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Optimization of in vitro cultivation for enhanced biochemical properties of geranium (*Pelargonium graveolens*) for functional use

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Submission Date: July 1st, 2025; **Acceptance Date:** July 29th, 2025; **Publication Date:** August 6th, 2025

Please cite this article as: Harutyunyan M., Melyan G., Martirosyan Y., Hovhannisyan M., Dangyan K., Terteryan K., Arajyan G., Khazaryan K., Sahakyan A. Optimization of *In Vitro* cultivation for enhanced biochemical properties of Geranium (*Pelargonium graveolens*) for functional use. *Functional Food Science* 2025; 5(8): 364 – 377.
DOI: https://doi.org/10.31989/ffs.v5i7.1697

ABSTRACT

Background: *Pelargonium graveolens* is a valuable aromatic and medicinal plant native to southern Africa, widely recognized for its essential oils rich in bioactive compounds with antiseptic, anti-inflammatory, and antioxidant properties. Its increasing cultivation in Armenia underscores its potential for natural health products and functional foods, where antioxidant-rich phytochemicals are in high demand.

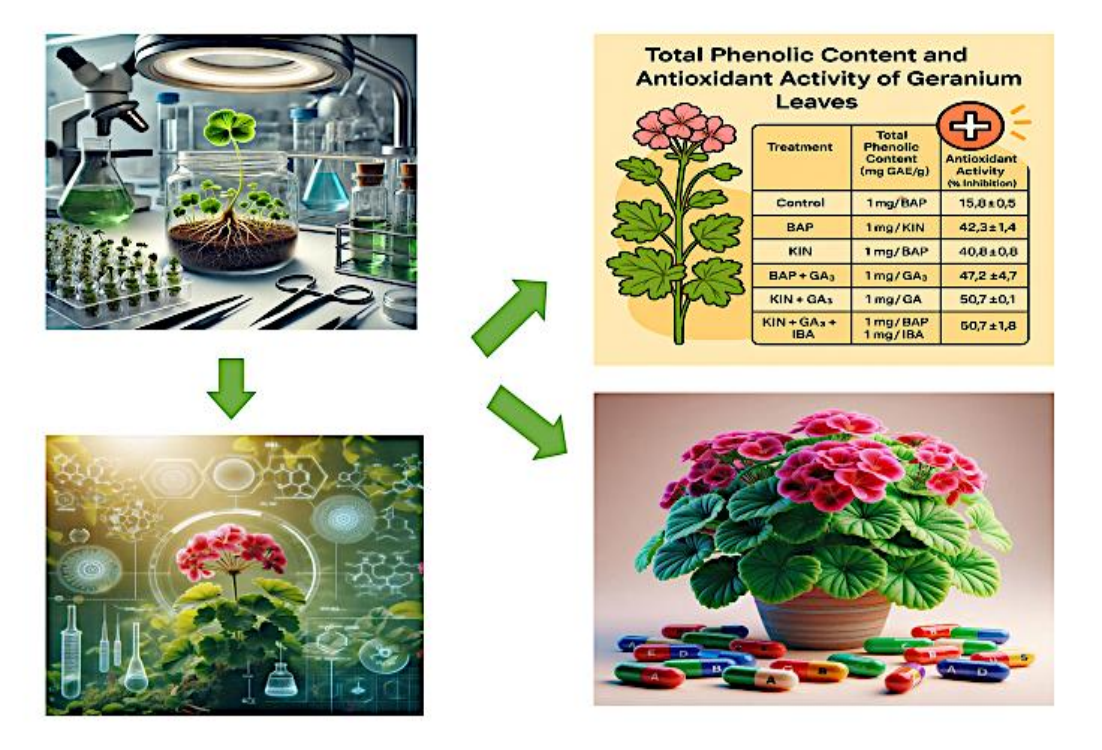
Objective: This study aimed to optimize *in vitro* propagation protocols for *P. graveolens* by evaluating the effects of various plant growth regulators (PGRs) on morphogenic response, phenolic accumulation, antioxidant activity, and essential oil composition—specifically focusing on citronellol, geraniol, and linalool.

Materials and Methods: Nodal explants were cultured on Murashige and Skoog (MS) medium supplemented with different PGRs, including 6-benzylaminopurine (BAP), kinetin (KIN), gibberellic acid (GA_3), and indole-3-butyric acid (IBA). After six weeks, growth parameters, chlorophyll content, total phenolic content (TPC), antioxidant activity, and essential oil profiles were analyzed. TPC was assessed using the Folin–Ciocalteu method, antioxidant activity via DPPH assay, and essential oils were identified by GC-MS. Data were statistically analyzed using one-way ANOVA ($p < 0.05$).

