SHST
Horticulture and Forestry
Society of Transylvania

Agüero-Esparza M *et al.* (2022)
Notulae Scientia Biologicae
Volume 14, Issue 3, Article number 11261
DOI:10.15835/nsb14311261
Research Article

NSB
Notulae Scientia
Biologicae

Effectiveness of foliar application of biostimulants and nanoparticles on growth, nitrogen assimilation and nutritional content in green bean

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Abstract

The use of biostimulants, such as salicylic acid (SA) and chitosan, are a sustainable strategy to solve stress problems in plants. Its use has been shown to have synergy with metallic microelements, which are very important for the development of crops under stress situations. An advance in the application of these nutrients is the use of nanoparticles, which emerge as a more precise alternative to achieve optimal plant development. The objective of this study was to evaluate the effect of foliar application of biostimulants, iron (Fe) and zinc (Zn) nanoparticles on growth, nitrogen assimilation, and nutritional content in green bean cv. 'Strike'. Three treatments were used where complete nutrient solution was applied via foliar, the combination of chitosan (Q) plus SA and nanoparticles of Fe and Zn plus Q and SA. The application of nutrient solution favoured biomass content and carotene content. While the Q+SA treatment increased the nitrate reductase enzymatic activity, the mineral content in the root and the amino acid content, which places it as a viable alternative in situations where the supply of nutrients is limited or the plant cope with stressful situations. For its part, the application of nanoparticles of Fe and Zn plus biostimulants generated an increase in the mineral content of the aerial part, indicating that the application of this type of compound generates a greater mobility of nutrients within the plant.

Keywords: chitosan; nanofertilizers; *Phaseolus vulgaris* L.; salicylic acid

Introduction

The common bean (*Phaseolus vulgaris* L.) is the third most important legume for human consumption and is considered a good source of protein, vitamins, carbohydrates and minerals, with high concentrations of

Received: 27 Apr 2022. Received in revised form: 30 Jun 2021. Accepted: 29 Jul 2022. Published online: 23 Sep 2022.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

iron (Fe) and zinc (Zn), as well as thiamin and folic acid (De Ron *et al*., 2015). Mexico is the third largest producer of beans worldwide, with a harvested area of 1,596 million hectares, obtaining a yield of 1,196 million tons, concentrating most of the production in the states of Zacatecas, Sinaloa, Durango, Chihuahua and Nayarit (FIRA, 2019).

Currently, its production faces a series of challenges caused by climate change that is occurring worldwide, situations such as extreme cold, floods, hurricanes, droughts and intense heat waves have caused stress in the plants that generates yield losses (Hansen *et al*., 2012; Assad *et al*., 2019). The introduction of new technologies to agriculture have been shown to mitigate the effects on plants due to climate change (Smith *et al*., 2017). Within these technologies, a novel and sustainable strategy to solve problems caused by stress is the use of biostimulants, which are substances that promote plant growth without being nutrients and increase tolerance to biotic and abiotic stress. These products have begun to be used in agricultural systems with the aim of modifying physiological processes and increasing production, receiving significant attention in the last decade by scientific communities (Palacio-Márquez *et al*., 2022). Such is the case of salicylic acid (SA), classified in the group of phenolic compounds, which is a molecule that influences tolerance to abiotic stress through physiological and metabolic regulation, increasing photosynthetic properties and ion transport, generating an increase in growth, development, flowering, yield and quality of the fruits (Hasanuzzaman *et al*., 2017; Kareem *et al*., 2017).

Another biostimulant whose use in agriculture has increased in recent years is chitosan (Q), which is the second most abundant renewable polymer in nature, and can be found in the exoskeletons of crustaceans, cell walls of fungi and in the cuticle of the insects. It is considered an environmentally friendly compound due to its rapid degradation, low toxicity and easy obtaining (Vasconcelos, 2014; Morin-Crini *et al*., 2019). Several studies have shown its beneficial properties, improving the physiological response of the plant, by mitigating the effects produced by different types of stress, stimulating the photosynthetic rate, reducing transpiration and inducing the synthesis of antioxidant enzymes, through the production of sugars, amino acids and a series of metabolites (Pichyangkura and Chadchawan, 2015; Hidangmayum *et al*., 2019). The use of biostimulants, such as SA and Q, has been shown to have positive synergy with metallic microelements, which are very important for the development of crops in general, especially under stress situations. Nutrients such as Fe and Zn are key in enzyme reactions related to plant defense systems, in addition to participating in different processes that allow photosynthesis to take place. However, the absorption of these elements is limited by various factors (Marschner, 2011; Choudhary *et al*., 2017; Abdoli *et al*., 2020).

