Green Synthesis and Foliar Application of Copper Nanoparticles in Sunflower (*Helianthus annuus* L.) to Improve Physiological Parameters and Yield

Síntesis verde y aplicación foliar de nanopartículas de cobre en híbridos de girasol (*Helianthus annuus* L.) para mejorar parámetros fisiológicos y el rendimiento

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Abstract

Nanotechnology holds significant interest across various domains, including agriculture. The green synthesis of nanoparticles offers environmentally friendly solutions. This study aimed to synthesize copper nanoparticles (NPs) using *Aloe vera* extracts and evaluate their foliar application on two sunflower hybrids, Chané (Ch) and Calchaquí (Ca). The two types of *Aloe Vera* extracts used to produce nanoparticles were characterized by UV-vis spectral analysis and dynamic light scattering (DLS). The Np particles synthesized with *Aloe vera* Home (Np1) measured 242.8 nm (62.6%) and 74.87 nm (37.4%), while *Aloe vera* Commercial (Np2) resulted in sizes of 339.6 nm (90.7%) and 66.07 nm (9.3%). Two different doses of Np (150 ppm and 300 ppm) were applied to sunflower plants. We measured germination power (GP), plant height (PH), leaf number (LN), leaf area (LA), dry weight accumulation and achene yield. Chané's parameters improved at both nanoparticle doses, while Calchaquí only improved with the 300 ppm treatment. This research highlights the potential use of green nanotechnology to improve growth and yield in sunflower.

Keywords
plant physiology • oilcrops • agrotechnology • reducing agent • crop science

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Resumen

La nanotecnología es un área de gran interés en diferentes campos de la ciencia, entre ellos la agricultura. La síntesis verde de nanopartículas ofrece soluciones sostenibles para el medioambiente. El objetivo del presente trabajo fue sintetizar nanopartículas de cobre (NPs) utilizando como agente reductor el *Aloe Vera* y evaluar el impacto de su aplicación foliar en dos híbridos de girasol, Chané (Ch) y Calchaquí (Ca). Se utilizaron dos tipos de extractos de *Aloe Vera* como agente reductor, las cuales se caracterizaron mediante análisis espectral UV-vis y dispersión dinámica de luz (DLS). Las Nps sintetizadas con *Aloe Vera* Home (Np1) presentaron tamaños de partícula de 242,8 nm (62,6%) y 74,87 nm (37,4%), mientras que las obtenidas con *Aloe Vera* comercial (Np2) dieron como resultados tamaños de partícula de 339,6 nm (90,7%) y 66,07 nm (9,3%). Se midieron parámetros fisiológicos de la planta como fue el poder germinativo (PG), la altura de la planta (PH), el número de hojas (NL) y el área foliar (LA). Se aplicaron dos dosis diferentes de Nps (150 ppm y 300 ppm) a las plantas y se cuantifico la acumulación de materia seca en tallo, peciolo, hoja y capitulo. El híbrido Chané presentó una mejora respuesta con ambas dosis de nanopartículas, mientras que Calchaquí mostró una mejora en sus parámetros solo con el tratamiento de 300 ppm. Esta investigación destaca el uso potencial de la nanotecnología verde en girasol para mejorar el crecimiento y el rendimiento.

Palabras claves

fisiología vegetal • cultivos oleaginosos • tecnología agraria • agente reductor • ciencias de los cultivos

Introduction

Recently, nanotechnology has emerged as a novel field with far-reaching applications across diverse sectors, including agriculture. Nanoparticles, with unique physicochemical properties, have garnered significant attention in crop management strategies (35, 36). Among these nanoparticles, copper nanoparticles stand out for their multifaceted properties, such as high surface-area-to-volume ratio, excellent conductivity, and intrinsic antimicrobial attributes (8, 28, 33). Their application in agriculture, particularly increasing plant growth and defense mechanisms, has sparked immense interest (17).

The exploration of natural sources for nanoparticle green synthesis constitutes a focal point in this expanding field (3, 15). *Aloe vera*, popular for its medicinal and bioactive properties, is a compelling candidate for synthesizing copper nanoparticles, aligning with sustainable practices and offering biocompatible and eco-friendly nanomaterials for agriculture (31).

The green synthesis of copper nanoparticles using *Aloe Vera* provides various phytochemicals with significant electrochemical reducing power. *Aloe vera* contains active components like polysaccharides, flavonoids, phenolic compounds, and anthraquinones (21, 25, 31) with functional groups like hydroxyl (-OH) and carbonyl (-C=O), that possess reducing and stabilizing power. Polysaccharides, particularly mannose-rich polymers and acetylated mannans, reduce copper ions to copper nanoparticles (9, 23, 29). Hydroxyl groups also lead to the reduction of copper ions and subsequent formation of copper nanoparticles (4, 27). Additionally, synergistic effects among various bioactive compounds in these extracts help stabilization and control the synthesis of copper nanoparticles.

