TERRA LATINOAMERICANA

Impact of Hydroxyapatite Nanoparticles as a Biostimulant in Strawberry Crop
Impacto de las nanopartículas de hidroxiapatita como bioestimulante en el cultivo de fresa

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SUMMARY

Biostimulation is the process by which plants respond and activate components of secondary metabolism, allowing the accumulation of biologically active substances in fruits and thereby improving their organoleptic and nutritional quality. In this research, the biostimulant effect of hydroxyapatite nanoparticles (nHAp) on strawberry crops was evaluated. The treatments included foliar application of increasing doses of nHAp; 500, 1000, 1500, 2000, and 2500 mg L^{-1}, and a control (deionized water). Foliar application of nHAp on strawberries significantly improved yield, commercial quality, and content of bioactive compounds in the fruit. The dose of 500 mg L^{-1} showed the best yield and fruit firmness, while higher doses increased bioactive compounds, although with a decreasing trend in productivity. These results show the potential of nHAp as an agricultural biostimulant, highlighting the need to determine optimal doses specific to each crop.

Index words: *antioxidants, calcium phosphate, nanotechnology, phytochemical compounds.*

RESUMEN

La bioestimulación es el proceso mediante el cual las plantas responden y activan componentes del metabolismo secundario, lo que permite la acumulación de sustancias biológicamente activas en los frutos mejorando asi su calidad organoleptica y nutricional. En esta investigación se evaluó el efecto bioestimulante de nanopartículas de hidroxiapatita (nHAp) en cultivo de fresa. Los tratamientos consistieron en la aplicación foliar de dosis crecientes de nHAp; 500, 1000, 1500, 2000 y 2500 mg L^{-1}, y un control (agua desionizada). La aplicación foliar de nHAp en fresas mejora significativamente el rendimiento, calidad comercial y contenido de compuestos bioactivos en los frutos. La dosis de 500 mg L^{-1} mostró el mejor rendimiento y firmeza de los frutos, mientras que dosis más altas incrementaron compuestos bioactivos, aunque con una tendencia decreciente en productividad. Estos resultados evidencian el potencial de las nHAp como bioestimulantes agrícolas, destacando la necesidad de determinar dosis óptimas específicas para cada cultivo.

Palabras clave: *antioxidantes, fosfato cálcico, nanotecnología, compuestos fitoquimicos.*

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INTRODUCTION

The natural metabolism carried out by plants can be stimulated by applying biostimulants to accumulate and promote bioactive compounds, favoring the neutralization of reactive oxygen species associated with cell damage and aging of plant species (Caballero, Valero, and Guerra, 2022). Oxidative stress, caused by biotic or abiotic agents, is a harmful process, and in response to this, plants need to invest energy in defense mechanisms, which reduces their growth capacity, quality, and yield (Magnabosco, Masi, Shukla, Bansal, and Carletti, 2023).

Nanotechnology in sustainable agriculture is a promising alternative to enhance the efficiency of agricultural inputs (Yadav, 2021). Hydroxyapatite nanoparticles (nHAp) $Ca_{10}(PO_4)6(OH)_2$, stand out among the various nanomaterials because they can act as nanofertilizers or nanobiostimulants, without negative effects on the environment (Rocha-Santillan *et al*., 2024). Recent studies support the benefits of their use by reporting increases in seed germination, fresh biomass, bioactive compounds, and enzymatic activity in various crops (Maghsoodi, Najafi, Reyhanitabar, and Oustan, 2020; López-Martínez *et al*., 2023; Azpeitia-Castillo, 2022[1]). These findings confirm its usefulness in promoting higher yields and quality in crops sustainably (Rana, Kour, Yadav, and Yadav, 2020).

