

Impact of the foliar application of magnesium nanofertilizer on physiological and biochemical parameters and yield in green beans

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Abstract

One of the most significant challenges humanity will face is food production. In order to preserve the output, mineral fertilizers are essential. However, it's not a suitable option in the long term. Magnesium is a crucial macronutrient, but it is the most limiting element in agriculture. Nanotechnology, with the implementation of nanofertilizers, is an excellent alternative since it provides nutrients, supports growth, and improves production; this in low amounts is more sustainable than conventional fertilizers. Although there is a piece of limited information regarding the proper foliar application of this macronutrient, the study helped to validate the effect of the foliar application of Magnesium nano fertilizer on the physiological, biochemical responses and yield of bean plants. Bean plants ejotero cv. 'Strike' and magnesium nanoparticles were applied at doses of 0, 50, 100, and 200 ppm. The biomass accumulation, yield, activity of the enzyme nitrate reductase, and photosynthetic pigments were evaluated. The foliar application of Mg nanoparticles at 50 ppm generated the highest amount of biomass and photosynthetic pigments. The 100 ppm dose improved pods yield and allowed the increased activity of the Nitrate Reductase enzyme. The results obtained suggest that, when increasing the dose of magnesium in plants, the amount of carotenes decreases.

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

Introduction

The common bean (*Phaseolus vulgaris* L.), belonging to the Fabaceae family, is the most important legume for direct consumption in the world, mainly for the South American and African zones (de la Fuente

et al., 2012); It is found in tropical and subtropical regions (Cardona *et al.*, 1995). Beans are grown primarily to be consumed as dry grain and to a lesser extent it is used for food such as fresh seeds and tender pods (Silbernagel *et al.*, 1991; Esquivel *et al.*, 2006). In Mexico for the year 2018, about 66 % of bean production was concentrated in the states of Zacatecas, Sinaloa, Durango, and Chihuahua, occupying the third position in importance for the area planted and the thirteenth position for the value of the national agricultural production, with a total yield of 1,196 million tons (FIRA, 2019).

However, its large-scale application as a chemical fertilizer to increase the productivity of crops is not an appropriate option in the long term, since although, on the one hand, they increase the production of the crop, on the other they disturb the mineral balance of the soil and decrease fertility; Likewise, the use of most macronutrient fertilizers by the plant is very low due to its investment in insoluble form in the soil (Solanki *et al.*, 2015). Magnesium (Mg) is a key macronutrient, since it plays a crucial role in the manipulation of compounds such as ATP, RNA and DNA, however, it is one of the minerals lacking in human diets, positioning itself as the most limiting macronutrient in agriculture and to which less attention has been paid in recent decades (Shinde *et al.*, 2018). Magnesium is essential as a structural component or enzymatic cofactor in plants (Rathore and Tarafdar, 2015), substantial in the light and dark reactions of photosynthesis, because it is the central atom in the chlorophyll molecule (Singh *et al.*, 2017). Chlorosis of fully expanded leaves is the most visible symptom of Mg deficiency. Also, Mg deficiency results in a lower proportion of protein N. (Marschener, 2012). Therefore, foliar fertilization of mineral elements, like Mg, is an alternative approach to the use of fertilizers in the soil (White and Broadley, 2009); but this action supported by a growing science called nanotechnology.

Nanotechnology provides novel applications in biotechnology and agriculture, since the transformation of macromaterials to nano-sized particles leads to useful characteristics (Khan and Rizvi, 2014). For agriculture, it is a great alternative for sustainable practice, since it has developed nanofertilizers, these are materials that provide plants with one or more nutrients, support their growth and improve production (Liu and Lal, 2015). The application of nanofertilizers facilitates their absorption by plants due to their slow release, in addition to being effective in very low portions (Kaul *et al.*, 2012; Butt and Naseer, 2020). In recent years, the development and exploitation of nanofertilizers has gained much importance over traditional fertilizers due to their lower environmental impact macronutrient nanofertilizers have a high priority since they offer sustainable development by renewing management and conservation tactics with less waste of agricultural inputs, and in addition to increasing low levels of minerals, in particular Mg (Shang *et al.*, 2019; Butt and Naseer, 2020). In studies carried out with magnesium nanofertilizers in *Stevia rebaudiana* Bertoni, an increase in the production of steviosides was found; other research conducted on black-eyed pea or *Vigna unguiculata* ssp. (Delfani *et al.*, 2014), showed greater mobility and absorption capacity of the Mg nanofertilizer; the content of total chlorophyll in wheat or *Triticum aestivum* L. was improved (Rathore and Tarafdar, 2015). However, it has also been found that the excessive application of Magnesium can be detrimental to the membrane and can alter the functioning of the photosynthetic system (Shabala and Haiadi, 2005). Therefore, it is extremely important to carry out studies that compare the effect of different doses of magnesium nanofertilizer, since the literature on this research topic is scarce.

