Zinc sulphate or zinc nanoparticle applications to leaves of green beans

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ABSTRACT

The green bean (*Phaseolus vulgaris* L.) is a very widely grown food crop that contributes significantly to human dietary needs in many countries due to its high content of protein. This study evaluates foliar applications of ZnSO₄ versus that of zinc oxide nanoparticles (ZnO NPs) to leaves of the green bean cv. 'Strike' and records the plant responses in terms of Zn uptake and concentrations of photosynthetic pigments and bioactive compounds. The experiment was conducted under greenhouse conditions in Chihuahua, Mexico, with a completely randomised experimental design with 10 replicates. The two treatments were foliar applications of either an aqueous solution of ZnSO₄ or an aqueous suspension of ZnO NPs (both 150 mg · L⁻¹). The application of ZnO NPs significantly increased concentrations of Zn²⁺ in the leaflets, roots, stems and pods of chlorophylls *a* and *b* (values 15.40 µg · g⁻¹ and 11.64 µg · g⁻¹, respectively). Sucrose concentration was also increased by Zn²⁺ applications, but no differences were found in total phenols (TP), total flavonoids (TFI) or antioxidant capacity (AC). In the pods and seeds, Zn²⁺ application left sucrose and TFI concentrations unchanged, but the TP increase was significant. The AC was affected by both zinc sources and only in the pods. The applications of ZnSO₄ or ZnO NPs significantly increased the biomass accumulation (79.10 g · p⁻¹ and 84.70 g · p⁻¹ DW) and yield (55.64 g · p⁻¹ and 53.80 g · p⁻¹ FW). These results suggest that the application of ZnO NPs could represent a worthwhile biofortification strategy in the commercial production of green bean cv. 'Strike'.

Keywords: antioxidant capacity, efficiency, fertilisation, nanofertilisers, *Phaseolus vulgaris* L.

Abbreviations: AC, antioxidant capacity; BBCH Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie; Chl. Chlorophyll; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EDTA, Ethylenediaminetetraacetic acid; ROS, reactive oxygen species; SOD, superoxide dismutase; TAn, total anthocyanins; TC, total carotenoids; TFI, total flavonoids; TP, total phenols; ZnO NPS, zinc oxide nanoparticles; ZnSO₄, zinc sulphate.

INTRODUCTION

The productivity of agricultural crops is closely related to optimisation of the mineral nutrient availability in the root zone (Souri and Hatamian, 2019). To achieve this, fertilisers are applied to the soil (Raliya et al.,

2018); however, fertiliser performance can be reduced by various factors including soil–water content and irrigation practices, soil pH, soil texture and soil structure, water solubility of the fertiliser and fertilisation rates

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(Singh et al., 2017; Souri et al., 2017; Souri et al., 2018; Hatamian et al., 2018). Foliar fertilisation of mineral nutrients can also be an appropriate technique to overcome minerals deficiency or to increase their contents in agriculture products (Souri and Dehnavars, 2017; Aslani and Souri, 2018; Mazaheri-Tirani et al., 2019). The efficiency of foliar application of fertilisers can also be a function of the environmental factors (temperature, light intensity and relative humidity) and plant structure (the presence of waxes, cuticle thickness and composition). These environmental and plant factors can also interact to increase or decrease the foliar fertilisation efficiency (Bonomelli et al., 2021).

The green bean (or the ‘common bean’ or ‘French bean’, *Phaseolus vulgaris* L.) is a vegetable which enjoys widespread popularity. The popularity of its immature pods and seeds can be attributed to their high content of minerals (Ca^{2+} , Fe^{2+} , Zn^{2+} , Mg^{2+} and P) and also of carbohydrates, proteins, fibre, calories and vitamin B (Sida-Arreola et al., 2015; Souri and Aslani, 2018; Sálcido-Martínez et al., 2020). However, the cultivation of this species extends to areas of the world in which the soils are well suboptimal for it due to the presence of (e.g.) high levels of carbonates, or salinity, or adverse physical properties (Minnocci et al., 2018; Balafrej et al., 2020). Under adverse soil conditions, deficiencies of some micronutrients can increase, including that of Zn^{2+} which reduces both the yield and quality of the harvested product (Korkmaz et al., 2018). Zinc is an essential micronutrient for fruit set (Minnocci et al., 2018). It actively participates as a cofactor in the synthesis of DNA and proteins and plays an important role in gene expression in stress tolerance (Korkmaz et al., 2018; Ponce-García et al., 2019). For these reasons, Zn^{2+} is supplied mainly via edaphic or foliar applications of $\text{Zn}(\text{NO}_3)_2$, ZnSO_4 or chelates (e.g. EDTA-Zn) (Pedruzzi et al., 2020). However, their foliar uptake efficiency is relatively low because Zn^{2+} absorption by the leaf depends on the chemical nature of the source (García-López et al., 2019).

