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Research Article

Biofortification with magnesium nanofertilizer on bioactive compounds and antioxidant capacity in green beans

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Abstract

The use of nanofertilizers has the potential to be used to enrich edible organs with nutrients (biofortification) and improve the biosynthesis of bioactive compounds and their antioxidant capacity. Therefore, the objective of this study was to evaluate the effect of biofortification with magnesium (Mg) nanofertilizer on the accumulation of bioactive compounds and antioxidant capacity in green bean cv. Strike compared to a conventional fertilizer (Mg sulfate). Two sources of Mg were applied via foliar: Nanofertilizer and Mg Sulfate at doses of 0, 50, 100, 200, and 300 mg/L of Mg. The accumulation of total polyphenols, flavonoids, anthocyanins, bioactive compounds, and antioxidant capacity was evaluated in pods. The results obtained in this research confirm the effect of green bean pods biofortified with Mg nanofertilizers on the production and accumulation of bioactive compounds and antioxidant capacity, improving the nutrition and nutraceutical quality of green beans. The 50 mg/L dose of Mg nanofertilizer was the most effective treatment to increase bioactive compounds and antioxidant capacity compared to high doses of Mg sulfate (300 mg/L). This is one of the first studies focused on biofortification with Mg nanofertilizers and their effect on the nutraceutical quality of green beans.

Keywords: biofortification; green beans; nanofertilizer; nutraceutical quality; *Phaseolus vulgaris* L.

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Introduction

Magnesium (Mg) deficiency in humans is associated with diet quality and is exacerbated by Mg deficiency in soils (Cakmak *et al*., 2020). Mg is an essential element and is of fundamental importance to the functions of more than 300 enzymes and hormones in the human body. On the other hand, agricultural soils around the world face a problem of nutrient deficiency. Among the limiting macronutrients is Mg, which is key in the growth and development of plants, since it plays a crucial role in the manipulation of compounds such as ATP, RNA, and DNA. However, it is positioned as the most limiting macronutrient in agriculture and the one that has received the least attention in recent decades. Mg malnutrition is considered a global human health problem since Mg is an essential nutrient in food and plants are the main source of daily intake of this nutrient (Cedeño *et al*., 2022).

Agronomic biofortification is an innovative strategy to increase the concentration of nutrients in the edible part of crops. The biofortification of Mg has been little studied. However, few studies mention that it is feasible to agronomically biofortify with conventional Mg fertilizers and improve crop production and nutraceutical quality (Ciscomani *et al*., 2021). Mg is essential for plant nutrition and is a fundamental component of chlorophyll (central atom) and acts as a cofactor for many enzymes (RNA polymerase, ATPases, protein kinases, phosphatases, carboxylases, and glutathione synthetase). In addition, it is involved in photosynthesis reactions, also involved in Ca-based signal transduction processes, and the regulation of the carbohydrate portion through the Mg-ATP complex. It is a regulator of the cation-anion balance in cells and as an ion is osmotically active and thereby regulates cell turgidity together with the K ion (Desai *et al*., 2017; Guo *et al*., 2016).

Mg is the fourth most common mineral in the human body with a content that ranges from 25 to 50 g. It is essential for health and plays an important part in many processes carried out by the body in the physiological function of the brain, heart and skeletal muscles, nervous system, blood sugar levels, and blood pressure. In addition, it has anti-inflammatory properties, helps to form protein, bone mass, and DNA, and acts as a Ca ion antagonist (Jeroen *et al*., 2015). According to various reports, during the last 60 years, the content of this macronutrient in fruits and vegetables decreased between 20% and 30% (Jeroen *et al*., 2015).

In current agriculture, nanotechnology provides novel applications in agriculture, since it is considered a great alternative for sustainable practice. Therefore, through nanotechnology, nanofertilizers have been developed, these are materials that provide plants with one or more nutrients, supporting their growth and they improve production. In addition, it is effective in very low portions (Liu and Lal, 2015). The possible use of nanoparticles (NPs) as nanofertilizers has been considered an effective and innovative alternative compared to conventional fertilizers (Shebl *et al*., 2020). Nanofertilizers are products composed of nanoparticles that use nanotechnology to improve nutritional efficiency in plants. That is, formulations with some types of nanostructured material are used, with nanometric sizes equivalent to one billion parts of a meter (1 nm = 10^{-9} m) (Rojas and Perez, 2020).

