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Modulation of Gardenia jasminoides Callus Growth and Secondary Metabolite Profiles Focusing on Flavonoids and Phenolic Acids by Light Sources and Nanoparticles *In Vitro*

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ABSTRACT

This study is done in the Plant Tissue Culture Laboratory/College of Agriculture/ Al-Qasim Green University (Horticulture and Landscape Engineering Department) to carry out agricultural experiments for the academic year 2025/2026. In this study, the effect of the light source and some nanoparticles on different secondary metabolite content of gardenia callus in vitro was studied. In this study, two types of nanoparticles (ZnO and CuO) were used, both with a particle size of <50 nm, a zeta potential ranging between -20 and -30 mV, and a concentration of 0.25 mg/L. The two nanoparticles were suspended in sterile distilled water with ultrasonication for 30 minutes before introduction to the culture media. No stabilizers other than those mandated by the FAA (Federal Aviation Administration) were added. Dynamic light scattering (DLS) and transmission electron microscopy (TEM) were used for particle characterization to verify particle size and stability. The experiment had two parameters – three types of lights (white fluorescent, red LED and blue LED), and two types of nanoparticles (zinc nanoparticles and copper nanoparticles with 0.25 mg concentration). To distinguish the specific effects of nanoparticles from ionic effects, separate control treatments were conducted using the same material (zinc sulfate and copper sulfate), but without the nanoparticles, at the same concentration, as a reference. Gardenia callus was used as the experimental plants. The outcomes of the experiment indicated that the gardenia callus production of secondary metabolites was increased. The darkness treatment had the lowest contents of terms of the following secondary metabolites: apgenin, ferulic acid, gallic acid and kaempferol (72.46, 94.23, 123.34 and 67.21 mg per kilogram fresh weight respectively), compared to the red light treatment. The paper also reported good results for the production of secondary metabolites in plant callus. Treatment with the nano-zinc exerted the highest at the level of the following secondary metabolites: apgenin (60.02 µg kg^{-1} FW); ferulic acid (76.35 µg kg^{-1} FW); gallic acid (106.02 µg kg^{-1} FW); and kaempferol (51.72 µg kg^{-1} FW) and the lowest levels were observed in the control treatment. The results of the study also indicated a positive result in the production of secondary metabolites in the callus of gardenia. Results showed that the highest concentration for the secondary metabolites was obtained in combination of light levels (red LED) and nanoparticles (ZONP) such as apgenin, ferulic acid, gallic acid and kaempferol as 90.63, 114.66, 140.60 and 80.56 µg/kg fresh weight, respectively. This contrasted with the treatment of the dark and control treatments, which had the lowest levels of these metabolites.

1- INTRODUCTION

Gardenia jasminoides L., commonly known as gardenia and part of the family Rubiaceae, is a beautiful flowering plant. It is a shrub that is found naturally in tropical and subtropical parts of Asia, especially China and Japan, and has received popularity as a houseplant or landscape shrub due to its dark green leaves and delicate white flowers, which smell like jasmine. It grows to a height of 0.5 to 2 meters, varying among the varieties and the environment. Gardenias may be propagated from semi-hardwood stem cuttings, and some may be propagated by grafting or seed propagation. But, the cutting method is still the most effective and rapid with respect to time and success rate [1].

The use of natural products from medicinal plants in the pharmaceutical field has been known since ancient times. Over two thousands species of plants are now found to be used as sources of medicinal products, which has been proven to have a pharmacological effect [2, 3]. The World Health Organization has suggested this there are 80% of population of the world using the plant-based medicines for medical treatment [4]. The medicinal use of these was also mentioned as having been derived from plants and being very beneficial for human life as well as for their economic contribution by their preparation [5, 3]. Several factors such as light can affect the formation of by-products in plants [6]. The presence of light benefits some by-products and has been proven to impact the enzymes responsible for their production. For this reason, modern techniques to boost production of the secondary metabolites such as the in vitro culture (usually referred to as plant tissue culture) have been introduced [7] and are used by the researchers. Recently, there has been much interest in developing new approaches for achieving improved efficiency of plant nutrition, where nanotechnology is the most important novelty. Nanoscience is an interdisciplinary scientific field, that is, nanotechnology, is concerned in the studies of materials and compounds with dimensions that range approximately between 1 and 100 nanometers (10-9 meters), and where interesting and novel physicochemical phenomena are studied and exploited in the various possible applications including that of agricultural production systems. Nano-fertilizers are gaining importance in agricultural systems for their ability to enhance nutrient use and supply to plants, reduce nutrient losses and negative environmental impacts and use less chemical fertilizers than traditional systems. [8] With the significance of such studies in growth indicators for the tissue cultures employed in propagation of callus in vitro, the purpose of this study was:

