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Hydroxyapatite Nanoparticles as an Effective Fertilizer in Sunflower Crop Quality
Nanopartículas de Hidroxiapatita Utilizado como Fertilizante Eficaz en la Calidad del Cultivo de Girasol

Oscar Emmanuel Gómez-García[1], Eduardo Arón Flores-Hernández[1], Rubén López-Salazar[1], Alejandro Moreno-Reséndez[1], and Mercedes Georgina Ramírez-Aragón[2‡]

[1] Universidad Autónoma Agraria Antonio Narro, Periférico Raúl López Sánchez s/n. Colonia Valle Verde. 27054 Torreón, Coahuila, México; (O.E.G.G.), (E.A.F.H), (R.L.S), (A.M,R).
[2] Tecnológico Nacional de México (TecNM), Instituto Tecnológico Superior de Lerdo. Av. Tecnológico No. 1555 Sur, Periférico Lerdo km 14.5, Placido Domingo. 35150 Ciudad Lerdo, Durango, México; (M.G.R.A).
[‡] Corresponding author: ginaaragon13@hotmail.com

SUMMARY

Nanotechnology involves manipulating materials at the atomic or molecular scale, particularly those smaller than 100 nm, which display unique properties due to quantum effects and a high surface area-to-volume ratio. In agriculture, nanoparticles (NPs) enhance fertilizer and pesticide efficiency, improve crop yield, and enable early detection and treatment of plant diseases. This study evaluated the effect of hydroxyapatite nanoparticles (HANPs) as a foliar fertilizer on sunflower (*Helianthus annuus*) crop quality, alongside vermicompost, chemical fertilizer, and biofertilizer applied to soil. The HANPs ranged from 10 to 45 nm, with 53.1% between 15 and 25 nm, predominantly rod-shaped. Agronomic variables (plant height, stem and disc diameter) and phytochemical properties of sunflower oil (total phenolic compounds, flavonoid content, antioxidant capacity) were analyzed under three HANP doses: 2000, 4000, and 6000 mg L^{-1}. Results showed that vermicompost and chemical fertilizer had no significant differences in plant height, whereas biofertilizer (299.6 and 258.6 cm) differed significantly from the control. Phytochemical analyses yielded 17.9-35.4 mg GAE mL^{-1} of phenolic compounds, 29.8-60.7 mg QE mL^{-1} of flavonoids, and 18024.60-25159.52 µM TE mL^{-1} of antioxidant activity across treatments. The aim was to assess HANPs' influence on sunflower development and the antioxidant profile of its oil. Among the doses tested, 4000 mg L^{-1} proved optimal for enhancing total phenolic and flavonoid content, as well as antioxidant capacity, indicating its potential for improving the nutritional and functional quality of sunflower oil.

Index words: *agronomy, antioxidants, bioactive compounds, secondary metabolites, soil.*

RESUMEN

La nanotecnología implica la manipulación de materiales a escala atómica o molecular, particularmente aquellos menores a 100 nm, los cuales presentan propiedades únicas debido a los efectos cuánticos y a una alta relación superficie-volumen. En la agricultura, las nanopartículas (NPs) mejoran la eficiencia de fertilizantes y pesticidas, aumentan el rendimiento de los cultivos y permiten la detección y tratamiento temprano de enfermedades en las plantas. Este estudio evaluó el efecto de nanopartículas de hidroxiapatita (HANPs) aplicadas como fertilizante foliar sobre la calidad del cultivo de girasol (*Helianthus annuus*), en combinación con vermicomposta, fertilizante químico y biofertilizante aplicados al suelo. Las HANPs presentaron tamaños entre 10 y 45 nm, con un 53.1% en el rango de 15 a 25 nm y predominantemente de forma alargada. Se analizaron variables agronómicas (altura de planta, diámetro del tallo y del capítulo) y propiedades fitoquímicas del aceite

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de girasol (compuestos fenólicos totales, contenido de flavonoides y capacidad antioxidante) bajo tres dosis de HANPs: 2000, 4000 y 6000 mg L^{-1}. Los resultados mostraron que la vermicomposta y el fertilizante químico no presentaron diferencias significativas en la altura de planta, mientras que el biofertilizante (299.6 y 258.6 cm) sí mostró diferencias significativas respecto al control. Los análisis fitoquímicos arrojaron valores entre 17.9 y 35.4 mg GAE mL^{-1} para compuestos fenólicos, 29.8 a 60.7 mg QE mL^{-1} para flavonoides y entre 18024.60 y 25159.52 µM TE mL^{-1} para la actividad antioxidante en los distintos tratamientos. El objetivo fue evaluar la influencia de las HANPs en el desarrollo del girasol y el perfil antioxidante de su aceite. Entre las dosis evaluadas, la de 4000 mg L^{-1} resultó ser la más adecuada para incrementar los compuestos fenólicos totales, flavonoides y capacidad antioxidante, lo que indica su potencial para mejorar la calidad nutricional y funcional del aceite de girasol.