A recent study about Mg biofortification was carried out by Ciscomani *et al*. (2021) who found that the nutritional quality of green beans could be improved by biofortification with Mg chloride and sulfate applied through the soil. Likewise, they mention that it is feasible to carry out a biofortification program with Mg sulfate at a dose of 100 mg/L to reduce malnutrition. On the other hand, Salcido-Martínez *et al*. (2020) report that the most effective doses of Mg nanofertilizers were 50 and 100 mg/L applied by foliar route, which improves physiological and production responses. However, the number of studies about the influence of the application of Mg nanofertilizers on the production and accumulation of bioactive compounds and antioxidant capacity in green bean pods remains very limited. It seems that the relationship between biofortification with Mg and the accumulation of bioactive compounds and antioxidant capacity could be positive (Ciscomani *et al*., 2021). Therefore, the objective of this study was to evaluate the effect of biofortification with Mg nanofertilizer on the accumulation of bioactive compounds and antioxidant capacity in green bean cv. 'Strike' compared to a conventional fertilizer (Mg Sulfate).

Materials and Methods

Crop management

The crop was developed in a greenhouse covered with an anti-aphid mesh located in the Faculty of Agrotechnological Sciences of the Autonomous University of Chihuahua, in Chihuahua, Chihuahua, Mexico, with an average temperature of 32.5 °C and relative humidity of 20.35%, following the methodology of Palacio *et al.* (2021), with some modifications. Green bean seeds cv. 'Stike' (*Phaseolus vulgaris* L.) germinated in a germination tray and 12 days later transplanted into plastic pots. A mixture of vermiculite and perlite in a 2:1 ratio was used as substrate. Two plants per pot were transplanted and irrigated with 500 mL of a nutrient solution composed of: 6 mM NH_4NO_3, 1.6 mM K_2HPO_4, 0.3 mM K_2SO_4, 4 mM $CaCl_2$, 5 µM Fe-EDDHA, 2 µM $MnSO_4$, 0.25 µM $CuSO_4$, 0.3 Na_2MoO_4 µM and H_3BO_3 0.5 µM, this nutrient solution free of Mg because the latter was applied via foliar. The first 30 days of planting were irrigated with 500 mL of the nutrient solution and the subsequent 30 days with 1L of solution. The treatments were applied every 10 days after the appearance of the true leaves in a foliar way 2 days after the transplant, applying 5 treatments in total.

Experimental design and treatments

A completely randomized design with 9 treatments and four repetitions was used (Figure 1). The treatments applied were at 0, 50, 100, 200, and 300 mg/L of Mg sulfate ($MgSO_4$) and Mg nanofertilizer (Nano Mg) to each plant in foliar form.

Plant sampling

Sixty days after germination and when the plants reached physiological maturity, whole plants were sampled. Subsequently, the material was divided into aerial, root, and bean pod parts. Fresh plant material was used to quantify biomass, pod production, and analysis of total polyphenols, flavonoids, anthocyanins, and bioactive compounds, while dry material was used to determine aerial and root biomass. Four replicates per treatment were used to analyse each variable.

Plant analysis

<u>Extraction of polyphenols</u>

Following what was described by Yang *et al.* (2020), 0.5 g of fresh green bean pods were weighed, 10 mL of a 70% ethanol solution acidified to 0.5% with formic acid (v/v) were added and placed in an ultrasonic bath for 30 min with an ice bath and the sample was filtered and stored for later analysis.

<u>Quantification of total polyphenols</u>

Quantification was performed by the Folin-Ciocalteu method with some modifications (Yang *et al.*, 2020), and a spectrophotometer (Thermo Scientific Genesys 10S Vis) was used. 750 µL of the extract were placed in a glass tube and 3.75 mL of distilled water were added, then 250 µL of Folin reagent were added, then it was left to stand for 3 min and 1 mL of Na_2CO_3 was added, then the tubes were placed in a water bath at 60°C for one min and the sample was placed in a quartz cell and read at 750 nm following the methodology of Singleton and Rossi (1965). The calibration curve was made with gallic acid, and the results were expressed as gallic acid equivalents (GAE) mg /100 g of dry weight. The linear range of the calibration curve was 0.1 to 200 mg/L of GAE.

