

Effect of silver nanoparticles on the *in vitro* establishment of *Lilium* sp.

Efecto de nanopartículas de plata en el establecimiento *in vitro* de *Lilium* sp.

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ABSTRACT

El *Lilium* sp., o lirio, es una planta de gran importancia ecológica, The *Lilium* sp., or lily, is a plant of significant ecological, cultural, and commercial importance, valued in gardening, fragrances, and traditional medicine. Given the limitations of its natural reproduction, *in vitro* propagation is essential for its efficient and sustainable cultivation, producing disease-free plants with high genetic quality. A major challenge of this technique is contamination, which requires strict disinfection protocols. In this context, silver nanoparticles (AgNPs) stand out for their antimicrobial properties, reducing contamination and improving process efficiency, while also offering a sustainable alternative to harsh chemical treatments. In this study, AgNPs Argovit™ were evaluated at concentrations of 0, 25, 50, and 100 mg·L⁻¹ with immersion times of 5, 10, and 15 minutes. The best asepsis (94%) was achieved with 100 mg·L⁻¹ and 15 minutes of immersion, while the highest sprouting (7.03 microbulbs per explant) occurred with 25 mg·L⁻¹ and the same duration. AgNPs also reduced phenolic oxidation in explants, optimizing the *in vitro* propagation of *Lilium* sp. These findings highlight their potential for the commercial production and conservation of this ornamental species.

KEY WORDS: Bionanotechnology, microbicidal activity, micropropagation.



Please cite this article as/Como citar este artículo: Nuncio-Orta, G.L., Juárez-Maldonado, A., Benavides-Mendoza, A., González-García, Y., Lorenzo-Feijoo, J.C., Bogdanchikova, N., Pestryakov, A., Companioni-González, B. (2025). Effect of silver nanoparticles on the *in vitro* establishment of *Lilium* sp. *Revista Bio Ciencias*, 12, e1906. <https://doi.org/10.15741/revbio.12.e1906>.

Article Info/Información del artículo

Received/Recibido: February 11th 2024.

Accepted/Aceptado: September 03^h 2025.

Available on line/Publicado: September 25th 2025.

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RESUMEN

El *Lilium sp.*, o lirio, es una planta de gran importancia ecológica, cultural y comercial, apreciada en jardinería, fragancias y medicina tradicional. Dado que su reproducción natural enfrenta limitaciones, la propagación *in vitro* es esencial para su cultivo eficiente y sostenible, permitiendo obtener plantas libres de enfermedades y con alta calidad genética. Un reto significativo de esta técnica es la contaminación, que demanda protocolos estrictos de desinfección. En este contexto, las nanopartículas de plata (AgNPs) destacan por sus propiedades antimicrobianas, reduciendo la contaminación y mejorando la eficiencia del proceso, además de ser una alternativa sostenible frente a químicos agresivos. En este estudio, las AgNPs Argovit™ se evaluaron en concentraciones de 0, 25, 50 y 100 mg•L⁻¹ con tiempos de inmersión de 5, 10 y 15 minutos. La mejor asepsia (94%) se obtuvo con 100 mg•L⁻¹ y 15 minutos de inmersión, mientras que la mayor brotación (7.03 microbulbos por explante) ocurrió con 25 mg•L⁻¹ y el mismo tiempo. Las AgNPs también redujeron la oxidación fenólica en los explantes, optimizando la propagación *in vitro* del *Lilium sp.* Estos hallazgos resaltan su potencial para la producción comercial y conservación de esta especie ornamental.

PALABRAS CLAVE: Actividad microbicida, bionanotecnología, micropropagación.