Palabras clave: *agronomía, antioxidantes, compuestos bioactivos, metabolitos secundarios, suelo.*

INTRODUCTION

By the year 2050, it's projected that the world's population will approach 9.6 billion individuals (Tripathi, Mishra, Maurya, Singh, and Wilson, 2019). Consequently, agricultural output needs to rise by 70-100% to satisfy the growing food requirements (Zhao *et al*., 2020). Nevertheless, challenges such as diminishing arable land, water scarcity, climate change effects, and the inefficiency of current agrochemicals exacerbate both abiotic and biotic stresses on crops, leading to decreased yields (Rodrígues *et al*., 2017). In this view, the urgent challenge was to significantly increase food production to meet the growing demands of a rising global population and address concerns about food security. Consequently, there is a need for innovative technologies or strategies to shield plants from stress and optimize the utilization of agrochemicals, thereby ensuring food security in a safe and sustainable manner (Zhao *et al*., 2020).

Nanotechnology spans various fields, with increasing attention being paid to its potential in agriculture by enhancing soil fertility, improving nutrient delivery, reducing environmental impact through targeted pesticide application, and fostering sustainable agricultural practices (Lowry, Avellan, and Gilbertson, 2019). Specifically, in promoting plant growth and enhancing crop yield (Giraldo, Wu, Newkirk, and Kruss, 2019), engineered nanoparticles have demonstrated promising outcomes in seed treatment, germination, plant development, pathogen detection, and identifying harmful agrochemicals (Nuruzzaman, Rahman, Liu, and Naidu, 2016). Nanoparticles (NPs) exhibit remarkable physicochemical characteristics, including reduced size, high surface area to volume ratio, heightened reactivity, strong ionizing potential, enhanced chemical stability, greater absorbability, improved pH tolerance, and extended thermal stability, these qualities provide greater efficiency when added to crops (Fatima, Hashim, and Anees, 2021). Consequently, the utilization of plant nanobiotechnology holds the potential to foster sustainable agricultural practices through mechanisms that differ from traditional chemical and genetic approaches, such as targeted delivery of nutrients and agrochemicals, enhanced stress resistance, improved crop yield, and reduced environmental impact by minimizing waste and overuse of resources (Zhao *et al*., 2020). Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] nanoparticles (HANPs) and their combinations have garnered considerable attention due to their significance across various disciplines such as material science, biology, medicine, and agronomy (De Silva *et al*., 2022). In recent times, hydroxyapatite nanoparticles (HANPs) have been studied in depth to determine their viability as substitutes for conventional phosphorus fertilizers due to offer a controlled and sustained release of phosphorus, ensuring efficient nutrient uptake by plants while minimizing losses due to leaching or runoff (Xiong, Wang, and Kopittke, 2018). According to what was mentioned above, these particles exhibit distinct properties attributable to their physical and chemical characteristics at the nanoscale, differing from those of the bulk material (Dakal, Kumar, Majumdar, and Yadav, 2016).

Nanoparticles show promise as a new type of non-living substance that can trigger the production of beneficial compounds in plant cells and tissues (Hatami, Naghdi-Badi, and Ghorbanpour, 2019). Numerous studies have explored how nanoparticles can act as triggers to activate genes responsible for creating secondary metabolites (Yarizade and Hosseini, 2015). While the exact mechanism is not fully understood yet, some suggest that nanoparticles prompt the generation of reactive oxygen species (ROS) and other signaling molecules, which then regulate gene expression in plant metabolism (Marslin, Sheeba, and Franklin, 2017).

According to Wu and Li (2022), interfacing NPs with plants in the field is done mainly through foliar delivery or root application (Figure 1). The main problem with sunflower cultivation is its low profitability, caused by low yields and high production costs. Sunflower profitability can be increased through studies on irrigation schedules, insect control, genotypes, nitrogen and phosphorus doses, and plant density (Moreno, Cruz, Herrara y Turrent, 2012).

