

# Silver Nanoparticles in the In Vitro Aseptic Establishment of Poinsettia (*Euphorbia Pulcherrima* Willd. Ex Klotzsch) Var. Belén

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## Research Article

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# Abstract

Poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) is the worldwide symbol of Christmas, and its demand constantly grows. The main challenge for the poinsettia *in vitro* propagation protocol is to maintain aseptic conditions in the culture, as the latex present in poinsettia stems significantly contributes to carbohydrates and other molecules that promote the development of endogenous phytopathogens in the culture medium after establishment. Therefore, the primary objective of this work is to assess the efficacy of AgNPs in achieving an aseptic *in vitro* culture using six concentrations of AgNPs (0, 100, 200, 300, 400, 500, and 600 mg L<sup>-1</sup>) added to the MS culture medium. Entirely aseptic nodal segments were obtained with the addition of 300 to 600 mg/L of AgNPs. The best shoot induction and growth promotion without compromising explant viability was obtained with the addition of 400 mg L<sup>-1</sup> to the medium. It is essential to note that shoot induction and growth promotion were achieved without the addition of 6-benzylaminopurine (BA) or naphthalene acetic acid (NAA), which have previously been reported as the optimal conditions for poinsettia micropropagation. These results highlight the potential of AgNPs to accomplish both tasks for *in vitro* establishment of poinsettia cultures, eliminating bacterial and fungal contamination, and promoting conditions for shoot induction and growth.

## Introduction

The poinsettia plant is one of the most popular ornamental plants in North America, Australia, and various European countries (Castellanos et al. 2010). It is the symbol of Christmas (Clarke et al. 2008) and is one of the most economically significant ornamental plants worldwide (Jacobo Villegas et al. 2015). The popularity of the plant relies on its bright, colorful bracts, which range from red to white, being fresh and unbroken for three to four months (Vilperte et al.,).

The shrub is a winter-flowering species native to the states of Guerrero and Morelos in the South-central region of Mexico, growing more than 3 m in this habitat (Trejo et al. 2012). However, it is essential to note that the cultivation of poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) for commercial purposes depends on varieties generated abroad, with a constant demand for new varieties of this species (Canul-Ku et al. 2018). In Mexico, there are nine varieties of sun poinsettia registered in the National Catalogue of Plant Varieties: “Valenciana” (red), “Juan Pablo” (pink), “Amanecer navideño” (white), and “Rehilete” and “Belén” (red) (González-García et al. 2022). Currently, there are approximately 300 cultivars resulting from genetic improvement and biotechnology (Trejo et al. 2018). Unfortunately, not enough attention has been paid to the preservation of the native *Euphorbia pulcherrima*. Particularly for variety Belén, genetic resources produce a limited number of commercial plants in their natural habitat, hence the need to explore and exploit the species.

The poinsettia can be propagated conventionally by seeds and cuttings. However, seeds lose their viability during storage, while propagation through cuttings is seasonal. Furthermore, rooting of these cuttings takes between six and eight weeks (Danial and Ibrahim 2016). Moreover, as not all cuttings

survive, it is worth mentioning that this method requires improved propagation techniques, which limit the available plant material for both commercial purposes and the conservation of plant genetic resources.

In vitro propagation is an alternative to conventional propagation methods, as it increases the multiplication rate and generates pathogen-free material (Chávez-García et al. 2020). It also produces genetically homogeneous and invigorated plants, achieved by culturing tissues in an artificial medium rich in nutrients and organic compounds for multiplication under laboratory conditions (Bello-Bello and Spinoso-Castollo 2023). However, despite the advantages of this technique, the success of biotechnology plant propagation systems largely depends on controlling and preventing microbial contamination. This fact is particularly evident in contamination produced by endogenous species in latex-producing plants, which exploit the abundance of nutrients present in the culture medium (Cassells 1991; Szewczyk-Taranek et al. 2020).