Aloe Vera as green reducing agent may influence the synthesis of copper nanoparticles (31). Homemade extracts (self-grown plants) may exhibit composition variations due to cultivar differences, growth conditions, extraction methods, and storage, potentially affecting concentrations of bioactive compounds. Alternatively, commercial products are subjected to standardized processing methods, potentially containing stabilizers or additives (21) that influence concentration and quality compared to homemade extracts (18). These variations in chemical composition and concentrations of bioactive compounds in *Aloe Vera* extracts might lead to differences in their reducing potential and, consequently, affect the synthesis of copper nanoparticles. Such differences can result in varied nanoparticle sizes, shapes, and stability, impacting their potential applications (34).

Parallely, the study of nanoparticle-induced responses in crop plants represents a promising alternative in agricultural research (17). Sunflower (*Helianthus annuus* L.) is an emblematic oilseed crop known for its adaptability to various environments and the capacity to provide oil, seeds, and biomass (1, 7, 39). Beyond their economic significance, sunflowers play a key role in phytoremediation and agricultural ecosystems (9, 20). Understanding the influence of *Aloe Vera*-based copper nanoparticles on growth, development, and nutrient dynamics of sunflower hybrids may optimize crop management strategies and contribute to sustainable agricultural practices.

This research aims to describe the mechanisms underlying nanoparticle-plant interactions, and how *Aloe Vera*-based copper nanoparticles affect growth, biomass accumulation and partitioning in two sunflower hybrids. This will expand our understanding of nanoparticle-mediated plant responses, for potential tailored nanoparticle-based strategies to optimize sunflower productivity and sustainability.

Materials and Methods

Synthesis and Characterization of *Aloe Vera*-based Copper Nanoparticles

Synthesis

Copper nanoparticles (Np) were synthesized using two *Aloe Vera* extracts as reducing agents. The first *Aloe Vera* extract (AVH) was obtained and characterized at the Biocolloids and Nanotechnology Laboratory of the Facultad de Ingeniería Química (FIQ), Instituto de Tecnología de Alimentos (ITA), Universidad Nacional del Litoral (UNL) in Santa Fe (Argentina). The second extract (AVC) was commercial (Jual Aloe Calchaquí SRL.). Np synthesis used two solutions containing copper sulfate pentahydrate ($CuSO_4.5H_2O$) (Anedra-Research AG) at [0.1 M]. The AVH and AVC were added to this solutions in a 8:2 ratio of copper salt and reducing agent. The obtained solutions were shaken (Fisatom-Model 753A) for 15 minutes at moderate and constant speed. Then, both solutions were placed in a thermal bath (Dalvo-Model BTMP) for 4 h at 85°C. Subsequently, they were left for 2 h at room temperature (25°C). The resulting solutions were oven-dried (Dalvo-Model FHR/I) at 90°C for 24 h, obtaining a green powder. This powder was weighed with a high-precision digital balance (Ohaus-Model PA 214), reconstituted with the same amount of evaporated water and stirred until a homogeneous solution was obtained. Then, solutions were centrifuged (Neofuge 18R Heal Force) at 3000 rpm or 1.016 g for 20 min at 20°C. Finally, supernatants were collected and stored, obtaining liquid Np1 ($CuSO_4.5H_2O$ with *Aloe Vera* Home) and Np2 ($CuSO_4.5H_2O$ with *Aloe Vera* commercial) (13).

Characterization

The Np were spectrally characterized using UV-vis spectroscopy (Perkin Elmer Lambda 20) to determine surface plasmon resonance (SPR) characteristic of metallic nanoparticles (35). Additionally, the percentage conversion was calculated using the normalized spectrum equation (12), and particle size was determined using the dynamic light scattering (DLS) technique (ZetaNano ZS Malvern UK) (37).

Plant Culture and Growth Conditions

Plant Material and Growth Conditions

This study was conducted under field conditions with summer rainfall and controlled irrigation at Donnet field, Facultad de Ciencias Agrarias (FCA-UNL) in Esperanza (31°26’34.4” S 60°56’26.3” W, Santa Fe, Argentina). Soil was a Mollisol subgroup typical Argiudol of the Esperanza Series, with 29% sand, 66% silt, and 5% clay in the Ap horizon (0 to 0.27 m deep) (7). A total of 440 sunflower (*Helianthus annuus* L.) seeds were sown, comprising 25 plants of the Chané (Ch) hybrid and 25 plants of Calchaquí (Ca) hybrid. The seeds underwent pre-germination treatment with dynasty-metalaxil-imida (DMI) to prevent fungal growth. Seeds were supplied by Dr. Daniel Álvarez (Estación Experimental Agropecuaria, Instituto Nacional de Tecnología Agropecuaria, EEA-INTA-Manfredi).

