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# Enhanced Tomato Seed Germination and Seedling Growth Through Oxidative Stress Using Biogenic Silver Nanoparticles
# Nanopartículas Biogénicas de Plata Incrementan la Germinación y Crecimiento de Tomate Mediante Estrés Oxidativo

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## SUMMARY

In this study, silver nanoparticles (AgNP) were successfully biosynthesized from *Larrea tridentata* leaf extract. X-ray Diffraction (XRD) and Transmission Electron Microscopy (TEM) analysis confirmed that AgNP are crystalline and hemispherical, with sizes between 4 and 26 nm. In addition, the effect of nanoparticles on tomato (*Solanum lycopersicum*) seed germination and seedling growth was examined. Seeds were pre-soaked with different concentrations of AgNP (0, 4.03, 6.72, 18.66, 51.84, and 86.4 mg $L^{-1}$). Up to 90% seed germination was achieved at a concentration of at least 4.03 mg $L^{-1}$, while the control reached below 60%. The highest vigor was obtained at a concentration of 51.84 mg $L^{-1}$, 2.67 times higher than the vigor of untreated seeds. The synthesized nanoparticles induced activation of tomato seedlings' defense system against reactive oxidative species, increasing CAT and APX enzyme activities and seedling's growth. Plumule and radicle length rose 1.49 and 1.98-fold compared to the control. The seedlings were susceptible at 86.4 mg $L^{-1}$ of AgNP, which affected all growth parameters and induced oxidative stress response through increased hydrogen peroxide ($H_2O_2$) formation and proline content. These provide further insight into the effects of biogenic silver nanoparticles on plants during their initial stages of development, demonstrating that these nanoparticles can promote early germination.

***Index words:*** *antioxidant mechanisms, biosynthesis, Larrea tridentata, nanomaterials, seed priming.*

## RESUMEN

En este estudio, se biosintetizaron nanopartículas de plata (AgNP) a partir del extracto de hoja de *Larrea tridentata*. El análisis de difracción de rayos X (DRX) confirmó que las AgNP son cristalinas, y el análisis de microscopía electrónica de transmisión (TEM) reveló que son hemisféricas con un tamaño promedio de 4 a 26 nanómetros. Además, se examinó el efecto de las AgNP sobre la germinación de semillas y el crecimiento de plántulas de tomate (*Solanum lycopersicum*). Las semillas se remojaron previamente con diferentes concentraciones de AgNP (0, 4.03, 6.72, 18.66, 51.84 y 86.4 mg $L^{-1}$). Se logró incrementar hasta un 90% la germinación de las semillas a una concentración de 4 mg $L^{-1}$ de AgNP, mientras que el control alcanzó menos del 60%. El mayor vigor se obtuvo con una concentración de AgNP de 51. 84 mg $L^{-1}$, 2.67 veces mayor que el vigor de las semillas no tratadas. Las AgNP indujeron la activación del sistema de defensa de las plántulas de tomate contra



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especies reactivas de oxígeno, aumentando las actividades de las enzimas CAT y APX; y el crecimiento de las plántulas. La longitud de la plúmula y la radícula aumentó 1.49 y 1.98 veces en comparación con el control. Las plántulas fueron susceptibles a 86.4 mg $L^{-1}$ de AgNP, lo que afectó todos los parámetros de crecimiento e indujo una respuesta al estrés oxidativo a través de una mayor formación de peróxido de hidrógeno ($H_2O_2$) y contenido de prolina. Estos proporcionan más información sobre los efectos de las AgNP biogénicas en las plantas durante sus etapas iniciales de desarrollo, lo que demuestra que estas nanopartículas pueden promover la germinación temprana.