Advances in bionanotechnology provide modern alternatives for pathogen control, particularly the use of metallic nanoparticles. Among all metallic nanoparticles, silver nanoparticles (AgNPs) have shown excellent antifungal and antimicrobial properties (Alfosea-Simón et al. 2025). In our research group, an AgNP formulation with a specific Ag: coating agent ratio has demonstrated a broad spectrum of antimicrobial activity on vegetal systems (Vazquez-Muñoz et al. 2014; Andújar et al. 2020; Stephano-Hornedo et al. 2020) with minimal phytotoxic effects evaluated in the reference model, *Allium cepa* (Casillas-Figueroa et al. 2020). Additionally, this AgNPs formulation has excellent potential in plant tissue culture, for plant health, *in vitro* germplasm conservation, metabolite production, genetic improvement, biotechnology, and micropropagation (Bello-Bello et al. 2017; Pérez-Caselles et al. 2023; Cabrera-Ramírez et al. 2024; Mendoza et al. 2025).

It is well known that the primary challenge in an *in vitro* propagation protocol for poinsettia is obtaining an aseptic culture, as the latex present in poinsettia stems serves as a source of endogenous phytopathogens, which can be expressed or manifested in the culture medium after establishment (Leifert and Cassells 2001; Perera and Trader 2010; Almzori et al. 2023). Therefore, the primary objective of this work is to evaluate the efficacy of NPsAg in achieving an aseptic in vitro culture, by assessing six concentrations of AgNPs added to the MS culture medium. Furthermore, we assess the effect of AgNPs on the length, diameter, and number of shoots generated, as well as the number of leaves on each shoot, as indicators of explant viability.

## Materials and Methods

The research was carried out in the biotechnology laboratory of the Escuela de Estudios Superiores de Xalostoc (UAEM), Morelos, Mexico (18° 44' 39" N and 98° 54' 34" W, 1 294 m.a.s.l.). The climate in the region is warm semi-humid, with an average temperature of 21–24°C, low relative humidity, and average rainfall ranging between 720 and 820 mm.

# Silver nanoparticles

Argovit-C™ is a commercial formulation of silver nanoparticles, provided by Vector-Vita Ltd. Research and Production Center, Novosibirsk, Russia. Argovit-C is a stable suspension of silver nanoparticles with a final concentration of 200 mg/L. The formulation composition corresponds to 12 mg/ml of metallic silver stabilized with 188 mg/L HP/PVP ratio 7/3 (HP = hydrolyzed protein, PVP = polyvinylpyrrolidone). These AgNPs exhibited a spherical morphology with an average diameter of  $14.95 \pm 10.1$  nm (based on 333 particles), spanning a size distribution range of 1 to 55 nm. The UV-visible spectrum for AgNPs at 0.03 mg/ml revealed the surface plasmon resonance at 454 nm. The hydrodynamic diameter distribution of AgNPs in ultrapure water yielded two size distributions (44 and 164 nm), with a zeta potential of  $+9.6 \pm 0.6$ . The complete characterization of Argovit C was previously reported (Garcia Garcia et al. 2023).

## Plant specimens and disinfection protocol

Nodal segments with an axillary bud devoid of leaves, approximately 1.0 cm, were taken from the middle part of the Belén poinsettia mother plants. In a laminar flow hood, the nodal segments were immersed in a solution containing 4g/L of Captan® (N-(trichloromethylthio) cyclohex-4-ene-1,2-dicarboximide, broad-spectrum fungicide) and 1 g/L Agri-micyn 500® (Copper 42.4% (tribasic copper sulfate) + streptomycin 1.755% + oxytetracycline 0.176%, broad-spectrum bacteriocide and fungicidal) diluted in distilled water. The nodal segments were kept under constant stirring for 30 min and subsequently rinsed three times with sterile distilled water. A 3% sodium hypochlorite (NaOCl) solution with 0.5 ml/L of detergent (escudo®) was used for surface disinfection. The samples were kept under constant stirring for 10 min and rinsed three times with sterile distilled water. Finally, the samples were immersed in 70% (v/v) ethanol for 2 min and rinsed three times with sterile distilled water.