Results: The PGR combination of 1.0 mg/L KIN + 1.0 mg/L GA_3 + 1.0 mg/L BAP + 1.0 mg/L IBA yielded the highest shoot number (3.9 ± 0.2) and length (6.3 ± 0.2 cm). Rooting was most effective with 1.0 mg/L IBA alone. The same combination significantly enhanced TPC (20.3 ± 0.9 mg GAE/g FW) and antioxidant activity (50.7 ± 1.8%). GC-MS analysis revealed increased concentrations of key essential oil constituents with known therapeutic and antioxidant properties.

Conclusion: Targeted PGR combinations effectively improve *in vitro* regeneration and biochemical quality of *P. graveolens*, boosting its value as a source of natural antioxidants and functional ingredients. These findings demonstrate the potential of optimized tissue culture methods for the sustainable production of bioactive plant compounds with health-promoting applications.

Keywords: *Pelargonium graveolens*, *in vitro* propagation, plant growth regulators, essential oils, antioxidant activity, bioactive compounds, functional foods



Graphical abstract: *In Vitro* Cultivation and Biochemical Profiling of Geranium (*Pelargonium spp*.)

INTRODUCTION

Pelargonium graveolens, commonly known as rose-scented geranium, is widely cultivated for its essential oil, which holds significant value in the fragrance, cosmetics, and aromatherapy industries [2–4]. The oil is particularly rich in aromatic compounds such as citronellol and geraniol, known for their strong antimicrobial, anti-inflammatory, and antioxidant properties [5–7]. In addition to its industrial applications, *P. graveolens* essential oil has demonstrated therapeutic potential in managing conditions such as Parkinson's disease, tendonitis, and rheumatoid arthritis, contributing to inflammation reduction, pain relief, and improved joint mobility [8,9].

Beyond essential oils, *P. graveolens* is also a valuable source of vitamin C, a vital nutrient that supports immune function and skin health by mitigating oxidative stress [10]. In Armenia, the cultivation of geranium dates back to 1938, particularly in the Hoktemberyan and Echmiadzin regions, where it has traditionally been grown for essential oil production. Additionally, the plant has long been used in traditional medicine to treat fractures, respiratory diseases, fevers, and skin disorders. The therapeutic value of *P. graveolens* stems largely from its bioactive compounds, including both nutrient and non-nutrient metabolites. These compounds play key roles in promoting health and preventing chronic diseases, thereby forming the scientific basis for the development of functional foods and nutraceuticals [11–20].

To fully harness the potential of *P. graveolens*, ensuring a stable and high-quality supply of virus-free planting material is crucial. In vitro cultivation, particularly micropropagation, has emerged as an essential technique for the rapid and consistent production of healthy, genetically uniform plants [21–24].

A major limitation in geranium cultivation lies in its susceptibility to viral and fungal diseases, which can significantly impair plant health, reduce yield, and compromise essential oil quality. Viral infections, in particular, are known to reduce oil content and disrupt the production of bioactive metabolites [25–30]. The most common diseases affecting geranium include:

- Bacterial blight – *Xanthomonas hortorum* pv. *pelargonii*: Causes leaf spots, wilting, and can lead to complete plant collapse; a major threat to commercial cultivation [31].
- Pelargonium leaf curl virus (PLCV) – Transmitted by aphids; induces leaf curling, distortion, and reduced vigor; significant in virus indexing programs [32].
- Downy mildew – *Peronospora pelargonii-zonalis*: Leads to yellowing, leaf curling, premature drop, and reduced essential oil yield; highly destructive in humid conditions [33].
- Fusarium wilt – *Fusarium oxysporum* f. sp. *pelargonii*: A soil-borne vascular wilt pathogen; causes root rot, stem collapse, and dramatic yield losses, especially in nursery settings [34].

Given these challenges, *in vitro* propagation not only enables the rapid multiplication of high-quality *P. graveolens* plants but also offers a strategic approach for virus elimination and improved disease resistance [35–38]. Recent biotechnological advances have demonstrated that combining micropropagation with virus elimination techniques can significantly improve plant performance and essential oil composition in *Pelargonium* species [30]. Moreover, recent studies on the chemical and biological profiles of *Pelargonium roseum* essential oil underscore its potential as a sustainable and effective source of functional bioactive compounds [39].

The present study aimed to optimize *in vitro* propagation protocols for *P. graveolens* using various

PGRs, with a focus on improving both morphogenic responses and biochemical characteristics. Emphasis was placed on enhancing the accumulation of high-value bioactive compounds, particularly phenolics, antioxidants, and essential oil constituents such as citronellol, geraniol, and linalool, for potential applications in the pharmaceutical, cosmetic, and functional food industries.

MATERIALS AND METHODS

Plant Material and Culture Conditions: *In vitro* cultures of *Pelargonium graveolens* (rose-scented geranium) were initiated using nodal segments obtained from healthy, 1-year-old greenhouse-grown plants. Explants were cultured on Murashige and Skoog (MS) basal medium supplemented with 3% (w/v) sucrose and solidified with 0.6% agar (Duchefa Biochemie). The pH was adjusted to 5.8 ± 0.1 before autoclaving at 121 °C for 20 minutes. Cultures were maintained in a growth chamber at 25 ± 2 °C under a 16-hour light / 8-hour dark photoperiod, with a light intensity of approximately 50 µmol m^{-2} s^{-1} provided by cool-white, fluorescent lamps. Each treatment consisted of 15 explants per replicate, with three replicates (total n = 45 per treatment).

