Uluslararası Fen Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Science Research 2025, Vol. 9 (4), 111-120
https://doi.org/10.29329/ijiasr.2025.1386.4

Original article

A Study on the Effects of *In Vitro* Elicitor Applications on Shoot Development in Rose-Scented Geranium (*Pelargonium graveolens* L.)

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Abstract

One of the most widely produced essential oil–bearing plants in the world, rose-scented geranium (*Pelargonium graveolens* L.), is extensively used in industry. Although more than two hundred and ninety components have been identified in its essential oil, the plant is considered a high value-added species, particularly preferred in the perfumery sector due to the increasing demand for its essential oil, its broad industrial applications, and its rose-like aroma. For this reason, the development of economically feasible, environmentally friendly, and sustainable practices that can enhance productivity is of great importance. This study was conducted to determine the effects of elicitor applications on shoot development of rose-scented geranium under in vitro conditions. In the study, the effects of different doses of melatonin, methyl jasmonate (MeJA), and pectin elicitors on in vitro plant growth were comparatively evaluated. As a result of the findings obtained, significant changes were observed in various parameters depending on the type and dose of the elicitor applied. Pectin treatments, particularly at doses ranging from 50 to 150 mg/L, supported regenerative growth by producing values close to or higher than the control group in terms of root formation and shoot number. This indicates that pectin is an elicitor with balanced effects on plant development and low phytotoxicity. In melatonin treatments, an increase in shoot length and dry weight was observed at a low dose (10 µM); however, with increasing doses, marked decreases occurred in rooting, shoot number, and biomass production. MeJA treatments suppressed root and shoot development at all doses and exhibited inhibitory effects on shoot growth, especially at medium and high doses. Overall, it was concluded that pectin may be preferred as a suitable elicitor in in vitro studies focused on regeneration and growth, melatonin may be preferred in studies aiming to enhance shoot length and biomass at low doses, whereas MeJA suppresses shoot development across all evaluated parameters and may yield unfavorable results in regeneration-oriented studies.

Keywords: Rose-scented geranium, Regeneration, Melatonin, Methyl jasmonate (MeJA), Pectin

Received: 11 November 2025 * **Accepted:** 31 December 2025 * **DOI:** https://doi.org/10.29329/ijiasr.2025.1386.4

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INTRODUCTION

Rose-scented geranium (*Pelargonium graveolens* L.) is one of the top twenty essential oil–producing plants worldwide and is widely used in the perfumery, cosmetic, food, and flavor industries due to its rose-like fragrance (Kumar et al., 2022). Because its aroma can substitute for the much more expensive rose oil, it is colloquially referred to as "the poor man's rose oil" in some countries (Aydınlık and Yücer, 2021). More than two hundred and ninety biochemical constituents have been identified in rose-scented geranium, including terpenoids, flavonoids, steroids, alkaloids, and other compounds (Amel et al., 2022).

Although its origin is South Africa, rose-scented geranium is a perennial or annual plant commercially cultivated in tropical and subtropical regions of Europe and Asia, including France, Belgium, Spain, Egypt, Morocco, Algeria, Réunion Island, Russia, China, Madagascar, Israel, and India. The plant can reach up to 1.5 m in height and 1 m in width and possesses a strong stem. Its leaves are pubescent, the flowers are white or pink in color, and the flowering period lasts approximately six months. The main commercially available geranium oil types include Bourbon, China, Algeria, Egypt, and Morocco.

Due to the wide range of applications of its essential oil across different industries, global demand is very high (Benazir et al., 2013; Kumar et al., 2022). The essential oil is obtained from the entire above-ground parts of the plant. The major constituents identified in the essential oil are citronellol (33.6%), geraniol (26.8%), linalool (10.5%), citronellyl formate (9.7%), and p-menthone (6.0%) (Rana et al., 2002). Global production of geranium essential oil ranges between 500 and 750 tons annually, with China being the leading producer, followed by Egypt, Morocco, and India. Due to the increasing demand for essential oil derived from rose-scented geranium (*P. graveolens*), there is a need to develop economically feasible, environmentally friendly, and sustainable agrotechnologies capable of significantly enhancing productivity (Maazed et al., 2023).