Experimental Design

Two plots were prepared, each undergoing two mechanical weed control sessions. Plot "A" was 6.26 m long and 4.30 m wide, with Ch hybrid sown in the western part and Ca hybrid in the eastern part. Similarly, plot "B" measured 6.47 m in length and 4.50 m in width. Ch was planted in the eastern section while Ca was planted in the western section of this plot. A total of 16 rows were created, 8 rows per plot, with 4 rows for each hybrid in both plots. Externally, plots were surrounded by three rows of plants with the same density to reduce edge effect. Sowing density was 3-4 seeds per linear meter, plus 15% for potential seed loss.

Soil Preparation, Germination, and Transplanting

Germination was carried out as previously described (5, 6, 7, 19). Briefly, seeds were washed with a 30% commercial bleach solution for 20 min, followed by three washes with distilled water and drying with inert paper. Subsequently, *in vitro* germination was conducted using 20 sterilized Petri dishes conditioned with inert paper and saturated with distilled water. Each Petri dish contained 22 seeds of each hybrid germinated under controlled conditions of saturated humidity and 27.2°C in a germination oven (Bioelec-Model RE-41.1). Temperature was monitored using a Data Logger (Cavadevices SATM), recording measurements every 15 minutes. After 72 h in the germination oven, seeds were transplanted, considering visible radicle without necrotic tissue.

Morphological and Physiological Parameters

We measured germination energy (GE), germination power (PG), plant height (PH), leaf number (LN), and leaf area (LA) of the 15^{th} and 18^{th} leaves. Parameters a, b, and X_0 were obtained by fitting Leaf expansion curves to a sigmoidal equation.

Harvest was carried out when plants reached physiological maturity, corresponding to stage R9 on the Scheiter and Miller scale (32). Dry weight accumulation was measured for the two copper nanoparticles (Np) at three doses (control, C; 0ppm; D1, 150 ppm of Np AVC and AVH; and D2, 300 ppm of AVC and AVH, respectively). Dry weight was partitioned into Heads, Stems, Petioles, and Leaves, then stored and oven-dried (DALVO-Model XHRF 6189) at 60°C until constant weight. Hybrids were harvested at 2141.7°C d^{-1} (5, 12).

Leaf Growth Analysis

Leaf Area (*LA*) was estimated as described in Eq.1 (6, 24) from length and width measurements as follows (2):

$$LA = L \times W \times 0.65 \quad (1)$$

Relative rate of leaf expansion was calculated as described in Eq.2 (6, 24) as the slope of the regression curve between LA natural logarithm and thermal time.

Leaf expansion dynamics were analyzed by sigmoid curves with three parameters (*a, b,* and x_0):

$$y = a / (1 + \exp\{- [(x - x_0) / b]\}) \quad (2)$$

Final LA was determined as the upper asymptote (a).

Maximum expansion velocity value (V_{max}) was calculated as follows (6, 24):

$$V_{max} = [\, a * (1/b)] /4 \quad (3)$$

Absolute leaf expansion rates (AER) were calculated as the slopes of the linear regression between leaf area and thermal time between two consecutive measurements for the entire experimental time (6, 24).

Leaf relative expansion rates (RER) were calculated as the ratios between the differences in leaf area logarithms and thermal time interval between two successive measurements (hn-1 and hn), (Eq.4) (5, 24):

$$RER= (\ln LAhn-\ln LAhn-1)/(°Cdhn-°Cdhn-1) \quad (4)$$

Nanoparticle Foliar Application

The Np were applied to leaves 15 and 18 in each hybrid (Ch and Ca) using a trigger spray applicator, at two different thermal moments (1507.4°C d^{-1} and 1645.9°C d^{-1}), with temperatures of 25.6 and 24.1°C, respectively. Both applications were carried out in the morning, ensuring open stomata and no wind. A volume of 12.5 mL of Np solution was applied to each leaf at each thermal time, totaling 25 mL per plant. Np were applied at two different doses in both hybrids: D1, 150 ppm of Np per plant and D2, 300 ppm of Np per plant (14).

Statistical Analysis

Data were analyzed by ANOVA and Fisher's least significant difference (LSD) test for 5 % significance level. ANOVA assumptions were verified by Shapiro-Wilks and Levene tests (7). Statistical analyses were run using InfoStat Professional software (Universidad Nacional de Córdoba) (7).

