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Can magnesium nanofertilizers enhance magnesium use efficiency, biomass and yield in green bean plants?

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Abstract

Crop productivity has been compromised due to nutrient deficiencies, especially magnesium (Mg). Although conventional fertilizers with Mg can improve crop growth, they are often not considered in fertilization programs and are inefficient to meet current agricultural needs and reduce eutrophication and groundwater contamination. Considering this, nanofertilizers can enhance crop growth and lessen environmental impact due to their small size, low fertilization rates, high nutrient efficiency and high specific surface area. Therefore, this study aims to evaluate two different Mg fertilizers in green bean plants grown in vermiculite/perlite substrate. Green bean plants were grown under three distinct treatments: control (no Mg fertilization), Mg nanofertilizer (Nano Mg®) and magnesium sulfate ($MgSO_4$). Each Mg source was applied at three different doses (50, 100 and 200 ppm). The parameters evaluated were biomass, yield and efficient use of Mg. The results obtained indicate that the Nano Mg® and $MgSO_4$ treatments at 200 ppm increased biomass by 6.61 and 8.38 g plant^{-1} DW, yield by 60.7 and 49.84 plant^{-1} FW, respectively. Mg use efficiency parameters were also increased by both fertilizers, which were comparable with each other. Thus, the application of Mg in the form of nanofertilizer is an efficient and innovative alternative, comparable to the application of magnesium sulfate.

Keywords: efficient use of magnesium; magnesium sulfate; nanoparticles; nanotechnology; *Phaseolus vulgaris* L.

Introduction

In recent decades, crop productivity has been compromised due to environmental stress and nutrient deficiencies (Ponce-García *et al.*, 2022). Due to this problem, conventional fertilizers have been used to address these issues, especially nutrient deficiencies. However, priority has been given to the application of nitrogen (N), phosphorus (P) and potassium (K) to obtain higher crop yields, leaving aside other macronutrients such as sulfur (S), calcium (Ca) and magnesium (Mg) (Chaudhry *et al.*, 2021).

Among these macronutrients, Mg plays a key role for plant growth development due to its involvement in numerous metabolic processes (Mitra, 2015). These include photophosphorylation, protein synthesis and

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the formation of chlorophyll (Ciscomani-Larios *et al.*, 2021). Consequently, its deficiency hinders carbon metabolism, carbon fixation and chlorophyll contents, as well as limiting nutrient and water uptake (Mitra, 2015). Despite this, Mg is frequently overlooked in agronomic fertilization programs, although several studies show that Mg improves crop productivity by generating favorable physiological results, increasing the yield and quality of crops such as peanut, tobacco, rice and corn (Lu *et al.*, 2020).

To correct Mg deficiency, many types of compounds have been used, the most common being magnesium sulfate ($MgSO_4$). However, their low efficiency generates various environmental problems such as eutrophication and groundwater contamination, as well as imbalance of soil minerals. In addition, volatilization and leaching of these products further reduce their efficiency. (Dhlamini *et al.*, 2020). Another option to address Mg deficiency is through the use of nanofertilizers. This nanotechnology shows great potential for sustainable use in crop production due to their lower cost, submicroscopic size (1-100 nm), high surface to area to volume ratio and higher nutrient efficiency which increases productivity (Majumdar and Keller, 2020). Moreover, the use of nanofertilizers reduces the overuse and potentially dangerous effects of conventional sources (Muñoz-Márquez *et al.*, 2022).

Recent studies report favorable responses after the application of Mg nanoparticles on total chlorophyll content in wheat, as well as on yield in green bean cv. 'Strike' (Rathore and Tarafdar, 2015). In general, there are few studies on the use and efficiency of Mg nanofertilizer application on growth and yield, so the objective of the present study was to evaluate the efficiency of magnesium nanofertilizer and magnesium sulfate application on biomass, yield and parameters of efficient use of Mg in green bean cv. 'Strike'.

Materials and Methods

Crop management

Two seeds of green bean (*Phaseolus vulgaris* L.) cv. Strike were germinated and grown in plastic pots of 13.4 L capacity, filled with vermiculite and perlite substrate in a 2:1 ratio (w/w). The experiment was conducted under shade net conditions in Delicias, Chihuahua, Mexico during the August-October period of 2022.

The plants were irrigated every third day with 500 mL per pot of nutrient solution composed of 6 mM NH_4NO_3, 1.6 mM K_2HPO_4, 0.3 mM K_2SO_4, 4 mM $CaCl_2$, 1 µM $ZnSO_4$, 5 µM Fe-EDDHA, 2 µM $MnSO_4$, 0.25 µM $CuSO_4$, 0.3 µM Na_2MoO_4, 0.5 µM H_3BO (Sánchez et al., 2006); maintaining a pH of 6.0-6.1 and an electrical conductivity of 1.938 dS m^{-1}. Once the flowering stage was reached, approximately 30 days after planting, irrigation was increased to 1000 mL per pot.