An advance in the application of these nutrients is the use of nanoparticles, which emerge as a sustainable and precise alternative to achieve optimal plant development. Its main characteristic is its size less than 100 nm. This size reduction generates an increase in the specific surface that causes a greater contact area allowing plants to absorb them more efficiently than traditional sources of these nutrients (Subbaiah *et al*., 2016; Ismail *et al*., 2017; Raliya *et al*., 2017). The use of nanoparticles has shown great effectiveness through foliar applications, with positive effects on growth, development, and production in different crops, as well as positive effects against stress caused by abiotic factors, enhancing the antioxidant system, mainly superoxide dismutase, ascorbate peroxidase and catalase enzymes (El-Ramady *et al*., 2018; Medina-Velo *et al*., 2018). Despite the promising results when applying these new technologies, there is little information regarding the combined use of these products, so the objective of this research work was to evaluate the effectiveness of foliar application of biostimulants and Fe and Zn nanoparticles, and on growth, nitrogen (N) assimilation and nutritional content in green bean cv. 'Strike'.

Materials and Methods

Crop management

The experiment was carried out in a greenhouse located in the facilities of the Food and Development Research Center in Delicias, Chihuahua, Mexico, with an average temperature of 32.9 °C. Green bean plants cv. 'Strike' which were planted in plastic pots with a diameter of 30.5 cm and a volume of 13.4 L, which were filled with a substrate composed of vermiculite and agricultural perlite in a 2:1 ratio. Four plants were placed per pot and watered with one L of 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 had a pH of 6.0 ± 0.1 and was applied every third day.

Experimental design and treatments

A completely randomized experimental design was used, with three treatments and four repetitions per treatment. The first treatment being the foliar application of the nutrient solution (previously described) and taken as a plant reference with correct development, the second treatment was the combination of chitosan (Q) at a dose of 50 ppm of the commercial brand Quitofyt® plus 13.8 ppm of Sigma® brand reagent grade SA and the third treatment was the combination of nanoparticles of iron oxide (NPsFeO) and zinc oxide (NPsZnO) at a dose of 25 ppm each, plus Q and SA. The treatments were applied via foliar 2 times every 10 days from the appearance of the first true leaves.

Characterization of nanoparticles

Zn oxide nanoparticles were obtained by wet chemical methodology in the form of Wurtzite crystals, with a size of approximately 50 nm, with a purity of 99.7% (Figure 1), with a density of 5.61 $g.cm^{-3}$ and a molecular weight of 81.40 g/mol. The morphology of the sample was obtained by scanning and transmission electron microscopy (Figure 2).

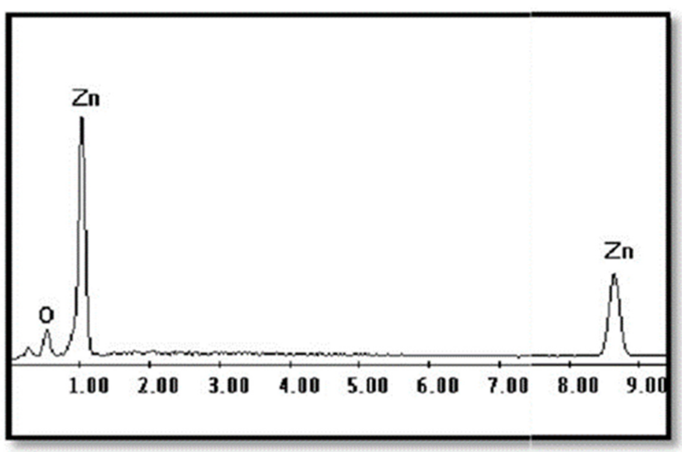

Figure 1. Elemental analysis (Chemical composition) of zinc oxide particles by energy dispersive X-rays (EDX)

On the other hand, the Fe oxide nanoparticles were obtained by the wet chemistry method in the form of Magheite crystals, with an average size <50 nm, a purity of 99.9% (Figure 3) and a molecular weight of 159.69 g/mole Figure 4 shows the morphology of the sample by scanning and transmission electron microscopy. Both materials were provided by the company "Investigación y Desarrollo de Nanomateriales S.A. of C.V." located in San Luis Potosi, Mexico.

