Physiological and Metabolic Responses of *Euphorbia pulcherrima* to Silver Nanoparticles under *in vitro* Conditions

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Abstract

The findings on *E. pulcherrima* revealed that the hormonal combination of 1 mg L^{-1} BAP with 0.1 mg L^{-1} IAA was the most effective for in vitro biomass induction, achieving the highest response rate of 86.67%. In parallel, the application of Silver Nanoparticles (AgNPs) demonstrated a clear stimulatory effect at low concentrations: the 1.0 mg L^{-1} treatment produced the highest dry weight and bud number, while concentrations of 0.5–1.5 mg L^{-1} significantly enhanced fresh weight compared to the control. Conversely, higher concentrations (2.0–2.5 mg L^{-1}) caused a slight reduction in growth indices.

Notably, AgNPs markedly improved the qualitative and quantitative profiles of secondary metabolites, increasing the number of detected compounds from 21 in the mother plant and 28 in the control to 36 compounds under the 2.5 mg L^{-1} treatment. This enhancement included the induction of new metabolites such as Cyclotetradecane, Linoleic acid, and Farnesol isomer a, along with elevated peak intensities of key compounds like Hexanoic acid and Palmitic acid. Moreover, all six targeted metabolites Diosgenin, α-Tocospiro B, Apocynin, Cyclotetradecane, Farnesol isomer a, and Germanicol acetate were either uniquely induced or reached their highest levels under the various AgNPs treatments. together, these results underscore the pivotal role of AgNPs as an effective elicitor capable of modulating plant defense pathways and enhancing secondary metabolite biosynthesis in vitro.

Keywords: Biomass induction, Elicitors, In vitro culture, Metabolites, Nanobiotechnology and Poinsettia

Introduction

The Euphorbiaceae family, is one of the largest, most diverse, and most widespread families of flowering plants. This family includes poinsettia (*Euphorbia pulcherrima*), a vital and rich source of highly bioavailable secondary metabolites (Basnett *et al*., 2023). These compounds have demonstrated multiple pharmacological properties, including antimicrobial activity (Gemici *et al*., 2025), antioxidant and anti-inflammatory activity, anticancer activity (Gao *et al*., 2016), and calming and analgesic effects (Venkatachalam *et al*., 2018). This diversity in therapeutic effects is attributed to the plant containing major classes of biologically active compounds such as alkaloids, terpenoids, and flavonoids (Mishra *et al*., 2020).

Plant tissue culture is a pivotal biotechnology based on the cultivation of plant parts (explant) in sterile artificial environments, It provides a strategic tool for producing homogeneous plants

and conserving genetic resources, and, most importantly, for stimulating the production of bioactive byproducts with medicinal and industrial value (Nordine, 2025), The success of poinsettia micropropagation depends on the genetic makeup, the type of plant part, and the composition of the nutrient medium, particularly the crucial role of plant growth regulators, a concentration of 6-Benzyl aminopurine (BAP) in the range of 2.2–8.8 μmol achieved the highest rates of axillary bud formation from terminal buds (Pickens *et al*., 2005). Furthermore, culturing stem nodules on MS medium containing 1.0 mg L^{-1} BAP and 0.2 mg L^{-1} NAA resulted in 100% regeneration (Danial & Ibrahim, 2016). Regarding indirect organic synthesis, the optimal hormonal combination for stimulating callus formation from leaf buds consisted of 1.0 mg L-6-BAP, 0.4 mg L-kinetin, 0.1 mg L-NAA, and 0.05 mg L-2,4-D (Qiu *et al*., 2023). Tissue culture provides an effective solution for increasing the sustainable production of secondary compounds (Mehbub *et al*., 2022).

Elicitation is the most successful method for enhancing the production of these compounds, as it stimulates the plant to activate its defense mechanisms, leading to increased biosynthesis of the active compounds (Selwal *et al*., 2025). In this context, silver nanoparticles (AgNPs), ranging in size from 1 to 100 nm (Khan *et al*., 2023), are a promising catalyst in tissue culture systems, as they have demonstrated the ability to enhance the production of secondary metabolites by stimulating cellular defense responses (Marslin *et al*., 2017; Fazal *et al*., 2016), in addition to their role in improving plant tissue growth and formation (Spinoso-Castillo *et al*., 2017). Salih *et al*. (2024) demonstrated that low concentrations of AgNPs stimulate growth, and that the optimal concentration (1.0 mg L^{-1}) is most suitable for inducing cell differentiation. AgNPs also led to changes in the levels of pigments such as chlorophyll, carotenoids, anthocyanins, and phenolic compounds (Tymoszuk & Kulus, 2020). Therefore, stimulated tissue culture using silver nanoparticles represents a sustainable and effective method for increasing the production of biomass and bioactive compounds from poinsettias.

The study aimed to employ in vitro propagation techniques and induction using silver nanoparticles (AgNPs) to produce biomass and secondary metabolites of medical importance.

Materials and Methods

Source of Plant Parts and Sample Preparation

The experiment on biomass production and the induction of secondary metabolites in poinsettia (*Euphorbia pulcherrima*) was conducted in the Plant Tissue Culture Laboratory, Department of Life Sciences, Faculty of Science, from October 25, 2023, to September 1, 2024. Poinsettia (*E. pulcherrima*) seedlings were collected from local nurseries. The foliage (tender shoots and leaves) was collected from the mother plant, washed, and dried in a shaded, well-ventilated area at room temperature (drying time ranged from 3 to 7 days). After drying, the plant samples were manually crushed into a fine powder and stored in airtight containers for extraction.

Preparing Samples for Tissue Culture and Surface Sterilization

Terminal tender buds were excised for use in biomass production. This process was carried out under sterile conditions within a laminar airflow hood after sterilization with 70% ethanol, thus minimizing the risk of microbiological contamination. Surface sterilization of plant parts is a crucial step for successful in vitro tissue culture. The selected plants were thoroughly washed while still in their natural environment (pots) using a water spray pump to remove dust and surface impurities. After the plants dried, the tender plant parts intended for tissue culture were carefully excised and then gently wiped with cotton wool and 70% ethanol. The excised plant parts were then immersed in a sterile solution of 0.1% mercuric chloride with two drops of Tween-20 added as a surfactant for 5 minutes. This was followed by five consecutive rinses with sterile distilled water to remove any residual sterilizing agent. These precise steps were performed in a sterile laminar airflow cabinet to ensure the samples were protected from atmospheric contamination. The sterile parts were then inoculated directly into the pre-prepared nutrient medium.

Preparation of the Culture Medium

Murashige & Skoog (1962) MS medium supplemented with 4.33 g L^{-1} of vitamins, 30 g L^{-1} of sucrose, 80 mg L^{-1} of adenine sulfate, and 120 mg L^{-1} of acidic sodium orthophosphate was used. Plant growth regulators (auxins and cytokinins) were added after being dissolved in 70% dimethyl sulfoxide (DMSO). The pH of the medium was adjusted to 5.7–5.8 using a pH meter and 0.1 N NaOH and HCl solutions. 5 g L^{-1} of agar was added to solidify the medium and distributed at a rate of 25 mL per glass jar. The medium was then sterilized in an autoclave for 20 minutes at 121°C and pressure. 1.5 bar.