Experimental design and treatments

A completely randomized design was used with seven treatments, six pots per treatments and one plant per pot, hence 12 plants per treatment were grown. The treatments began once the first true leaf appeared and consisted of two different Mg sources: magnesium nanofertilizer (NanoMg) and magnesium sulfate ($MgSO_4$) with three different doses each: 50, 100 and 200 ppm (Ciscomani-Larios *et al.*, 2021) and a control treatment without any Mg fertilization (Table 1). Treatments were applied once a week during 60 days after the germination.

Table 1. Description of treatments: source and dose

Mg source	Dose (ppm)	Replicates	Code
Control	0	6	0
Magnesium sulfate	50	6	50 $MgSO_4$
Magnesium sulfate	100	6	100 $MgSO_4$
Magnesium sulfate	200	6	200 $MgSO_4$
Magnesium nanofertilizer	50	6	50 NanoMg
Magnesium nanofertilizer	100	6	100 NanoMg
Magnesium nanofertilizer	200	6	200 NanoMg

Characterization of magnesium sources

Magnesium nanofertilizer

The material that was applied as nanofertilizer is the commercial product PHC® Nano Mg, which is a suspension, where Mg is available in ionic form. The Mg content is 30% w/v.

Magnesium sulfate

The material applied was magnesium sulfate ($MgSO_4$), reagent grade of the JT Baker brand. It has a molecular weight of 246.48 g/mol and a purity of 99.45%.

Plant sampling

60 days after the germination, all plants were sampled and the grains were harvested. plants were sectioned into root, stem, leaf and fruit. The fresh material was used to determine yield, while the dry material was used to determine root, fruit, leaf and stem biomass, as well as for Mg concentration analysis. The plant material was washed twice with distilled water.

Plant analysis

Biomass and yield

The plant organs (leaf, stem, fruit and root) were placed in a drying oven (Shell) at a temperature of 70 ^{0}C until they were completely dried (24 h). The total biomass was obtained with the sum of the dry weights of each organ analyzed, using an analytical balance (AND HR-120, San Jose, California, USA). Total biomass was expressed as grams per plant of dry weight (g plant^{-1} DW)

Yield was obtained with the average weight of fresh pods per plant. Total yield was expressed as grams per plant fresh weight (g plant^{-1} FW).

Determination of Mg concentration

Mg concentration was determined by atomic absorption using the ICE 3000 SERIES spectrophotometer (Thermo SCIENTIFIC®). For this purpose, 1 g of dry material sample was subjected to a mineralization process with nitric acid and hydrogen peroxide (Wolf, 1982), then measured in the Atomic Absorption spectrophotometer at a wavelength of 285.2 nm. Mg concentration was expressed in mg kg $^{-1}$ dry weight (DW).

Calculation of Mg efficiency parameters (MgUE)

MgUE parameters were calculated as follows: total Mg accumulation (TMgA) was calculated as total Mg concentration multiplied by total plant biomass

Mg uptake efficiency (MgUpE) was calculated as TMgA divided by root dry weight (mg g^{-1} DW)

$$MgUpE = \frac{TMgA}{root\ DW} \quad (1)$$

Mg utilization efficiency (MgUtE) was calculated as leaf tissue (g DW) divided by Mg concentration (g^2 DW mg^{-1} Mg) (Siddiqi and Glass, 1981).

$$MgUtE = \frac{leaf\ tissue\ DW}{Mg\ concentration} \quad (2)$$

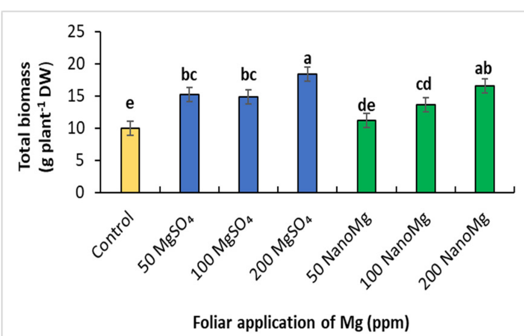
Statistical analysis

The data was subjected to an analysis of variance (ANOVA) and significant differences in means were determined using Fisher's LSD test (Least significant difference) with a significance of 95%, using the SAS version 8 statistical package.

