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Research Article

Biofortification with nanoparticles and zinc nitrate plus chitosan in green beans: effects on yield and mineral content

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Abstract

Approximately 33% of the world's population is affected by Zinc (Zn) deficiency, making it the fifth leading cause of human disease and mortality. An innovative strategy to this problem in the food diet is biofortification. Therefore, the use of nanotechnology emerges as a possible way to achieve the optimal development of plants in a sustainable and precise way. The objective of the present study was to increase the Zn content in bean plants cv. 'Strike', through the application of nanoparticles versus Zn nitrate plus chitosan. Two sources of Zn were applied via foliar: Zn nanoparticles and Zn nitrate at doses of 0, 25, 50 and 100 ppm with and without chitosan. The results indicate that the application of Zn favours the biofortification process, finding increases for all the treatments used. The treatments that stood out were Zn nitrate plus chitosan at 50 and 100 ppm, which increased the Zn content in fruits by more than 110%. The application of Zn nanoparticles at 25 ppm and Zn nitrate at 50 ppm favoured biomass accumulation and production. Furthermore, the addition of chitosan helped biomass and yield, especially when combined with Zn nitrate. Finally, indicate that a greater number of studies are required regarding the use of nanoparticles and chitosan in horticulture to determine with certainty their effect on the physiology and nutrition of plants.

Keywords: biofortification; bioregulators; nanofertilizers; *Phaseolus vulgaris*; zinc

Introduction

Globally, malnutrition affects more than 2 billion people around the world, causing problems such as heart disease, cancer, stroke, and diabetes. There are currently around 821 million people who suffer from hunger (WHO, 2018; FAO, 2019). Malnutrition has become a serious child health problem, being the cause of death of about 3.1 million children under 5 years of age, which represents 45% of all child deaths annually. It also contributes to increased infectious mortality, neurological disability, physical and mental growth

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retardation; In addition, early malnutrition increases the risk of obesity in adulthood. One of the causes of this problem is the deficiency of vitamins and nutrients such as iron (Fe) and zinc (Zn) (Bouis and Welch, 2010; Kane *et al.*, 2015; Bourke *et al.*, 2016; WHO, 2018).

Zn is an essential nutrient for almost all organisms and plays a key role as a metal cofactor in more than 300 proteins within the human body. In addition, it plays an essential role as a stabilizer and hormone receptor; It also acts as a regulator, being essential for the synthesis of biomolecules such as DNA and binds to nuclear proteins and forms complexes called "Zn fingers" (Caro *et al.*, 2016; Taboada-Lugo, 2017). The requirement for Zn in adults is 11 mg.day^{-1} and it is present in an amount of 2 to 3 g, being the second most abundant micronutrient in the human body. Zn deficiency is very common in countries where the diet is unbalanced, mainly in places with cereal-based diets and low protein intake. Approximately 33% of the world population is affected by Zn deficiency, being the cause of 5% of deaths in children under 5 years of age; while, in developing countries, Zn deficiency is the fifth leading cause of human disease and mortality (Bilski *et al.*, 2012; Singh and Prasad, 2014; Reed *et al.*, 2015).

Currently, an innovative strategy to the problem of micronutrient malnutrition in the diet is "Biofortification". Which has been defined as the process of increasing the bioavailable concentrations of essential elements in edible portions of cultivated plants through agronomic management (fertilization) or genetic improvement (White and Broadley, 2005; Cakmak and Kutman, 2018). The common bean (*Phaseolus vulgaris* L.) is one of the five species that have been considered for biofortification programs because it is the legume with the highest direct consumption in the world, since it is the main food for more than 300 million people in regions of Africa and Latin America. In nutritional terms, beans are the main source of plant-based protein, in addition to their high content of minerals, especially Fe and Zn, and their great contribution of vitamins (Blair, 2013; Petry *et al.*, 2014).

To carry out the process of increasing the concentration of minerals in plant tissues, the exogenous application of these nutrients through fertilization is necessary. An important tool to combat micronutrient deficiencies in crops is foliar fertilization, which has been shown to improve crop yield and quality. However, the application of fertilizers in a foliar way can be limited by the formulation, the source, and the particle size (Fernández *et al.*, 2013; Blasco, 2015). Based on the above, the use of nanotechnology emerges as a possible way to achieve the optimal development of plants in a sustainable and precise way; given their size, plants can absorb them with different dynamics, which presents an additional advantage. However, the excessive use of these fertilizers can cause stress damage to crop, so the study of nanoparticles and their interaction with plants requires further studies to determine the extent of their benefits (Raliya *et al.*, 2017; Sturikova *et al.*, 2018). An alternative to mitigate stress situations caused using this new technology is the use of chitosan, which favors root growth and nutrient absorption, in addition to helping the plant to face stress situations and against pathogen attacks (Pichyangkura and Chadchawan, 2015; Saharan *et al.*, 2016; Deshpande *et al.*, 2017).

In general, there is little information on the use and application of nanoparticles together with organic substances such as chitosan in biofortification works. Based on the above, the objective of this research work was to increase the Zn content in bean plants (biofortification) through the application of nanoparticles versus Zn nitrate plus chitosan, as well as to evaluate the effect of these compounds on the mineral content in fruits of the bean.