Preparation of the Liquid Medium and Bioreactors

The same steps were followed for preparing the liquid medium for the bioreactors, with the exception of adding agar. The medium was distributed in Plantform Bioreactors at a rate of 500 ml per reactor. The reactors were sterilized and equipped with a dynamic and controlled aeration system consisting of an air pump that pushed the nutrient medium upwards to feed the plant parts periodically and intermittently (5 minutes of pumping every 4 hours) through sterilization filters (0.22 µm) to ensure regular aeration and feeding.

Plant Explant Culture

The culture process was carried out in a UV-sterilized laminar airflow cabinet 70% ethanol.

Stage 1: Establishing Primary Tissue Cultures: Terminal buds were inoculated into glass containers containing the nutrient media specific to each plant. The cultured media were transferred to a plant growth chamber under controlled incubation conditions: a light intensity of 40.5 µMm-2s-1 for 12 hours (day-1) and a constant temperature of 25 ± 2°C.

Stage 2: Applying the Silver Nanoparticle Experiment in Bioreactors: After determining the optimal medium for biomass production, the expanded tissues were transferred to bioreactors to apply the nanoparticle experiment (Figure 1).

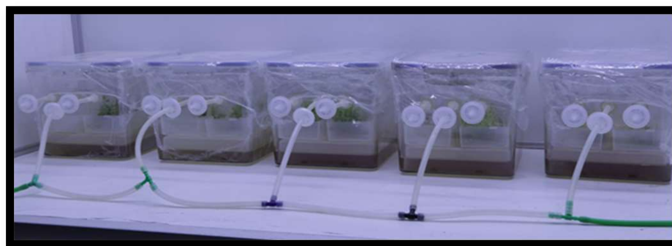


Figure1: Bioreactors

Biomass Production Stimulation and Growth Regulators

Eighteen combinations of plant growth regulators (NOA, IAA, NAA, TDZ, BAP, and KIN), as shown in Table 1, were tested to stimulate biomass production. The percentage of stimulation was used to determine the most efficient combination:

Percentage of Biomass Stimulation = Number of Plants with Biomass Formed / Total Number of Plants Cultivated × 100

Table (1): Table of plant growth regulator supplements to stimulate *in vitro* biomass production in poinsettias

Treatments	Plant growth regulators					
	NOA	IAA	NAA	TDZ	BAP	KIN
1	0	0	0.1	0	0	1
2	0	0	0.1	0	1	0
3	0	0	0.1	1	0	0
4	0	0.1	0	0	0	1
5	0	0.1	0	0	1	0
6	0	0.1	0	1	0	0
7	0.1	0	0	0	0	1
8	0.1	0	0	0	1	0
9	0.1	0	0	1	0	0
10	0	0	0.2	0	0	1
11	0	0	0.2	0	1	0
12	0	0	0.2	1	0	0
13	0	0.2	0	0	0	1
14	0	0.2	0	0	1	0
15	0	0.2	0	1	0	0
16	0.2	0	0	0	0	1
17	0.2	0	0	0	1	0
18	0.2	0	0	1	0	0

Application of Silver Nanoparticle (AgNPs) Induction

After obtaining sufficient biomass and maintaining the cultures, the multiplied plant parts were transferred to agar-free bioreactors and left for one week to acclimatize. Subsequently, they underwent multiple treatments using silver nanoparticles (AgNPs) to stimulate growth and the production of secondary compounds for four weeks.

The silver nanoparticle solution was prepared by dissolving 25 mg of the nanoparticles in 50 mL of sterile distilled water preheated to 35°C (so that each mL of solution contained 0.5 mg of the solute). Magnetic stirring was used to ensure complete dissolution and homogeneity of the solution within a sterile culture chamber to minimize the risk of contamination. To enhance sterilization, the resulting solution was filtered using a 0.1 µm pore filter and added by injection through the aeration tube of the bioreactors at levels of (0, 0.5, 1.0, 1.5, 2.0, and 2.5) mg L^{-1}

The following indicators were calculated after the treatment period:

Fresh Weight: The biomass was washed, dried, and weighed using a sensitive balance.

Number of Buds: The number of buds or shoots formed on each biomass was counted.

Dry Weight: The plant samples were completely dried and weighed using a precision balance.

Extraction of Secondary Compounds:

A modified plant extraction method based on Harborne (1998) was adopted.

Five grams of dry powder were weighed and placed in a thimble extraction bag. The sample was treated with 50 mL of petroleum ether using a Soxhlet apparatus to remove fats, then dried. The plant powder was soaked in a mixture of 50 mL of acidified solution (10% acetic acid in 90% ethyl alcohol) for 24 hours using a laboratory shaker. The extracts were concentrated using a rotary evaporator, and the pH was adjusted to 9 by adding concentrated ammonium hydroxide. After filtration, the secondary compounds were separated using a separation funnel and chloroform.

GC-MS Instrument and Analytical Conditions:

Qualitative analysis of plant extracts was performed using gas chromatography-mass spectrometry (GC-MS) (Figure 12) at the Basra Oil Company laboratories, in an analytical environment equipped according to the highest technical standards. An Agilent Technologies 7890B Gas Chromatograph was used, connected to an advanced Agilent Technologies 5977A Mass Selective Detector (MSD) equipped with an electron impact ionization (EI) detector, providing high-resolution analytical capabilities. The analysis relied on an HP-5ms capillary column with a composition of 5% phenyl and 95% dimethylpolysiloxane, measuring 30 m × 250 µm × 0.25 µm, specifically designed for the separation of complex organic compounds.

The furnace temperature was initially set at 40°C for 5 minutes to ensure the stability of volatile substances. This was followed by a thermal gradient program at a rate of 8°C per minute until reaching 300°C, with the final temperature held for 20 minutes to ensure the analysis of compounds with high boiling points. High-purity helium gas was used as the carrier gas at a flow

rate of 1 mL/min-1, with a purge flow rate of 3 mL/min-1, to ensure separation accuracy and minimize interference.

Injection and Spectroscopic Analysis Conditions:

The injection process was performed using the Splitless pulse injection method at 290°C to ensure complete sample entry into the column. The injection volume was 0.5 µL per run. The electron ionization source was set to 230°C, and the scanning speed was 1562 amu/sec within a mass range of 44–750 m/z, allowing for broad coverage of the spectrum of potential secondary compounds, the spectral data were analyzed using the NIST 2020 standard compound libraries to ensure the identification of the extracted chemical compounds.