Shoot Proliferation under PGR Treatments: To evaluate the effects of PGRs on shoot induction and elongation, the MS medium was supplemented with various PGR combinations (Table 1). All PGRs—6-benzylaminopurine (BAP), kinetin (KIN), gibberellic acid (GA_3), and indole-3-butyric acid (IBA)—were filter-sterilized and added to the medium after autoclaving. Cultures were maintained for six weeks, after which the number and length of regenerated shoots were recorded.

Table 1. PGR treatments used for shoot proliferation in *Pelargonium graveolens*.

Treatment No.	PGR Composition (mg/L)
1	No PGR (Control)
2	BAP (1.0)
3	KIN (1.0)
4	BAP (1.0) + GA_3 (1.0)
5	KIN (1.0) + GA_3 (1.0)
6	BAP (1.0) + KIN (1.0) + GA_3 (1.0) + IBA (1.0)

Rooting Experiment: Regenerated shoots (2–3 cm in length) were excised and transferred to MS medium supplemented with various auxin treatments (Table 2) to induce rooting. The basal medium composition remained unchanged. Each treatment included 15 shoots per replicate, with three replicates (n = 45). After six weeks, the number of roots per shoot and average root length were recorded.

Table 2. Auxin treatments used for root induction in *Pelargonium graveolens* shoots.

Treatment No.	Auxin Type	Concentration (mg/L)
1	None (Control)	–
2	Indole-3-acetic acid (IAA)	0.5
3	Indole-3-acetic acid (IAA)	1.0
4	IBA	0.5
5	IBA	1.0

Biochemical Analyses: Fully expanded leaves from plantlets grown under various PGR treatments were harvested for biochemical analysis. All measurements were performed in triplicate.

Chlorophyll Content: Chlorophyll *a*, chlorophyll *b*, and total chlorophyll were extracted using 80% (v/v) acetone and quantified spectrophotometrically at 645 and 663 nm, following the method of Lichtenthaler and Wellburn (1983) [42]. Results were expressed as mg/g of fresh weight.

- Total Phenolic Content (TPC): TPC was quantified using the Folin–Ciocalteu reagent as described by Singleton et al. (1999) [43], and expressed as mg gallic acid equivalents (GAE) per gram of fresh weight.
- Antioxidant Activity: Antioxidant activity was evaluated using the DPPH radical scavenging assay, following Brand-Williams et al. (1995) [44]. Results were reported as percentage inhibition.
- Essential Oil Extraction and Analysis: Essential oils were extracted from 50 g of fresh leaves by hydrodistillation for 3 hours using a Clevenger-type apparatus, according to standard protocols [45]. Extracted oils were dried over anhydrous sodium sulfate and stored in sealed amber vials at 4 °C until analysis.
- GC–MS Composition Analysis: Essential oil composition was analyzed by gas chromatography–mass spectrometry (GC–MS) using an Agilent 7890A GC system coupled with a 5975C MS detector. An HP-5MS column (30 m × 0.25 mm, 0.25 µm film thickness) was employed, following the method described by Adams (2007) [46].

Statistical Analysis: All experiments were conducted in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical differences among treatments were evaluated using one-way analysis of variance (ANOVA) at $p < 0.05$. Statistical analyses were performed using GraphPad software.

RESULTS AND DISCUSSION

The effects of various PGRs on the *in vitro* regeneration of *Pelargonium graveolens* (geranium) were assessed by evaluating shoot number, shoot length, and root number after six weeks of cultivation. As shown in Table 3, PGR treatments significantly influenced these growth parameters. No shoot formation was observed in the control group.

Table 3. Effect of Various PGRs on Shoot Number and Shoot Length of *Pelargonium graveolens*

Treatment	Shoot Number (Mean ± SD)	Shoot Length (cm, Mean ± SD)
Control	No shoots formed	No shoots formed
1.0 mg/L BAP	2.2 ± 0.3	4.3 ± 0.3
1.0 mg/L KIN	1.1 ± 0.4	4.0 ± 0.3
1.0 mg/L BAP + 1.0 mg/L GA_3	2.7 ± 0.3	5.5 ± 0.2
1.0 mg/L KIN + 1.0 mg/L GA_3	1.7 ± 0.2	4.7 ± 0.2
1.0 mg/L KIN + 1.0 mg/L GA_3 + 1.0 mg/L BAP + 1.0 mg/L IBA	3.9 ± 0.2	6.3 ± 0.2

