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### ***Effect of putrescine and type of light in callus of Gardenia Jasminoides L. content from some effective medical compounds***

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**Abstract.** The experiment was carried out in the laboratory of tissue culture of Plant Production Techniques Department, at Musaib Technical College to study the effect of different concentrations of putrescine and the type of light on the content of the Gardenia from some effective medical compounds. The experiment included two factor the first consisted of two sources of light, namely light (fluorescent), LED light (18 red: 2 blue) and the second factor, the addition of Putrescine with three concentrations (0.5, 1 and 1.5) mg.l-1. Some effective medical substances (Coumaric, Ferulic, Caffeoylquanic, Sinapic acid and Tannic) acids was measured in Callus Of Gardenia using high-performance liquid chromatography (HPLC). The results were analyzed using the statistical program Genstat and the mean was measured according to the least significant difference of LSD at the probability level of 5%. The results showed superior treatment of LED lighting in the concentration of all measured active compounds, The highest concentration of compounds (Ferulic, Caffeoylquanic and Sinapic) acids was given with a concentration of 0.5 mg.l-1. The highest concentration of compounds (Coumaric and Tannic) acids. The interaction (LED + Putrescine at 0.5) gave the highest concentration of compounds (Ferulic, Caffeoylquanic and Sinapic) in Callus of Gardenia.

#### **1. Introduction**

*Gardenia jasminoides* L. shrub belonging to the Rubiaceae and the Gardenia species has more than 200 species, named after the American world Alexander Garden (1730-1791), China is the homeland of the original plant. Plant height ranges between 1 - 2 m and flower period from mid - May until mid - July to give white flower with an aromatic scent of waxy bivalent textures. Their petals are often made up of several layers [1]. Leaves of the plant are spherical to oval inverted up to a length of about 10 cm dark green gloosy prominent veins opposite in triangular pools, and is one of the beautiful ornamental shrubs that adorn the gardens of the house and the public used as flowers in the extraction of perfume as well



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as used as flowers picking, which is one of the most important ornamental plants abundant in the world, The main method of reproduction is the propagation by cutting or vaccination or the installation of strong assets resistant to nematodes [2,3]. The method of tissue propagation outside the organism is also preferred because it gives a high propagation rate through organogenesis [4]. gardenia is a plant under the direct sunlight that is necessary to achieve the best floral output [5]. The techniques of tissue culture played an important role in multiplying many plants, including trees and shrubs that are difficult to multiply by normal vegetative methods. Among these plants is gardenia [6]. Was the first to use tissue culture technology in the propagation of gardenia, which succeeded in rooting the modern growth rates in the pipes of agriculture by 75% to get rid of the low output to reproduce this bush in the traditional way. The addition of industrial growth hormones to the food media is essential to stimulate and stimulate plant parts to grow, develop and form roots, so tissue culture cannot succeed without the use of growth regulators [7,8]. The amino acids are represented by Putrescine are very low molecular weight organic compounds containing two or more effective amino acids It has several functions in physiological processes within the plant and is present in all its parts. It is one of the secondary growth organizations that has recently been introduced into research and studies for effective management in most plant development processes [9,10]. The Putrescine and its plant formula  $C_4H_{12}N_2 \cdot 2H_2O$  is one of the polyamine types and the primary source of other species (spirmidine and spermine). It is also the least amine of molecular weight, which makes it faster to move between the cell or plant components. It is found to play an important role in cell division, flowering and morphological formation [11]. Several studies have pointed to the role of multilayered in the formation of spin roots, as they play an important role in the stage of root formation in many wood plants, including those found by [12,13]. When multiplying different types of citrus origin outside the living body. The growth of tissue culture requires the regulation of many conditions such as light, heat, humidity and carbon dioxide in the growth room [14].

Low or excessive light is a barrier to plant growth or leads to excessive growth respectively, and the quality of lighting affects morphological characteristics such as plant length and leaf area [15]. They are used in different colors, including white, red, blue, yellow and green, or a mixture of them and each property color is characterized by the fact that the function of the red LED light is to induce chlorophyll to manufacture food by photosynthesis process and the blue light on the plant morphology [16]. Through research, [17] found that a mixture of red and blue LEDs (18 red - 4 blue) gave the highest number of branches and leaves in the tissue cultivars of Rosa compared to white fluorescent light. The aim of the research is to increase the production of effective medical compounds through the treatment of different types of lighting and the various concentrations of multi-amino-Putrescine.

