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BIOSYNTHESIS OF SILVER NANOPARTICLES MEDIATED FROM AZOLLA SPP. AND THEIR EVALUATION AS A POTENTIAL BIOSTIMULANT IN THE DEVELOPMENT OF RAPHANUS SATIVUS L

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ABSTRACT

Green nanoparticle synthesis is a biotechnological approach that uses plant extracts, offering important advantages and allowing the synthesis of nanoparticles with remarkable properties for use in numerous scientific fields. In particular, its application in agriculture stands out for the benefits it offers to plants, improving their development. The objective of this study was to synthesize silver nanoparticles (AgNPs) using aqueous extract of Azolla spp. with the purpose of applying them in the in vitro cultivation and field sowing of radish (Raphanus sativus). Indeed, to synthesize the nanoparticles, 9 mM silver nitrate solutions were mixed with 5% Azolla aqueous extract. The pH was adjusted to 7.5 with 0.1 mM NaOH and the solution was stored for 5 days. Finally, it was centrifuged (5500 rpm for 15 min) and the nanoparticles were dried in a muffle (45°C for 12 h). According to the characterisation of AgNPs, UV-VIS spectroscopy revealed an absorbance peak maximum of 2,651 at 430 nm, the presence of functional groups determined by FT-IR and phytochemical screening, and a nanoparticle size between 25 - 75 nm based on AFM data. In addition, the application of AgNPs in radish showed a remarkable effect on the in vitro germination percentage of 95% after 6 days of cultivation; however, the average number of seeds that developed their cotyledons and there in vitro growth was not favored, being lower than the control and Azolla extract treatments, respectively. The field evaluation of the plants treated with nanoparticles, on the other hand, showed a slightly higher growth than the control reflected in the average plant height, leaf development and weight of the radish tuber, but it was not significantly different and presented lower values compared to the other treatments applied.

KEYWORDS: Azolla, Growth, Germination, Synthesis, Silver Nanoparticles, Radish.

1. INTRODUCTION

Technological advancement has been a fundamental component in the development of viable alternatives to address the various limitations currently faced by the agricultural sector. As the global population increases, greater food production is required to meet the nutritional demands of the human population. In fact, Rea et al. (2015) and Cota et al. (2016) mention that "according to the latest UN calculations, the world population will increase from 6.8 billion to 9.1 billion by 2050."

Nanotechnology has emerged as a technological and scientific advancement capable of transforming agriculture through the provision of innovative tools for the molecular detection of biotic and abiotic stress, as well as for the rapid identification of phytopathogenic diseases and the enhancement of plant capacity to absorb water, nutrients, and pesticides (Lateef et al., 2013; Lira et al., 2018). Moreover, nanobiotechnology enhances and complements knowledge of crop biology and, therefore, can potentially optimize crop yield and nutritional value (Fraceto et al., 2016).

Silver nanoparticles (AgNPs) are nanomaterials with sizes ranging from 1 to 100 nm in at least one dimension (Alvear, 2019). As size decreases, the surface area-to-volume ratio increases, leading to significant modifications in their physical, chemical, and biological properties. Due to their extremely small size, these nanoparticles can penetrate biological tissues more effectively (Sánchez, 2017; Esquivel and Mas, 2021).

Azolla spp., a plant belonging to the Azollaceae (Salviniaceae) family, is known for its symbiotic association with the cyanobacterium *Anabaena*. It is an aquatic fern native to temperate regions of the American continent (Samad et al., 2020). This plant is widely used as a nutrient-rich organic fertilizer to improve soil fertility and is particularly noted for its ability to fix atmospheric nitrogen (N_2) and accumulate heavy metals. Additionally, *Azolla* biomass represents a promising resource for the green synthesis of silver nanoparticles (AgNPs) for agricultural applications (López et al., 2022).

In Ecuador, approximately 15,000 hectares of radish are cultivated in the inter-Andean region (Vera, 2024). Radish (*Raphanus sativus* L.) is a horticultural crop with strong market potential, characterized by a diverse nutritional profile that benefits consumer health and moderate commercial value.

The present study aims to qualitatively characterize metabolites with reducing capacity in *Azolla* spp. extract and to identify the formation of silver nanoparticles synthesized from this extract. Subsequently, the effect of these nanoparticles will be evaluated under in vitro conditions and in field cultivation of radish (*Raphanus sativus* L.) to assess their potential for improving crop development.

Based on the above, this study was conducted to evaluate the benefits of synthesizing silver nanoparticles (AgNPs) using *Azolla* spp. biomass and their effect on radish (*Raphanus sativus* L.) seedlings.

2. METHODOLOGY

The study involved the synthesis of silver nanoparticles (AgNPs) from the aqueous extract of *Azolla spp.* and their application in radish cultivation. The experimental design relevant to the study is a completely randomized block design (CRBD) for in vitro and field evaluation. In this sense, for the in vitro cultivation, 4 treatments with 4 repetitions were established, each containing 10 certified seeds of the Crimson Giant radish variety per replica (Table 1).

Table 1: Experimental Design for the In Vitro Cultivation of Radish.

Treatment	Replicates (n)	Seeds per replicate	Applied treatment	Concentration	Dose per replicate
T0 (Control)	4	10	Agar medium	N/A	N/A
T1	4	10	Azolla extract	0.05 g mL^{-1}	1 mL
T2	4	10	Silver nanoparticles	50 mg L^{-1}	1 mL
T3	4	10	Murashige and Skoog medium (MS)	4.54 g L^{-1}	0.181 g

Note: The Treatments Were Applied Prior To In Vitro Planting in the Laminar Flow Chamber.

The field cultivation carried out after transplantation was constituted with 20% of the plants cultivated in vitro for each treatment, such that the application of 4 treatments was evaluated in 8 experimental units for each, selecting the in vitro plants randomly (Table 2).

Table 2: Experimental Design for Field Cultivation of Radish (Raphanus Sativus L.).

Treatment	Replicates (n)	Plants per replicate	Applied treatment	Concentration	Application dose per plant
T0 (Control)	8	1	Control (no treatment)	N/A	N/A
T1	8	1	Aqueous extract of *Azolla*	0.05 g mL^{-1}	25 mL
T2	8	1	Silver nanoparticles	50 mg L^{-1}	25 mL
T3	8	1	Granular fertilizer	150 kg ha^{-1}	0.165 g

Note: Treatments Were Applied Every Two Weeks, And Irrigation Was Performed Every Three Days Throughout the Experiment.

In this way, the total population evaluated in the in vitro culture amounts to 160 radish seeds, while in the field culture, 20% of the seedlings germinated in vitro were evaluated, totaling 32 radish plants. It should be noted that the data from this study were processed using the statistical program Infostat version 2020, and an analysis of variance (ANOVA) was performed using the Tukey test with a p-value >0.05 to determine if there were significant differences between the treatments used.

2.1. Synthesis Of Nanoparticles

To synthesize silver nanoparticles, silver nitrate ($AgNO_3$) was purchased from La Casa del Químico in Quito, Ecuador. The *Azolla* used in this study was obtained from a local supplier in Loja, Ecuador. The first step consisted of preparing an aqueous extract. For this purpose, *Azolla* was washed with tap water to remove roots and residual impurities. After cleaning, 5 g of *Azolla* were weighed and crushed using a mortar and pestle. The material was then transferred, using a funnel, into a 100 mL volumetric flask and filled with distilled water to the mark. Subsequently, the metabolites were extracted by placing the solution in a water bath at 80 °C for 20 min. After extraction, the solution was removed from the water bath and allowed to cool to room temperature. It was then filtered under vacuum, transferred into 100 mL glass vials, and stored at 4–8 °C until use. The obtained aqueous *Azolla* extract was used as both a reducing and stabilizing agent for the synthesis of silver nanoparticles. In parallel, a 9 mM silver nitrate solution was prepared by dissolving 1.528 g of $AgNO_3$ in 1 L of distilled water. Once both the aqueous extract and the silver nitrate solution were prepared, 1 L of the $AgNO_3$ solution was transferred to an Erlenmeyer flask and heated to 80 °C. Upon reaching this temperature, 50 mL of the *Azolla* extract were added under continuous stirring to ensure homogeneity. The pH of the mixture was then measured at room temperature and adjusted to 7.5 using 0.1 M sodium hydroxide. Finally, the solution was transferred to a 1 L glass container, covered with aluminum foil, labeled, and stored at room temperature for 5 days. After this period, the supernatant was partially removed, leaving approximately 200 mL containing the precipitate. The mixture was homogenized by agitation and transferred into centrifuge tubes, followed by centrifugation at 5500 rpm for 15 min. Subsequently, the supernatant was discarded, and the resulting pellet was collected and stored in microcentrifuge (Eppendorf) tubes. The nanoparticles were dried in a drying oven at 45 °C for 12 h, obtaining a dry sample (see Figure 1).

Figure 1: Precipitation Of Silver Nanoparticles.