Statistical Analysis of Growth Indicators:

The experiment was designed according to a Complete Randomized Design (CRD) system, where it was carried out as single-factor experiments, with 9 replicates per treatment, represented by three bioreactors, each containing 3 plant masses. The biomass induction experiments and the biomass growth indicators (fresh weight of biomass, number of vegetative buds, and dry weight of biomass) were subjected to statistical analysis using one-way ANOVA to verify the presence of significant differences between treatments. SPSS version 26 was used to perform the analysis, and significant differences between means were determined using the Least Significant Difference (LSD) test at a statistical significance level of ($P \leq 0.05$).

Results and discussion:

Biomass induction ratio of *E. pulcherrima*:

Results shows the significant difference achieved by the (BAP + 0.1IAA1) combination over all other combinations. Panel (1) recorded (86.67%), the highest percentage of in vitro biomass induction for E. pulcherrima, followed by the combinations 1KIN + 0.1 IAA and 11TDZ + 0.1 IAA, both at (73.33%). The combinations (1TDZ + 0.1 NAA, 1KIN + 0.2 IAA, and 1TDZ + 0.2 NOA) all recorded the lowest percentage of *in vitro* biomass induction for E. pulcherrima, at 13.33%. Figure (2).

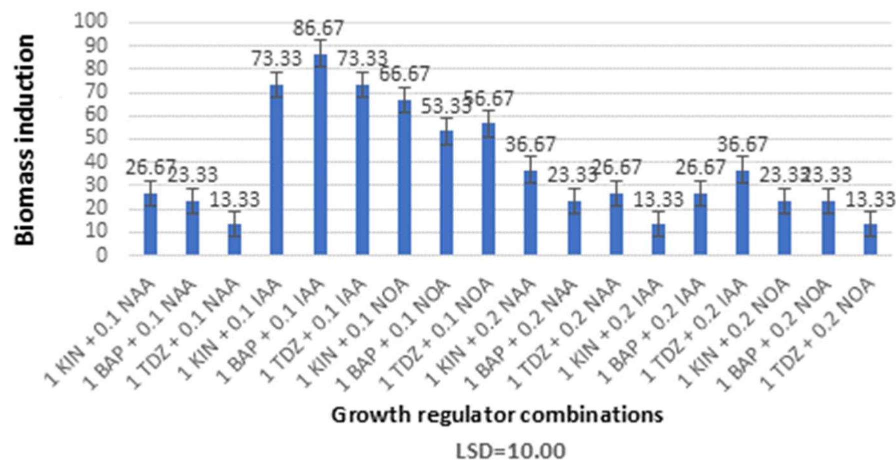
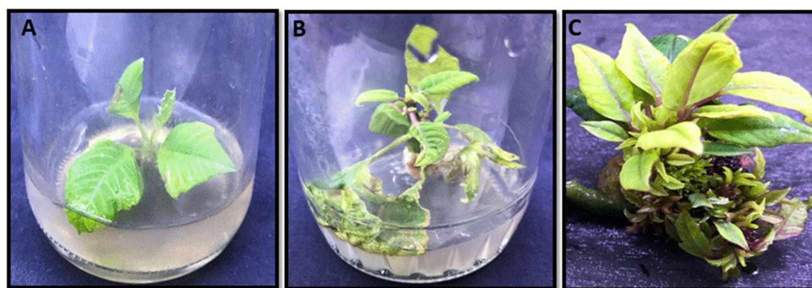


Figure (2): Percentage of *in vitro* biomass induction of *E. pulcherrima*



Panel (1): Stages of tissue culture propagation of *E. pulcherrima*: A: Terminal bud culture, B: Multiplication stage, C: Biomass production stage on multiplication medium 1 BAP + 0.1 IAA

The results showed that the combination of BAP + 0.1 IAA1 achieved the highest rate of vegetative bud mass induction in ***E. pulcherrima*** (86.67%) compared to the other combinations, indicating that a simple hormonal balance in favor of cytokinin's with a low auxin content supports the process of cell division directed towards budding. This observation is consistent with the principle that cytokinin's, specifically BAP, stimulate apical differentiation and the formation of new buds when auxin is present at low levels. In contrast, some combinations that contained NAA or a higher concentration of IAA led to a significant reduction in the rate of bud induction (13.33%), reflecting the role of the type and concentration of auxin in diverting the cell pathway towards radical differentiation or the formation of unwanted tissue instead of budding. The efficacy of TDZ and KIN was also significant, but less than that of BAP under the current experimental conditions. These results are consistent with Grzegorczyk-Karolak *et al*. (2015), who confirmed that BAP is the most effective cytokinin for bud proliferation and growth in Scutellaria alpina bud cultures, particularly at concentrations of 2 and 4 µM in conjunction with 0.57 µM IAA. These concentrations produced the highest number of buds (23 to 25 buds per plant part) within five weeks. This also aligns with Almzori (2022) finding that BAP effectively enhanced tissue

culture indices in poinsettia in vitro, with a concentration of 1.0 mg/L being the most effective in increasing bud number and length, while a concentration of 2.0 mg/L resulted in the highest number of leaves.

BAP is one of the most widely used and successful cytokinin's in plant tissue culture, due to its high efficacy in promoting cell division, bud proliferation, and increasing biomass in various plant cultures. Its high efficacy stems from its ability to break apical dominance and stimulate lateral bud growth, which directly translates into increased vegetative propagation rates. This tissue response contributes to increasing the number of resulting buds, thereby enhancing the plant's overall biomass in terms of both fresh and dry weight. Furthermore, BAP's role appears more pronounced during primary growth, as it specifically supports cell proliferation and increases plant size, unlike some other plant regulators that contribute more significantly to later stages, such as regulating the production of secondary metabolites, as noted by Mahardhini *et al*. (2023)

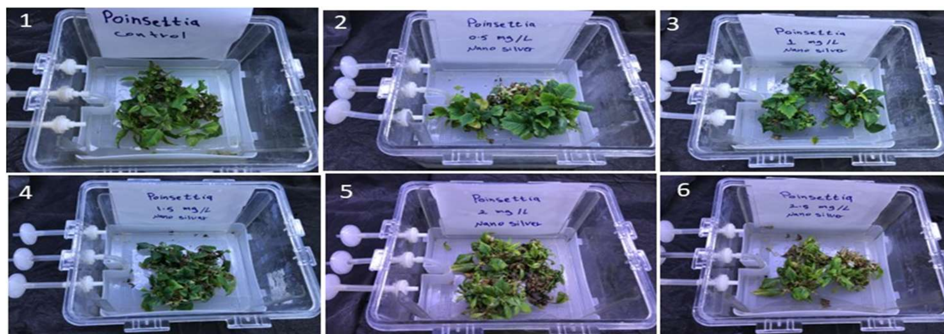
This differentiation in hormonal functions explains the superiority of BAP in inducing vegetative buds and increasing biomass in tissue-cultured poinsettia, while the effect of TDZ and KIN may be more related to other processes or different concentrations. These results confirm that the efficiency of biomass induction depends on the type and level of growth regulators and their relative balance, as the synergy between cytokinin's and auxins plays a pivotal role in determining the pathway of cell differentiation and the response of plant tissue under in vitro culture conditions. (Schaller *et al*., 2015).