A one-way ANOVA revealed a statistically significant effect of PGR treatments on shoot number ($p < 0.05$), indicating substantial differences among the groups. The highest shoot proliferation (3.9 ± 0.2) occurred with the combined treatment of 1.0 mg/L KIN, 1.0 mg/L GA_3, 1.0 mg/L BAP, and 1.0 mg/L IBA, while the lowest (1.1 ± 0.4) was recorded with 1.0 mg/L KIN alone. The absence of shoot formation in the control group further confirmed the necessity of PGRs for regeneration. The enhanced shoot proliferation under combined cytokinin (KIN and

BAP), gibberellin (GA_3), and auxin (IBA) treatments likely results from their synergistic interaction. Cytokinins are known to stimulate cell division and shoot initiation, while auxins promote elongation and morphogenesis [47,48]. BAP alone (2.2 ± 0.3) and its combination with GA_3 (2.7 ± 0.3) also significantly increased shoot number compared to the control, underlining the role of gibberellins in enhancing shoot proliferation. Shoot length varied significantly across treatments ($p < 0.05$), with the longest shoots (6.3 ± 0.2 cm) observed under the combined KIN, GA_3, BAP, and IBA treatment. The BAP + GA_3 treatment also resulted in relatively long shoots (5.5 ± 0.2 cm), whereas the shortest shoots (4.0 ± 0.3 cm) were recorded under KIN alone. These findings reinforce the distinct yet complementary roles of PGRs: cytokinins mainly initiate shoot formation, while gibberellins facilitate elongation. This trend aligns with earlier studies reporting cytokinin–gibberellin interactions that enhance shoot development in various species [49]. The effects of different auxin treatments on root number and root length in *in vitro*-cultivated *P. graveolens* plants are shown in Figure 1. Data are expressed as mean ± standard deviation (SD) and highlight the role of auxins in promoting root initiation and elongation across treatments.

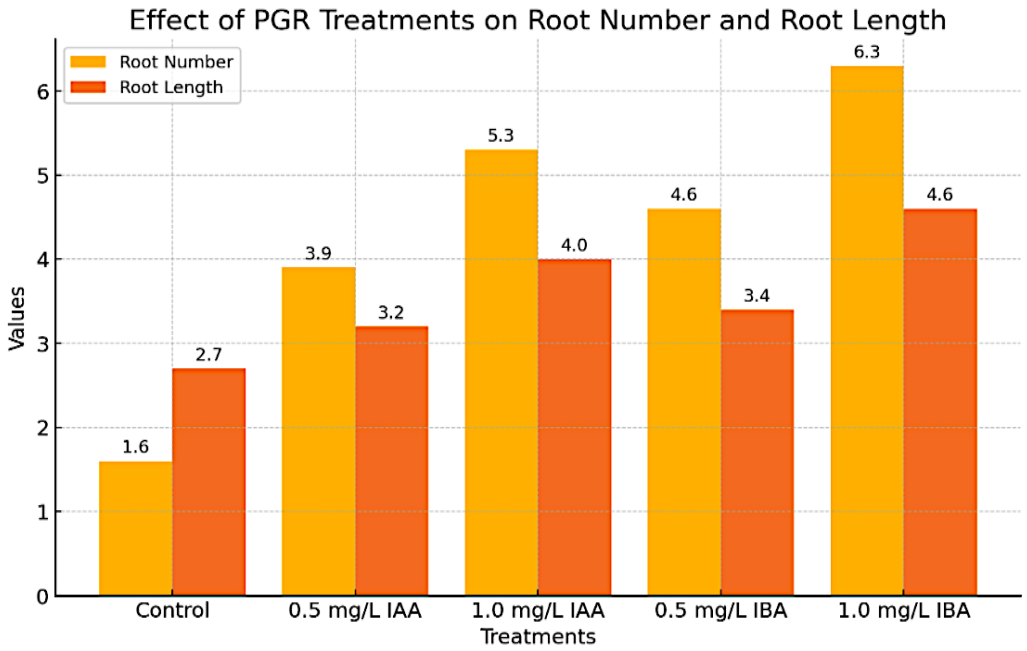


Figure 1. Effect of different PGR treatments on *in vitro* rooting of *Pelargonium graveolens* (Geranium).

One-way ANOVA revealed significant differences in root number among auxin treatments ($F = 533.13$, $p < 0.001$), confirming the pivotal role of auxins in root initiation. The highest root number was observed with 1.0 mg/L indole-3-butyric acid (IBA) (6.4 ± 0.5), followed by 1.0 mg/L indole-3-acetic acid (IAA) (5.3 ± 0.3). Lower concentrations—0.5 mg/L IBA (4.6 ± 0.4) and 0.5 mg/L IAA (3.9 ± 0.5)—also significantly increased root numbers compared to the untreated control (1.6 ± 0.2), where root formation was minimal. These findings demonstrate the superior efficacy of IBA over IAA in promoting root development under *in vitro* conditions. Kumar et al. (2023) [50] similarly reported that 2000 mg/L IBA was the most effective concentration for enhancing root formation in *Pelargonium graveolens* stem cuttings.