2.2. Characterization Of the Nanoparticles

The silver nanoparticles synthesized from the aqueous extract of Azolla were characterized and sent for three analyzes at the Laboratory of Services and Products (OSP) of the Faculty of Chemical Sciences at the Central University of Ecuador in Quito, Ecuador. Thus, analyzes were conducted using ultraviolet-visible spectroscopy (UV-Vis), Fourier-transform infrared spectroscopy (FT-IR), and to determine the structure and size of the silver nanoparticles, atomic force microscopy (AFM) analysis was performed.

2.3. Phytochemical Screening of the Aqueous Extract of Azolla

A 75 mL sample of aqueous *Azolla* extract was qualitatively analyzed at SSV Consulting in Guayas, Ecuador, to determine the metabolites present in the extract used as a reducing and stabilizing agent in the synthesis of silver nanoparticles. The Liebermann–Burchard test was performed to detect triterpenes and steroids; the Mayer and Wagner tests for alkaloids; the Shinoda test for flavonoids; the ferric chloride test for tannins and phenols; the foam test

for saponins; the Fehling test for reducing sugars; and the Bornträger test for quinones.

2.4. Preparation Of In Vitro Culture

For in vitro culture, a total of 16 sterilized 370 mL flasks were used, each containing 40 mL of 1% (w/v) agar as a solid support for all treatments. Four flasks corresponding to the Murashige and Skoog (MS) treatment (T3) were additionally supplemented with 0.724 g of MS medium along with the agar (Table 3). Once the agar was dispensed into each flask, the flasks were sterilized in an autoclave at 121 °C for 15 min and allowed to solidify at room temperature. Subsequently, under aseptic conditions in a previously sterilized laminar flow hood, all flasks containing agar, the sterilized seeds, a 50 mg L^{-1} silver nanoparticle solution prepared in sterile distilled water, and the aqueous Azolla extract were arranged to initiate in vitro culture. A total of 10 seeds were aseptically placed in each flask using sterile forceps. The flasks were then sealed with aluminum foil and Parafilm. Prior to planting, 1 mL of Azolla extract was added to each flask corresponding to the Azolla treatment (T1). Similarly, for the nanoparticle treatment (T2), 1 mL of AgNPs solution was added to each flask, after which planting was carried out (see Figure 2).

Figure 2: In Vitro Planting of Radish Seeds.

The data on germination and in vitro growth were taken every 24 hours. Among the evaluated data, the germination percentage, growth rate (cotyledon emergence), root length, and plant size were considered.

Table 3: Experimental Design for In Vitro Seed Culture Using Agar Medium.

Treatment	Replicates (n)	Support medium	Medium volume per replicate (mL)	Applied treatment	Dose per replicate
T0 (Control)	4	Agar medium	40 mL	Agar (no additive)	N/A
T1	4	Agar medium	40 mL	*Azolla* extract	1 mL
T2	4	Agar medium	40 mL	Silver nanoparticles (AgNPs)	1 mL
T3	4	Agar medium + MS	40 mL	Murashige and Skoog medium (MS)	0.181 g

Note: The Agnps Solution Was Applied At 50 Mg L^{-1}, The *Azolla* Extract At 0.05 G Ml^{-1}, And the MS Medium At 4.54 G L^{-1}.

2.5. Field Cultivation

The field evaluation was conducted by transplanting radish seedlings grown in vitro. For this purpose, 8 plants per treatment were transplanted, initially into seedbeds containing peat as substrate. Once the seedlings developed true leaves, they were transferred to individual 1 L pots containing prepared soil. The plants were maintained under irrigation every 3 days. According to the treatments established in Table 6, edaphic applications were performed every 14 days. Data were recorded throughout the cultivation period until harvest at 55 days. The evaluated parameters included leaf number, emergence of new shoots, tuber weight, and plant height (see Table 4).

Table 4: Experimental Treatments Used in the Field Cultivation of Radish (Raphanus Sativus L.).

Treatment	Replicates (n)	Applied treatment	Concentration	Application dose per replicate
T0 (Control)	8	Water	N/A	N/A
T1	8	*Azolla* extract	0.05 g mL^{-1}	25 mL
T2	8	Silver nanoparticles (AgNPs)	50 mg L^{-1}	25 mL

T3	8	Granular fertilizer	150 kg ha^{-1}	0.165 g

Note: In Treatment T3, The Commercial Granular Fertilizer Yaramila™ Complex (N-12%-P2O5-11%-K2O-18%) Was Applied.

3. RESULTS

3.1. Phytochemical Screening of the Aqueous Extract of Azolla Spp.

The aqueous extract of *Azolla spp.* used as a reducing and stabilizing agent in the synthesis of silver nanoparticles showed the presence of essential metabolites in this synthesis process. Thus, through qualitative analysis, the presence of alkaloids, flavonoids, anthocyanins, tannins, phenols, saponins, and amino acids was identified (Table 5).

Table 5: Qualitative Phytochemical Screening of the Aqueous Extract of Azolla Spp.

Phytochemical group	Test / reagent	Result
Triterpenes / Sterols	Liebermann–Burchard test	−
Alkaloids	Mayer test	+
	Wagner test	+
	Dragendorff test	+
Flavonoids / Anthocyanins	Shinoda test	+
Phenols / Tannins	Ferric chloride test	+
Reducing sugars	Fehling test	−
Saponins	Foam test	+
Amino acids	Ninhydrin test	+

Note: (+) Presence of the Metabolite; (−) Absence. Results Provided by SSV Consulting.

3.2. Characterization Of Agnps

The UV-Vis absorption spectrum of silver nanoparticles synthesized using an aqueous extract of Azolla after 5 days revealed a single band with a maximum peak corresponding to the surface plasmon resonance (SPR) of the nanoparticles within the ultraviolet-visible range, measured between 350 and 700 nm. In this regard, an absorbance of 2.651 was recorded with a maximum peak at 430 nm (Figure 3).

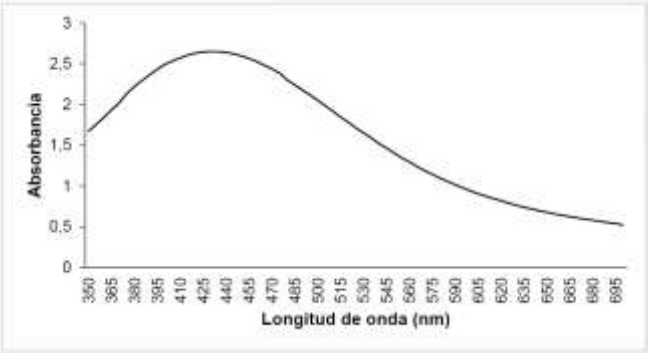


Figura 3: UV-Vis Spectrum of Silver Nanoparticles Synthesized with Aqueous Extract of Azollanote. The UV-Vis Spectrum Was Measured Between 350 To 700 Nm and A Maximum Absorbance Peak Of 2.651 Was Recorded At 430 Nm.

3.3. Fourier Transform Infrared Spectroscopy (Ft-Ir)

The presence of functional groups in the Azolla extract facilitated the synthesis of silver nanoparticles. In this regard, the FT-IR analysis confirmed the presence of various vibrations between 400 and 4000 cm^{-1} corresponding to a great diversity of functional groups. Among them, the region between 3000 and 4000 cm^{-1} stands out, with peaks indicating the presence of C-H, -OH, and N-H bonds, mainly associated with alcohols and amines. In the lower region, between 2000 and 3000 cm^{-1}, vibrations of C≡C and C≡N bonds were found. In the region from 1000 to 2000 cm^{-1}, on the other hand, bands corresponding to double bonds C=O, C=C, C=N, and single bonds C-O and C-N were detected, which may indicate the presence of functional groups such as alcohols, carboxylic acids, amines, amides, and esters. On the other hand, in the lower range between 400 and 1000 cm^{-1}, there are vibrations of simple bonds such as C-C, C-O, and C-N, which may be associated with aromatic groups and other functional groups that allowed for the stabilization and coating of the nanoparticles (Figure 4).