***Palabras clave****: mecanismos antioxidantes, biosíntesis, Larrea tridentata, nanomateriales, cebado de semillas.*

## INTRODUCTION

At present, recent advances in nanotechnology are creating opportunities for the synthesis of metallic nanoparticles (NP) and their use in environmental remediation (Guerra, Attia, Whitehead, and Alexis, 2018) and agriculture (Shang *et al*., 2019). In agriculture, nanotechnology can solve multiple crop production problems by efficiently using fertilizers, reducing the number of agrochemicals required to control pests and treat diseases, and increasing crop quality and yield (Chen *et al*., 2020; Rajan, Chandran, Shahena, and Mathew, 2020; Zahedi, Karimi, and Teixeira, 2020). The characteristics of NP, such as size, shape, and chemical composition, determine their properties for agronomic applications, such as uptake, translocation, and toxicity to plant systems (Tripathi *et al*., 2017a). Therefore, it is important to evaluate the toxicity of NP using seed germination assays since stress tolerance is regulated at different germination phases (Vishal and Kumar, 2018).

Silver nanoparticles (AgNP) can be synthesized using physical and chemical methods (Yaqoob, Umar and Ibrahim, 2020). However, biogenic synthesis is an eco-friendly alternative to preparing AgNP; it has advantages over physical and chemical methods, such as lower process time and cost production. The natural compounds from plant extracts can act as reducing and stabilizing agents. Their interaction with these agents strongly influences the size, morphology, stability, and properties of metallic NPs (Yaqoob *et al*., 2020). The adsorption of amino acids, sugar, organic acids or fatty acids, and secondary metabolites onto the surface of NPs from the plant extracts made them biocompatible (Zhang *et al*., 2021). *Larrea tridentata* is a shrub that grows in the desert areas of the Southwestern United States and Northern Mexico (Abou-Gazar, Bedir, Takamatsu, Ferreira, and Khan, 2004). Their phytochemical compounds include phenols, triterpene glycosides, lignans such as nordihydroguaiaretic acid, and flavonoids (Jitsuno and Mimaki, 2010; Abou-Gazar *et al*., 2004). Their biological activities include antioxidants, anti-VIH, antitumor, anti-inflammatory, anti-hyperglycemic, and antimicrobial actions against plant pathogenic fungi (Martins, Aguilar, Teixeira, and Mussatto, 2012; Osorio *et al*., 2010; Abou-Gazar *et al*., 2004). Also, some phytoconstituents produced by plants may exhibit beneficial effects in cells at biochemical and molecular levels or have a synergic antagonist effect against phytopathogens (Karmous, Pandey, Haj, and Chaoui, 2019; Song, Wu, Wang, and Han, 2019).

In seed science, Ag (Roy and Anantharaman, 2018), Au (Ndeh, Maensiri, and Maensiri, 2017), ZnO (Raja *et al*., 2019), and $TiO_2$ (Rafique *et al*., 2019) NP obtained from plant extracts have been used to enhance seed germination and seedling growth. They can increase the activity of enzymes such as amylases, proteases, and lipases involved in the growth and development of the embryo (Acharya, Jayaprakasha, Crosby, Jifon, and Patil, 2020). Additionally, these nanoparticles show fewer toxic effects on seed germination than NP obtained from chemicals (Garg *et al*., 2020). The AgNP offers protection against phytopathogenic microorganisms and positively affects plant germination, growth, and development of crop plants (Biba *et al*., 2020; Singh, Handa, and Manchanda, 2020). However, the size, morphology, and concentration of AgNP generate adverse effects at the biochemical and physiological levels in the plant´s growth stage (Prażak, Święciło, Krzepiłko, Michałek, and Arczewska, 2020). Therefore, it is essential to investigate its effects on the critical steps for successful plant growth.

In the present study, silver NP (AgNP) were biosynthesized using *Larrea tridentata* leaf extract and characterized using X-ray diffraction (XRD) and transmission electron microscopy (TEM). Furthermore, the effect of the biosynthesized AgNP on tomato seed germination and seedling growth was investigated.

## MATERIALS AND METHODS

### Preparation of Leaf Extract

Fresh *Larrea tridentata* leaves were collected from Jaguey de Ferniza, Saltillo, Coahuila, 25° 13′ 03.7″ N; 101° 03′ 48.7″ W, and 2198 meters of altitude. The leaves were dried by lyophilization and ground. Dried leaf powders (10 g) were placed in 100 mL of deionized water and boiled for 60 min at 60 °C. The extract was filtered through a glass funnel with a sintered glass disc (0.22 µm), and the filtered extract solution was stored at 4 °C for further use (Méndez-Andrade, 2019[1]).