In plants produced through tissue culture, the synthesis of desired secondary metabolites can be achieved under suitable conditions, and their levels can be positively altered through various treatments; moreover, the production of novel secondary metabolites is also possible. In addition, the rapid large-scale production of highly standardized plant material contributes to balancing supply and demand (Vuran and Türker, 2021).

The antioxidant activity of rose-scented geranium essential oil is directly correlated with its chemical composition as well as its phenolic and flavonoid contents (Cavar and Maksimovic, 2012). Substances that stimulate secondary metabolism in plants are referred to as elicitors. Elicitors are classified as biotic or abiotic according to their origin. In tissue culture systems, secondary metabolite production is influenced by both biotic and abiotic stress factors. The concentration of elicitors, duration

of application, developmental stage, and timing of application are critical parameters that must be considered to enhance secondary metabolite production (Vuran and Türker, 2021). Within the scope of this study, three different elicitors (melatonin, methyl jasmonate, and pectin) were applied to evaluate their effects on shoot regeneration.

MATERIALS and METHODS

The research was conducted between 2024 and 2025 at the Department of Field Crops Medicinal & Aromatic Plants Laboratory and Tissue Culture Laboratory of Akdeniz University, as well as the Tissue Culture Laboratory of the Güney Agripark R&D Center. Axillary buds of *P. graveolens* obtained from the Tissue Culture Laboratory of the Güney Agripark R&D Center were used as plant material, and in vitro micropropagation was performed. During the study, a laminar airflow cabinet, climate chamber, growth room, autoclave, ultrapure water system, drying oven, and Clevenger apparatus were utilized.

Plant material

Rose-scented geranium (*Pelargonium graveolens*) Bourbon cultivar was used as the plant material in the study.

Micropropagation

During micropropagation, 200 mL glass jars were used, and three explants were placed in each jar. Sucrose at a concentration of 3% was used as the carbon source in all media, which were solidified with 0.7% agar, and the pH was adjusted to 5.7–5.8. Media were sterilized in an autoclave at 121 °C under 1.2 atm pressure for 15 minutes. Cultures were incubated at 24 °C under a 16/8 h photoperiod with a light intensity of 4000 lux.

Sterilization of plant material

Axillary buds of *P. graveolens* were washed under running tap water and then immersed in 70% ethanol for 30 seconds with gentle agitation, followed by rinsing once with autoclaved water. Subsequently, sterilization solutions containing 10%, 15%, and 20% commercial sodium hypochlorite supplemented with two drops of Tween-20 were prepared, and the explants were agitated for 5, 10, and 15 minutes. After treatment, samples were rinsed twice with autoclaved distilled water and subjected to sterilization trials with three replicates. Successful sterilization was achieved at all applied concentrations and durations. In this study, sterilization with 10% sodium hypochlorite for 15 minutes was selected as the preferred method.

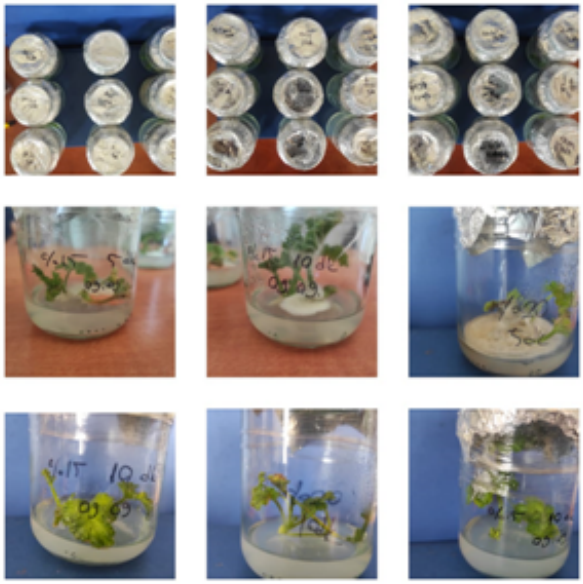


Figure 1. Representative images from the sterilization experiment

Determination of Shoot Culture Medium Composition

During the micropropagation stage, Murashige and Skoog (MS) basal medium was used as the shoot culture medium, supplemented with benzylaminopurine (BAP) at a concentration of 0.25 mg/L and indole-3-butyric acid (IBA) at a concentration of 0.025 mg/L as plant growth regulators.