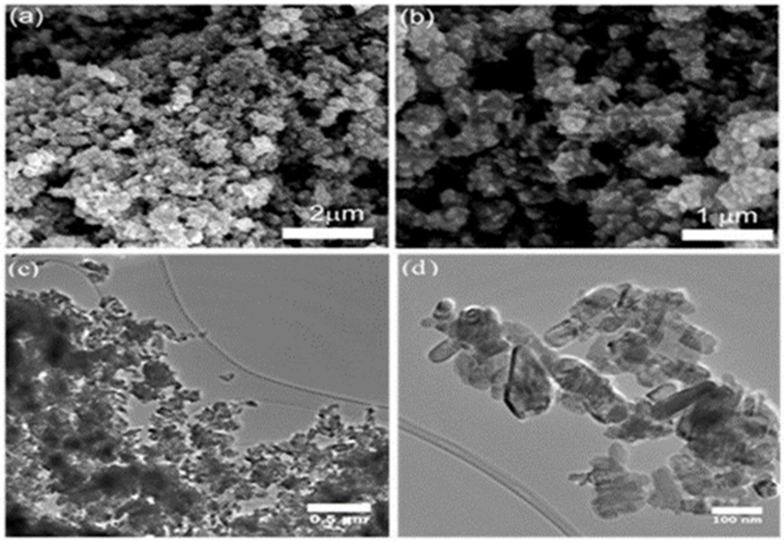

Figure 2. (a, b) NPsZnO morphology by scanning electron microscopy. (c, d) Morphology of NPsZnO by utilizing transmission electron microscopy

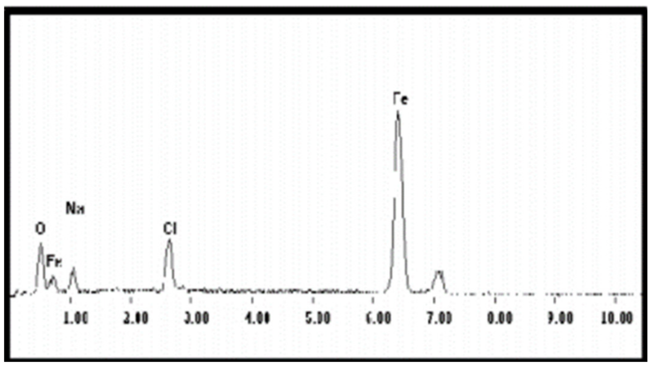

Figure 3. Elemental analysis (chemical composition) of iron oxide by energy dispersive X-rays (EDX)

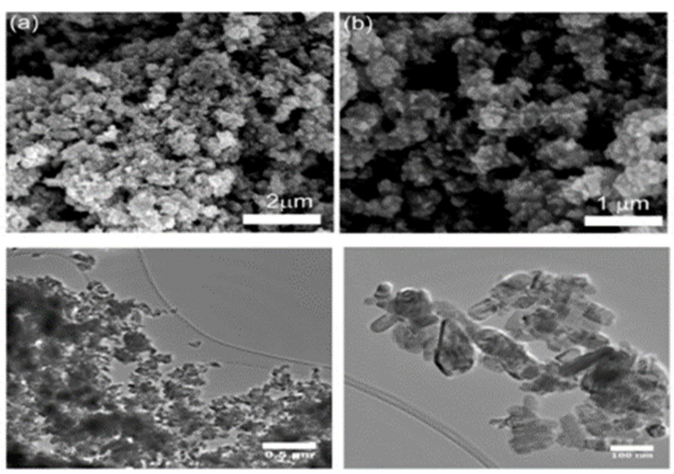

Figure 4. (a, b) Morphology of NPsFeO by scanning electron microscopy (SEM), (c, d) Morphology of the sample by transmission electron microscopy (TEM)

Plant sampling

Thirty days after planting, plant sampling was carried out, the crop was in the pre-flowering phenological stage, samples were taken and separated into two parts: root part and aerial part; subsequently, they were washed 3 times with distilled water and a 1% non-ionic detergent.

Plant analysis

Biomass

The weight of the aerial part and the root was obtained with the help of an analytical balance (AND HR-120, San José, California, USA). The results were expressed in grams per plant based on fresh weight.