Effect of Silver Nanoparticles on Biomass Growth Indices of *E. pulcherrima* In Vitro

The highest fresh weight values were recorded at treatments 0.5, 1.0, and 1.5 mg L^{-1}, at 8.82, 9.17, and 8.43 g, respectively, as shown in Table (2). These values were very close to each other, with no significant difference between them, but they were clearly superior to the control treatment, which reached 6.68 g. The higher concentrations of 2.0 and 2.5 mg L^{-1} decreased to 6.72 and 6.45 g, respectively, and were close to the control in terms of significance. The highest dry weight value was recorded at 1.0 mg L^{-1} (0.94 g), followed by the 0.5 mg L^{-1} treatment (0.88 g), both of which were significantly higher than the control (0.66 g). In contrast, the 1.5 mg/L treatment showed an average value of 0.78 g, which did not differ significantly from the control. The higher concentrations of 2.0 and 2.5 mg/L resulted in a decrease to 0.59 and 0.56 g, respectively, with significant differences compared to the other treatments. The 0.5, 1.0, and 1.5 mg/L treatments also achieved the highest average number of buds, reaching 21.11, 22.78, and 19.00 buds, respectively, significantly exceeding the control's 15.00 buds. At the higher concentrations of 2.0 and 2.5 mg/L, the number decreased to 17.89 and 15.89 buds, respectively, and these values were close to the average number of buds in the control group, showing no significant differences (Figure 2).

Table (2) Effect of silver nanoparticles on biomass growth indicators of *E. pulcherrima* in vitro

Silver nanoparticle mg L^{-1}	Growth indicators ± standard deviation		
	Fresh weight gm	Dry weight gm	Number of buds
Control	6.68 ± 1.02 ^{b}	0.66 ± 0.10 ^{c}	15.00 ± 3.32 ^{d}
0.5	8.82 ± 0.75 ^{a}	0.88 ± 0.09 ^{a}	21.11 ± 1.83 ^{ab}
1.0	9.17 ± 0.48 ^{a}	0.94 ± 0.10 ^{a}	22.78 ± 2.39 ^{a}
1.5	8.43 ± 0.75 ^{a}	0.78 ± 0.08 ^{b}	19.00 ± 1.73 ^{bc}
2.0	6.72 ± 0.75 ^{b}	0.59 ± 0.07 ^{cd}	17.89 ± 2.15 c
2.5	6.45 ± 0.57 ^{b}	0.56 ± 0.07 ^{d}	15.89 ± 2.71 ^{c}
LSD	1.71	0.10	3.11



Panel (2): *E. pulcherrima* biomass, cultured in liquid medium with varying concentrations of silver nanoparticles using a bioreactor. 1: Control (no silver nanoparticles), 2: 0.5 mg L^{-1} silver nanoparticles, 3: 1 mg L^{-1} silver nanoparticles, 4: 1.5 mg L^{-1} silver nanoparticles, 5: 2 mg L^{-1} silver nanoparticles, 6: 2.5 mg L^{-1} silver nanoparticles

The results of the current study indicate that the concentration of silver nanoparticles (0.5–1.5 mg L^{-1}) was the most effective in promoting vegetative growth in poinsettias in vitro, leading to a significant increase in fresh and dry weight and the number of shoots compared to the control. This can be explained by the fact that silver nanoparticles, at optimal concentrations, stimulated cell division and tissue growth through their direct effect on regulating metabolic activity, thus contributing to increased efficiency of physiological processes. This, in turn, led to increased biomass accumulation and improved nutrient uptake and distribution in the developing tissues (Hegazi *et al*., 2021). However, with increasing concentrations (2–2.5 mg L^{-1}), a gradual decrease in all indicators was observed, with fresh and dry weight and the number of shoots falling below the control levels. This may be attributed to the induction of oxidative stress as a result of the accumulation of silver particles inside the cells and the formation of reactive oxygen species (ROS), inhibiting basic biological processes such as respiration and potentially having a toxic

effect on meristematic cells, which weakened the formation of new buds and inhibited their growth (Jalil *et al*., 2019).

The application of silver nanoparticles represents an effective technology for promoting plant growth and development, enabling them to act as biostimulants, especially under abiotic stress conditions. This is attributed to their ability to penetrate cell barriers and, due to their small size, exhibit high solubility and efficient absorption by plants, which greatly improves the qualitative and quantitative parameters of plants when exposed to various biotic and abiotic stresses. It has been shown that exposing plant cells to silver nanoparticles promotes bud regeneration and development in vitro, and this is what the results of the current study have concluded, in agreement with many researchers in this field (Aqeel *et al*., 2022; Hegazi *et al*., 2021; Al-Khayri *et al*., 2023). This may be because silver nanoparticles also induce stress in plants, leading to a decrease in CO2 assimilation rates and alterations in the performance of the second photosynthetic system. This is considered a protective response to stress conditions, as confirmed by Dewez *et al*. (2018). Furthermore, silver nanoparticles inhibit chlorophyll synthesis, reducing photosynthetic efficiency and disrupting water balance within leaves. This can lead to a decrease in stomatal conductance, resulting in reduced gas exchange and impaired carbon dioxide assimilation (Xalxo *et al.*,2021).

In contrast, nanoparticles can enhance plant defense mechanisms, mitigating the harmful effects of abiotic stress due to their small size, which allows for improved absorption and targeted chemical delivery. However, silver nanoparticles may also contribute to the production of reactive oxygen species (ROS), leading to phytotoxicity (Falco *et al*., 2020; Marslin *et al*., 2017). Increased levels of reactive oxygen species (ROS) associated with nanoparticle exposure are likely to activate stress signaling pathways, stimulating defense responses and enhancing the plant's ability to adapt to stress (Jalil *et al.*, 2019). When plant cells sense stress, a variety of secondary messengers, including calcium ions, reactive oxygen species (ROS), phospholipids, and nitric oxide (NO), transmit and amplify stress signals. This process often involves specific protein kinases (Zhang *et al*., 2023), including the mitogen-activated protein kinase (MAPK) pathway. MAPK activation drives plant antioxidant components to sequentially come into contact with ROS. Plant secondary compounds then shed ROS, and as a result, cellular responses, such as cell division and differentiation, are regulated to achieve normal plant cell growth and development. This early activation of stress signaling mechanisms is important for enhancing tolerance to abiotic stresses (Mohanta *et al*., 2018).

The results of the current study are consistent with those of Hassan *et al.* (2019), who reported that nanoparticles at low concentrations improved the growth and development of olive plants *in vitro*, and the results of Castro-González *et al*. (2019) on *Stevia rebaudiana* B tissues, and another study that showed that exposure to high concentrations of silver nanoparticles in pearl millet (*Pennisetum glaucum*) led to significant reductions in both fresh and dry mass (Khan *et al*., 2019). They attributed this effect to the inhibition of ethylene synthesis or action (Pérez-Llorca *et al*., 2023). a study by Salih *et al*. (2024), who confirmed that a nutrient medium supplemented with 1.0 mg L^{-1} of silver nanoparticles significantly stimulated callus formation and somatic

embryogenesis in in vitro date palms, noting that higher concentrations of the particles reversed these effects.

