

Biostimulant effect of nanoparticles of hydrogen titanate with chitosan in Frailescano beans

Edwin Giuliani Roblero-Torres¹

Verónica Castro-Velázquez²

Vicente Rodríguez-González²

Magín González-MoscOSO^{1,§}

¹ Departamento de Nanotecnología-Universidad Politécnica de Chiapas. Carretera Tuxtla Gutiérrez-Portillo Ñ Zaragoza km 21+500, Colonia Las Brisas Suchiapa, Chiapas, México. CP. 29150.

² División de Materiales Avanzados-Instituto Potosino de Investigación Científica y Tecnológica. Camino a la Presa de San José 2055, San Luis Potosí, México. CP. 78216.

[§]Autor para correspondencia: mgonzalez@in.upchiapas.edu.mx.

Abstract

The excessive use of fertilizers and agrochemicals in agriculture has caused environmental deterioration. In this context, nanotechnology emerges as a sustainable alternative to modern agriculture. The purpose of this study was to evaluate the biostimulant effect of compounds formed by nanoparticles of hydrogen titanates and chitosan biopolymer, in formulations with 10 and 90%chitosan in the crop of beans (*Phaseolus vulgaris* L.), Frailescano variety, under greenhouse conditions. The research was carried out in a greenhouse of the Polytechnic University of Chiapas in 2024. The experimental design used was randomized complete blocks with five treatments: T1 (control), hydrogen titanate, chitosan, HT10CS (hydrogen titanate-10% chitosan), and HT90CS (hydrogen titanate-90% chitosan). The study revealed that treatments with hydrogen titanate and HT10CS significantly improved crop yield, increasing the number of pods, leaves, and flower buds. Regarding the content of chlorophyll (c α and c β) and β -carotenoids, there was an increase due to the application of nanoparticles. This study demonstrates that using titanate and chitosan nanomaterials has the potential to positively impact the development of bean crops, showing a significant effect on key variables of growth and chlorophyll content. This approach allowed for the development of innovative strategies that improve agricultural productivity and reduce environmental impact.

Keywords:

Phaseolus vulgaris L., chlorophyll, hydrogen titanates, modern agriculture, nanomaterials, plant stimulation.



Introduction

Global warming, together with the increase in population, has encouraged the use of agrochemicals to optimize crop yields and ensure food security in a context of growing global demand (Arora *et al.*, 2022). Chemical fertilizers have been crucial to increasing agricultural production. However, their widespread use has sparked debate about their environmental and human health impacts (Tudi *et al.*, 2021). This is because the chemical residues of these products can contaminate the air, water, and soil (Bernardes *et al.*, 2015).

Beans (*Phaseolus vulgaris* L.) are a legume of great importance worldwide, grown in various regions of the world and various climates and environments (Vázquez-Herrera and Taboada-Gaytán, 2023). They are one of the most important crops because they provide proteins, starch, dietary fiber, B vitamins, and essential minerals (Morales-Santos *et al.*, 2017). Beans are essential in the diet of the Mexican population, and their production is key to food sovereignty. Nevertheless, they face a decrease in production due to multiple factors (Sangerman-Jarquín *et al.*, 2010).

In this context, nanotechnology has emerged as an innovative tool in various sectors, including the food industry and agriculture (Jeyaraman and Eltzov, 2025). Employing nanotechnology has been shown to be a promising strategy for optimizing plant growth and improving crop production (Senthamizh *et al.*, 2025). There is currently a wide range of nanomaterials used in agricultural crops that have various impacts on plants (Khan *et al.*, 2024).

This range includes metallic, non-metallic, carbon-based, and polymeric nanomaterials (Manimegalai *et al.*, 2023). Among the metallic ones, titanium dioxide has attracted attention due to its positive effects on plants, as it improves photosynthetic efficiency by increasing the absorption and utilization of light (Lian *et al.*, 2020). Nanostructured titanium dioxide has been reported to promote plant growth in *Zea mays* L. by enhancing photosynthesis, transpiration, and beneficial soil microorganism activity (Kumari *et al.*, 2024).

On the other hand, polymeric materials, such as chitosan, are widely used in agriculture due to their key properties, such as the degree of deacetylation and their molecular weight, which determine their functionality in different agricultural applications (Castro Velázquez *et al.*, 2025). Therefore, chitosan in nanoform emerges as an alternative to improve agricultural practices in a sustainable way (Wang *et al.*, 2024).