After Fe, Zn is the most abundant transition metal element in living organisms (Castillo-González et al., 2018). It plays a major role in plant metabolism because it is an essential component of several key enzymes, including oxidoreductases, lyases, isomerases, transferases, hydrolases and ligases (Ojeda-Barrios et al., 2019; Akanbi-Gada et al., 2019). Zinc is also an essential micronutrient for the growth and development of cultivated plants and generally requires concentrations in the range of 15–20 mg · kg⁻¹ (Ewais et al., 2017). Moreover, Zn is involved in tryptophan synthesis, auxin metabolism, cell division and chlorophyll synthesis. It is also involved in pollen development and fruit set (Pedruzzi et al., 2020; Balafrej et al., 2020; Balandran-Valladares et al., 2021).

A further advance in foliar fertilisation is the use of nano-technologies, since the transformation of macro minerals to nano-sized particles (NPs, size <100 nm)

leads to useful characteristics for the application of micronutrients (Ghidan et al., 2020). Foliar spraying of ‘nanofertilisers’ (Nfs) increases the nutrient availability, thus reducing disease incidence and increasing crop quality and yield (Sálcido-Martínez et al., 2020). Compared with conventional fertilisers, NPs can reduce product wastage and so minimise impacts on the environment (soil and groundwater contamination) (Fatollahpour-Grangah et al., 2020). However, the use of nanosuspensions can lead to their deposition on the leaf surface, generate toxicity problems and this affects their uptake (Akanbi-Gada et al., 2019).

A number of studies have demonstrated the effectiveness of applications of zinc oxide nanoparticles (ZnO NPs) on germination, biomass accumulation, photosynthetic pigments and enzyme activity (Singh et al., 2017; Rossi et al., 2019). A further advantage is that NPs can be supplied in smaller concentrations with similar or better results than the larger doses used for conventional soil-surface fertilisation (Mazaheri-Tirani et al., 2019). However, excessive use of NPs can cause stress to plants, affecting the synthesis of proteins, carbohydrates and DNA (Abbasifar et al., 2020). The uptake efficiency of NPs is determined by their chemical composition, shape, reactivity, pH, degree of adsorption and the area of leaf surface covered (Marzouk et al., 2019).

Foliar applications of NPs of ZnSO_4 and ZnO have been reported to be practical measures that can achieve better physiological and biochemical plant responses, while also minimising environmental impacts by reducing the number of applications (Pullagurala et al., 2018; Nafady et al., 2019). With an increasing demand for beans as a staple of human nutrition, the generation of further information on the use of Zn^{2+} , including its uptake and impact on biofortification are essential to meet the needs of both the producers and consumers of this legume (Sida-Arreola et al., 2015; Ewais et al., 2017). The objective of this study was to evaluate foliar applications of ZnSO_4 and ZnO NPs to compare responses in terms of Zn uptake, concentrations of the photosynthetic pigments and bioactive compounds in the bean cv ‘Strike’.

MATERIALS AND METHODS

Experimental site, crop management and experimental design

For this study, plants of the green bean (*P. vulgaris* L.) cv. ‘Strike’ (short cycle variety, sensitive to salinity and adapts to greenhouse conditions) were grown in polyethylene bags filled with 3 kg of soil (sandy loam, pH 6.8, electrical conductivity 0.51 dS · m⁻¹, organic matter 1.5%, water saturation 48%) (Figure 1). The soil composition contained the micronutrients in mg · kg⁻¹: 0.22 Zn^{+2} , 0.4 Cu^{+2} , 5.4 Fe^{+2} and 1.3 Mn^{+2} . Plants were raised in an experimental greenhouse in Chihuahua, Mexico (28°41′ 29.8″ N, 106°06′58.6″ W) with a mean

Figure 1. Green beans cv. 'Strike' plants under greenhouse conditions at Universidad Autónoma of Chihuahua, Mexico.

temperature of 30 °C. Mineral nutrition was carried out using a solution prepared with distilled water and containing the macronutrients (mM): 7.5 NH_4NO_3 , 1.0 K_2HPO_4 , 1.05 K_2SO_4 , 2.5 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.0 MgSO_4 and the micronutrients (μM): 44.6 $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 1.2 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.185 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.06 $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ and 0.5 H_3BO_3 (Ponce-García et al., 2019).