Effect of silver nanoparticles on the induction of secondary metabolites in *E. pulcherrima* cultures

Table (3) shows the results of the metabolic chemical compounds detected using gas chromatography-mass spectrometry in the mother poinsettia, the control treatment, and the silver nanoparticle treatments. The results indicate a significant effect (both quantitative and qualitative) of silver nanoparticles on the production of chemical compounds in tissue-cultured poinsettias treated with different levels of silver nanoparticles (0.5, 1, 1.5, 2, and 2.5 mg L^{-1}). The analysis identified 39 compounds, 21 of which were detected in the mother poinsettia treatment. The number of compounds produced by the poinsettia biomass increased when using in vitro tissue culture techniques, reaching 28 compounds in the control treatment. The number of compounds in the tissue-enriched poinsettia biomass treated with different levels of silver nanoparticles increased, with the numbers reaching (35, 33, 33, 33, and 36) compounds when treated with (0.5, 1, 1.5, 2, and 2.5) mg L-1 respectively.

As can be seen from Table (3), the compounds Hexanoic acid, 2-Decanone, 4-Vinylphenol, Nonanoic acid, Propyl-2-methoxy-4-propylphenol, Dihydroactinidiolide, L-Pyroglutamic acid, 2-Pentadecanone, 6,10,14-trimethyl-, Loliolide, 1-Octadecene, Palmitic acid, Octadecanal, 1-Octadecanol, Oleic acid, Stearic acid, ethyl ester, and Germanicol were present in all treatments (mother, control, and silver nanoparticle treatments), while the mother poinsettia was the only one to produce 2,4-Dimethylfuran and Phenylacetamide, while the control and mother had a mark in the production of the compound 2-Methoxy-4-vinylphenol.

While the silver nanoparticle treatments and the control co-produced Dimethyl Sulfoxide (DMSO), Dimethyl sulfone, Hexamide (Hexanamide), DL-Proline, 5-oxo-, ethyl ester, Apocynin, Palmitic acid, methyl ester, Phytol, Octadecanamide, and Diosgenin, the two treatments (0.5 and 2.5) mg L-1 of silver nanoparticles and the control co-produced γ-Sitosterol. Cyclotetradecane, Farnesol isomer a, Linoleic acid, Linolenic acid, Ethyl Oleate, and α-Tocospiro B were present in all silver nanoparticle treatments, while the (0.5, 1.0, and 2.5) mg L-1 treatments produced Germanicol acetate, and the 2.5 mg L-1 treatment alone produced β-Amyrin in this experiment.

The highest peak areas were observed for the adult compounds 2-Decanone, 4-Vinylphenol, Nonanoic acid, 2-Methoxy-4-vinylphenol, Dihydroactinidiolide, 2-Pentadecanone, and 6,10,14-trimethyl (16.07, 3.41, 6.26, 6.57, and 97.37, respectively) in the mother poinsettia plant. In the control group, the highest peak values were recorded for the compounds propyl-2-methoxy-4-propylphenol, loliolide, oleic acid, dimethyl sulfoxide (DMSO), dimethyl sulfone, hexamide, palmitic acid, methyl ester, and phytol (9.08, 257.37, 6.05, 105.63, 95.73, 45.58, 8.55, and 10.56, respectively). Ethyl oleate reached the highest peak areas. The peak concentration in the 0.5 mg/L treatment was 82.60, while the highest peak for Farnesol isomer A in the 1.0 mg/L treatment was 7.64. The highest peaks for 2-Pentadecanone, 6,10,14-trimethyl stearic acid, ethyl ester, DL-

Proline, 5-oxo-ethyl ester, and Diosgenin were achieved in the 1.5 mg/L treatment, at 46.97, 3.27, 99.90, and 8.33, respectively. The highest peaks for hexanoic acid, apocynin, cyclotetradecane, linolenic acid, and germanicol acetate were achieved in the 2.0 mg/L treatment (131.59, 23.39, 21.91, 32.62, and 3.99, respectively). L-Pyroglutamic acid, Octadecene-1, Palmitic acid, Octadecanal, 4-Amino-2,6-dihydroxypyrimidine, Germanicol, β-Sitosterol, Octadecanamide, Linoleic acid, and α-Tocospiro B peaked at the 2.5 mg L-1 treatment, registering 8.00, 2.69, 39.02, 160.90, 72.93, 23.04, 38.51, 19.80, 19.43, and 2.64, respectively.

Table (3) Secondary metabolites identified in gas chromatography-mass spectrometry analysis of ex vivo poinsettia exposed to different levels of silver nanoparticles