Despite the few studies, nanofertilizers are already in commercial form, so the aim of this study was to validate the effect of the foliar application of Magnesium nanofertilizer on the physiological, biochemical responses and yield of plants of green beans cv. 'Strike'.

Materials and Methods

Crop management

The study was carried out at the facilities of the Center for Research in Food and Development (CIAD), located in cd. Delicias, Chihuahua, Mexico. For the experiment, seeds of green beans (*Phaseolus vulgaris* L.), Var: 'Strike', a shrub plant with determined growth and straight pods were used, this variety was selected for

being were short-cycle and easy to handle. In the first place, four seeds were sown in plastic pots with a capacity of 13.4 L, provided with an inert substrate, vermiculite, and perlite, in a 2:1 ratio. The pots were left exposed to the environmental conditions of the field. After germination, only two plants were left per pot. These were watered every third day with 500 mL of nutrient solution, increasing the volume to 1000 mL once the flowering stage arrived, maintaining a pH of 6.0 ± 0.1 , and with the formulation presented in table 1.

Table 1. Formulation of nutritive solution, used by Sánchez (2006)

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

Experimental design and treatments

A completely randomized experimental design was used with four PHC® Nano Mg treatments at doses of 0, 50, 100 and 200 ppm and four repetitions each, generating a total of 16 experimental units. The treatments were applied by foliar application every 10 days from the appearance of the true leaves.

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

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, 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.

Nitrate reductase activity "in vivo"

In a reduction process, the enzyme Nitrate Reductase transforms nitrates (NO_3^-) into nitrites (NO_2^-). To quantify it, the method proposed by Sánchez (2006) 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 + NO₃⁻), following the same method but using a modified incubation buffer containing 50 mM KNO₃. The NR induced by NO₃⁻ and Mo (NR + NO₃⁻ + Mo), and the NR induced by NO₃⁻ and Mo (NR + NO₃⁻ + Mo), were also determined using a modification of the incubation buffer containing 20 mM NaMoO₄ and KNO₃ plus 50 mM NaMoO₄ 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 color and free of damages was selected. A concentration of 400 µmol per mole of CO₂ was used in the reference cell, while the sample cell was maintained at approximately 380 µmol per ml of CO₂. 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 CO₂ m²·s⁻¹.

SPAD values

Chlorophyll readings were performed using the SPAD index (Soil Plant Analysis Development), using the SPAD-502 portable chlorophyllometer, which quantitatively evaluates the intensity of the green of the leaf (Cunha, 2015). Measurements were made in hours of high light, obtaining five random measurements per experimental unit. Two readings were made with the SPAD-502 meter, one on September 18, 2019 corresponding to the pre-flowering stage and the second on October 7, 2019 (Figure 5), and equivalent to the filling stage of pods.

Photosynthetic pigments

The method explained by Wellburn (1994) was used for the extraction and quantification of leaf pigments. Therefore, 10 leaf discs of seven millimeters 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 this time, 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.

Statistical analysis

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

Results and Discussion

Biomass and production

Biomass is an indicator of the nutritional status of plants, since the yield of a crop is given by the ability to accumulate biomass in the organs that are destined for harvest (Peil and Gálvez, 2004). In the present experiment, significant differences were observed in the total biomass in dry weight (Figure 1), highlighting the foliar application dose of 50 ppm of Nano Magnesium with the highest total biomass, with an increase of 25%, 19% and 9% in relation to the Control and the doses of 200 and 100 ppm of Nano Magnesium respectively. The production of pods increased with the dose of 100 ppm, showing a higher level of average yield of 62%, in relation to the dose of 50 ppm where the minimum values were presented (Figure 2). For their part, the control plants and doses of 200 ppm were kept 52 and 51% below the level of the best dose, respectively.