<u>Flavonoid quantification</u>

500 μL of the sample were taken and 3.5 mL of ultrapure water were added, then 150 μL of 0.5 M $NaNO_2$ were added and allowed to react for 6 min, then 150 μL of 0.3 M $AlCl_3$ were added and left to stand for 5 min, then 1 mL NaOH 1 M was added, after which time it was read in a UV spectrophotometer at a wavelength of 510 nm, following the methodology of Adom and Liu (2002). The calibration curve was made using Catechin and the results were expressed in mg of catechin/100 g of fresh weight. The range of the calibration curve was from 100 to 4000 mg/L.

<u>Quantification of antioxidant capacity</u>

The procedure described by Brand *et al.* (1995) was followed with some modifications. 100 μL of the polyphenolic extract was placed and 2.9 mL of a DPPH solution was immediately added (6 mg of DPPH measured to 100 mL of 80% methanol). After adding the DPPH solution, it was incubated at room temperature for 30 min and measurements were recorded at a wavelength of 517 nm. The calibration curve was performed using a Trolox solution as a standard with a range of 0.133 to 0.799 μmol ETrolox mL^{-1}, the results obtained were reported as μmol ETrolox/100g FW.

<u>Anthocyanin analysis</u>

The methodology was carried out as reported (Guisti and Wrolstad, 2001), in a test tube 200 μL of the poly-phenolic extract was placed and 2800 μL of potassium chloride 0.025 M pH 1 was added, in another tube the same amount of extract was placed but now 2800 μL of sodium acetate 0.4 M pH 4.5 was added, after performing the reaction with the two reagents, both reactions were read in a spectrophotometer at 530 and 700 nm, to calculate the absorbance per sample the following equation was used: $A = (A_{530} - A_{700})_{pH\ 1.0} - (A_{530} - A_{700})_{pH\ 4.5}$, already having the absorbance (A), the following formula is used to calculate the monomeric anthocyanin of the sample: Monomeric anthocyanin (mg/L) = $(A \times MW \times DF \times 1000)/(\varepsilon \times 1)$, where A= sample absorbance, MW= anthocyanin molecular weight, DF= dilution factor, ε= molar absorptivity.

<u>HPLC analysis</u>

The methodology described by Villarreal *et al.* (2022) was followed with some modifications, using the UltiMate 3000 HPLC equipment, with a UV-DAAD Diode array detector (Dionex Softron GmbH Part of Thermo Fisher Scientific Inc. Germering, Germany) with an Agilent Phoro Shell 120 C18 column, a pore size of 2.7 μm, with two solvents in a gradient system; the solvents were A (tridistilled water) and B (methanol trifluoroacetic acid (TFA) 0.1% v/v); a linear gradient from 5% to 50% for 17 min, an isocratic elution for 5 min and from 50% B to 5% B for 3 min was used. The flow rate was 0.5 mL/min, with an injection volume of 1 μL and a run time of 25 min. The column temperature was maintained at 35 °C. All samples were filtered through a 0.45 μm nylon syringe filter, as described by Dewanto *et al.* (2002).

Results

Growth of green bean plants cv. 'Strike'

After the foliar application of the selected treatments to the green bean plants during their growth, a difference was observed in the plants at the time of harvest. The determined differences are shown in Figure 1. It was observed in the control plants (plants without application of Mg) that presented yellow leaves, while in the plants to which the Mg sulfate treatment was applied, yellow, dry leaves and smaller plants were observed as well as the stems presented an inclination in their growth, which may be related to Mg deficiency. On the other hand, in the plants where Mg nanofertilizer was applied, larger plants were observed with mostly green leaves, with a totally firm stem.