Materials and Methods

Crop management

The experiment was carried out in a greenhouse covered with shade mesh located at the CIAD facilities in Cd. Delicias, Chihuahua, Mexico, with an average temperature of 29.4 °C and an average relative humidity of 22.38%. Green bean plants cv. 'Strike' which were transplanted 10 days after emergence in plastic pots with a diameter of 30.5 cm and a volume of 13.4 L, a substrate formed by vermiculite and agricultural perlite was used in a 2:1 ratio. Two plants were placed per pot and watered with the following nutrient solution: 6 mM NH_4NO_3, 1.6 mM K_2HPO_4, 0.3 mM K_2SO_4, 4 mM $CaCl_2$, 1.4 mM $MgSO_4$, 5 µM Fe-EDDHA, 2 µM $MnSO_4$, 0.25 µM $CuSO_4$, 0.3 µM Na_2MoO_4 and 0.5 µM H_3BO_3; which was applied every third day during the first 30 days and daily the following 30 days.

Experimental design

A completely randomized experimental design (DCA) was used with a 2*3*2 factorial arrangement plus an absolute control (control without application) and one with the application of chitosan with four repetitions, being factor A the Zn sources: Zn nitrate(7% N and 17% Zn) of commercial use brand GoZinc® (NZN) and nanoparticles of Zn oxide (ZnO) added with urea to equalize the nitrogen supply; factor B, corresponded to the doses: 0, 25, 50 and 100 ppm and factor C, was the addition of the bioregulator: with commercial use chitosan brand Quitofyt® (Poly-D-glucosamine) at a dose of 50 ppm and without the addition thereof; for a total of 14 treatments (Table 1) with four repetitions. The treatments were applied via foliar four times every 10 days from the appearance of the first true leaves.

Table 1. Description of the zinc plus chitosan treatments

Source of Zn	Dose (ppm)	Chitosan	Abbreviation
Control	0	No	Control
Control	0	Yes	Control + Q
ZnO	25	No	ZnO25
ZnO	50	No	ZnO50
ZnO	100	No	ZnO100
ZnO	25	Yes	ZnO25+Q
ZnO	50	Yes	ZnO50+Q
ZnO	100	Yes	ZnO100+Q
NZN	25	No	NZN25
NZN	50	No	NZN50
NZN	100	No	NZN100
NZN	25	Yes	NZN25+Q
NZN	50	Yes	NZN50+Q
NZN	100	Yes	NZN100+Q

Characterization of nanoparticles

Zinc oxide (ZnO) nanoparticles produced by wet chemistry methodology were used in the form of Wurzite crystals, with a purity of 99.7% and an average size of 50 nm free of contaminants (Figure 1). The morphology of the sample was obtained by scanning and transmission electron microscopy (Figure 2). The material was provided by the company "Investigación y Desarrollo de Nanomateriales S.A. de C.V", located in San Luis Potosí, Mexico.

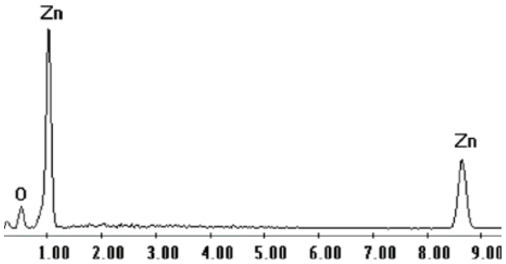

Figure 1. Elemental analysis Elemental analysis (chemical composition) of zinc oxide (ZnO) nanoparticles by energy dispersive X-rays (EDX)

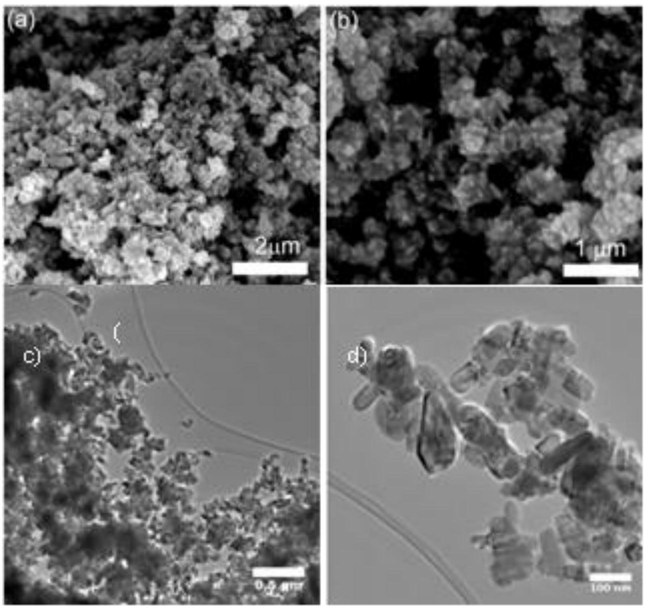

Figure 2. (a, b) ZnO morphology by Scanning Electron Microcopy (SEM); (c, d) ZnO morphology by Transmission Electron Microscopy (TEM)

Plant sampling

Once the plants reached maturity (60 days after sowing), the samples were taken and separated into 4 parts: root, stem, leaf, and fruit and washed 3 times with distilled water and a non-ionic detergent at 1%. The samples were dried in an oven at 70 °C for 48 h for biomass, mineral content, and protein analysis.

Variables evaluated

Biomass

The dry weight per plant was obtained with the help of an analytical balance (AND HR-120, San José, California, USA). The results were expressed in grams per plant on a dry weight basis.

Yield

The weight of the total fruits per plant was determined based on fresh matter and expressed in grams per plant.

Nitrogen (N), sulphur (S) and protein content

The content of N, S and protein was determined using a Flash 2000 unit (Thermo Scientific, Waltham, MA, USA), using the methodology proposed by Calvo *et al.* (2008). The results were expressed as a percentage for the three variables.