The data obtained in the present work are very similar to those found by Stagnari *et al.* (2009), where foliar fertilization with magnesium in green beans (*Phaseolus vulgaris* L.) increased aerial and root biomass, as well as pod yield. In the research of Mahawa *et al.* (2017), where they worked with MgO nanoparticles in mung beans (*Vigna radiata* L.), the data were also similar, finding that the doses of 50 and 100 mg.L⁻¹ of magnesium nanoparticles stimulated performance. Insufficient levels of Mg, inhibit protein synthesis, generate lower content of chlorophyll and carotenoids, in addition to affecting the transport of assimilates to chloroplasts, sensitivity of demand organs and an increase in susceptibility to heat stress, among others; This leads to a decrease in performance (Mengel and Kirkby, 2000; Delfani *et al.*, 2014; Singh *et al.*, 2017). Several studies show that magnesium is an essential macronutrient in the photosynthetic process, likewise, an increase in its content increases the yield and quality of crops, in addition to being involved in carbohydrates and protein synthesis (Neuhaus and Mühling, 2014), as well as, in the translocation of assimilates towards sinks (Fischer and Bremer, 1993; Cakmak *et al.*, 1994; Fischer *et al.*, 1998; Shabala and Hariadi, 2005; Stagnari *et al.*, 2009).

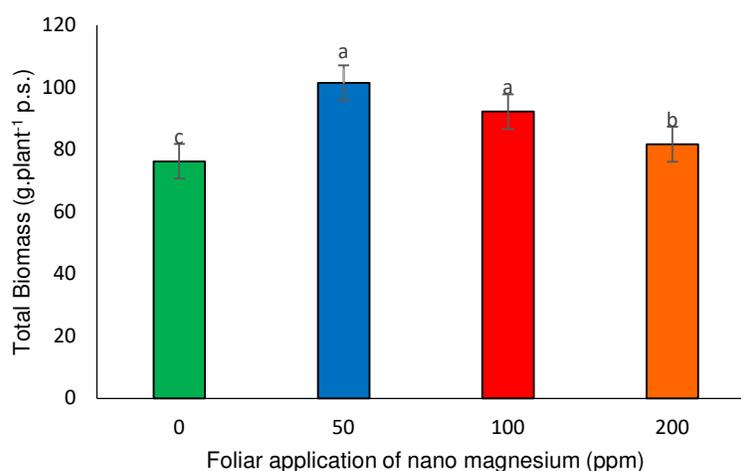


Figure 1. Total biomass in dry weight in the different treatments of Nano Magnesium via foliar route in bean plants cv. 'Strike'

The letters show significant statistical differences

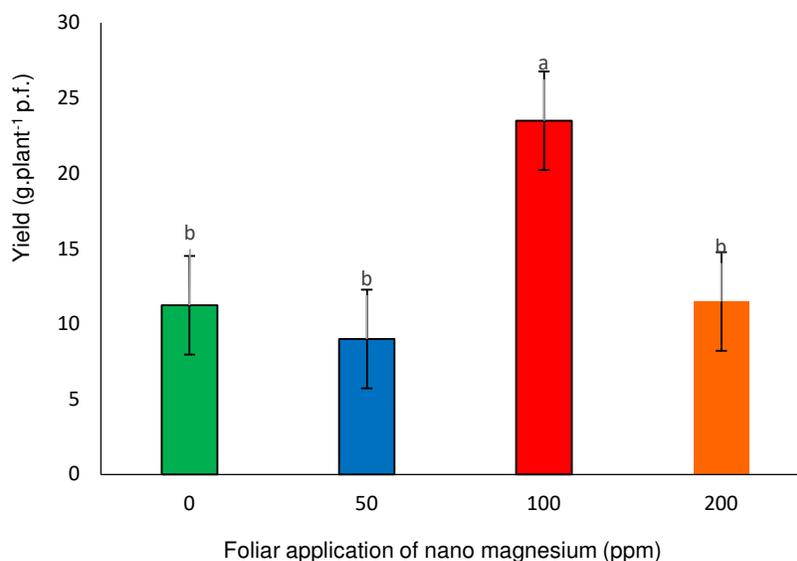


Figure 2. Effect of the application of Magnesium Nano fertilizer on the production of fruits in fresh weight in bean plants cv. 'Strike'
Letters show significant statistical differences