We used a completely randomised experimental design with 10 replicates, where the experimental unit consisted of one plant. There were two Zn treatments and a control (without Zn). The first Zn treatment used an aqueous solution of ZnSO_4 (150 $\text{mg} \cdot \text{L}^{-1}$, Merck®, Germany) and the second an aqueous suspension of ZnO NPs (Investigación y Desarrollo de Nanomateriales S.A. de C.V., Mexico). Both treatments were applied before sunrise using a soft brush to the upper and lower surfaces of the first trifoliate leaves. To avoid any contact with the soil, each plant was sprayed separately, and a plastic film was used to cover the top of each pot before spraying. Foliar application was done every 10 days for a total of three applications. After physiological maturity of the plants, at 60 DAG, the samples were taken and separated into four parts: root, stem, leaf, and fruit and washed thrice with distilled water and 1% non-ionic detergent.

Characterisation of the Nfs

The material applied as Nfs was ZnO obtained by wet chemistry method in the form of wurtzite crystals with an average size of 50 nm with negligible contaminants, a purity level of 99.7% and a density of 5.61 $\text{g} \cdot \text{cm}^{-3}$ (Figure 2 and 3) (Ponce-García et al., 2019).

Parameters evaluated

Zinc concentration (leaflet, stem, root and pods)

Prior to analysis, the plant was separated into leaflets, stems, roots and pods. The determination of Zn^{2+} concentration in these plant organs was carried out following the method of Rossi et al. (2019) with slight modifications. Briefly, the different plant organs were placed in a container with 150 mL of distilled water and

left to stand for 5 min before washing again, but with a 4N HCl solution (1 $\text{mL} \cdot \text{L}^{-1}$) and rinsed with distilled water to remove any superficial Zn residues. This wash water was then made up to 200 mL and filtered through Whatman Paper No. 1.

Organ samples were then dried in a Heratherm VCA 230® (Thermo Scientific, USA) oven at 70°C for 72 h (leaflets, stems and roots) or for 144 h (pods). Each sample was then homogenised in a Willey R-TE-650/1 mill with a 1-mm mesh (Tecnal, Piracicaba, Brazil) and the homogenate was placed in an airtight container and held at -20 °C for further analysis. Subsamples of 1 g of fresh weight of homogenate were then taken and digested in 25 mL of a triacid mixture (HNO_3 , HCl and H_2SO_4), filtered with Whatman Paper No. 1 and made up to 50 mL with tri-distilled water. Zinc concentrations were then determined with an atomic absorption spectrophotometer (Thermo Scientific, USA). The results are expressed in $\text{mg} \cdot \text{kg}^{-1}$ dry weight.

Chlorophyll and total carotenoids

The concentrations of chlorophyll *a* (chl *a*) and chlorophyll *b* (chl *b*) and total carotenoids were determined by the method of Ponce-García et al. (2019), which requires weighing 0.2–0.3 g of fresh photosynthetic material (leaflets) to which 10 mL of pure methanol (CH_3OH) was added. The samples were then incubated at room temperature in darkness for 24 h. After this period, absorbance was measured at 470 nm (carotenoids), 653 nm (chl *b*) and 666 nm (chl *a*). The results are expressed as $\mu\text{g} \cdot \text{g}^{-1}$.

Bioactive compounds (leaflet, pod and seed)

Extract preparation and determination

Extraction of bioactive compounds was carried out following the method of Cera-Campos et al. (2019) with slight modifications. Fresh samples (0.5 g) were homogenised with 5 mL of 80% (v:v) methanol. The homogenised sample was centrifuged at 6,000 rpm for 10 min at 4 °C. The supernatant was used to quantify

Figure 2. (A and B) Sample morphology of ZnO NPs by scanning electron microscopy and (C and D) transmission electron microscopy (Ponce-García et al., 2019). NPs, nanoparticles.