Chitosan has been reported to improve the growth of corn (*Zea mays* L.), tomato (*Solanum lycopersicum*), and potato (*Solanum tuberosum*) crops (Khairy *et al.*, 2022; Hassan *et al.*, 2022); it even stimulates chlorophyll concentration and improves antioxidant activity in plants (Zayed *et al.*, 2017; Abdel-Maksoud *et al.*, 2022). At present, as far as we know, there is no published information on the combined effect of hydrogen titanates and chitosan to induce stimulation in bean plants; this combination could have a biostimulant effect.

This research aimed to evaluate the biostimulant effect of nanoparticles of hydrogen titanates with chitosan and their combinations on the growth, yield, and chlorophyll content in bean plants of the Frailescano variety.

Materials and methods

Description of the study area

The research work was carried out in a greenhouse of the Polytechnic University of Chiapas, located at 16° 36' 56" north latitude and 93° 05' 22" west longitude in the municipality of Suchiapa, Chiapas. At an altitude of 460 m, the average annual temperature was 24–35 °C, the climate was warm sub-humid and annual rainfall ranged between 1 200 and 3 000 mm (INEGI, 2024).

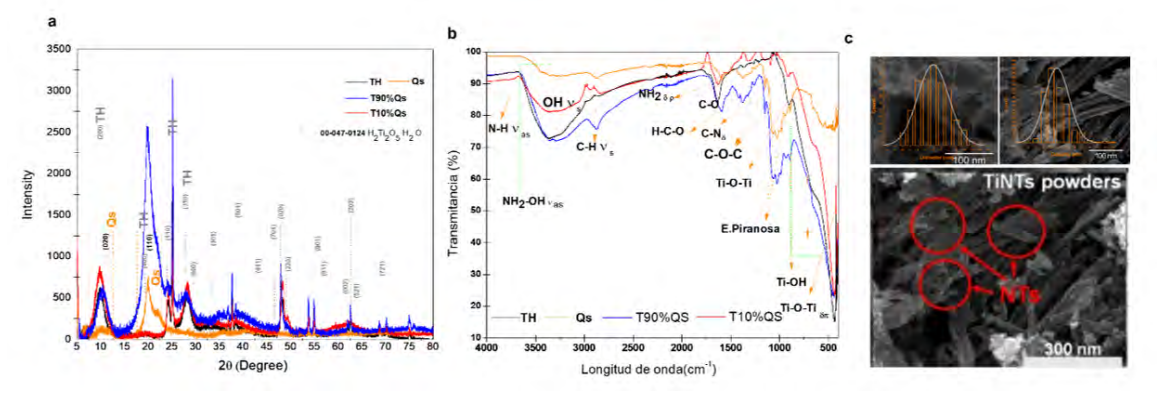
Soil

The soil was collected in the first 30 cm of the arable layer on the ranch of the Aceros family, in the municipality of Suchiapa, Chiapas, located at the geographical coordinates 16° 36' 58.4" west longitude and 93° 06' 07.3" north latitude. The soil, used for agriculture, is grown with crops such as soybeans, corn, beans and others. The agricultural plot is subjected to mechanized tillage using a tractor for soil rotation.

Nanomaterials

The hydrogen titanate and chitosan nanoparticles were synthesized in the Heterogeneous Catalysis Laboratory (Lanocat), for its acronym in Spanish of the Advanced Materials Division (DMAv), for its acronym in Spanish of the Potosino Institute of Scientific and Technological Research AC (IPICYT, for its acronym in Spanish). These were synthesized using the microwave-assisted hydrothermal method (Liu *et al.*, 2011). The sizes of the nanoparticles ranged from 6 to 26 nm (Figure 1).

Figure 1. Characterization of treatments using the following techniques: a) X-ray diffraction (XRD); b) Fourier-transform infrared spectroscopy (FTIR) and c) scanning electron microscopy (SEM) (Castillo *et al.*, 2023; Castro *et al.*, 2025).



Experiment establishment

The experiment was set up with a randomized block design. Five treatments were included, each with 18 experimental units (one plant as an experimental unit), resulting in a total of 90 experimental units. The treatments were T1: control, T2: hydrogen titanates (TH), T3: chitosan (CS), T4: hydrogen titanates/chitosan 10% CS (T10% CS), T5: hydrogen titanates/chitosan 90% CS (T90% CS). A total of 200 mg of nanomaterial was used for each treatment.