The Murashige and Skoog (MS) culture medium was supplemented with 100 mg/L of myo-inositol, 1 mg/L of thiamine HCl, 1 mg/L of pyridoxine HCl, 1 mg/L of niacin, 1 mg/L of nicotinic acid, 1 mg/L of glycine, 3% sucrose, 0.6% agar-agar, and 2.0 g/L of activated charcoal. The pH was adjusted to 5.7.

The medium was dispensed in 100 mL flasks, into which 20 mL of culture medium was placed and sterilized in a Stik® MJ-54 A autoclave (Provedor de Laboratorios, S.A. de C.V., Tlajomulco de Zúñiga, Mexico) for 18 minutes at 120°C and 1.5 kg cm<sup>-2</sup>.

Six treatments of silver nanoparticles (AgNPs) Argovit-C were evaluated (0, 200, 300, 400, 500, 600 mg/L), added to the MS culture medium, and subsequently sterilized. The AgNPs concentrations reported in this work are expressed in two forms: 1) metallic silver + coating agent concentration (mg/L) and 2) the metallic silver contained in the AgNP formulation (mg/L) (Table 1).

Table 1

AgNPs concentrations used for each treatment. Concentrations correspond to metallic silver + coating agent concentration (mg/L) and the metallic silver contained in the AgNP formulation (mg/L)

| AgNPs treatment | Metallic silver + coating agent (mg/L) | Metallic silver (Ag) (mg/L) | Metallic silver (mM) |
|-----------------|----------------------------------------|-----------------------------|----------------------|
| Control         | 0                                      | 0                           | 0                    |
| Treatment 2     | 200                                    | 11.97                       | 0.11                 |
| Treatment 3     | 300                                    | 17.96                       | 0.16                 |
| Treatment 4     | 400                                    | 23.95                       | 0.22                 |
| Treatment 5     | 500                                    | 29.94                       | 0.27                 |
| Treatment 6     | 600                                    | 35.92                       | 0.33                 |

## Establishment of treatments

Working in a laminar flow hood, we placed three poinsettia nodal segments per flask containing culture medium for each AgNPs treatment. The flasks containing the explants were incubated in a climate chamber at 25°C, with a 16/8 h light/dark photoperiod, and a light intensity of 29  $\mu\text{E}/\text{m}^2\text{s}^{-1}$ . A completely randomized experimental design was used with six AgNPs treatments and 30 replicates. Each flask containing three Belén poinsettia nodal segments represents an experimental unit. After 45 days, the explants were assessed for aseptic efficacy and expressed as percentage survival (%). The number of shoots, shoot length (mm), shoot diameter (mm), and number of leaves per shoot were also evaluated.

## Statistical analysis

The data obtained were processed using analysis of variance and a comparison of means test (Tukey,  $P \leq 0.05$ ). Statistical analysis was performed using SAS software (SAS Institute Inc 2013). Figures were generated with GraphPad Prism 10.

## Results and discussion

### Effect of AgNPs on asepsis of culture media

The asepsis of the nodal segments of poinsettia was evaluated after 45 days of exposure to different concentrations of AgNPs. To our knowledge, this is the first time that AgNPs are used to obtain an aseptic nodal segment of poinsettia for micropropagation. The results are reported in Table 2 and Fig. 1. The asepsis of the control group under the experimental conditions of this study was practically nonexistent; a 0.33% asepsis rate was observed. The lower assessed concentration of AgNPs produces just 20% asepsis. On the other hand, concentrations from 300 to 600 mg/L produce a 100% asepsis. The AgNPs concentration to achieve 50% asepsis is 213.8 mg/L.

Sterilization of culture media by autoclaving can lead to toxic decomposition products; meanwhile, chemical sterilization using NaOCl, Ca(OCl)<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>, or HgCl<sub>2</sub> can be harmful to the explants and affect propagation efficiency (Tung et al.,. Previous works reported the chemical disinfection of the nodal segment of poinsettia, achieving asepsis rates of up to 60% using 20% NaOCl for 15 to 25 minutes (Perera and Trader 2010; Rangel-Estrada et al. 2017). On the other hand, Rangel-Estrada and co-workers reported that the best average survival rate of explants was 50%, achieved with explants aged two months and immersion time in NaOCl of 20 min (Rangel-Estrada et al. 2017).