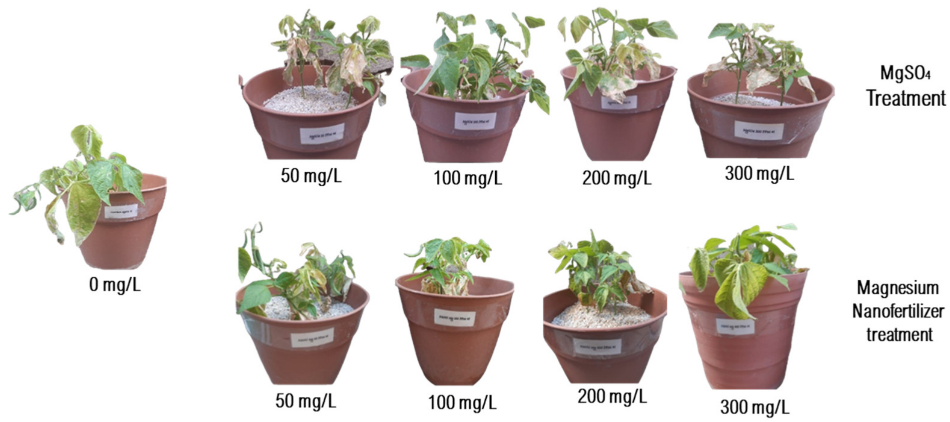

Figure 1. Growth of the string beans cv. Strike treated with Mg sulfate and Mg nanofertilizer

Table 1 shows the values obtained from the determination of aerial and root biomass, as well as the production of green bean pods. As can be seen in Table 1, a greater amount of aerial biomass (2.470 g) was obtained with the dose of 300 mg/L of Mg sulfate, while for the Mg nanofertilizer it was 22.930 g at a concentration of 50 mg/L. Similarly, it is observed that the highest root biomass values were obtained at a concentration of 100 mg/L for the Mg sulfate treatment, obtaining the amount of 19.662 g. While in the Mg nanofertilizer treatment, the greatest accumulation of root biomass was at the dose of 50 mg/L with 20.410 g. On the other hand, the values obtained in the production of green bean pods for the treatment with Mg sulfate were 6.948 g at a dose of 200 mg/L and for the Mg nanofertilizer treatment, it presented a production of pods of 5.707 g at a dose of 200 mg/L 50 mg/L dose.

Table 1. Aerial and radicular biomass, and pod production in green bean plants cv. 'Strike'

Mg doce (mg/L)	Aerial biomass (g)	Root biomass (g)	Pod production (g)
Control	14.797±0.02	5.481±0.01	1.901±0.02
Mg sulfate			
50	17.576±0.01	16.440±0.02	4.767±0.01
100	21.560±0.01	19.662±0.02	7.176±0.02
200	21.592±0.01	19.262±0.02	6.948±0.02
300	27.470±0.01	17.410±0.02	4.662±0.01
Mg nanofertilizer			
50	22.930±0.02	20.41±0.02	5.707±0.02
100	19.582±0.01	16.39±0.01	4.401±0.02
200	16.741±0.02	14.72±0.01	3.101±0.02
300	14.531±0.02	13.54±0.01	1.930±0.02

Quantification of total polyphenols

The results obtained from the quantification of total polyphenols after the application of the Mg sources are shown in Table 2. As can be seen, in all the treatments where Mg sulfate was applied, the values increased concerning the control, where this treatment was not used, going from 78.162 mg GAE/ 100 mg FW of the control to 133.376 mg GAE/ 100 mg FW obtained in the dose of 200 mg/L. However, it is important to point out that a linear increase or decrease was not observed from the values, when increasing the doses of Mg sulfate, that is, if the dose of Mg sulfate is increased, an increase in the concentration of total polyphenols is not necessarily observed. In the case of the Mg nanofertilizer, it was observed that the highest concentration of total polyphenols was obtained at the dose of 50 mg/L with 131.667 g. Likewise, in all the doses of nanofertilizers used (50 mg/L, 100 mg/L, 200 mg/L, and 300 mg/L) the values obtained for total polyphenols were higher than those obtained in the control (without application of Mg). However, with this treatment, it is observed that when increasing the doses of Mg nanofertilizers, the concentration of total polyphenols decreased. These results allow us to affirm that, as in the case of the determination of biomass and production of green bean pods, when low doses of Mg nanofertilizers are used, the most significant results are obtained. With this treatment, it is observed that when increasing the doses of Mg nanofertilizers, the concentration of total polyphenols decreased.

