

Impact of the foliar application of potassium nanofertilizer on biomass, yield, nitrogen assimilation and photosynthetic activity in green beans

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Abstract

The agricultural areas of the world face problems that create difficulties when producing food and the excessive use of fertilizers is generating a negative environmental impact. An alternative that appears as a solution to this problem is the use of nanofertilizers. Within nanofertilizers an area of opportunity is the application of macronutrients, which report an increase in absorption efficiency of 19% compared to conventional fertilizers. Potassium (K) is one of the three macronutrients most used in agriculture and its deficiency affects key processes in plant development, limiting crop production. However, the number of publications where K is used as a nanofertilizer is limited, despite this, products in this form are already on the market. Therefore, the aim of this research work was to study the effect of the foliar application of K nanofertilizer on biomass, yield, nitrogen assimilation and photosynthetic activity in green beans cv. 'Strike'. K was applied in the form of a nanofertilizer in doses of 0, 50, 100 and 200 ppm. The biomass accumulation, yield, nitrate reductase enzyme activity, photosynthetic activity and photosynthetic pigments were evaluated. The dose of 100 ppm of K nanofertilizer obtained a higher accumulation of biomass, nitrate reductase activity, photosynthetic activity, SPAD values and total chlorophyll content. While the 200-ppm dose obtained a higher increase in yield. The results obtained suggest that the application of K nanofertilizers benefits the physiological development of plants. However, more studies are required to compare the application of nanofertilizers with traditional fertilizers.

Keywords: nanoparticles; nanotechnology; *Phaseolus vulgaris* L.; photosynthetic activity

Introduction

The common bean (*Phaseolus sp.*) belongs to the legume family (*Fabaceae*) and due to its nutritional contributions, mainly its high protein content, it is one of the legumes with the highest consumption in the

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world with a per capita consumption of 2.51 kg, reaching up to 11 kg per capita in rural areas of Central and South America (Lara-Flores, 2015; Ramírez-Jaspeado *et al.*, 2020; HelgiAnalytics, 2021). In Mexico the consumption of beans is high, however the consumption of green beans (green bean pod) is relatively low, this despite its high content of proteins, carbohydrates, fiber, calcium and vitamins. In addition, bean production in general is limited since it does not satisfy national needs (Salinas-Ramírez *et al.*, 2012).

In general, the agricultural areas of the world face a series of problems that generate difficulties when producing food, such as stagnation in crop yields, low efficiency in the use of nutrients, degradation of arable soils and the scarce availability of water. In turn, the excessive use of fertilizers is generating a negative environmental impact, destabilizing ecosystems, causing eutrophication and even some health-related problems (Chhipa, 2017; Raliya *et al.*, 2017). One of the alternatives that appear as a solution to these problems is the use of nanotechnology in agriculture, especially the use of nanofertilizers, which are defined as those nano-sized particles that provide one or more nutrients to the plant; nanofertilizers could be used as a more precise and effective option in order to increase crop yields and minimize the environmental impact generated by traditional fertilizers (Feregrino-Pérez *et al.*, 2018).

Within nanofertilizers an area of opportunity is the application of macronutrients in the form of nanoparticles. A study published by Kah *et al.* (2018) report an increase in absorption efficiency of 19% compared to conventional fertilizers that contain macronutrients. Potassium (K) is one of the three macronutrients most used in agriculture, it is also the most abundant cation inside plant cells and is key for osmoregulation, activation of various enzymes and the transport of nutrients through membranes, so its deficiency affects key processes in plant development such as photosynthesis, protein synthesis and consequently limits crop production (Marschner, 2011; Wang and Wu, 2013).

In a study carried out with K nanoparticles in alfalfa plants (*Medicago sativa* L.), increases in growth and mineral content were found, as well as an increase in the response mechanisms to stress-related problems (El-Sharkawy *et al.*, 2017). For their part, Asgari *et al.* (2018), applied a K nano fertilizer to daffodil plants (*Narcissus tazetta* L.), finding significant differences in biomass production, water absorption and anthocyanin content in the petals. However, according to Liu and Lal (2015), the number of publications where K is used as a nanofertilizer is limited, despite this, products in the form of K nanofertilizers are already on the market. Based on the above, the aim of this research work was to study the effect of foliar application of K nanofertilizer on biomass, yield, nitrogen assimilation and photosynthetic activity in green beans cv. 'Strike'.