Nitrate reductase enzyme activity "in vivo"

Photosynthetic nitrate reductase activity is an enzyme that participates in the assimilation of nitrogen, since it is in charge of reducing nitrates (NO_3^-) to nitrites (NO_2^-); It has been postulated by researchers as an important indicator of nitrogen nutrition status (Villalobos and Carvajal, 1978; Ruiz and Azcón, 1996; Neubauer *et al.*, 1999; Maldonado, 2013; Meloni *et al.*, 2017). In the present investigation, no significant differences were observed between the studied doses (Figure 3) that could be explained because the control plant did not have an Mg deficiency; however, the data obtained by the 100-ppm dose stand out with respect to the others by an average of 5%. Meanwhile, Rathore and Tadarfar (2015) applied 20 ppm of magnesium nanoparticles in wheat and found significant results, increasing the nitrate reductase activity by 65%. The activity of the enzyme nitrate reductase in plants is correlated with growth and yield (Srivastava, 1980). When applying the 100-ppm dose, an important relationship was found between the nutritional status of magnesium and nitrogen assimilation. Marschener (2012) mentions that a good level of the macronutrient Magnesium generates a higher proportion of protein and activates the enzyme glutamine synthetase; this is important for the efficient use of nitrogen. The existence of abundant nitrogen contributes to the formation of chlorophyll, which increases photosynthetic activity and therefore plant development (Raigón *et al.*, 2006).

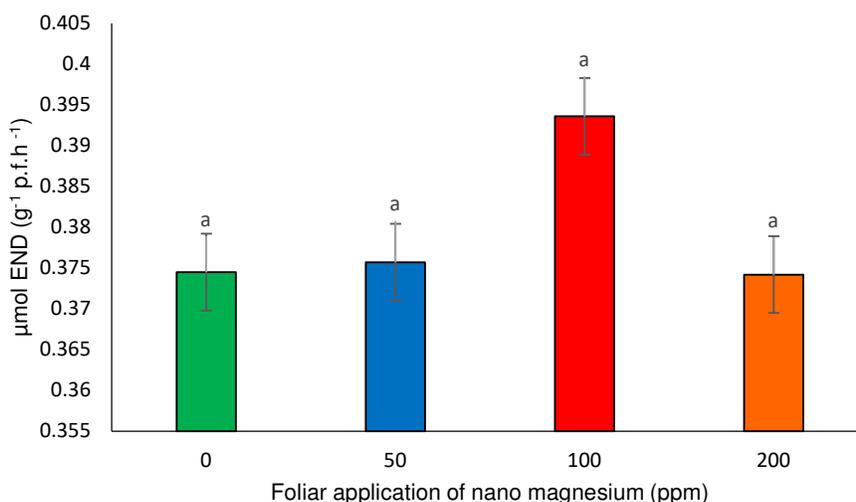


Figure 3. Endogenous Nitrate Reductase enzymatic activity because of the application of magnesium nanofertilizer in green bean cv. 'Strike'

Photosynthetic activity

Photosynthetic activity is a fundamental process for the development of crops because it is the main source of energy for plants, however, deficiencies in the magnesium supplement can affect this process and affect productivity (Azcón-Bieto *et al.*, 2008; Farhat *et al.*, 2016). In the present research work, no significant differences were obtained in terms of photosynthetic activity (Figure 4). However, the application of magnesium at doses of 50, 100 and 200 ppm obtained increases of 16.31%, 53.94% and 61.36% respectively, in relation to the control. Several studies mention that a deficiency of mg affects the photosynthetic activity of plants, because it acts negatively on the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) involved in the fixation of CO₂, also, it has been shown that Mg deficiency induces a lower performance of the electron transport chain involved in photosystems, this being a very important factor in the decrease in CO₂ uptake (Yang *et al.*, 2012; Farhat *et al.*, 2016). Finally, despite not obtaining the highest values in terms of photosynthetic activity, the dose of 100 ppm obtained a higher production of pods, which may indicate that the plants were starting a maturation process as reported by Chávez-Simental and Álvarez-Reyna (2012) indicate that photosynthetic activity in beans decreases as the harvest period approaches.