Preparation and Application of Elicitors under In Vitro Conditions

Table 1. Elicitor doses applied in the study

Number	Applications	Doses
1	Control	-
2	Melatonin	10 µM
3	Melatonin	20 µM
4	Melatonin	40 µM
5	Methyl Jasmonate	10 µM
6	Methyl Jasmonate	20 µM
7	Methyl Jasmonate	40 µM
8	Pektin	50 mg/L
9	Pektin	100 mg/L
10	Pektin	150 mg/L

Preparation and Application of Elicitors under In Vitro Conditions

Preparation of Elicitors

The elicitors were sterilized using a 0.22 µm Millipore filter and incorporated into culture media that had been autoclaved at 121 °C for 20 minutes.

Preparation of Melatonin-Containing Media

The melatonin stock solution (Cayman, ≥98%) was dissolved in 96% ethanol and subsequently brought to the desired volume with dH_2O (Simlat et al., 2018). The solution was then sterilized through a 0.22 µm Millipore filter and added to the autoclaved culture medium (rooting medium) to achieve final concentrations of 10 µM, 20 µM, and 40 µM. The media were dispensed as 30 mL aliquots into 200 mL glass jars.

Preparation of Methyl Jasmonate-Containing Media

The methyl jasmonate (MeJA) stock solution (Cayman, ≥98%) was dissolved in 96% ethanol and adjusted to volume with dH_2O (Ali et al., 2019). After sterilization through a 0.22 µm Millipore filter, the stock solution was added to the autoclaved culture medium (rooting medium) to obtain final concentrations of 10 µM, 20 µM, and 40 µM. The media were dispensed as 30 mL aliquots into 200 mL glass jars.

Preparation of Pectin-Containing Media

Pectin (Merck) was dissolved in distilled water, sterilized through a 0.22 µm Millipore filter, and added to the autoclaved culture medium (rooting medium) to obtain final concentrations of 50 mg/L, 100 mg/L, and 150 mg/L (Gadzovska et al., 2014). The media were dispensed as 30 mL aliquots into 200 mL glass jars.

Application of Elicitors

Elicitor treatments consisted of 10 µM, 20 µM, and 40 µM for melatonin and methyl jasmonate, and 50 mg/L, 100 mg/L, and 150 mg/L for pectin. Elicitors were applied by directly incorporating them into the in vitro shoot culture medium at the specified concentrations.