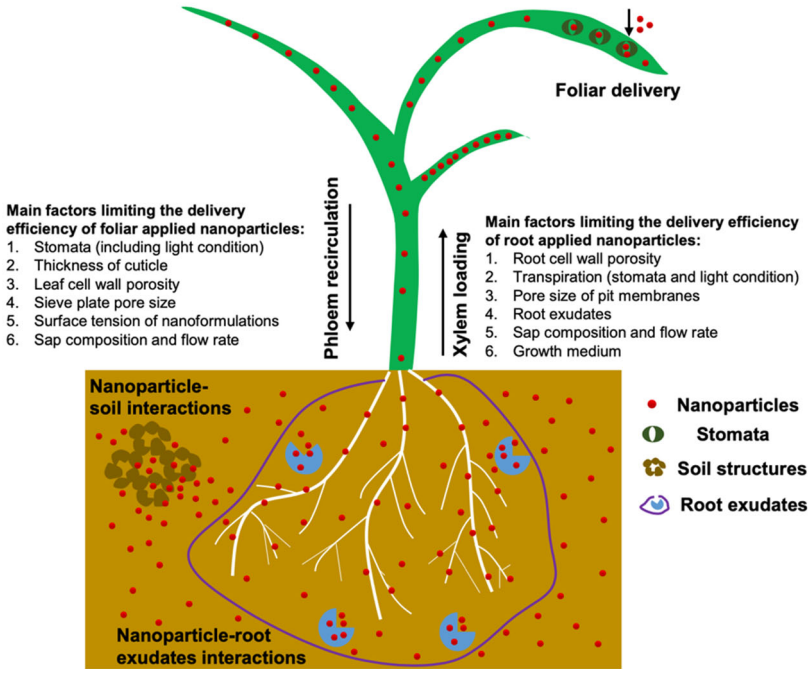

Figure 1. Primary factors that restrict the efficiency of nanoparticle delivery to plants (Wu and Li, 2022).

Sunflowers hold a prominent status among oilseed crops globally, prized for their high-quality oil and dietary fiber, which play crucial roles in human health. With the persistent rise in the world's population, the demand for edible sunflower seeds, oil, and related products has surged, necessitating intensified efforts to bolster sunflower production (Adeleke and Babaloba, 2020).

The primary utilization of nanomaterials in crop cultivation aims to minimize the reliance on agrochemicals while simultaneously enhancing yield through improved pest and nutrient management (Prasad, Bhattacharyya, and Nguyen, 2017). Recent research has underscored the prospective applications of nanotechnology in crop production, emphasizing its role in augmenting yield and enhancing the nutritional and nutraceutical value of crops (Fraceto *et al*., 2016).

Based on the aforementioned points, this research focused on examining the impact of hydroxyapatite nanoparticles (NPs) on sunflower cultivation, particularly on the antioxidants found in sunflower oil.

MATERIALS AND METHODS

Characterization of Nanoparticles Using Transmission Electron Microscopy

The morphology and microstructure of the hydroxyapatite nanoparticles were determined using a FEI-TITAN 80-300 kV transmission electron microscope (Fisher Scientific, Hillsboro), operated at an acceleration voltage of 300 kV. Samples for analysis were prepared by depositing and evaporating a drop of the colloidal solution onto carbon-coated copper grids. High-resolution imaging was performed using conventional transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM). Electron diffraction (ED) was utilized to examine the crystallographic properties of the nanoparticles.

Transmission electron microscope micrographs were processed and analyzed using Digital Micrograph 3.7.0 software (Gatan Inc, 2005) for detailed structural interpretation.

For particle size analysis, Image Pro-10 Version 10.0.3 (Media Cybernetics Inc., 2021) was employed to measure and calculate the size distribution of nanoparticles.

A dataset of at least 300 nanoparticles was analyzed to ensure statistical relevance. A) The primary diameters of individual nanoparticles were measured from the CTEM images. B) The average diameter was calculated, and the absolute frequency for defined diameter ranges was determined to generate the particle size distribution profile.

This comprehensive methodology allowed for the precise determination of the size, shape, and crystallinity of the hydroxyapatite nanoparticles, which are critical parameters for their application in agriculture.

Experiment Location

The present study was conducted during the summer season of 2023 at the experimental field of the Universidad Autónoma Agraria Antonio Narro Unidad Laguna (UAAAN-UL), located at Periférico and Santa Fe Highway, km 1.5, Torreon, Coahuila, Mexico. The geographical coordinates are 25° 31′ 11″ N, 103° 25, 75″ W, with 1123 meters of altitude.

Land Preparation

The land preparation consisted of fallowing, harrowing, leveling, and marking the furrows. A 6000-caliber tape irrigation system with emitters at 30 cm was installed.

Sowing

The soil was prepared by leveling and creating furrows to facilitate a simple furrow sowing system, ensuring proper drainage and ease of seed placement.

The sowing was performed manually, with each furrow carefully opened to the required depth to accommodate the seeds. Two sunflower seeds were deposited at each sowing point along the furrow. A spacing of 30 cm between sowing points was maintained to provide adequate space for root development and canopy expansion, thereby minimizing competition for nutrients, water, and light.

The furrows were lightly covered with soil to ensure good seed-to-soil contact, which is critical for germination.

Establishment of the Experiment

Sunflower seeds acquired from the germplasm bank of UAAAN were utilized. Four different soil treatments were conducted: two kilograms per square meter of vermicompost (VC) with established characteristics (Table 1), chemical fertilizer (MAP 11-52-00 and Urea), biofertilizer (Biotlakualli®), and a control. Sunflower seeds were sown in each of these areas. These treatments were subjected to varying doses of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] nanoparticles (2000, 4000, and 6000 mg L^{-1}) used as fertilizer during irrigation.

Table 1. Nutrient Composition of Vermicompost (VC).