Materials and Methods

Crop management

The research work was carried out in a greenhouse covered with shade mesh located at the Center for Research in Food and Development located in Delicias, Chihuahua, México, with an average temperature of 29.4 °C and an average relative humidity of 22.38%. Green bean seeds cv. 'Strike' (*Phaseolus vulgaris* L.) were sown, in plastic pots with a diameter of 30.5 cm and a volume of 13.4 L, filled with inert vermiculite and perlite in a 2:1 ratio. Two plants were placed per pot, these were watered with 500 ml of the following complete nutrient solution: 6 mM NH₄NO₃, 1.6 mM K₂HPO₄, 0.3 mM K₂SO₄, 4 mM CaCl₂, 1.4 mM MgSO₄, 5 μM Fe-EDDHA, 2 μM MnSO₄, 0.25 μM CuSO₄, 0.3 μM Na₂MoO₄ y 0.5 μM H₃BO₃; which was applied every third day for the first 30 days and daily for the next 30 days, maintaining a pH 6.0 ± 0.1.

Experimental design and treatments

A completely randomized experimental design was used with four treatments of PHC® NANO K in doses of 0, 50, 100 and 200 ppm with 4 repetitions each, generating a total of 16 experimental units.

The material applied as nanofertilizer was the commercial product PHC® NANO K, which is a suspension, where K is available in ionic form with nanoparticle size between 500 and 2000 nanometers, which

allows it to be quickly absorbed by the plant. The treatments were applied foliarly every 10 days from the appearance of the true leaves.

Plant sampling

At the physiological maturity of the plants, 60 days after germination, the plant samples were collected and rinsed twice, once with distilled water and the second with tri-distilled water; the material was divided into two parts, dry material, and fresh material. The dry material was used for the analysis of biomass in dry matter, while the fresh material was used to determine the yield, biomass in fresh matter, and the analysis of physiological and biochemical indicators (enzymatic activity).

Plant analysis

Biomass

The total biomass production of the different organs was determined, they were first weighed fresh at harvest time, later they were subjected to a decontamination process, and for this they were cleaned with abundant deionized water. Then, they were introduced to a drying oven (Shell) at a temperature of 70/80 °C and until they had completely dried (16/24 h), to finally proceed to weigh and obtain the dry weight (g) of each organ.

Yield

The plant yield was expressed as the average weight of fresh fruit per plant. The green pods collected from each plant were weighed at sampling. Total production was reported as grams per plant.

Assay of the enzymatic activity of nitrate reductase (EC 1.6.6.1)

In a reduction process, the enzyme Nitrate Reductase transforms nitrates (NO_3^-) into nitrites (NO_2^-). To quantify it, the method proposed by Sánchez *et al.* (2004) was used. The leaves were cut into cylindrical sections of 5 mm in diameter and the sample was placed in 10 ml of incubation buffer (10 mM of 100 mM potassium phosphate buffer, pH 7.5) and 1% (v/v) of propanol. The samples were infiltrated, and the intracellular spaces of the tissues were washed with buffer using a vacuum (0.08 MPa). After five minutes, the vacuum was released, and the samples were re-evacuated; They were incubated at 30 °C in the dark for one hour and then placed in boiling water to interrupt Nitrate Reductase (NR) activity. NO_2^- were determined by spectrophotometry at 540 nm in a reaction mixture consisting of 2 cm³ of extract, 2 cm³ of 1 % sulfanilamide (m/v) in 1.5 M HCl and 2 cm³ 0.02 % (m/v) N-(1-Naphthyl dihydrochloride)-ethylenediamine in 0.2 M HCl (NR + NO_3^-), following the same method but using a modified incubation buffer containing 50 mM KNO_3 . The NR induced by NO_3^- and Mo (NR + NO_3^- + Mo), and the NR induced by NO_3^- and Mo (NR + NO_3^- + Mo), were also determined using a modification of the incubation buffer containing 20 mM NaMoO_4 and KNO_3 plus 50 mM NaMoO_4 20 mM, respectively. The resulting nitrate concentration was also determined spectrophotometrically.

