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Research Article

Efficacy of the application of boron nanofertilizer on biomass, yield, nitrogen assimilation and photosynthetic activity in green beans

Cristina L. FRANCO-LAGOS[1], Esteban SÁNCHEZ[2*],
Alejandro PALACIO-MÁRQUEZ[2], Sandra PÉREZ-ÁLVAREZ[1],
Marina TERRAZAS-GÓMEZ[1], Octavio VILLALOBOS-CANO[1],
Carlos A. RAMÍREZ-ESTRADA[2]

[1]*Universidad Autónoma de Chihuahua, Facultad de Ciencias Agrícolas y Forestales, Km. 2.5 carretera a Rosales, Poniente, 33000 Delicias, Chihuahua, Mexico; francolarissa96@gmail.com; spalvarez@uach.mx; miterrazas@uach.mx; ovc@uach.mx*
[2]*Centro de Investigación en Alimentación y Desarrollo A.C. Unidad Delicias, Av. Cuarta Sur 3828, Fracc. Vencedores del Desierto. 33089. Delicias, Chihuahua, México; esteban@ciad.mx (*corresponding author); alexmarquezmj8@gmail.com; carlosramirez0021@gmail.com*

Abstract

Boron (B) nanofertilizers are an innovative alternative with great potential to make nutrient application more efficient and thereby improve crop growth and productivity. However, nowadays there is little literature on the effects of boron nanofertilizers on physiological and biochemical processes in plants. Therefore, the objective of the present research was to study the efficacy of foliar application of a boron nanofertilizer on biomass, yield, nitrogen assimilation and photosynthetic activity in green beans plants cv. Strike. The B nanofertilizer was foliar applied at 0, 25, 50 and 100 ppm. Biomass accumulation, yield, nitrate reductase enzyme activity, photosynthetic activity, stomatal conductance and photosynthetic pigment content were evaluated. The results obtained indicate that the application of B nanofertilizer at dose of 25 ppm were more effective in improving biomass, while the dose of 100 ppm favoured nitrate reductase activity and stomatal conductance. The results suggest that the application of B nanofertilizers stimulated the development of green bean plants. Finally, more studies are needed to evaluate the possible phytotoxic effects of high doses of B nanofertilizers and to compare their effects with conventional B fertilizers already used in the market.

Keywords: micronutrient; nanotechnology; nanoparticles; *Phaseolus vulgaris* L.

Introduction

Beans (*Phaseolus vulgaris* L.) belong to the Fabaceae family and are the third most important legume for human consumption, in addition to being an important source of protein, fiber, minerals and phenolic compounds for millions of people in developing countries (De Ron *et al.*, 2015). In Mexico, it is considered a strategic product, since it represents a whole tradition of production and consumption, fulfilling diverse functions both of a nutritional nature and for socioeconomic development. By 2020, Mexico was the eighth

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largest producer in the world with a production of 1,056,071 tons and an average yield of 700 kg.ha^{-1} (SIAP, 2021; Borja-Bravo and García-Salazar, 2022).

Currently, agriculture faces the challenge of ensuring food security without neglecting quality food, so it is important to optimize fertilization doses to maintain crop profitability, but in an environmentally sustainable way (FAO *et al.*, 2021; Caamal-Pat *et al.*, 2014). Micronutrients emerge as an alternative to this problem because, despite the small concentrations required, they are necessary to enhance yield and fruit quality (Tripathi *et al.*, 2015).

Among the micronutrients, boron (B) plays a fundamental role in the physiological development of plants, forming an essential part of the structural components of the cell wall (Shireen *et al.*, 2018; Mitra, 2015). In addition, it participates in the process of photosynthesis, sugar transport, uracil synthesis, pollination and fruiting. While its deficiency, affects chlorophyll content, photosynthetic activity, reduces nitrate reductase enzyme activity and fruit production (Shireen *et al.*, 2018; Moreno *et al.*, 2016). B requirements in plants range from 10 to 100 mg.kg^{-1}, and the range between deficiency and toxicity of this nutrient is very narrow (Reguera, 2009).