Mineral content

The mineral content was determined using the method proposed by Wolf (1982). In which, one gram of dry sample was weighed and 25 mL of triacid mixture (88.9% HNO_3, 8.9% HCl and 2.2% H_2SO_4) were added and placed in a digester oven at 300 °C. The resulting sample was made up to 50 ml with distilled water (main sample). The reading of potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), manganese (Mn), copper (Cu) and nickel (Ni) was performed using an atomic absorption apparatus (AAS, iCE 3000 Series, Thermo Scientific, Waltham, MA, USA.). In the case of K, Ca, and Mg, a 1:100 dilution was made for reading.

For its part, the reading of phosphorus (P) was carried out following the ammonium metavanadate method (NH_4VO_3). 0.5 mL of the main sample was taken in a test tube to which 1 mL of phosphorus reagent plus 3.5 mL of distilled water was added, stirred, and left to stand for one hour for later reading. The reading was made in a spectrophotometer (Genesis 10s UV/Vis, Thermo Scientific, Waltham, MA, USA.) at 430nm against a K_2HPO_4 standard curve. The concentration of P was expressed as a percentage.

Degree of biofortification and distribution pattern

The degree of biofortification was expressed as a percentage and was determined according to the following formula:

$$GB = \left(\frac{ZnF * 100}{ZnC}\right) - 100$$

Where:

GB: degree of biofortification

ZnF: Zn content in the fruit of plants with foliar application of Zn

ZnC: Zn content in the fruit of the control treatment

While the distribution pattern consisted of the fraction of Zn that each organ of the plant contained based on the total Zn of the same and was expressed as a percentage.

Statistical analysis

An analysis of variance, a mean separation test using the LSD method, and a Pearson correlation analysis using the SAS statistical package (SAS, 2004) were performed on the data obtained.

Results and Discussion

Biomass

The food industry seeks sustainable solutions in modern agriculture for biomass production, due to the excess resources it consumes to maintain the required food production (Joshi *et al.*, 2018). In the present investigation, no changes were found regarding the Zn source used (Figure 3), placing nanoparticles as a viable alternative for biomass production, compared to a widely used traditional fertilizer. In addition, a better performance was observed when the doses of nanoparticles were lower, with ZnO25 being the treatment that presented the highest value with an increase of 29.67% compared to the control without application. Various authors report similar effects when ZnO was used in a foliar manner as a Zn source. Garcia-Lopez *et al.* (2019) applied ZnO nanoparticles in habanero pepper plants and obtained an increase in biomass of 10.21% compared to their control with their lowest dose and when their dose doubled, biomass was reduced. For their part, Elizabath *et al.* (2017) found greater growth in carrot plants when they applied 50 ppm of ZnO and when the dose was increased, this growth was reduced. Previous studies mention that Zn excess may cause stress in plants, affecting their development and yield (Sturikova *et al.*, 2018).

In relation to the addition of chitosan, positive effects were found with respect to the treatments without its application for the doses of 50 and 100 ppm of ZnO and for the doses of 25 and 50 ppm of NZN, the last two being the only ones that passed of 26 g per plant of dry weight. Previously, Salimi *et al.* (2019), reported that by combining chitosan with Zn and applying it foliarly, increases in the biomass of tomato plants were obtained. Furthermore, foliar applications of chitosan improved vegetative growth under stress conditions in bean plants (Abu-Muriefah, 2013). The increase in dry matter production can be related to studies where the stimulating action of chitosan against oxygen free radicals was demonstrated, mitigating possible stress situations generating greater vegetative growth (Pichyangkura and Chadchawan, 2015).

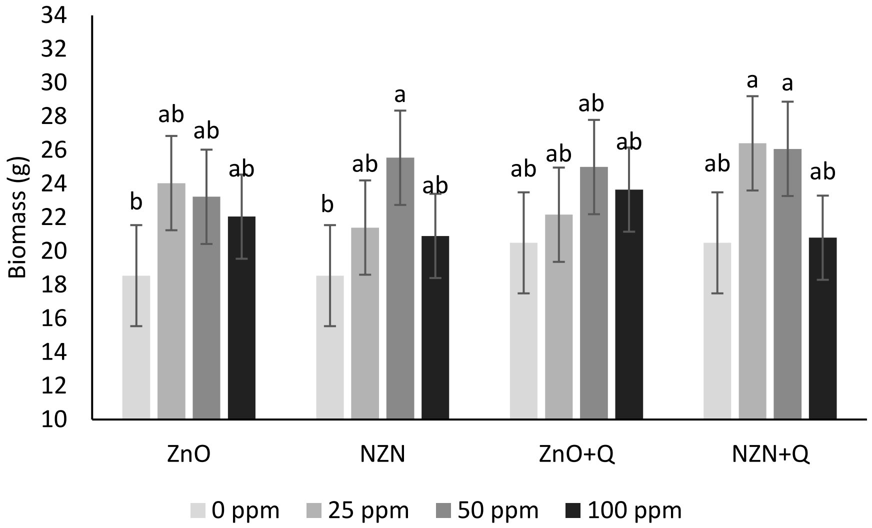

Figure 3. Effect of the application of nanoparticles and Zn nitrate in combination with chitosan on the biomass (dry weight) of bean plants cv. 'Strike'

Different letters indicate significant differences. Vertical bars indicate ±S.D.