### Synthesis of AgNP Using *Larrea tridentata*

The extract solution was heated at 80 °C, and 0.5 mM $AgNO_3$ solution was slowly dropped (1 mL $min^{-1}$) until the extract: $AgNO_3$ ratio was 1:5 (v/v). The reaction mixture was incubated at 80 °C for 2 h. AgNP biosynthesis was accompanied by an observable color change from deep yellow to reddish black. Next, the AgNP suspension was centrifuged at 10 000 rpm for 10 min, and pellets were washed thrice with methanol (Méndez-Andrade *et al*., 2022).

### Characterization of AgNP

Phase identification was performed by powder XRD with a Rigaku Ultima IV diffractometer using CuKα radiation (λ = 1.5418 Å) at 40 kV and 44 mA and a scan rate of 0.02 (2θ/s). The size and morphology of AgNP were studied by TEM using the FEI Titan 80-300 microscope.

### Seed Germination Assay

Seed germination assays followed the ISTA protocol (ISTA, 2004). Tomato seeds were placed in Petri dishes with 100 seeds per each. Six treatments were applied; control seeds were treated with distilled water, and test seeds were treated with AgNP suspensions at concentrations of 0, 4.03, 6.72, 18.66, 51.84, and 86.4 mg $L^{-1}$; the doses are according to Méndez-Andrade *et al*. (2022) the effectiveness of inhibiting the growth bacteria *Clavibacter michiganensis*. AgNP suspensions (10 mL) of different concentrations were added to seeds for imbibition for 18 h in a growth chamber (Equitec model EGCS 301 3SHR) at 25 °C ± 1 °C and 30% relative humidity under a 16:8-h photoperiod. Once the seed imbibition period was completed, four replicates of 25 seeds for each treatment were placed on Anchor papers (Seedburo Equipment Company, Des Plaines, IL, USA). Germination rate (%) was determined as the percentage of germinated seeds from the total number of seeds at the end of 14 days. Germination vigor (%) was calculated as the percentage of normal seedlings from the total number of seedlings at seven days after seeding. Plumule and radicle lengths were measured with a ruler. Seedling dry biomass was weighed with an analytical balance.

### Vigor Index

The first count of normal seedlings was carried out on the sixth day after sowing and was expressed as a percentage. This variable is an indicator of the vigor that the seed possesses to germinate in less time and that the plants can be properly established under field conditions. The equation used to calculate this variable is the one indicated by García-López *et al*. (2018):

$$\text{Vigor index} = (\text{Normal seedling (first counting)})/(\text{Total seeds sown})*100 \quad (1)$$

### Germination Percentage

At the end of the bioassay, a count of normal seedlings was performed and the information obtained was expressed as GP (%). The equation used to calculate this variable was the following:

[1] Méndez-Andrade, R. (2019). Efectividad antibacterial in vitro e in vivo de nanopartículas de plata biosintetizadas a partir de extractos de *Larrea tridentata* contra *Clavibacter michiganensis* en plantas de *Solanum lycopersicum*. Tesis para obtener el grado de Maestro en Ciencias. Centro de Investigación en Química Aplicada. Saltillo Coahuila. Disponible en https://ciqa.repositorioinstitucional.mx/jspui/bitstream/1025/631/1/Tesis_RolandoMendez%202019.pdf

Germination percentage = (Normal seedling (second counting))/(Total seeds sown)*100 (2)

### Biochemical Parameters

### Proline and Hydrogen Peroxide ($H_2O_2$) Content

The proline content was determined by the method of Abraham, Hourton, Erdei, and Szabados, (2010). Plant fresh tissue (500 mg) was homogenized in 3% sulfosalicylic acid. After centrifugation, 0.2 mL of the supernatant was collected, and 0.2 mL was mixed with glacial acetic acid (28.6%) and acid ninhydrin reagent (6 M) and incubated for 1 h at 96 °C. The reaction was terminated in an ice bath, followed by the extraction of the colored chromophore in toluene. The absorbance of the chromophore was measured at 520 nm. The proline concentration in the samples was computed from a standard curve of L-proline. $H_2O_2$ content was determined by the method of Velikova, Yordanov, and Edreva (2000). Fresh tissues (0.25 g) were homogenized in 2 ml of 0.1% (w/v) TCA. The homogenate was centrifuged at 12 000 rpm for 15 min. The supernatant was mixed with 10 mM phosphate buffer pH 7.0 in a relation of 1:1 (v/v) and 1 ml of 1 M KI. The $H_2O^2$ content of the supernatant was evaluated by comparing its absorbance at 390 nm with a standard calibration curve.