Components	VC
Chemical composition	mg kg^{-1}
Nitrogen (N)	32.1
Nitrates (NO_3^{-1})	128.33
Ammonium (NH_4^+)	149.3
Phosphorus (P)	54.67
Potassium (K)	461.0
Calcium (Ca)	39.85
Sulfate (SO_4^{-2})	-
Magnesium (Mg)	1.60
Manganese (Mn)	4.36
Copper (Cu)	0.92
Iron (Fe)	1.28
Zinc (Zn)	1.37
Additional properties	
Physical appearance	Solid dark brown
Organic material (%)	8.5
pH	8.2
Electrical conductivity (dS m^{-1})	6.18
Cation exchange capacity (cmol L^{-1})	

Harvest

The harvest was carried out by hand 120 days after sowing, harvesting all the plants from each treatment. Subsequently, agronomic measurements were made (height, stem diameter, and disc flower diameter) of four plants from each treatment taken at random. Afterward, the seed of the four different flowers harvested from the cultivation of each treatment was recovered for the extraction of oil that was analyzed.

Oil Extraction

The seeds were dehulled to separate the kernel from the outer shell, as the hull can reduce oil yield and quality. After that, the seeds were dried to reduce the moisture content to 8–10%, ensuring optimal pressing conditions and preventing microbial contamination.

The seeds were lightly crushed or flaked to rupture cell walls and facilitate better oil release during pressing. A hydraulic press was used to extract the oil. During pressing, temperatures were maintained between 50 °C and 55 °C to ensure efficient extraction while preserving oil quality. This moderate heat softened the oil-containing cells without degrading the nutritional properties of the oil.

The pressing operation applied a pressure of 10–12 MPa (100-120 bar), which was sufficient to extract a significant amount of oil from the seeds.

The extracted oil flowed out of the press, collected in a container, and filtered to remove solid impurities. The crude oil was passed through fine filters to remove any remaining solid particles, resulting in clarified oil. The extracted sunflower seed oil was stored in dark, airtight containers at 4 °C to prevent oxidation and maintain quality until analysis.

Measurement of Variables

Synthesis of Hydroxyapatite Nanoparticles

The hydroxyapatite nanoparticles were obtained through a wet chemical precipitation reaction using mechanical stirring at 1000 rpm. The particle sizes ranged from 10 to 45 nm; however, 53.1% of the NPs were within the range of 15 to 25 nm, predominantly rod-shaped. These conditions are desirable for the study and application in agriculture. Table 2 shows the size distribution and Figure 2 morphology of hydroxyapatite nanoparticles.

Agronomical Variables

It was quantified based on four random plants as the distance from the base of the plant to the node where the flower head begins for height. The stem diameter was measured in each plant at the same time as the diameter of the disc flower of the crop; these variables were measured in centimeters.

Table 2. Size distribution of hydroxyapatite nanoparticles.

Nanoparticles diameter	Absolute frequency	Percentage
nm		%
10 - 15	43	12.2
15 - 20	95	27
20 - 25	92	26.1
25 - 30	71	20.2
30 - 35	36	10.2
35 - 40	13	3.7
40 - 45	2	0.6
TOTAL	352	100

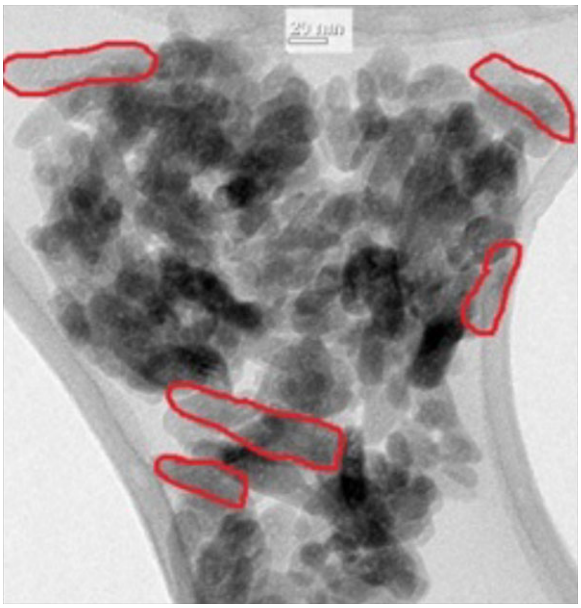

Figure 2. Micrographs of the synthesized hydroxyapatite nanoparticle sample.