On the other hand, strawberry cultivation holds great socioeconomic relevance in Mexico, as it represents 14.83% of global exports (Cossio and Flores, 2021). For this reason, the purpose is to increase its production and improve the development of this crop. In addition to its commercial relevance, strawberries are valued for containing secondary metabolites, vitamins, minerals, carotenoids, ascorbic acid, phenolic compounds, and other antioxidants that benefit health (Martínez-Pérez, Ruíz, and Jacobo, 2023). The present study aimed to evaluate the biostimulant effects of nHAp on yield and nutraceutical quality in strawberry fruits.

MATERIALS AND METHODS

Site and Experimental Conditions

This research was conducted in a greenhouse within the facilities of the Instituto Tecnológico de Torreón, Mexico (25° 36’ 36.54” N and 103° 22’ 32.28” W, with an altitude of 1123 m. Inside the greenhouse, the daytime temperature averaged 29.5 °C, while the nighttime temperature was 18.4 °C. Strawberry plants (*Fragaria* x *ananassa* Duch.) were transplanted in a previously disinfected sand/perlite (80/20, v/v) based substrate, in 10 L black polyethylene bags; plastic bags were placed as pots in double rows with a density of nine plants per m^2, establishing one plant per pot. Irrigation was given with Steiner nutrient solution based on the water requirements of the crop, maintaining pH 5.5 and EC 2.0 deciSiemens per meter.

Treatments and Experimental Design

The nHAp was donated by the Centro de Investigación en Química Aplicada de la Ciudad de Saltillo, Coahuila, México. Particle size was 15 to 25 nm, with predominantly rod-shaped morphology (Flores-Hernández *et al*., 2021). The treatments consisted of the foliar application of five increasing doses of nHAp: 500, 1000, 1500, 2000, and 2500 mg L^{-1} and a control treatment (deionized water). For their preparation, an initial concentration of 2000 mg L^{-1} was used with the support of a Tianjin Autoscience sonicator (AS2060 Instrumental Factory®), sterile deionized water in suspension for 30 minutes. Each treatment was replicated six times, resulting in a total of 36 experimental units. The doses were foliar applied between 8 and 10 h using manual sprinklers, every 15 days after transplanting, until the end of the crop cycle. The research was established with a completely randomized experimental design.

Yield and Fruit Quality

Yield per plant was recorded by weighing the fruit of each plant on an analytical balance (VE-CB2000, Velab). Harvesting began 59 days after transplanting, when the fruit presented a maturity index level of five and six according to NMX-Ff-062-SCFI-2002 (2002). To determine fruit size, the polar diameter from calyx to apex and equatorial diameter at the middle of the fruit were measured using a digital vernier (ASK-500-196-30; Mitutoyo), total soluble solids (°Brix) were determined with a manual refractometer (Atago Master 2311) on fully red fruit. Fruit firmness was determined with an Extech penetrometer (FHT200), using a 5 mm diameter plunger on opposite sides of the fruit, taking an average of two measurements in Newton.

[1] Azpeitia-Castillo, H. M. (2022). E*valuación de calcio, silicio y nanopartículas de hidroxiapatita sobre la calidad de higo (Ficus carica L.) producido en dos densidades, en invernadero.* (Doctoral dissertation). Universidad Autónoma de Nuevo León. Available on http://eprints.uanl.mx/23989/1/1080328562.pdf

Using ethanolic extracts of the samples, total phenols (mg AG equivalents 100 g^{-1} fresh weight (FW), flavonoids (mg QE g^{-1} FW) and antioxidant capacity (µM equiv Trolox 100 g^{-1} FW) were determined, according to the methodology of Molina-Quijada, Medina, González, Robles, and Gámez (2010). 2 g of fresh pulp were mixed in 10 mL of ethanol in a screw-capped plastic tube, which was placed in a rotary shaker (HZ-300) for 6 h, at 5 °C and 20 rpm. Subsequently, the tubes were centrifuged at 3000 rpm for 5 min, and the supernatant was extracted for analytical tests. They were performed per treatment and triplicate each repetition.