Photosynthetic activity

Photosynthetic activity was measured in leaves when the plant reached its physiological maturity (Kocal *et al.*, 2008). A portable LI-COR 6400 meter (Lincoln, Nebraska, USA) was used, in each plant a healthy leaf of homogeneous colour and free of damages was selected. 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 air in the sample chamber was less than 1.5 and the block temperature that housed the sheet was 25 °C. Photosynthetic activity was expressed as μmol of CO_2 $\text{m}^2 \cdot \text{s}^{-1}$.

Photosynthetic pigments

The method explained by Wellburn (1994) was used for the extraction and quantification of leaf pigments. Therefore, 10 leaf discs of seven millimetres in diameter of fresh photosynthetic plant material were required. 10 ml of pure methanol (CH₃OH) were added. The samples were incubated for 24 h in the dark at room temperature. After, the absorbance was measured at 666 nm (chlorophyll a, chl a), 653 nm (chlorophyll b, chl b) and 470 nm (carotenoids). A blank containing only methanol was prepared. Pigment concentrations were expressed as mg.ml⁻¹ fresh weight.

SPAD values

The chlorophyll index was obtained by means of a SPAD 502 equipment (Konica Minolta Sensing, Inc., Osaka, Japan) for which 5 completely expanded and healthy leaves were taken for each plant with 4 plants per treatment. The results obtained were expressed in SPAD units (Shrestha *et al.*, 2012).

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

Biomass and production

The yield of a crop is generally related by the ability to accumulate biomass as fresh and dry matter in the organs of importance for the harvest (Barrientos-Llanos *et al.*, 2015). In the present study, no significant differences were observed between the different doses of K nanofertilizer on biomass (Figure 1). However, the dose of 50 ppm favoured the accumulation of biomass, obtaining increases of 23.05% and 1.18% with respect to the doses of 200 ppm and 100 ppm of K nanofertilizer, respectively. In addition, the three doses applied obtained increases above 35.6% in relation to the control without application.

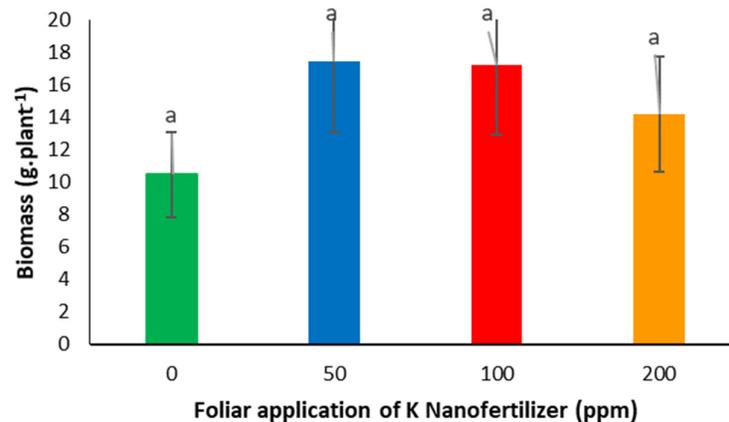


Figure 1. Effect of the foliar application of potassium nanofertilizer on the total biomass in dry weight of green bean plants cv. 'Strike'

Different letters indicate significant differences.

Previously published results do not use K in the form of nanoparticles individually; However, there are studies such as those published by Zahedifar and Najafian (2017), which obtained increases in biomass of more than 40% in relation to the control without application, when combining nano K in low doses with biochar

applied in an edaphic way in basil plants. In turn, Hasaneen *et al.* (2016), found that when applying NPK nanofertilizer formulations in combination with chitosan at low doses in French bean plants, they obtained increases in relation to the control without application, but when increasing the concentrations, the values decreased below the control, concluding that nanofertilizers perform was better at small doses.