### Statistical Analysis Antioxidant Enzymes

For catalase (CAT) and ascorbate peroxidase (APX) activities, an enzymatic extract was done, according to Elavarthi and Martin (2010). Fresh tissues (0.25 g) were grounded with 1% (w/v) polyvinylpyrrolidone in a prechilled mortar and pestle. The samples were homogenized in phosphate buffer (100 mM, pH 7.0) and added with EDTA (0.1 mM). The homogenate was centrifuged at 15 000 × g for 15 min at 4 °C. The supernatant was stored at -20 °C until analysis. APX assay was determined as described by Nakano and Asada (1981) by monitoring the ascorbate decomposition per minute at 25 °C and was calculated using an extinction coefficient of 2.8 $mM^{-1}$ $cm^{-1}$. For enzyme assay, 0.1 mL of enzyme extract was added to 1.84 mL of assay buffer (100 mM phosphate buffer pH 7.0, 0.1 µM EDTA), and 0.60 mL of 0.5 M $H_2O_2$ was added to start the reaction. CAT activity was determined following the method of Aebi (1984) by monitoring the disappearance of $H_2O_2$ at 240 nm and calculated using an extinction coefficient of 0.036 $mM^{-1}$ $cm^{-1}$. For the CAT assay, 0.1 mL of the extract was mixed with 950 mL of 10 mM $H_2O_2$, and the absorbance at 240 nm was recorded for 2 min. The protein concentration was determined by Bradford assay (Kruger, 2009). The enzyme-specific activity was expressed as enzyme units per milligram protein.

The data obtained was analyzed in the statistical program INFOSTAT (Di Rienzo *et al*., 2001), significant differences between the control and treatments were analyzed with one-way ANOVA, and means were compared using the Tukey test ($P \leq 0.05$).

## RESULTS AND DISCUSSION

### Characterization of AgNP

The AgNP presented characteristic diffraction peaks at 38.2°, 44.2°, 64.5°, and 77.2° (2θ), which correspond to the lattice planes (111), (200), (220), and (311), respectively, reflecting the FCC structure of Ag (JCPDS card 04-0783), (Figure 1). Smaller peaks were also observed at 27.8°, 32.2°, 46.2°, 54.9°, and 57.6°, which correspond to the lattice planes (111), (200), (220), (311), and (222), respectively, reflecting the AgCl phase (FCC structure JCPDS No. 31-1238). TEM revealed crystalline and hemispherical AgNP with a 4-26 nm particle size (Figures 2a, b).

### Effect of AgNP on tomato seeds

The germination rate was increased in all treatments compared with control, without significant differences among different concentrations (Figure 3a). The germination rate was over 90% in seeds treated with AgNP and 54% without treatment. The highest seed vigor (85.60% ± 3.25%) was achieved at an AgNP concentration of 51.84 mg $L^{-1}$, which was 2.67 times higher than the vigor of control seeds (32% ± 0.02%) (Figure 3b). In this study, the AgNP-treated seedlings showed markedly increased plumule and radicle lengths and dry seedling biomass compared with control seedlings (Table 1); however, at 86.4 mg $L^{-1}$, all variables were significantly decreased.