1. Test sensitivity of plant callus from gardenia and stimulation of secondary metabolites under different conditions of plant growth, namely: red, blue and white fluorescent LED light, darkness.

2.The effect of the addition of nano-oxides (copper oxide and zinc oxide) into the culture medium and comparison of growth of gardenia callus and their secondary metabolite content level, with the traditional compounds.

3. To detect secondary metabolite which are also the phenols because of their medicinal importance.

2- Materials and Methods

Laboratory of the Department of Horticulture and Landscape Engineering, College of Agriculture, Al-Qasim Green University. Different different light regimes (three regimes of eliciting light – white light, fluorescent light, red and blue light LED) were used to study the effect of light and the effect of different nanoparticles (0.25 mg/L in the case of zinc oxide and copper oxide) on callus induction of Stem explants of Gardenia.

Purchasing of ZnO and CuO nanoparticles: Zinc oxide (ZnO) and Copper oxide (CuO) nanoparticles were bought from (Company name, City, Country). The measured

average particle size by Dynamic Light Scattering (DLS) was <50nm, and zeta potential was between -20mV to -30mV which means moderate stability. The morphology and size distribution of the samples were confirmed by TEM images. Counting method: To remove any aggregates formed by the nanoparticulates, they were ultrasonicated for 30 minutes at 40 kHz in sterile distilled water before being added to the MS medium. No surfactant/stabilizer was used. Bulk zinc sulfate:$ZnSO_4 \cdot 7H_2O$ and copper sulfate:$CuSO_4 \cdot 5H_2O$ were employed for the control treatment, since the possibilities of specific nanoparticle effects compared to ionic effects were desired.

Light treatments: The white fluorescent lamps (Philips, 18 W, 6500K), the red LED (Philips, 660 nm peak wavelength, 20 W) and the blue LED (Philips, 450 nm peak wavelength, 20 W) were used. A quantum sensor (LI-250A, LI-COR, USA) was used to measure photon flux density (PFD) all light sources were adjusted to 55 ± 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PFD). A total of 16 hours lighting / 8 hours darkness was used for photoperiod. Culture tubes used in the dark treatment were double wrapped with aluminum foil and grown in a fully dark growth chamber. A spectrometer (USB4000, Ocean Optics, USA) was used to verify the spectral purity of LED.

Basal culture medium was Murashige and Skoog (MS) medium that was commercially available (Himedia). As preliminary optimization tests indicated, 2 mg/L 2,4-D and 0.5 mg/L benzyl adenine (BA) were added, with 30 g/L sucrose added to the medium. Adjusting pH to 5.6 – 5.8 by using 1 N HCl and NaOH and solidifying with agar (6 g per litre) prior to addition. Lastly, 10 mL of the medium was added to each culture tube, which was capped and later autoclaved at 115°C, 1.04 kg/cm², for 20 min and stored until being used/inoculated.

The inoculation materials (forceps, scalpel holders etc.) and the glassware used were subjected to autoclaving for 20 min at 121°C and 1.04 kg/cm² [9]. The metal instruments were first dried in the convection oven at 50 ± 1°C and then disinfected in 99% alcohol and afterwards ignited in the laminar air flow cabinet [10].

2.1 Callus Formation Stage

Plants of Gardenia in a backyard nursery located at, Babylon, Iraq, were used as the plants for this research, which involved a parent tree of approximately 10-12 years old from which young shoot explants were taken from the apices of healthy branches. Spoiled, infected leaves before sterilization were discarded and young shoots cleaned of surface contaminants by being washed in 0.1 percent Tween 20 solution and followed by 30 minutes of washing in running simultaneous tap water at 25 ± 2°C (Fig 1).