Figure 3. (A) Elemental analysis (chemical composition) by energy dispersive X-ray scattering and (B) crystalline structure by X-ray diffraction of the sample ZnO NPs (Ponce-García et al., 2019). NPs, nanoparticles.

the phenols, flavonoids, anthocyanins and antioxidant capacity (AC). Total phenols (TP) were quantified according to the Folin–Ciocalteu method described by Monroy-Gutiérrez et al. (2017). Two samples were taken with 0.250 mL of the supernatant to which 0.750 mL and 0.250 mL of 2% and 50% (v:v) Na_2CO_3

and Folin–Ciocalteu were added, respectively. The mixture was incubated for 1 h and the absorbance was measured at 725 nm on a Lambda 25[®] UV-visible spectrophotometer (PerkinElmer, Waltham, USA). The results are expressed in $\text{mg GAE} \cdot 100 \text{ g}^{-1}$. Total flavonoids (TFI) were quantified according to the method

of Chang et al. (2002). Here, 0.250 mL of supernatant was added to 0.075 mL of NaNO_2 , the mixture was shaken vigorously for 5 min, then 0.150 mL, 0.500 mL and 2.025 mL of AlCl_3 , NaOH and deionised water were added, and the mixture was left to stand for 40 min, after which the absorbance was determined at 510 nm (PerkinElmer, Waltham, USA). The results are expressed as $\text{mg EC} \cdot 100 \text{ g}^{-1}$. The total anthocyanins (TAn) were determined using the differential pH method described by Giusti and Wrolstad (2001) with slight modifications. Two aliquots of 0.500 mL of supernatant were taken. To one aliquot was added 2 mL of KCl and to the second, 2 mL of CH_3COONa , and shaken vigorously for 1 min. After this, the absorbances were measured at 460 nm and 710 nm, respectively (PerkinElmer, Waltham, USA). The results are expressed as $\text{mg ocyanidin-3-glucoside} \cdot 100 \text{ g}^{-1}$. AC was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method described by Cera-Campos et al. (2019) with some modifications. To 0.3 mL of extract, 5.7 mL of the DPPH compound was added (DPPH, concentration $0.0375 \text{ g} \cdot \text{L}^{-1}$). The mixture was kept in the dark for 30 min. The decrease in the DPPH radical was measured at 515 nm (PerkinElmer, Waltham, USA). The results are expressed as % DPPH inhibition.

Sucrose (leaf, pod and seed)

The non-structural carbohydrate was quantified according to the method of Irigoyen et al. (1992) with slight modifications: Briefly, 0.5 g of fresh plant material was taken and homogenised thrice, the first time with 5 mL of 96% ethanol and then twice with 5 mL of 70% ethanol. The homogenate was centrifuged at 3,740 g for 10 min at 4 °C. An amount of 0.1 mL was taken and 3 mL of anthrone solution dissolved in H_2SO_4 (1 g anthrone in 100 mL H_2SO_4) was added. It was then placed in a water bath for 10 min, allowed to cool and the absorbance was determined at 650 nm (PerkinElmer, Waltham, USA). The results are expressed in $\text{mg} \cdot \text{g}^{-1}$.

Yield and biomass

Fruit yield was determined at the end of the phenological cycle. The results are expressed in grams of fresh weight per plant ($\text{g} \cdot \text{p}^{-1} \text{FW}$). Biomass was determined according to the method indicated by Sida-Arreola et al. (2015). In each sampling, the fresh weight of leaflets, stems and roots were taken and dried in a Heratherm VCA 230® (Thermo Scientific, USA) oven at 70 °C for 72 h. The results are expressed in grams of dry weight per plant ($\text{g} \cdot \text{p}^{-1} \text{DW}$).

Statistical analyses

Prior to the statistical analysis, Levene's test for homogeneity of variances was carried out for each group of data per variable (Sokal and Rohlf, 1995). Subsequently, the analysis of variance was carried out and when a significant treatment effect was detected, Tukey's multiple comparison of means test was applied ($p \leq 0.05$). In all cases, the statistical program IBM SPSS Statistics 25 was used.

