale* Makino grown *in vitro* under white, blue, red and red : blue lights recorded ranges between 92− 93% without any significant difference among them. Our results opposes what was reported with **Jang *et al.*,[33])** who declared that the DPPH radical scavenging activity in *Camellia japonica* callus culture showed its highest activity (82.4%) with the calli cultures grown under a mixture of red and blue light while the lowest DPPH activities were detected in calli cultures grown under white (23.2%) and blue (25.0%) lights. As for shoots cultures, our results opposes what was declared **El Ashry *et al,* [34],** who found that, the highest DPPH percentage was recorded after 30 days of exposing the red beet shoots to the blue light (42.27%) while its lowest value (14.30%) was recorded with exposing the shoots cultures to the green light for 10 days. As for that the extracts of shoots exposed to white LED light recorded the highest reducing efficiency (74.40 %) it could be justified as the shoots that exposed to the white light recorded the highest cholorogenic acid (CGA) compared to all the other treatments for the two different cultures.

Determination of phenols and flavonoids contents by High Performance Liquid Chromatography (HPLC).

Among different phenolic and the flavonoids compounds which were tested in the calli and shoots cultures exposed to the different LED light quality (red, blue , white), only the chlorogenic acid was detected in the different treatments in both cultures with varying quantities.

Table (3): Effect of exposing the calli and shoots cultures to different light LED quality on chlorogenic acid content (µg / g dry), after one month of culturing.

Treatment	Explant	Chlorogenic acid (µg / g dry weight)
Red LED	Calli	12.90
Blue LED	Calli	4.91
White LED	Calli	52.43
Red LED	Shoots	1353.23
Blue LED	Shoots	7537.71
White LED	Shoots	8783.53

The data in table (3) declared that chlorogenic acid content varied greatly among the two different types of cultures. Its content was measured in micrograms in calli cultures with the different treatment, whilst it reached milligram in the shoots cultures with all the treatments. It was found that, the white LED light recorded the highest content of chlorogenic acid with both cultures, since it scored 52.43 µg / gdw with calli cultures versus 8783.53 µg / gdw with shoots cultures. Chlorogenic acid (CGA) is recognized as cinnamic acid derivatives with a highly biological effects due to their anti-inflammatory activities. **Steck,(1968) [35]** suggested two different pathways for the synthesis of chlorogenic acid from cinnamate which are the first route :cinnamic acid ----------p-cuomaric acid ----------p - coumaroylquinic acid ---------- chlorogenic acid while the secondary route; cimamic acid ---------p - coumaricacid ---------- caffeic acid---------- chlorogenic acid. Recently chlorogenic acid shows various pharmaceutical benefits like it could reduce the risk of Alzheimer’s disease , diabetes type 2 and cardiovascular disease [**6–8]**, and it also owes different anti-bacterial and anti -inflammatory activities[**9- 10]**.

Among the different factors affecting plant development, physiology and its cellular differentiation the light is considered the most important one **[36].** Light spectrum type which is considered one of the environmental factors is essential signaling component affecting plant physiology and its metabolism **[37- 38]** Recently, LEDs have been increasingly applied *in vitro* which return to their advantages over conventional light sources. Since, LEDs are consuming lower energy which leads to longer life cycle, and also they have higher spectral specificity than standard fluorescent lamps [FL] **[24].** Moreover, the usage of monochromatic light is important in research centers **[16]**. FL light is composed of a range of wavelengths (350–750 nm) and thus it is more preferable as a light

source for different plant species; however, even though it has some disadvantages which are, the higher consumption of electricity and the much more heat emission than LEDs **[39].** LED irradiation has been proved to be much more effective in stimulating plant metabolite production [**40]**. The light spectrum of the LEDs has a very strong effect on plant growth, its development and the production of metabolites. Light spectra affects plant physiology in different ways among species [**41].** As for the effect of light on chlorogenic acid content, our results are on line with what was found with **Bach *et al.*, [42]** who declared that, the chlorogenic acid was found in the shoots of lachenalia 'Rupert' grown under white light compared with that grown under complete darkness , red or blue light.Whereas our results opposes what was detected with **Szopa and Ekiert, [43]** on their study on *Schisandra chinensis* since they reported that the highest overall amount of phenolic compounds was obtained in extracts from the cultures maintained under blue light. The most dominant compounds were: chlorogenic acid (15.33 mg/100 g DW), protocatechuic acid (13.11 mg/100 g DW) and gallic acid (6.27 mg/100 g DW) they added that a high satisfactory total amount (41.62 mg/100 g DW) was also detected in the cultures which was maintained in complete darkness.

Finally it could be concluded that chlorogenic acid is much more in the shoots cultures compared with calli cultures also, the exposure to the white light increase the chlorogenic acid content. This results could be explained as the increase in phenolic compounds has been found always in plants exposed to environmental stresses such as UV-radiation or high light intensity **[44- 45].** Higher content of phenolics in the leaves may be explained as the leaves are exposed to these factors greatly. Also, **Afzalpurkar and Lakshminarayana [46]** described changes in the content of endogenous chlorogenic, caffeic, and quinic acid during maturation of sunflower seeds. This process was found to be accompanied by decreasing content of chlorogenic acid and increasing concentration of caffeic . Which proves that there is an opposite relation between chlorogenic and caffeic acid.

4.Conclusions

Finally it could be concluded that chlorogenic acid is much more in the shoots cultures compared with calli cultures also, the exposure to the white light increase the chlorogenic acid content.

5. Conflicts of interest

There are no conflicts to declare.

6. Formatting of funding sources

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