Table 2. Content of total polyphenols, flavonoids, and antioxidant capacity in green bean pods cv. 'Strike'

Mg dose (mg/L)	Total polyphenols (mg GAE/ 100 mg FW)	Flavonoids (mg catechin/100 g FW)	DPPH (μmol ETrolox/100g FW)
Control	78.162 ± 0.03	NA	25.682±0.02
Mg sulfate			
50	104.829 ± 0.02	NA	34.562±0.03
100	88.590 ± 0.01	NA	29.214±0.01
200	133.376 ± 0.01	NA	47.514±0.02
300	126.197 ± 0.01	NA	40.357±0.01
Mg nanofertilizer			
50	131.667 ± 0.01	970.001 ± 0.02	46.876±0.03
100	90.128 ± 0.00	1703.330 ± 0.01	31.215±0.02
200	79.017 ± 0.01	NA	25.102±0.03
300	72.094 ± 0.02	NA	23.132±0.03

Total flavonoid quantification

The quantification of total flavonoids present in the pods of green bean cv. Strike are shown in Table 2. The results obtained showed that in the plants treated with foliar Mg sulfate, values were obtained below the quantification limit established in the calibration curve. While with the doses of Mg nanofertilizers, quantifiable values were obtained at concentrations of 50 and 100 mg/L with an amount of 970.001 and 1703.330 mg/L, respectively.

Antioxidant capacity

The antioxidant capacity of the polyphenolic extracts, shown in table 2 showed that for the treatments with Mg sulfate, the concentrations of 200 and 300 mg/L were the ones that obtained the highest antioxidant capacity with 47.514 and 40.357 μmol ETrolox/g FW, respectively. As well as for the treatments with Mg nanoparticles in which the concentration of 50mg/L was the one that showed the highest antioxidant capacity with 46.876 μmol ETrolox/g FW, demonstrating, as in the quantification of total polyphenols and flavonoids, the same concentrations with the best results.

Anthocyanin analysis

Six anthocyanins are present in the polyphenolic extract of green bean pods cv. Strike, and the concentrations obtained for each one of them are shown in Table 3. These concentrations were obtained from the equations mentioned in the methodology and the values of the dilution factor, molecular structure in weight of the anthocyanins. It was determined that in the treatment with Mg sulfate it was possible to identify the six molecules of anthocyanins used as standards, where the highest concentration was obtained with malvidin 3 glucoside with 2.85 mg/100 g fresh extract as well as pelargonidin with 1.14 mg/100 g fresh extract, the other anthocyanins were found in amounts less than 1 mg. For the treatments with Mg nanofertilizers, the same anthocyanins were identified, at two concentrations of 100 and 300 mg/L.

Table 3. Monomeric anthocyanins present in green bean pods cv. 'Strike'

Mg dose (mg/L)	Cyanidin	Cyanidin-3-gal	Delphinidin	Malvidine	Malvidin-3-glu	Pelargonidin
	mg/100 g fresh extract					
Mg sulfate						
200	0.88	0.98	0.66	0.67	2.85	1.14
Mg nanofertilizer						
100	0.88	0.98	0.66	0.67	2.85	1.14
300	0.53	0.59	0.39	0.4	1.71	0.69

HPLC analysis

The results obtained in the HPLC analysis of the polyphenolic extracts of green bean pods cv. Strike are shown in Table 4. In the control, the presence of cyanidin and chlorogenic acid was identified, while in the treatments in which Mg sulfate was used, the presence of these same compounds was observed in the different doses studied, highlighting that at a dose of 50 mg/L of Mg, the amount of Cyanidin present was 25.022 mg/L, which was higher than the concentration obtained in the control. For chlorogenic acid, in all doses evaluated with Mg sulfate, the values obtained were lower than the control. For the treatments with Mg nanofertilizers, it is observed that there is no presence of cyanidin in comparison to the control. However, the treatment with Mg nanofertilizer at a dose of 100 mg/L found pelargonidin, chlorogenic acid and quercetin; while the treatment with Mg nanofertilizer at a dose of 300 mg/L of pelargonidin, chlorogenic acid and cinnamic acid was found. Chlorogenic acid concentrations in both treatments are lower than those obtained in the control but higher than those obtained in treatments with Mg sulfate.