Results and Discussion

Characterization of Copper Nanoparticles (Np)

Spectral Characterization and Particle Size

Other studies report that the green synthesis method also enabled the observation of the SPR phenomenon at a wavelength of 398 nm for copper nanoparticles (35). Additionally, other peaks were observed at wavelengths ranging from 277 to 305 nm, similar to those observed in the present work (26). Figure 1A (page 192), shows comparable peaks in copper nanoparticles synthesized using different reducing agents (*Aloe Vera* Home and *Aloe Vera* Commercial), potentially attributed to the small nanoparticles. Understanding particle size is crucial as it directly influences physical and chemical properties of nanoparticles (37). Employing the DLS technique (figure 1B and figure1C, page 192), Yugandhar *et. al.* (2018) observed synthesized copper nanoparticles of 61.1 nm. Furthermore, Sánchez Gómez *et al.* (2018) documented a size distribution of 50 nm for their synthesized copper nanoparticles. These findings can be compared to the second population observed in Np2. Notably, both nanoparticle sets synthesized using *Aloe Vera* exhibited sizes as those reported in the previously mentioned studies.

The different compositions of *Aloe Vera* extracts, whether Home or Commercial, might affect the reduction mechanisms or stabilize the nanoparticles differently during synthesis, potentially influencing particle size, as detected with DLS technique.

Plant Analysis

Physiological Parameters Before Nanoparticles Applicacion

Germinative Energy (GE) and Germinative Power (GP)

Germination energy in Ch was 94.0%, similar to that in *Pisum sativum* L. seeds with Treatment 1 (control) at 3 days (16). This implies that the Ca sunflower hybrid possesses a lower GE at 67.3% (figure 2A, page 192), while the Ch hybrid shows an even lower GE at 30.4%. Sánchez Gómez *et al.* (2018) analyzed GE at 7 days for Huaxyacac seeds *cv.* Cunningham (*Leucaena leucocephala* (Lam.) de Wit. treated with IA24 (water immersion at 24°C for 12 h), finding GE of 31.7%. In comparison, Ch sunflower hybrid seeds display a higher value (45.9%), while Ca seeds achieve 80.0% (figure 2b, page 192).

(Green line) *Aloe Vera* Home (AVH); (Blue line) Commercial *Aloe Vera* (AVC); (Black line) UV-Visible spectra of copper nanoparticles (Np1); (Red line) UV-Visible spectra of copper nanoparticles (Np2); and (Purple line) pentahydrated copper sulfate ($CuSO_4.5H_2O$).

(Línea verde) *Aloe Vera* Home (AVH); (Línea azul) *Aloe Vera* Comercial (AVC); (Línea negra) espectros de UV-Visible de nanopartículas de cobre (Np1); (Línea roja) espectros de UV-Visible de nanopartículas de cobre (Np2); y (Línea púrpura) sulfato de cobre pentahidratado ($CuSO_4.5H_2O$).

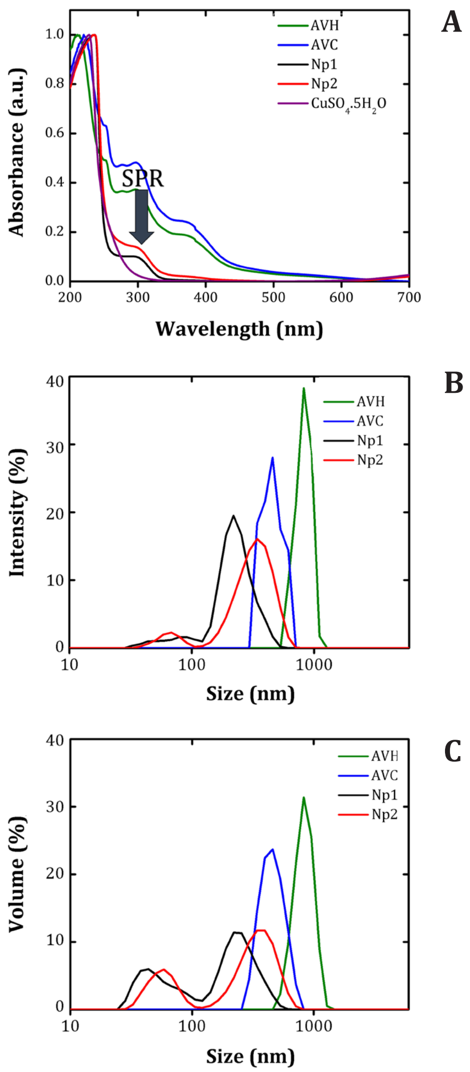


Figure 1. UV-visible spectra (A), Particle size distribution (PSD) based on Intensity (B) and Volume percentage (C) of the systems.

Figura 1. Espectros de UV-Visible (A), Distribución del tamaño de las partículas (PSD) en función de la intensidad (B) y del porcentaje de volumen (C) de los sistemas.

(A). Germination Energy at 72 h (EG) and (B) Germination Power (PG) at 168 h evaluated in two sunflower hybrids (*Helianthus annuus* L.) Chané (Ch) and Calchaquí (Ca).