Root length analysis showed a comparable trend, with significant differences among treatments ($F = 67.13$, $p < 0.001$). The longest roots (4.6 ± 0.5 cm) were recorded

with 1.0 mg/L IBA, followed by 1.0 mg/L IAA (4.0 ± 0.5 cm). Lower auxin concentrations also promoted root elongation relative to the control (2.7 ± 0.5 cm). These results further affirm the positive role of auxins, particularly IBA, in enhancing both root number and length—likely due to IBA's greater chemical stability and sustained activity, which facilitate cell division and elongation in root primordia [51].

The effects of various PGRs on the total phenolic content and antioxidant activity of *Pelargonium graveolens* leaves are presented in Table 4.

Table 4. Effect of different PGR treatments on total phenolic content and antioxidant activity in *Pelargonium graveolens* leaves.

Treatment Combination	Total Phenolic Content (mg GAE/g) (Mean ± SD)	Antioxidant Activity (% Inhibition) (Mean ± SD)
Control (No PGR)	10.2 ± 0.5	35.5 ± 1.2
Single PGR		
1 mg/L BAP	15.8 ± 0.7	42.3 ± 1.4
1 mg/L KIN	14.2 ± 0.6	40.8 ± 1.3
Combined PGRs		
1 mg/L BAP + 1 mg/L GA_3	18.4 ± 0.8	47.2 ± 1.6
1 mg/L KIN + 1 mg/L GA_3	16.5 ± 0.7	45.0 ± 1.5
1 mg/L KIN + 1 mg/L GA_3 + 1 mg/L BAP + 1 mg/L IBA	20.3 ± 0.9	50.7 ± 1.8

The results demonstrated a significant effect of PGR treatments on both total phenolic content and antioxidant activity in *Pelargonium graveolens* leaves. The control treatment (no PGRs) exhibited the lowest phenolic content (10.2 ± 0.5 mg GAE/g). Application of 1 mg/L BAP significantly increased phenolic content to 15.8 ± 0.7 mg GAE/g, while 1 mg/L KIN resulted in a moderate increase to 14.2 ± 0.6 mg GAE/g. Combined treatments further enhanced phenolic accumulation: 1 mg/L BAP + 1 mg/L GA_3 raised phenolic content to 18.4 ± 0.8 mg GAE/g, and 1 mg/L KIN + 1 mg/L GA_3 to 16.5 ± 0.7 mg GAE/g. The highest phenolic content (20.3 ± 0.9 mg GAE/g) was observed with the full combination of 1 mg/L KIN, 1 mg/L GA_3, 1 mg/L BAP, and 1 mg/L IBA. These findings suggest a synergistic effect of multiple PGRs in promoting phenolic biosynthesis more effectively than individual treatments [52].

Antioxidant activity exhibited a similar trend. The control showed the lowest inhibition percentage (35.5 ± 1.2%). Treatments with 1 mg/L BAP (42.3 ± 1.4%) and 1 mg/L KIN (40.8 ± 1.3%) moderately enhanced antioxidant potential. Combination treatments produced further improvements: 1 mg/L BAP + 1 mg/L GA_3 elevated antioxidant activity to 47.2 ± 1.6%, and 1 mg/L KIN + 1 mg/L GA_3 reached 45.0 ± 1.5%. The highest antioxidant activity (50.7 ± 1.8%) was achieved with the full combination of all four PGRs. These results highlight synergistic interactions among PGRs in enhancing the antioxidant capacity of *P. graveolens* leaves [53]. Comparing individual and combined treatments, combined applications had a more pronounced effect on both phenolic content and antioxidant activity. For example, 1 mg/L BAP alone increased phenolic content by approximately 55% compared to the control, while its combination with 1 mg/L GA_3 resulted in an 80% increase. The full combination treatment nearly doubled phenolic content, underscoring the additive effect of multiple PGRs.

Antioxidant activity followed the same pattern: the full combination treatment enhanced antioxidant activity by approximately 43% compared to the control, further supporting the synergistic benefits of multiple growth regulators. The observed synergy may be attributed to the interplay between cytokinins, gibberellins, and auxins, collectively regulating phenolic biosynthesis pathways and antioxidant enzyme activity [52-53].

To further explore the relationship between total phenolic content and antioxidant activity in *Pelargonium graveolens* leaves, a correlation analysis was performed across the different PGR treatments. The results are presented in Figure 2, which illustrates the strength and nature of this association.