Table 4. Bioactive compounds analyzed by HPLC of the polyphenolic extract of green bean pods cv. 'Strike'

Mg dose (mg/L)	Cyanidin	Pelargonidine	Chlorogenic acid	Cinnamic acid	Quercetin
	mg/100 g fresh extract				
Control	22.711	-	54.569	-	-
Mg sulfate					
50	25.022	-	4.247	-	-
100	9.578	-	2.155	-	-
200	6.867	-	10.638	-	-
300	3.311	-	9.086	-	-
Mg nanofertilizer					
50	-	4.551	29.707	-	-
100	-	0.373	20.776	-	2.228
200	-	2.398	28.69	-	-
300	-	1.407	19.621	28.654	-

Discussion

Biomass is the main indicator of proper plant growth, and that the fertilization carried out was correct (Sánchez *et al*., 2016). According to the data corresponding to the amount of aerial and root biomass in the study carried out, it is shown that at doses of 50 mg/L of Mg nanofertilizer, said amounts of biomass are higher compared to other treatments with the same nanofertilizers. Salcido-Martínez *et al*. (2020) worked with Mg nanoparticles in green bean plants and reported that at 50 mg/L a higher total biomass content was recorded than other applied treatments, such as in the production of green beans, they report a higher production of green beans in plants treated with Mg nanoparticles at a dose of 100 mg/L. However, in the present research work, it is highlighted that a dose of 50 mg/L of Mg nanofertilizer was where a higher production of pods per plant was obtained. Indicate that in general terms, application of Mg nanoparticles would be more effective than the application of Mg sulfate in obtaining a higher biomass due to the great absorption and mobility of Mg nanofertilizers (Rathore and Tarafdar, 2015; Salcido-Martínez *et al.*, 2020).

Magnesium participates in several biochemical processes (Cakmak *et al*., 2020). One of them is the secondary metabolism, through which plants synthesize molecules such as polyphenols, through two important primary routes, the shikimic acid route, and polyacetals, the latter part of an initial molecule of acetyl CoA and through a series of condensations, polyacetals are formed. The shikimic acid pathway is light dependent and, through various transformations, reaches β-coumaroyl CoA, which is the active precursor of most plant-derived phenols. The routes combine precursors of both routes and said a combination of these biologically active molecules are called flavonoids, anthocyanins, and condensed tannins (Quinones *et al*., 2012).

After applying the two sources of Mg (Mg Sulfate and Nanofertilizer) to the green bean plants cv. Strike, the results obtained from the number of polyphenols in the treatments with Mg nanofertilizers were similar to those reported by Jiratanan and Hai (2004), who report 80 mg EAG 100 g^{-1} in their study of antioxidant activity of processed green beans. Likewise, they carried out quantification of flavonoids where they reported content of 50 mg of catechin 100 g^{-1} fresh weight, which corresponds to the fact that in this research work the Mg nanofertilizers in low concentrations such as those mentioned above were the ideal ones to ensure that the number of flavonoids present was higher than that reported in other works. This means that the Mg was absorbed and transported by the plant more easily due to its size and the plant was able to use it correctly and carry out its biochemical processes.

Morales *et al.* (2020) report that the antioxidant capacity goes hand in hand with the amount of total polyphenols present in the plant, especially in fruits and vegetables. In the present study, the application of Mg nanofertilizers at a dose of 50 mg/L showed the best antioxidant capacity with 46,876 μmol Etrolox/100g FW, obtaining a similar antioxidant capacity with the treatments with Mg sulfate at doses of 200 and 300 mg /L.

Other bioactive compounds present in green beans are anthocyanins. These compounds are a class of flavonoids widely present in the vacuoles of flowers, fruits, stems and leaves of plants (Aquino *et al*., 2021; Sande *et al*., 2016). The values reported in other research works (Hu *et al*., 2015), vary from 0.42 to 1.05 mg/g of dry weight in the total content of anthocyanins present in green beans, being the main anthocyanin, cyanidin and malvidin glycoside. 3. In our study, these anthocyanins present values similar to those found and to the highest concentration. Likewise, in this aspect, it is reaffirmed that the best concentrations of bioactive compounds are found at low doses of Mg nanofertilizer used.

The main polyphenol profiles described in beans are flavonoids, including flavonols, an-thocyanins, proanthocyanidins, and isoflavones, and on the other hand, phenolic acids, and their derivatives. Rodríguez and Suárez (2020) recently reported the presence of cyanidin, pelargonidin, and quercetin in bean samples. In our study, we detected the presence of cyaniding, pelargonidin, and quercetin, as well as the presence of chlorogenic acid and cinnamic acid.

