Effect of Putrescine and the Lighting type on *Gardenia jasminoides* L. callus content of some active medical compounds

Ali S. Hassoon[1] , Inas Abdulsattar A.jabbar[1*] , Ahmed A. Kadhim[1] , "Madeha H. Hussien[2]

[1]*Al-Musaib Technical College/ Al-Furat Al-Awsat Technical University, Iraq*
[2]*Medical Institute Technical Mansour / Middle Technical University, Iraq*

E-mail: *inasabdulsattar7@gmail.com*[*] , *dr.inas.abdulsattar@atu.edu.iq* , *com.hs.ali@atu.edu.iq*

DOI: 10.46617/icbe7003

Abstract

The role and importance of medicinal and aromatic plants is due to the antioxidant properties of its components, usually associated to a wide range of amphipathic molecules, broadly termed Phenolic compounds. Therefore, the research aims to increase the production of active medicinal compounds through the treatment of callus *Gardenia jasminoides* L plantlets with two different types of lighting and different concentrations of polyamine, and putrescine. The experiment was conducted in the tissue culture laboratory belonging to the department of plant production techniques, AL-Musaib technical college, the experiment included two factors, the first included two sources of Lighting type, the regular light (fluorescent) and LED light (18 red: 2 blue), and the second factor adding putrescine in three concentrations (0.5, 1, 1.5) $mg.L^{-1}$ in addition to the control concentration, Some phenolic substances (Coumaric, Ferulic, Caffeorylquanic, Sinapic acid, Tannic acid) were estimated in *Gardenia jasminoides* L callus using the high-performance liquid chromatography technique Hplc. Data was analyzed using Genstat statistical program, and the averages were compared according to the LSD test at 0.05. The results showed that the LED lighting treatment was significantly excelled in the concentration of all the measured compounds, and the putrescine treatment at a concentration of 0.5 $mg.L^{-1}$ gave the highest concentration of the compounds (Ferulic, Caffeory lquanic, Sinapic), while the concentration 1 $mg.L^{-1}$ gave the highest concentration of the compounds (Coumaric, Tannic acid). Also, the Bi-interaction (LED + Putrescine at concentration 0.5 $mg.L^{-1}$) gave the highest concentration of compounds (Ferulic, Caffeory lquanic, Sinapic) in *Gardenia jasminoides* L callus.

Keywords: *Gardenia jasminoides* L, HPLC, Phenolic Compounds

1) Introduction

Gardenia jasminoides L An evergreen branched woody shrub belonging to the Rubiaceae family, the *Gardenia* genus includes more than 200 species, It is named belonging to the American scientist Alexander Garden (1730 - 1791 AD), China is the original country, the plant height ranges between 1-2 meters and it flowering from mid-May to mid-July to give white flowers with a waxy aromatic smell of bisexual strength and their petals are often composed of several rows (ketmera) [1]. The leaves of the plant are lanceolate to an inverted Ovate, their