NO.	RT	Compound	Peak Area (10^6)						
			Mother plant *E. pulcherrima*	Silver nano particles mg. L^{-1}					
				0.0 Control	**0 .5**	**1**	**1.5**	**2**	**2.5**
1	**6.01**	**2,4-Dimethylfuran**	**1.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
2	**8.87**	**Hexanoic acid**	**1.12**	**92.31**	**92.69**	**98.06**	**109.70**	**131.59**	**89.68**
3	**13.02**	**2-Decanone**	**16.07**	**1.14**	**1.14**	**1.49**	**1.26**	**1.88**	**2.31**
4	**13.55**	**4-Vinylphenol**	**3.41**	**2.08**	**1.20**	**1.56**	**1.32**	**2.49**	**3.30**
5	**14.65**	**Nonanoic acid**	**6.26**	**4.01**	**2.88**	**3.24**	**3.00**	**3.32**	**3.44**
6	**15.23**	**2-Methoxy-4-vinylphenol**	**1.32**	**1.02**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
7	**15.65**	**Phenylacetamide**	**1.42**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
8	**16.35**	**Propyl-2-methoxy-4-propylphenol**	**1.21**	**9.08**	**1.26**	**1.12**	**1.15**	**1.08**	**1.27**
9	**18.73**	**Dihydroactinidiolide**	**6.57**	**1.95**	**4.72**	**3.32**	**3.40**	**3.33**	**3.53**
10	**19. 22**	**L-Pyroglutamic acid**	**3.97**	**6.75**	**7.47**	**7.80**	**7.88**	**7.81**	**8.00**
11	**20.41**	**2-Pentadecanone, 6,10,14-trimethyl**	**97.37**	**44.05**	**46.56**	**46.89**	**46.97**	**44.31**	**43.22**
12	**21.92**	**Loliolide**	**54.60**	**257.37**	**154.16**	**143.18**	**187.73**	**137.90**	**102.39**
13	**22.21**	**1-Octadecene**	**1.32**	**1.25**	**1.47**	**1.80**	**1.87**	**2.27**	**2.69**
14	**23.49**	**Palmitic acid**	**31.96**	**32.53**	**34.47**	**23.46**	**20.54**	**27.60**	**39.02**
15	**24.08**	**Octadecanal**	**1.01**	**4.79**	**4.44**	**15.65**	**1.32**	**3.21**	**160.90**
16	**24.62**	**1-Octadecanol**	**3.21**	**1.49**	**1.55**	**1.57**	**1.65**	**1.92**	**2.14**
17	**26.01**	**Oleic Acid**	**1.13**	**6.05**	**1.77**	**1.79**	**1.87**	**1.64**	**1.75**
18	**27.02**	**Stearic acid, ethyl ester**	**1.22**	**1.79**	**3.18**	**3.20**	**3.27**	**3.04**	**3.16**
19	**28.87**	**4-Amino-2,6-dihydroxypyrimidine**	**1.1**	**0.00**	**14.28**	**43.90**	**31.37**	**14.48**	**72.93**
20	**30.85**	**Germanicol**	**7.41**	**2.26**	**1.31**	**10.44**	**1.05**	**1.03**	**23.04**
21	**33.62**	**β-Sitosterol**	**1.09**	**9.72**	**11.10**	**0.91**	**0.93**	**0.00**	**38.51**
22	**6.26**	**Dimethyl Sulfoxide**	**0.00**	**105.63**	**20.79**	**1.02**	**9.33**	**21.20**	**88.61**
23	**8.38**	**Dimethyl sulfone**	**0.00**	**95.73**	**64.25**	**61.27**	**64.35**	**85.35**	**58.72**
24	**10.33**	**Hexamide**	**0.00**	**45.58**	**1.25**	**1.03**	**1.04**	**1.20**	**0.99**
25	**16.83**	**DL-Proline, 5-oxo-, ethyl ester**	**0.00**	**3.60**	**89.80**	**89.83**	**99.90**	**90.50**	**74.91**
26	**17.66**	**Apocynin**	**0.00**	**2.09**	**9.63**	**17.47**	**17.87**	**23.39**	**11.14**
27	**23.15**	**Palmitic acid, methyl ester**	**0.00**	**8.55**	**7.20**	**7.53**	**7.60**	**8.00**	**8.42**
28	**25.15**	**Phytol**	**0.00**	**10.56**	**1.81**	**1.83**	**1.91**	**1.68**	**1.77**
29	**27.94**	**Octadecanamide**	**0.00**	**3.95**	**9.91**	**11.03**	**8.00**	**5.94**	**19.80**
30	**32.01**	**Diosgenin**	**0.00**	**4.32**	**2.17**	**8.30**	**8.33**	**1.01**	**2.04**
31	**34.35**	**γ-Sitosterol**	**0.00**	**1.09**	**3.68**	**0.00**	**0.00**	**0.00**	**1.08**
32	**19.55**	**Cyclotetradecane**	**0.00**	**0.00**	**18.32**	**20.85**	**21.18**	**21.91**	**11.76**
33	**20.83**	**Farnesol isomer a**	**0.00**	**0.00**	**1.07**	**7.64**	**1.39**	**4.08**	**3.86**
34	**25.95**	**Linoleic acid**	**0.00**	**0.00**	**1.92**	**2.19**	**3.54**	**3.31**	**19.43**
35	**26.25**	**Linolenic acid**	**0.00**	**0.00**	**14.31**	**12.61**	**2.26**	**32.62**	**31.85**
36	**26.67**	**Ethyl Oleate**	**0.00**	**0.00**	**82.60**	**52.62**	**32.70**	**22.14**	**2.56**
37	**31.02**	**α-Tocospiro B**	**0.00**	**0.00**	**1.03**	**1.05**	**1.12**	**0.90**	**2.64**
38	**34.91**	**Germanicol acetate**	**0.00**	**0.00**	**0.99**	**0.00**	**0.00**	**3.99**	**1.07**
39	**35.31**	**β-Amyrin**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**2.12**

The list includes only the compound that has been identified; some compounds may appear on the gas chromatography–mass spectrometry (GC–MS) chromatogram but are not included in the list due to insufficient data for their identification. RT = retention time, Relative areas were calculated according to the total area of the listed compounds, ND = not detected

Table (4) summarizes the percentages of compounds identified by gas chromatography-mass spectrometry (GC-MS). In the mother poinsettia, the highest percentage was recorded at 39.94%, while the lowest percentages were 41% for 2,4-dimethylfuran and octadecane. The control group recorded the highest percentage for loliolide at 33.83% and the lowest percentage for 2-methoxy-4-vinylpheno at 0.13%. Loliolide achieved its highest percentage at 21.52% in the 0.5 mg L^{-1} treatment, while the lowest percentages were 0.14% for α-Tocospiro B and Germanicol acetate. Loliolide also recorded its highest percentage at 20.29% in the 1.0 mg L^{-1} treatment, while the lowest percentage was 0.13% for β-sitosterol. Loliolide also had the highest percentage in the treatment. The highest percentage was 26.56% for the 1.5 mg L-1 treatment, while the lowest percentage was for the compounds Germanicol and Hexamide, at 0.15%. Loliolide again achieved the highest percentage in the treatment, at 19.26% for the 2.0 mg L-1 treatment, while the lowest percentage was for the compound α-Tocospiro B at 0.13%. Octadecanal achieved the highest percentage at 17.04%, and Germanicol acetate achieved the lowest percentage at 0.11%.

Table (4) Percentage of secondary metabolites detected in poinsettia cultured in vitro and exposed to different levels of silver nanoparticles