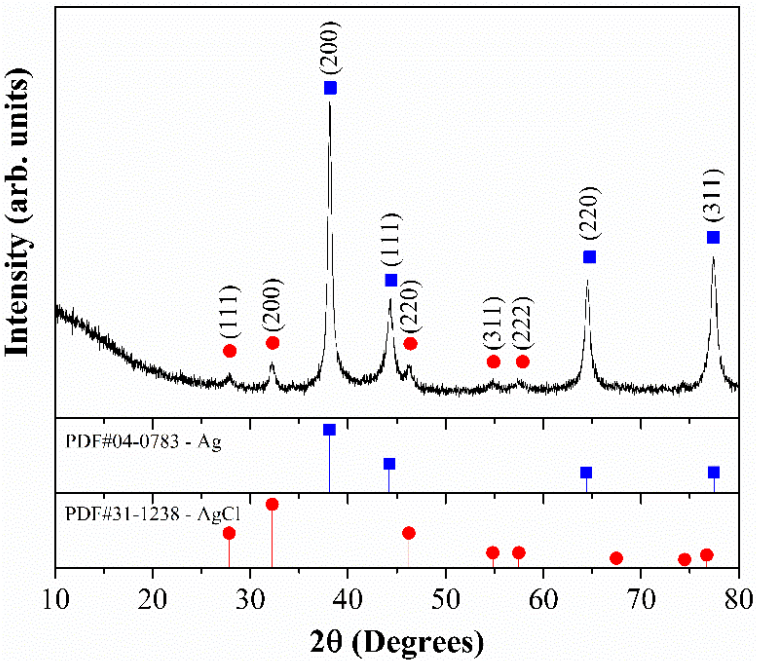


**Figure 1. X-ray diffraction pattern of biosynthesized silver nanoparticles.**

A slight decrease of $H_2O_2$ was observed with the dose of 4 to 51.4 mg $L^{-1}$ (Table 2); however, at 86.6 g $L^{-1}$, it reached an increment of 2.75 and 1.1fold (92±0.98 µM $g^{-1}$ FW, radicle; 184±2.04 µM$g^{-1}$ FW, plumule) to respect to control (88±2.41 µM $g^{-1}$ FW, radicle; 167±1.53 µM $g^{-1}$ FW, plumule). A similar effect was observed in the proline content, which was reduced in both tissues (plumule and radicle) treated with concentrations below 86.6 mg $L^{-1}$ of Ag NPs. The results showed a decrease in proline content with a maximal of 0.78-fold less (15.97±0.92 mg $g^{-1}$ FW) at 6.7 mg $L^{-1}$ concerning control (20.33±0.89 mg $g^{-1}$ FW), (Table 2), and a decrease in proline content related to the low level of $H_2O_2$ but increased with the higher dose of AgNP in a 10.5 and 14.6% (22.71±0.7 mg $g^{-1}$ FW; 32.85±0.61 mg $g^{-1}$ FW) concerning control (20.33±0.89 mg $g^{-1}$ FW).

The above results were accomplished by enzyme antioxidant activation (Figure). Plumule showed higher CAT activity than plumule compared to control. CAT activity increased at 18.7 mg $L^{-1}$ of AgNP in both tissues, reaching 1.75 and 1.5-fold (1.93±0.14 UI $mg^{-1}$ of protein, radicle; 2.5±0.14 U $mg^{-1}$, plumule). at 18.7 mg $L^{-1}$respect to control (1.1±0.16 UI $mg^{-1}$ of protein, radicle; 1.6 0.1 UI $mg^{-1}$ of protein, plumule). CAT activation is according to the lower $H_2O_2$ content. On the other hand, APX activity increased in both tissues related to AgNP concentration, and the increase was statistically significant compared to the control (Figure 4). The higher APX activity reached 86.6 mg $L^{-1}$, rising 1.8 and 1.3-fold concerning control (3.5±0.2 UI $mg^{-1}$ of protein, radicle; 5.3±0.14 UI $mg^{-1}$ of protein).