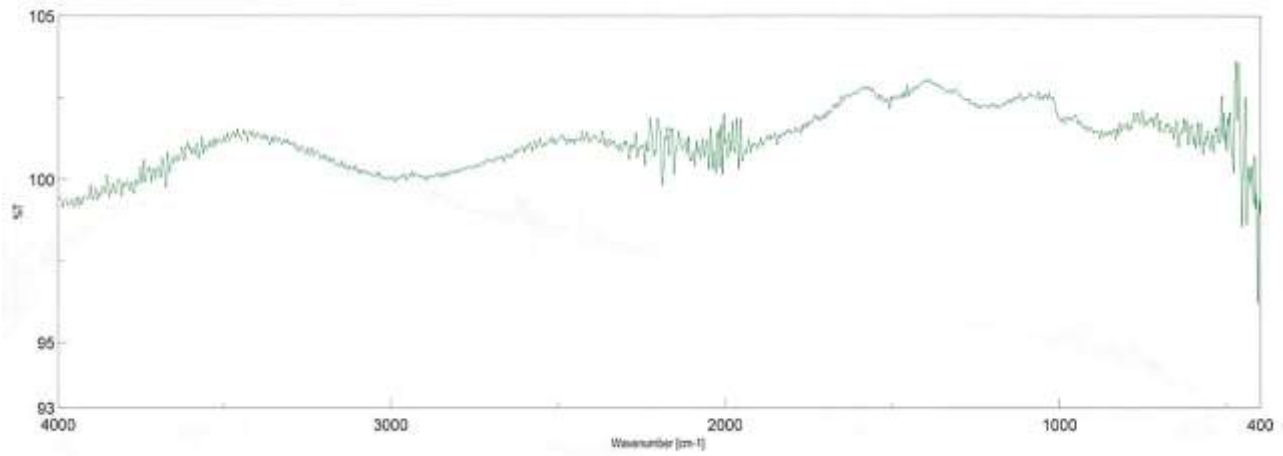
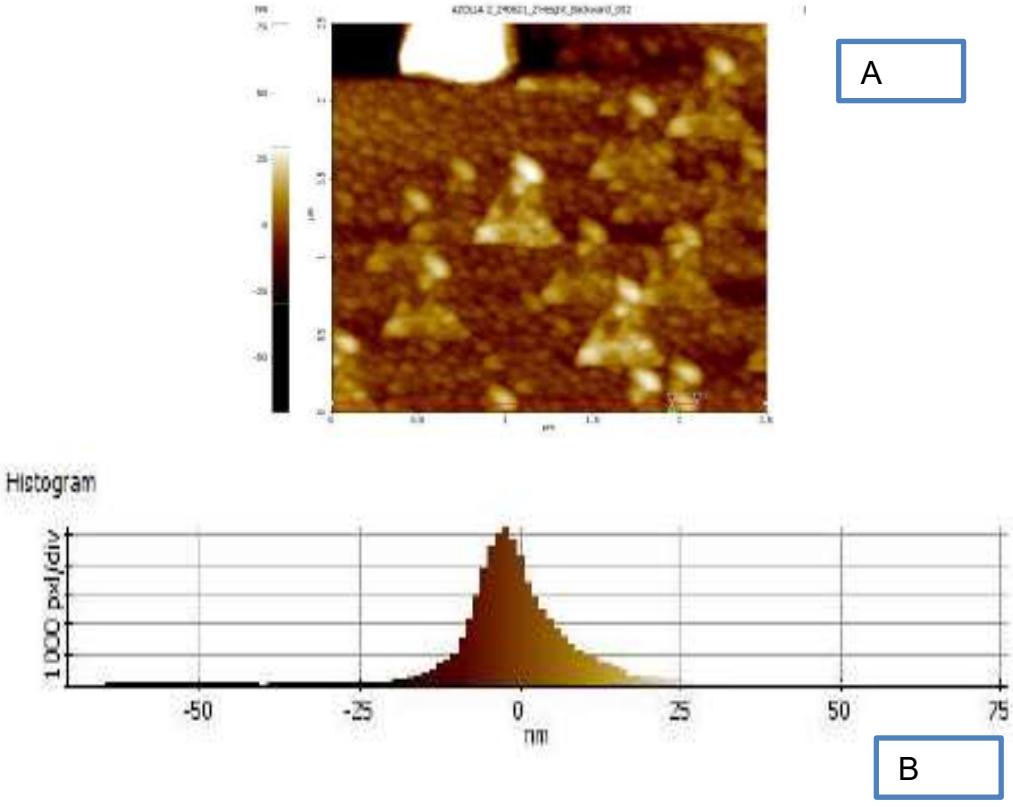


Figure 4: FT-IR Spectrum of Agnps Biosynthesized with Azolla Spp. Extract.
Note: The Infrared Spectrum Was Measured Between 400 To 4000 Cm¯1.

3.4. Atomic Force Microscopy (Afm)

AFM was used to examine the morphology of the nanoparticles, such that the 2D horizontal cross-section (Figure 5A) showed that the AgNPs are poorly defined, with an irregular shape and sizes ranging from 25 to 75 nm. In a three-dimensional view (Figure 5B), the roughness and lack of homogeneity in the formation of nanoparticles could be observed, allowing the distinction of certain agglomerations; and the line profiling (Figure 5C) showed an AgNPS height of approximately 25 nm.



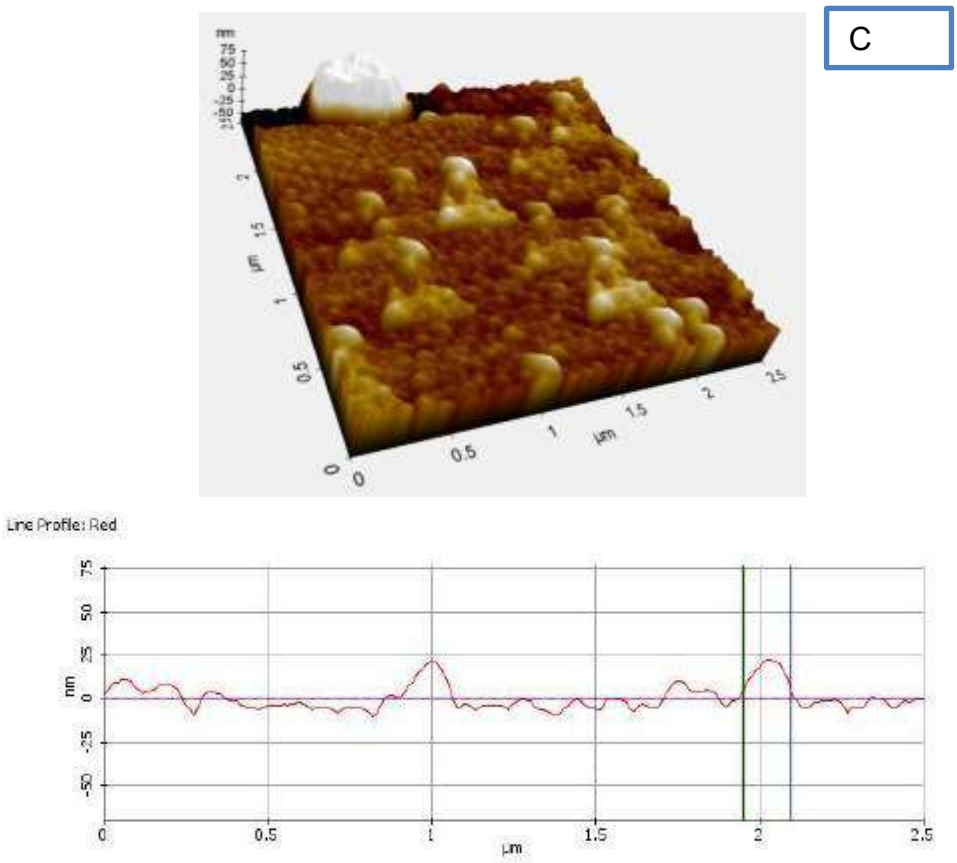


Figure 5: Result Obtained by Atomic Force Microscopy of Agnpsnote. The Height and Size Distribution of Silver Nanoparticles Are Shown By AFM. (A) 2D Image of the Horizontal Cross-Section of Agnps, (B) 3D Image, And (C) Line Profiling.

3.5. Evaluation Of In Vitro Culture

The observations made during the in vitro cultivation, especially in the germination percentage and growth rate based on the average number of germinated seeds, grown plants, and the appearance of cotyledons, revealed minimal differences among the 4 treatments applied.

3.5.1. Germination Percentage

At the end of the in vitro evaluation (6 days), no significant differences were found according to the analysis of variance of the data based on the Tukey test ($p>0.05$) shown in Table 6. Thus, the treatments of Nanoparticles (T2), Control (T0), and Azolla (T1) did not show a significant difference, but in comparison to the Murashige and Skoog (T3) treatment, they were significantly different.

Table 6: Comparison Of Treatment Means Using the Tukey Test (A = 0.05).

Treatments	Means	n	S.E.	
T0	8.75	4	0.91	A
T1	7.50	4	0.91	A B
T2	6.75	4	0.91	A B
T3	4.00	4	0.91	B

Note: Means With a Common Letter Are Not Significantly Different (P > 0.05).

However, in Figure 6a, the germination data show that the use of silver nanoparticles (T2) stimulates greater seed germination over time, with 15 seeds germinated at 24 hours and 38 seeds germinated at 124 hours of cultivation, achieving a total of 37% initially and 95% at the end of the evaluation (6 days). Compared to the other treatments, T0 also showed a high germination percentage of 37% and 92% at the beginning and end of the evaluation, respectively. The T1 treatment, on the other hand, had a germination percentage lower than the previous two treatments, with a total of 15 and 82%. On the contrary, treatment T3 showed the lowest germination percentages, with 5% and 67%,

respectively (Figure 6b).