length reaches about 10 cm, dark green, lustrous, prominent veins confronting in triple assemblages. It is considered one of the beautiful ornamental shrubs that decorate the home and public gardens, where its flowers are used to extract perfumes as well as use as cut flowers, and it is one of the most important ornamental plants spread abundantly in the world, the main method of reproduction is the cutting, Grafting on strong rootstock resistant to nematodes [2]; [3]. The method of tissue culture in vitro is preferred as it gives a high average of propagation through organogenesis [4]. Gardenia is a Heliophytes and Hygroscopic plant that grows in direct sunlight that is necessary in order to achieve the best flowering production [5]. The tissue culture techniques play an important role in the propagation of many plants, including trees and shrubs, which are difficult to multiply by the usual vegetative methods. Among these plants is the Gardenia. [6] was the first to use tissue culture technology to propagate Gardenia where it succeeded in rooting the recent growth resulting in culture tubes by 75%, To get rid of this low product to multiply this shrub in the traditional methods. The addition of industrial growth hormones to the nutritional media is considered one of the basic and important matters in order to stimulate the plant parts to grow, develop and root formation. Therefore, tissue culture does not succeed without the use of growth regulators [7]; [8]. The polyamines represented by putrescine (Put) are organic compounds of very low molecular weight that contain two or more active amine groups with multiple functions in physiological processes within the plant and are present in all parts and are among the secondary growth organizations that have been recently introduced in research and studies for their effective generation in Most plant development processes [9]; [10]. Putrescine and its plant formula C4H12N2.2H2O are a kind of polyamine and the primary source for the formation of other types (spermidine and spermine). It is also the least amines molecular weight, which gives it a rapid transition between cell components or plant members, where it was found that it plays an important role in cell division, flowering processes and morphological formation [11]. Numerous studies have indicated a multi-amine role in the formation of adventitious roots, where they play an important role in the stage of root development in many woody plants, including those found by [12]; [13], when multiplying different types of citrus origins *in vitro*. The growth of tissue cultures requires the regulation of many conditions such as light, heat, moisture, and carbon dioxide inside the growth rooms [14]. where little or excessive light impedes plant growth or leads to excessive growth, respectively, The quality of lighting also affects morphological characteristics such as stem length and leaf size [15]. In recent decades, Light Emitting Diode (LED) lamps have been used worldwide in agriculture as a new lighting source because of its advantages, the most important of which are small size, little drainage for electric energy, high efficiency and long operating hours (50 to 100,000 hours) with little heat production. These lamps are used in different colors, including white, red, blue, yellow, green, or a mixture thereof, and each colour has its own characteristic, where the function of the red LED light is to induce chlorophyll to manufacture food by photosynthesis, while blue light affects the morphology of the plant [16]. Through research, [17] found that using a mixture of red and blue LED lights (18 red - 4 blue) gave the highest number of branches and leaves in the tissue culture of Rosa *Kordesii*, compared to white fluorescent light.

2) Materials and methods

The experiment was conducted in the tissue culture laboratory belonging to the department of plant production techniques, AL-Musaib technical college, to study the effect of different concentrations of putrescine and the Lighting type in the content of *Gardenia jasminoides* L. callus from some active medical compounds.

Plants material

Gardenia plants took Ellis cultivar from a good growing mother plant grown in a private nursery and it was taken into consideration that it is free from any insect or disease infection at the age of 3 - 4 years and it was approved as a source for taking the plant parts to be propagated tissue cultured in the laboratory after removing all the open leaves.

Sterilizing the used working tools

All tools used in tissue culture were sterilized for the current study from tweezers, scalpels, Petri dishes and filter paper after wrapping them with aluminum leaves and placed in the conductor for 20 minutes at a temperature of 121 C° pressure 1.04 kg/cm^2. During the culture process, the tools were covered with ethanol with a concentration of 96%, and then exposed to direct fire flame during the culture process. As for the sterilization of hands and workbenches, it was by using ethyl alcohol with a concentration of 70% before and during the culture process. The laminar flow cabinet was also sterilized by spraying its internal walls and floors with ethanol at a concentration of 70% and wiped with blotting paper, and it was filled 30 minutes before its use [12].

Sterilizing plant materials (Explants)

After separating the new growths from the mother's plants, they were washed with regular tap water and sterilized with 70% ethyl alcohol and liquid soap, where parts of the leaf blade that were taken from fully leaves were located directly after the apical meristem with a length of less than 1 cm and a width of 0.5 cm container on the middle vein. Then the parts are cleared by leaving them under tap water for 30 minutes. Then it is immersed in an antioxidant solution to get rid of tissue brown damage, Which consisted of ascorbic acid (150 mg.L^{-1}) and citric acid (100 mg.L^{-1}) for 30 minutes and then transferred to the pentomyl solution (fungicide) for 2-3 minutes, followed by rinsing the explants with distilled and sterile water for 3 Minutes. Surface sterilization was then performed for the selected explants parts after they were transferred to the laminar airflow cabinets in $HgCl_2$ solution at a concentration of 0.1% (w/v) for 5 minutes with adding two drops of Tween 20 diffuser with continuous shaking to remove the air bubbles formed on the final parts. In the end, it was washed with distilled and sterile water three times for 3 minutes each time in order to get rid of any harmful effects of sterilization and to preserve the vitality of the explants parts [18].