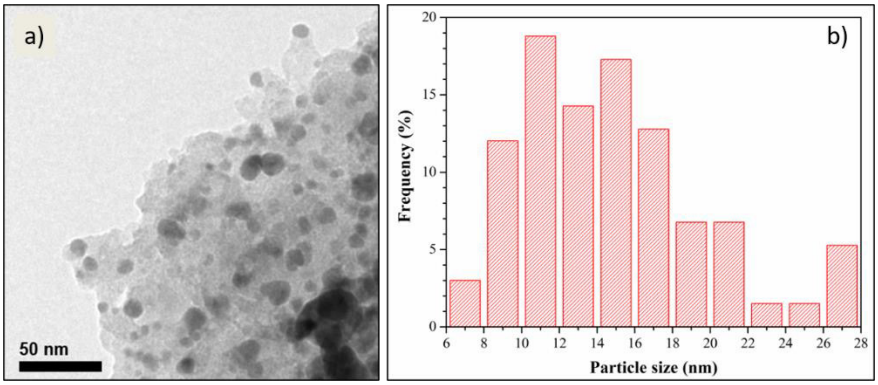


**Figure 2. Transmission electron microscopy image (a) and particle size distribution (b) of silver nanoparticles.**

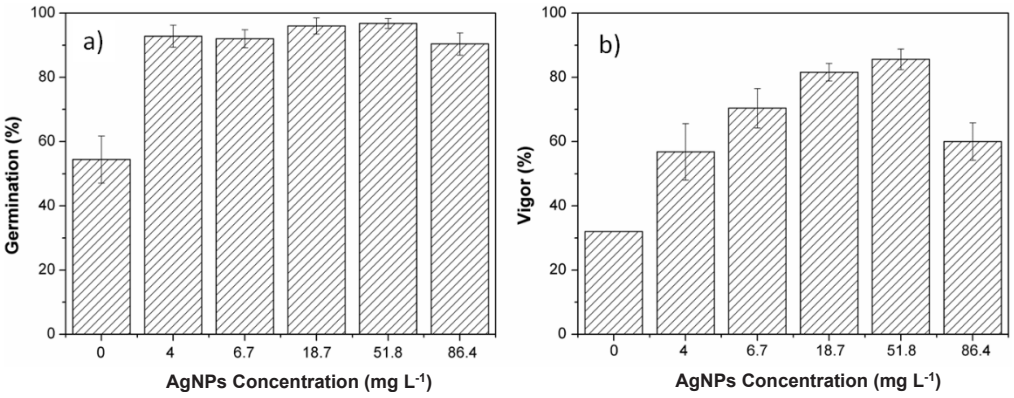


**Figure 3. Effect of silver nanoparticles on the germination (a) and vigor (b) of tomato seeds Bars represent mean ± standard deviation (n = 4).**

Seed priming is a pre-sowing seed treatment that enhances and accelerates germination through physiological activities and morphogenesis, which begins with physical water absorption (Fu *et al*., 2024). AgNP have been reported to improve water absorption by activating enzymes such as α-amylase, which are necessary for germination. Moreover, NP increased reactive oxygen species production and affected starch hydrolysis for maintaining embryonic growth, producing vigorous seedlings (Mahakham, Sarmah, Maensiri, and Theerakulpisut, 2017; Laware and Raskar, 2014). In *Withania somnifera* seeds treated with deoxycholic acid, capped AgNP broke physical dormancy, thus increasing seed germination by 50%, which was superior to the performance of other compounds used to induce germination (Thangavelu, Munisamy, and Krishnan, 2019). Previous studies on tomatoes, rice, barley, beans, and turnip have reported similar dose-dependent effects of AgNP, with high doses showing negative effects (Noori *et al*., 2020; Thuesombat, Hannongbua, Akasit, and Chadchawan, 2014; Thiruvengadam, Gurunathan, and Chung, 2015). In *Arabidopsis thaliana*, a reduction in radicle length was related to ethylene biosynthesis inhibition via aminocyclopropane-1-carboxylic acid activation (Siddiqui, Al-Whaibi, Firoz, and Al-Khaishany, 2015). The effects of AgNP on the morphology and physiology of plants have been attributed to the accumulation of essential proteins involved in cell division and glycolysis (Syu, Hung, Chen, and Chuang, 2014). Moreover, AgNP induce indoleacetic acid production and activate biochemicals related to oxidative stress and immune response (chlorophyll, phenols, carotenoids, antioxidant enzymes, and genes involved in defense), (Noori *et al*., 2020; Sadak, 2019; Latif, Ghareib, and Tahon, 2017). Also, NP increase nitrate reductase enzyme activity, improving the ability of the seeds to absorb and utilize water and other nutrients (Shinde, Paralikar, Ingle, and Rai, 2020). Proline has been considered an antioxidant due to its ability to neutralize the reactivity of $O^2$, $OH^-$, $O^{2-}$ and $H_2O_2$ and to stabilize/activate enzymes with antioxidant functions such as CAT, APX, and POX activity, which are highly dependent on proline concentration (Szabados and Savoure, 2010). Proline is an indicator of stress in the plant, and its metabolism influences signaling pathways by ROS formation in the mitochondria via the electron transport chain; some studies suggested that proline level decreases after relief stress (Agrawal *et al*., 2015; Al-Khayri, Jain, and Johnson, 2016). In our results, proline accumulation decreases at lower doses of NPs.