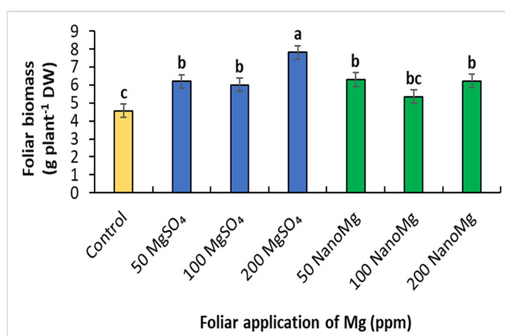
Results and Discussion

Total biomass, foliar biomass, root biomass and pod biomass

Mg has an important impact on crop growth and development due to its influence in various physiological and metabolic process such as photosynthesis and enzyme activation (Wang *et al*., 2020). Figure 1A shows that all NanoMg and $MgSO_4$ doses, except for 50 NanoMg, had a considerable impact on total biomass production. However, 200 $MgSO_4$ had the highest value, showing a 100% increase compared to the control conditions. Even so, this increase was not significantly different with the 200 NanoMg treatment. These results agree with those reported by Neuhaus *et al*. (2014), where foliar application of $MgSO_4$ at 200 mM concentration on wheat plants significantly increased total biomass.) Mg is engaged in the synthesis of proteins, chlorophyll, and nucleic acids, all which are vital for the accumulation of biomass in plants (Kumari *et al*., 2022). Furthermore, Mg stimulates the absorption of other vital nutrients such as N, P, K and Ca that promote plant growth (Ding *et al*., 2012). Other important roles of Mg include its impact on the transport systems of the essential nutrients and compounds inside the plant, maintaining a continuous supply to ensure proper development and metabolic functions (Ahmed *et al*., 2023). All these processes increase biomass accumulation.



A)



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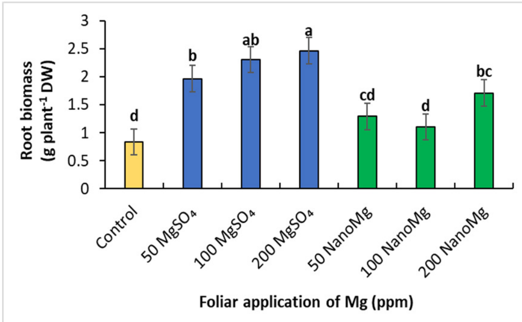

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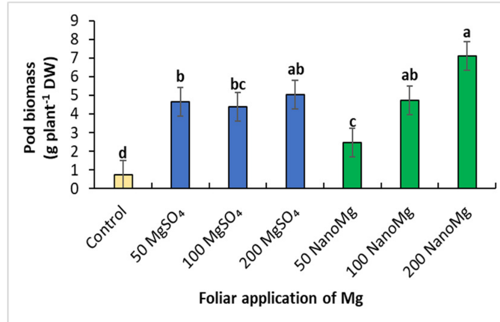

D)

Figure 1. Effect of the application of different treatments of NanoMg® and $MgSO_4$ applied to the leaf on green bean plants cv. Strike, on: A) Total biomass in dry weight, B) Foliar biomass in dry weight, C) Root biomass in dry weight and D) Pod biomass in dry weight. Different letters show significant statistical differences according to LSD test ($P<0.05$)

Regarding the leaf biomass, both Mg sources generated similar results, but once again the 200 $MgSO_4$ showed the greatest biomass accumulation with a 78% increase when compared with the control plants (Figure 1B) This biomass buildup could be attributed to the redox-regulatory network that occurs in S metabolism. In this regulatory system, the reductive power provided by electrons is balanced with the oxidizing power of reactive oxygen species generated (ROS) as by products from the photosynthetic and respiratory electron chains (Telman and Dietz, 2019). If reducing conditions exist, the secondary metabolism activates and favor the synthesis of secondary metabolites or hormones. Conversely, oxidizing conditions lead to the activation of the primary S-metabolism and the creation of antioxidants, amino acids (such as cysteine and methionine) and other compounds (Buchanans *et al*., 2016). In other words, S compounds function as the crosslink connecting the reductive assimilation process of photosynthesis and the ROS (Zenda *et al*., 2021). This is especially important in organelles with great electron flow rates such as the chloroplasts and mitochondria, where the protection from ROS allows for better photosynthetic performance, and, biomass buildup not only in the leaves but in all the plant.

As for root biomass the $MgSO_4$ treatments obtained significant increases in root weight, where 200 $MgSO_4$ showed the highest value than the other Mg sources and the control treatment, with a 400% biomass increase (Figure 1C). Besides its role in plant defense against ROS, S is important in N uptake and an essential component of amino acids promoting plant and root growth (Mengel and Kirby, 2000; Magnucka *et al*., 2023). In general, crop growth rate is modified by nitrogen application, and its use can be highly dependent on the supply of Mg and S to plants (Karooki *et al*., 2021).