Yield

Studies related to the application of nanoparticles and their effects on plants are of great importance to determine the exact amount of nutrients that should be applied and standardize the productivity and growth of crops (Feregrino-Pérez *et al.*, 2018). The production results showed significant differences (Figure 4), standing out the treatments of ZnO25, NZN50, NZN25+Q and NZN50+Q, which had increases of 18, 19.25, 30.75 and 29.5 g, respectively in relation to the control. In turn, these 4 treatments exceeded the average of 78 g per green bean plant reported by Salinas-Ramírez *et al.* (2012). In contrast, the source factor did not obtain significant differences, however, the ZnO25 treatment obtained the same performance as NZN50. These results support the theory that the application of nanofertilizers can reduce the amount of conventional fertilizer to be used, without affecting crop production (El-Ramady *et al.*, 2018).

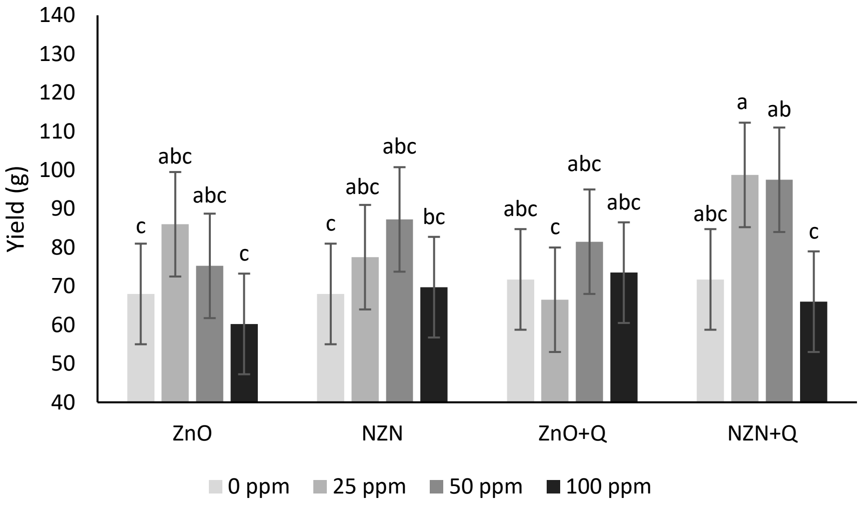

Figure 4. Effect of the application of nanoparticles and Zn nitrate in combination with chitosan on the yield of bean plants cv. 'Strike'

Different letters indicate significant differences. Vertical bars indicate ±S.D.

The foliar application of chitosan did not present significant differences, however, in the treatment of 100 ppm of nanoparticles, which was the one with the lowest production, an increase of 21.99% was observed when chitosan was applied. In the same way, the 25 and 50 ppm NZN treatments increased their production by 27.42 and 11.75%. The results obtained in the present study demonstrate positive effects of the foliar application of chitosan as well as those published by Shehata *et al.* (2012), who obtained increases in the yield of cucumber plants during 2 cycles when applying chitosan foliarly; likewise, Mahmood *et al.* (2017), through the application of chitosan increased the weight, diameter, and number of fruits in chili plants. Several studies show positive effects when applying chitosan in a foliar way in numerous crops, mainly when they are under stress situations (Pichyangkura and Chadchawan, 2015; Malerba and Cerana, 2018).

Zinc content

Zn fertilization has increased in recent years both in soil and foliar, with the objective of increasing production. However, its use as an alternative to increase the content of this nutrient in edible parts remains little studied (Cakmak and Kutman, 2018). The present research work presented significant differences for the Zn content in the different organs of bean plants (Figure 5), because all treatments obtained increases in relation to the control without application. While the source used did not present differences, placing nanoparticles as an equally effective alternative to correct Zn deficiencies; while the dose factor showed a linear trend in Zn content as the dose increased. These results agree with those published by Salama *et al.* (2019), who applied ZnO in increasing doses up to 40 ppm and obtained an increase in the Zn content in bean leaves as the dose increased. In the same way, Akanbi-Gada *et al.* (2019), mentions that when applying Zn nanoparticles in an edaphic way, the same result is obtained, that is, a linear increase in the Zn concentration. However, she mentions that the translocation of Zn is lower in this way, so this may explain why the control treatment did not present a high concentration of Zn in leaves. For their part, Broadley *et al.* (2012), mentions that plants can develop with a concentration of 20 mg.kg^{-1} in mature leaves, presenting symptoms of deficiencies below 15 mg.kg^{-1}, while Mitra (2015), considers a range of 25 to 150 ppm of Zn as the optimal range for the correct development of plants in general.

On the other hand, the application of chitosan did not favour the total content of Zn in relation to its counterparts without application, but it presented a better result when combined with NZN, slightly increasing the content of Zn in fruit, root and stem, only decreasing in leaf, but without affecting the total Zn content (less than 1% difference), which indicates a greater translocation; these results differ from those published by Mirbolook *et al.* (2021), which indicate that chitosan favoured the absorption of Zn when applied to the roots, but the translocation factor was lower than when applying Zn only in the form of Zn sulphate ($ZnSO_4$).

Degree of biofortification and distribution pattern

The agronomic biofortification process can increase the content of a specific nutrient in the fruit of the plants without genetically affecting their composition (Sida-Arreola *et al.*, 2017). In the present study, significant differences were obtained between treatments, finding that the treatments that included Zn applied foliarly increased the Zn content in the green bean (Table 2), in a range of 32.96 to 129.05 % in relation to the control without application. Cambraia *et al.* (2019), indicate that the foliar application of Zn can increase the content in bean grains from 4 to 40%, while in other crops the increases can reach up to 420%; however, there is no previous reference of the increase that the bean in the form of green beans can reach.

