Role of Nano-Oxides and Light Sources in Secondary Metabolite Production of Gardenia Callus Cultured In Vitro

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Abstract

This study was conducted at the Plant Tissue Culture Laboratory, Department of Horticulture and Landscape Design, College of Agriculture, Al-Qasim Green University, during the 2025–2026 research season. The objectives were to evaluate the effects of different light sources and selected nano-oxide compounds on the growth of Gardenia jasminoides L. cv. Veitchii callus and its accumulation of secondary metabolites under in vitro conditions.

The experiment was arranged as a two-factor completely randomized design (CRD). The first factor comprised three light regimes—white fluorescent, red LED (610–700 nm), and blue LED (400–500 nm)—together with a complete darkness treatment. The second factor included zinc oxide nanoparticles (ZnO NPs) and copper oxide nanoparticles (CuO NPs), each at 0.25 mg·L^{-1}, alongside an untreated control.

Results showed that red LED irradiation significantly outperformed all other light treatments in accumulating secondary metabolites, recording the highest means for quercetin (110.811 mg·g^{-1} FW), rutin (91.389 mg·g^{-1} FW), caffeic acid (80.333 mg·g^{-1} FW), and total phenolic content (247.01 mg·g^{-1} FW), while the darkness treatment consistently yielded the lowest values. ZnO NPs at 0.25 mg·L^{-1} similarly surpassed CuO NPs and the control for all four traits. The interaction of red LED × ZnO NPs produced the highest concentrations across all secondary metabolites, while white fluorescent light combined with ZnO NPs yielded the highest callus fresh weight (4.229 g) and dry weight (0.226 g).

Keywords: light regimes, nanoparticles, callus culture, Gardenia jasminoides, secondary metabolites, quercetin, rutin, ZnO NPs, LED irradiation, HPLC

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1-Introduction

medicines as a primary healthcare resource (Ober, 2003). The biologically active constituents of such medicines are fundamentally of plant origin, and their industrial-scale production contributes substantially to both human welfare and global economic revenue (Hamish and Sue, 1998; Hartmann, 2004).

The biosynthesis of plant secondary metabolites is markedly stimulated by a broad range of environmental stimuli, among which light plays a particularly pivotal role (Huang and Liu, 2002). Accordingly, researchers have increasingly turned to plant tissue culture to enhance secondary metabolite productivity, given its reliability as a scalable source of high-value metabolites through undifferentiated callus cell cultures (Peter, 2003). In recent years, nanotechnology has also attracted considerable interest in plant nutrition and growth regulation, with nano-fertilizers demonstrated to enhance nutrient uptake efficiency while mitigating environmental risks associated with conventional fertilizer application (Monreal et al., 2016).

Gardenia jasminoides L. cv. Veitchii is an ornamental flowering shrub belonging to the family Rubiaceae. Originating from the tropical and subtropical regions of Asia principally China and Japan this species is recognized for its lustrous dark-green foliage and intensely fragrant white flowers. Plant height typically ranges from 0.5 to 2.0 m depending on cultivar and prevailing growth conditions. Propagation is most commonly achieved through semi-hardwood stem cuttings, seed germination and grafting are occasionally employed, though stem cuttings remain the most efficient method in terms of time and success rate (Lee et al., 2021).

The use of plant-derived natural products in pharmaceutical applications has been documented since antiquity. More than 2,000 plant species are currently recognized as legitimate sources of bioactive medicinal compounds with clinically confirmed therapeutic efficacy (Swerdlow, 2000; Hartmann, 2004). The World Health Organization (WHO) has estimated that approximately 80% of the global population relies on plant-based

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throughout. The medium was supplemented with 2,4-D at 2.0 $mg \cdot L^{-1}$ and BA at 0.5 $mg \cdot L^{-1}$. Sucrose was incorporated at 30 $g \cdot L^{-1}$, the pH adjusted to 5.6–5.8, and agar–agar added at 6 $g \cdot L^{-1}$. Medium was dispensed in 10 mL aliquots into culture tubes, sealed, and sterilized at 115°C under 1.04 $kg \cdot cm^{-2}$ pressure for 20 minutes. All instruments were autoclaved at 121°C for 20 minutes (Salman, 1988), then dry-heat sterilized at 50°C for 24 hours and surface-sterilized with 99% ethanol in the laminar airflow cabinet (Al-Dahimi, 2009).