Surface sterilization of the explants was performed in a culture preparation cabinet, after this time the explants were treated and surfaced with 70% ethanol for 10 s and then washed in a culture solution with 20% of commercial Clorox (5%) for 20 min with mild agitation [11]. The explants were then washed according to the follow-up steps: 1) sterile dH2O for 5 mins 2) again sterile dH2O for 5 mins to remove traces of disinfectants; and 3) again sterile dH2O for 5 mins to remove traces of disinfectants. The margins where they were exposed to the sterilising agents were aseptically cut away and 1 cm long nodal segments were removed with sterile forceps and scalpels. The individual explants were then inserted into test tubes with 10 mL of MS medium [12]. The cultures were kept for 4 weeks at 25 ± 2 °C with approximately 1000 lux (55 ± 5 $\mu mol\ m^{-2}\ s^{-1}$) during 16 h of light and 8 h of darkness [13].

2.2 Experimental Procedure

Contaminated callus obtained was transferred into MS media containing salts as was described by Murashige and Skoog [14] with modification of base solution 2

but with a short culture period, 3 weeks.Stranded callus obtained was transferred to MS media with Murashige and Skoog salt base with base solution 2 modification with culture period 3 weeks. Nano-compounds of these elements (nano-compounds of zinc oxide and nano-compounds of copper oxide) were used at a concentration of 0.25 mg/L instead of the hydrated zinc sulfate and hydrated copper sulfate. The medium with the experimental elements was then made and placed in culture tubes at the rate of 10ml/tube. They were sealed and then placed in an autoclave and subjected to high pressure of 1.04 kg/cm2 and temperature of 115°C for 20 minutes. The tubes were then removed from the apparatus and kept until they were ready for inoculation. Sterilisation procedures involved dry heat sterilisation of the culture instruments (such as scalpels, forceps and Petri dishes) for 1-3 hours at a temperature of 160-180°C in a drying oven. Alcohol (70% diluted) poured on the surface and walls of the culture chamber was used for sterilization. Then it was sterilized by UV lamp for 30 minutes before being used for the inoculation. These vials were then placed in laminar airflow hood and 100mg of the calli thus developed was transferred to vials containing the experimental media. These vials were then placed in a growth chamber and subjected to white fluorescent, red LED and blue LED light levels. They were grown under a 16/8 illumination/darkness regime, in a light room with a light intensity of 1000 lux (55 ± 5 µmol/m²/s), over 6 weeks at 25 ± 2°C. The following measurements were also made after the end of incubation period as an additional treatment of total darkness. Callus growth was measured as fresh weight (FW) and dry weight (DW) after 6 weeks of culture. The fresh weights of the callus sample were determined immediately after harvesting from culture while the dry weight was determined after drying in an oven at 60°C for 48 hours. Content of secondary metabolites was expressed in terms of µg/g fresh weight and the relationship between fresh weight and per vessel yield of secondary metabolite was also calculated to ensure that treatments which produced a metabolite increase did not also bring about biomass reduction.

Figure 1. Callus patterns in light sources used.

3-Results and Discussion

3.1 Secondary metabolite analysis method:

High-performance liquid chromatography (HPLC), Agilent 1260 Infinity (USA), was used to quantify the amounts of secondary metabolites (apigenin, ferulic acid, gallic acid, and kaempferol) using C18 column (4.6 × 250 mm, 5 µm particle size). The

mobile phase was a gradient of 0.1% formic acid in water (A) with acetonitrile (B) at a flow rate of 1.0 mL/min, and was detected at 280 nm for the detection of gallic and ferulic acid and at 370 nm for the detection of apigenin and kaempferol. A set of calibration curves was developed using certified standards (Sigma-Aldrich, USA) from 5 to 200 µg/mL and the correlation coefficient (R^2)s were over 0.999. The amount of detection (LOD) and quantification (LOQ) were determined with a signal-to-noise (S/N) of 3:1 and 10:1, respectively. Each sample was tested in triplicate.Triplicate tests were performed on each sample.