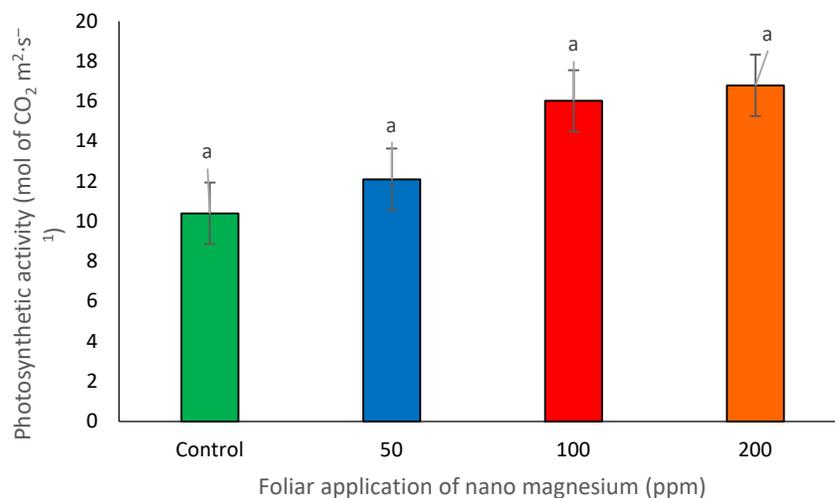


Figure 4. Photosynthetic activity because of the application of Magnesium nanofertilizer in green beans cv. 'Strike'

SPAD values

The SPAD-502 Minolta portable device measures the transmission of red light at 650 nm, and infrared light, at 940 nm, which is highly correlated with chlorophyll content (Markwell *et al.*, 1995; Guimarães *et al.*, 1999; Gil *et al.*, 2002). Magnesium is an essential element for chlorophyll, since it is found in the center of the tetrapyrrolic ring of the molecule (Romero, 1995). In the present study, for both cases, no significant statistical differences were found. However, it is observed that in the control the data were maintained; in the 100 ppm treatment there was a 3% drop; the 50 ppm treatment had an increase of 5%; and the 200 ppm dose increased by 12%. Possibly the variability between the data of the different doses was generated as a consequence of the sudden changes in the environmental conditions (drastic increases and decreases in temperature, heavy rains) that occurred during the establishment of the experiment, which could have generated this variability in the data. It is noticeable that, in all the treatments, the readings are lower than 40 units, which indicates, according to what Hendry and Price (1993) said, the beginning of a possible deterioration of the photosynthesis process, due to disturbances caused in the plants by environmental factors.

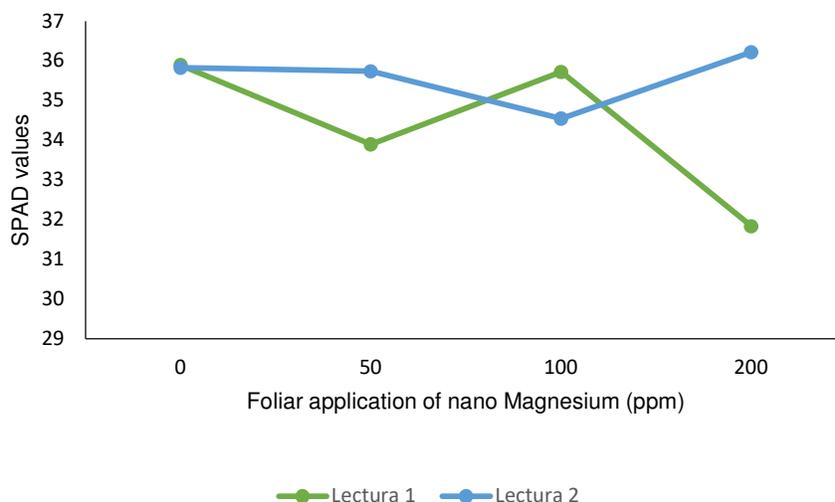


Figure 5. SPAD values, because of the application of Magnesium nanofertilizer in green bean cv. 'Strike' Reading 1, September 18, 2019 pre-flowering; reading 2, October 7, 2019 filled with pods

Photosynthetic pigments

The determination of the concentration of photosynthetic pigments is a useful parameter to evaluate the physiological state of plants (Casierra *et al.*, 2012).