Conclusions

The results obtained in this research confirm the effect of green bean pods biofortified with Mg nanofertilizers on the production and accumulation of bioactive compounds and antioxidant capacity, improving the nutrition and nutraceutical quality of green beans. The 50 mg/L dose of Mg nanofertilizer was the most effective treatment to increase bioactive compounds and antioxidant capacity compared to high doses of Mg sulfate (300 mg/L). This is one of the first studies focused on foliar agronomic biofortification with Mg nanofertilizers and their effect on the nutraceutical quality of green beans. Finally, indicate that a greater number of studies are required regarding the use of Mg nanofertilizers in horticulture to elucidate the mechanisms of action in the physiology and biochemistry of the synthesis and accumulation of bioactive compounds and antioxidant capacity and their final effect in the nutraceutical quality of biofortified crops.

Authors' Contributions

E.S., L.H-O and N.I. A-O designed the study. D.L. O-B, G.D. A-Q. and M.A. F-C. analyzed the data. E.S., L.H-O and N.I A-O prepared the manuscript, while N.I. A-O., A.S-M., C.A. R-E, D. C-F., and J.G. A-S. conducted the experiments. N.I.A-O., L.H-O., D.C-F., J.G. A.S., 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.

References

Adom KK, Liu RH (2002). Antioxidant activity of grains. Journal Agriculture Food Chemistry 50:6182-6187. *https://doi.org/10.1021/jf0205099*

Aquino EN, Garzon AK, Alba JE, Chavez JL, Vera AM, Carrillo JC, Santos MA (2021). Physicochemical characterization and functional potential of *Phaseolus vulgaris* L. and *Phaseolus coccineus* L. landrace green beans. Agronomy Journal 11:803. *https://doi.org/10.3390/agronomy11040803*

Brand W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology 28:25-30. *https://doi.org/10.1016/S0023-6438(95)80008-5*

Cakmak I, White PJ (2020). Magnesium in crop production and food quality. Plant and Soil 457:1-3. *https://doi.org/10.1007/s11104-020-04751-6*

Cedeño JR, García JV, Solórzano CM, Jiménez LAJ, Ulloa SM, López FX, Sánchez AB (2022). Fertilization with magnesium in plantain 'Barraganete' (Musa AAB) Ecuador. Life Sciences Journal 35:8-19. *https://doi.org/10.17163/lgr.n35.2022.01*

Ciscomani JP, Sánchez E, Jacobo JL, Sáenz HK, Orduño N, Cruz O, Ávila GD (2021). Biofortification efficiency with magnesium salts on the increase of bio-active compounds and antioxidant capacity in snap beans. Rural Science 51(6):e20200442. *https://doi.org/10.1590/0103-8478cr20200442*

Desai B, Desai V, Desai P, Singh D, Suthar H (2017). Effect of magnesium nanoparticles on physiology and stevioside in *Stevia rebaudiana* Bertoni. European Journal of Biomedical Science 4:642-646. *https://www.ejbps.com/ejbps/abstract_id/3085*

Dewanto V, Wu KK, Liu RH (2002). Thermal processing improves the nutritional value of tomatoes by increasing total antioxidant activity. Journal Agriculture and Food Chemistry 50:3010-3014. *https://doi.org/10.1021/jf0115589*

Guisti MM, Wrolstad RE (2001). Characterization and measurement of anthocyanins by UV-Visible spectroscopy. Current Anal food protocol Chemistry 47:4631-4637. *https://doi.org/10.1002/0471142913.faf0102s00*

Guo W, Nazim H, Liang Z, Yang D (2016). Magnesium deficiency in plants: An urgent problem. The Crop Journal 4(2):83-91. *https://doi.org/10.1016/j.cj.2015.11.003*

Hu J, Chen G, Zhang Y, Cui B, Yin W, Yu X, Zhu Z, Hu Z (2015). Anthocyanin composition and expression analysis of anthocyanin biosynthetic genes in kidney bean pod. Plants and Biochemistry 97:304-312. *https://doi.org/10.1016/j.plaphy.2015.10.019*

Jeroen HF, de Baaij, Joost GJ, Hoenderop, René JM (2015). Magnesium in Man: Implications for Health and Disease. Physiological Reviews 95(1):1-46. *https://doi.org/10.1152/physrev.00012.2014*