Polyethylene bags with a capacity of 3 kg were used and filled with previously sampled agricultural soil. Seed beans of the Frailescano variety, collected in the Fraileasca region, were used. Sowing was done in the bags with moistened soil at a depth of 3–5 cm, using four seeds per bag. After germination, the most vigorous seedling was selected, and the rest were removed.

Fertilization with the nanomaterials was applied via drench (directly to the soil), dosing individually in each experimental unit with a frequency of 15 days after germination: 5 ml plant⁻¹, 15 days after the first application: 10 ml plant⁻¹, 15 days after the second application: 15 ml plant⁻¹ and finally, 15 days after the third application: 20 ml plant⁻¹.

Variables evaluated

Agronomic variables were controlled to monitor growth and determine the interaction of the nanomaterial with the plant and its effects on its growth. The agronomic variables that were evaluated in this work were plant height, stem diameter, number of leaves, flower buds, fresh root biomass, fresh and dry aboveground biomass, root length, and number of pods.

Determination of chlorophylls and β -carotenoids

A sampling of plant material was carried out per experimental unit of each treatment. The leaves were collected during the flowering period of the bean plant and stored in properly labeled vacuum bags. These were stored in refrigeration at -2°C until the extraction was carried out. The chlorophyll of the leaves was determined using the technique described by Senthilkumar *et al.* (2021) with modifications to standardize to 10 ml. Using 100 g of fresh tissue, an 80% acetone solution was prepared, adding 20 ml of distilled water to 80 ml of acetone.

The mixture was then crushed in a mortar to a homogeneous consistency and transferred to a Falcon tube for centrifugation on a Frontier™, 5000 Series, model FC5707+R05, at 3 000 RPM for 5 min. The supernatant was extracted with a micropipette and stored in a 10 ml volumetric flask. The sediment was washed with 5 ml of 80% acetone and subjected to centrifugation again. Finally, the samples were calibrated with 80% acetone and analyzed in an Agilent Technologies Cary 5000 Series UV-Vis spectrophotometer, measuring absorbance at 643, 645, and 470 nm.

The values obtained were processed in Excel to determine the concentration of chlorophyll α , chlorophyll β , and β -carotenoids expressed in mg 100 g of fresh weight, and the calculations were made using the following equations: Chlorophyll (C α) ($\mu\text{g ml}^{-1}$) = $[(12.25 A_{663} - 2.79 A_{645}) (1\ 000\ v)] / [1\ 000w] \cdot 1$. Chlorophyll β (C β) ($\mu\text{g ml}^{-1}$) = $[(21.5 A_{645} - 5.10 A_{663}) (1\ 000\ v)] / [1\ 000w] \cdot 2$. Carotenoids (β) c(x+c) ($\mu\text{g ml}^{-1}$) = $[(1\ 000 A_{470} - 1.82\ ca - 85.02\ cb) / (198)] (1\ 000\ v) / [1\ 000w] \cdot 3$.

Data analysis

An analysis of variance (Anova) was carried out to determine the differences between treatments, and a mean test was performed according to Fisher's least significant difference ($\alpha = 0.05$). The InfoStat statistical package (v2019) was used to perform the analyses. The design of the figures was made with the SigmaPlot software (V12.0).

Results and discussion

Growth

Plant height, stem diameter and number of leaves showed statistically significant differences due to the application of nanomaterials compared to the control; in addition, a tendency of stimulation was observed in the three response variables due to the application of HT and T10CS (Table 1). The height of the bean plant increased 243.82% when exposed to HT, while the T10CS treatment managed to increase the height of the plant by 99.48%.

Table 1. Effect of nanomaterials on the growth variables of bean plants.