2.1 Callus Induction

Shoot tips were harvested from actively growing branches of a 10–12-year-old Gardenia jasminoides cv. Veitchii plant from a private nursery in Babylon Province, Iraq. Explants were surface-washed with 0.1% (v/v) Tween-20, rinsed under running tap water for 30 minutes, then surface-sterilized by immersion in 70% ethyl alcohol for 10 seconds followed by 20% (v/v) sodium hypochlorite for 20 minutes under continuous magnetic agitation (Nayyef et al., 2022). Sterilized explants were rinsed three times with sterile distilled water and inoculated into culture tubes containing 10 mL MS medium. Cultures were incubated for four weeks at 25 ± 2°C under 1,000 lux (55 ± 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$) with a

Accordingly, the present study was designed with the following specific objectives:

1. To evaluate the efficacy of red LED, blue LED, and white fluorescent light sources, together with a complete darkness treatment, on callus proliferation and secondary metabolite production in Gardenia jasminoides.
2. To investigate the influence of nano-oxide supplements (ZnO NPs and CuO NPs) incorporated into the culture medium on callus growth and secondary metabolite content, in comparison with conventional mineral sources.
3. To qualitatively and quantitatively identify phenolic secondary metabolites in Gardenia callus by HPLC, in recognition of their considerable medicinal and pharmaceutical significance.

2-Materials and Methods

The study was carried out at the Plant Tissue Culture Laboratory, Department of Horticulture and Landscape Design, College of Agriculture, Al-Qasim Green University, Babylon, Iraq, during the 2025–2026 research season. A commercially prepared Murashige and Skoog (1962) basal medium (HiMedia, India) was used

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for 30 minutes. Approximately 100 mg of callus tissue were aseptically transferred into experimental vials and distributed across light treatments: white fluorescent, red LED, blue LED, and complete darkness. All cultures were incubated for six weeks at 25 ± 2°C under 1,000 lux with a 16-hour photoperiod, or under total darkness as applicable. Upon completion of incubation, secondary metabolites underwent qualitative and quantitative analysis via HPLC.

16-hour light / 8-hour dark photoperiod (Smith, 2013).

2.2 Experimental Treatment Application

Following successful callus establishment, conventional $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$ in the MS micronutrient stock solution were replaced with ZnO NPs and CuO NPs at 0.25 mg·L⁻ ¹. The laminar airflow cabinet was decontaminated with 70% (v/v) ethanol followed by UV irradiation

3.Results and Discussion

3.1Quercetin Content in Callus (mg.g^{-1}fw)

was obtained under complete darkness (25.433 mg·g⁻ ¹ FW). ZnO NPs yielded the highest nano-oxide mean (91.492 mg·g⁻ ¹ FW), compared with the lowest in the untreated control (68.383 mg·g⁻ ¹ FW).

Statistical analysis of the data presented in Table 1 revealed a significant effect of light source on quercetin accumulation in Gardenia callus cultures. The highest mean quercetin content was recorded under red LED irradiation (110.811 mg·g⁻ ¹ FW), while the lowest value

Table 1. Effect of light source and nano-oxide supplements on quercetin content in Gardenia jasminoides callus cultured in vitro (mg·g⁻ ¹ FW).