Prepare the MS nutritional medium

MS was used as the basic medium in the current study. The medium was prepared in the laboratory from nutrient salts according to the recommended concentrations and for preparing one litre of medium dissolving 6 g (Agar-Agar) in 400 ml of distilled water at 1 degree. Boiling

and mixing the ingredients using a Magnetic stirrer on the hot plate with the addition of Macro and Micronutrients, vitamins, sucrose and Myoinositol after dissolving it with distilled water to the nutrient medium each according to the required concentration and then complete the volume to 1 L and distribute it in the 200 ml bakeries with the addition of the growth regulator according to the aim of the experiment conducted. Then the baker's nozzles were covered with heat-resistant aluminum foil and sterilized at 121 C° and pressed 1.04 kg/cm^3 with an autoclave for 20 minutes, then the tubes were removed from the autoclave and left to cool at room temperature [19].

Prepare the explants

After performing superficial sterilization of the explant, they were cut into smaller parts (the length of the nodes is about 1.2 - 1.5 cm), where they were transferred to pre-sterilized Petri dishes using pointed end tweezers with sharp surgical blades and thus ready for transplantation [4].

The stage of initiation and multiplication

The first stage 4 weeks after the culture is considered initiation stage, where the leaf blade parts were culture after the sterilization process was completed in test tubes containing 10 ml of pre-prepared solid MS medium on the response of the leaf blade parts containing the middle vein to the callus initiation and its differentiation. The cultures were incubated in the growth room at a temperature of 25 ± 2 C° and the light intensity 1000 lux with 16 hours of light followed by 8 hours of darkness. And then these explants were re-culture for an additional four weeks in the same medium of the initiation stage and under the same conditions and it was considered a multiplication stage [18].

Callus Cultures initiation

When the callus volume reached the appropriate size, i.e., after 8 weeks of culture, it was transferred to a new culture medium after removing the callus mass from the tubes by sterile forceps and placed inside a sterile petri dish and the callus cut into small pieces, For the purpose of re-culture with the aim of studying the effect of the presence of putrescine in three concentrations (0.5, 1, 1.5) mg.L $^{-1}$ in addition to the control concentration in the culture medium under the influence of two sources of lighting type are the regular light (fluorescent) and LED light (18 red: 2 blue) in order to Determine the best combination to influence the type of active substance [17].

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 µm 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-1. temp: 40 C [15].

The sequences of the eluted fatty acids standard were as follow (Table 1), each standard was 25ug.ml-1

Table (1) Retention Time and the area of fatty acids

Seq	Subjects	Retention time minute	Area	Concentration
1	Tannic acid	2.13	123026	25 $mg.l^{-1}$
2	Coumaric	4.40	130370	25 $mg.l^{-1}$
3	Ferulic acid	5.50	117958	25 $mg.l^{-1}$
4	Caffeoylquinic	6.70	85243	25 $mg.l^{-1}$
5	Sinapic acid	7.35	93515	25 $mg.l^{-1}$

Quantitative determinations 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 [20]:

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

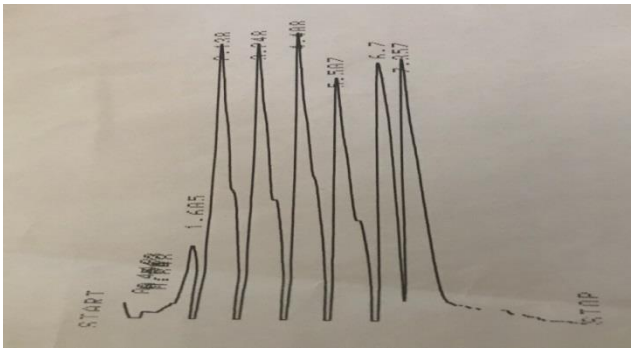

Fig (1) Separation chromatogram of compounds under the optimum separation condition

Experimental design and statistical analysis

A factorial experiment using according to Completely Randomized Design (CRD) [21]. Data was analyzed using the GenStat statistical program, and the averages were compared according to the LSD test at 0.05. Ten replicates were used for each treatment, with one culture vial, for each frequency of multiplication and initiation experiments.

3) Results and discussion

The results of the statistical analysis are presented in Table (2). They indicate a significant difference in the concentration of Coumaric acid in the Gardenia callus, where the LED Lighting treatment gave the highest average of 156.8 µg /mL while the florescent treatment gave the lowest average of 148.0 µg /mL.