RESULTS AND DISCUSSION

Concentration of Zn^{2+} (leaflets, roots, stems, pods)

The application of ZnO NPs significantly increased the Zn^{2+} concentrations in the leaflets, roots, stems and pods with values of $94.66 \text{ mg} \cdot \text{kg}^{-1}$, $80.30 \text{ mg} \cdot \text{kg}^{-1}$, $87.18 \text{ mg} \cdot \text{kg}^{-1}$ and $66.29 \text{ mg} \cdot \text{kg}^{-1} \text{DW}$, respectively (Figure 4). Except for the stems, the differences between the two treatments were significant. Ranked in order of efficacy, it can be expressed as $\text{ZnO NPs} > \text{ZnSO}_4 > \text{control}$. In this regard, Ponce-García et al. (2019), when evaluating the edaphic application of $100 \text{ mg} \cdot \text{kg}^{-1}$ of Zn^{2+} (sulphate) in the green bean (*P. vulgaris* L.), reported a significant increase in the concentrations of Zn in the roots and leaflets with values of $31 \text{ mg} \cdot \text{kg}^{-1}$ and $5 \text{ mg} \cdot \text{kg}^{-1}$, respectively.

Figure 4. Zn^{2+} concentrations in leaflet, stem, root and pods of the green bean cv. 'Strike' sprayed with ZnSO_4 and ZnO NPs. The data shown are mean values $\pm$ standard deviations ($n = 10$). Bars with the same letter are not significantly different according to Tukey's test ($p \leq 0.05$). NPs, nanoparticles.

In other crops, such as *Coffea arabica* L., applications of ZnO NPs increased the concentration of Zn^{2+} in the leaves but not in the stems or roots (Rossi et al. 2019). The plants used in this study did not show symptoms of Zn^{2+} deficiency, which could explain the foliar increase due to Zn micronutrient. On the other hand, it has been reported that the dissolution of ZnO NPs in deionised water is relatively slow, so in 24 h only 2% is dissolved, hence its effect can be better observed after 30 days (Singh et al 2017).

In this study, the application of ZnO NPs to the leaves indicates a significant concentration of Zn^{2+} to the pods, stems and roots. In contrast, in another study (Ponce-García et al. 2019), applications of 25 mg · kg⁻¹, 50 mg · kg⁻¹ or 100 mg · kg⁻¹ of ZnO NPs on the green bean ‘Strike’ showed enhancement in the roots but not in the stems. This variation in behaviour may be associated with the phenological stage, nutrient antagonism or applied dose, as indicated by Rossi et al. (2019). On the other hand, pods harvested from plants treated with ZnO NPs presented higher Zn^{2+} concentrations at 66.29 mg · kg⁻¹ (Figure 5). This suggests that ZnO Nfs could help in the biofortification of green bean of this variety. Similar results were reported for this variety by Ponce-García et al. (2019) with applications of 100 mg · kg⁻¹. In this sense, the use of ZnO NPs improves crop productivity by increasing the uptake efficiency, application accuracy and with less environmental impact (Shang et al., 2019). It has been shown that the level of uptake and translocation of ZnO NPs is associated with the applied dose and the phenological stage (Singh et al., 2017). However, little information is available on the physiological and biochemical effects of ZnO NPs application with this species.

Photosynthetic pigment concentrations

Reduced synthesis photosynthetic pigment and accumulation are good indicators of stress or suboptimal growth conditions (Marzouk et al., 2019; Zargar Shooshtari et al., 2020; Ebrahimi et al., 2021). The concentration of photosynthetic pigments (chlorophyll, carotenoids and TAn) are presented in Table 1. The application of ZnO NPs increased the concentrations of chl *a* and chl *b* (15.40 µg · g⁻¹ and 11.64 µg · g⁻¹, respectively) with respect to ZnSO₄. Previous studies on the broad bean (*Vicia faba* L.) with foliar applications of 50 mg · L⁻¹ showed positive results with respect to the total chlorophyll concentration (Ghidan et al., 2020). It is known that most micronutrients such as Fe²⁺, Mn²⁺ Cu²⁺ and Zn^{2+} are involved in activation of various metabolic enzymes, including protein and chlorophyll biosynthesis (Sida-Arreola et al., 2015; Castillo-González et al., 2018; Souri et al., 2019; Aghaye-Noroozlo et al., 2019). These results contrast with those reported by Ponce-García et al. (2019) who, when applying ZnO NPs and chelates (25, 50 and 100 mg · kg⁻¹) in nutrient solution, report no significant effects. These authors point out an inverse behaviour between the doses of ZnO NPs and chlorophyll concentration, attributed to the stress generated by the possible toxicity caused by the application of these Nfs to the soil. In our study, no visible symptoms of Zn^{2+} toxicity (wilting, curling, waving, chlorotic and necrotic spots) were detected (Pullagurala et al., 2018), which could be linked to the slow dissolution of ZnO NPs in deionised water (Rossi et al., 2019).