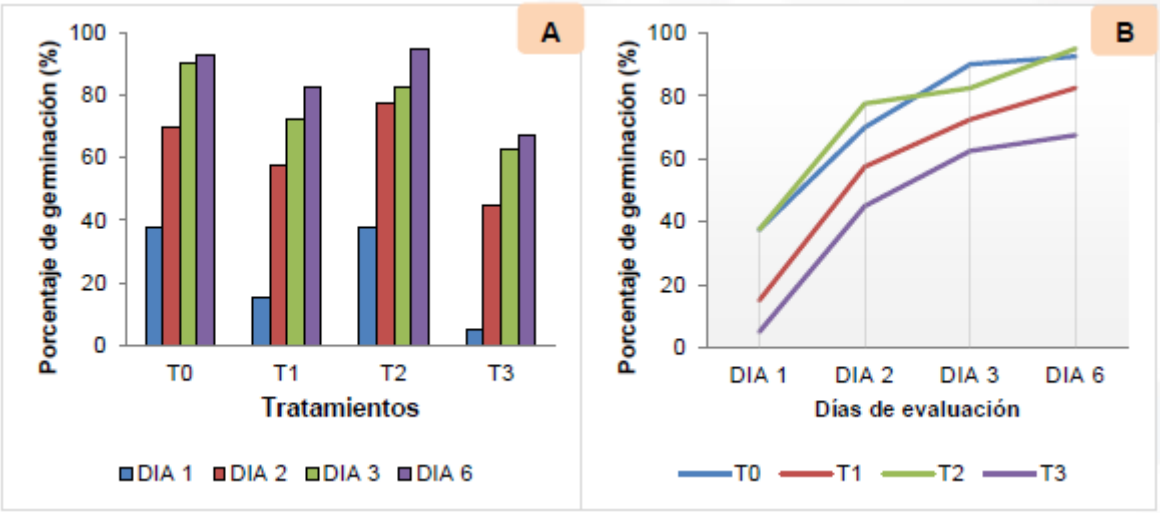


Figure 6: Percentage Of Seeds Germinated In Vitro.

Note: Bar Chart (Left) And Frequency Polygon (Right) Of the Percentage of Seeds Germinated In Vitro. Control Treatment (T0), Azolla (T1), Nanoparticles (T2), And MS (T3).

3.5.2. Growth Rate

The growth rate of radish plants was evaluated based on the emergence of cotyledons and plant size, showing differences between treatments. In this regard, based on the average number of seeds that developed their cotyledons during in vitro cultivation at 3 and 6 days, it was revealed that the best treatment was the control (T0), followed by the Azolla (T1) and Nanoparticles (T2) treatments, with Murashige and Skoog (T3) showing the lowest average (Figure 7).However, through the analysis of variance, it was discovered that the results among the three treatments T0, T1, and T2 were not significantly different, but they were with respect to treatment T3. Thus, after 6 days of in vitro cultivation, it was found that 87.5% of the seeds germinated in T0 developed to form their primordial leaves. On the contrary, the T1 treatment resulted in a growth of 75%, while T2 produced a growth of 67.5%, in contrast to T3, which only resulted in a growth of 40%.

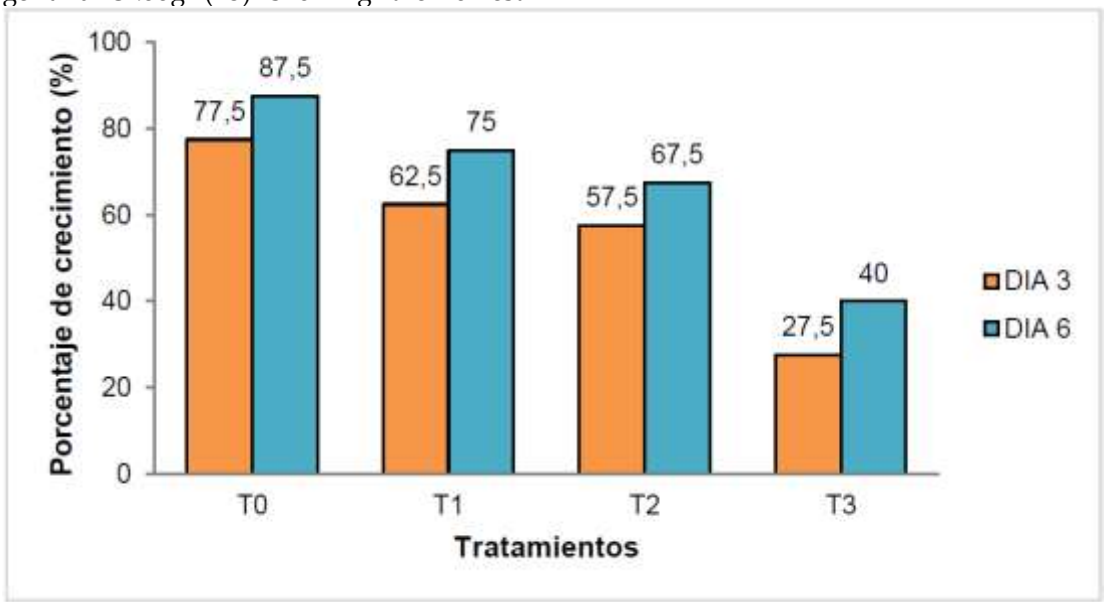


Figure 7: Percentage Of Plants Developed in In Vitro Culturenote. Data From the Control Treatment (T0), Azolla (T1), Nanoparticles (T2), And MS (T3) Are Shown.

The data from the measurements of root and pseudostem length of radish plants in in vitro culture established that the treatment with Azolla (T1) showed the highest average with 5.66 and 6.06 cm for root and stem, respectively, followed by T0 with values of 4.5 and 5.31 cm and T2 with 4.31 and 5.56

cm corresponding to root and stem length. However, treatment T3 showed the lowest values, with 2.97 and 4.18 cm (Figure 8). It should be noted that the statistical analysis of these data revealed a significant difference between treatment T1 and treatment T3, but no differences were found with treatments T2 and T0.

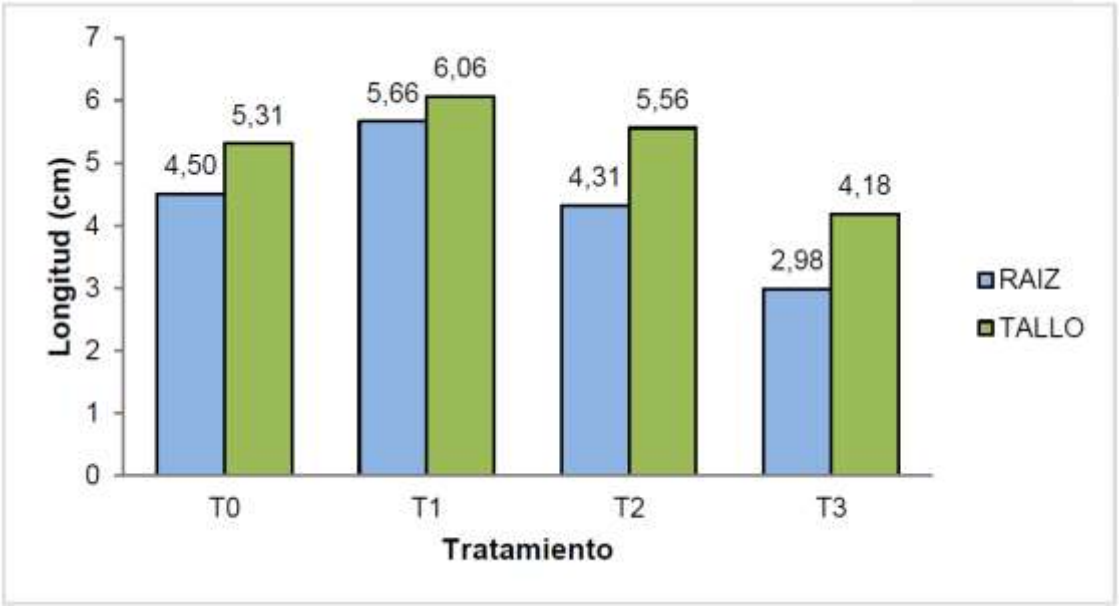


Figure 8: Average Root and Stem Length of Radish Plants in In Vitro Culture At 6 Daysnote. Data From the Control Treatment (T0), Azolla (T1), Nanoparticles (T2), And MS (T3) Are Shown.

3.6. Field Crop Evaluation

At the end of the field evaluation of the radish crop, it was demonstrated that T3, corresponding to the fertilizer treatment, showed the best average plant height with 17.06 cm (Table 7). Thus, the analysis of variance (ANOVA) revealed that it is significantly different from the values observed in treatments T0, T1, and T2 corresponding to the control, azolla, and nanoparticles treatments, with the latter two having averages close to 14.81 and 14.15 cm, respectively. These values were significantly different and higher than those obtained in the control treatment, which was 12.65 cm. Therefore, the T3 treatment demonstrated a differential and elevated growth compared to the other treatments, while the T1 and T2 treatments were slightly superior to T0 (Figure 9a).