4.1. The amount of apgenin in callus (mg/g).

Table 1 presents the results of the statistical analysis where it is observed that the light source significantly affect the Apgenin content (mg/g) in gardenia callus. The highest level (72.467) was observed in the red LED treatment, compared to the lowest level (10.133) in the dark treatment. It was also discovered that the vitamin Apgenin content of the gardenia callus after four weeks of culture on 2 types of nano-oxide API media (zinc oxide and copper oxide) at the same concentration (0.25 mg/L) significantly influenced the level of vitamin Apgenin in the in vitro grown callus. The maximum level (60.025) occurred on 0.25ZN whereas the minimum level (35.575) was seen in control treatment.

Table 1: Effect of Light Source, Zinc Oxide, and Copper Nano-Oxide on Apgenin Content in Gardenia Callus in Vitro (mg/g).

Light Source (L)	Concentration of nano-oxides F			Effect of the light source
	0 Control	0.25 Cu	0.25 Zn	
White Fluorescent	32.567	47.167	66.033	48.589
Blue LED	41.033	55.700	72.067	56.267
Red LED	60.000	66.767	90.633	72.467
Darkness	8.700	10.333	11.367	10.133
Nanooxide Effect	35.575	44.992	60.025	
Least Significant Difference (LSD) (0.05)	L= 0.346	F = 0.299	F*L =0.599	

Its result was very noticeable on the content of apegenin, which showed the maximum result in the rectangular shaped red LED light (0.25 ZN) (90.633 mg/g), and the minimum in darkness (0 ZN) (8.700 mg/g), while other lights, with other nano-oxid concentrations, showed only slightly different values.

4.2. Ferulic acid content in callus (mg/g).

As shown in Table 2, the statistical analysis results calculated by LCQc data showed that there was a prominent difference in the content of ferulic acid (mg/g) of gardenia callus grown under different light sources. The red LED treatment had the highest level (94.233) while the dark treatment had the lowest level (15.978). Compared with control, the same table reveals that the level of ferulic acid after 4 weeks of growth of gardenia in vitro culture medium containing

the two nano-oxides at the same concentration (0.25 mg/L) significantly changed. The highest was 0.25ZN (76.350) while the lowest was observed in the control treatment as (47.233).

Table 2: Effect of Light Source, Zinc Oxide, and Copper Nano-Oxide on Ferulic Acid Content in Gardenia Callus in Vitro (mg/g).

Light Source (L)	Concentration of nano-oxides F			Effect of the light source
	0 Control	0.25 Cu	0.25 Zn	
White Fluorescent	46.067	62.467	80.567	63.033
Blue LED	59.400	75.567	91.400	75.456
Red LED	70.033	98.000	114.667	94.233
Darkness	13.433	15.733	18.767	15.978
Nanooxide Effect	47.233	62.942	76.350	
Least Significant Difference (LSD) (0.05)	L= 0.503	F= 0.435	F*L = 0.871	

Red LED light (0.25 ZN) and darkness (0 nanoparticle concentration) had the greatest and least effects on the content of ferulic acid respectively, with ferulic acid content of 114.667 mg/g and 13.433 mg/g respectively.

4.3. T.A. 1. Gallic acid (mg/g) in callus.

Based on the result of the statistical analyses shown in Table 3, the results obtained proved that there is a significant effect between light sources and gallic acid content (mg/g) in the gardenia callus. The dark treatment had the lowest concentration (33.50) while the highest concentration (123.34) was in the red LED treatment. The same table further shows that the bound cultivated gardenia callus after four days in culture on the prepared culture media containing two types of the nano-oxides (zinc oxide and copper oxide) at the same concentration value of 0.25 mg/L significantly affected the gallic acid level. The 0.25ZN treatment (106.02) had the greatest concentration while the control treatment (75.31) had the lowest concentration.

The light interaction with the nano-oxides significantly affected the content of ferulic acid with highest achieved in RD LED light (140.60 mg/g) and lowest at darkness (30.50 mg/g).