Total Phenolic Concentration

Total phenolic concentration (TPC) was determined by modifying the Folin-Ciocalteu reagent according to the procedure described by Ramírez-Aragón, Borroel, López, Nieto, and García (2024). The various oil samples were blended with hexane before analysis. First, 50 µL of each prepared sample was combined with 3 mL of distilled water in a test tube. Subsequently, 250 µL of the Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis MO, USA) was added to the mixture. After vortexing for 10 seconds, the mixture was allowed to rest for 3 minutes to facilitate the reaction. Following this, 750 µL of sodium carbonate solution (20% w/v) was added to each sample tube and vortexed for 10 seconds, followed by the addition of 950 µL of distilled water for further vortexing. The samples were then incubated for 2 hours at room temperature in a dark environment. After incubation, the samples were transferred to a UV spectrophotometer (Genesys, USA) and measured at an absorbance of 765 nm. All analyses were conducted in triplicate. The total phenolic compounds were quantified using a calibration curve with gallic acid as the standard, and the results are expressed in milligrams of gallic acid equivalents per milliliter of the sample (mg GAE mL^{-1}).

Total Flavonoids Concentration

Initially, 50 µL of extract obtained from the sample was placed into a test tube, followed by adding ethanol to achieve a final volume of 1 mL. Subsequently, the samples were mixed with 4 mL of distilled water and 0.3 mL of 5% NaNO3 solution, followed by adding 0.3 mL of 10% AlCl3 solution. The resulting mixture was then incubated for 5 minutes. After this incubation period, 2 mL of NaOH solution at 1 M was added, and the volume was adjusted to 10 mL with double-distilled water. The mixture was allowed to react for 15 min. Total flavonoid (TF) quantification was conducted using spectrophotometry at an absorbance of 510 nm. The total flavonoid content is expressed in milligrams of quercetin equivalents per milliliter of sample (mg QE mL^{-1}) (Baba, and Malik, 2015). Analyses were conducted in triplicate.

Total Antioxidant Activity

Trolox equivalent antioxidant capacity (TEAC) assay was developed according to the method of Domínguez, and Ordoñez (2013), utilizing ABTS (2.2′-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) as the radical source (Sigma-Aldrich®, USA). In the assays, 10 µL of the prepared sample was mixed with 990 µL of the adjusted ABTS radical solution. After a 30 minutes incubation period, the absorbance was measured at 734 nm using spectrophotometry. The calibration curve was established using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as the standard antioxidant agent. Results were expressed as µM TEAC mL^{-1} of sample.

Statistical Analysis

A completely randomized design was employed, with three replications. The evaluated variables included plant height, stem diameter, head diameter, total phenolic compounds, flavonoid content, and antioxidant capacity. The data were statistically analyzed using ANOVA to compare means. The Tukey test was used to verify statistically significant differences between treatments with a confidence level of 95% ($P < 0.05$). Data was analyzed using Statistica 6.0® software (Statsoft, 2000).

RESULTS AND DISCUSSION

In the present study, significant differences ($P < 0.05$) were observed among soil treatments and the various doses of hydroxyapatite nanoparticles added to sunflower cultivation. The results showed that the plants treated with vermicompost and chemical fertilizer were the tallest, with values of 299.6 and 289.4, respectively. In contrast, the plants treated with biofertilizer did not show statistically significant differences ($P < 0.05$) compared to the control treatment (Figure 3).

Regarding the results obtained for the height variable, comparing the doses of hydroxyapatite nanoparticles added to the different soil treatments ranges between 2.68 - 2.87 m were obtained. The doses that yielded the greatest height were 4000 mg L^{-1} and 6000 mg L^{-1} of nanoparticles, where a significant statistical difference was not shown, unlike the 2000 mg L^{-1} dose, where the lowest results were reported for this variable (Table 3).

For the agronomic variables concerning stem diameter and head diameter of the different sunflower plants treated with varying doses of hydroxyapatite nanoparticles, results ranged between 3.83 - 3.34 cm for stem diameter and 23.9 - 21.3 cm for head diameter. It was found that the highest concentration of hydroxyapatite (6000 mg L^{-1}) yielded the best results for all three agronomic variables measured (Table 3).

On the other hand, the measured variables between stem diameter and head diameter showed results where there is no significant difference for stem diameter, but there is for the head diameters of the sunflower crop flowers. The results with higher values are those presented when the soil was treated with vermicompost, and the lowest value for the biofertilizer treatment (Figure 4).

Studies have shown that fertilizers containing hydroxyapatite nanoparticles (HANPs) can offer improved phosphorus nutrient delivery in agricultural contexts, leading to enhanced crop yield and plant biomass production. The enhanced physical and chemical properties of nanoparticles (NPs) hold significant potential for mitigating environmental consequences, such as nutrient loss, associated with conventional fertilizers (Madanayake, Adassooriya, and Salim, 2021). At a concentration of 1000 mg L^{-1} of HANPs, Bala, Dey, Das, Basu, and Nandy (2014) and Liu *et al*. (2015) noted enhanced seed germination and plant growth in chickpeas (*Cicer arietinum L*.), as well as an increased germination percentage in cucumber seeds (*Cucumis sativus L*.). These results refer to the positive outcomes of HANPs on various plant variables, which align with the findings reported in this experiment.