Table 7: Initial And Final Mean Values of Plant Height, Number of Leaves, Number of Shoots, And Tuber Weight in Radish Plants Under Different Treatments.

Treatment	Initial height (cm)	Initial leaves	Initial shoots	Final height (cm)	Final leaves	Final shoots	Tuber weight (g)
T0	5.81	1.50	0.50	12.65	6.37	0.62	19.01
T1	6.93	1.87	0.50	14.81	6.75	0.87	26.70
T2	6.55	1.75	0.62	14.15	6.50	0.87	23.52
T3	5.12	1.75	0.50	17.06	7.62	1.00	32.53

Note: Values Correspond to the Averages Recorded at Day 15 (Initial Evaluation) And Day 35 (Final Evaluation).

Similarly, the evaluations of the number of leaves and shoots revealed that the fertilizer treatment (T3) was the most effective for the leaf development of the radish plant in field cultivation (Figures 9c and 9d), with values of 7.62 and 1, respectively, which were significantly different from T0. In contrast, the Azolla (T1) and Nanoparticles (T2) treatments recorded lower values, but slightly higher than the control (T0), with averages of 6.75 and 6.50 for the number of leaves and 0.87 and 0.87 for the number of sprouts, respectively.

The weight of the tuber harvested at 55 days also showed a similar pattern (Figure 9b), where the fertilizer treatment (T3) achieved the best average with 32.53 g, but it was not significantly different from the others ($p>0.05$). However, it emerged as the best treatment followed by T1 and T2, which were not significantly different compared to the control treatment (T0), despite recording a higher average than it.

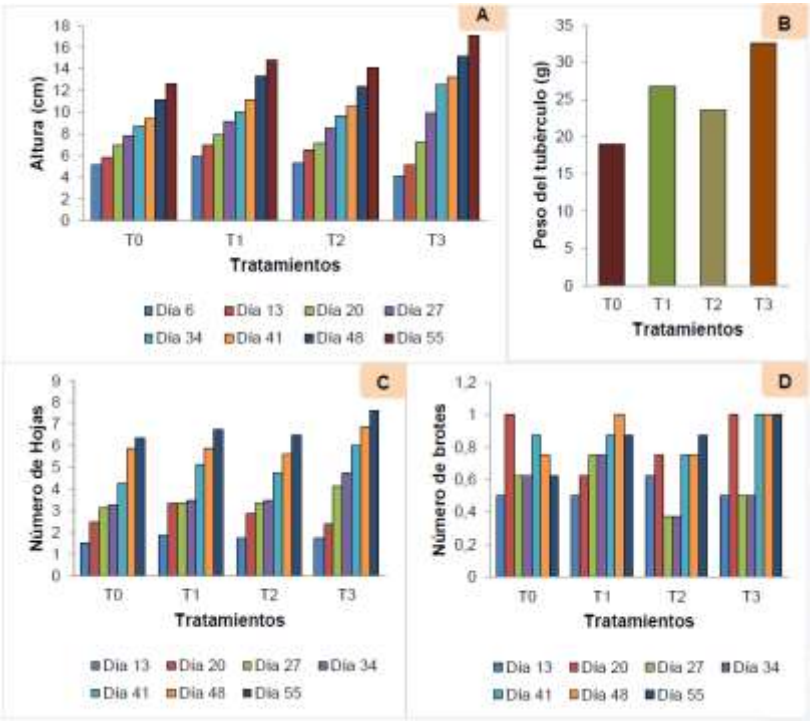


Figure 9: Evaluation of Field Results of Radish.

Note: The Graphs Show the Results Obtained on the Days of Evaluation of the Radish Crop in the Field, So That the Average Plant Height (A), Tuber Weight (B), Number of Leaves (C), And Number of Shoots (D) Can Be Observed.

4. DISCUSSION

The qualitative phytochemical screening of the aqueous extract of Azolla spp. revealed the presence of alkaloids, flavonoids, anthocyanins, tannins, phenols, saponins, and amino acids. The presence of these metabolites enables the reduction of silver ions (Ag^+) into metallic silver (Ag^0), acting as both reducing and stabilizing agents. Recent studies have further clarified the mechanistic role of plant-derived metabolites in the green synthesis of silver nanoparticles. Phenolic compounds and flavonoids act as electron donors, facilitating the reduction of Ag^+ ions, while simultaneously forming a capping layer that stabilizes the nanoparticles and prevents aggregation. This dual role has been widely reported in recent literature, where plant extracts rich in secondary metabolites enhance nanoparticle stability and bioactivity (Goswami et al., 2024). Additionally, the presence of functional groups such as hydroxyl and carbonyl groups contributes to strong metal–ligand interactions, which are essential for nanoparticle formation and long-term stability. Espinoza et al. (2022) reported that the synthesis of AgNPs mediated by plant extracts is attributed to organic compounds, mainly phenols, terpenoids, and flavonoids, which promote silver reduction through redox reactions. Berta et al. (2021) indicated that phytochemicals serve dual functions as reducing agents and stabilizers of nanoparticle formation, involving functional groups such as carboxyl, carbonyl, and amine groups. Additionally, plant-mediated nanoparticles are considered more stable and diverse in shape and size. Majumdar et al. (2023) also states that these phytochemicals play a key role in coating nanoparticles and preventing their agglomeration, thereby enhancing their biological activity. Therefore, the extract used in this study is suitable for nanoparticle synthesis due to the presence of metabolites capable of reducing silver ions (Ag^+) provided by silver nitrate, which acts as a precursor.

Regarding ultraviolet–visible (UV–Vis) spectroscopy, the silver nanoparticles synthesized using aqueous Azolla extract exhibited a characteristic surface plasmon resonance (SPR) band, with a maximum absorbance of 2.651 at 430 nm within the range of 350–700 nm, confirming the formation of AgNPs. Previous studies have established that UV–Vis spectra are indicative of nanoparticle formation, as this phenomenon is associated with the excitation of surface plasmons, resulting in light absorption in the range of 400–450 nm (Ledezma et al., 2014). These surface plasmons are directly correlated with nanoparticle size, shape, crystallinity, and chemical composition (Cruz et al., 2013). Similarly, studies using plant extracts have reported SPR peaks between 399 and 439 nm (Pardo et al., 2022), which is consistent with the results

obtained in this study.

The UV–Vis absorption peak observed at 430 nm is consistent with recent reports on plant-mediated synthesis of AgNPs, where surface plasmon resonance typically occurs between 420 and 450 nm depending on particle size and morphology (Akhter et al., 2024). Similarly, the size range obtained in this study (25–75 nm) aligns with values reported in recent studies using plant extracts, which commonly produce nanoparticles below 100 nm with diverse morphologies and moderate agglomeration (Haridas et al., 2025). These findings confirm that the synthesis conditions employed in this study are comparable to current green nanotechnology approaches.

FTIR analysis of the Azolla extract revealed the presence of functional groups (C–H, O–H, N–H, C=O, C=C, C–O, and C–N) within the range of 400–4000 cm^{-1}, associated with biomolecules such as alcohols, amines, and carboxylic acids. These groups are involved in the synthesis, reduction, and stabilization of silver nanoparticles. The identified functional groups correspond to biomolecules responsible for nanoparticle coating and stability (Anjana et al., 2019). In particular, the carboxyl and hydroxyl groups of flavonoids play a key role in the reduction of Ag^+ ions through chelation mechanisms and electron transfer processes (Akram et al., 2021). Furthermore, an increase in pH promotes the ionization of –OH and –COOH groups, enhancing nanoparticle stabilization under alkaline conditions (Amaladhas et al., 2013). These findings suggest that Azolla extract provides the necessary functional groups for the bioreduction process.

AFM analysis showed that the AgNPs exhibited irregular morphology with sizes ranging from 25 to 75 nm. Three-dimensional visualization revealed surface roughness and slight agglomeration, indicating a non-homogeneous distribution. Line profiling showed an approximate nanoparticle height of 25 nm. These results are consistent with previous studies reporting nanoparticle sizes within a similar range. For instance, Moosa et al. (2015) reported AgNPs with sizes between 66 and 117 nm, while Majeed et al. (2016) observed nanoparticles ranging from 30 to 50 nm. Likewise, Anjana et al. (2019) reported AgNPs synthesized using Azolla extract with an average size of 23.6 ± 8.18 nm. Therefore, the synthesis performed in this study yielded nanoparticles within the expected nanometric range, with limited agglomeration.