Regarding the effect of foliar application of K nanofertilizer on production, the data obtained in the present research work for the production of pods based on fresh weight did not obtain significant differences between treatments (Figure 2). Unlike the biomass variable, in the case of production, the dose of 50 ppm is below the control treatment with a decrease of 5.36%, while the outstanding treatments are 100 and 200 ppm of K nanofertilizer with increases of 42.86% and 102.24% respectively in relation to the control without application. Despite the favourable results obtained for the high doses of Nano K, the results did not exceed the general average for 'Strike' beans of 54.24 g reported by Salinas-Ramírez *et al.* (2012).

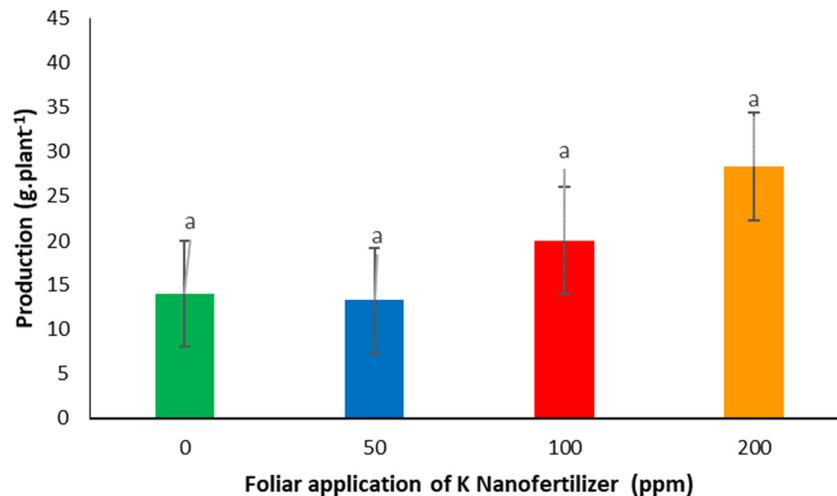


Figure 2. Effect of the foliar application of potassium nanofertilizer on the production in fresh weight of green bean plants cv. 'Strike'
Different letters indicate significant differences.

Unlike our study, Abdel-Aziz *et al.* (2018), found that the yields in wheat plants produced in clayey and clayey-sandy soils were higher when the doses of nanofertilizers containing the 3 main macronutrients (nitrogen, phosphorus, and potassium) were lower and tended to decrease when the dose was increasing. For their part, Farnia and Ghorbani (2014) found better results when they applied intermediate doses of a commercial K-based chelate nanofertilizer (Khazraa K[®]) in red bean plants and, like our study, obtained the lowest yields. at the lowest dose. Various studies show that fertilization with nanofertilizers favours plant performance, and as an advantage it was found that, compared to conventional fertilizer, the loss of nutrients is substantially reduced (Attia *et al.*, 2016).

Nitrate Reductase enzyme activity "in vivo"

Nitrate reductase activity is an enzyme that participates in nitrogen assimilation, since it is responsible for reducing NO₃⁻ to NO₂⁻ (Maldonado, 2013). The results obtained for the nitrate reductase enzyme activity do not show significant differences (Figure 3), being the dose of 100 ppm the only one that obtained values above the control without application with an increase of 2.16%. In general, the application of K nanofertilizer in the present study did not show great variations for this variable, despite the fact that K plays a fundamental role in the synthesis of the enzyme nitrate reductase (Hasanuzzaman *et al.*, 2018).