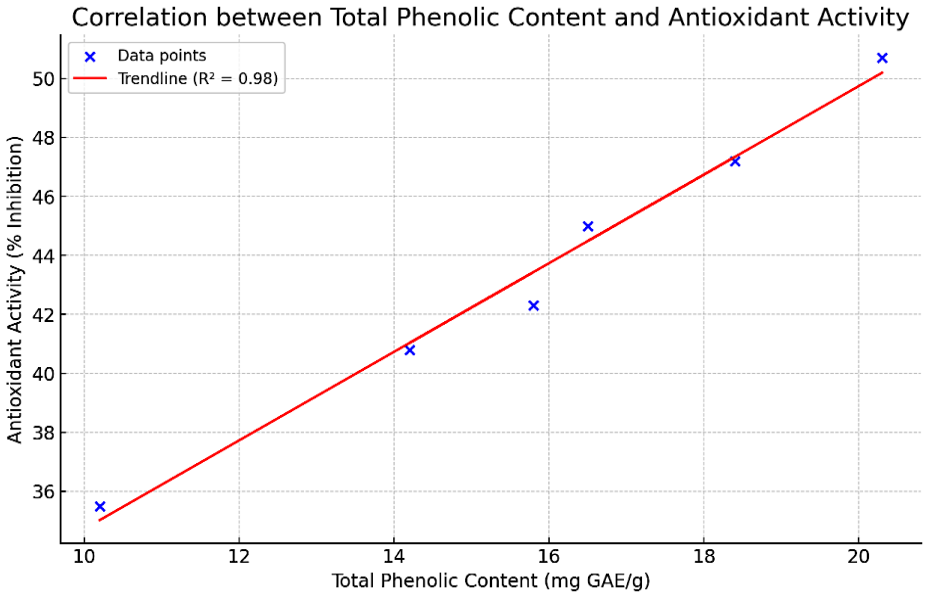


Figure 2: Correlation between Total Phenolic Content and Antioxidant Activity in *Pelargonium graveolens* leaves

Figure 2 illustrates a strong positive correlation between total phenolic content (mg GAE/g) and antioxidant activity (% inhibition) in *P. graveolens* leaves under different PGR treatments. Each point represents the mean value for a specific treatment. The red trendline indicates this relationship, with a coefficient of determination (R^2) of 0.98, suggesting that increased phenolic content is strongly associated with enhanced antioxidant activity. The scatter plot shows that treatments yielding higher total phenolic content also demonstrate greater antioxidant activity. Notably, combined PGR treatments—such as 1 mg/L BAP + 1 mg/L GA_3 and the full combination of 1 mg/L KIN + 1 mg/L GA_3 + 1 mg/L BAP + 1 mg/L IBA—cluster in the upper right quadrant of the plot, reflecting improved biochemical responses. This trend indicates a synergistic effect of multiple PGRs in promoting both phenolic biosynthesis and antioxidant potential. Overall, the data presented in Figure 2 strongly support the hypothesis that optimized combinations of PGRs significantly enhance the biochemical quality of *Pelargonium graveolens* by increasing both total phenolic content and antioxidant activity. To further explore the biochemical effects of PGR treatments, the chemical composition of essential oils extracted from treated leaves was analyzed using GC-MS. The major monoterpenoid constituents identified and their relative concentrations under different treatments are summarized in Table 5 and illustrated in Figure 3.

Table 5. Essential Oil Composition of *Pelargonium graveolens* Leaves under Different PGR Treatments

Component	Control (µg/g) (Mean ± SD)	1 mg/L BAP (µg/g) (Mean ± SD)	1 mg/L KIN (µg/g) (Mean ± SD)	1 mg/L BAP + 1 mg/L GA_3 (µg/g) (Mean ± SD)	1 mg/L KIN + 1 mg/L GA_3 (µg/g) (Mean ± SD)	1 mg/L KIN + 1 mg/L GA_3 + 1 mg/L BAP + 1 mg/L IBA (µg/g) (Mean ± SD)
Citronellol	270.5 ± 10	350.0 ± 12	324.9 ± 11	390.8 ± 13	410.5 ± 15	429.5 ± 16
Geraniol	151.4 ± 7	178.0 ± 8	163.2 ± 7.5	198.5 ± 9	187.9 ± 8.5	210.8 ± 10
Linalool	30.5 ± 2	40.5 ± 2.5	37.6 ± 2.3	50.5 ± 3	51.0 ± 3.1	65.0 ± 4
Isomenthone	22.2 ± 1.5	25.5 ± 1.8	24.9 ± 1.7	27.1 ± 1.9	25.9 ± 1.6	28.2 ± 2
Geranyl formate	20.2 ± 1	22.9 ± 1.2	23.6 ± 1.3	25.8 ± 1.4	24.9 ± 1.3	25.7 ± 1.4
Menthone	31.4 ± 2	33.8 ± 2.2	32.5 ± 2	35.4 ± 2.3	35.1 ± 2.2	36.6 ± 2.4