Jiratanan T, Hai R (2004). Antioxidant activity of processed table beet (*Beta vulgaris* var. *conditiva*) and green beans (*Phaseolus vulgaris* L.). Journal Agriculture and Food Chemistry 52:2659-2670. *https://doi.org/10.1021/jf034861d*

Liu R, Lal R (2015). Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. Science of the Total Environment 514:131-139. *https://doi.org/10.1016/j.scitotenv.2015.01.104*

Morales A, Alvarado CJ, Andueza RH, Tun JM, Medina KB (2020). Nutritional and nutraceutical quality of cowpea green bean (*Vigna unguiculata* [L] Walp.) from the Yucatan peninsula. Ecosistemas y Recursos Agropecuarios 7(3):e2541. *https://doi.org/10.19136/era.a7n3.2541*

Palacio A, Ramírez CA, Gutiérrez NJ, Sánchez E, Ojeda DL, Chávez C, Sida JP (2021). Efficiency of foliar application of zinc oxide nanoparticles versus zinc nitrate complexed with chitosan on nitrogen assimilation, photosynthetic activity and production of green bean (*Phaseolus vulgaris* L.). Scientia Horticulturae 288:110297. *https://doi.org/10.1016/j.scienta.2021.110297*

Quinones M, Michael M, Aleixandre A (2012). Polyphenols, naturally occurring compounds with healthy effects on the cardiovascular system. Nutr Hosp 27:76-89. *https://doi.org/10.1590/S0212-16112012000100009*

Rathore I, Tarafdar JC (2015). Perspectives of biosynthesized magnesium nanoparticles in foliar application of wheat plant. Journal of Bionanoscience 9(3):209-214. *https://doi.org/10.1166/jbns.2015.1296*

Rodríguez R, Suárez B (2020). Development and validation of the ultrasound-assisted extraction (UAE) and HPLC-DAD method for the determination of polyphenols in dried beans (*Phaseolus vulgaris*). Journal of Food Composition and Analysis 85:103334. *https://doi.org/10.1016/j.jfca.2019.103334*

Rojas C, Perez A. (2020). Nanofertilizantes para cereales: situación actual y perspectivas futuras. Terras 1:62-66. *https://dialnet.unirioja.es/servlet/articulo?codigo=8288779*

Salcido-Martínez A, Sánchez E, Licón- Trillo LP, Pérez-Álvarez S, Palacio-Márquez A, Amaya-Olivas NI, Preciado-Rangel P (2020). Impact of the foliar application of magnesium nanofertilizer on physiological and biochemical parameters and yield in green beans. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 48(4):1-15. *https://doi.org/10.15835/nbha[12090*

Sánchez E, Ruiz JM, Romero L (2016). Nitrogen compounds stress indicators in response to toxic doses of Nitrogen and deficient in green beans. Nova Science 8:228-244.

Sande D, Diaz M, Milian YE, Castro I, Source L, Altunaga N, Lugo Y, Colenc G, Aparecida J (2016). Mulberry (*Morus alba*) roots natural and hybrid varieties: phenolic content and nutraceutical potential as an antioxidant Journal. App. Farmacia Ciencia 6:63-69. *https://doi.org/10.7324/JAPS.2016.601110*

Shebl A, Hassan AA, Salama DM, Abd ME, Abd MS (2020). Template-free microwave-assisted hydrothermal synthesis of manganese zinc ferrite as a nanofertilizer for squash plant (*Cucurbita pepo* L). Heliyon 26:3:e03596. *https://doi.org/10.1016/j.heliyon.2020.e03596*

Singleton V, Rossi JA (1965). Colorimetry of total phenolic compounds with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16:144-158.

Villarreal G, Escajeda J, Amaya N, Chavez D, Neder D, Ayala JG, Quintero A, Ruiz T, Hernández L (2022). Determination of phenolic compounds in blue corn flour (*Zea mays* L.) produced and/or metabolized by *Colletotrichum gloesporioides* in a fermentation process Fermentation 8:2-11. *https://doi.org/10.3390/fermentation8060243*

Yang QQ, Farha A, Chen L, Kim G, Zhang T, Corke H (2020). Phenolic content and antioxidant activity in vitro in common beans (*Phaseolus vulgaris* L.) are not directly related to antiproliferative activity. Food Bioscience 36:100662. *https://doi.org/10.1016/j.fbio.2020.100662*