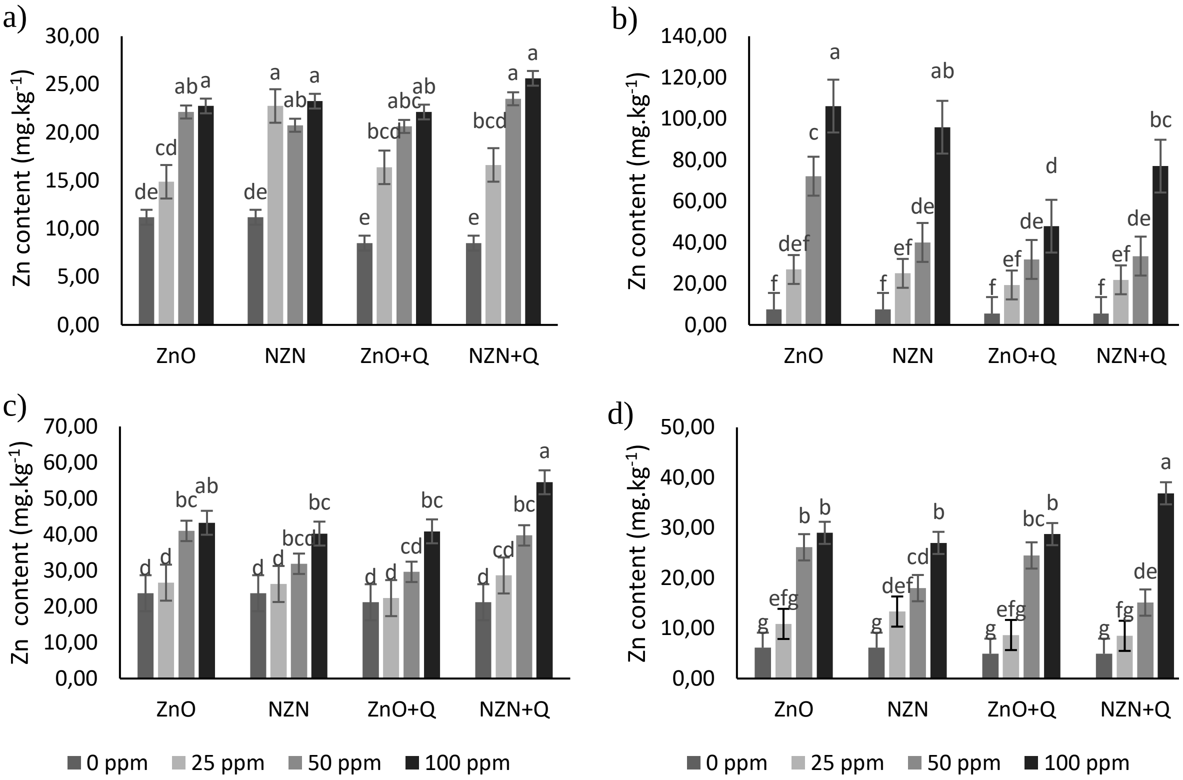

Figure 5. Effect of the application of nanoparticles and Zn nitrate in combination with chitosan on the zinc content in bean plants cv 'Strike'.
a) fruit, b) leaf, c) root, d) stem; Different letters indicate significant differences. Vertical bars indicate ±S.D.

Table 2. Degree of biofortification achieved by the application of nanoparticles and Zn nitrate in combination with chitosan in green bean cv. 'Strike'

Treatment	Degree of biofortification (%)
ZnO25	32.96 c
ZnO50	97.77 ab
ZnO100	103.35 a
NZN25	103.35 a
NZN50	85.47 abc
NZN100	107.82 a
ZnO25+Q	46.37 bc
ZnO50+Q	84.36 abc
ZnO100+Q	97.77 ab
NZN25+Q	48.60 bc
NZN50+Q	110.06 a
NZN100+Q	129.05 a

Different letters indicate significant differences.

For their part, Haider *et al.* (2021), report a range of 11.81% to 106% in various varieties of mung bean with the soil application of Zn. Alternatively, Poblaciones and Rengel (2016), report an increase in Zn content of more than 300% in pea fruits with their foliar application treatment in relation to the control without Zn application. In addition, they report that this increase was reduced when foliar application was combined with a soil application, in agreement with various studies that foliar Zn application may be a more effective way to achieve biofortification than soil application and priming in legume seeds (Jha and Warkentin, 2020). This could be explained by Fernández *et al.* (2013), which indicate that Zn tends to move from the leaves and stems to the fruits when the senescence stage of the plants begins.

Within the treatments, the one that obtained the best result was NZN100+Q, increasing the Zn content in the fruit by 129.05%. However, among the treatments that are in the same range of significance, the NZN50+Q treatment should be highlighted, which increased the Zn content in the fruit by 110.06% in relation to the control and stands out in the biomass and yield variables (Figures 3 and 4). In addition, when comparing it with products found on the market, it had an increase of 21.89% in relation to the values reported by Fernández-Valenciano and Sánchez-Chávez (2017), for green beans obtained in supermarkets in Mexico.

Regarding the distribution pattern, a marked difference is observed due to the foliar application of Zn (Figure 6), generating a higher concentration in the leaf in relation to the control treatments, which had a higher concentration in the root. These results were expected because Zn is considered a medium or conditionally mobile element, for which it is logical to find a higher concentration of Zn in the organs where it was applied, however, various studies indicate that despite its reduced mobility these applications have a significant benefit in the development of the plant (Broadley *et al.*, 2012; Fernández *et al.*, 2013). Concerning the source used, a slight change in the distribution pattern can be observed, especially in the concentration of Zn in the fruit, increasing by 3.84% when NZN was applied compared to the application of ZnO. Fernandez *et al.* (2013), mentions that the chemical form in which a product is applied can influence its foliar absorption, but it is not confirmed that it can influence its mobilization within the plant, so more in-depth studies are necessary to determine whether the chemical source can influence the distribution pattern of nutrients in the plant.