Table (2) The effect of the Lighting type and putrescine on the *Gardenia jasminoides* L. callus content of Coumaric acid

Lighting Type	Putrescine ($mg.L^{-1}$)				Mean
	0	0.5	1	1.5	
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	
$L.S.D_{(0.05)}$	Lighting 16.01	Putrescine N.s	Lighting *Putrescine 32.01		

The treatment with putrescine significantly affected the concentration of Coumaric acid in *Gardenia jasminoides* L. callus, where it gave the treatment at a concentration of 1 mg/L, the highest average of 170.2 µg /mL, while the control treatment gave the lowest average of 129.2 µg /mL. The results indicate that there was a significant bi-interaction between the study factors in Coumaric acid concentration in *Gardenia jasminoides* L. callus where the interaction (LED + Putrescine with a concentration 1 mg/L) gave the highest mean average of 176.9 µg /mL while the combination (Florescent + 0) gave the lowest average of 122.4 µg /mL.

The results of the statistical analysis in Table (3) indicate a significant difference in the concentration of Ferulic acid in the *Gardenia jasminoides* L. callus, where the LED lighting treatment gave the highest average of 116.2 µg /mL, while the Florescent treatment gave the lowest average of 109.4 µg /mL. The treatment with Putrescine significantly affected the concentration of Ferulic acid in *Gardenia jasminoides* L. callus, where it gave the treatment at a concentration of 0.5 mg/L, the highest average of 120.9 µg /mL, while the control treatment gave the lowest average of 97.8 µg /mL. The results indicate that there was a significant bi-interaction between the study factors in the Ferulic acid concentration in the *Gardenia jasminoides* L. callus, where the interaction (LED + Putrescine at a concentration of 0.5 mg/L) gave the highest average of 126.0 µg /mL while the combination (Florescent + 0) gave the lowest average of 94.9 µg /mL.

Table (3) The effect of the Lighting type and putrescine on the *Gardenia jasminoides* L. callus content of Ferulic acid

Lighting Type	Putrescine (mg.L^{-1})				Mean
	0	0.5	1	1.5	
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$_{(0.05)}$	Lighting 116.2	Putrescine 20.17	Lighting *Putrescine 28.52		

The results of the statistical analysis in Table (4) indicate the presence of significant differences in the concentration of Caffeoylquinic acid in *Gardenia jasminoides* L. callus, where the LED lighting treatment gave the highest average of 291.4 µg/mL while the Florescent treatment gave the lowest average of 276.3 µg/mL.

Table (4) The effect of the Lighting type and putrescine on the *Gardenia jasminoides* L. callus content of Caffeoylquinic acid

Lighting Type	Putrescine (mg.L^{-1})				Mean
	0	0.5	1	1.5	
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$_{(0.05)}$	Lighting	Putrescine	Lighting *Putrescine		

	24.06	34.03	48.13	

The treatment with Putrescine significantly affected the concentration of Caffeoylquinic acid in *Gardenia jasminoides* L. callus, where the Putrescine treatment at a concentration of 0.5 mg/L was given at the highest average of 297.6 µg/mL, while the control treatment gave the lowest average of 263.5 µg/mL The results indicate that there was a significant bi-interaction between the study factors in the concentration of Caffeoylquinic acid in the *Gardenia jasminoides* L. callus, where the interaction (LED + Putrescine at a concentration of 0.5 mg/L) gave the highest average of 307.7 µg/mL while the combination (Florescent + 0) gave the lowest average of 263.5 µg/mL.

The results of the statistical analysis in Table (5) indicate a significant difference in the concentration of Sinapic acid in *Gardenia jasminoides* L. callus, where the LED lighting treatment gave the highest average of 262.8 µg/mL, while the Florescent treatment gave the lowest average of 257.7 µg/mL. The Putrescine treatment significantly affected in the concentration of Sinapic acid in *Gardenia jasminoides* L. callus, where the treatment at a concentration of 0.5 mg/L was given at the highest average of 274.5 µg/mL, while the control treatment gave the lowest average of 236.8 µg /mL. The results indicate that there was a significant bi-interaction between the study factors in the concentration of Sinapic acid in the *Gardenia jasminoides* L. callus, where the interaction (LED + Putrescine at a concentration of 0.5 mg/L) gave the highest average of 285.7 µg /mL while the combination (Florescent + 0) gave the lowest average of 232.9 µg /mL.