Introduction

Lilium sp., commonly known as lily, plays a vital role across various domains. Ecologically, it serves as a food source for pollinators such as bees and butterflies, contributing to ecosystem health (García-Pérez *et al.*, 2023; Silva & Rocio, 2024). Culturally, it holds deep symbolic value, often representing purity and rebirth in traditions, art, and religions (Mantilla *et al.*, 2023; Arévalo-Galarza *et al.*, 2024). In horticulture, lilies are highly prized for their beauty and wide range of colors, enhancing both indoor and outdoor spaces (Imbago-Lanchimba & Gómez-Cabezas, 2021; Winarto *et al.*, 2024). They also have commercial applications in the fragrance industry due to their distinctive aroma, and certain species are used in traditional medicine to alleviate many ailments (Khan *et al.*, 2021; Anisah *et al.*, 2023). Thus, *Lilium sp.* combines aesthetic appeal, functional utility, and cultural significance, making it a truly exceptional plant.

Accelerated *in vitro* propagation of *Lilium sp.* is essential due to limitations in its natural vegetative reproduction (Patil *et al.*, 2021; Zuo *et al.*, 2024). The species often struggles to reproduce efficiently via bulbs, which restricts its commercial production and availability. *In vitro* techniques overcome these barriers by enabling the rapid generation of large numbers of plants under

controlled conditions (Kamalashree & Nayaka, 2023; Pałka et al., 2023). Moreover, this approach ensures disease-free plants with uniform genetic traits, enhancing crop quality (Dhorajiwala, 2022). *In vitro* propagation also supports the conservation of rare or endangered species and enables large-scale commercial production to meet ornamental and agricultural market demands (Mosonyi et al., 2022). For these reasons, *in vitro* propagation is an indispensable strategy for optimizing *Lilium sp.* cultivation and ensuring its sustainability.

However, *in vitro* cultivation of *Lilium sp.* faces several challenges, with contamination during the establishment phase being one of the most critical (Patil et al., 2021; Zuo et al., 2024). Contaminants often originate from microorganisms present in plant tissues or the laboratory environment, even under sterile conditions (Gong et al., 2023; Basit & Lim, 2024). The difficulty of completely eliminating fungi, bacteria, and other pathogens from explants can jeopardize the success of the culture. Improper handling of tools and culture media further increases contamination risks. This issue not only reduces process efficiency but also raises costs due to repeated cleaning efforts and media preparation (Fan & Sun, 2024; Winarto et al., 2024). Therefore, rigorous disinfection and sterilization techniques, along with strict protocols in controlled environments, are essential to minimize contamination and maximize *in vitro* outcomes.

Silver nanoparticles (AgNPs) have emerged as a promising tool for controlling contamination during the *in vitro* establishment of *Lilium sp.* (Singh et al., 2023; Zeng et al., 2024). Thanks to their antimicrobial properties, AgNPs can inhibit the growth of bacteria, fungi, and other microorganisms that typically compromise culture success (Lashin et al., 2021; Orlikowski et al., 2023). Their incorporation into culture media significantly reduces contamination rates, improving process efficiency and reducing reliance on harsher chemical treatments (Noori et al., 2024; Alfosea-Simón et al., 2025). Furthermore, AgNPs offer a sustainable and effective alternative, as they can be used at low concentrations without harming plant tissue development. Recent studies have explored their integration into disinfection and sterilization protocols, yielding promising results in the *in vitro* propagation of ornamental plants like *Lilium* (Hernández-Díaz et al., 2021; Okoroafor, 2022). This innovative approach represents a significant advancement in plant biotechnology, enhancing the production of high-value ornamental and commercial species. The objective of this study was to determine the effect of Argovit™ silver nanoparticles (AgNPs) on the *in vitro* establishment of *Lilium sp.*

Material and Methods

Plant material

Explants were obtained from commercially graded bulbs of *Lilium sp.*, oriental hybrid 'Concador Select' (grade 20/22; Ø 6.1–6.8 cm). The bulbs were stored for two months at 4 °C to overcome dormancy.

Silver nanoparticles (AgNPs) Argovit™

Silver nanoparticles were provided by the Scientific Production Center Vector-Vita Ltd. (Novosibirsk, Russia, <http://vector-vita.com/>) and are marketed under the name *Argovit*™. Characterization of *Argovit*™ AgNPs was previously conducted as described by Pastelín-Solano *et al.* (2020).