At the end of the in vitro evaluation (6 days), ANOVA followed by Tukey's test ($p > 0.05$) indicated that treatments T0 (control), T1 (Azolla), and T2 (AgNPs) did not differ significantly among themselves but differed significantly from T3 (Murashige and Skoog medium). Germination data showed that T2 produced the highest response, reaching 37% at 24 h and 95% at 124 h, followed by T0 (37% and 92%) and T1 (15% and 82%). Treatment T3 exhibited the lowest germination percentages (5% and 67%). These results are consistent with previous studies. Zuverza et al. (2016) reported a germination rate of 96% at 125 mg L^{-1} compared to 93% in the control. Similarly, Tymoszuk (2021) observed germination rates of 97% and 98% at concentrations of 50 and 100 mg L^{-1}, respectively, compared to 96% in the control. These findings suggest that silver nanoparticles slightly enhance germination, likely due to improved water absorption during early stages.

The growth rate of radish plants under in vitro conditions was evaluated based on cotyledon emergence and plant size. Results showed that T0, T1, and T2 did not differ significantly among themselves but differed from T3, which exhibited the lowest growth. At 6 days, the development of true leaves was 87.5% in T0, 75% in T1, 67.5% in T2, and 40% in T3. Regarding morphological parameters, T1 showed the highest average root length (5.66 cm) and pseudostem length (6.06 cm), whereas T3 recorded the lowest values. These findings partially agree with Wang et al. (2015), who reported lower root length in nanoparticle-treated plants compared to the control. Tymoszuk (2021) also observed variable growth responses depending on nanoparticle concentration. Overall, these results indicate that nanoparticle effects on in vitro growth are inconsistent and depend on multiple factors.

Field evaluation showed that the fertilizer treatment (T3) exhibited the highest growth, with an average plant height of 17.06 cm, significantly higher than T0, T1, and T2 according to ANOVA. T3 also recorded the highest number of leaves (7.62) and shoots (1). Treatments T1 and T2 showed intermediate values, slightly higher than the control. Regarding tuber weight, T3 showed the highest average (32.53 g), although no significant differences were observed ($p > 0.05$). Similar trends have been reported in previous studies. Çakmakci et al. (2022) observed slight increases in leaf number with increasing nanoparticle concentrations. Khan (2023) reported improvements in growth parameters in fenugreek treated with AgNPs. However, Krishnappa et al. (2022) found that higher concentrations inhibited growth, indicating a dose-dependent effect.

From an applied perspective, the use of plant-mediated silver nanoparticles represents a promising

alternative for sustainable agriculture. Green-synthesized AgNPs have demonstrated antimicrobial and antioxidant properties, which can contribute to plant protection and improved crop performance (Lieu et al., 2024). However, their effectiveness depends strongly on concentration and environmental conditions, as excessive accumulation in plant tissues may lead to phytotoxic effects. Therefore, while AgNPs offer advantages over conventional agrochemicals, their application must be carefully optimized to avoid adverse effects on plant growth and soil health.

These findings suggest that AgNPs can modulate plant growth, either by stimulation or inhibition, depending on concentration and environmental conditions (Molina, 2015). The effect of silver nanoparticles on radish may be related to their interaction with nutrient uptake mechanisms. Although the exact mechanism remains unclear, it has been suggested that nanoparticles may interfere with diffusion pathways or active transport channels, as silver ions can disrupt membrane integrity (Zuverza, 2023). Sener and Saygun (2023) also reported that low concentrations of AgNPs stimulate growth, whereas higher concentrations exert inhibitory effects. This behavior is associated with the high surface area of nanoparticles, which enhances their interaction with cell surfaces. At high concentrations, metal accumulation may induce toxicity through oxidative stress and enzymatic interactions.

Future research should focus on evaluating a broader range of nanoparticle concentrations to determine optimal doses for agricultural applications. In addition, studies integrating physiological, biochemical, and molecular approaches are required to better understand the interaction between AgNPs and plant systems. Recent evidence suggests that silver nanoparticles can induce oxidative stress and alter membrane integrity in plants, highlighting the need for deeper investigation into their mechanisms of action (Tripathi et al., 2024). Moreover, exploring the combined use of nanoparticles with biofertilizers or organic amendments could provide innovative strategies for improving crop productivity while maintaining environmental sustainability.

Overall, the results indicate that the effect of silver nanoparticles on radish development depends on concentration and underlying physiological mechanisms that are not yet fully understood. In this study, the applied concentration (50 mg L^{-1}) showed slight improvements over the control but was less effective than fertilizer and Azolla treatments. Therefore, further studies are required to elucidate the mechanisms involved and to evaluate different concentrations to better understand the effects of AgNPs on plant development.

5. CONCLUSIONS

The aqueous extract of *Azolla* spp., acting as a reducing and stabilizing agent due to its functional groups, enabled the effective synthesis of silver nanoparticles (AgNPs) with undefined morphology and sizes ranging from 25 to 75 nm. These nanoparticles exhibited slightly enhanced effects on both in vitro and field cultivation of radish. Seeds of the Crimson Giant radish variety treated with AgNPs at a concentration of 50 mg L^{-1} showed a higher germination percentage (95%) after 6 days compared to the control (92%), although a lower proportion of seeds developed cotyledons.

Under in vitro conditions, AgNP-treated seedlings did not exhibit significant differences in growth compared to the control and Murashige and Skoog (MS) treatments, and their performance was lower than that of seedlings treated with aqueous *Azolla* extract in terms of root and stem length. Under field conditions, AgNP-treated plants showed improved growth relative to the control in parameters such as plant height, tuber weight, leaf number, and shoot production; however, these differences were not statistically significant when compared to the other treatments.

Overall, this study demonstrates that plant-based extracts, such as *Azolla* spp. biomass, constitute a viable and sustainable alternative for the green synthesis of silver nanoparticles. While AgNPs exhibited a positive effect on seed germination, their influence on plant growth under both in vitro and field conditions was limited. These findings highlight the importance of optimizing nanoparticle concentration and application strategies to enhance their agronomic potential.

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original draft preparation, Cesar Gavin; writing—review and editing, Steven Ramos; visualization, Arreaga Ronnie, Barzola Jordano; supervision, Steven Ramos; project administration, Cesar Gavin. All authors have read and approved the published version of the manuscript. Please refer to the CRediT taxonomy for the explanation of the term. Authorship should be limited to those who have substantially contributed to the reported work.

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REFERENCES

Acevedo, L., Ossa, O. y Torres, L. (2023). Evaluación de protocolos de síntesis de nanopartículas de cobre u óxidos de cobre. *Ingeniería*, *28*(2), 17-29.

Aguilar, A., Guerrero, J., Morales, T., López, S., Chirino, G. y Palomar Morales, M. (2019). Estudio comparativo del cultivo de rábano (Raphanus sativus L.) en condiciones de invernadero con aplicación de hidroponía, lombricompost y Azotobacter sp. *Artificial Cells, Nanomedicine, and Biotechnology, 28*(1), 261–372.

Akhter, N., Batool, M., Yaqoob, A. *et al.* Potential biological application of silver nanoparticles synthesized from *Citrus paradisi* leaves. *Sci Rep* **14**, 29028 (2024).

Akram, W., Chakraborty, S. y Owens, G. (2021). Una revisión de la síntesis de AgNP (nanopartícula de plata) mediada por fitoquímicos: la partícula maravilla de la última década. *Applied Nanoscience, 11*(2), 2625–2660.

Alvear, A. (2019). *Evaluación del efecto biocida de nanopartículas de plata a partir de papa (Solanum tuberosum) sobre Colletotrichum spp con prospectiva en post cosecha* [Tesis de Grado, Universidad de las Américas].

Amaladhas, T., Sivagami, S., Devi, T. y Narayanan, A. (2013). Síntesis biogénica de nanopartículas de plata mediante extracto de hoja de Cassia angustifolia. *Advances in Natural Sciences Nanoscience and Nanotechnology, 3*(4), 45-53.

Ambrusi, R., Sánchez, M. y García, S. (2018). Efecto electrocatalítico de nanopartículas bimetálicas de Ag y Cd hacia la reducción de iones nitrato y/o nitrito. Matéria (Rio de Janeiro), 23, 12141.

Anjana, V., Joseph, M., Francis, S., Joseph, A., Koshy, E. P., y Mathew, B. (2021). Síntesis ecológica asistida por microondas de nanopartículas de plata para aplicaciones ópticas, catalíticas, biológicas y electroquímicas.

Anjana, V., Koshy, E. y Mathew, B. (2020). Síntesis fácil de nanopartículas de plata utilizando Azolla caroliniana, su citotoxicidad, actividad catalítica, óptica y antibacteriana. *Materials Today: Proceedings, 5*(1), 163-168.

Arteaga, L., Montero, I., Almendárez, M., Anguiano, M., Castillo, A. y Ruiz, F. (2020). *Actividad fungicida y bacteriana de nanopartículas de plata contra Fitopatógenos del tomate, evaluación in vitro.* [Tesis de grado, Universidad Autónoma de San Luis Potosí]

Asuad, S. y Sánchez, J. (2018). Un análisis exploratorio y comparativo entre los enfoques de bottom-up y top-down para una construcción espacial de una matriz regional Input-Output. *Revista mexicana de economía y finanzas*, *13*(2), 137-174.