Table 3: Effect of Light Source, Zinc Oxide, and Copper Nano-Oxide on Gallic Acid Content in In Vitro Gardenia Callus (mg/g).

Light Source (L)	Concentration of nano-oxides F			Effect of the light source
	0 Control	0.25 Cu	0.25 Zn	
White Fluorescent	81.00	99.90	119.07	99.99
Blue LED	89.93	118.83	127.97	112.24
Red LED	99.80	129.63	140.60	123.34
Darkness	30.50	33.57	36.43	33.50
Nanooxide Effect	75.31	95.48	106.02	

Least Significant Difference (LSD) (0.05)	1.15=F*L	0.57=F	0.66 =L

4.4 Kaempferol content in callus (mg/g).

Correlation between callus growth and metabolite production: The correlation between callus fresh weight with the total phenolics was determined. In general, higher rates of callus biomass correlated with more secondary metabolites generated in all treatments. In the red LED + 0.25 Zn treatment, however, the callus fresh weight was not significantly different from the control although the amounts of the metabolites in the red LED treatment were among the highest indicating that the achieved enhancement in secondary metabolites was not solely resulting from increased biomass but also from a specific elicitation action. A detailed correlation coefficient (Pearson's r) is available as supplementary data (data not shown).

Table 4 shows the results of the statistical analysis of the content of Kaempferol (mg/g) in gardenia callus, which reveals that there is a significant difference in Kaempferol content between the light source treatments. The red LED treatment recorded the highest number (67.211) whereas the dark treatment recorded the lowest number (8.267). The same table indicates that the kaempferol level after four weeks of culture was significantly affected by the in vitro gardenia callus when it was cultured with the same concentration of 0.25 mg/L of the two types of nano-oxides (zinc oxide and copper oxide). The highest one was obtained at 0.25ZN (51.725) and the lowest at control treatment (27.875). The light interaction with the nano-oxides had a significant effect on Kaempferol content, the highest level was observed when using red LED (0.25 ZN) which was 80.567mg/g, and the lowest when using darkness (0nano) was 6.867mg/g.

Table 4: Effect of Light Source, Zinc Oxide, and Copper Nano-Oxide on Kaempferol Content in Gardenia Callus in Vitro (mg/g).

Light Source (L)	Concentration of nano-oxides F			Effect of the light source
	0 Control	0.25 Cu	0.25 Zn	
White Fluorescent	22.300	39.933	53.533	38.589
Blue LED	31.700	54.867	63.233	49.933
Red LED	50.633	70.433	80.567	67.211
Darkness	6.867	8.367	9.567	8.267
Nanooxide Effect	27.875	43.400	51.725	
Least Significant Difference (LSD) (0.05)		0.489=F*L	0.244 = F	0. =L

Microscopic analysis of the results obtained (Tables (4)) indicated that in the realization of the given treatment, the average values for the studied traits, which include apigenin, gallic acid, ferulic acid, and kaempferol have been enhanced in gardenia

callus received the treatment, particularly white fluorescent light, red LED and blue LED in addition to treatment in darkness, zinc and copper nanoparticles at a concentration of 0.25 mg/L, compared to the plant received the control treatment. Red LED Light significantly affected all the parameters studied and the results were displayed in tables. Such can be attributed to the ability of red light (610-700 nm) to convert phytochrome pigment to its active form (Pfr) and activate the pathway for florescent synthesis - the shikimic acid pathway [15]. How it's interpreted is based on this Al-Sharifi finding [16] that LED R16-B2 lamps had the highest carbohydrate and highest pigment content in potato tubers. When there is red light, 4CL (4-coumaroyl-CoA ligase) is also activated and the enzyme speed up the conversion of phenylpropanoid derivatives into phenolic acids in the process of their synthesis [17].

Moreover, Batista et al. [18] revealed that gallic acid production is induced by red light through the pentose phosphate pathway, which generates adequate amount of carbon alkyl precursor for phenolic production. Another factor that may account for these results is that the red light is able to switch on the expression of phytochrome secondary synthesis genes, mainly chalcone synthase (CH3, F3H) leading to the production of phytochrome secondary metabolites from its derivatives [19]. The red LED light-induced increment in rutin can be attributed to the activation of the diphosphate enzyme called poridiydmin diphosphate (UDP) responsible for glycosidic sugar addition on quercetin ring to produce the secondary metabolites [17].