However, current fertilization techniques are unsustainable due to the high degree of contamination and degradation they cause in ecosystems, so sustainable alternatives must be sought to maintain agricultural productivity. A novel alternative is the use of nanotechnology, which in the form of nanofertilizers, can reduce nutrient losses and provide slow-release and localized fertilization, giving greater profitability to the producer and in turn reducing environmental problems (Romero-Méndez, 2018; Singh *et al.*, 2021).

Several studies indicate that the application of nanofertilizers increase plant growth, increasing chlorophyll content, photosynthetic activity, total biomass, yield, among other things (Zulfiqar *et al.*, 2019). Specifically, in a work with B nanoparticles, Meier *et al.* (2020) report increases in root and aerial biomass in lettuce and zucchini plants. Meanwhile, Davarpanah *et al.* (2016), when applying a B nanofertilizer found increases in the yield of pomegranate trees, and when combined with a zinc nanofertilizer increased the quality of the fruit. In turn, Ibrahim *et al.* (2019), obtained significant differences in plant height, number of pods and total yield when applying B nanoparticles foliarly in mung bean (*Vigna radiata* L.) plants.

Although nanotechnology is still an emerging field, in constant research and the studies that have been presented so far are not yet sufficient to determine that the use of this new technology is superior to the use of traditional fertilizers (Kah *et al.*, 2018). Based on the above, the objective of this research work was to study the efficacy of foliar application of a B nanofertilizer on biomass, yield, nitrogen assimilation and photosynthetic activity in green bean cv. ‘Strike’ plants.

Materials and Methods

Crop management

The study was conducted at the Centro de Investigación en Alimentación y Desarrollo (CIAD), in Delicias, Chihuahua, Mexico, geolocated at 28°10'21.6" N Latitude, 105°27'16.5" W Longitude, with an average altitude of 1280 masl, an average temperature of 29.4 °C and an average relative humidity of 22.38%. Seeds of green bean cv. Strike (*Phaseolus vulgaris* L.) were used. First, 4 bean seeds were sown in plastic pots with a capacity of 13.4 L, provided with vermiculite and perlite as substrate, in a 2:1 ratio, respectively. The pots were left exposed to field conditions. After germination, only 2 plants were left per pot. They were watered every third day with 500 mL of nutrient solution (Table 1), increasing the volume to 1000 mL once flowering arrived, and maintaining a pH 6.0 ± 0.1.

Table 1. Nutrient solution applied on the substrate (Sánchez *et al.*, 2004)

Macronutrients	Micronutrients
6 mM NH_4NO_3	1 µM $ZnSO_4$
1.6 mM K_2HPO_4,	5 µM Fe-EDDHA
0.3 mM K_2SO_4	2 µM $MnSO_4$
4 mM $CaCl_2$	0.25 µM $CuSO_4$
1.4 mM $MgSO_4$	0.3 µM Na_2MoO_4
	0.5 µM H_3BO_3

Experimental design and treatments

A completely randomized experimental design was used with 4 treatments of PHC ® Nano Boron at doses of 0, 25, 50 and 100 ppm and four replications, giving a total of 16 experimental units. The treatments were applied by foliar application every 10 days from the appearance of the first true leaves.

The commercial product applied was PHC ® Nano B, a liquid inorganic fertilizer with a B composition of 10% (w/v) based on ammonium borate.

Plant sampling

Once the plants reached physiological maturity, 60 days after the appearance of the first true leaves, plant samples were collected and 2 washes were performed for each sample. The first wash was carried out with distilled water and the second with tridistilled water. After washing, the plants were divided into four organs: root, stem, leaf and fruit. The material was separated into two parts, dry and fresh material. The dry material was used for the analysis of biomass in dry matter and physicochemical properties, while the fresh material was used for the determination of yield, biomass in fresh matter, and for the analysis of physiological and biochemical indicators (enzymatic activity).