Determination of Phytochemical Compounds

Total phenolic content was measured by a modification of the Folin-Ciocalteu method (Singleton, Orthofer, and Lamuela, 1999). Antioxidant capacity was evaluated using the DPPH+ in vitro method, with modifications to the method published by Brand-Williams, Cuvelier y Berset (1995). Total flavonoid content was determined by a colorimetric method (Zhishen, Mengcheng, and Jianming, 1999). Vitamin C concentration was determined by the method of Klein and Perry (1982). The results were expressed as milligrams per 100 grams of fresh weight.

Statistical Analysis

The data of the response variables were analyzed by analysis of variance and comparison of means with Tukey's test ($P \leq 0.05$), using the Statistical Analysis System Institute (SAS) version 9.4 statistical package (SAS Institute, 2013)

RESULTS AND DISCUSSION

Yield and Fruit Quality

The results indicate that foliar application of nHAp in strawberry crops significantly improves yield and commercial quality. Plants treated with 500 mg L^{-1} showed the highest yield values (240.6 %) compared to the control treatment. However, as the concentration of nHAp sprayed increases, crop productivity decreases (Figure 1). This improvement in yield is explained by the greater amount of fruit achieved during the crop cycle, followed by the doses of 1000 and 1500 mg L^{-1}. Previous studies have shown that nHAp has a slow and sustained release of P, its small size and large surface area allow a release to specific sites, facilitating its entry into the cells (Fellet, Pilotto, Marchiol, and Braidot, 2021). The increase in yield can be explained by the fact that nHAp in solution releases phosphate ions that can be used in the primary metabolism of plants (Szameitat *et al*., 2021).

These P ions are essential in the formation of ATP, responsible for storing and transferring energy for different metabolic processes such as respiration, photosynthesis, and nutrient transport within plant cells (Bernal and Cabrales, 2022). Similar findings are reported in Italian Parsley, where the dose of 500 mg L^{-1} of nHAp achieved the highest yield, tripling that obtained by the control treatment (Sabry *et al*., 2023). As in the present investigation, increasing the dose of nHAp decreases the yield with values similar to the control treatment; an additional advantage of nHAp is the contribution of calcium, stimulating the elongation of plant cells and strengthening the cell walls, which is a desirable process for the postharvest life of the fruits (Farhan, Khairo, Islam, and Ortas, 2021).

The commercial quality of strawberries is determined by the firmness, size, and color of the fruit (Villarroel and Albornoz, 2024), and fruit firmness was improved with the foliar application of nHAp. Firmness is associated with the mechanical resistance of the cell wall and is linked to the physical, chemical, and structural changes that occur during fruit development and ripening (Tian and Xu, 2023). In this study, the foliar application of nHAp improved the firmness and size of strawberries compared to the control treatment (Table 1). This improvement in commercial quality is attributed to Ca, which acts by stabilizing and enhancing the integrity of the cell membrane, thereby conferring greater firmness to the fruit (Gamboa-Angulo *et al*., 2020).

Total soluble solids increased by 35.88% with a dose of 2000 mg L^{-1} compared to the control (Table 1). The fruits of plants sprayed with nHAp exhibited greater sweetness, which is attributed to biostimulation by nHAp, due to the active participation of phosphorus (P) in the photosynthesis process, where sugar levels are transported to the fruit (Alvarado-Cepeda, Mendoza, Sandoval, Vega y Franco, 2020). By increasing the dose of P, the plant improves its photosynthetic process, optimizing sugar production (Partida, Díaz, Cortegaza, Zazueta, and Flores, 2022). However, a negative trend in yield and commercial quality is observed with increasing doses of nHAp, which, according to Guo, Liu, Chen, Zhu, and Zhang (2022), may be because the effect of nanoparticles (NPs) is different according to the species cultivated. The phenomenon of hormesis can occur, behaving favorably at lower doses and causing adverse effects at high doses (López-Martínez *et al*., 2023), which reinforces the need to determine the optimum dose of NPs for each type of crop.