Nitrate reductase activity "*in vivo*"

Nitrate reductase activity "*in vivo*" (NR) (EC 1.6.6.1) was determined using the method proposed by Sánchez et al. (2004), 0.1 g of fresh material was weighed into 7 mm diameter leaf discs and placed in 10 ml of incubation buffer (100 mM K-phosphate buffer, pH 7.5 and 1% (v/v) propanol). The samples were infiltrated at a pressure of 0.8 bars. They were incubated at 30 °C in the dark for 1 h and finally placed in a boiling water bath for 15 min to stop NR activity. Then, 1 ml of enzyme extract was taken and 2 ml of 1% (p/v) sulfanilamide in 1.5 M HCl and 2 ml of 0.02% (p/v) N- (1-naphthyl-dihydrochloride)-ethylenediamine were added in 0.2 M HCl. The resulting nitrite concentration was determined by spectrophotometry at 540 nm, against a standard curve of NO_2^-.

Chlorophyll index

The chlorophyll index was measured using the method proposed by Shrestha et al. (2012), using a Minolta SPAD 502 chlorophyll reader (Konica Minolta Sensing, Inc., Osaka, Japan) for which fully expanded leaves without physical damage and in parts free of veins were taken. The results obtained were expressed in SPAD units.

Photosynthetic pigments

They were analyzed following the methodology proposed by Wellburn (1994), for which foliar taleolae of 7 mm in diameter, weighing approximately 0.125 g, were collected and placed in test tubes. 10 ml of methanol were added to each sample and left to stand for 24 h in the dark. After this time, the reading was taken in a Genesis 10S UV-VIS spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA) at wavelengths of 666, 653 and 470 nm. The results were expressed in mg g^{-1} of fresh weight and were calculated using the following formulas:

$$Chl\ a = [15.65(A666) - 7.34(A653)]$$

$$Chl\ b = [27.05(A653) - 11.21(A666)]$$

$$Carotenoids = [(1000*A470) - 2.86(Chl\ a) - 129.2(Chl\ b)]/ 221$$

Soluble amino acids and proteins

For the quantification of amino acids and soluble proteins, 0.5 g of fresh material was weighed and homogenized in a 50 mM KH_2PO_4 buffer at pH 7 on a layer of ice to keep the sample cold. They were then centrifuged at 12,000 g for 15 min at 4 °C. The supernatant obtained was used for the determination of total amino acids by the ninhydrin method with slight modifications (Sánchez *et al*., 2004); total free amino acids were expressed as mg glycine g^{-1} fresh weight (FW). Soluble protein content was measured with the Bradford reagent (Kruger, 2009) and expressed as mg g^{-1} fresh weight, using bovine serum albumin as standard.

Mineral content

The N and S content 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. While for the other nutrients the method proposed by Wolf (1982) was used. 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. The reading of potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), zinc (Zn), iron (Fe), manganese (Mn), copper (Cu) and nickel (Ni) was performed by an atomic absorption spectrophotometer (AAS, iCE 3000 Series, Thermo Scientific, Waltham, MA, USA.). For its part, the reading of phosphorus (P) was carried out following the method of ammonium metavanadate (NH_4VO_3) against a standard curve of K_2HPO_4. The concentration of P was expressed as a percentage.

Statistical analysis

The data obtained were subjected to an analysis of variance, a mean separation test by Tukey with a confidence interval of 95%, using the SAS version 9 software (SAS, 2004).

Results and Discussion

Biomass

A physiological indicator of the level of environmental stress can be observed in the biomass of plants (Durigon *et al*., 2019). In the present study, significant differences in aerial and root biomass were observed in response to foliar application of nutrient solution, Q, SA and NPsFeO and NPsZnO (Figure 5). Highlighting the foliar application of nutrient solution with the highest aerial biomass, with an increase of 75 and 64.7% in relation to Q+SA and Q+SA + NPsFeO and NPsZnO, respectively. Similarly, for root biomass, the application of nutrient solution stood out with an increase of 76 and 83.3% in relation to Q+SA and Q+SA + NPsFeO and NPsZnO, respectively.