Chlorophyll

The present study revealed that the content of chlorophyll "a", "b" and total in bean leaves (Figures 6, 7 and 8, respectively) is not statistically significant. However, a similar behaviour was observed in all three, with the highest data obtaining the dose with 50 ppm, followed by the dose of 200 ppm, and the lowest level was for the studies with 100 ppm and 0 ppm. These results are quite similar to those found by Mahawar *et al.* (2017), where the measurement of the chlorophyll "a" content in mung bean plants (*Vigna radiata* L.) exposed to concentrations of 50 and 100 mg.L⁻¹ revealed a significant increase. In turn, Neuhaus *et al.* (2013), found that in *Vicia faba*, both chlorophyll and photosynthesis concentrations increased after the application of Mg.

Magnesium is essential in the photosynthetic process (for both light and dark reactions), specifically for the activity of two important CO₂ binding enzymes, ribulose phosphate carboxylase and phosphoenolpyruvate carboxylase (Seftor *et al.*, 1986; Stagnari *et al.*, 2009; Romero, 1995). The increase in pigment content due to the foliar application of Nano Magnesium is explained as a consequence of an improvement in the size, structure and function of chloroplasts (including the transfer of electrons in photosystem II) (McSwaine *et al.*, 1976), as well as a favourable absorption of light, photosynthetic activities and a greater release of enzymes to achieve the mobilization of nutrients, resulting in a better absorption of these and therefore in higher yield. Emphasizing, magnesium is the structural component of chlorophyll and many coenzymes; a small variation in its level strongly affects key photosynthetic enzymes (Mitra, 2015).

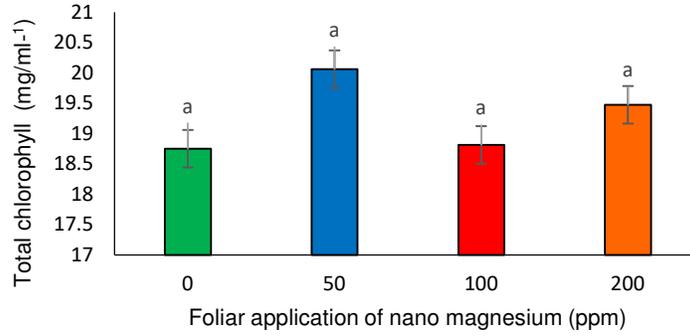


Figure 6. Total chlorophyll concentration in green bean leaf cv. 'Strike' as a result of foliar application of Magnesium Nanofertilizer

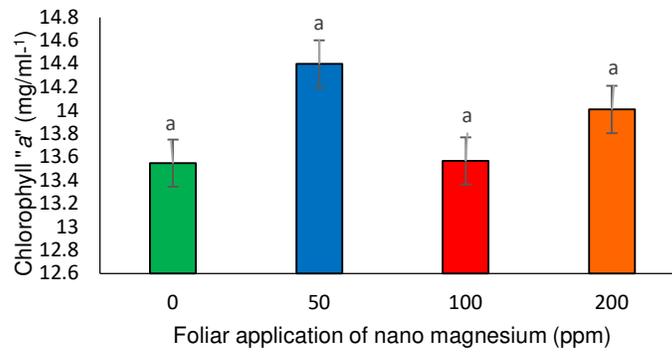


Figure 7. Chlorophyll concentration "a" as a result of foliar application of Magnesium Nanofertilizer on green bean leaf cv. 'Strike'