## **2. Methods and Materials**

The experiment was carried out in the Laboratory of tissue culture of Plant Production Techniques Department at Musaib Technical College to study the effect of different concentrations of Putrescine and the type of light in the content of the Gardenia jasminoides L. from some effective medical compounds.

### *2.1. Plant Material*

Gardenia plants (Ellis variety). took from good-growing native plants in one of the nursery nurseries. It was considered safe to be free from any insect or pathogenic infection at the age of 3-4 years and was adopted as a source for taking the plant parts to be culturally propagated inside the laboratory after removing all open leaves.

### *2.2. Sterilization of the Work Tools*

All the tools used in the textile culture. of the current study were sterilized from tweezers, scalpels, petri dishes and filter paper after they were wrapped with aluminum foil and placed in the sterilizer for 20 minutes at 121 ° C pressure 1.04 kg .cm 2 during the cultivation process, the tools were bathed with 96% ethanol solution and then exposed to direct fire flame during the cultivation process. The. sterilization

of hands and work tables was done by using ethyl alcohol at a concentration of 70% before and during the cultivation process. The airflow cabin was sterilized with a spray of its inner walls and ground with 70% ethanol and was wiped off the blotting paper and operated about 30 minutes before use.

### 2.3. *Preparation of Sterilization of Explant*

After the separation of modern plants from the mother plants washed with ordinary tap water and then sterilized by the alcohol ethyl concentration of 70% liquid soap, taking parts of the leaf blade taken from the leaves of full bloom occurring after the summit developing directly less than 1 cm and width 0.5 cm container on the middle race. It was then immersed in an anti-oxidant solution to remove tissue damage from acidic ascorbic acid (150 mg.l<sup>-1</sup>) and citric acid (100 mg.l<sup>-1</sup>) for 30 minutes and then transferred to a benomyl solution (fungicide) for 2 - 3 minutes, followed by rinsing the plant parts with distilled and sterile water for 3 minutes [18]. The surface sterilization of the selected plant parts was then carried out to the class flow air chamber in HgCl<sub>2</sub> solution at 0.1% (weight/volume) concentration for 5 minutes with the addition of two Tween 20 droplets with continuous stirring to remove the air bubbles formed on the final parts. In the end, it was separated by distilled and sterilized water three times for 3 minutes at a time to eliminate any harmful effects of sterilization and to preserve the vitality of the plant parts.

### 2.4. *Preparation of tissue culture media*

MS [19] was used as the primary medium in the current study. The laboratory medium was prepared from the nutrient salts according to the recommended concentrations and to prepare one liter of the medium. Agar was dissolved in 400 ml distilled water at boiling point. Mix the ingredients using magnetic vibrators over the hot plate with the addition of the major and minor nutrients, vitamins, sucrose and mayoicitol after dissolving them with distilled water to the food medium, depending on the concentration required, and then complete the volume to one liter and be distributed in 200 ml bakers with the growth regulator added to the target of the experiment. The baker's nozzles were covered with heat resistant aluminum foil and sterilized at 121 ° C and pressed 1.04 kg. cm<sup>3</sup> with autoclave for 20 minutes. The tubes were then removed from the stock and left to cool at room temperature.

### 2.5. *Preparation of the Explant*

After the superficial sterilization of the plant parts, they were cut into smaller pieces (the length of the stem node is about 1.2 - 1.5 cm). They were transferred to sterile petri dishes using end pointed tweezers with sharp surgical blades and thus ready for cultivation.

### 2.6. *The stage of formation and multiplication*

The first phase, four weeks after planting, was the stage of emergence. The parts of the paper blade were implanted after the sterilization process was completed in test tubes containing 10 ml of the pre-prepared MS medium on the response of parts of the leaf blade containing the central sweat of the callus formation and differentiation. Incubators were incubated in the growth chamber at 25 ± 2 ° C and a light intensity of 1000 lux with 16 hours light followed by 8 hours of darkness. These parts were then replanted for an additional four weeks in the same culture of the upbringing and under the same circumstances, they promised the doubling phase [18].

### 2.7. *Cultivation of callus plantations*

After eight weeks of planting, was transferred to a new agricultural medium after removing the mass of callus from the tubes by sterile forceps and placed inside a sterile petri dish and section callus to small pieces for the purpose of re-planting in order to study the effect of the presence of biotersin three concentrations 0.5, 1, 1.5) mg .l<sup>-1</sup>. In addition to the concentration of comparison in the middle of agriculture under the influence of two sources of the type of lighting are the usual light (fluorescent) and LED light (18 red: 2 blue) to determine the best combination of the effect type of active substance.