Table 2

Asepsis on nodal segments of poinsettia observed after exposure to different concentrations of AgNPs.

| AgNPs concentration in mg/L* | 0                        | 200                    | 300                     | 400                  | 500                  | 600                  |
|------------------------------|--------------------------|------------------------|-------------------------|----------------------|----------------------|----------------------|
|                              | (0)                      | (11.97)                | (17.96)                 | (23.95)              | (29.94)              | (35.92)              |
| Asepsis (%)                  | 0.33 ± 0.30 <sup>c</sup> | 20 ± 0.40 <sup>b</sup> | 100 ± 0.37 <sup>c</sup> | 100 ± 0 <sup>a</sup> | 100 ± 0 <sup>a</sup> | 100 ± 0 <sup>a</sup> |

\*Concentration in parentheses corresponds to metallic silver within the silver nanoparticle formulation. Different letters in the same columns correspond to statistically significant differences. Data expressed as average value ± standard deviation.

The major problem in achieving complete asepsis in the nodal segments of the poinsettia may be associated with the high microbial activity in the latex due to the abundance of organic compounds. The richer the latex in carbohydrates, the higher the microbial population (Salomez et al. 2014). Latex plays a significant role in plant defense due to the concentration of bioactive metabolites and defense-related enzymes, which control the growth of foreign microorganisms. Still, no damage is produced to endogenous bacteria (Salomé-Abarca et al. 2021). Endogenous bacteria take advantage of the opportunity provided by a nutrient-rich culture medium and the lack of competition from other microorganisms to grow rapidly, competing with the explant for nutrients and compromising its development (Leifert and Cassells 2001).

In this work, the use of a broad-spectrum fungicide (Captan-500), a broad-spectrum bactericide (Agri-mycin 500), and NaOCl 3% in the disinfection protocol were useless in avoiding the proliferation of endogenous bacteria. The control group exhibits 99.7% contamination (Table 2). Conversely, the presence of AgNPs in the culture media controls the contamination in a dose-dependent manner, reaching complete elimination at 300 mg/L. The continuous presence of AgNPs in the culture media allows their interaction with the endogenous bacteria, affecting several molecular processes, including ROS overproduction, cytoskeleton compromise, and direct interaction with DNA and DNA repair enzymes, which lead to the elimination of fungi and bacteria (Guerra et al. 2020; Rodrigues et al. 2024).

The effectiveness of AgNPs to produce aseptic cultures for micropropagation has been demonstrated in several vegetative systems. AgNPs with a size of < 20 nm with β-chitosan as stabilizer exhibit outstanding disinfection results of chrysanthemum plantlets using a pretreatment with AgNPs

concentration from 125–500 mg/L and a few drops of Tween 80 for 5–30 minutes. Subsequently, 4–5 mg/L of AgNPs were added to the culture media to evaluate the asepsis percentage every week for four weeks. This method achieves 100% asepsis from the first week by simply adding AgNPs to culture media without autoclaving (Tung et al. 2021).

In gladiolus apices disinfection, 91.67% of asepsis was achieved with pretreatment of 50–100 mg/L of PVP-AgNPs of 35 nm and immersion time of 10 minutes, followed by the addition of 25–200 mg/L of AgNPs to the culture medium. In this case, AgNPs were used as an auxiliary in the disinfection process. Before exposure to AgNPs, the disinfection process involves immersion in 3% NaOCl + 0.5g/l of detergent for 5 minutes (Chávez-García et al. 2020).