(A) Energía Germinativa a las 72 h (EG) y (B) Poder Germinativo (PG) a las 168 h en dos híbridos de girasol (*Helianthus annuus* L.) Chané (Ch) y Calchaquí (Ca).

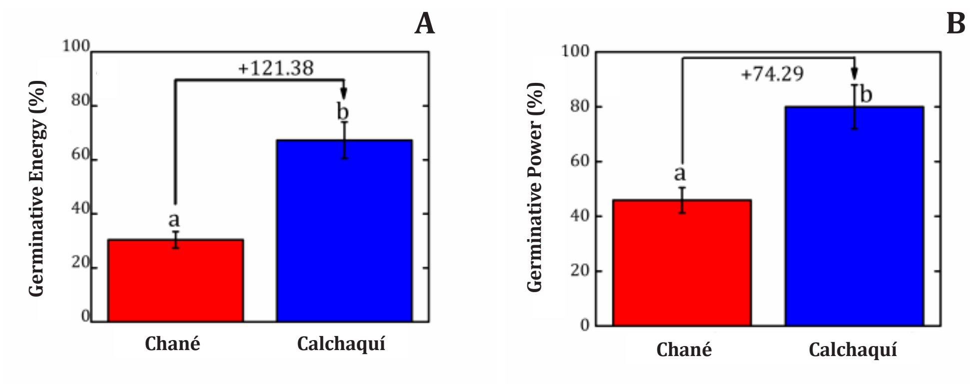


Figure 2. Germinative energy (%) and Germinative power (%) in two sunflower hybrids.

Figura 2. Energía germinativa (%) y Poder Germinativo (%) en dos híbridos de girasol.

Plant Height (PH) and Leaf Number (LN)

Figure 3A shows statistically significant differences in PH, except at 996.7 and 1283.7°C d^{-1}, probably since PH under controlled growth conditions is genotype dependent (24).

A research conducted by Ortis *et al.* (2005) involving 20 sunflower inbred lines found the KLM 295 hybrid exhibited similar behavior in PH as Ch and Ca hybrids, measuring 170 cm. Similarly, two sunflower hybrids PARSUN-1 and SMH-9707 (10), were shorter than Ch and Ca hybrids (136.61 cm and 137.63 cm, respectively). As previously described, this could be genotype-dependent. However, differences in PH can also be explained by internode elongation as a response to sowing density (1).

Figure 3B shows LN of Ch and Ca at eight different thermal times, starting from 528.3°C d^{-1}. At this moment, both hybrids had 18 visible leaves. Similarly, at 571.3°C d^{-1}, Ch exhibited 21 leaves while Ca had 22 leaves. Furthermore, at 611.7, 676.6, 731.5, and 884.1°C d^{-1}, Ch showed 23, 25, 27, and 29 true leaves, respectively. In contrast, during these days, Ca had 25, 27, 29, and 31 leaves, indicating an average difference of 2 extra leaves for Ca. Lastly, both hybrids had equal number of leaves (30 and 31) at 996.7 and 1283.7°C d^{-1} of plant development. As with PH, this difference in LN may be genetic (24).

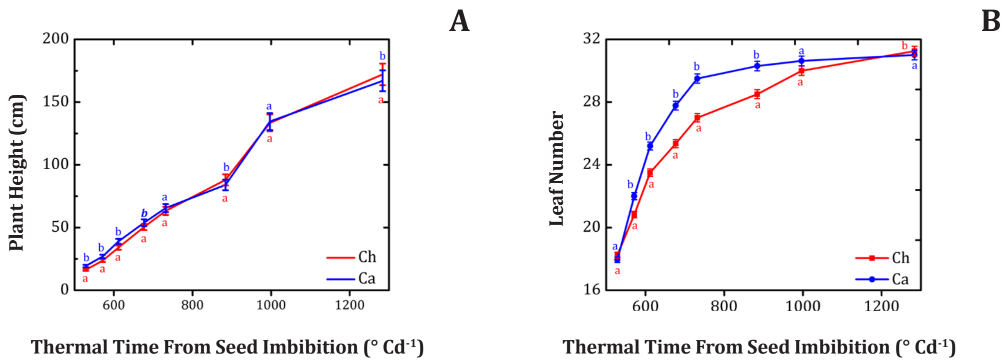


Figure 3. (A) Average plant height (cm) and (B) Leaf Number of two sunflower hybrids (*Helianthus annuus* L.), Chané (Ch), and Calchaquí (Ca).

Figura 3. (A) Altura media de planta (cm) y (B) número de hojas de dos híbridos de girasol (*Helianthus annuus* L.), Chané (Ch) y Calchaquí (Ca).