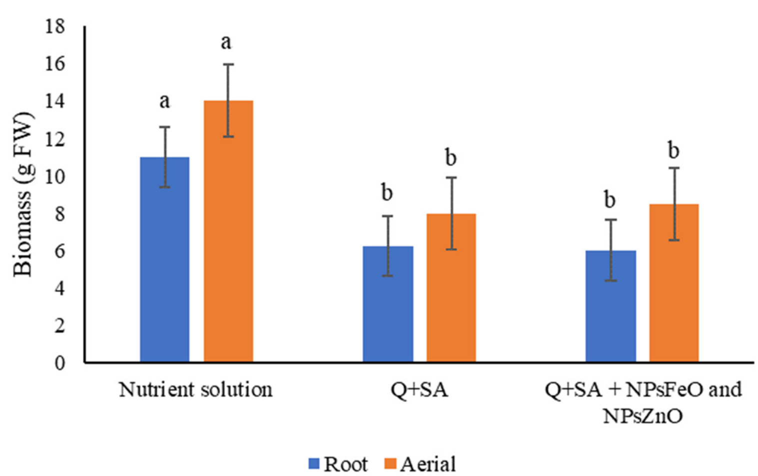

Figure 5. Effect of foliar application of nutrient solution, chitosan, salicylic acid, NPsFeO and NPsZnO on green bean plants cv. 'Strike' on biomass

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

The results obtained agree with previous studies that have shown that the foliar application of macronutrients and micronutrients improve biomass accumulation and generate an increase in the growth of bean plants, especially if they are applied in critical stages of crop development (El -Bassiony *et al*., 2010; Waheed *et al*., 2019). Probably, these results are due to the fact that foliar applications of nutrients generate a greater mobilization of the same within the plant, compared to exclusively edaphic applications (Kannan, 2010; Roosta and Hamidpour, 2013).

Regarding the addition of Fe and Zn nanoparticles, a slight increase in aerial biomass of 6.25% was observed in relation to the treatment of biostimulants without nanoparticles. The results obtained agree with those published by Dhoke *et al*. (2013), who obtained increases in mung bean biomass by combining nanoparticles of Fe, Zn and Cu, however, their application was in a nutrient solution through hydroponics. For their part, Mahmoud *et al*. (2019), found similar results when combining Fe and Zn in the form of nanoparticles with an organic amendment, obtaining a 6.06% increase in fresh aerial biomass in relation to the treatment where only the organic amendment was applied.

Enzymatic activity Nitrate Reductase "in vivo"

The NR enzyme participates as a precursor in N assimilation, although it is in turn affected by different environmental stimuli (Abbasifar *et al*., 2020). In the present investigation, no significant differences were observed in the activity of the NR enzyme (Figure 6). However, the Q+SA treatment obtained an increase of 22.1% in relation to the nutrient solution treatment. Various studies indicate positive effects on NR activity when applying biostimulants such as chitosan and SA. For example, Mondal *et al*. (2013), found similar results when applying Q in *Vigna radiata* L. plants, obtaining an increase of 11.98% in the NR enzymatic activity with a dose of 50 ppm. For their part, Hayat *et al*. (2012), obtained increases of up to 32% when applying SA in a foliar way in chickpea plants. While, Zanganeh *et al*. (2019), reported an increase of 3.45% when applying SA in the form of priming in corn seeds.

Previous studies have shown that the application of biostimulants increases the activity of key enzymes in N metabolism such as nitrate reductase, in addition to an increase in biomass and parameters related to photosynthesis (Latique *et al*., 2013; Esyanti *et al*., 2019).

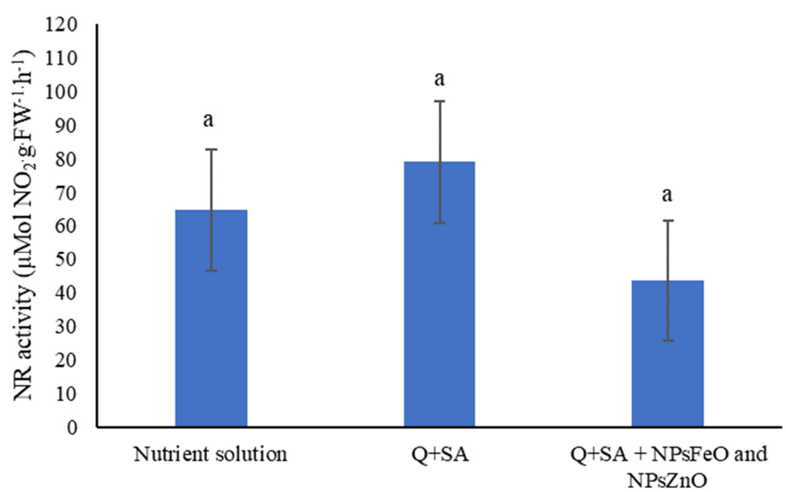

Figure 6. Effect of foliar application of nutrient solution, chitosan, salicylic acid, NPsFeO and NPsZnO on green bean plants cv. 'Strike' on NR enzyme activity