NO.	Compound	Formula	Chemical Classification	% Peak Area						
				E. pulcherrima	Control	Silver nano particles mg. L^{-1}				
						0 .5	1	1.5	2	2.5
1	**2,4-Dimethylfuran**	C_6H_8O	**Furan derivative (heterocyclic compound)**	**0.41**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
2	**Hexanoic acid**	$C_6H_{12}O_2$	**Fatty acid (carboxylic acid)**	**0.46**	**12.13**	**12.94**	**13.90**	**15.52**	**18.38**	**9.50**
3	**2-Decanone**	$C_{10}H_{20}O$	**Ketone**	**6.59**	**0.15**	**0.16**	**0.21**	**0.18**	**0.26**	**0.24**
4	**4-Vinylphenol**	C_8H_8O	**Phenolic compound**	**1.40**	**0.27**	**0.17**	**0.22**	**0.19**	**0.35**	**0.35**
5	**Nonanoic acid**	$C_9H_{18}O_2$	**Fatty acid (carboxylic acid)**	**2.57**	**0.53**	**0.40**	**0.46**	**0.42**	**0.46**	**0.36**
6	**2-Methoxy-4-vinylphenol**	$C_9H_{10}O_2$	**Phenolic derivative**	**0.54**	**0.13**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
7	**Phenylacetamide**	C_8H_9NO	**Amide**	**0.58**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
8	**Propyl-2-methoxy-4-propylphenol**	$C_{12}H_{18}O_2$	**Alkylated phenol**	**0.50**	**1.19**	**0.18**	**0.16**	**0.16**	**0.15**	**0.13**
9	**Dihydroactinidiolide**	$C_{11}H_{16}O_2$	**Norisoprenoid / lactone**	**2.69**	**0.26**	**0.66**	**0.47**	**0.48**	**0.47**	**0.37**
10	**L-Pyroglutamic acid**	$C_5H_7NO_3$	**Amino acid**	**1.63**	**0.89**	**1.04**	**1.11**	**1.11**	**1.09**	**0.85**
11	**2-Pentadecanone, 6,10,14-trimethyl**	$C_{18}H_{36}O$	**Ketone**	**39.94**	**5.79**	**6.50**	**6.64**	**6.64**	**6.19**	**4.58**
12	**Loliolide**	$C_{11}H_{16}O_3$	**Monoterpenoid**	**22.40**	**33.83**	**21.52**	**20.29**	**26.56**	**19.26**	**10.85**
13	**1-Octadecene**	$C_{18}H_{36}$	**Alkene**	**0.54**	**0.16**	**0.21**	**0.25**	**0.27**	**0.32**	**0.29**
14	**Palmitic acid**	$C_{17}H_{34}O_2$	**Fatty acid ester**	**13.11**	**4.28**	**4.81**	**3.32**	**2.91**	**3.85**	**4.13**
15	**Octadecanal**	$C_{18}H_{36}O$	**Fatty aldehyde**	**0.41**	**0.63**	**0.62**	**2.22**	**0.19**	**0.45**	**17.04**
16	**1-Octadecanol**	$C_{18}H_{38}O$	**Fatty alcohol**	**1.32**	**0.20**	**0.22**	**0.22**	**0.23**	**0.27**	**0.23**
17	**Oleic Acid**	$C_{18}H_{34}O_2$	**Monounsaturated fatty acid**	**0.46**	**0.80**	**0.25**	**0.25**	**0.26**	**0.23**	**0.19**
18	**Stearic acid, ethyl ester**	$C_{20}H_{40}O_2$	**Fatty acid ester**	**0.50**	**0.24**	**0.44**	**0.45**	**0.46**	**0.42**	**0.33**
19	**4-Amino-2,6-dihydroxypyrimidine**	$C_4H_5N_3O_2$	**Pyrimidine derivative**	**0.45**	**0.00**	**1.99**	**6.22**	**4.44**	**2.02**	**7.72**
20	**Germanicol**	$C_{30}H_{50}O$	**Triterpenoid**	**3.04**	**0.30**	**0.18**	**1.48**	**0.15**	**0.14**	**2.44**
21	**β-Sitosterol**	$C_{29}H_{50}O$	**Phytosterol (sterol)**	**0.45**	**1.28**	**1.55**	**0.13**	**0.13**	**0.00**	**4.08**

22	**Dimethyl Sulfoxide**	C_2H_6OS	**Sulfoxide**	**0.00**	**13.88**	**2.90**	**0.15**	**1.32**	**2.96**	**9.39**
23	**Dimethyl sulfone**	$C_2H_6O_2S$	**Sulfone**	**0.00**	**12.58**	**8.97**	**8.68**	**9.10**	**11.92**	**6.22**
24	**Hexamide**	$C_6H_{13}NO$	**Amide**	**0.00**	**5.99**	**0.17**	**0.15**	**0.15**	**0.17**	**0.10**
25	**DL-Proline, 5-oxo-, ethyl ester**	$C_7H_{11}NO_3$	**Amino acid derivative (ester)**	**0.00**	**0.47**	**12.54**	**12.73**	**14.13**	**12.64**	**7.93**
26	**Apocynin**	$C_9H_{10}O_3$	**Phenolic compound)**	**0.00**	**0.28**	**1.34**	**2.48**	**2.53**	**3.27**	**1.18**
27	**Palmitic acid, methyl ester**	$C_{16}H_{32}O_2$	**Saturated fatty acid**	**0.00**	**1.12**	**1.01**	**1.07**	**1.08**	**1.12**	**0.89**
28	**Phytol**	$C_{20}H_{40}O$	**Diterpene alcohol**	**0.00**	**1.39**	**0.25**	**0.26**	**0.27**	**0.23**	**0.19**
29	**Octadecanamide**	$C_{18}H_{37}NO$	**Fatty acid amide**	**0.00**	**0.52**	**1.38**	**1.56**	**1.13**	**0.83**	**2.10**
30	**Diosgenin**	$C_{27}H_{42}O_3$	**Triterpenoid**	**0.00**	**0.57**	**0.30**	**1.18**	**1.18**	**0.14**	**0.22**
31	**γ-Sitosterol**	$C_{29}H_{50}O$	**Phytosterol**	**0.00**	**0.14**	**0.51**	**0.00**	**0.00**	**0.00**	**0.11**
32	**Cyclotetradecane**	$C_{14}H_{28}$	**Cycloalkanes**	**0.00**	**0.00**	**2.56**	**2.95**	**3.00**	**3.06**	**1.25**
33	**Farnesol isomer a**	$C_{15}H_{26}O$	**Sesquiterpene alcohol**	**0.00**	**0.00**	**0.15**	**1.08**	**0.20**	**0.57**	**0.41**
34	**Linoleic acid**	$C_{18}H_{32}O_2$	**Polyunsaturated fatty acid**	**0.00**	**0.00**	**0.27**	**0.31**	**0.50**	**0.46**	**2.06**
35	**Linolenic acid**	$C_{18}H_{30}O_2$	**Polyunsaturated fatty acid**	**0.00**	**0.00**	**2.00**	**1.79**	**0.32**	**4.55**	**3.37**
36	**Ethyl Oleate**	$C_{20}H_{38}O_2$	**Fatty acid ester**	**0.00**	**0.00**	**11.53**	**7.46**	**4.63**	**3.09**	**0.27**
37	**α-Tocospiro B**	$C_{30}H_{50}O_2$	**Tocopherol derivative**	**0.00**	**0.00**	**0.14**	**0.15**	**0.16**	**0.13**	**0.28**
38	**Germanicol acetate**	$C_{32}H_{52}O_2$	**Triterpenoid ester**	**0.00**	**0.00**	**0.14**	**0.00**	**0.00**	**0.56**	**0.11**
39	**β-Amyrin**	$C_{30}H_{50}O$	**Triterpenoid (pentacyclic)**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.22**

Regarding the metabolic profile, previous studies have indicated that AgNPs act as biostimulants that can enhance the production of secondary metabolites in plants. A study investigating the effect of AgNPs in fennel *(Pimpinella anisum* L.) cells showed an increase in the concentrations of essential fatty acids such as palmitic and linoleic acids, as well as phenolic compounds such as phenylalanine and salicylic acid, particularly at low concentrations (1 and 5 mg L^{-1}) (Ulusoy *et al*., 2024). Another study demonstrated that AgNPs can stimulate alkaloid production in catnip tissue (Fouad *et al*., 2021). These studies are consistent with the findings of the current study, which revealed qualitative and quantitative changes in the production of secondary metabolites. Some of these compounds exceeded their levels in the parent plants and the control treatment. This effect was clearly evident in the plant studied, as treatment with silver nanoparticles showed a significant stimulation of secondary metabolic pathways, with the abundance of some compounds exceeding their levels in the parent plants and the control treatment.