On the other hand, the concentrations of total carotenoids and anthocyanins were not affected by the application of ZnO NPs. Previous studies indicate a positive effect on plant productivity by increasing

Figure 5. Effect of application of ZnSO₄ and ZnO NPs on biomass accumulation and yield (fresh pods) in the green bean cv. ‘Strike’. NPs, nanoparticles.

Table 1. Changes in concentrations of photosynthetic pigments, bioactive compounds and antioxidant capacities in leaflets of the green bean cv. ‘Strike’ following applications of ZnSO₄ and ZnO NPs.

Treatment	Chl		TC	TAn	Sucrose	TP	TFI	AC
	a	b						
Control	11.41 b	7.70 b	2.91 a	1.05 a	5.06 b	3.37 a	0.32 a	62.90 a
ZnSO ₄	12.62 b	8.24 b	2.14 a	1.24 a	6.38 a	3.55 a	0.42 a	72.31 a
ZnO NPs	15.40 a	11.64 a	1.58 a	1.50 a	5.53 ab	3.20 a	0.44 a	56.57 a

Data are expressed on a fresh-weight basis. Values with the same letter within a column are not significantly different according to Tukey's test ($p \leq 0.05$).

AC, antioxidant capacity (% DPPH inhibition); Chl, chlorophyll ($\mu\text{g} \cdot \text{g}^{-1}$); NPs, nanoparticles; TC, total carotenoids ($\mu\text{g} \cdot \text{g}^{-1}$); TAn, total anthocyanins (mg cyanidin-3-glucoside $\cdot 100 \text{ g}^{-1}$); Sucrose (mg $\cdot \text{g}^{-1}$); TP, total phenols (mg GAE $\cdot 100 \text{ g}^{-1}$); TFI, total flavonoids (mg CE $\cdot 100 \text{ g}^{-1}$); ZnO NPs, zinc oxide nanoparticles.

photosynthesis (Pullagurala et al., 2018; Marzouk et al., 2019); however, there are no specific references indicating changes in the concentrations of carotenoids and TAn for green beans. In this sense, it would be necessary to conduct evaluations with other doses, application times and growth stages to improve our understanding of the effects of NPs on the behaviour of accessory pigments.

Bioactive compounds in leaflets

In this study, the application of ZnSO₄ slightly increased the sucrose concentration (6.38 mg $\cdot \text{g}^{-1}$), but it did not significantly exceed that observed in the treatment with ZnO NPs (5.53 mg $\cdot \text{g}^{-1}$). The increase in photosynthetic activity is associated with the synthesis and activity of chlorophylls, which allows the accumulation of carbohydrates, including sucrose. Previous reports on green bean indicate that increase in the leaf concentrations of Mg²⁺, Fe²⁺ and Zn²⁺ affect carbohydrate accumulation (Sálcido-Martínez et al., 2020; Balafrej et al., 2020), which is associated with the participation of these micronutrients in the metabolism of proteins linked to the synthesis and accumulation of chlorophylls (Korkmaz et al., 2018). In general, a gradual increase in the concentration of this soluble sugar was observed in this research. This could be due to the simultaneous need to accumulate and translocate sucrose as a source of carbon and energy to the shoots and pods, as part of the overall growth and development (García-Caparrós et al., 2021).

On the other hand, treating leaflets with ZnSO₄ or ZnO NPs did not affect the concentrations of TP or TFI. To date, there are no reports comparing the effects of ZnO NPs with ZnSO₄ on the accumulations of bioactive compounds in green beans. However, according to our results we infer that the application of ZnO NPs and ZnSO₄ did not significantly modify the physiological and biochemical mechanisms of the plants. The small differences found can be associated with the solubility characteristics of ZnSO₄, which when applied to leaves can drop rapidly; therefore, the bioavailability of Zn²⁺ ions over a prolonged period is uncertain (Doolette et al., 2018). In addition to its physical properties (size, structure and particle shape), previous studies with foliar applications of ZnO NPs varying between 1,000 mg $\cdot \text{L}^{-1}$