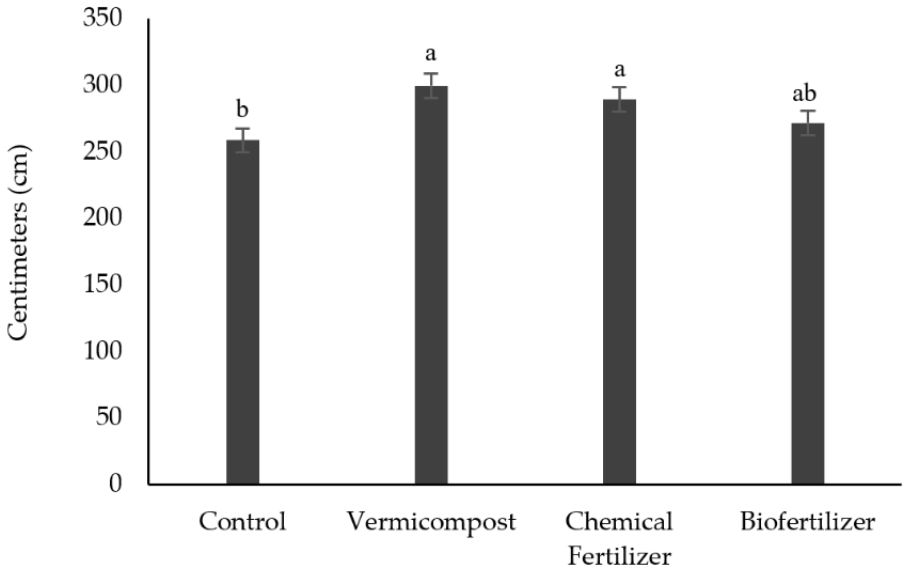

Figure 3. Determination of plant height using different soil treatments. [a-b] Different letters above the bars indicate significant differences according to the Tukey test with a significance level of $P < 0.05$ (n = 3).

Table 3. Results of the effect on agronomic variables by hydroxyapatite nanoparticle doses applied.

Doses of NPs of hydroxyapatite	Height	Stem diameter	Head diameter
mg L^{-1}	m	- - - - - - - - - - - - - - - - - cm - - - - - - - - - - - - - - - - -	
2000	2.68±0.10 b	3.34±0.27 b	21.37±1.36 b
4000	2.87±0.07 a	3.80±0.22 a	23.53±1.53 a
6000	2.84±0.11 a	3.83±0.31 a	23.93±0.91 a

[a-b] Different letters in each column indicate significant differences according to the Tukey test ± standard deviation with a significance level of $P < 0.05$.

Maghsoodi, Ghodszad, and Lajayer (2020) investigated the impact of HANPs at low concentrations (5 g kg^{-1}) in soybean plants; they were cultivated in a greenhouse using an inert medium composed of 50% perlite and 50% moss peat over 12 weeks. The phosphorus concentration in both the nHAP treatments and the phosphorus fertilizer was maintained at 28.1 mg L^{-1}. The findings revealed that applying nanoparticles enhanced plant growth and grain yield by 33% and 20%, respectively, compared to using conventional phosphorus fertilizer ($Ca(H_2PO_4)_2$). Additionally, biomass production increased by 18% in the shoot and 41% in the root. The tallest plants were observed in the HANPs treatment group, with an average height of 121 cm, approximately 30% taller than those receiving conventional phosphorus fertilizer (monocalcium phosphate).

According to the results obtained in this study, for the TPC and TF variables, the dose of 4000 mg L^{-1} of HANPs showed the best results in the different sunflower oils, but not for the TEAC variable, where the results showed that the effect of the highest concentration dose of nanoparticles (6000 mg L^{-1}) had the best response in sunflower oil (Table 4).

The results reported in this research for the different soil treatments yielded data ranging from 17.9 to 35.4 mg GAE mL^{-1} for TPC and 29.8 to 60.7 mg QE mL^{-1} for TF, where the lowest concentration data corresponded to the chemical fertilizer treatment, and the highest values were reported in biofertilizer treatment (Figure 5).

Regarding the antioxidant activity obtained, data ranging from 18024.60 to 25159.52 µM TE mL^{-1} were reported. These results were for the control treatment and vermicompost, respectively (Figure 6).

Nanoparticles show promise as a new abiotic elicitor for stimulating the production of bioactive compounds in plant cells and tissue (Hatami *et al*., 2019). In recent years, numerous studies have explored the use of nanoparticles as elicitors to induce the expression of genes responsible for secondary metabolite biosynthesis (Hu *et al*., 2020). Although further research is needed to understand the mechanism fully, some authors suggest that nanoparticles trigger the production of reactive oxygen species (ROS) and secondary signaling molecules, resulting in transcriptional regulation in plant secondary metabolism (Rivero-Montejo, Vargas, and Torres, 2021).