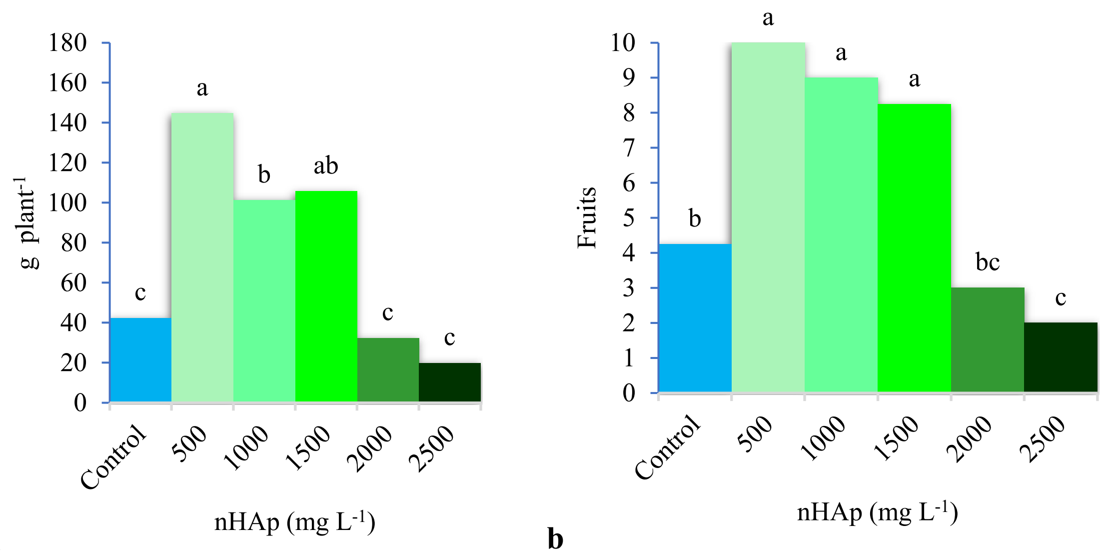

Figure 1. Effect of nHAp on yield (a), fruits per plant (b). Means with different letters differ statistically from each other (Tukey *P* ≤0.05).

Bioactive Compounds

Fruits rich in phenolic compounds, flavonoids, and vitamin C are essential for human health due to their antioxidant properties, which protect cells against damage caused by oxidative stress and can help prevent various chronic degenerative diseases (Otero *et al*., 2020). Foliar application of nHAp increased the biosynthesis of bioactive compounds in strawberry fruits; phenolic compounds had an increase of 11.99% with doses of 2500 mg L^{-1}, compared to the control treatment (Figure 2a). On the other hand, flavonoids reached their highest concentration at 1000 mg L^{-1}, exceeding (131.53%) the concentration observed in the control (Figure 2b). The application of high doses of NPs induces abiotic stress (Memari-Tabrizi, Yousefpour, and Babashpour 2021), during the stress, the biosynthesis of flavonoids that are concentrated in the vacuole helps the detoxification of H_2O_2 (Shomali *et al*., 2022), these compounds have antioxidant properties that counteract the excessive production of reactive oxygen species (ROS) in the plant and repair the damage caused by them (Pérez-Hernández *et al*., 2024). The biostimulant effect caused by nHAp on strawberry fruit coincides with previous research on fig cultivation, where nHAp application resulted in a considerable increase in phenolic compounds (Azpeitia-Castillo, 2022[1]). Phenolic compounds are directly related to the improvement of sensory characteristics such as color, aroma, and postharvest flavor of fruits (Villarroel and Albornoz, 2024)

Table 1. Effect of nHAp on polar diameter, equatorial diameter, firmness, and total soluble solids in strawberry fruit.