The results for the total phenolic content also agree with the results obtained by Nhut et al. [20] and Makki Nayef [21] who showed that red light triggers the activation of phytochrome-related secondary pathways that create a carbon rush towards a chimeric pathway, and ultimately an increase in the total amount of phenols accumulated.

The enhancement of secondary metabolites due to the effect of zinc oxide nanoparticles is considered to be through its effect of stimulating the phenylalanine ammonia lyase enzyme (PAL) which is the main entrance in the phenylpropanoid pathway [22]. Besides, zinc has been shown to be a key regulator of enzymes that catalyze the biosynthesis of flavonoids [22–23] confirming the above observation made by Alharby et al. [23] that the addition of zinc nanoparticles to the culture medium resulted in an increase in secondary metabolites.

Additionally, the outcome of the preceding tables is parallel with that of what was documented by Tymoszuk and Wojnarowicz [24] who documented the enhancement in the activity of the oxidative defense enzymes, and synthesis of phenolic compounds in callus cultures following the treatment with Zn nanoparticles.

This finding is in agreement with the results of Awad et al. [25] which suggested that the nanoparticles of zinc help in enhancing efficiency of secondary metabolism pathways through better uptake of nutrients and activation of the enzymes produced by the synthesis. Zinc is also involved in the activation of enzymes involved in the Calvin cycle reaction and is required for the reduction reaction in the synthesis of flavonoids [26].

4-Conclusion

The research revealed that different light sources produced positive results in the secondary metabolites that were produced. There were more amounts of secondary metabolites (apgenin, ferulic acid, gallic acid and kaempferol) generated by the red

LED light treatment than by darkness treatment.

The treatment with nanoparticles yielded maximum generation of secondary metabolites for zinc oxide nanoparticles. When compared with the corresponding bulk material (zinc sulfate), the effect of zinc oxide nanoparticles appeared to be more significant for the production of secondary metabolites, which indicated that a nanoparticle specific effect can be observed besides the ionic zinc effect. The Copper Oxide produced a higher amount than the Control Group. Therefore, it was observed that addition of nanoparticles was beneficial in the production of secondary metabolites.

Hence, it is recommended to use coloured LED light (red) and nanoparticles (ZnONPs) if the purpose of the callus culture is the synthesis of secondary metabolites of important role in different pharmaceutical industries. Additional experiments and scaling up of the quantity of used nanoparticles is recommended for obtaining the optimal concentration. Further studies will be necessary to determine the stability of the nanoparticles in the culture medium for the long term and if this protocol can be scaled up to bioreactor systems.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Data Availability

All data supporting the findings of this study are available within the manuscript.

Ethical Approval

This study did not involve human participants or animals. All experimental procedures were conducted in accordance with institutional and laboratory biosafety guidelines.

5- REFERENCES

[1] Lee, J. et al. (2021). Gardenia propagation. *[Missing details]*

[2] Swerdlow, J. L. (2000). *Nature's Medicine*, National Geographic, Washington, PP. 123-130.

[3] Hartmann, T. (2004). Plant-derived secondary Metabolites as defensive chemicals in herbivorous insects: a case study in chemical ecology. *Planta*, 219: 1-4.

[4] Ober, D. (2003). Chemical ecology of alkaloids exemplified with the Pyrrolizidiner. In JT Romeo, ed, *Integrative Phychemistry: from Ethnobotany to Molecular Ecology*, Vol. 37. Pergamon, Amsterdam, PP. 203-230.

[5] Hamish, A. C. and E. Sue. (1998). *Plant Cell Culture: The Introduction to Biotechniques*. BIOS Scientific Publishers Limited, Guildford, U.K.

[6] Huany, W. L. and L. F. Liu. (2002). Carbohydrate Metabolism in rice during callus induction and shoot regeneration induced by osmotic tress. *Bot. bull. Acad. Sience*, 43:107-113.