Plant analysis

Biomass

The total biomass production in the different organs was determined. First, they were weighed fresh, immediately after being harvested, then they were subjected to a decontamination process, for which they were cleaned with abundant deionized water. Then they were placed in a drying oven (Shell) at a temperature of approximately 70-80 °C and until they were completely dried (16/24 h), to finally proceed to weigh and obtain the dry weight (g) of each organ.

Yield

Plant yield was expressed as mean fresh fruit weight per plant. Green pods collected from each plant were weighed at sampling. Total yield was reported as grams per plant.

Photosynthetic activity and stomatal conductance

Photosynthetic activity and stomatal conductance were measured in leaves when the plant reached physiological maturity (Kocal *et al.*, 2008). A portable LI-COR 6400 meter (Lincoln, Nebraska, USA) was used; a healthy leaf of homogeneous colour and free of damage was selected on each plant. A concentration of 400 µmol per mole of CO_2 was used in the reference cell, while the sample cell was maintained at approximately 380 µmol per mL of CO_2. The vapor pressure deficit of the sample chamber air was less than 1.5 and the block temperature that housed the leaf was 25 °C. Photosynthetic activity was expressed as µmol of CO_2 $m^2.s^{-1}$ and stomatal conductance was reported as mmol of CO_2 $m^2.s^{-1}$

In vivo Nitrate Reductase enzyme activity

The *in vivo* activity of the enzyme nitrate reductase (NR, EC 1.6.6.1) was determined by the method proposed by Sánchez *et al.* (2004). We weighed 0.1 g of fresh material in the form of 7 mm diameter leaf discs and placed them 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% (w/v) sulphanilamide in 1.5 M HCl and 2 mL of 0.02% (w/v) N-(1-naphthyl)-ethylenediamine dichlorohydrate in 0.2 M HCl were added. The resulting nitrite concentration was determined spectrophotometrically at 540 nm against a NO_2^- standard curve.

SPAD values

Chlorophyll readings were performed using the SPAD (Soil Plant Analysis Development) index, using the SPAD-502 portable chlorophyllometer, which quantitatively evaluates leaf green intensity (Cunha *et al.*, 2015). Measurements were taken at approximately midday (hours of high light intensity), obtaining five random measurements per experimental unit.

Photosynthetic pigments

They were analysed according to the methodology proposed by Wellburn (1994), for which leaf stalks 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 a reading was taken in a Genesis 10S UV-VIS spectrophotometer (Thermo Sientific, 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 according to the following formulas:

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

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

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

$Total\ chlorophyll = Chl\ a + Chl\ b$

Statistical analysis

An analysis of variance and a mean separation test were performed using the LSD method with a confidence interval of 95%, using the SAS statistical package (SAS, 2004).

Results and Discussion

Biomass

The application of B influences root elongation and intervenes in the synthesis of nucleic acids, proteins, amino acids, starch, auxins and phenols, so its deficiency causes the meristematic tissues to stop growing, affecting development and biomass accumulation (Lizarazo *et al.*, 2013). The results obtained in the present research work presented significant differences ($p<0.05$), highlighting the 25-ppm treatment, which obtained increases of 101.44% and 97.1% in relation to the control without application and the 100-ppm treatment, respectively (Figure 1).

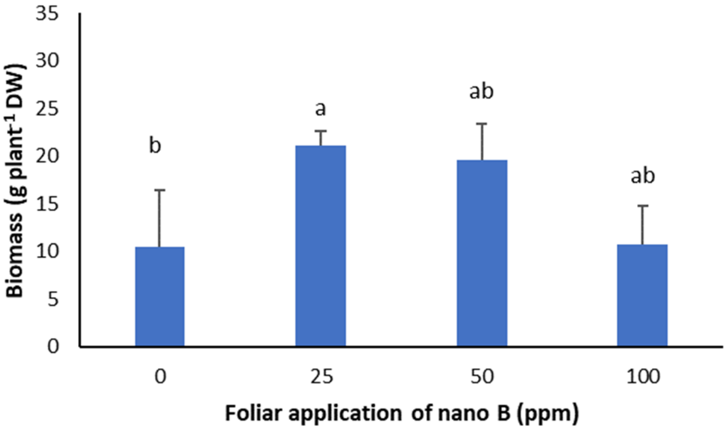

Figure 1. Effect of B nanoparticle application on biomass (dry weight) of bean plants cv. 'Strike'
Different letters indicate significant differences.