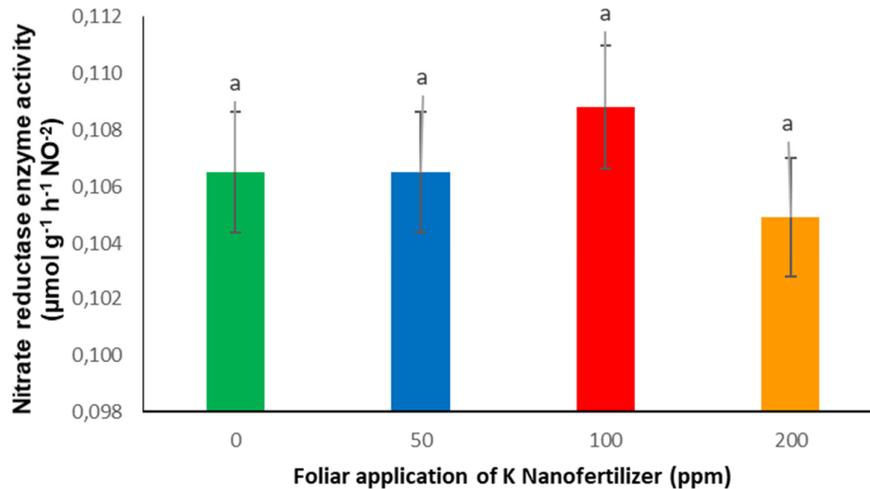


Figure 3. Effect of foliar application of potassium nanofertilizer on nitrate reductase enzyme activity in green bean plants cv. 'Strike'
Different letters indicate significant differences.

The results obtained agree with those published by Lavres-Junior *et al.* (2010), who found that applying K individually in guinea grass plants did not generate large changes in the enzymatic activity of nitrate reductase, but when combined with nitrogen the activity increased significantly. Similarly, Jabeen and Ahmad (2011), found that when applying potassium nitrate in sunflower plants the levels of the enzyme nitrate reductase increased even under conditions of saline stress, so in future research it is advisable to combine the application of nano-fertilizer of K with some nitrogen source.

Photosynthetic activity

Potassium is essential for the photosynthesis process to take place because a K deficiency reduces the leaf area, which leads to a lower photosynthetic rate and therefore a lower production of photosynthetic assimilates (Hasanuzzaman *et al.*, 2018). In the present research work, significant differences were obtained in photosynthetic activity due to the application of different doses of K nanofertilizer (Figure 4), finding increases of 5.20%, 25.76% and 42.69% when 100 ppm, 50 ppm and 200 ppm were respectively applied in relation to the control. Several studies indicate that the application of K increases the photosynthetic activity and the higher the application dose, the greater the net photosynthetic activity. Zahoor *et al.* (2017), found that when applying doses of 150 and 300 kg ha⁻¹ of K₂O in cotton plants, they increased net photosynthesis both in normal situations and under situations of water stress, reaching the highest values in the dose of 300. Likewise, Ibrahim *et al.* (2012) applied a range of 0-270 kg ha⁻¹ of K and obtained similar results, increasing net photosynthesis as the fertilization dose was higher.

Although, the use of potassium nanofertilizers and their effects on parameters related to photosynthesis has not been much studied, a trend in the general use of nanofertilizers is that high doses tend to affect plant development (Feregrino-Pérez *et al.*, 2018). This trend may explain the results obtained in our study, since increasing the dose from 100 to 200 ppm significantly reduced photosynthetic activity.