Light Source (L)	0 Control	0.25 CuO NPs	0.25 ZnO NPs	Light Source Effect
White Fluorescent	77.433	86.367	100.533	88.111
Blue LED	80.867	98.333	110.533	96.578
Red LED	92.900	114.167	125.367	110.811
Darkness	22.333	24.433	29.533	25.433
Nano-oxide Effect	**68.383**	**80.825**	**91.492**	
LSD (0.05): L = 0.567 F = 0.491 F×L = 0.983				

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Analysis of variance for Table 2 indicated a significant influence of light source on rutin accumulation. The highest mean rutin content was obtained under red LED irradiation (91.389 mg·g⁻ ¹ FW), while complete darkness produced the lowest value (19.878 mg·g⁻ ¹ FW). ZnO NPs supplementation yielded the highest nano-oxide mean (80.900 mg·g⁻ ¹ FW), markedly exceeding the untreated control (45.242 mg·g⁻ ¹ FW).

The interaction between light source and nano-oxide treatment was significant. The highest mean was recorded under red LED × ZnO NPs (125.367 mg·g⁻ ¹ FW), while the lowest was observed under darkness × control (22.333 mg·g⁻ ¹ FW).

3.2 Rutin Content in Callus (mg.g⁻¹fw)

Table 2. Effect of light source and nano-oxide supplements on rutin content in Gardenia jasminoides callus cultured in vitro (mg·g⁻ ¹ FW).

Light Source (L)	0 Control	0.25 CuO NPs	0.25 ZnO NPs	Light Source Effect
White Fluorescent	46.167	60.267	88.500	64.978
Blue LED	52.433	78.867	99.767	77.022
Red LED	65.000	96.333	112.833	91.389
Darkness	17.367	19.767	22.500	19.878
Nano-oxide Effect	**45.242**	**63.808**	**80.900**	
LSD (0.05): L = 0.475 F = 0.412 F×L = 0.824				

caffeic acid content. Red LED irradiation produced the highest mean (80.333 mg·g⁻ ¹ FW), while complete darkness resulted in the lowest accumulation (18.667 mg·g⁻ ¹ FW). ZnO NPs at 0.25 mg·L⁻ ¹ yielded the highest caffeic acid content (66.450 mg·g⁻ ¹ FW) compared with the untreated control (46.592 mg·g⁻ ¹ FW).

The light source × nano-oxide interaction was significant. Red LED × ZnO NPs produced the highest mean (112.833 mg·g⁻ ¹ FW), while darkness × control yielded the lowest (17.367 mg·g⁻ ¹ FW).

3.3 Caffic Acid Content in Callus (mg.g⁻¹fw)

The results in Table 3 demonstrate a significant effect of light source on

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Table 3. Effect of light source and nano-oxide supplements on caffeic acid content in Gardenia jasminoides callus cultured in vitro (mg·g⁻ ¹ FW).

Light Source (L)	0 Control	0.25 CuO NPs	0.25 ZnO NPs	Light Source Effect
White Fluorescent	48.833	55.400	70.933	58.389
Blue LED	53.900	61.700	80.133	65.244
Red LED	66.600	80.667	93.733	80.333
Darkness	17.033	17.967	21.000	18.667
Nano-oxide Effect	**46.592**	**53.933**	**66.450**	
LSD (0.05): L = 0.421 F = 0.361 F×L = 0.730				

significant effect of light source. Red LED irradiation produced the highest total phenolic content (247.01 mg·g⁻ ¹ FW), while complete darkness yielded the lowest (126.67 mg·g⁻ ¹ FW). ZnO NPs at 0.25 mg·L⁻ ¹ recorded the highest nano-oxide mean (217.55 mg·g⁻ ¹ FW) relative to the untreated control (188.68 mg·g⁻ ¹ FW).

The interaction of light source and nano-oxide was significant. The highest mean was recorded under red LED × ZnO NPs (93.733 mg·g⁻ ¹ FW), while darkness × control recorded the lowest (17.033 mg·g⁻ ¹ FW).

3.4Total Phenolic Content in Callus (mg.g^{-1}fw)

Statistical analysis of total phenolic content (Table 4) confirmed a

Table 4. Effect of light source and nano-oxide supplements on total phenolic content in Gardenia jasminoides callus cultured in vitro (mg·g⁻ ¹ FW).