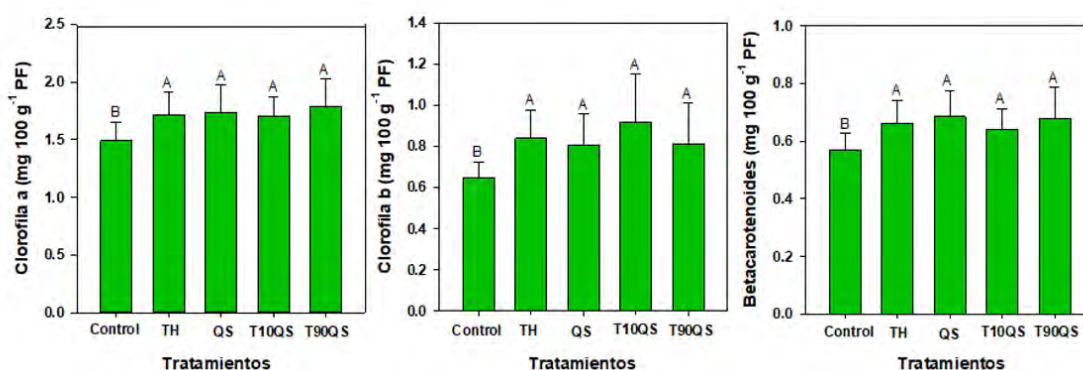
Treatments	Height (cm)	Stem diameter (mm)	Num. of leaves	Root length (cm)
Control	30.83 $\pm$ 8c	3.08 $\pm$ 0.2b	25.33 $\pm$ 7c	34.17 $\pm$ 11.84a
HT	106.17 $\pm$ 20.4a	4.08 $\pm$ 0.2a	47.17 $\pm$ 8.2a	28.33 $\pm$ 11.08a
CS	35 $\pm$ 9.8c	3.58 $\pm$ 0.8ab	27.5 $\pm$ 6.4c	33.67 $\pm$ 5.01a
T10CS	61.5 $\pm$ 25.7b	3.83 $\pm$ 0.7a	41.83 $\pm$ 15a	28.67 $\pm$ 14.26a
T90CS	31 $\pm$ 4.5c	3.42 $\pm$ 0.8ab	32.67 $\pm$ 18.4bc	30 $\pm$ 9.76a
CV (%)	30.05	17.27	34.51	34.37

Means with different letters in the same column are statistically different according to Fisher's least significant difference test ($p \leq 0.05$). $\pm$ = standard deviation; CV = coefficient of variation; HT = hydrogen titanates; CS = chitosan; T10CS = hydrogen titanates/chitosan 10%; T90CS = hydrogen titanates/chitosan 90%.

Concentrations of 100 mg L^{-1} of TiO_2 nanoparticles have been reported to increase the plant height of *Dracocephalum moldavica* significantly (Gohari *et al.*, 2020). Titanium in its nanostructured form increases plant growth because it stimulates the production of chlorophylls and improves the absorption of magnesium, iron and nitrogen (Farahi *et al.*, 2023).

The increased growth of bean plants in this study could be directly related to the higher concentration of chlorophyll, as shown in Figure 2. In addition, the application of titanium in nanoform has stimulative effects that are reflected in plant growth and leaf size due to an improvement in physiological and molecular processes (Trela-Makowej *et al.*, 2024).

Figure 2. Concentration of chlorophylls a and b and β -carotenoids in leaves of bean plants subjected to nanomaterial treatments. The error bars represent the standard deviations, while the different capital letters by bar indicate statistical differences according to Fisher's least significant difference test ($p \leq 0.05$, $n = 6$). PF= fresh weight; TH= hydrogen titanates; QS= chitosan; T10QS= hydrogen titanates/chitosan 10%; T90QS= hydrogen titanates/chitosan 90%.



On the other hand, in this study, chitosan applied alone did not show a significant effect; nonetheless, when combined with 10% hydrogen titanates, there was a biostimulant effect. Chitosan can stimulate plant growth because it provides nutrients and can promote physiological and biochemical processes of plants (Ramírez-Rodríguez *et al.*, 2024).

Regarding stem diameter, plants treated with HT and T10CS showed an increase of 32.46% and 24.35%, respectively, compared to the control. Regarding the number of leaves, the application of HT in bean plants resulted in an 86.22% increase, while the treatment with T10CS achieved a 65.14% increase, evidencing a positive effect of both treatments on leaf development.

The drench application of nanomaterials did not have a statistically significant effect on root length. Titanium in its nanoform with soil and foliar applications has beneficial effects at low concentrations; therefore, it indicates its potential as a growth-promoting agent in agriculture (Machanuru *et al.*, 2024). In root length, there was no impact from the application of nanomaterials; however, plant responses can range from biostimulation to toxicity or zero impact (Benavides-Mendoza *et al.*, 2021).