Leaf Expansion Dynamics of Leaves 15 and 18

Figure 4A (page 194), describes leaf expansion dynamics of the 15th and 18th leaves of Ch and Ca hybrids. Leaf 15 in Ch grew faster than Ca. Comparing these results with figure 3B, we concluded that Ch had fewer leaves but a bigger 15th leaf at all recorded thermal times. Figure 4A (page 194), shows that at 758.20°C d^{-1}, the Ch hybrid reached 50% of its final leaf area, while the Ca hybrid reached this value at 762.89°C d^{-1}.

Figure 4B (page 194), describes leaf expansion dynamics of the 18th leaf in both hybrids. When compared, both genotypes showed similar results in parameters "a" and "x_0", 34,307.21 and 808.31 for Ch, and 33,410.21 and 809.19 for Ca. Additionally, leaf expansion ceased at 979.08°C d^{-1} and 963.88°C d^{-1} in Ch and Ca, respectively. In conclusion, leaf growth dynamics of the 18th leaf were the same between hybrids and comparable to Céccoli *et al.* (2012).

Leaf area calculated by Eq. 2 (page 190).

El área foliar, para cada hoja y tiempo termal, fue calculado como lo indica la Eq. 2 (pág. 190).

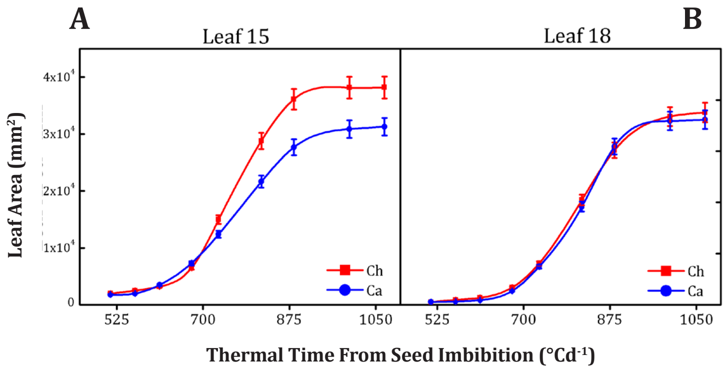


Figure 4. (A) Leaf area (LA) of the 15th leaf in Chané (Ch) and Calchaquí (Ca) hybrids; (B) Leaf area (LA) of the 18th leaf in Chané (Ch) and Calchaquí (Ca) hybrids.

Figura 4. (A) Área foliar (LA) de la hoja 15 en Ch y Ca; (B) Área (LA) de la hoja 18 en los híbridos Ch y Ca.

Leaf Growth Analyses

Figure 5 (page 195), compares different lea physiological parameters between leaves 15th and 18th, in Ch and Ca. Parameter "a" had statically lower area in leaf 15 of Ca (-12.97%; figure 5A, page 195), but no differences were seen for leaves 18 (figure 5A, page 195). Regarding parameter "b" in the 15th leaf, Ca had a significantly higher curvature in the sigmoid curve compared to Ch (figure 5B, page 195) while, the18th leaf showed no significant differences (figure 5B, page 195).

No significant differences were found for "x0" in the 15th leaf of any hybrid. Ca showed -0.42% (figure 5C, page 195), and Ch reached 50% leaf expansion in less thermal time compared to Ca (figure 5C, page 195). No statistically significant differences were found for leaf expansion cessation (figure 5D, page 195).

Leaf growth duration and Vmax increase were not statistically significant (figure 5F, page 195). Absolute leaf expansion rate (AER) was -33.82% in the 15th leaf of Ca with respect to Ch, with significant differences (figure 5G, page 195). The 18th leaf showed no significant differences (figure 5G, page 195). Finally, Leaf Relative Expansion Rate (RER) was -50.0% lower in Ca with respect to Ch in the15th leaf (figure 5H, page 195), and -33.33% considering the 18th leaf (figure 5H, page 195).

Physiological and Productive Parameters After Foliar Application of Copper Nanoparticles

Finally, Plant DW accumulation in Ch hybrid with Np1D1 and Np2D1 increased by 48.74% and 38.26%, respectively, compared to control plants. These Nps resulted in more benefits for this hybrid than for Ca, which decreased by 8.95% and 11.36% with Np1D1 and Np2D1, respectively (figure 6, page 196).