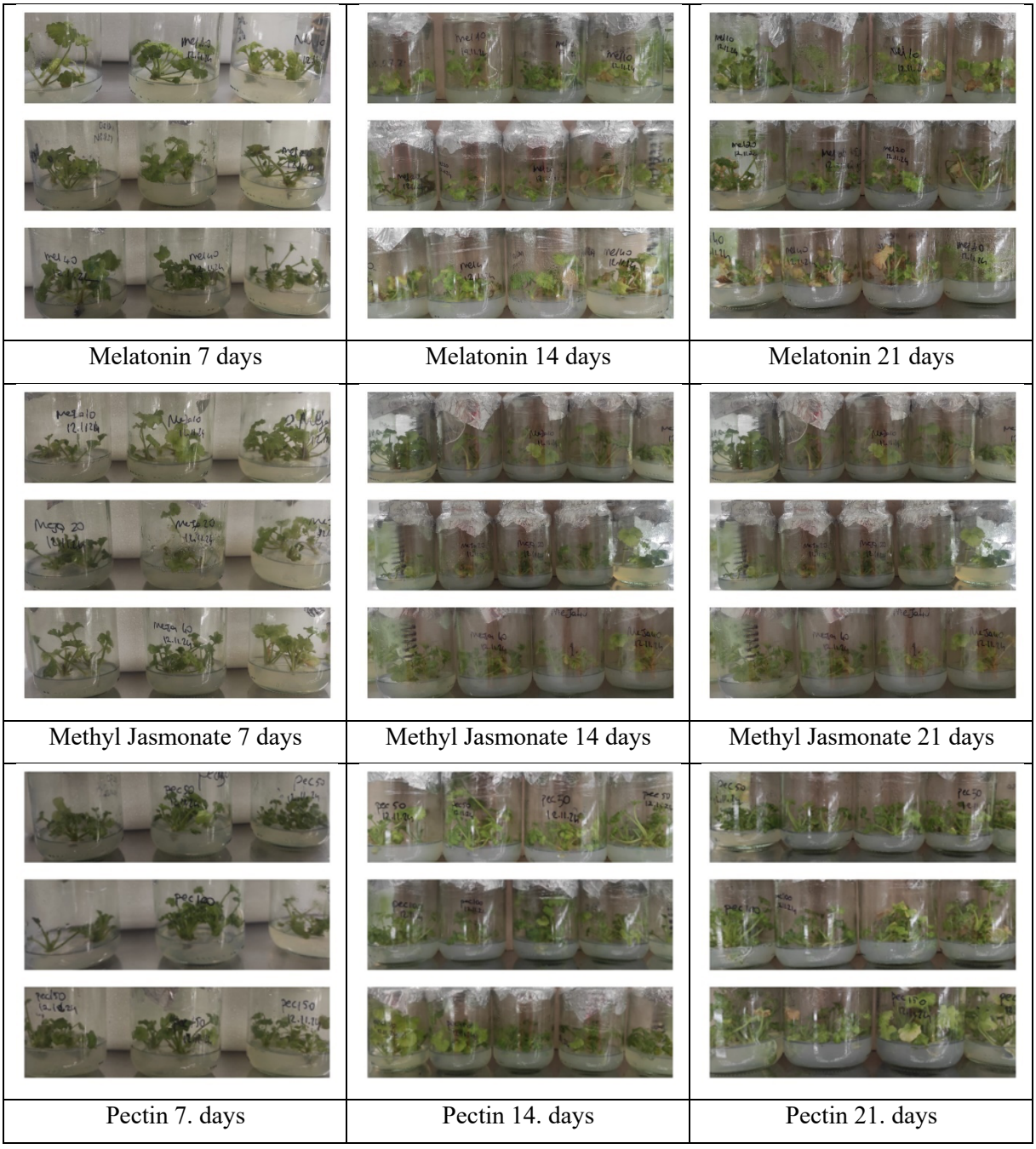
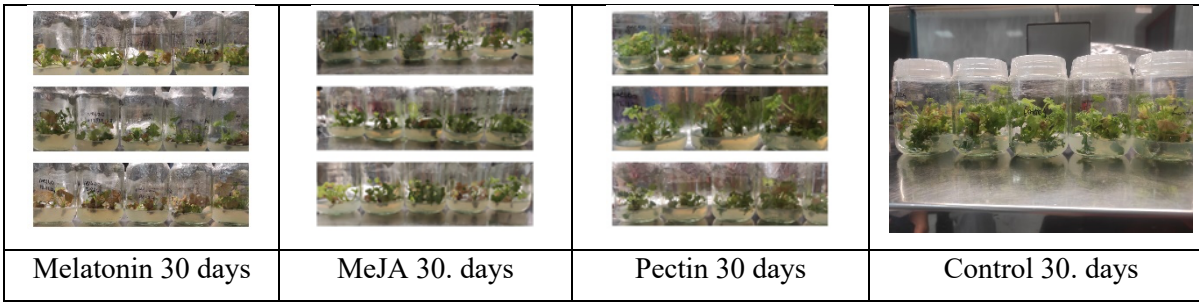


Figure 2. Images of elicitor treatments at 7, 14, and 21 days

After 30 days of culture on the shoot induction medium, the number of shoots per explant, shoot length, and fresh weight were measured; the explants were then dried at room temperature, and their dry weights were recorded.



Melatonin 30 days	MeJA 30. days	Pectin 30 days	Control 30. days

Figure 3. Images of elicitor and control group treatments at 30 days

RESULTS and DISCUSSION

In this study, the effects of different doses of various elicitors (melatonin, methyl jasmonate, and pectin) on plant growth and developmental parameters under in vitro conditions were investigated. Evaluation of the obtained data indicated that significant differences in morphological parameters occurred depending on the type and dose of the elicitor applied.