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

Mineral content

Macronutrients

Plants require essential macronutrients in larger amounts for optimal plant development, as well as for a better nutritional status (Fageria *et al*., 2009). In the present study, there were significant differences in the content of macronutrients in the root, while for the aerial part only significant differences were obtained for P and Mg (Table 1). The results obtained for the N content agree with the range published by Millis et al. (1996), which mentions a range that oscillates between 3 and 6% of N as the optimal concentration for the correct development of bean plants, with N being the most important element in plant nutrition since it is the main component of amino acids, proteins, purines and pyrimidine rings of nucleic acids, chlorophyll and enzymes (Mitra, 2015). The Q+SA treatment obtained an increase of 13.3% in relation to the treatment used as reference. These results could explain the result obtained for the NR activity (Figure 6), since the application of biostimulant compounds generates an increase in the content of nitrogenous compounds and in the total N content (Esyanti *et al*., 2019). In the same way, the Q+SA treatment favored root P content with increases of more than 100% compared to the nutrient solution and biostimulant plus nanoparticle treatments.

Table 1. Effect of foliar application of nutrient solution, chitosan, salicylic acid, NPsFeO and NPsZnO on green bean plants cv. 'Strike' on the macronutrient content

Treatments	Plant part	Macronutrients (%)				
		N	P	K	Ca	Mg
Nutrient solution	Aerial	3.57a	0.05b	5.94a	0.61a	0.07b
Q+SA		3.61a	0.06b	5.26a	0.63a	0.20a
Q+SA + NPsFeO and NPsZnO		3.67a	0.18a	7.11a	0.73a	0.05c
Nutrient solution	Root	3.46b	0.09b	4.99a	0.51a	0.07a
Q+SA		3.92a	0.19a	3.73b	0.47b	0.02c
Q+SA + NPsFeO and NPsZnO		3.91a	0.07b	1.54c	0.35c	0.06b

Different letters indicate significant differences.

Regarding the foliar application of Fe and Zn nanoparticles, favorable results were found in the content of N, P, K and Ca in the aerial part of the plant, with increases of 2.8, 260, 19.7 and 19.7% respectively in relation with nutrient solution treatment. These results are similar to those published by Pérez-Velasco et al. (2021), where the macronutrient content increased significantly when Zn nanoparticles were applied foliarly and edaphically in tomato. Similarly, Yang et al. (2020), obtained increases in the content of macronutrients when applying Fe nanoparticles in combination with fulvic acids in soybean plants. For their part, Dimkpa et al. (2017), found that applying Zn in the form of nanoparticles improves the efficiency in the absorption and mobility of macronutrients in sorghum plants, especially in situations where the supply of these macronutrients is limited. These results indicate a possible effect of Q+SA and nanoparticles on the mobility of nutrients in the plant. This hypothesis had been reported by Choudhary et al. (2019), who obtained clues that Zn released from a Q matrix applied to maize plants obtained greater mobility within the plant. However, the information about the use of SA, Q, NPsZnO and NPsFeO together is limited, so more in-depth studies are required to verify these effects.

Micronutrients

The application of micronutrients plays a very important role in plant nutrition, despite being required in small amounts (Janmohammadi *et al*., 2016). In the present research work, significant differences were obtained for all the micronutrients evaluated both in the root and in the aerial part, with the exception of the Cu content in the aerial part (Table 2). The results continued with the trend found in the macronutrients, where the Q+SA treatment obtained the highest values in the root part for the content of Zn, Fe, Mn and Cu with significant increases in relation to the nutrient solution treatment and with the treatment of Q+SA + NPsFeO and NPsZnO. Various studies report that the application of biostimulants improves the absorption, translocation and utilization of nutrients (Van Oosten *et al*., 2017; Kocira *et al*., 2020). As an example, Amiri *et al*. (2017) found increases in the content of micronutrients in safflower seeds through foliar application of SA + Q, without affecting yield or quality parameters.