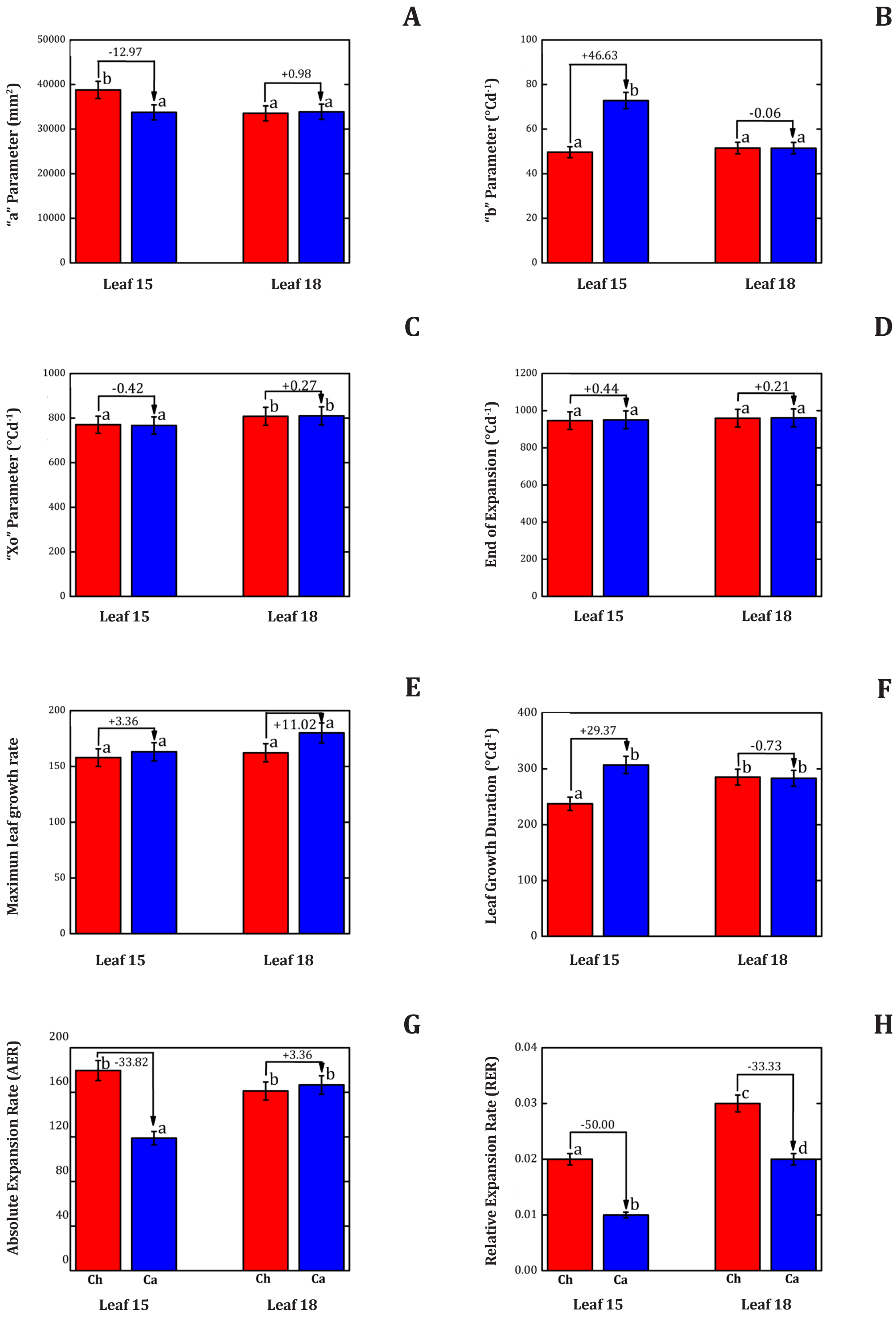
We conclude that dry weight accumulation using Np1 and Np2 at two doses suggests an interaction between the treatments and genotypes used (1, 7, 10, 11).

Foliar application of copper nanoparticles at 300 ppm is beneficial for plant development under saline stress, preventing biomass loss, while enhancing the levels of various bioactive compounds (17). These reported results can be compared with the present research, indicating positive effects of copper nanoparticles on leaf growth dynamics and dry weight accumulation in sunflower.

Significant increases in dry weight accumulation of head, stem, petiole, and leaf in Ch hybrid with Np1D1, Np2D1, Np1D2, and Np2D2 may indicate a better response to those specific doses or *Aloe Vera* genotypes. Conversely, the Ca hybrid showed varied responses, indicating diverse sensitivity upon *Aloe Vera* extracts (36, 38).

Differences in extract composition may lead to variations in synthesis or delivery of nanoparticles, altering their efficacy. The applied doses might have triggered diverse metabolic pathways, resulting in distinct responses between hybrids (1, 7, 10, 11).

Understanding these intricate relationships between nanoparticles, *Aloe Vera* extracts, and plant physiology requires further investigation to optimize nanoparticle application for enhanced agricultural production.



(A) Parameter "a" (mm^2), (B) parameter "b", (C) parameter "x0", (D) End of leaf expansion (°C d^{-1}), (E) parameter Vmax, (F) Duration of leaf expansion (°C d^{-1}), (G) Absolute rate of leaf expansion (AER, mm^2.°Cd^{-1}), and (H) Relative rate of leaf expansion (RER °Cd^{-1}) in two sunflower hybrids (*Helianthus annuus* L.), Chané (Ch) and Calchaquí (Ca) for leaf 15 and 18.