Table 3. Mean values of root, shoot, and biomass parameters in response to elicitor treatments

	Average root number	Average shoot number	Average shoot length	Average fresh weight	Average dry weight
Control	0,33	5,75	3,04	7,08	0,35
Melatonin 10	0,44	2,53	3,88	5,67	0,37
Melatonin 20	0,16	2,67	3,45	2,22	0,19
Melatonin 40	0,16	1,87	2,23	2,44	0,10
MeJA 10	0,33	2,27	2,96	2,59	0,22
MeJA 20	0	1,60	2,57	1,40	0,15
MeJA 40	0,13	1,60	2,31	2,26	0,22
Pektin 50	0,53	5,11	2,47	4,51	0,31
Pektin 100	0,20	4,87	2,63	4,55	0,32
Pektin 150	0,55	4,89	3,07	3,60	0,24

When Table 3 is evaluated in terms of average root number, pectin treatments—particularly at 50 and 150 mg/L—exhibited higher values compared to the control group. In contrast, melatonin and MeJA treatments suppressed root formation with increasing doses, and no root development was observed at the 20 µM MeJA dose. These findings indicate that MeJA and melatonin negatively affect root regeneration.

With respect to average shoot number, pectin at 50 mg/L produced shoot numbers close to those of the control group, whereas a pronounced decrease in shoot number was observed with increasing doses of melatonin and MeJA. These results demonstrate that elicitors exert an inhibitory effect on regeneration at higher doses.

In terms of average shoot length, melatonin at 10 and 20 µM and pectin at 150 mg/L produced longer shoots compared to the control. However, increasing melatonin concentration resulted in a

progressive reduction in shoot length, with the lowest shoot length observed at the highest melatonin dose. These results indicate that melatonin promotes shoot elongation at low doses but restricts the formation of new shoots. While the pectin elicitor produced lower average shoot lengths than the control at low doses, it generated longer shoots than the control at the highest dose; however, similar to melatonin, pectin also limited shoot number.

Regarding average fresh weight, the control group exhibited the highest mean value, while melatonin at 10 µM and pectin at 50 and 100 mg/L yielded results close to the control. In melatonin and MeJA treatments, significant reductions in average fresh weight were observed at higher doses. Similar trends were noted for average dry weight values; melatonin at 10 µM resulted in higher average dry weight than the control, whereas high-dose melatonin treatments caused marked decreases. These findings suggest that low-dose melatonin may enhance cellular metabolic activity and dry matter accumulation, while increased doses may induce phytotoxic effects that negatively affect shoot development.

Mazrou et al. (2023) investigated the effects of melatonin under drought stress in rose-scented geranium using a 100 µM melatonin treatment and reported that melatonin alleviated the adverse effects of drought—such as reductions in plant growth, leaf yield, and total chlorophyll content—by enhancing antioxidant enzyme activities (ascorbate peroxidase, catalase, and glutathione reductase), reducing oxidative damage, and maintaining cell membrane integrity. Additionally, melatonin improved essential oil components and supported plant stress tolerance by increasing total phenol, proline, and glutathione accumulation, thereby contributing to the preservation of essential oil yield and improvement of product quality even under drought conditions.

Khetsha et al. (2018) examined the effects of foliar application and frequency (7 and 14 days) of bioinhibitors—abscisic acid (ABA) and methyl jasmonate (MeJA)—on yield and wound-healing response time in hail-damaged rose-scented geranium grown under controlled greenhouse conditions. The applied doses were ABA (0, 75, and 150 µM L^{-1}) and MeJA (0, 10, and 20 mM L^{-1}). Hail damage was simulated by 100% defoliation. The authors reported that MeJA application alone significantly reduced leaf area ($P = 0.05$), plant recovery rate ($P = 0.01$), leaf fresh mass ($P = 0.01$), and oil yield ($P = 0.05$) in hail-damaged plants. In the present study, MeJA negatively affected average fresh weight even at low doses compared to other elicitors. The findings of previous studies are consistent with the results obtained in the current research.

Conclusion

In conclusion, pectin applications supported shoot development and growth, whereas melatonin applications exhibited both inhibitory and promotive effects depending on dose, demonstrating the potential to positively influence shoot development and biomass accumulation at lower concentrations under in vitro conditions. In contrast, MeJA applications were observed to suppress shoot development under in vitro conditions.

Acknowledgements

Plant materials of *P. graveolens* were obtained from the Tissue Culture Laboratory of the Güney Agripark R&D Center in Antalya.

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