Yield

The function of B in plants has been related to the processes of cell division and growth, germination, hormone regulation, among others. Therefore, its deficiency affects the productivity of more than 132 cultivable species (Azcón-Bieto and Talón, 2013; Mitra, 2015). In the present research work, no significant differences were found for the yield variable ($p<0.05$), however, a decrease was presented when applying 100 ppm of nano B of 64.13, 61.02 and 54.16% in relation to the treatments of 50 ppm, 25 ppm and the control without application, respectively (Figure 2). The results indicate that, when a high dose of nano B is applied, both biomass and yield are reduced. Previous results reported by Ibrahim *et al.* (2019) indicate a similar trend by finding a decrease of 29.87% of *Vigna radiata* L. plants when they raised the dose from 90 to 180 ppm of B nanoparticles. These results can be explained because an increase in B content in plant tissues causes chlorosis in leaves affecting fruit development and yield (Marschner, 2011).

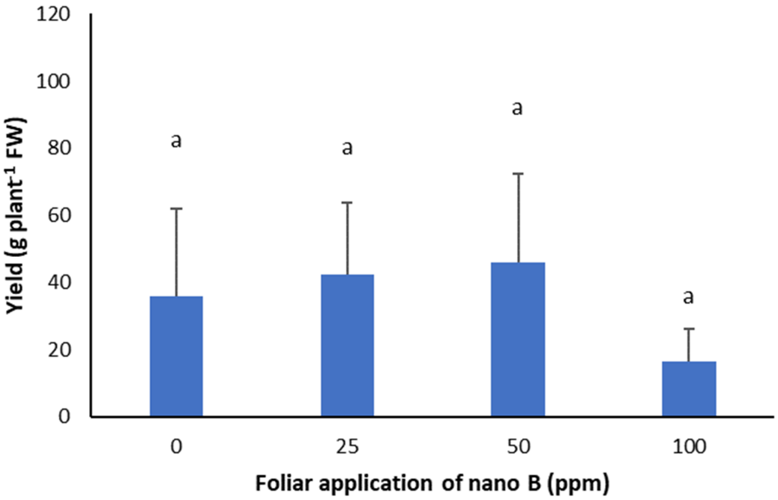

Figure 2. Effect of B nanoparticle application on the yield of bean plants cv. 'Strike'
Different letters indicate significant differences.

The application of B nanoparticles has obtained contradictory results in terms of crop yield, for example, Davarpanah *et al.* (2016), found an increase of 30.44% in the yield of pomegranate trees when applying a commercial brand B nanoparticle fertilizer at a dose of 6.5 mg L^{-1} foliarly. Genaidy *et al.* (2020), on the other hand, found slight increases in the yield of olive trees by applying doses of 20 ppm of B_2O_3 in the form of nanoparticles during two growing seasons, however, they found no statistical differences with respect to their control. On the other hand, Vishekaii *et al.* (2019), found increases of 229.78 and 157.57% relative to the control when applying 200 and 300 ppm of nano B in olive trees during two productive seasons.

Photosynthetic activity and stomatal conductance

B levels above or below the optimum range cause significant changes in plant metabolism, affecting photosynthetic capacity and overall crop quality (Bañón *et al.*, 2012). The results obtained in the present study do not indicate statistical differences ($p<0.05$) in response to the application of different doses of B for the photosynthetic activity variable. However, slight increases of 8.15, 4.68 and 15.9% were obtained for the doses of 25, 50 and 100 ppm respectively in relation to the control (Figure 3). On the other hand, stomatal conductance presented significant differences among treatments ($p<0.05$), finding an increase in the 100 and 50 ppm treatments of 107.14 and 35.71% respectively in relation with the control without application (Figure 4).