Light Source (L)	0 Control	0.25 CuO NPs	0.25 ZnO NPs	Light Source Effect
White Fluorescent	198.33	218.00	230.20	215.51
Blue LED	205.33	230.00	244.43	226.59
Red LED	229.47	247.03	264.53	247.01
Darkness	121.60	127.37	131.03	126.67
Nano-oxide Effect	**188.68**	**205.60**	**217.55**	
LSD (0.05): L = 1.16 F = 1.00 F×L = 2.01				

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callus fresh weight. White fluorescent light produced the highest mean fresh weight (3.858 g), while complete darkness yielded the lowest (2.098 g). ZnO NPs at 0.25 mg·L^{-1} recorded the highest fresh weight among nano-oxide treatments (3.358 g), compared with the lowest value under CuO NPs (2.365 g).

The interaction effect was statistically significant. The highest mean was consistently recorded under red LED × ZnO NPs (264.53 mg·g^{-1} FW), while darkness × control produced the lowest value (121.60 mg·g^{-1} FW).

3.5 Callus Fresh Weight (g)

Analysis of variance revealed a significant effect of light source on

Table 5. Effect of light source and nano-oxide supplements on callus fresh weight of Gardenia jasminoides cultured in vitro (g).

Light Source (L)	0 Control	0.25 CuO NPs	0.25 ZnO NPs	Light Source Effect
White Fluorescent	4.212	3.134	4.229	3.858
Blue LED	2.322	2.154	3.167	2.548
Red LED	3.535	2.035	3.989	3.186
Darkness	2.114	2.136	2.045	2.098
Nano-oxide Effect	**3.046**	**2.365**	**3.358**	
LSD (0.05): L = 0.237 F = 0.205 F×L = 0.410				

callus dry weight. White fluorescent light produced the highest mean dry weight (0.2060 g), while complete darkness yielded the lowest (0.1257 g). ZnO NPs at 0.25 mg·L^{-1} recorded the highest dry weight among nano-oxide treatments (0.1818 g), compared with the lowest value under CuO NPs (0.1285 g).

The light source × nano-oxide interaction was significant. The highest mean was recorded under white fluorescent × ZnO NPs (4.229 g), while the lowest was observed under darkness × control (2.114 g).

3.6 Callus Dry Weight (g)

Statistical analysis revealed a significant effect of light source on

Table 6. Effect of light source and nano-oxide supplements on callus dry weight of Gardenia jasminoides cultured in vitro (g).

Light Source (L)	0 Control	0.25 CuO NPs	0.25 ZnO NPs	Light Source Effect
White Fluorescent	0.2250	0.1670	0.2260	**0.2060**
Blue LED	0.1240	0.1150	0.1690	0.1360
Red LED	0.1880	0.1080	0.2130	0.1697
Darkness	0.1340	0.1240	0.1190	0.1257
Nano-oxide Effect	**0.1678**	**0.1285**	**0.1818**	
LSD (0.05): L = 0.0080 F = 0.0069 F×L = 0.0139				

route for phenylpropanoid-derived metabolites including flavonoids and hydroxycinnamic acids (Taiz and Zeiger, 2010). This interpretation is corroborated by Al-Sharifi (2020), who reported that LED R16:B2 irradiation produced the highest carbohydrate and pigment contents in potato microtubers. Furthermore, red LED promotes upregulation of 4-coumaroyl-CoA ligase (4CL) activity toward hydroxycinnamic acid biosynthesis (Landi et al., 2020), while Batista et al. (2018) demonstrated that red light stimulates phenolic anabolism through the photosynthetic pentose phosphate pathway. Red LED has further been shown to regulate the expression of chalcone synthase (CHS), F3H, and related flavonoid biosynthetic genes via Phytochrome-mediated signaling cascades (Elshafie and Mohamed, 2023). The elevated rutin content is mechanistically explained by

The light source × nano-oxide interaction was significant. The highest mean was recorded under white fluorescent × ZnO NPs (0.2260 g), while the lowest was observed under red LED × CuO NPs (0.1080 g).