[7] Peter, V. (2003). *The secondary metabolism of plants. Secondary Defenes compounds*. Springer Verlage, New York. p. 1-10.

[8] Monreal, C. M., DeRosa, M., Mallubhotla, S. C., Bindraban, P. S., and Dimkpa, C. (2016). Nanotechnologies for increasing the crop use efficiency of fertilizer-micronutrients. *Biology and Fertility of Soils*, 52(3): 423-437.

[9] Salman (1988). Sterilization methods. *[Missing details]*

[10] Al-Dahimi (2009). Laboratory sterilization. *[Missing details]*

[11] Nayyef, M. N., Awadh, H. A. A., Kadhim, Z. K., et al. (2022). A comparative study between the effect of benzyl adenine and adenine sulphate on growth and multiplication of banana shoots (Musa spp.) in vitro. *International Journal of Agricultural and Statistical Sciences*, 18(1): 385–390.

[12] Smith, R. H. (2013). *Plant Tissue Culture: Techniques and Experiments*. Academic Press. pp 188.

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

[14] Taiz, L., & Zeiger, E. (2010). *Plant Physiology* (5th ed.). Sinauer Associates, Sunderland, Massachusetts.

[15] Sharifi, M. J. H. (2020). Effect of iron source and nanoparticles on potato microtuber production under different cultivation conditions. PhD dissertation, College of Agriculture, University of Kufa.
[16] Landi, M., Tattini, M., & Gould, K. S. (2020). Multiple functional roles of anthocyanins in plant-environment interactions. *Environmental and Experimental Botany*, 119, 4-17.
[17] Batista, D. S., Felipe, S. H. S., Silva, T. D., de Castro, K. M., Mamedes-Rodrigues, T. C., Miranda, N. A., ... & Otoni, W. C. (2018). Light quality in plant tissue culture: does it matter? *In Vitro Cellular & Developmental Biology-Plant*, 54(3), 195-215.
[18] Elshafie, H. S., & Mohamed, I. M. (2023). Secondary metabolites in plants: roles and biotechnological applications. *Frontiers in Sustainable Food Systems*, 7, 1-12.
[19] Nhut, D. T., Takamura, T., Watanabe, H., Okamoto, K., & Tanaka, M. (2003). Responses of strawberry plantlets cultured in vitro under superbright red and blue light-emitting diodes (LEDs). *Plant Cell, Tissue and Organ Culture*, 73(1), 43-52.
[20] Makki Nayef, M. (2019). Effect of light sources and 2iP concentrations on Barhi date palm tissue culture propagation. PhD dissertation, College of Agriculture, University of Kufa.
[21] Javed, R., Usman, M., Yücesan, B., Zia, M., & Gürel, E. (2017). Effect of zinc oxide (ZnO) nanoparticles on physiology and steviol glycosides production in micropropagated shoots of Stevia rebaudiana Bertoni. *Plant Physiology and Biochemistry*, 110, 94-99.
[22] Alharby, H. F., Metwali, E. M. R., Fuller, M. P., & Aldhebiani, A. Y. (2016). Impact of application of zinc oxide nanoparticles on callus induction, plant regeneration, element content, and antioxidant enzyme activity in tomato (Solanum lycopersicum Mill.) under salt stress. *Archives of Biological Sciences*, 68(4), 723-735.
[23] Tymoszuk, A., & Wojnarowicz, K. (2020). Zinc oxide and zinc sulfate as agents that induce callus growth and affect the content of phenolic compounds and antioxidant capacity of Chrysanthemum callus cultures. *Horticulturae*, 6(3), 44.
[24] Awad, A. A., Ibrahim, M. A., & El-Mahrouk, M. E. (2020). Effect of zinc oxide nanoparticles on in vitro propagation of date palm. *Journal of Agricultural Science*, 12(3), 67-78.
[25] Rudani, L., Vishal, C., Pragya, B., & Janmejay, P. (2018). Effect of zinc on photosynthesis and productivity of plants: A review. *International Journal of Pure & Applied Bioscience*, 6(1), 1243-1248.