and 2,500 mg $\cdot \text{L}^{-1}$ on *Brassica nigra* and *Capsicum chinense* indicate a significant effect on FT and TFI concentrations (Zafar et al. 2016; García-López et al., 2019). On the other hand, Abbasifar et al., (2020) when applying 1,000 mg $\cdot \text{L}^{-1}$, 2,000 mg $\cdot \text{L}^{-1}$ or 4,000 mg $\cdot \text{L}^{-1}$ report a significant increase in the concentration of flavonoids in basil leaves (*Ocimum basilicum* L.). However, it is important to note that the ZnSO₄ and ZnO NPs doses applied in these studies exceed those used in our study by 10 times. According to Zafar et al. (2016), these could induce oxidative stress and stimulate the synthesis of non-enzymatic compounds (phenolic compounds, flavonoids, carotenoids, among others). It is known that under optimal conditions, Zn²⁺ plays an important role in the synthesis and activation of superoxide dismutase (SOD), the main enzyme associated with the removal of reactive oxygen species (ROS) (Korkmaz et al. 2018). This behaviour changes radically when the concentration of this micronutrient is at phytotoxic levels, causing oxidative stress (García-López et al., 2019). In this sense, the response to foliar Zn²⁺ application is a function of the species, nutrient status, phenological stage, dose supplied and number of applications (Shang et al., 2019; Abbasifar et al., 2020).

The applications of ZnSO₄ and ZnO NPs showed no effects on AC performance. This result was similar to that observed with foliar concentration of TFI (Table 2). In contrast, significant increases in AC values have been reported in other species, including basil (*O. basilicum* L.) (Abbasifar et al., 2020), potato (*Solanum tuberosum* L.) (Korkmaz et al., 2018) and tobacco (*Nicotiana tabacum* L.) (Mazaheri-Tirani et al., 2019). Korkmaz et al. (2018) report significant variations in AC resulting from the interaction between dose and time of application, suggesting that Zn²⁺ plays a key role in the synthesis and activation of the plant antioxidant system in mitigating oxidative stress (Balafrej et al., 2020).

Bioactive compounds in pods and seeds

The applications of ZnSO₄ and ZnO NPs showed no significant variation in relation to sucrose concentration in the pod and seeds of green bean. However, TFI concentrations were significant for the seed (Table 2).

Table 2. Concentrations of sucrose, TP, TFI and AC in pods and seeds of the green bean cv. ‘Strike’ by supplying ZnSO₄ and ZnO NPs.

Organ	Treatment	Sucrose (mg · g ⁻¹)	TP (mg GAE · 100 g ⁻¹)	TFI (mg CE · 100 g ⁻¹)	AC (% DPPH inhibition)
Pod	Control	10.87 a	1.96 b	0.061 a	12.36 b
	ZnSO ₄	10.89 a	1.80 b	0.074 a	18.60 a
	ZnO NPs	15.06 a	2.53 a	0.092 a	21.39 a
Seed	Control	12.90 a	2.18 b	0.109 a	71.15 a
	ZnSO ₄	14.92 a	2.16 b	0.077 b	70.96 a
	ZnO NPs	17.87 a	4.15 a	0.136 a	70.48 a

Data are expressed on a dry-weight basis. Values with the same letter within a column are not significantly different according to Tukey’s test ($p \leq 0.05$).

AC, antioxidant capacity; DPPH, 2,2-diphenyl-1-picrylhydrazyl; NPs, nanoparticles; TP, total phenols; TFI, total flavonoids; ZnO NPs, zinc oxide nanoparticles.

These results may be related to the dose and number of Zn²⁺ applications. Usually, the application of Zn²⁺ increases the concentrations of nutritional and bioactive compounds, however, information on the effects of applications of ZnSO₄ and ZnO NPs on the concentrations of sucrose and TFI in pods and seeds of green bean is limited. One of the factors offered to explain the high consumption of green beans is their high nutritional value, mineral, fibre, protein, carbohydrate and vitamin contents (Minnocci et al., 2018). However, this crop is highly susceptible to Zn²⁺ deficiency in soils with high carbonate contents (Doolette et al., 2018; Souiri and Aslani, 2018). There is evidence only for leaves, as indicated by Fatollahpour-Grangah et al. (2020); when applying ZnO NPs (1.5 mg · L⁻¹) on pinto beans – cvs ‘Coosha’, ‘Ghaffar’ and ‘Sadri’ – significantly increased the concentrations of soluble sugars. Abbasifar et al. (2020) report a significant increase from 11.09 mg · g⁻¹ to 25.27 mg · g⁻¹ DW in leaf concentration of TFI by applying 1,000 mg · L⁻¹ and 4,000 mg · L⁻¹ of ZnO NPs, respectively.