### 2.8. *Analysis of Phenols*

The main compounds were separated on m FLC (Fast Liquid Chromatographic) on reversed phase 3 µm particle size, (50 x 2.0 mm I.D) C-18DB column, separation occurred on liquid chromatography

Shimadzu 10AV-LC equipped with binary delivery pump model LC-10A Shimadzu, the eluted peaks were monitored by shimadzu SPD 10A vp, the data were recorded on shimpack C-R8A integrator (Shimadzu, koyota, Japan). The optimum separation condition as follow :

Column: FLC (Fast Liquid Chromatographic ) column , 3  $\mu$ m particle size, (50 x 2.0 mm I.D ) C-8DB column.

Mobile phase were :acetonitrile : tetrahydrofuran (THF):0.1 % acetic acid (6 : 3 : 1, V/V)

detection : UV set at 254 nm, flow rate 1.2 ml.min<sup>-1</sup>.

temp: 40 C.

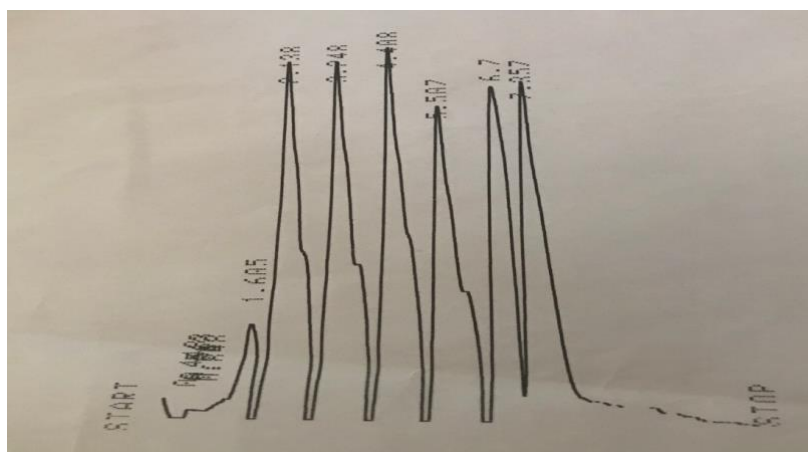
The sequences of the eluted fatty acids standard were as follow, each standard was 25ug.ml<sup>-1</sup>

**Table1:** The Retention Time and the area of fatty acids

Seq	Subjects	Retention time minute	Area	Concentration
1	Tannic acid	2.13	123026	25mg.l <sup>-1</sup>
2	Coumaric	4.40	130370	25 mg.l <sup>-1</sup>
3	Ferulic acid	5.50	117958	25 mg.l <sup>-1</sup>
4	Caffeoylquanic	6.70	85243	25 mg.l <sup>-1</sup>
5	Sinapic acid	7.35	93515	25 mg.l <sup>-1</sup>

The HPLC separation profile revealed the presence of various chromatographic peaks in the studied sunflower seeds sample extract. The assayed of the separated compounds representing the major detected peaks and summarizing the obtained data for each of the detected chromatographic peak are discussed below. Quantitative determination of fatty acids were done by comparison the peak area of authentic standard with that of sample peaks under the same optimum separation condition, by using the following equation:

$$\text{Concentration of sample } \mu\text{g/ml} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{conc. of standard} \times \text{dilution Factor}$$



**Figure (1):** Separation chromatogram of compounds under the optimum separation condition

### 3. Experimental design and statistical analysis

Perform the research as a factorial experiment using the complete random design C.R.D [20]. The data were analyzed using the statistical program Genstat and the mean was measured according to the least significant difference of LSD at the probability level of 0.05. Ten

replicates were used for each treatment and one vial for each replicate in multiplication experiments.

#### 4. Results and Discussion

The results of the statistical analysis in Table (2) showed significant differences in the concentration of Coumaric acid in Gardenia Callus. The LED light treatment gave the highest mean of 156.8  $\mu\text{g}/\text{ml}$  while the Florescent treatment gave a mean of 148.0  $\mu\text{g}/\text{ml}$ .