Application of PGRs significantly altered the essential oil composition of *Pelargonium graveolens* leaves (Table 5, Figure 3). Key monoterpenoid components—citronellol, geraniol, linalool, isomenthone, geranyl formate, and menthone—were notably elevated in all treated samples compared to the control. Among these, citronellol showed the largest increase, reaching 429.5 µg/g under the combined treatment of 1 mg/L KIN + 1 mg/L GA_3 + 1 mg/L BAP + 1 mg/L IBA, representing a 58.7% increase over the control level of 270.5 µg/g. This suggests a synergistic enhancement of citronellol biosynthesis by the combined application of cytokinins, gibberellins, and auxins, which proved more effective than single PGR treatments. Geraniol also followed this trend, increasing by 39.2% to 210.8 µg/g in the combined treatment group, underscoring the role of PGRs in elevating this aromatic compound. Linalool exhibited the most dramatic relative increase, more than doubling from 30.5 µg/g in controls to 65.0 µg/g under combined PGR treatment—an increase of 113%—demonstrating its strong responsiveness to synergistic hormonal effects. Isomenthone and geranyl formate increased moderately by approximately 27%, while menthone showed the smallest increase of 16.5%. The low standard deviations across treatments confirm the reliability and reproducibility of these findings. The observed effects of PGR treatments on *Pelargonium graveolens* highlight the intricate hormonal regulation underlying both morphogenesis and secondary metabolism. The superior shoot proliferation and elongation seen with combined cytokinin (BAP, KIN), gibberellin (GA_3), and auxin (IBA) application reflect the importance of hormonal crosstalk during *in vitro* regeneration. Cytokinins stimulate cell division and shoot initiation, gibberellins enhance cell elongation, and auxins promote root formation and tissue differentiation, collectively driving improved plantlet growth [54]. Among auxins tested, IBA outperformed IAA in stimulating root development, likely due to its greater chemical stability and slower breakdown rate, consistent with findings in other species such as *Ruta graveolens* [55–57]. The increase in total phenolic content and antioxidant activity under combined PGR treatments suggests activation of the phenylpropanoid pathway—a key route for secondary metabolite biosynthesis. These results align with previous studies showing that environmental and hormonal cues can enhance phenolic and essential oil production in *P. graveolens* [58]. The strong positive correlation (R^2 = 0.98) between phenolic content and antioxidant activity further supports their key role in plant defense mechanisms.

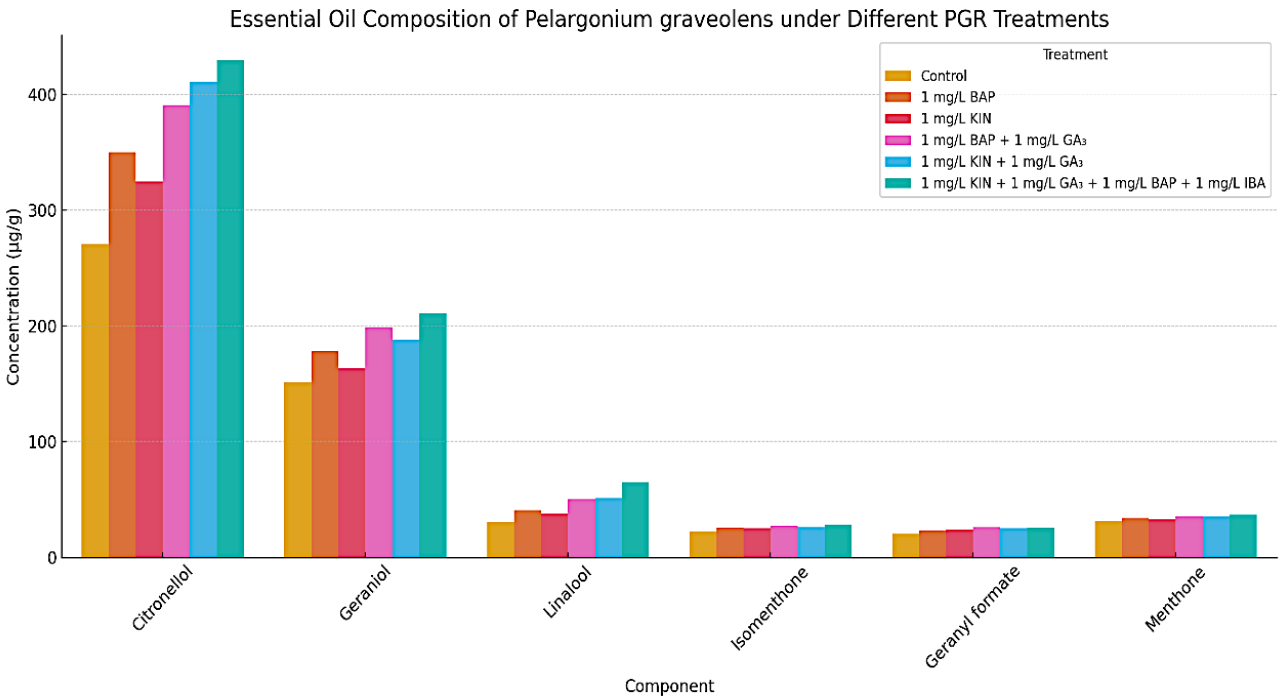


Figure 3. Essential oil composition of Pelargonium graveolens leaves under different plant growth regulator (PGR) treatments. The bar chart shows the mean concentrations (µg/g fresh weight) ± standard deviation (SD) of major monoterpenoids identified by GC-MS, including citronellol, geraniol, linalool, isomenthone, geranyl formate, and menthone. Combined PGR treatment (KIN + GA_3 + BAP + IBA) notably enhanced the levels of citronellol and linalool.