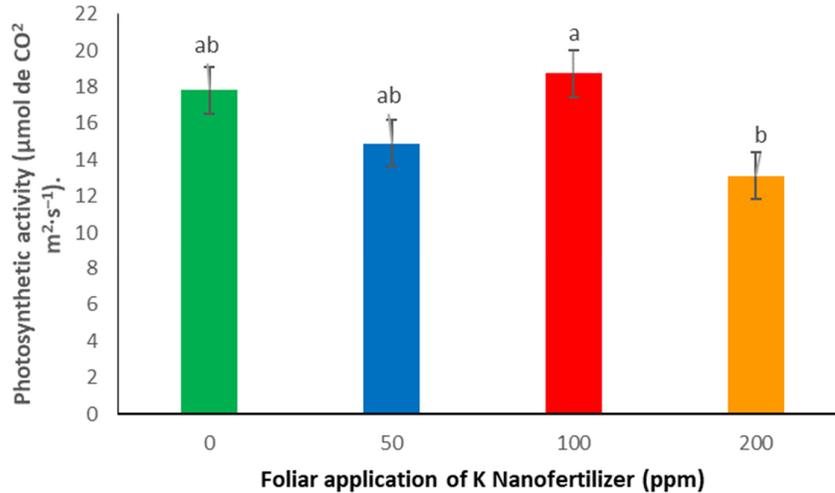


Figure 4. Effect of foliar application of potassium nanofertilizer on photosynthetic activity in green bean plants cv. 'Strike'
Different letters indicate significant differences.

SPAD values

The SPAD-502 quantitatively evaluates the intensity of the green of the leaf, measuring the light transmissions at 650 nm, where light absorption occurs by the chlorophyll molecule. It is a fast and non-destructive way to detect chlorophyll levels in plants and thus form an idea of its nutritional situation and is also highly correlated with the chlorophyll content in the leaf (Shrestha *et al.*, 2012; Cunha *et al.*, 2015). In the present study, two readings were made with the SPAD-502 meter, one on September 18, 2019 in the pre-flowering stage and the second measurement was made on October 7, 2019, corresponding to the filling of pods (Table 1). For both cases, no significant statistical differences were found.

Table 1. SPAD values of the application of Potassium nanofertilizer in green bean cv. 'Strike'. Reading 1, September 18, 2019 pre-flowering (Spad1); reading 2, October 7, 2019 (Spad2).

Treatment	Spad1	Spad2
0 ppm	32.94 a	31.20 a
50 ppm	36.30 a	35.12 a
100 ppm	36.71 a	31.55 a
200 ppm	32.51 a	36.31 a

Different letters indicate significant differences

In the first reading, the 50 and 100 ppm treatments reached values within the range of 35-50 units reported by Medina-Pérez *et al.* (2018), for bean plants (*Phaseolus vulgaris* L.); while the second reading the treatments of 50 and 200 ppm were those that were in the previously mentioned range. The second reading showed a decrease in 3 of the 4 treatments, this can be explained since the reading of the SPAD apparatus is performed on the leaf and the plant at the beginning of its reproductive process tends to move nitrogen to the demand organs, therefore the chlorophyll index may decrease (Cunha *et al.*, 2015).

Photosynthetic pigments

Photosynthetic pigments are substances capable of capturing light energy and transforming it into chemical energy through photosynthesis and are key to determining the physiological state of plants (Casierra-Posada *et al.*, 2011).

Chlorophyll

In the present study, the results obtained for the total chlorophyll parameter did not show significant differences (Figure 5), the doses of 50 and 100 ppm of K nanofertilizer obtained similar results and an increase in relation to the control of 1.05%, while the 200-ppm treatment obtained a decrease of 12.99%. The results are similar to those obtained by Al-Fahdawi and Allawi (2019), who applied 0.75 and 1.5 gL⁻¹ of K nanofertilizer in eggplant plants. It is worth mentioning that their results are below their control with traditional fertilization, while when combined with beneficial microorganisms, it increased significantly in relation to the individual application and to the control.