(A) Parámetro "a" (mm^2), (B) parámetro "b", (C) parámetro "x0", (D) Fin de la expansión foliar (°C d^{-1}), (E) parámetro Vmax, (F) Duración de la expansión foliar (°C d^{-1}), (G) Tasa absoluta de expansión foliar (AER mm^2.°Cd^{-1}), y (H) Tasa relativa de expansión foliar (RER °Cd^{-1}) en dos híbridos de girasol (*Helianthus annuus* L.), Chané (Ch) y Calchaquí (Ca) para la hoja 15 y la hoja 18, respectivamente.

Figure 5. Leaf Growth Analyses.
Figura 5. Análisis del crecimiento de las hojas.

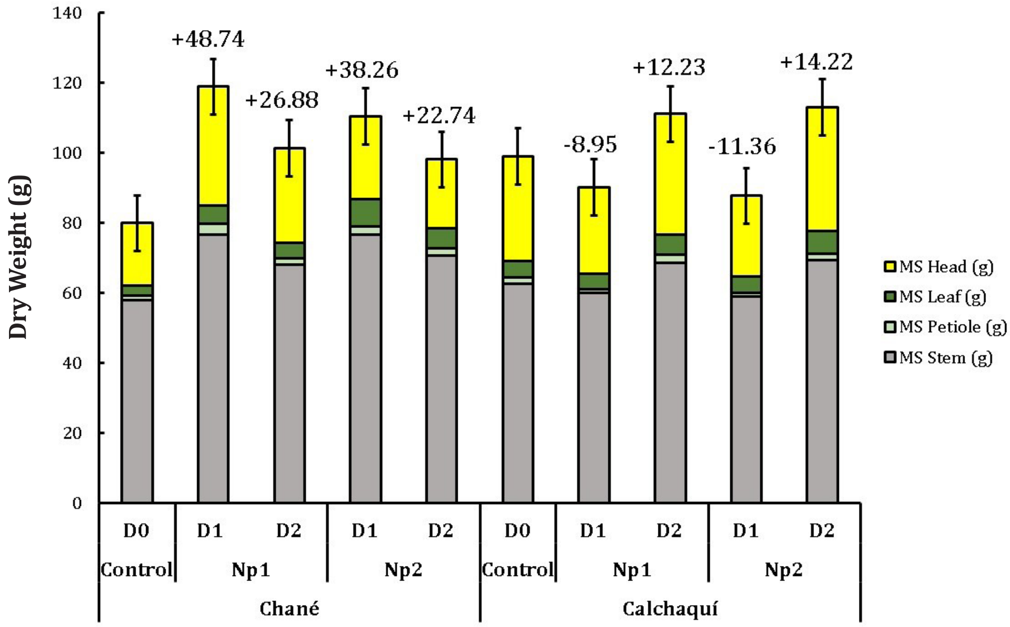


Figure 6. Dry weight accumulation of two sunflower hybrids (*Helianthus annuus* L.), Chané (Ch), and Calchaquí (Cal), partitioned into head, stem, petiole and leaf blade, after application of copper nanoparticles at harvest.

Figura 6. Peso de materia seca de los dos híbridos de girasol (*Helianthus annuus* L.), Chané (Ch) y Calchaquí (Cal), particionados en capitulo, tallo, pecíolo y lámina, luego de la aplicación de nanopartículas de cobre. La cosecha se realizó en madurez fisiológica.

Conclusions

This study assessed physiological responses in two sunflower (*Helianthus annuus* L.) hybrids, Chané (Ch) and Calchaquí (Ca), after foliar application of two types and doses of copper nanoparticles.

Different particle sizes of copper nanoparticles were observed employing *Aloe Vera* homemade extracts (Np1). The DLS technique allowed detecting two peaks at 242.8 nm and 74.87 nm, constituting 62.6% and 37.4% of the particles, respectively.

A comparison between sunflower hybrids showed that Calchaquí (Ca) had a higher Germinative Energy (GE) and Germinative Power (GP) by +121.38% and +74.29% respectively, than Chané. Leaf number was higher in the Calchaquí hybrid at all thermal times, except for the last measurement (1283.7°C d^{-1}). The Chane hybrid had higher expansion and relative expansion rates on leaf 15. Leaf 18 had similar parameter values in both hybrids.

Finally, Np1 ($CuSO_4.5H_2O$ with *Aloe Vera* Home), at 150 ppm (D1) for Chané (Ch), increased "Plant DW" accumulation by 48.74%. The study lays groundwork for further optimization of nanoparticle application to different sunflower hybrids.

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