Plant defence systems include antioxidant enzymes (peroxidases, SOD, catalase and ascorbate peroxidase) and antioxidant compounds (phenols, flavonoids, glutathione, carotenoids, among others) that can inhibit or reduce the production and effects of ROS (Ahmadi et al., 2019; Abbasifar et al., 2020; Hatamian et al., 2020). In this study, ZnO NPs sprays affected TP values in pods and seeds; however, only in the pods was the AC significantly affected with both Zn²⁺ sources (Table 2). Recent studies have demonstrated the positive effects of Zn²⁺ application in the form of ZnO NPs and conventional ZnO on the AC of tomato (*Solanum lycopersicum* L.) (Akanbi-Gada et al., 2019) and habanero pepper (*Capsicum chinense* Jacq.) fruits (García-López et al., 2019). On the other hand, it has been observed that the application of ZnO NPs alone promotes the generation of ROS near the particle surface; however, their lifetime is short and they are eliminated by antioxidant agents (Pedruzzi et al., 2020). In this experiment, a low dose of Zn²⁺ (ZnSO₄ and ZnO NPs) was used, which did not allow observation of the characteristic symptoms of phytotoxicity (necrosis and reduction of photosynthetic

activity in leaflets), which confirms the importance of dose, number of applications and plant species to obtain positive effects on the physiology and biochemistry.

P. vulgaris L. is one of the most important crops for human nutrition worldwide (Ewais et al., 2017). In this study, applications of ZnSO₄ and ZnO NPs significantly increased both biomass (79.10 g · p⁻¹ and 84.70 g · p⁻¹ DW) and yield (55.64 g · p⁻¹ and 53.80 g · p⁻¹ FW) compared with the control (Figure 3). In this regard, it is important to note that Zn²⁺ is a micronutrient that plays important roles in photosynthesis, because it alters the chlorophyll concentrations and modifies (Ewais et al., 2017; Pullagurala et al., 2018; Shang et al., 2019). These results agree with those of Ponce-García et al. (2019) who, when evaluating soil applications of 25 mg · L⁻¹ (ZnO NPs) and 50 mg · kg⁻¹ (ZnSO₄) reported increases of 42% and 35% of biomass respectively, compared with the control. On the other hand, the yield values were found to exceed those reported by Ponce-García et al. (2019) when applying soil doses of 50 mg · kg⁻¹ and 25 mg · kg⁻¹ of ZnSO₄ and ZnO NPs, respectively. It has been reported that the efficiency of NPs is associated with the particle size, chemical structure, surface area covered and application rate (Aguilar-Carpio et al., 2018; Rossi et al., 2019; Ghidan et al., 2020). However, in this study the application of ZnO NPs and ZnSO₄ showed similar behaviours. These behaviours may be associated with the solubilities and stabilisation of the acidic pH of the solution presented by ZnSO₄ (Minnocci et al., 2018).

CONCLUSIONS

Foliar application of ZnO NPs increased the concentration of Zn²⁺ in leaflets, stems, roots and fruits. It also improved the foliar concentration of chlorophyll (*a* and *b*), without variation in carotenoids and TAN. ZnSO₄ was significant for sucrose, however, it did not exceed what was observed with the application of ZnO NPs. ZnO NP spraying affected the total phenolic values in pods and seeds, while AC was significant with both Zn²⁺ sources but only in pods. Plants treated with ZnSO₄ and ZnO NPs accumulated a higher biomass (79.1 g · p⁻¹ and 84.70 g · p⁻¹ DW) and yield

(55.64 g · p⁻¹ and 53.80 g · p⁻¹ FW). Foliar application of ZnO NPs could be an agronomic management strategy for biofortification with Zn²⁺ in green bean ‘Strike’ production.

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AUTHOR CONTRIBUTIONS

J.B.-D., O.C.-A., D.L.O.-B., E.S.-C., and O.A.H.-R. designed experiments and performed analytical measurements. J.B.-D., J.L.J.-C., D.L.O.-B., G.D.A.-Q., and P.P.-R. collection and statistical analysis of data. O.C.-A., D.L.O.-B., E.S.-C., and J.L.J.-C. writing and review of this paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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