The treatment of Putrescine non significantly affected the concentration of Coumaric acid in Gardenia Callus. The results showed that there was a significant overlap between the study factors in the concentration of Coumaric acid in Gardenia Callus. the interference (LED + Putrescine at a concentration of 1) gave the highest mean of 176.9  $\mu\text{g}/\text{ml}$  while (Florescent + 0) gave the lowest mean of 122.4  $\mu\text{g}/\text{ml}$ .

**Table (2):** Effect of the type of light and Putrescine in the content of Gardenia Callus from Coumaric acid

Putrescine Lighting Type	0	0.5	1	1.5	Mean
Florescent	122.4	154.2	163.4	151.9	148.0
LED	136.0	160.9	176.9	153.4	156.8
Mean	129.2	157.5	170.2	152.6	
L.S.D <sub>(0.05)</sub>	Lighting 16.01	Putrescine n.s	Lighting *Putrescine 32.01		

The results of the statistical analysis in Table (3) showed significant differences in the concentration of Ferulic acid in Gardenia Callus. The LED light treatment gave the highest mean of 116.2  $\mu\text{g}/\text{ml}$  while the Florescent treatment gave a mean average of 109.4  $\mu\text{g}/\text{ml}$ .

The treatment of Putrescine significantly affected the concentration of Ferulic acid in Gardenia Callus. The treatment was given at a concentration of 0.5  $\mu\text{g}/\text{ml}$  at an mean of 120.9  $\mu\text{g}/\text{ml}$  while the comparison treatment gave a mean mean of 97.8  $\mu\text{g}/\text{ml}$ . The results showed that there was a significant overlap between the study factors in the concentration of Ferulic acid in Gardenia Callus. the interference (LED + Putrescine at a concentration of 0.5) gave the highest mean of 126.0  $\mu\text{g}/\text{ml}$  while (Florescent + 0) gave the lowest mean of 94.9  $\mu\text{g}/\text{ml}$ .

**Table (3):** Effect of the type of light and Putrescine in the content of Gardenia Callus from Ferulic acid

Putrescine Lighting Type	0	0.5	1	1.5	Mean
Florescent	94.9	115.7	117.5	109.4	109.4
LED	100.7	126.0	123.8	114.5	116.2
Mean	97.8	120.9	120.7	111.9	
L.S.D <sub>(0.05)</sub>	Lighting 116.2	Putrescine 20.17	Lighting *Putrescine 28.52		

The results of the statistical analysis in Table (4) showed significant differences in the concentration of Caffeoylquanic acid in Gardenia Callus. The LED light treatment gave the highest mean of 291.4  $\mu\text{g}/\text{ml}$  while the Florescent treatment gave a mean average of 276.3  $\mu\text{g}/\text{ml}$ .

The treatment of Putrescine significantly affected the concentration of Caffeoylquanic acid in Gardenia Callus. The treatment was given at a concentration of 0.5  $\mu\text{g.ml}^{-1}$  at an mean of 297.6 $\mu\text{g.ml}^{-1}$  while the comparison treatment gave a mean mean of 267.8 $\mu\text{g.ml}^{-1}$ .

The results showed that there was a significant overlap between the study factors in the concentration of Caffeoylquanic acid in Gardenia Callus. the interference (LED + Putrescine at a concentration of 0.5) gave the highest mean of 307.7 $\mu\text{g.ml}^{-1}$  while (Florescent + 0) gave the lowest mean of 263.5 $\mu\text{g.ml}^{-1}$ .

**Table (4) :** Effect of the type of light and Putrescine in the content of Gardenia Callus from Caffeoylquanic acid

Putrescine Lighting Type	0	0.5	1	1.5	Mean
Florescent	263.5	287.6	276.4	277.7	276.3
LED	272.1	307.7	296.0	290.1	291.4
Mean	267.8	297.6	286.2	283.9	
L.S.D <sub>(0.05)</sub>	Lighting 24.06	Putrescine 34.03	Lighting *Putrescine 48.13		

The results of the statistical analysis in Table (5) showed significant differences in the concentration of Sinapic acid in Gardenia Callus. The LED light treatment gave the highest mean of 262.8 $\mu\text{g.ml}^{-1}$  while the Florescent treatment gave a mean average of 257.7 $\mu\text{g.ml}^{-1}$ .

The treatment of Putrescine significantly affected the concentration of Sinapic acid in Gardenia Callus. The treatment was given at a concentration of 0.5  $\text{mg.ml}^{-1}$  at an mean of 274.5 $\mu\text{g.ml}^{-1}$  while the comparison treatment gave a mean of 267.8 $\mu\text{g.ml}^{-1}$ .