Ávalos, A., Haza, A. y Morales, P. (2013). Nanopartículas de plata: Aplicaciones y riesgos tóxicos para la salud y el medio ambiente. *Revista Complutense de Ciencias Veterinarias, 7*(2), 1-23.

Becking, J. y Donze, M. (2021). Distribución de pigmentos y fijación de nitrógeno en Anabaena azollae. *Plant and Soil, 61*(2), 203-226.

Bello, B. y Spinoso, C. (2023). Utilización de nanopartículas de plata en la micropropagación de plantas. *Mundo nano. Revista interdisciplinaria en nanociencias y nanotecnología*, *16*(30).

Berta, L., Coman, N. A., Rusu, A. y Tanase, C. (2021). Una revisión de la síntesis de nanopartículas bimetálicas mediada por plantas, su caracterización y sus aplicaciones biológicas. *Materials, 14*(24), 16-22.

Blandón, L., Vázquez, M. V., Boannini, E., y Ballarin, B. (2015). Síntesis electroquímica de nanopartículas de plata en presencia de un surfactante neutro. Afinidad, 72(569).

Brito, K. (2013). *Síntesis de compuestos híbridos nanoestructurados y evaluación de su comportamiento supramolecular*

y actividad biológica [Tesis de grado, Universitat de les Illes Balears].

Bueno, T. (2017). *Estudio de las aplicaciones biomédicas de las nanopartículas de plata* [Tesis de grado, Universidad de Sevilla].

Çakmakcı, Ö., Çakmakcı, T. y Şensoy S. (2022). Efectos de las nanopartículas de plata en los parámetros de crecimiento del rábano (Raphanus sativus l. var. radicula). cultivado bajo riego deficitario. *Current Trends in Natural Sciences, 1*(21), 37-44.

Chávez, G., Parra, I., Luzardo, M., Bravo, B. y Márquez, N. (2013). Caracterización de cristales líquidos por microscopía óptica en sistemas surfactante polietoxilado-alcano-agua. Química Nova, 36, 1343-1347.

Colonia, R., Martínez, V., Solís, J. y Gómez, M. (2013). Síntesis de nanopartículas de ZnO2 empleando ultrasonido: caracterización estructural y morfológica para aplicaciones bactericidas. *Revista de la Sociedad Química del Perú*, *79*(2), 126-135.

Corzo, L. (2014). Síntesis de nanopartículas de oro obtenidas por reducción de H [AuCl4]. *Revista de la Sociedad Química del Perú*, *78*(2), 79-90.

Cota, O., Cortez, M., Burgos, A., Ezquerra, J., y Plascencia, M. (2016). Matrices de liberación controlada y micro/nanopartículas de quitosano con potencial antimicrobiano: desarrollo de nuevas estrategias para el control microbiano en la agricultura. *Journal of the Science of Food and Agriculture*, *93*(7), 1525-1536.

Cruz, D., Rodríguez, M., López, J., Herrera, V., Orive, A. y Creus, A. (2013). Nanopartículas metálicas y plasmones de superficie: una relación profunda. *Avances en Ciencias e Ingeniería, 3*(2), 67-78.

De Silva, C., Noor, A., Abd, K., Gunasekaran, B., Abd, G., Cabrera, M. y Ahmad, S. (2020). La síntesis verde y caracterización de nanopartículas de plata de Serratia spp. *Revista Mexicana de Ingeniería Química, 19*(3), 1327-1339.

Espinoza, K., Sánchez, A. y Castañeda, A. (2022). Bio-síntesis de nanopartículas de Plata mediante el extracto de Geranium (Pelargonium spp). *Afinidad, 79*(1), 245-250.

Esquivel, F., Ramón, D. y Diego, S. (2021). Síntesis biológica de nanopartículas de plata: revisión del uso potencial de la especie Trichoderma. *Revista Cubana de Química, 33*(2), 23-45.

Europea, U. (2009). Reglamento (CE) nº 1107/2009 del Parlamento Europeo y del Consejo, de 21 de octubre de 2009, relativo a la comercialización de productos fitosanitarios y por el que se derogan las Directivas 79/117/CEE y 91/414/CEE del Consejo. *Diario Oficial de la Unión Europea L*, *309*(7), 1-50.

Fan, L., Wang, Y., Xu, L., Tang, M., Zhang, X., Ying, J., ... y Liu, L. (2020). Un estudio de asociación de todo el genoma descubre un papel fundamental del gen RsPAP2 en Raphanus sativus L de piel roja. *Horticulture research*, *7*(2), 14-25.

Fraceto, L., Grillo, R., de Medeiros, G., Scognamiglio, V., Rea, G. y Bartolucci, C. (2016). Nanotecnología en Agricultura: ¿qué potencial de innovación tiene? Nanotecnología en Agricultura: ¿qué potencial de innovación tiene?. *Frontiers in Environmental Science, 4*(20), 1-5.

Galindo, D. (2019). Una revisión de la síntesis de nanopatrículas de plata por reducción biológica. *Contactos, Revista de Educación en Ciencias e Ingeniería, 25*(114), 5-10.

García, M. (2018). El fin de la resistencia bacteriana: Nanopartículas de plata. *MoleQla: Revista de Ciencias de la Universidad Pablo de Olavide*, 7(31), 20-32.

Goswami, M. J., Hati Boruah, J. L., Saikia, R., Dutta, U., & Kakati, D. (2024). *Synthesis, antimicrobial and antioxidant bioevaluation of silver nanoparticles using leaf extract of Sarcochlamys pulcherrima*. *Next Nanotechnology*. 5, 1 -11.

Gutiérrez, M. y Castellanos, M. (2014). Síntesis por el método sol-gel aplicado al estudio del polimorfismo en nanopartículas de TiO2. Mundo nano. Revista interdisciplinaria en nanociencias y nanotecnología, 4(1), 67-73.

Hall, D., Markov, S., Watanabe, Y. y Krishna, R. (2020). Las posibles aplicaciones de la fotosíntesis de cianobacterias para tecnologías limpias. *Photosynthesis Research, 46*(1-2), 159-167.

Haridas, E.S.H., Varma, M.K.R. & Chandra, G.K. Bioactive silver nanoparticles derived from *Carica papaya* floral extract and its dual-functioning biomedical application. *Sci Rep* **15**, 9001 (2025).

Jesús, Y. y Carbajal, F. (2017). El uso de radiación de microondas para la síntesis de Nanopartículas. *Revista de Innovación Sistemática*, *10*(2), 46-56.

Jiménez, M., Ramírez, H., Orozco, C. y González, D. (2024). Biosíntesis de nanopartículas de plata con actividad antimicrobiana por Pseudomonas aeruginosa ambiental. *Mundo nano. Revista interdisciplinaria en nanociencias y nanotecnología*, *17*(32), 11-22.

Khan, S., Zahoor, M., Sher, R., Ikram, M. y Islam, N. (2023). El impacto de las nanopartículas de plata en el

crecimiento de las plantas: Las aplicaciones agrícolas. *Heliyon, 9*(6), 16-25.

Kobayashi, H., Shirasawa, K., Fukino, N., Hirakawa, H., Akanuma, T., y Kitashiba, H. (2020). Identificación de polimorfismos de un solo nucleótido en todo el genoma entre accesiones de rábano geográficamente diversas. *DNA Research*, *27*(1), 1-13.

Krishnappa, S., Kalikeri, S., Garampalli, R., Lingaju, H. y Kumar, C. (2022). A brief review of the impact of silver nanoparticles on agriculture and certain biological properties: A case study certain biological property: A case study. *International Journal of Health and Allied Sciences, 11*(1), 63-69.

Kumar, U., Nayak, A., Panneerselvam, P., Kumar, A., Mohanty, S., Shahid, M., ... y Nayak, P (2019). Diversidad de cianobiontes en seis Azolla spp. y relación con el perfil de nutrientes de Azolla. *Planta*, *249*(4), 1435-1447.

Kunjiappan, S., Chowdhury, R. y Bhattacharjee, C. (2014). Un enfoque de química verde para la síntesis y caracterización de nanopartículas de oro bioactivas utilizando extracto de metanol de Azolla microphylla. *Fronteras de la ciencia de los materiales, 8*(1), 123-135.

Lateef, A., Nazir, R., Jamil, N., Alam, S., Shah, R., Khan, M., y Saleem, M. (2013). Síntesis y caracterización de nanocompuestos a base de zeolita: un fertilizante de liberación lenta respetuoso con el medio ambiente. M*ateriales microporosos y mesoporosos, 232*(6), 174-183.

Ledezma, A., Romero, J., Hernández, M., Moggio, I., Arias, E. y Torres, S. (2014). Síntesis biomimética de nanopartículas de plata utilizando extracto acuoso de nopal (Opuntia sp.) y su electrohilado polimérico. *Superficies y vacío, 27*(4), 133-140.