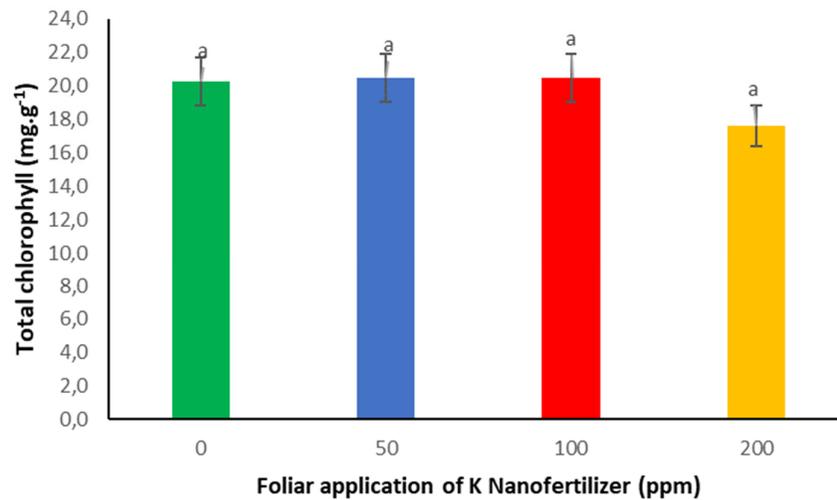


Figure 5. Effect of foliar application of potassium nanofertilizer on photosynthetic activity in green bean plants cv. 'Strike'

The foliar application of nano K and in general of nanofertilizers has been reported to contribute to the stimulation of metabolic activities and therefore increases the area of the leaves because it has a greater contact area which increases physical and chemical activity (Naderi and Abedi, 2012; Subbaiah *et al.*, 2016). Furthermore, Ahmad *et al.* (2018), mention that the K in the cell can help protect the structure of chlorophyll, so the application of this under stress situations can have positive effects on the content of chlorophyll and other variables related to the physiological development of the plants.

Carotenoids

The results obtained in the present research work for the carotenoids variable did not obtain significant differences (Figure 6). However, the results obtained in the present study indicate that the application of K nanofertilizer reduced the carotenoid content by 43.78, 53.86 and 35.51% for doses of 50, 100 and 200 ppm, respectively. The increase in carotenoids tends to be related to the stress of the plant and its state of physiological maturity (Bramley, 2013), which indicates that the control may have suffered stress during the development of the crop since the other variables do not indicate that it had an increase in physiological maturity. These results agree with those published by Adhikari *et al.* (2020), which obtained a decrease in the carotenoid content when they applied different forms of K in a foliar way in soybean plants, however, when the plants were under saline stress, the carotenoid content increased.

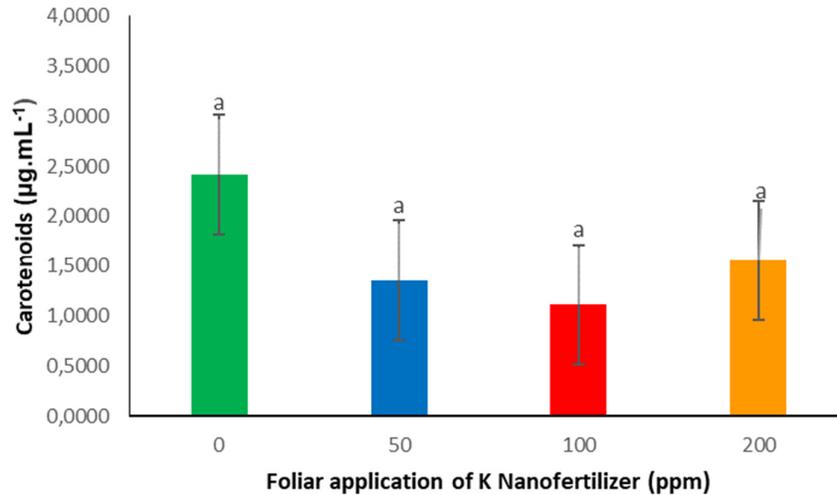


Figure 6. Effect of the foliar application of potassium nanofertilizer on the content of carotenoids in green bean plants cv. 'Strike'

Conclusions

The dose of 100 ppm of K nanofertilizer favoured the growth and development of bean plants cv. Strike, because it obtained a greater accumulation of biomass, nitrate reductase activity, photosynthetic activity, SPAD values and total chlorophyll content. On the other hand, the production variable obtained greater increases when the 200-ppm dose was applied. The results obtained in the present research work suggest that the application of K nanofertilizers benefit the physiological development of the plants since in all the variables evaluated there was a dose that had an increase in relation to the control. However, more studies are required where the application of nanofertilizers is compared with traditional fertilizers, in addition to working with doses between 100 and 200 ppm to find the ideal dose for the development and production of green bean plants cv. 'Strike'.

Authors' Contributions

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