In the T90CS treatment, the high proportion of chitosan (90%) may explain the slight difference compared to the control. Although chitosan acts as a biostimulant, improving photosynthesis, growth and stress tolerance, its effect tends to stabilize the physiological state under non-stressful conditions, without inducing significant visible changes (Ahmed *et al.*, 2020).

Its action is mainly manifested at the biochemical level, a parameter not evaluated in this work, stimulating defenses such as phytoalexins and antioxidant enzymes, which does not always translate into increases in biomass or height in the short term (Ithape *et al.*, 2024). In addition, the low content of titanates (10%) may not have been enough to enhance the effects of chitosan or generate additional responses. Therefore, the treatment showed a similar response to the control in the measured parameters.

Yield components

The application of nanomaterials did not stimulate the production of aboveground and root dry biomass; nevertheless, a slight increase was observed due to the T10CS treatment, although it was statistically equal to the control (Table 2). The application of HT increased the production of flower buds by 192.71%, and the rest of the treatments were statistically equal to the control. Regarding the number of pods, HT treatment stimulated 84.4% of pod production, whereas the application of T10CS managed to increase this variable by 57.7%.

Table 2. Components of bean plant yield as a function of the application of nanomaterials.

Treatments	Aboveground dry biomass (g)	Dry root biomass (g)	Flower buds	Num. of pods
Control	25.17 ±5.49a	6.67 ±0.52a	4.67 ±2.5b	7.5 ±3.1bc
HT	25.33 ±6.35a	6.17 ±0.75a	13.67 ±7.4a	13.83 ±4.2a
CS	24 ±6.51a	7.83 ±4.02a	5.83 ±1.7b	6.17 ±1.7c
T10CS	29.17 ±10.36a	7 ±1.1 a	8.5 ±4.1b	11.83 ±7.6a
T90CS	21.83 ±4.75a	6 ±0.89a	4.33 ±2.8b	4.83 ±0.98c
CV (%)	27.19	28.49	57.35	49.13

Means with different letters in the same column are statistically different according to Fisher's least significant difference test ($p \leq 0.05$). $\pm$ =standard deviation; CV= coefficient of variation; HT= hydrogen titanates; CS= chitosan; T10CS= hydrogen titanates/chitosan 10%; T90CS= hydrogen titanates/chitosan 90%.

Nanoparticles improve crop productivity because they act as signaling molecules, activating genes, improving defense mechanisms, and improving nutrient uptake (Francis *et al.*, 2024). The increase in growth and yield of a plant exposed to nanomaterials is due to their high surface-to-volume ratio and their unique physical and chemical properties (Jie *et al.*, 2024). As for the increase in flower buds resulting from the application of HT and T10CS, this may be related to the regulation of phytohormones, as nanomaterials can regulate the synthesis of specific hormones through cross-signaling communication (Tripathi *et al.*, 2022).

Photosynthetic pigment concentration

The concentration of chlorophylls (α - β) and β -carotenoids in bean plants exposed to HT and CS nanoparticles and their combination with different percentages showed statistically significant differences compared to the control (Figure 1). Interestingly, all the treatments evaluated increased the concentration of chlorophylls. The chlorophyll # content increased by 19.79% when the plants were treated with T90CS.

As for chlorophyll β , the treatment of T10CS increased it by up to 42.68%. On the other hand, CS managed to increase β -carotenoids by 21.42% in bean plants. Nanomaterials have been reported to stimulate the production of chlorophylls and carotenoids (Li *et al.*, 2024).

In the case of titanium-based nanomaterials, they have shown a positive effect on light absorption due to their photocatalytic properties and thermal conductivity (Ebrahimi *et al.*, 2016); in addition, titanium-based nanomaterials can accelerate the electron transfer process in photoreaction centers, reduce NADPH, and stimulate water photolysis (Xinyi *et al.*, 2024). This light absorption capacity in chlorophyll will generate biostimulant effects on photosynthesis (Medina-Pérez *et al.*, 2018).

Conclusions

The application of HT alone and in combination with 10% CS generated a stimulating effect on crop yield variables. The application of nanomaterials in bean crops of the Frailescano variety had a stimulating effect on the content of photosynthetic pigments. Nanomaterials in agriculture can be a sustainable technology to improve agricultural productivity.

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