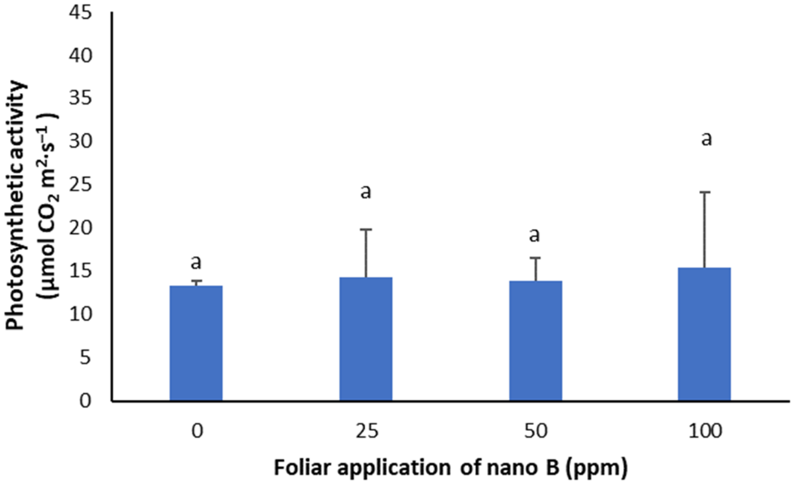

Figure 3. Effect of B nanoparticle application on photosynthetic activity of bean plants cv. 'Strike'
Different letters indicate significant differences.

These results agree with those mentioned by Andreon-Viçosi *et al.* (2020), who found no significant differences for photosynthetic parameters when B was foliarly applied as Borax in green beans plants; however, they obtained a trend like the results of the present study, since when they applied B at the flowering stage, they obtained slight increases as the B dose increased, both for photosynthetic activity and stomatal conductance.

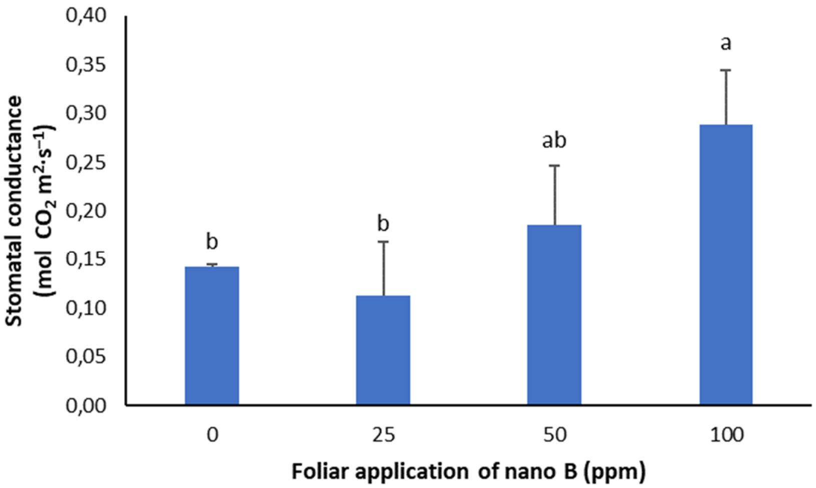

Figure 4. Effect of B nanoparticle application on stomatal conductance of bean plants cv. 'Strike'
Different letters indicate significant differences

Landi *et al*. (2012) reported that the absence of B in leaves of different crops negatively affects CO_2 assimilation, whereas high B levels can increase intracellular CO_2 content without affecting stomatal conductance. However, the results of B application on photosynthetic parameters remain contradictory, for example, Wang et al. (2011), found that as B dosage increased in pear trees, photosynthetic activity decreased as did stomatal conductance. Similarly, Bañón *et al*. (2012) found a decrease in photosynthetic activity and stomatal conductance when B excess was applied as fertigation in two species of ornamental shrubs.