Moreover, the significant increases in essential oil monoterpenoids—particularly citronellol, geraniol, and linalool—indicate hormonal regulation of terpenoid biosynthesis pathways, likely mediated by cytokinin-sensitive mechanisms such as the methylerythritol phosphate (MEP) pathway. Similar cytokinin-induced enhancements in linalool and related compounds have been observed in other geranium species [59], highlighting the potential of plant growth regulator (PGR) manipulation to improve the commercial quality of essential oils. Roman et al. (2024) demonstrated that the essential oil composition of *Pelargonium graveolens* varies considerably depending on drying and extraction methods, with citronellol and linalool levels influenced by environmental factors [60]. Our study builds on these findings by identifying hormonal regulation as an additional factor driving monoterpenoid biosynthesis under highland cultivation conditions. Furthermore, Santos et al. (2025) provided a comprehensive review of *P. graveolens* essential oil, emphasizing that advanced extraction and encapsulation techniques enhance the stability, bioactivity, and food application potential of key monoterpenoids such as citronellol and geraniol [61].

Practical Implications and Future Directions: These findings provide valuable insights for optimizing micropropagation protocols aimed at simultaneously enhancing biomass yield and phytochemical accumulation in *Pelargonium graveolens*. The strategic application of combined cytokinins, gibberellins, and auxins not only supports robust morphogenesis but also significantly enhances the synthesis of pharmacologically and commercially important aromatic compounds.

Future research should aim to elucidate the molecular mechanisms underlying PGR-regulated secondary metabolism, particularly through gene expression analysis of key biosynthetic enzymes. Additionally, validating the effectiveness of these PGR combinations during the *ex vitro* acclimatization phase

will be essential for confirming their applicability in large-scale cultivation and essential oil production.

Together, the observed biochemical and morphogenic enhancements underscore the effectiveness of integrated PGR treatments for improving both growth performance and essential oil profiles in *P. graveolens*. Furthermore, the demonstrated role of IBA in promoting root development under *in vitro* conditions supports its inclusion in optimized protocols. By situating these findings within the Armenian highland context—characterized by distinctive ecological and climatic conditions—this study contributes to the development of region-specific micropropagation strategies for aromatic and medicinal plants with high economic value.

CONCLUSION

The synergistic application of kinetin, gibberellic acid, benzylaminopurine, and indole-3-butyric acid significantly enhanced *in vitro* regeneration, root development, and the biochemical profile of *Pelargonium graveolens*. This combined treatment promoted robust shoot and root formation while markedly increasing phenolic content and antioxidant capacity, thereby strengthening the plant's functional bioactivity. Moreover, the optimized treatments enriched the essential oil composition, with notable increases in citronellol, geraniol, and linalool—compounds valued for their therapeutic properties. The strong positive correlation between phenolic content and antioxidant activity underscores the potential to produce high-value plant material with enhanced medicinal qualities. To fully harness these advantages, future studies should assess the *in vivo* bioavailability and health-promoting effects of these enriched compounds, paving the way for their incorporation into functional foods and natural antioxidant products.

Abbreviations: ANOVA – Analysis of Variance; BAP – 6-Benzylaminopurine; DPPH – 2,2-Diphenyl-1-picrylhydrazyl; GA_3 – Gibberellic Acid; GAE – Gallic Acid Equivalent; GC-MS – Gas Chromatography–Mass Spectrometry; IAA – Indole-3-acetic Acid; IBA – Indole-3-butyric Acid; KIN – Kinetin; MS – Murashige and Skoog (medium); PGR – Plant Growth Regulator; PLCV – *Pelargonium* Leaf Curl Virus; SD – Standard Deviation.

Conflicts of Interest: The authors declare that they have no conflict of interest.

Authors' Contributions: MH – conceptualization, methodology, validation, resources, data curation, writing-original draft preparation, writing-review and editing; GM – methodology, data curation, writing-review and editing; YM – validation, resources, writing-review and editing; MHv – methodology, data curation, writing-review and editing; KD – methodology, data curation, writing-review and editing; KT – resources, writing-review and editing; GA – methodology, data curation, resources, writing-review and editing; KK – data curation, resources, writing-review and editing; AS – conceptualization, validation, resources, writing-review and editing. All authors read and approved the final version of the manuscript.

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