Effect of AgNPs on disinfection and *in vitro* establishment of *Lilium sp.*

To evaluate the effect of *Argovit*™ AgNPs on the disinfection of *Lilium sp.* scales during the *in vitro* establishment phase, different concentrations (0, 25, 50, and 100 mg•L⁻¹) and immersion times (5, 10, and 15 minutes) were tested. Middle and inner scales of the bulbs (Figure 1A and B) were selected as explants. These were first washed with commercial detergent and rinsed thoroughly with water. They were then immersed in 0.2 % (w/v) Captan® 50 PH for 15 minutes, followed by a rinse with sterile distilled water.

Under laminar flow conditions, explants were immersed in 70 % (v/v) ethanol for one minute, then disinfected with 0.25% mercuric chloride (HgCl₂) solution containing one drop of Tween 20 for seven minutes, followed by three rinses with sterile distilled water.

Finally, *Argovit*™ AgNPs were applied as previously described, followed by three additional rinses with sterile distilled water. The scales were trimmed at the proximal end and placed adaxially on Murashige & Skoog (1962) basal medium supplemented with 1.0 mg•L⁻¹ 6-BAP, 1.0 mg•L⁻¹ thiamine, 100 mg•L⁻¹ myo-inositol, and 30 g•L⁻¹ sucrose, solidified with 3.5 g•L⁻¹ Phytigel™ (pH adjusted to 5.7 ± 0.1).

During the experiment, explants were maintained in a growth chamber under dark conditions for 42 days at 25 ± 2 °C. At the end of incubation, the following parameters were evaluated across all treatments: contamination rate, phenolic oxidation, and number of microbulbs sprouted per *Lilium sp.* 'Concador Select' scale. A completely randomized bifactorial experimental design was used, with five jars per treatment and three explants per jar, totaling 15 replicates per treatment. Data were analyzed using InfoStat software version 2020, applying Fisher's LSD test at a significance level of $p \leq 0.05$.

Results and Discussion

Table 1 shows the effect of AgNPs on the disinfection and *in vitro* establishment of *Lilium sp.* scales from the oriental hybrid 'Concador Select'. The treatment with AgNPs at a concentration of 100 mg•L⁻¹ and 15 minutes of immersion achieved the highest level of asepsis (94%), showing significant differences compared to the other treatments. This demonstrates that silver nanoparticles can provide effective microbicidal activity, reducing *in vitro* contamination of explants—a key factor for successful *Lilium sp.* propagation. Additionally, the treatment with 25 mg•L⁻¹ of *Argovit*™

AgNPs and 15 minutes of immersion resulted in the highest sprouting per explant, with an average of 7.03 microbulbs, also showing significant differences from the other treatments. These findings suggest that AgNPs may enhance scale sprouting at specific concentrations, promoting plant development in *in vitro* cultures (Figure 1). Furthermore, a positive effect of AgNP disinfection was observed on the phenolization response of *Lilium sp.* explants (Table 1), with the highest values of absence of phenolic oxidation recorded across various AgNP concentrations and immersion times, showing marked significant differences compared to the control treatment.