Exposure of plants to silver nanoparticles leads to oxidative stress. Oxidative stress plays an important role in the production of reactive oxygen species (ROS), which act as key signaling molecules in stress responses. In order for plants to grow and produce under stress conditions, they must use ROS not only to modify metabolic pathways, but also to develop adaptive phenotypes capable of activating detoxification mechanisms in plants to reduce damage caused by ROS. Therefore, the biosynthesis of secondary compounds activates a complex network of defense mechanisms to counteract it (Fallah *et al*., 2024; Movahedi *et al*., 2024).

The findings indicate that supplementing the culture medium of *Nerium oleander* callus with low concentrations of silver nanoparticles produces clear and effective stimulatory effects (Alaa,

2016). This treatment enhanced the production of several key secondary metabolites, reflecting an activation of the plant's natural defense mechanisms and an overall improvement in its adaptive responses**.**

Furthermore, AgNPs influence cellular response by modulating membrane permeability and ionic imbalance, contributing to changes in the levels of secondary metabolic end products (Marslin *et al*., 2017). They can also affect the gene expression of enzymes involved in secondary metabolism, leading to alterations in intracellular plant metabolic pathways (Babele *et al*., 2019). Ulusoy *et al*. (2024) successfully stimulated the synthesis of important food and pharmaceutical by products from anise callus (*Pimpinella anisum* L.). This was achieved using MS medium supplemented with plant growth regulators and 1–5 mg L^{-1} of silver nanoparticles. In parallel with this finding, Fatima *et al*. (2021) observed that adding different concentrations of nanoparticles to MS medium increased the accumulation of phenolic content and antioxidant activity in callus derived from the leaves, roots, and apical meadowsweet (*Artemisia annua* L.).These results are consistent with those obtained in our current study.

E. pulcherrima, as well as control plants and various stress treatments, were characterized by the presence of other compounds of notable biological interest, including vinylphenol-4, nonanoic acid, L-pyroglutamic acid, Germanicol, apocynin, and Germanicol acetate. which are known for their diverse pharmacological and medicinal properties.

Vinylphenol-4 is a phenolic compound that has demonstrated broad-spectrum biological activity, particularly in the field of cancer prevention. It has been shown to induce apoptosis in breast cancer cell lines (MDA-MB-231), inhibit angiogenesis, and suppress tumor growth in animal models. This compound also possesses antioxidant and antimicrobial properties, making it a potential contributor to cell protection against free radical damage and enhancing the antimicrobial efficacy of certain plant extracts (Leung *et al*., 2018).

Nonanoic acid, also known as pelagronic acid, is a medium-chain saturated fatty acid, and recent studies are highlighting growing interest in its biological and medicinal properties. It has demonstrated clear activity against a range of fungi and bacteria, and its role in supporting mitochondrial metabolism is being investigated, particularly in pathological conditions associated with impaired long-chain fatty acid oxidation, which may contribute to reducing the accumulation of toxic metabolites. Some preliminary studies suggest its potential use in treating epileptic seizures and promoting neuronal differentiation, as well as its potential effects in modulating the biological behavior of small intestinal neuroblastomas, where it is believed to play a role in remodeling the phenotype of cancer cells (Almobarak *et al*., 2023).

Meanwhile, L-Pyroglutamic acid, also known as 5-oxyproline, is an amino acid derivative that plays a pivotal role in the glutathione cycle, one of the most important cellular defense mechanisms against oxidative stress and detoxification. Elevated levels of L-pyroglutamic acid may indicate metabolic disorders or depletion of intracellular glutathione stores. In addition, this compound contributes to supporting cognitive function and improving memory through its effect on the

neurotransmitter acetylcholine, and some research suggests its potential role as an anti-anxiety agent. It is widely used in cosmetics as a component of the skin's natural moisturizing factor (Zhang *et al*., 2021).

Germanicol is a five-cyclic triterpene naturally found in the leaves and stems of certain plants, such as figs and blackberries. Laboratory studies have demonstrated its potent anti-inflammatory and antioxidant activity, as well as its inhibitory effects on cancer cell growth. It achieves this by inducing apoptosis (programmed cell death), halting the cell cycle, and preventing cell migration in certain human colon cancer cell lines (Dong, 2016). similarly, apocynin is a naturally occurring organic compound that has garnered significant attention as a potent inhibitor of NADPH oxidase, the enzyme responsible for producing reactive oxygen species (ROS) in cells. Studies indicate that this compound can reduce inflammation and oxidative stress, suggesting potential therapeutic value in spinal cord injuries and ischemic/reperfusion injury syndromes affecting the heart, kidneys, and brain. Some evidence suggests its potential use in treating neurodegenerative diseases such as Parkinson's disease and amyotrophic lateral sclerosis (ALS) by reducing neuroinflammation and enhancing neuroprotection (Boshtam *et al*., 2021).

Finally, Germanicol acetate is a triterpene derivative, a five-cyclic compound extracted from the bark and leaves of certain plants. Preliminary studies have shown it possesses anti-inflammatory and antifungal activity, and possibly anti-gastrointestinal properties. Germanicol and its derivatives have also been shown to exhibit multiple biological activities, including inhibiting oxidative stress and influencing cellular functions, in addition to their toxic effects, indicating the diversity of their biological roles and their potential for future applications (Riaz *et al*., 2019).

Conclusion

The results obtained in this study highlight that the diversity of compounds discovered in poinsettia, whether common compounds such as phytol, β-sitosterol, and palmitic acid, or less common compounds such as vinylphenol-4, nonanoic acid, L-pyroglutamic acid, germanicol, and its derivatives, reflects an advanced adaptive response of the plant to various stress conditions. These compounds are not merely byproducts of metabolism, but rather potent defensive components that work in close synergy to protect plant cells from oxidative stress and regulate metabolic and hormonal balance, in addition to possessing valuable pharmacological properties of importance in medical and pharmaceutical applications.

The accumulation of these compounds under stress conditions may reflect the plant's tendency to reprogram its metabolic pathways to enhance its survival capacity by stimulating the production of bioactive molecules associated with defense and resistance. The presence of compounds with antioxidant, antimicrobial, or even anticancer activity suggests that these metabolic changes may extend to form a biological bridge between the plant's physiological responses and its medicinal

benefits. Thus, poinsettia not only exhibits high physiological resilience to stress but also promising pharmacological potential that warrants further research to explore its future pharmaceutical and therapeutic applications.

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