Nitrate reductase activity

NR activity is considered an essential variable to understanding the nitrogen status in plants and the number of studies related to B levels are limited focusing on the deficiency status (Seth and Aery, 2017). The results obtained in the present research work indicate significant differences between treatments ($p<0.05$), finding increases of 465.16, 160.28% and for the 100 and 50 ppm treatments respectively in relation to the control (Figure 5).

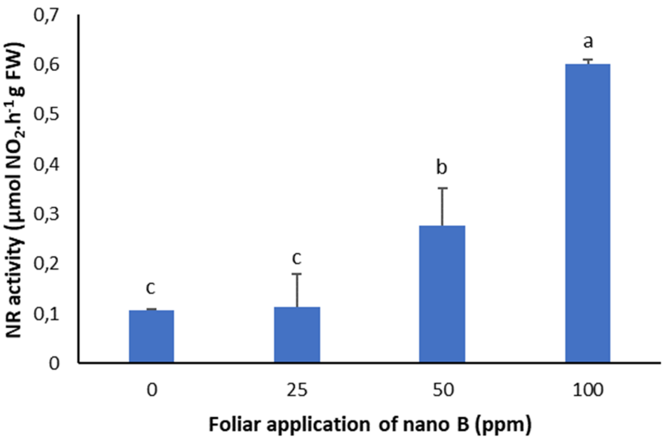

Figure 5. Effect of B nanoparticle application on nitrate reductase activity of bean plants cv. 'Strike'
Different letters indicate significant differences.

There is scarce evidence of the application of B nanoparticles on the NR activity parameter, however, foliar application of B has obtained results similar to those obtained in the present study. Bellaloui *et al.* (2010), found a significant increase in NR activity by applying B in the form of boric acid at a dose of 0.45 kg ha^{-1} on soybean plants. Similarly, Kumar *et al.* (2015) found statistical differences when applying B foliarly at doses of 0.4 and 0.8 ppm in seven rice genotypes. Similar results were reported when applying 2 kg of B edaphically per hectare in cowpea plants, obtaining an increase of 58.44% in relation to the control without application (Bhupenchandra *et al.*, 2021). According to Marschner (2011), B is related to nitrogen metabolism and both its deficiency and toxicity can affect the activity of the NR enzyme, and he also mentions that the effect of B on this enzyme can be mediated by an interruption of membrane transport processes.

Chlorophyll index

Chlorophyll index quantification is a quick and non-destructive way to detect chlorophyll levels in plants and thus form an idea of their nutritional status, in addition it is highly correlated with leaf chlorophyll content (Cunha *et al.*, 2015). In the present work, no significant differences ($p<0.05$) were found between treatments for any of the chlorophyll index readings. However, in both readings the treatment that obtained the highest values was the 25-ppm dose of B, obtaining slight increases of 14.88 and 23.46% in relation to the control without application for the pre-flowering and fruit filling readings, respectively (Table 2).

Table 2. Effect of B nanoparticle application on chlorophyll index in bean plants cv. 'Strike'

Reading	Foliar Application of nano B (ppm)			
	0	25	50	100
Pre-flowering	32.94 ±3.31 a	37.84 ±2.78 a	35.37 ±4.42 a	32.54 ±3.21 a
Pod filling	31.20 ±7.68 a	38.52 ±1.10 a	35.71 ±7.90 a	34.75 ±5.10 a

*Results are expressed in SPAD units. Different letters indicate significant differences.

The results obtained for the 25 and 50 ppm treatments on both dates agree with the range published by Medina-Pérez et al. (2018) of 35 to 50 SPAD units for *Phaseolus vulgaris* L. cv pinto. Similarly, Alves-Flores et al. (2018), obtained values between 35 and 39 SPAD units and found no differences between treatments when applying various B sources edaphically in common bean plants Cv BRS-Estilo. Regarding the application of B nanoparticles, the results are limited for beans, however, Sutulienė *et al.* (2022) found a similar trend to the results obtained in the present study, but in pea plants when applying commercial brand nano B, since the highest value of chlorophyll index was obtained with the smallest dose and as the dose increased the values decreased. Several authors have reported that the application of nanoparticles has a greater impact on crop development when smaller doses are applied compared to a traditional fertilizer (Singh *et al.*, 2021; El-Ramady *et al.*, 2018).