**Table 1. Effects of AgNP on plumule and radicle length and dry seedling biomass in tomato seedlings.**

| Concentration | Plumule length | Radicle length | Seedling dry biomass |
|---|---|---|---|
| mg $L^{-1}$ | - - - - - - - - - - - - - - - - - - cm - - - - - - - - - - - - - - - - - - | | mg |
| 0 | 3.23±0.15 b | 4.45±0.30 cd | 16.80±0.00 c |
| 4 | 3.75±0.12 b | 4.96±0.29 d | 20.40±3.07 bc |
| 6.7 | 4.83±0.14 a | 8.33±0.30 a | 26.66±3.30 ab |
| 18.7 | 4.78±0.14 a | 8.19±0.26 a | 27.02±1.92 ab |
| 51.8 | 4.63±0.13 a | 6.96±0.22 b | 34.36±1.70 a |
| 86.4 | 3.80±0.11 b | 3.34±0.19 e | 22.40±2.44 bc |

Different letters indicate significant differences (Tukey test, $P \leq 0.05$) between treatments.

**Table 2. Effects of AgNP on proline and hydrogen peroxide ($H_2O_2$) content on plumule and radicle length in tomato seedlings.**

| | Proline | | $H_2O_2$ | |
|---|---|---|---|---|
| Concentration | Radicle | Plumule | Radicle | Plumule |
| mg $L^{-1}$ | - - - - - - - - - - - mg $g^{-1}$ fw - - - - - - - - - | | - - - - - μM $g^{-1}$ fw - - - - - | |
| 0 | 20.33±0.89 c | 28.67±0.91 ab | 88±2.41 | 167±1.53 |
| 4 | 17.94±1.07 ab | 26.98±1.08 a | 75±3.15 | 156±1.02 |
| 6.7 | 15.97±0.92 a | 23.05±0.32 ab | 63±1.56 | 142±2.01 |
| 18.7 | 16.29±0.52 a | 19.19±0.32 ab | 68±3.22 | 139±0.85 |
| 51.4 | 17.45±0.76 ab | 25.08±0.62 ab | 78±1.91 | 162±0.93 |
| 86.6 | 22.71±0.7 c | 32.85±0.61 a | 92±0.98 | 184±2.04 |

Different letters indicate significant differences (Tukey test, $P \leq 0.05$) between treatments.

This decrease could be related to the participation of AgNP in improving electron exchange efficiency, resulting in seedling growth through antioxidant status at lower concentrations. (Sharma *et al*., 2016; Sami, Siddiqui, and Hayat, 2020). Meanwhile, Yadu *et al*. (2018) demonstrated that AgNP addition improves the oxidative injuries caused by fluoride stress, enhancing the levels of proline and up-regulated stress response genes Nevertheless, AgNP can increase ROS levels at higher doses that harm plant growth, as demonstrated in our results at 86.4 mg $L^{-1}$ (Vinković *et al*., 2018).