For shrubby specimens, such as those of the Rosaceae family, the use of 200 mg/L AgNPs and an immersion time of 20 minutes yields the highest reduction in contamination without affecting explant survival (Shokri et al. 2015). Contamination control of G x N15 (Garnem), a hybrid of *Prunus amygdalus* (Garfi) × *Prunus persica* (Nemared), was achieved under micropropagation conditions using 100 mg/L of AgNPs by either immersion or addition to the culture medium (Arab et al.,. Similarly, biogenically produced AgNPs eliminate contamination of culture media used for *Rumex nervosus* micropropagation at a concentration of 40 mg/L (Alfarraj et al.,. On the other hand, it has been reported that the shoot tip disinfection of *P. friedrichsthalianum* (O. Berg) Nied. partially eliminates contamination using 50 mg/L PVP-AgNPs (Andújar et al. 2020). In all cases, the disinfection process includes the use of NaOCl or HgCl<sub>2</sub>. The above data demonstrates that the effectiveness of AgNPs relies on the nature of the AgNPs, the concentration, and the vegetal system used.

In our system, AgNPs concentrations of 300 to 600 mg/L efficiently eliminate the bacterial and fungal contamination in the in vitro establishment of poinsettia cultures.

## Effect of AgNPs on poinsettia micropropagation

The effect of different concentrations of AgNPs on poinsettia was evaluated by measuring the response in the number of shoots per explant, the length and diameter of the shoots, and the number of leaves produced per shoot. Results of the parameters mentioned above are shown in Table 3 and Fig. 2.

Table 3

Effect of AgNPs administration on shoots per explant, length and diameter of shoots, and leaves per shoot obtained *in vitro* from nodal segments of poinsettia.

| Concentration AgNPs<br>(mg L <sup>-1</sup> ) * | Shoots per<br>explant (#) | Shoot length<br>(mm)      | Shoot diameter<br>(mm)   | Leaves per<br>shoot (#)  |
|------------------------------------------------|---------------------------|---------------------------|--------------------------|--------------------------|
| 0 (0)                                          | 0.10 ± 0.31 <sup>c</sup>  | 0.76 ± 2.4 <sup>c</sup>   | 0.07 ± 0.21 <sup>d</sup> | 0.10 ± 0.31 <sup>c</sup> |
| 200 (11.97)                                    | 0.20 ± 0.41 <sup>c</sup>  | 2.82 ± 5.9 <sup>c</sup>   | 0.16 ± 0.36 <sup>d</sup> | 0.33 ± 0.53 <sup>c</sup> |
| 300 (17.96)                                    | 0.83 ± 0.38 <sup>b</sup>  | 30.63 ± 13.2 <sup>b</sup> | 1.16 ± 0.42 <sup>c</sup> | 2.5 ± 0.60 <sup>b</sup>  |
| 400 (23.95)                                    | 1.06 ± 0.19 <sup>a</sup>  | 46.04 ± 7.4 <sup>a</sup>  | 1.74 ± 0.28 <sup>a</sup> | 4.2 ± 0.25 <sup>a</sup>  |
| 500 (29.94)                                    | 1.01 ± 0.05 <sup>a</sup>  | 50.29 ± 6.4 <sup>a</sup>  | 1.34 ± 0.11 <sup>b</sup> | 2.76 ± 0.25 <sup>b</sup> |
| 600 (35.92)                                    | 1.00 ± 0.00 <sup>ab</sup> | 46.35 ± 5.9 <sup>a</sup>  | 1.10 ± 0.10 <sup>c</sup> | 2.50 ± 0.26 <sup>b</sup> |

\* Values in parentheses correspond to the amount of metallic silver administered. Different letters in the same columns correspond to statistically significant differences. Data expressed as average value ± standard deviation.