nHAp	Polar Diameter	Equatorial Diameter	Firmness	Total Soluble Solids
mg L^{-1}	- - - - - - - - - - - - - - - mm	- - - - - - - - - - - - - - -	N	°Brix
Control	29.74±0.99 b*	26.98±0.54	0.16±0.01 b	7.051±0.30 c
500	36.42±3.24 a	30.44±3.4	0.18±0.01ab	9.282±1.11 ab
1000	33.90±4.10 ab	27.01±3.30	0.20±0.02 a	8.745±0.26 ab
1500	33.88±4.02 ab	28.24±3.66	0.20±0.04 a	8.815±0.20 ab
2000	33.99±3.09 ab	27.17±3.73	0.22±0.04 a	9.5825±1.38 a
2500	31.90±2.04 ab	26.35±3.04	0.22±0.04 a	8.2925±0.67

* Values with different letters are significantly different (Tukey $P \leq 0.05$). Mean ± standard deviation (n=20).

The content of non-enzymatic antioxidant compounds has great relevance as the antioxidant capacity, which refers to the capacity of fruits to neutralize free radicals; these molecules can irreparably damage cells. Here lies the importance of including fruits rich in antioxidants in the human diet (Montoya-Vizuete, Castillo, Cajas, and García, 2022). The doses of 500, 1,000, 1500, and 2000 mg L^{-1} were higher (1.8, 1.5, 2.1, and 2.4%) than the control treatment and the dose of 2500 mg L^{-1} (Figure 2c). The increase in antioxidant capacity is directly related to the fact that it is composed of hydrophilic compounds, such as vitamin C, and polyphenols, which encompass the broad group of flavonoids, as well as lipophilic compounds, including carotenoids and vitamin E (Gulcin, 2020). The greater accumulation of vitamin C results in better fruit quality, as it is considered a natural free radical scavenger. The importance of consuming fruits rich in this compound is because plants and animals synthesize ascorbic acid from glucose, and this process is not possible in humans, so it is obtained from the diet (Ovando, Reyes, Cabrera, and Ovalle, 2022). Ascorbic acid in strawberry fruits increased to 150.69% with the dose of 1500 mg L^{-1} in the control treatment (Figure 2d). The mechanism of action of this vitamin protects and inhibits the formation of superoxide radicals during digestion through the release of a hydrogen atom to form a stable and unreactive radical called ascorbyl (Cervantes-Valencia, López, Rojas, Rivera, and Pedraza, 2024). This component is part of the first line of defense for plants, inducing secondary metabolism and participating in the activity of other exogenous antioxidants, such as polyphenols (Zwolak, 2020). This indicates the relationship between the increase of phenolic compounds with the application of high doses of nHAp and the increase of vitamin C. Currently, the consumption of ascorbic acid is used to mitigate diseases, mainly cancer (Rojas-Lemus, Bizarro, González, López, and Rivera, 2022).