It is well known that nanomaterials can induce oxidative stress, though plants have different mechanisms of defense against stress enzymatic/non-enzymatic to control the excess of reactive oxygen species (ROS). CAT and APX are the principal components of the enzymatic defensive systems that increase activities to reduce excess toxic oxidants such as $H_2O_2$ (Pandhair and Sekhon, 2006). The decreased CAT activity indicates that AgNP affected the cellular homeostasis of tomato seedlings at non-optimized concentrations, which could result in a growth reduction due to an excess of ROS (Tripathi *et al*., 2017b). Our results do not reveal the toxicity of AgNP at the doses assayed. The presowing treatment with AgNP had superior values in the germination and growth development of the tomato seeds than the control. It showed an activated antioxidant defense system due to the oxidative burst stimulated during germination (Shinde *et al*., 2020). The high antioxidant properties can enhance the tolerance of seedlings and plants to biotic and abiotic stress during post-priming germination, reducing losses in field production (Pereira, Caixeta, Fraceto, and Santaella, 2021). Recent research has demonstrated the potential of NP for seed nano-priming due to the improvement in root and shoot development through a signaling pathway that involves the activation of the antioxidant system and genes of modulate metabolic processes, such as phytohormones and others related to plant stress resistance (Pereira *et al*., 2021). Nonetheless, at 86.4 mg $L^{-1}$, the plumule length and percentage of vigor decreased, indicating the possibility that a higher dose had adverse effects on the seedling.

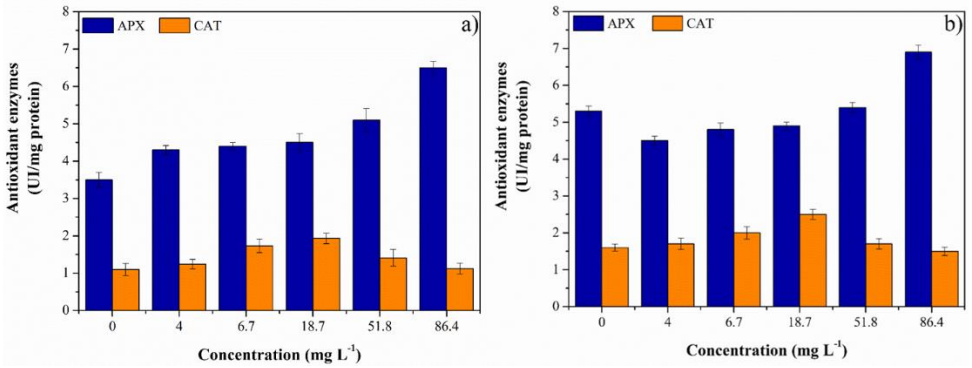


**Figure 4. Activities of antioxidant enzyme, ascorbate peroxidase (APX), and catalase (CAT) in tomato seedlings.**

## CONCLUSIONS

We biosynthesized AgNP using *Larrea tridentata* leaf extract. The biosynthesized AgNP were hemispherical and crystalline, with an average size of 4-26 nm. Exposing tomato seeds to 51.84 mg $L^{-1}$ of AgNP improved germination and seed vigor through moderate stress to seeds while soaking and stimulating stress responses, such as ROS and enzymatic and non-enzymatic antioxidants. Applying this dose as a treatment before sowing will improve seed uniformity, break dormancy, support effective plant growth, and lead to a higher yield. This technology is an economically viable option for most field crops However, seed germination is a complex process involving coordinated signaling pathways and molecular regulation, where hormones and nitric oxide are essential molecules involved. Nevertheless, whether these processes rely on a unique dominant signaling pathway or the overlap of many is unknown. Additional research using tools in -omics science could give us a comprehensive view of the mechanisms while NP promote germination.

## ETHICS STATEMENT

No potential conflict of interest was reported by the authors.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF SUPPORTING DATA

The data that support the findings of this study are available from the corresponding author, IVR upon reasonable request.

## COMPETING OF INTERESTS

The authors declare that they have no competing interests.

## FINANCING

Not applicable.

## AUTHORS' CONTRIBUTIONS

Author contribution statement: R.M.A., and I.V.R. Conceived and designed the work: I.V.R., and L.A.G.C. Provided the plant materials: R.M.A., and N.A.R.T. Performed the experiments and analyzed the data: R.M.A., I.V.R., and L.A.G.C. Wrote the original draft: Y.W., I.V.R., N.A.R.T., and L.A.G.C. Analyzed the data and revised the manuscript: R.M.A. All authors read and approved the manuscript.

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