Photosynthetic pigments

The application of B to crops has been shown to significantly affect photosynthetic pigment levels, especially in plants deficient in this micronutrient (Keshavarz *et al.*, 2011; Genaidy *et al.*, 2020). In the present study, no significant differences were obtained for the chlorophyll variable ($p<0.05$), however, a similar trend was obtained with the chlorophyll index variable for the applied treatments, obtaining a decrease of 9.85 and 12.14% as the B dose was increased, but in this case the chlorophyll content was lower than the control without application (Figure 6). Andreon-Viçosi *et al.* (2020) report similar results, as they found no significant differences for total chlorophyll content when B was foliar applied as Borax on ejo bean plants, obtaining minimal variation between their treatments and the control without B application. A similar trend in pear trees was reported by Wang *et al.* (2011), who obtained a significant decrease of 37.60% as they increased the dose of B applied hydroponically, taking as control the dose of 10 ppm and increasing it up to 500 ppm. These results can be explained by the findings of Flores *et al.* (2018), which indicate that an increase in B fertilization has no effect on nitrogen metabolism, which is strongly related to chlorophyll content.

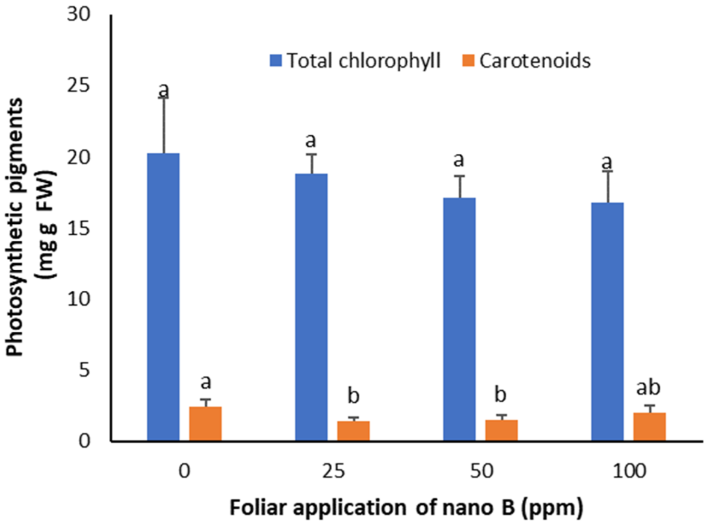

Figure 6. Effect of B nanoparticle application on photosynthetic pigment content of bean plants cv. 'Strike' Different letters indicate significant differences.

Regarding carotenoid content, in the present study significant differences were found between treatments ($p<0.05$), finding decreases of 42.3, 36.9 and 18.3% for the 25, 50 and 100 ppm treatments, respectively, in relation to the control (Figure 6). Studies where they evaluate carotenoid content under the effects of foliar application of nano B are very limited. However, similar trends have been found in other crops, for example, Genaidy *et al.* (2020) found significant differences in relation to their control and a decrease of 7.14% with their lowest dose of B in the form of B_2O_3 nanoparticles, and when they doubled the dose the carotenoid content increased by 35.9 %. For their part, Deswal and Pandurangam (2018) found no significant differences when applying B foliarly in maize plants. On the other hand, Wang *et al.* (2011) obtained a significant decrease of 22.2% as they increased the dose of B applied hydroponically; it should be noted that the B dose reached 500 ppm, which is considered phytotoxic for most crops.

Conclusions

Foliar application of B nanofertilizer at dose of 25 ppm were more effective in improving biomass, while the dose of 100 ppm favoured nitrate reductase activity and stomatal conductance of green beans plants cv. Strike. The results suggest that the application of nanofertilizers of B stimulated the development of the green beans plants. Finally, more studies are needed to evaluate the possible phytotoxic effects of high doses of B nanofertilizers and to compare their effects with conventional B fertilizers already used in the market.

Authors' Contributions

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

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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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