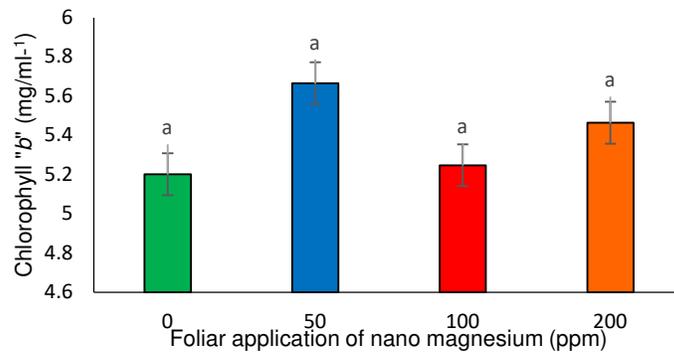


Figure 8. Chlorophyll "b" concentration in green bean leaf cv. 'Strike' as a result of the foliar application of Magnesium Nanofertilizer

Carotenes

In the case of carotenes (Figure 9), significant statistical differences were found, observing a decrease as the dose was higher, obtaining the dose of 200 ppm, 38 % disadvantage with respect to the control. Meléndez

et al. (2004) mention that these pigments are found in the chloroplasts of green tissues, where they are masked by chlorophyll until the tissue ages; the carotenoid content increases and the chlorophyll content decreases during the maturation of the plants. This means that, by increasing the dose of magnesium in the plants, the amount of carotenoids decreased, since the plants were less stressed against oxidative and photolytic damage, compared to the control that lacked Magnesium. Which agrees with what was said by (Casierra *et al.*, 2012) in their work with calendula leaves, where the higher values found in the carotene/chlorophyll ratio, under high lighting conditions, would be the reflection of a greater synthesis of carotenes than chlorophylls, in order to protect tissues from oxidative stress caused by highly reactive oxygen radicals (ROS). These pigments fulfill the function of being photon collectors and act as accessory pigments in photosystems and as photoprotective agents that limit the damaging effects of high lighting (Johnson *et al.*, 1993).

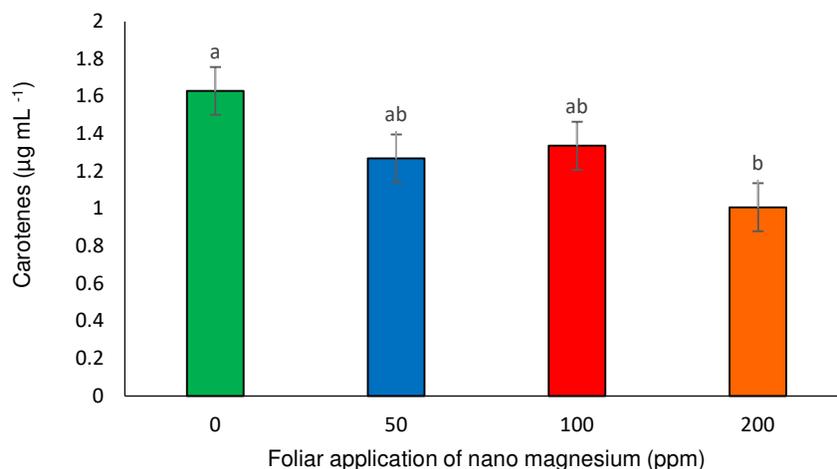


Figure 9. Carotene content as an effect of the application of Magnesium nanofertilizer in green bean cv. 'Strike'

The letters show significant differences

Conclusions

The magnesium nanofertilizer doses that allowed obtaining the best parameters were 50 and 100 ppm. The first generated the highest amount of biomass and photosynthetic pigments, while the dose of 100 ppm had better pods yield and a higher activity of the nitrate reductase enzyme, in addition to a higher photosynthetic activity. The present research work sought the dose of Nano Magnesium that offered a better physiological and production response, so the most appropriate application would result in 100 ppm. However, an investigation is suggested where a dose between 50 and 100 ppm is included, in order to find the most appropriate dose, and work with a dose greater than 200 ppm to find the values that are phytotoxic.

Authors' Contributions

Conceptualization: ES; Methodology: ASM, NIAO, APM; Validation: LPLT, SPA; Formal analysis: APM, PPR; Investigation: ASM, APM, NIAO; Data curation: ES, PPR; Funding acquisition: ES; Project administration: ES; Writing: ASM, ES; Review and editing: ASM, APM, ES; All authors read and approved the final manuscript.

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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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