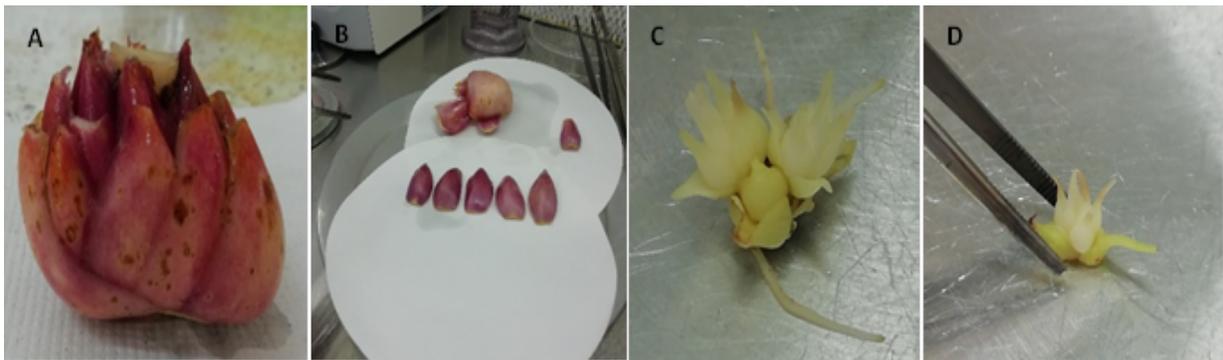


Figure 1. *In vitro* regeneration of *Lilium sp.* from the use of Argovit™ silver nanoparticles in the *in vitro* establishment stage (concentration of 25 mg·L⁻¹ and 15 minutes of immersion with silver nanoparticles).

Donor bulb (A), scale sections (B), microbulb induction (C), and differentiated microbulb at 42 days of age (D).

Table 1. Effect of Argovit™ silver nanoparticles on the disinfection and *in vitro* establishment of *Lilium sp.* scales.

Time (minutes)	Immersion with AgNPs (mg·L ⁻¹)	Percentage of contamination (%)	Absence of phenolic oxidation (%)	Number of sprouted microbulbs per explant
5	0	23 ^b	65 ^d	3.61 ^d
	25	33 ^b	100 ^a	6.70 ^b
	50	27 ^b	100 ^a	5.70 ^b
	100	23 ^b	80 ^b	4.80 ^c
10	0	27 ^b	63 ^d	3.42 ^d
	25	27 ^b	100 ^a	6.20 ^b
	50	33 ^b	87 ^b	5.90 ^b
	100	33 ^b	67 ^d	4.50 ^c
15	0	27 ^b	70 ^c	3.55 ^d
	25	27 ^b	100 ^a	7.03 ^a
	50	33 ^b	85 ^b	5.84 ^b
	100	6 ^a	74 ^c	4.66 ^c

Means with the same letter indicate that there are no statistically significant differences, LSD Fisher, $p \leq 0.05$.

As observed in this study, silver nanoparticles have shown positive effects on *in vitro* plant cultivation due to their antimicrobial and growth-promoting properties. These nanoparticles can reduce microbial contamination in culture media, ensuring a more sterile and suitable environment for plant tissue development. Moreover, in species such as *Brassica juncea* (Indian mustard), nanoparticles have been found to enhance shoot and root elongation. Similarly, in *Solanum tuberosum* (potato), they have improved plant regeneration under laboratory conditions. These benefits position silver nanoparticles as a promising tool for plant biotechnology and crop improvement (Chávez-García *et al.*, 2020; Bello-Bello & Castillo, 2023; Martínez-Martínez *et al.*, 2024; Vázquez-Flores, 2024).

Conclusions

The microbicidal effect of Argovit™ silver nanoparticles was demonstrated in the disinfection and *in vitro* establishment of *Lilium sp.* from the oriental hybrid 'Concador Select'. Additionally, *in vitro* sprouting was successfully promoted at a concentration of 25 mg•L⁻¹ with 15 minutes of immersion in silver nanoparticles. A positive effect of AgNP disinfection was also observed in the response of *Lilium sp.* explants to the phenomenon of phenolization.

Author contributions

Nuncio-Orta: Investigation, data management. Juárez-Maldonado: Conceptualization, experimental validation, and data analysis. Benavides-Mendoza: Conceptualization, data analysis. González-García: Experimental validation, conceptualization. Lorenzo-Feijoo: Data analysis, review, and editing. Bogdanchikova: Conceptualization, review. Pestryakov: Conceptualization. Companioni-González: Methodology development, writing, review, and editing.

All authors have read and approved the published version of this manuscript.

Funding

This research was funded by the Research Directorate of the Universidad Autónoma Agraria Antonio Narro, Saltillo, Coahuila (Project Code: 38111-425405001-2241).

Ethics Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments

The authors gratefully acknowledge the support and scientific exchange provided by the International Network on Bionanotechnology with Impact in Biomedicine, Food, and Biosecurity – CONAHCYT, Universidad Nacional Autónoma de México, Ensenada, Baja California, Mexico.

Conflict of Interest

The authors declare no conflict of interest.

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