Table (5) The effect of the Lighting type and putrescine on the *Gardenia jasminoides* L. callus content of Sinapic acid

Lighting Type	**Putrescine (mg.L^{-1})**				**Mean**
	0	**0.5**	**1**	**1.5**	
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$_{(0.05)}$	Lighting 26.43	Putrescine 37.38	Lighting *Putrescine 52.86		

The results of the statistical analysis in Table (6) indicate the presence of significant differences in the concentration of Tannic acid in *Gardenia jasminoides* L. callus, where the LED lighting treatment gave the highest average of 87.9 µg /mL, while the Florescent treatment gave the lowest average of 80.8 µg /mL.

Table (6) The effect of the Lighting type and putrescine on the *Gardenia jasminoides* L. callus content of Tannic acid

Lighting Type	Putrescine (mg.L^{-1})				Mean
	0	0.5	1	1.5	
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		

The treatment with Putrescine significantly affected the concentration of Tannic acid in *Gardenia jasminoides* L. callus, where the treatment was given at a concentration of 1 mg /L at the highest average of 89.1µg / mL, while the control treatment gave the lowest average of 78.2 µg /mL. The results indicate that there was a significant bi-interaction between the study factors in the concentration of Tannic acid in the *Gardenia jasminoides* L. callus, where the interaction (LED + Putrescine at a concentration of 1 mg/L) gave the highest average of 94.6 µg /mL, while the combination (Florescent + 0) gave the lowest average of 75.5 µg /mL.

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

Of the results achieved, the increase in the concentration of phenolic compounds in *Gardenia jasminoides* L. 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. It was found the does not consume energy emitted in heat and 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 [22], [23] 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 [24] 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 [25]. The results of the present study were consistent with

those found by [26], which obtained the best growth in the development of the plant parts of the *Alternanthera sessilis* 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-bioterase 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],[27] 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.

4) Conclusions

From the results obtained, we can conclude that the treatment with LED light and the adding of Putrescine led to a clear increase in the concentration of phenolic compounds measured in the *Gardenia jasminoides* L. callus according to the conditions of the experiment. We recommend conducting further studies on other plants containing active substances and increasing the concentration of phenolic compounds under different conditions.

5) References

[1] Kobayashi, K. D. and Kaufma, A. J. 2006. Common Gardenia . College of tropical agriculture and human resources. University of Hawaii at Manoa, Cooperative Extension Service, Ornamentals and Flowers. 32: 1 – 7.

[2] Sultan, S. M.; T. M. Chalabi and Mohammed D.1992. Ornamental plants, Dar Al Kuttab Printing Press, Mosul University. The Republic of Iraq. 464 pages.

[3] Albtil, N. 2003. External ornamental plants. Publications of the University of Damascus. Syrian Arab Republic. 362 pages.

[4] Jarar, A. and Rola B. 2011. The effect of some growth hormones on the multiplication of *Gardenia jasminoides* (ellis cultivar) and its rooting *in vitro*. Damascus University Journal for Agricultural Sciences. 27 (1): 129 - 142.

[5] Kent, D. K. and Andrew, J. K. 2006. Common gardenia. Cooperative Extension Service, Collage of Tropical Agriculture and Human Resources. University of Hawaii and Manoa. Ornamental and Flowers Fact Sheet. O. F. (32).

[6] Dumanois, C.; B. Godin,; J. Lebofuf and Bigo, C. 1984. Multiplication vegetative *in vitro* de *Gardenia jasminoides* ellis. P.H.M. Revue Horticolen., 249: 19 – 30.

[7] Fahmy, F. J. 2003. Plant tissue culture. Scientific Books House for Publishing and Distribution. Faculty of Agriculture, Assiut University. Arab Republic of Egypt.

[8] Rifai, A.T. & Samir A. 2007. Tissue culture and plant propagation. Egyptian Library for Printing and Publishing. Arab Republic of Egypt.

[9] Martin-Tanguy, J. 2001. Metabolism and function of polyamines in plants: recent development (new approaches). Journal of Plant Growth Regulation, 34(1): 135 – 148.