The administration of AgNPs produces a concentration-dependent increase in the number of shoots in the nodal segments of poinsettia. The highest number of shoots was obtained with a concentration of 400 mg/L (1.06 ± 0.19 shoots per explant) and remains unchanged with the increase in AgNPs concentration (Fig. 2a). A concentration of 300 mg/L exhibits no statistically significant difference in shoots per explant compared to the concentration of 400 mg/L (Table 3). The length of the shoots also shows an AgNPs concentration-dependent response from 200 to 500 mg/L. The maximum length observed was 50.29 ± 6.4 mm (Table 3). Conversely, although there is no statistically significant difference, with a concentration of 600 mg/L, the length of the shoot decreases to 46.35 ± 5.9 mm (Fig. 2b). A similar trend to that described for shoot length was observed for the shoot diameter and the number of leaves per shoot; however, the values for the latter parameters decreased by 500 and 600 mg/L. The administration of AgNPs to the culture media at 400 mg/L results in a 10-fold increase in shoot production, a 66-fold increase in shoot length, a 25-fold increase in shoot diameter, and a 42-fold increase in leaves per shoot, compared with the control (Table 3 and Figs. 2c and 2d).

It is essential to note that the longest shoots obtained with 500 and 600 mg/L of AgNPs also exhibit rolled and curly leaves with a significant decrease in the leaf area (Fig. 3).

Leaf rolling is a typical plant response to environmental stresses, such as the overproduction of reactive oxygen species (ROS) (Kadioglu et al. 2012). The high concentration of AgNPs could produce the leaf rolling observed on poinsettia due to an overproduction of ROS. It has been reported that PVP-AgNPs Argovit enhances ROS production, inducing a growth-promoting response in plants at low doses (Bello-Bello et al. 2017; Casillas-Figueroa et al. 2020; Chávez-García et al. 2020; Mendoza et al. 2025). ROS

concentration exhibits a dose-dependent response, producing an increase in polyphenols as a defensive response in various plant systems, including vanilla (Spinoso-Castillo et al. 2017), sugarcane (Bello-Bello et al. 2017), gerbera (Mosqueda-Frómeta et al. 2023), and *Allium cepa* (Casillas-Figueroa. A hormetic response is observed when the molecular and enzymatic antioxidant response of plants are overwhelmed. The toxic effects of AgNPs on the mentioned plant systems are observed in the range of 50 to 250 mg/L, depending on the plant species.

In this work, the hormetic response is observed with concentrations above 400 mg/L (Figs. 2c, 2d, and 3). The macroscopic effects that enable us to propose the phytotoxic impact associated with high concentrations of AgNPs are the decrease in shoot length and diameter, alongside a reduction in the number of leaves per explant (Table 3, Fig. 2), and browning of the shoots (data not shown). Conversely, AgNPs concentrations of 300 and 400 mg/L enhance the length, diameter, and number of leaves per shoot without evident change in color or shape of the leaves (Table 3, Figs. 2 and 3).

Interestingly, shoot induction and growth promotion were achieved in this work without the addition of 6-benzylaminopurine (BA) or naphthalene acetic acid (NAA), which have previously been reported as the optimal conditions for poinsettia *in vitro* propagation (Almzori et al.,. Results from Table 3 show that one shoot per explant, with a length of 46 mm and 4.2 leaves per explant, were obtained with an AgNPs concentration of 400 mg/L (0.22 mM of metallic silver). Almzori reports 2.62 shoots per explant and 21.6 mm with the addition of 1 mg/L of BA. To obtain the maximum number of leaves per explant (20.41 leaves per explant), the BA concentration needs to increase to 2 mg/L (Almzori et al. 2023). Perera reports that BA between 0.9 and 2.7 mg/L was the most efficient cytokinin for poinsettia multiplication during micropropagation processes (Perera and Trader 2010). Pickens developed axillary shoots and organogenesis protocols for poinsettia using 0.36–1.5 mg/L of BA and 0.49 mg/L of indole-3-acetic acid (IAA), producing 2.5 shoots per explant (Pickens et al. 2005). Additionally, five shoots per explant were obtained by adding 0.99 mg/L  $\mu$ M of BA and 0.08 mg/L of NAA to the culture medium (Castellanos et al.,. In summary, adding AgNPs without cytokinin or auxins to culture media yields half the number of shoots compared to using cytokinin and auxins, which are considered optimal conditions for shoot production and length. In our work, it was shown that the use of 400 mg/L of AgNPs led to an increase in shoot production and their length by 10 and 66 times, respectively. These results highlight the potential of AgNPs in promoting shoot induction and growth for the *in vitro* establishment of poinsettia cultures.