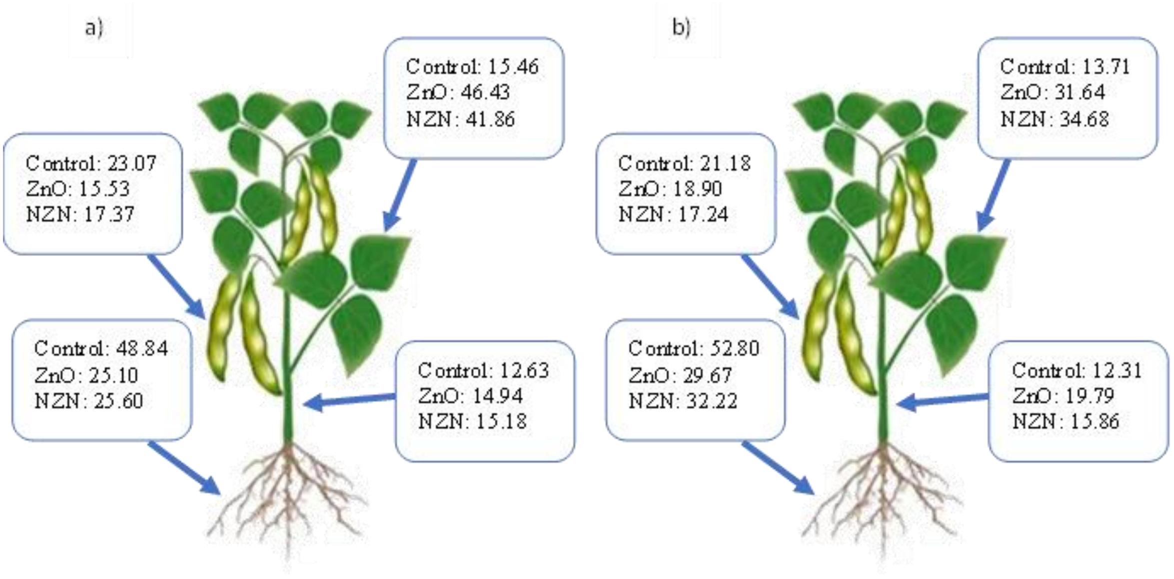

Figure 6. Zn distribution pattern in response to the application of nanoparticles and Zn nitrate in combination with chitosan in bean plants cv.

a) without chitosan. b) with chitosan; The results are expressed in percentage

The application of chitosan, when combined with Zn sources, reduced the leaf percentage for ZnO by 14.79% and 7.18% for NZN, indicating a possible increase in Zn mobility. In addition, in the case of the combination with nanoparticles, increases in the distribution pattern of 3.37 % in fruit, 4.57 % in root and 4.58 % in stem were obtained; results that indicate a possible effect of chitosan on nanoparticles that allows a greater mobility of Zn in the plant. This hypothesis had been reported by Choudhary et al. (2019), whose results suggests that the Zn released from a chitosan matrix applied to maize plants moved from the leaf to the grain during the filling stage, increasing the Zn concentration.

Mineral content

Mineral content is essential for the correct development of plants; N and P are considered the elements that most affect plant growth, however, the content of other nutrients is just as important to achieve a maximum physiological response in plants (Weih *et al.*, 2018). In the present study, significant differences were obtained for the mineral content of the elements analysed except for Cu (Table 3). In the same way as Zn, all the treatments increased the N content in the leaf, obtaining a positive correlation between these two elements (Table 4). These results could be explained due to the nitrogenous contribution included in the treatments; In addition, various studies suggest a beneficial relationship between N and Zn, finding that N deficiency does not allow the ideal absorption of Zn (Hafeez *et al.*, 2013; Montoya *et al.*, 2020). On the other hand, González *et al.* (2019), mention that when the Zn content is sufficient or high, there is a positive effect of N in the biofortification process.

Table 3. Effect of the application of Nanoparticles and Zn nitrate in combination with chitosan on the foliar mineral content in bean plants cv. 'Strike'

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Fe (ppm)	Mn (ppm)	Cu (ppm)
Control	2.01 de	0.35 b	1.73 b	3.79 ab	2.39 abcd	0.12 c	144.31 ab	151.69 fg	18.94 a
Control + Q	1.58 e	0.39 a	2.10 a	2.16 ef	2.42 abc	0.10 c	151.94 a	166.13 def	15.63 a
ZnO25	3.18 bcd	0.19 cd	1.31 d	4.72 a	2.36 bcdef	0.17 c	127.50 bcd	142.88 fg	17.38 a
ZnO50	3.58 abc	0.20 cd	1.32 d	3.05 bcde	2.37 abcde	0.18 c	129.13 bc	156.13 efg	19.50 a
ZnO100	4.77 a	0.20 cd	1.25 d	3.76 abc	2.46 ab	0.32 ab	110.63 def	136.63 g	17.25 a
NZN25	4.58 ab	0.18 d	1.38 cd	1.63 f	2.38 abcde	0.15 c	105.00 f	184.00 cde	18.38 a
NZN50	4.13 abc	0.22 c	1.34 d	1.62 f	2.28 bcdefg	0.14 c	117.13 cdef	199.50 bc	20.38 a
NZN100	4.20 abc	0.19 cd	1.25 d	3.12 bcde	2.47 a	0.15 c	118.75 cdef	184.75 cde	18.63 a
ZnO25+Q	3.37 abcd	0.20 cd	1.50 bcd	3.43 bc	2.23 defg	0.17 c	107.88 ef	200.00 bc	16.63 a
ZnO50+Q	3.56 abc	0.22 c	1.35 d	2.80 bcde	2.18 fg	0.20 bc	130.13 bc	216.63 ab	17.88 a
ZnO100+Q	3.83 abc	0.22 c	1.35 d	3.26 bcd	2.38 abcd	0.35 a	109.63 ef	187.50 cd	15.63 a
NZN25+Q	2.81 cde	0.22 c	1.72 bc	2.76 cde	2.25 cdefg	0.11 c	122.88 cde	228.00 ab	17.38 a
NZN50+Q	3.58 abc	0.21 cd	1.57 bcd	2.26 cde	2.15 g	0.18 c	113.50 cdef	229.50 a	16.25 a
NZN100+Q	3.69 abc	0.22 c	1.50 bcd	3.36 bc	2.20 efg	0.19 c	119.38 cdef	208.25 abc	18.38 a