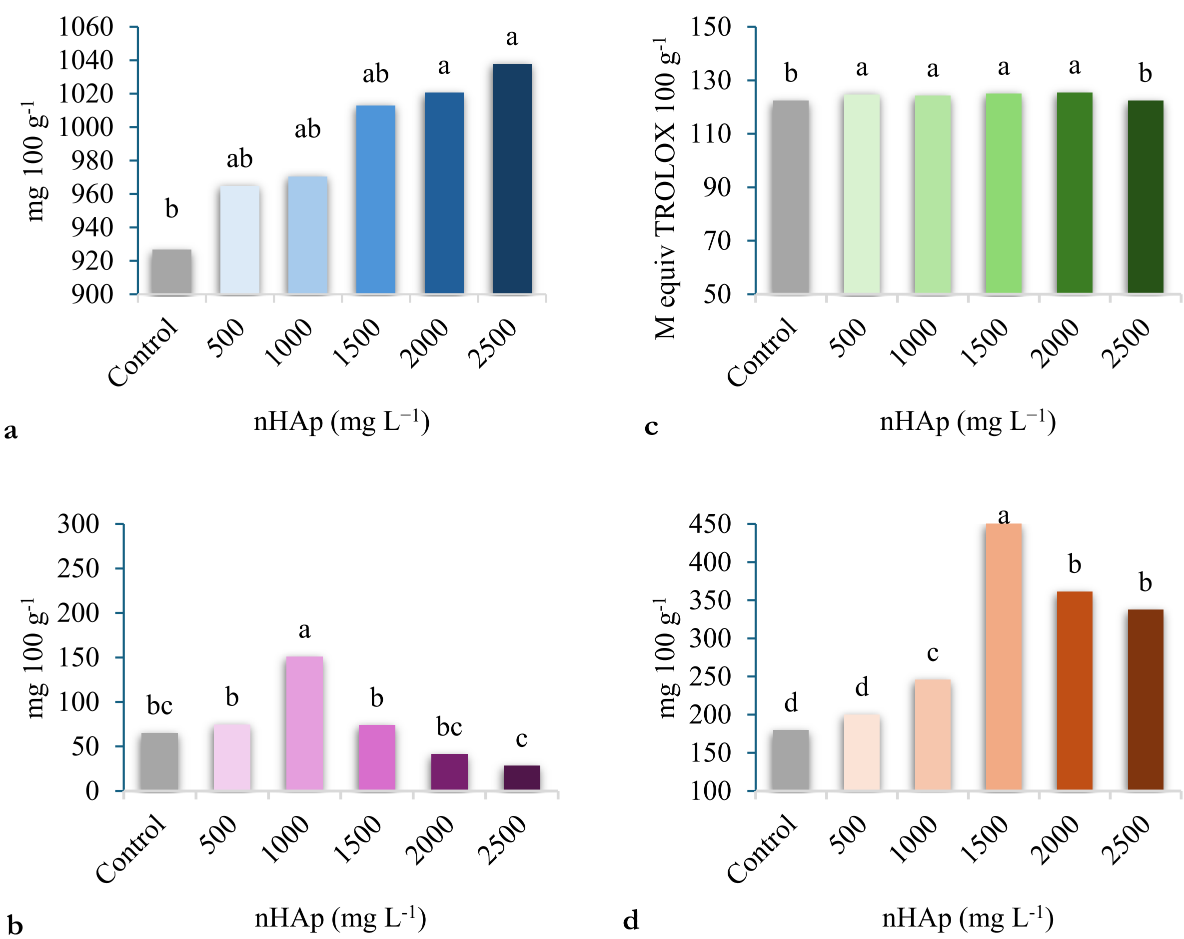

Figure 2. Effect of nHAp on the Content of (a) Phenolic (b) Flavonoids (c) Antioxidant capacity and (d) Vitamin C compounds in strawberry fruits. Means with different letters differ statistically from each other (Tukey, $P \leq 0.05$).

CONCLUSIONS

The use of hydroxyapatite nanoparticles, as a biostimulant, in strawberry cultivation increases strawberry yield and nutraceutical quality. The dose of 500 mg L^{-1} favors yield and its components, while the doses of 1000 and 1500 mg L^{-1} optimize nutraceutical quality in fruits. Hydroxyapatite nanoparticles represent an innovative strategy to increase strawberry yield and nutraceutical quality.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

The data used and analyzed in this study can be sent upon formal request by the interested party to the corresponding author.

COMPETING INTERESTS

The authors declare that there are no conflicts of interest related to this article.

FINANCING

Not applicable.

AUTHORS' CONTRIBUTIONS

Conceptualization: J.J.R.S., and P.P.R. Formal analysis: J.J.R.S., A.M.R., and E.A.F.H. Research: J.J.R.S., M.G.C., and E.A.F.H. Methodology: J.J.R.S., and A.G.T. Project management: P.P.R., A.G.T., M.G.C., and A.M.R. Supervision: P.P.R., A.G.T., E.A.F.H., M.G.C., and A.M.R. Validation: P.P.R., and E.A.F.H. Visualization: P.P.R. Writing - original draft: J.J.R.S., P.P.R., and A.M.R. Writing - review and editing: P.P.R. The authors read and approved the final manuscript.

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