General Discussion

The data presented in Tables 1–6 collectively demonstrate significant and consistent effects of both light regime and nano-oxide supplementation on all six traits examined in Gardenia jasminoides callus cultured in vitro.

The superior performance of red LED irradiation across all four secondary metabolite traits is attributed primarily to its capacity to photoconvert phytochrome to its physiologically active Pfr form, which activates the shikimate pathway—the principal biosynthetic

assimilation. Additionally, zinc facilitates NADPH regeneration through the Calvin cycle, providing the reductive equivalents required for flavonoid biosynthesis (Rudani et al., 2018).

The superiority of white fluorescent light in callus fresh weight and dry weight traits is attributed to its broad-spectrum emission, which activates both PS I and PS II simultaneously, generating optimal quantities of ATP and NADPH to drive H^+ -ATPase proton pumps. These pumps establish the electrochemical gradient that drives ion and water uptake through aquaporin channels, increasing cellular turgor—the physiological basis of fresh weight (George et al., 2008). This is consistent with findings of Gok et al. (2016), who reported that white fluorescent light yielded the highest fresh weight in Gerbera tissue cultures, and with Hernández and Kubota (2014) and Al-Sharifi (2020), who documented the advantages of broad-spectrum irradiation for biomass accumulation.

4.Conclusions

jasminoides callus cultures under in vitro conditions, while complete darkness consistently suppresses secondary metabolite production to minimum levels.

2. ZnO NPs at 0.25 $mg \cdot L^{-1}$ significantly enhanced the production of all four secondary metabolite classes investigated, surpassing both CuO NPs and the conventional mineral control.

activation of uridine diphosphate glucosyltransferase (UGT) (Landi et al., 2020). These findings are consistent with Nhut et al. (2003) and Nayef (2019), who documented that red-light-driven phytochrome signaling generates a pronounced carbon flux toward the shikimate pathway.

With respect to the stimulatory effect of ZnO NPs on secondary metabolite biosynthesis, the primary mechanism involves the role of zinc ions in activating phenylalanine ammonia-lyase (PAL), the regulatory gateway enzyme of the phenylpropanoid pathway (Javed et al., 2017). Zinc is also an essential cofactor for several flavonoid biosynthetic enzymes, as corroborated by Alharby et al. (2016) and Tymoszuk and Wojnarowicz (2020), who demonstrated that ZnO NPs enhance antioxidant enzyme activity and promote phenolic biosynthesis. Awad et al. (2020) reported that nano-zinc ions improve secondary metabolic pathway efficiency by enhancing nutrient

Based on the results of this study, the following conclusions are drawn:

1. Red LED irradiation constitutes the most effective light source for stimulating secondary metabolite biosynthesis—specifically quercetin, rutin, caffeic acid, and total phenolic content—in Gardenia

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for biomass production applications.

3. White fluorescent light combined with ZnO NPs achieved the highest callus fresh weight (4.229 g) and dry weight (0.226 g), demonstrating its superiority

5.Recommendation

medium for Gardenia callus culture, given its significant and comprehensive superiority across all measured indicators.

4. Future studies are warranted to test a wider range of ZnO NPs concentrations (0.1, 0.5, and 1.0 $mg \cdot L^{-1}$) to delineate the dose–response relationship and investigate interactions between ZnO NPs and phytohormones.

1. Red LED irradiation should be adopted as the primary light source in plant tissue culture laboratories oriented toward the production of pharmaceutical phenolic compounds from Gardenia callus.
2. White fluorescent light or dual-spectrum LED (red:blue) is recommended for mass propagation laboratories targeting vegetative growth and biomass accumulation.
3. ZnO NPs at 0.25 $mg \cdot L^{-1}$ should be adopted as a replacement for conventional zinc sulfate in MS

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