Different letters indicate significant differences.

Besides, the Zn application presented negative effects on the content of P, K and Fe, which had average decreases of 40, 19.08 and 18.49 %, respectively, in relation to the control without application. Various studies confirm an imbalance between Zn and P in the plant, explaining the negative correlation presented in this work; P being the main limiting factor for the absorption, translocation, and utilization of Zn in the aerial parts of the plant because high levels of P in the cells affect the specific Zn functions (Mousavi, 2011; Hafeez *et al.*, 2013). Similarly, the Zn-Fe and Zn-K ratio can be affected when the nutrients are in solution form and the way they are applied, especially the Fe content, which tends to decrease when the amount of Zn applied is older and vice versa (Hafeez *et al.*, 2013).

The application of chitosan individually presented significant differences (Table 3) for P, K and Ca; obtaining an increase of 11.43 and 21.39 % in the content of P and K respectively in relation to the control treatment. These results agree with those published by El-Miniawy *et al.* (2013), who obtained significant increases in the content of P and K when chitosan was foliarly applied to strawberry leaves; and as in the present work, they obtained slight decreases in the N content without obtaining significant differences. For his part, Vasconcelos (2014), mentions that, although promising results have been obtained with the application of chitosan on the mineral content, especially with metals, the results are still highly variable, so it is necessary to carry out more studies to validate the use of this biocompound in biofortification works or in cases of bioremediation.

Table 4. Pearson correlation analysis for 13 green bean variables

	Biomass	Produc.	N	P	K	Ca	Mg	S	Zn	Fe	Mn	Cu	Protein
Biomass	1	.719**	0.149	-0.16	-0.148	-0.061	-0.034	-0.002	-0.03	-0.096	.347**	-0.216	-0.243
		0	0.272	0.238	0.277	0.654	0.802	0.989	0.825	0.48	0.009	0.111	0.071
Produc.	.719**	1	0.064	-0.018	0.105	-0.128	-0.069	-0.171	-0.243	-0.028	.294*	-0.185	-0.162
	0		0.637	0.895	0.442	0.349	0.611	0.208	0.071	0.839	0.028	0.173	0.233
N	0.149	0.064	1	-.481**	-0.242	-0.03	0.026	.372**	.365**	-.396**	0.006	-0.046	0.12
	0.272	0.637		0	0.073	0.825	0.848	0.005	0.006	0.003	0.966	0.734	0.379
P	-0.16	-0.018	-.481**	1	.706**	-0.056	0.202	-0.174	-.452**	.621**	-0.186	-0.087	-.271*
	0.238	0.895	0		0	0.683	0.135	0.199	0	0	0.171	0.524	0.043
K	-0.148	0.105	-0.242	.706**	1	-0.057	-0.126	-.319*	-.465**	.424**	0.018	-0.164	-0.11
	0.277	0.442	0.073	0		0.675	0.356	0.016	0	0.001	0.895	0.227	0.42
Ca	-0.061	-0.128	-0.03	-0.056	-0.057	1	0.212	0.211	0.135	0.09	-0.261	0.011	-0.175
	0.654	0.349	0.825	0.683	0.675		0.116	0.118	0.32	0.511	0.052	0.938	0.196
Mg	-0.034	-0.069	0.026	0.202	-0.126	0.212	1	0.178	0.188	0.103	-.376**	0.133	0.05
	0.802	0.611	0.848	0.135	0.356	0.116		0.188	0.165	0.45	0.004	0.328	0.713
S	-0.002	-0.171	.372**	-0.174	-.319*	0.211	0.178	1	0.21	-.470**	-0.082	-0.004	0.113
	0.989	0.208	0.005	0.199	0.016	0.118	0.188		0.12	0	0.548	0.977	0.409
Zn	-0.03	-0.243	.365**	-.452**	-.465**	0.135	0.188	0.21	1	-0.159	-0.149	0.149	0.061
	0.825	0.071	0.006	0	0	0.32	0.165	0.12		0.243	0.273	0.273	0.655
Fe	-0.096	-0.028	-.396**	.621**	.424**	0.09	0.103	-.470**	-0.159	1	-0.12	0.183	-.360**
	0.48	0.839	0.003	0	0.001	0.511	0.45	0	0.243		0.379	0.176	0.006
Mn	.347**	.294*	0.006	-0.186	0.018	-0.261	-.376**	-0.082	-0.149	-0.12	1	0.174	0.004
	0.009	0.028	0.966	0.171	0.895	0.052	0.004	0.548	0.273	0.379		0.2	0.979
Cu	-0.216	-0.185	-0.046	-0.087	-0.164	0.011	0.133	-0.004	0.149	0.183	0.174	1	0.211
	0.111	0.173	0.734	0.524	0.227	0.938	0.328	0.977	0.273	0.176	0.2		0.118
Protein	-0.243	-0.162	0.12	-.271*	-0.11	-0.175	0.05	0.113	0.061	-.360**	0.004	0.211	1
	0.071	0.233	0.379	0.043	0.42	0.196	0.713	0.409	0.655	0.006	0.979	0.118	