The results showed that there was a significant overlap between the study factors in the concentration of Sinapic acid in Gardenia Callus. the interference (LED + Putrescine at a concentration of 0.5) gave the highest mean of 307.7 $\mu\text{g.ml}^{-1}$  while (Florescent + 0) gave the lowest mean of 236.8 $\mu\text{g.ml}^{-1}$ .

**Table (5):** Effect of the type of light and Putrescine in the content of Gardenia Callus from Sinapic acid

Putrescine Lighting Type	0	0.5	1	1.5	Mean
Florescent	240.7	263.3	264.5	262.2	257.7
LED	232.9	285.7	266.3	266.5	262.8
Mean	236.8	274.5	265.4	264.3	
L.S.D <sub>(0.05)</sub>	Lighting 26.43	Putrescine 37.38	Lighting *Putrescine 52.86		

The results of the statistical analysis in Table (6) showed significant differences in the concentration of Sinapic acid in Gardenia Callus. The LED light treatment gave the highest mean of 87.9 $\mu\text{g.ml}^{-1}$  while the Florescent treatment gave a mean average of 80.8 $\mu\text{g.ml}^{-1}$ .

The treatment of Putrescine significantly affected the concentration of Sinapic acid in Gardenia Callus. The treatment was given at a concentration of 1  $\text{mg/L}$  at an mean of 89.1 $\mu\text{g.ml}^{-1}$  while the comparison treatment gave a mean of 78.2 $\mu\text{g.ml}^{-1}$ .

The results showed that there was a significant overlap between the study factors in the concentration of Sinapic acid in Gardenia Callus. the interference (LED + Putrescine at a concentration of 1) gave the highest mean of 94.6 $\mu\text{g.ml}^{-1}$  while (Florescent + 0) gave the lowest mean of 75.5 $\mu\text{g.ml}^{-1}$ .

**Table (6):** Effect of the type of light and Putrescine in the content of Gardenia Callus from Tannic acid

Putrescine Lighting Type	0	0.5	1	1.5	Mean
Florescent	75.5	81.4	83.7	82.4	80.8
LED	80.8	83.7	94.6	92.7	87.9
Mean	78.2	82.6	89.1	87.6	
L.S.D(0.05)	Lighting 6.72	Putrescine 9.51	Lighting *Putrescine 13.45		

Of the results achieved. The increase in the concentration of phenolic compounds in Gardenia Callus can be attributed to the effect of the type of lighting used on LED lighting. The amount of light intensity resulting from it is higher than the normal light, turning 20% of the electric energy into light. Does not consume energy emitted in heat does not cause damage to the part outside the in vivo compared to the other type of fluorescence, which converts about 4% while the rest is dispersed as a heat, so the amount of light received by the part is projected on the food medium by taking the needs of light ideally It absorbs the maximum limits of the actual needs and therefore this is reflected on the nature of its growth [21,22] also explained the effect of light source on plants outside the organism to its involvement in the process of metabolism and form morphology, which is reflected in the formation of vegetative parts. also pointed out that light is an environmental factor that has a significant influence on the physiological responses of plants. It increases the ability to absorb growth regulators, especially cytokines, by the vegetative parts of plants. It also reduces the side effects that produce high levels of Oxytin and cytokines added to the dietary medium. In addition, also [23]. pointed out that is light has a direct effect on nutrient accumulation, which is positively reflected in increased vegetative growth as well as its role in increasing cell productivity of secondary compounds [24]. The results of the present study were consistent with those found by [25], which obtained the best growth in the development of the plant parts of the *Alternanthera versicolor* plant in a laboratory that used 16-hour LED lighting in red and blue of the solar spectrum. The significant increase in the multivariate amino-biodegrade effect may be due to its role in activating enzymatic antioxidants and increasing non-enzymatic antioxidant rates. It also plays a direct role in increasing the levels of nucleic acids and mineral nutrients. This increases the concentration of phenolic substances [10]. [26] have shown that bioterinsin plays an important role in cellular stimulation to increase the concentration of secondary compounds, including phenolic compounds. It also protects the plasma membrane by preventing the formation of free oxygen radicals, which cause severe damage to cellular membranes, nucleic acids and proteins inside Plant cells.

## 5. Conclusions

From the obtained results we can conclude that the treatment with LED light and the addition of Putrescine led to increase in the concentration of phenolic compounds measured in Gardenia Callus according to the experimental conditions.

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