## Conclusions

The results of this work demonstrate the outstanding capacity of AgNPs Argovit to eliminate bacterial and fungal contamination at concentrations of 300 and 400 mg/L, addressing one of the most significant challenges in the *in vitro* establishment of poinsettia micropropagation without compromising the viability of the explants. The concentration of 400 mg/L of AgNP, in addition to ensuring aseptic conditions in the culture, yields the best results in terms of shoot induction, length, and diameter, along with the highest number of leaves per shoot, without the addition of cytokinin or auxins, which have previously been reported as the optimal conditions for poinsettia micropropagation. To the

best of our knowledge, this is the first report to evaluate the efficiency of AgNPs in producing aseptic conditions and stimulating growth protocols for *E. pulcherrima*. The results obtained in this work on the micropropagation process of (*Euphorbia pulcherrima* Willd. ex Klotzsch) var. Belén will contribute to a rationally designed bionanotechnology-based protocol that enables the generation of high-quality plants to meet the demand for this Christmas symbol.

## Declarations

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### Declaration of Conflicting Interests

The authors report there are no conflicts of interest in this work.

### Author Contributions

TTRR, MAR, DG, and JCGR: Conceptualization, methodology, experimental design, formal analysis, data interpretation, and writing the original draft; TTRJ, NB, and AP: funding acquisition, supervision, manuscript editing; TTRJ, NB, AP, DG, and JCGR: editing and improvement of the manuscript. The manuscript was read and approved by all authors.

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**Data Availability** The data sets generated during the current study are available from the corresponding author upon reasonable request.

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## Figures

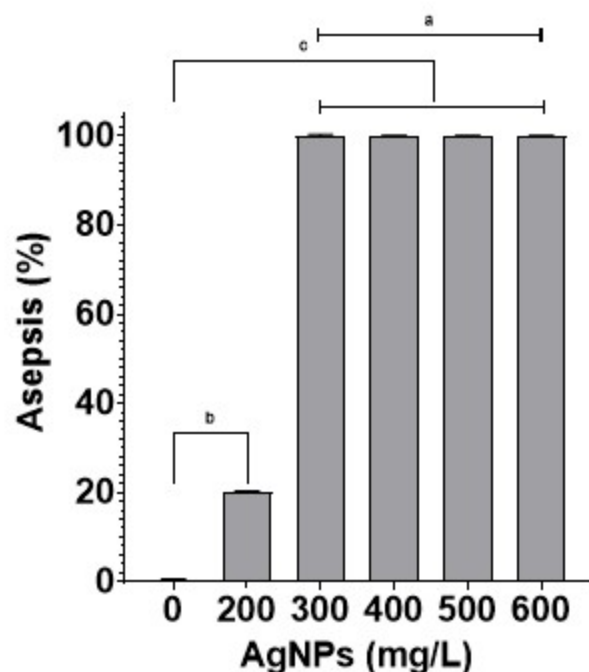


**Figure 1**

Asepsis (%) on poinsettia nodal segments exposed to different concentrations of AgNPs after 45 days of exposure. Values are expressed as the average  $\pm$  standard deviation ( $P < 0.05$ ).