Produc. - production

Protein content

Zinc (Zn) is a fundamental nutrient for the synthesis and structure of proteins due to its role in the translation and transcription of genetic material, its correct contribution being of great importance (Broadley *et al.*, 2012). The results obtained in the present study indicate significant differences in protein content in response to foliar application of Zn plus chitosan (Figure 7). Among the treatments, those of NZN25, ZnO100 and ZnO25+Q, obtained values above 20 g per 100 g of dry weight. Similar results were published by Salinas-Ramírez *et al.* (2012), who obtained a range from 17.9 to 22.3 g per 100 g of dry weight in green beans grown in Mexico. In the same way, these 3 treatments were located within the range obtained by Suarez-Martínez et al. (2016), which indicate that 100 g of different bean varieties contain between 20 and 30% protein.

Regarding the source of Zn used, the results do not indicate significant differences, finding an average increase in relation to the control of 7.86 g when ZnO was used and 9.49 g when NZN was applied. The use of nanoparticles as well as the foliar Zn content (Figure 5) presented a linear trend when the dose was increased. These results are like those obtained by Salama *et al.* (2019) who report a similar increase in protein content as they increase the dose of nanoparticles in the form of ZnO in common bean plants.

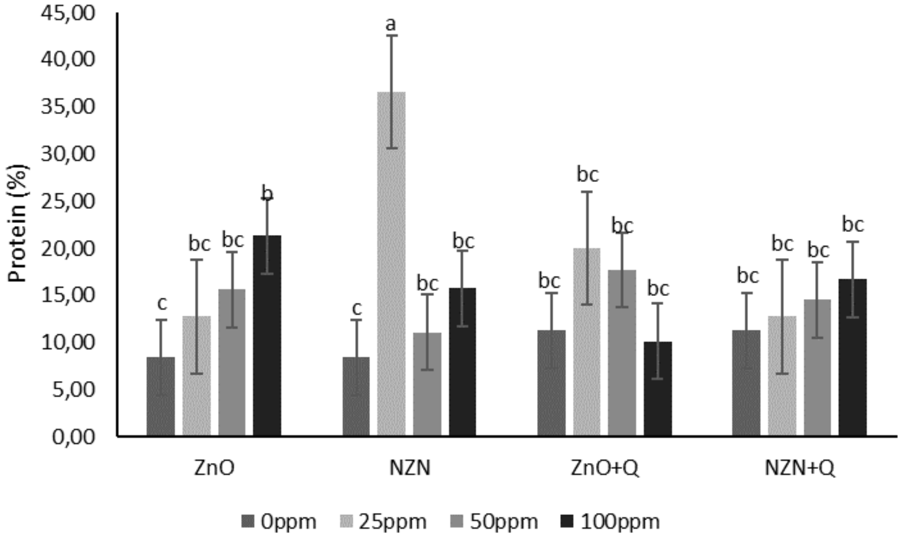

Figure 7. Effect of the application of nanoparticles and Zn nitrate in combination with chitosan on the protein content in bean plants cv. 'Strike'
Different letters indicate significant differences. Vertical bars indicate ±S.D.

While the application of chitosan individually favoured the protein content with an increase of 33.93% in relation to the control without application. Similar results were reported by Abu-Muriefah (2013) who obtained an increase in the content of soluble proteins when applying chitosan in short bean plants, however, the plants were under water stress. The positive effect of chitosan on protein content can be explained because various studies indicate an increase in the content of nitrogenous compounds because of chitosan application, generating greater development in the plant (Hidangmayum *et al.*, 2019).

Conclusions

The foliar application of Zn favours the biofortification process, finding increases in the content of Zn in the fruit for all the treatments used. The treatments that stood out were NZN50+Q and NZN100+Q, which increased the Zn content in fruits by more than 110%. In addition, the NZN50+Q treatment stood out in biomass production and exceeded the average yield reported for snap beans. In the present research work, no significant differences were found in production and biomass for the Zn source used, placing nanoparticles as a promising alternative for use in agriculture compared to a widely recognized fertilizer on the market. On the other hand, the application of chitosan plus nanoparticles did not present differences in the biofortification process, having similar results to the treatments without the application of this compound, however, the distribution pattern indicates a possible help in the translocation of Zn to the different plant organs. In the same way, the application of chitosan obtained favourable results when combined with NZN in biomass production, protein content, yield, and Zn content in fruit. Regarding the mineral content, a positive correlation between Zn and N was found, which indicates that, in biofortification works with Zn, a correct supply of N is essential. On the other hand, a negative correlation was obtained between P and Zn, and a similar trend between Zn and Fe, so it is important to be careful in the levels of these nutrients in future biofortification works. Finally, indicate that a greater number of studies are required regarding the use of nanoparticles and chitosan in horticulture to determine with certainty their effect on the physiology and nutrition of plants.

Authors' Contributions

ES and APM designed the study. APM and CRE analyzed the data. ES and APM. prepared the manuscript, while APM, CRE, CChM and DOB conducted the experiments. APM and ES organized the data and performed the statistical analysis. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

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

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