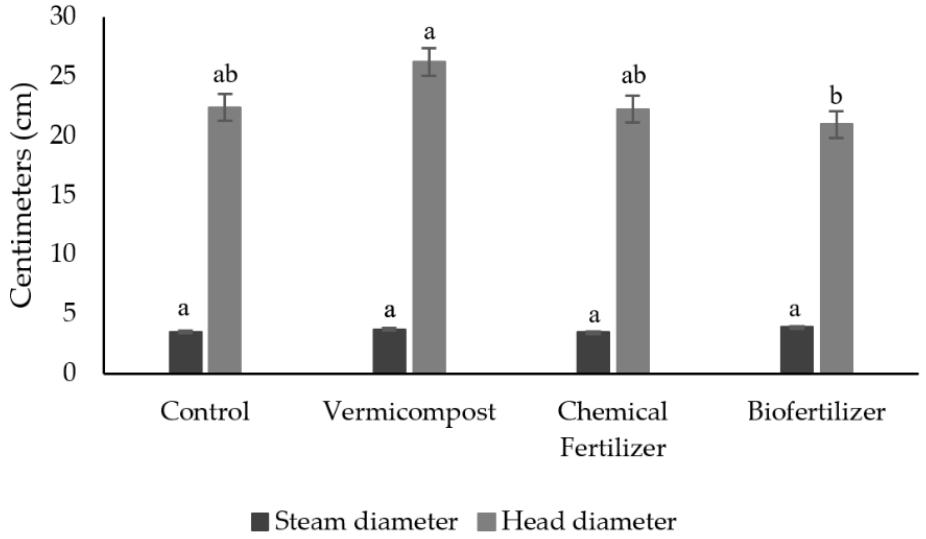

Figure 4. Determination of plant stem diameter and head diameter using different soil treatments. [a-b] Different letters above the bars indicate significant differences according to the Tukey test with a significance level of $P < 0.05$ (n = 3).

Table 4. Effect of hydroxyapatite nanoparticles on phytochemical variables.

Doses of NPs of hydroxyapatite	TPC	TF	TEAC
mg L^{-1}	mg GAE mL-1	mg QE mL-1	µM TE mL-1
2000	18.16±2.3 b	35.86±4.39 c	22945.23±570.74 b
4000	30.57±2.15 a	67.31±4.38 a	20344.04±832.86 c
6000	18.75±3.23 b	38.09±5.41 b	23329.16±429.61 a

[a-b] Different letters in each column indicate significant differences according to the Tukey test ± standard deviation with a significance level of $P < 0.05$. TPC = total phenolic concentration; TF = total flavonoid; TEAC = trolox equivalent antioxidant capacity; GAE = gallic acid equivalent; QE = quercetin equivalent; TE = trolox equivalent.

Diverse secondary metabolites are increased in plants when they are under the effect of NPs. Polyphenols have garnered significant interest from both scientists and consumers lately, owing to their potential medicinal attributes in preventing and treating various degenerative diseases, notably cancers, cardiovascular ailments, and neurodegenerative disorders (Tsao, 2010). Besides their recognized biological functions like antioxidants and anticancer properties, silver nanoparticles (AgNPs) also exhibit antimicrobial and antifungal activities, the latter showing promise in agriculture. Nanoparticles of silver have been recognized as novel and efficient elicitors in plant biotechnology that enhance the production of bioactive compounds (Rezaei, Ghanati, Behmanesh, and Mokhtari, 2011).

In a study conducted by Sabry *et al*. (2023), results were obtained when their treatments were developed with nanoparticles of hydroxyapatite. They evaluated components present in the essential oil of parsley plants, which showed differences among the various doses used for the treatments in that research. They obtained values with higher activity of bioactive compounds when the dose of the nanoparticle was 0.5 g L^{-1}. These researchers concur with the results reported in this experiment. The results reported in the different treatments under doses of hydroxyapatite nanoparticles indicate that there is a positive effect on the concentration of secondary metabolites.