Lieu, M. D., Dang, T. K. T., & Nguyen, T. H. (2024). *Green synthesized silver nanoparticles, a sustainable approach for fruit and vegetable preservation: An overview*. *Food Chemistry: X*, 23, 1–15.

Lira, S., Méndez, A., Santos, V. y Vera, R. (2018). Nanotechnology potential in sustainable agricultura. *Acta universitaria*, *28*(2), 9-24.

López, M., Robledo, P., Flores, H., Corona, T., Gutiérrez, E., Hernández, R., y García-de los Santos, G. (2022). Mutagénesis in vitro en anturio inducido por colchicina. *Revista Chapingo Serie horticultura, 28*(1), 17-34.

Majeed, S., Nanda, A., Abdullah, M. y Tahir, M. (2016). Estudio in vitro de las actividades antibacteriana y anticancerígena de nanopartículas de plata sintetizadas a partir de Penicillium brevicompactum (MTCC-1999). *Journal of Taibah University for Science, 10*(4), 7-15.

Majumdar, R. y Kar, P. (2023). Biosíntesis, caracterización y actividad antihelmíntica de nanopartículas de plata de un aislado de Clerodendrum infortunatum. *Scientific reports, 13*(1), 74-85.

Méndez, M., Pérez, T., Pérez, J. y Jimenez, V. (2018). Azolla sp., un alimento de alto valor nutricional para la acuicultura. Biotecnia, 20(1), 32-40.

Molina, M., Fernández, C., Mercado, C., Manquián, K. y Arancibia, N. (2015). Efecto de nanoparticulas de plata sobre el crecimiento de trigo (Triticum aestivum L.) y la disponibilidad de fósforo en presencia/ausencia de fertilización fosfatada en un andisol. *Convibra, 18*(2), 1-11.

Moosa, A., Ridha, A. y Hameed, M. (2015). Síntesis ecológica de nanopartículas de plata utilizando extracto de hojas de té gastadas con microscopía de fuerza atómica. *International Journal of Current Engineering and Technology, 5*(5), 47-61.

Moreno, V. (2021). *Síntesis verde de nanopartículas de plata para aplicaciones como fungicida en los fitopatógenos Fusarium solani y/o Rhizopus stolonifer que atacan la planta de aguacate* [Tesis de grado, Universidad Nacional de Colombia].

Mosquera, E., Rosas, N., Debut, A. y Guerrero, V. (2015). Síntesis y caracterización de nanopartículas de dióxido de titanio obtenidas por el método de sol-gel. Revista Politécnica, 36(3), 7-7.

Pardo, L., Arias, J. y Molleda, P. (2022). Elaboración de nanopartículas de plata sintetizadas a partir de extracto de hojas de romero (Rosmarinus officinalis L.) y su uso como conservante. *La Granja: Revista de Ciencias de la Vida, 35*(1), 45-58.

Planticulous. (2024). *Impactos y usos de Azolla en la agricultura* [Fotografía]. Planticulous:

Puca, P., Guerrero, A., Tacuri, C. y López, C. (2013). Síntesis y caracterización de nanopartículas superparamagnéticas obtenidas por precipitación en microemulsión inversa para aplicaciones biomédicas. *Revista de la Sociedad Química del Perú*, 79(2), 99-106.

Quezada, G., Aragón, M. y Meza, D. (2024). Implicaciones bioéticas en la aplicación de nanopartículas de plata (AgNPs) para el manejo de fitopatógenos. *Mundo Nano. Revista Interdisciplinaria en Nanociencias y Nanotecnología*, *17*(33), 1-16.

Rautela, A. y Rani, J. (2019). Síntesis verde de nanopartículas de plata a partir del extracto de semillas de Tectona

grandis: caracterización y mecanismo de acción antimicrobiana sobre diferentes microorganismos. *Revista de Ciencia y Tecnología Analítica*, *10*(1), 18-25.

Ravi, R., Ishak, I., Amin, M., Hamzah, Z., Rasat, M. y Salam, M. (2020). Extracto de AgNPs-Azolla Pinnata como larvicida contra Aedes Aegypti (Diptera: Culicidae). *In IOP Conference Series: Earth and Environmental Science, 596*(1), 12-27.

Rea, S., Maldonado, C. y Villao, S. (2015). Los Sistemas de Información para lograr un desarrollo competitivo en el sector agrícola. *Revista Ciencia Unemi*, *8*(13), 122-129.

Rojas, A., Piedra, C, Icaza, O. y Castillo, C. (2024). *Nuevos registros, nombre validado y redefinición en Azolla (Salviniaceae, Pteridophyta) de Costa Rica* [Tesis de grado, Universidad Nacional de Costa Rica].

Ruiz, L., Valladares, P. y Breedy, M. (2020). Actividad antimicrobiana de adhesivo ortodóntico con nanopartículas de plata sobre Streptococcus mutans. *Odontología*, *22*(2), 33-44.

Samad, F., Idris, L., Abu, H., Goh, Y. y Loh, T. (2020). Efectos de Azolla spp. como ingrediente alimentario sobre el rendimiento del crecimiento y la digestibilidad de los nutrientes del pollo de engorde. *Journal of animal physiology and animal nutrition, 104*(6), 1704-1711.

Sanchez, G. (2018). *Evaluación de la eficiencia de un biofertilizante de residuos orgánicos en relación a otras fuentes de fertilización en el desarrollo del cultivo de Rábano (Raphanus sativus L.)* [Tesis de grado, Universidad Peruana Unión].

Sánchez, M. (2017). *Nanopartículas de plata: Preparación, caracterización y propiedades con aplicación en inocuidad de los alimentos* [Tesis de grado, Universidad Peruana Unión].

Şener, S. y Sayğı, H. (2023). El papel de las nanopartículas de plata en la respuesta in vitro de las plantas Boysenberry al estrés por sequía. *Horticulturae, 9*(11), 10-23.

Tijani, S. (2019). Conocimiento de los agricultores sobre el cultivo, el valor económico y nutricional del cultivo de rábano en el área del gobierno local de Iseyin del estado de Oyo. *Agricultural and Food Science Journal of Ghana*, *12*(1), 1074-1084.

Tkach, V., Nechyporuk, V., y Yagodynets, P. (2013). Descripción matemática de la síntesis electroquímica de polímeros conductores en la presencia de surfactantes. *Avances en Química*, 8(1), 9-15.

Torres, M y Calderón, J. (2015). Evaluación de la resistencia a la corrosión erosión de recubrimientos de níquel modificados con nanopartículas de diamante. Revista Facultad de Ingeniería Universidad de Antioquia, (54), 42-48.

Tripathi, S., Mahra, S., Sharma, S., Mathew, S., & Sharma, S. (2024). *Interaction of silver nanoparticles with plants: A focus on the phytotoxicity, underlying mechanism, and alleviation strategies*. *Plant Nano Biology*, 1–10.

Tymoszuk, A. (2021). Efectos de las nanopartículas de plata en la germinación in vitro, el crecimiento y la actividad bioquímica de plántulas de tomate, rábano y col rizada. *Materials, 1*4(18), 53-60.

Valderrama, A., Carvajal, D., Peñailillo, P. y Tapia, J. (2016). Capacidad de acumulación de cadmio y cobre, y sus efectos en el desempeño fotosintético en Azolla filiculoides Lam. bajo rizofiltración aumentada. Gayana. *Botánica, 73*(2), 283-291.

Valdivia, J. C., Pérez, A., Rodríguez, G., Hurtado, G., Moggio, I., Arias, E., ... y Castañeda, A. (2021). Estudio espectroscópico de nanopartículas de plata pasivadas con el polímero conjugado PEDOT: PSS. Superficies y vacío, 34.

Vanlalveni, C., Lallianrawna, S., Biswas, A., Selvaraj, M., Changmai, B. y Rokhum, SL (2021). Síntesis verde de nanopartículas de plata utilizando extractos de plantas y sus actividades antimicrobianas: una revisión de la literatura reciente. *Avances RSC*, *11*(5), 2804-2837.

Vera, M. (2024). *Manejo agronómico del cultivo de rábanos (Rhapanus sativus) mediante hidroponía en el Ecuador* [Tesis de grado, Universidad de Babahoyo (UTB)].

Wang, L., Zhu, J., Qiuyun, W. y Huang, Y. (2015). Efectos de las nanopartículas de plata en la germinación de semillas y el crecimiento de plántulas de rábano (Raphanus Sativus L.). *International Conference on Civil, Materials and Environmental Science, 76*(1), 604-608.

Zuverza, N., Armendariz, R., Peralta, J. y Gardea, J. (2016). Efectos de las nanopartículas de plata en los brotes de rábano: Reducción del Crecimiento Radicular y Modificaciones en el Valor Nutricional. *Frontiers in plant science, 7(*2), 90-105.