Table 2. Effect of foliar application of nutrient solution, chitosan, salicylic acid, NPsFeO and NPsZnO on green bean plants cv. 'Strike' on the micronutrient content

Treatments	Plant part	Micronutrients (ppm)				
		Zn	Fe	Cu	Mn	Ni
Nutrient solution	Aerial	16.62b	81.66c	16.04a	7.33b	3.27b
Q+SA		18.85ab	121.70b	6.58a	8.055b	4.58b
Q+SA + NPsFeO and NPsZnO		29.13a	214.21a	37.59a	53.75[a]	8.06a
Nutrient solution	Root	10.80c	143.23b	10.05c	4.50c	5.71b
Q+SA		23.76a	270.18a	49.66a	75.82[a]	5.80b
Q+SA + NPsFeO and NPsZnO		17.16b	125.03c	29.96b	52.81b	6.74a

Different letters indicate significant differences.

In the same way as in the content of macronutrients, the treatment of Q+SA + NPsFeO and NPsZnO obtained significant results for the content of Zn, Fe, Mn and Ni in the aerial part of the plant, increasing by 75.27, 162.32, 633.29 and 146.48% in relation to the treatment taken as reference. The results published by Pérez-Velasco *et al*. (2021), agree with those obtained in the present study, because they also obtained increases in the content of micronutrients by applying NPsZnO foliarly to tomato plants. In turn, Yang *et al*. (2020), found similar results when applying Fe nanoparticles plus fulvic acids in soybean plants, increasing the Fe content by 523.93% and Zn by 302% in relation to their control. The results obtained for each micronutrient, with the exception of Cu, are within the optimal ranges proposed by Millis *et al*. (1996), for bean plants.

Chlorophyll index

SPAD values quickly express the chlorophyll index, an indicator that allows to quickly interpret if the plant presents some type of stress (Kumar and Sharma, 2019). In the quantification of SPAD values, significant differences were observed, in which the response to foliar application of Q+SA + NPsFeO and NPsZnO stands out with an increase of 3.69% and 7.76% in relation to foliar application of nutrient solution and Q+SA, respectively (Table 3). The results obtained agree with those published by Medina-Pérez et al. (2018), who found a range between 35-50 SPAD units for Pinto beans applying 1.3 and 6 g of ZnO through fertigation, however, they did not find a significant difference compared to the control. In turn, Mahmoud et al. (2019), found an increase of 15% when foliarly adding Fe and Zn nanoparticles to an organic amendment (chicken manure) in radish plants.

Previous studies have shown that the application of micronutrients such as Fe and Zn reflect UV rays, preventing chlorophyll degradation, in vegetative growth (Elemike *et al*., 2019). In this sense, Marzouk *et al*. (2019) reported an increase in SPAD values in two bean varieties through foliar applications of nanomaterials. Results that may be due to the fact that Fe is part of the essential synthesis of chlorophyll, in addition, Zn is required by many essential enzymes for chlorophyll biosynthesis (Nikolic and Kastori, 2000; Taiz and Zeiger, 2004). On the other hand, SA has shown its influence on physiological and biochemical processes, including some enzymatic activities related to chlorophyll production (Hayat *et al*., 2007). Finally, Q contributes to improving the chlorophyll content due to its endogenous ability to act on cytokinins (Basit *et al*., 2020).

Table 3. Effect of foliar application of nutrient solution, chitosan, salicylic acid, NPsFeO and NPsZnO on green bean plants cv. 'Strike' on the content of photosynthetic pigments, chlorophyll index, amino acids and soluble proteins

Treatments	Chlorophyl ($mg.gFW^{-1}$)	Carotenoids ($mg.gFW^{-1}$)	Proteins ($mg.gFW^{-1}$)	Amino acids ($mg.gFW^{-1}$)	SPAD units
Nutrient solution	28.06 a	2.24 a	19.39 a	3.40 b	45.52 ab
Q+SA	28.63 a	2.00 a	19.77 a	4.19 a	43.80 b
Q+SA + NPsFeO and NPsZnO	25.80 a	1.87 a	18.52 a	2.47 b	47.20 a

Different letters indicate significant differences.