[10] Ahmed, S. M. 2017. The effect of polyamines on growth, yield and quality of the antioxidant defense system for wheat plants grown under conditions of stress of heavy elements. PhD thesis, Faculty of Agriculture, Fayoum University. Arab Republic of Egypt.

[11] Taher, Maryam R. 2018. The response of the Rankenl plant to spraying with polyamines and salicylic acid before and after the harvest. Master Thesis, College of Agriculture - Diyala University. The Republic of Iraq.

[12] Amari, I. W. 2015. Effect of the type and components of the medium and the vegetative part of the native citrus cultivar Volcamariana and the exotica Cleopatra. Master Thesis. Faculty of Agriculture, University of Baghdad. The Republic of Iraq.16p.

[13] Khazali, S. R. and Hamad M. S. 2016. The effect of Auxin and polyamine on the rooting of branches of the origin of citrus fulgamaryana *in vitro*. Iraqi Journal of Agricultural Sciences. 737-732: (3) 47.

[14] Shin, K. S.; H. N. Murthy,; J. W. Heo,; E. J. Hahn and Paek, K.Y. 2008. The effect of light quality on the growth and development of *in vitro* cultured Doritaenopsis plants. Acta Physiol. Plant., 30:339–343.

[15] Yen, H. C.; S. Y. Liou and Hsieh, Y. C. 2011.Tissue culture of *Anaoectochilus formonasus* Hayata by combining fluorescent lamp R-LEDs as light source. Conference on Industrial Electronics and Applications., 312 – 315.

[16] Gupta, S. D. and Jatothu, B. 2013. Fundamentals and applications of light-emitting diodes (LEDs) *in vitro* plant growth and morphogenesis. Plant Biotechnology Rep., 7:211– 220.

[17] Azmi, N. S.; R. Ahmad and Rusli I. 2014. Effects of Red and Blue (RB) LED on the *in vitro* Growth of Rosa *kordesii* Multiplication Phase. International Conference on Agriculture and Biotechnology. 57: 86 – 99.

[18] Noah, K. M. 2009. Growing Gardenia bushes. Master Thesis. Faculty of Agriculture and Forestry, University of Mosul. The Republic of Iraq.138p.

[19] Murashige, T., & Skoog, F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia plantarum, 15(3), 473-497.

[20] Ninfa, A. J & Ballou, D. P. 1998. Fundamental Laboratory Approaches for Biochemistry and Molecular Biology, Fitzgerald Science Press, Inc., Bethesda, Maryland.

[21] Alrawi, K. M. and Abdul Aziz M. K. 2000. Design and analysis of agricultural experiments. University of Al Mosul. Directorate of the House of Books for Printing and Publishing. The Republic of Iraq.

[22] Al-Aradi, H. J. 2016. The effect of some growth regulators on the induction of callus and the multiplication of the *Alternanthera sessilis in vitro*. Iraqi Journal of Aquaculture. 13 (2): 151 - 164.

[23] Salihi, Q. J. & Kadhim M. 2013. Biotechnology, First Edition Translated by K. G. RAMAWAT, Technical Education Press, Ministry of Higher Education and Scientific Research. The Republic of Iraq. 265 pages.

[24] Al-Hamidawi, H. K. and Mohammed S. H. 2016. The effect of the optical spectrum and benzene adenine on some vegetative and chemical properties of two classes of Gerbera outside the living body. Iraqi Journal of Agricultural Sciences. 47 (3): 698 - 707.

[25] Sumaidaie, K. M. 2017. Applications in Plant Biotechnology, First Edition. part One . University House for Printing, Publishing and Translation. The two rivers university. Ministry of Higher Education and Scientific Research. The Republic of Iraq. 364 pages.

[26] De – Almeida, B. A.; A. M. Filho; M. J. Mendes and Rodrigues, P.M. 2004. *In vitro* organogenesis optimization and plantlet regeneration in Citrus sinensis and Citrus limonia. Scientia Agricola, 59: 229 – 34.

[27] Amri, E. and Shahsavar, A. R. 2010. Response of Lime Seedlings (*Citrus aurantifolia* L.) to Exogenous spermidine Treatments under Drought stress .Australian Journal of Basic and Applied Sciences, 4(9) : 4483 – 4489.