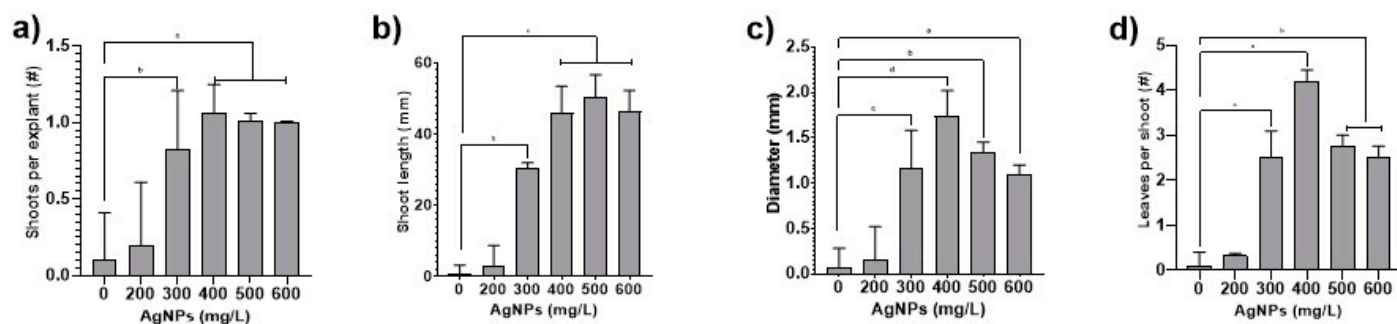


**Figure 2**

Effect of different concentrations of AgNPs (mg/L) on a) shoots per explant, b) shoot length, c) shoot diameter, and d) leaves per shoot obtained in vitro from nodal segments of poinsettia. Values are expressed as the average  $\pm$  standard deviation ( $P < 0.05$ ).

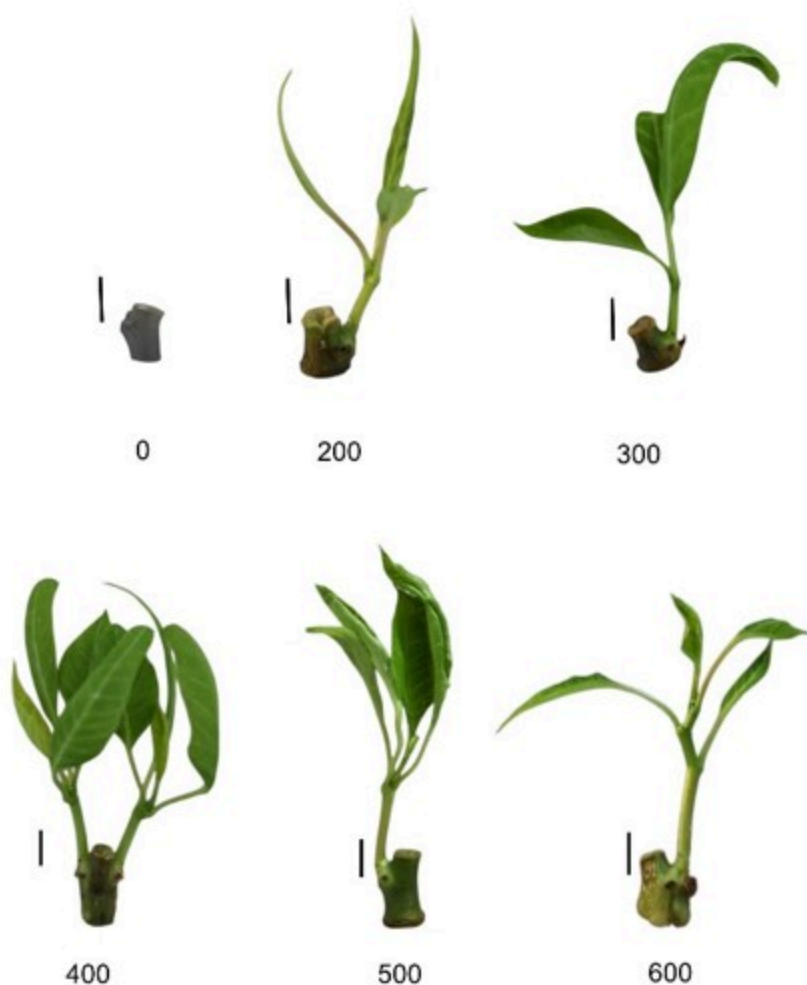


**Figure 3**

Representative images of poinsettia nodal segments development after 45 days of exposure to different concentrations of AgNPs. The concentration is indicated below each nodal segment. The black bar on the left represents 1 mm in length.