Photosynthetic pigments

Photosynthetic pigments are those compounds that have the capacity to capture light and convert it into energy, and also have a certain reducing power, which allows them to face stress situations (Jaleel *et al*., 2009). In the present project, no significant differences were observed in the quantification of photosynthetic pigments (Table 3). Regarding the total chlorophyll content, the foliar application of Q+SA stood out with an increase of 2% and 10.97% with respect to the foliar application of nutrient solution and Q+SA + NPsFeO and NPsZnO, respectively. In this sense, El-Kenawy (2017) found an increase of 13.31% when applying the combination of Q plus SA foliarly on grapevines. Jeyakumar *et al*. (2008) reported a tendency to improve chlorophyll content through foliar applications of biostimulants prior to flowering, coinciding with the results of this study; this may be due to the fact that the foliar application of Q and/or SA contributes to improving the endogenous levels of cytokinins, stimulating the synthesis of chlorophyll, in addition to the stimulatory effects on RUBISCO (Khodary, 2004; Malekpoor *et al*., 2016).

On the other hand, for the content of carotenoids, it can be highlighted the nutrient solution treatment, obtaining an average increase of 15.89% in relation to the treatments with biostimulants and Fe and Zn nanoparticles. These results coincide with those obtained for the biomass variable, which indicates that the plants to which the nutrient solution was applied had an accelerated development, since previous studies have related the increase in the content of carotenoids with the state of maturity of a plant (Bramley, 2013; Llorente *et al*., 2016). This may be due to the fact that the applied nutrients stimulate enzymes in the production of photosynthesis and, therefore, improve the production of carotenoids, which function as non-enzymatic antioxidants responsible for reducing the accumulation of reactive oxygen species in unfavorable conditions (Mahdi *et al*., 2021).

Soluble amino acids and proteins

Generally, proteins and amino acids are the main contributors to the nutritional content, as well as various metabolic activities (Yi-Shen *et al*., 2018). In the present study, significant differences were observed in the concentration of amino acids, but the content of soluble proteins did not present significant differences (Table 3). The Q+SA treatment increased the content of both variables with increases of 23.34% for amino acids and 1.96% for soluble proteins in relation to the nutrient solution treatment. Similar results were published by Farouk *et al*. (2011), who applied 100 and 200 ppm of Q in radish plants, increasing the content of amino acids and soluble proteins, with the difference that their treatments were applied to the soil solution and under cadmium stress. For his part, El-Kenawy (2017), applied the combination of Q plus SA in a foliar way and found an increase of 14.27% in the protein content in grapes.

In previous studies, it has been reported that foliar applications of biostimulants, especially Q, improve the concentration of amino acids, which act in different ways in metabolic processes and are considered an important component for the stimulation of plant growth and development. In addition, they act as osmoregulators, reducing cell transpiration, chlorophyll content, antioxidant activity, the absorption of different nutrients by the root and being fundamental in protein synthesis (Pichyangkura and Chadchawan, 2015; Tadros *et al*., 2019; Hidangmayum *et al*., 2019).

Conclusions

The application of nutrient solution in foliar way favored the content of aerial and root biomass, in addition there are indications that it accelerated the development of the plant, since increases in the content of carotenes were found. On the other hand, the Q+SA treatment increased the NR enzymatic activity, the mineral content in the root part and the amino acid content, in addition to slight increases in the content of total chlorophyll and soluble proteins, which places it as a viable alternative in situations where the supply of nutrients is limited or the plant faces stress situations. For its part, the application of Fe and Zn nanoparticles plus the combination of biostimulants generated an increase in the mineral content of the aerial part, indicating that the application of this type of compounds generates a greater mobility of nutrients within the plant, however, more in-depth studies are needed to corroborate this hypothesis, as well as the physiological and biochemical effects that may occur when using these technologies together.

Authors' Contributions

E.S. and M.A.-E. designed the study. O.V.-C. and A.P.-M. analyzed the data. E.S and A.P.-M. prepared the manuscript, while S.P.-A., J.P.S.-A., M.A.-E., and C.A.R.-E. conducted the experiments. M.A.-E., A.P.-M., and E.S. 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.

Acknowledgements

We would like to thank the Consejo Nacional de Ciencia y Tecnología (CONACyT—Mexico) for the support provided by means of the Convocatoria Atención a Problemas Nacionales: Project #1529 "Biofortification of basic agricultural crops representing the key to combat malnutrition and ensure food security in Mexico."

Conflict of Interests

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

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