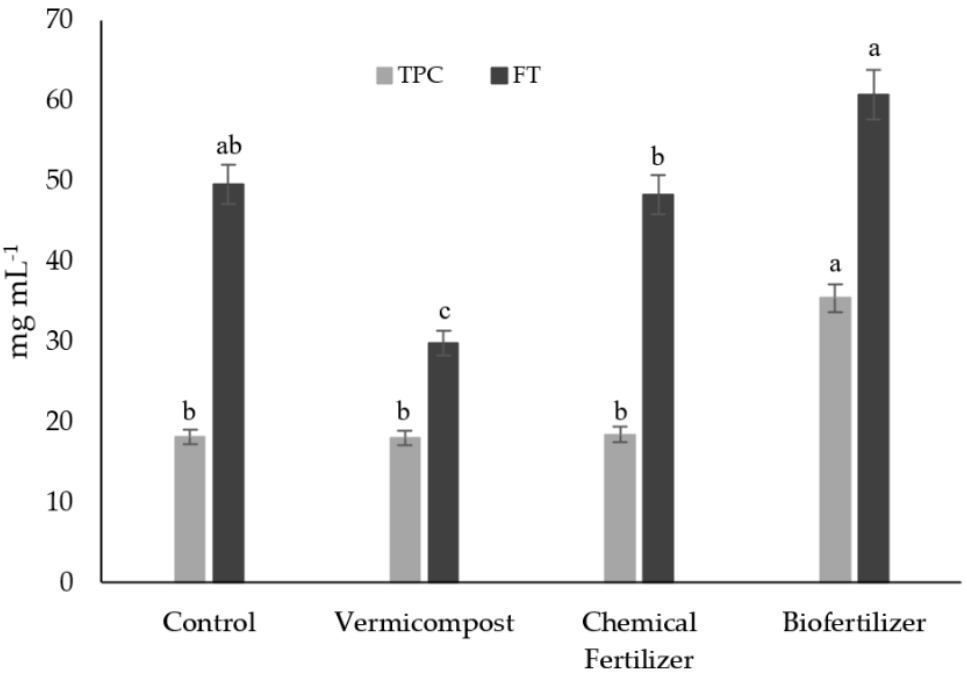

Figure 5. Determination of total phenolic compounds and total flavonoids in sunflower oil for different treatments in soil. [a-c] Different letters above the bars indicate significant differences according to the Tukey test with a significance level of $P < 0.05$ (n = 3).

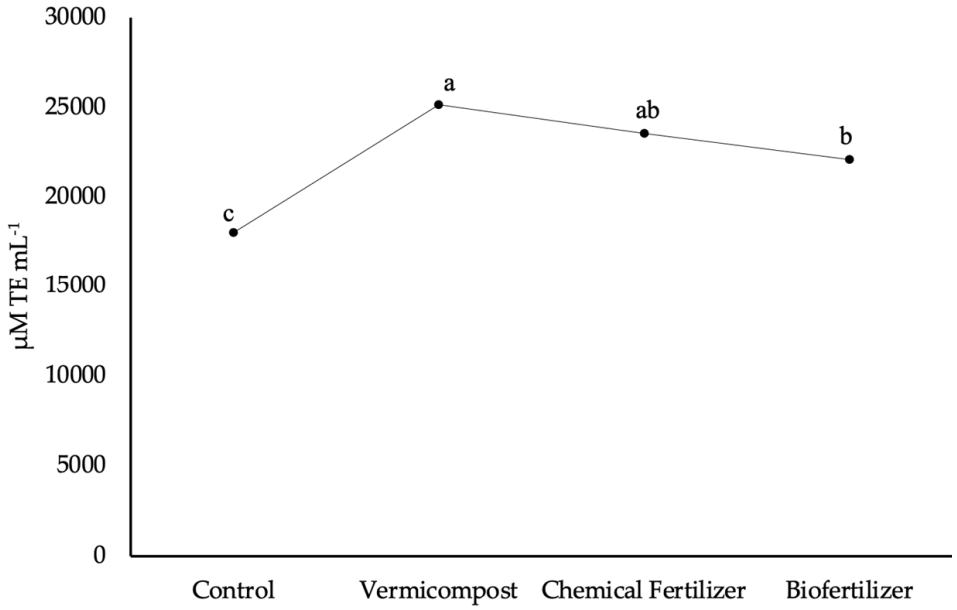

Figure 6. Trolox equivalent antioxidant capacity in sunflower oil for different treatments in soil. [a-c] Different letters above the bars indicate significant differences according to the Tukey test with a significance level of $P < 0.05$ (n = 3).

CONCLUSION

We conclude that hydroxyapatite nanoparticles do influence the concentration of secondary metabolites in Sunflower. Based on the physicochemical characteristics obtained for the different sunflower oils, significant differences were detected among the fertilization treatments and the application of hydroxyapatite; however, no significant differences in the agronomic variables measured were observed. It was demonstrated that the use of biofertilizer is the treatment that shows the best results for the variables of total phenolic compounds and total flavonoids, which are fundamental components of total antioxidant activity. As for the dose of nanoparticles used in the cultivation, it was demonstrated that 4000 mg L^{-1} is the appropriate concentration to obtain better results in the concentration of phenolic compounds, total flavonoids, and total antioxidant capacity. Therefore, a favorable impact was found in the addition of hydroxyapatite nanoparticles on sunflower oil with the support of soil biofertilizer.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FINANCING

Not Applicable.

AUTHORS' CONTRIBUTIONS

Conceptualization: O.E.G.G., and E.A.F.H. Methodology: M.G.R.A. Software: A.M.R. Validation: M.G.R.A., and E.A.F.H. Formal analysis: O.E.G.G. Investigation: O.E.G.G., and R.L.S. Resources: E.A.F.H., and O.E.G.G. Data curation: A.M.R. Writing-original draft preparation, M.G.R.A. writing-review and editing, O.E.G.G., and M.G.R.A. Visualization: E.A.F.H. Supervision: R.L.S. Project administration: O.E.G.G., and R.L.S. Funding acquisition: E.A.F.H.

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