With respect to pod biomass, all Mg fertilizers increased pod weight, however, the highest increment was obtained with the 200 NanoMg with a 90% increase when compared to control plants but did not show significant differences when compared to 100 NanoMg and 200 $MgSO_4$ (Figure 1D). Whereas the lowest dose of $MgSO_4$ contributed the lowest increment. As mentioned before, Mg can facilitate the transport of carbohydrates and photoassimilates from the leaves to sink organs as the pods to secure their proper growth (Jezek *et al*., 2015; Ishfaq *et al*., 2022). These results are consistent to those reported by Kanjana (2020), who reported the highest weight of cotton bolls when applying nano-sized Mg, compared to $MgSO_4$ fertilizer. Another advantage of nanofertilizers over the conventional forms is their smaller size allowing for a better nutrient delivery system to enhance crop growth (Kanjana, 2020).

Yield

Mg fertilization improves the yield and quality of crops due to its influence in various physiological processes, making its application an important measure to boost crop production (Wang *et al.*, 2020; Tian *et al.*, 2023). In the present experiment, the 200-ppm dose of NanoMg® (Figure 2), outperformed the control by 309 %, without being statistically different from the 200-ppm dose of $MgSO_4$. These results agree with the trends for pod biomass (Figure 1D).

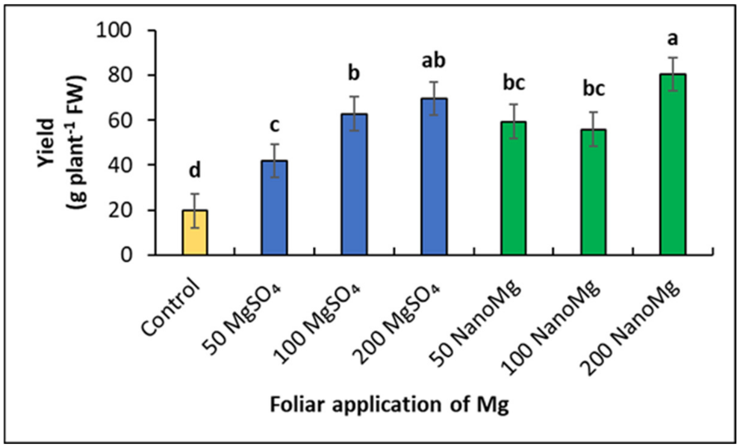

Figure 2. Effect of the application of different treatments of NanoMg® and $MgSO_4$ applied via foliar on green bean plants cv. Strike, on fresh weight yield. Different letters show significant statistical differences according to LSD test ($P<0.05$)

The increase in yield due to the application of NanoMg® at 200 ppm is because of the importance of Mg as a central element of chlorophyll; this position allows for the absorption of light and the start of photosynthesis (Tang *et al.*, 2023). The influence of Mg in the photosynthetic machinery also involves the transfer of energy via the formation of the ATP and NADPH required for the formation of organic compounds (Jiao *et al.*, 2023; Kleczkowski and Igamberdiev., 2023). Mg is also an essential cofactor for the activation of the RuBisCO enzyme, making possible the fixation and transformation of CO_2 into carbohydrates and other organic molecules (Douglas-Gallardo *et al.*, 2022). Another study by Gautam *et al.* (2023), indicates that MgO nanoparticles priming on mustard seeds enhanced vegetative parameters in plants, most notably the yield. This increased yield is due to increased photosynthetic pigments. On the other hand, Mg has a beneficial role in N absorption and assimilation, improving the contents of proteins and other structural compounds in plants (Peng *et al.*, 2020). The absorption and assimilation of N is reliant on H^+-ATP for energy, whereas Mg provides the ions necessary for the electron flow that synthetizes ATP (Tian *et al.*, 2021). He *et al.* (2023) reported that Mg fertilization had a positive effect on tea yield and N use efficiency, as well as promoting photosynthetic product formation rates and biomass. Several studies suggest that nanoscale delivery of Mg is more readily absorbed by plants and thus improve yield (Echeverría-Machado, 2019; Khalid et al., 2022).

Mg concentration in leaf, pod, root and stem and total Mg concentration

For plants to acquire and maintain high concentrations of Mg, a highly efficient transport system is necessary for its uptake, storage, and translocation (Chen *et al.,* 2018). The results of the present study showed significant differences, observing an increase in that element with doses of $MgSO_4$ and NanoMg (Table 2). The highest leaf concentrations were found in the 200 $MgSO_4$ treatment with 20.12% increase compared to the 100 NanoMg treatment. Nevertheless, there was no statistical difference with the 200 NanoMg treatment.

These results agree with Nehaus *et al.* (2014), where foliar application of 200 mM $MgSO_4$ on *Vicia faba* resulted in an increase in Mg concentration. With respect to the pods, as the dose increased in each treatment, the Mg concentration was higher (Table 2). The highest concentration was obtained in the 200 NanoMg treatment, exceeding the 200 $MgSO_4$ treatment by 29%.

Similarly, the results follow the trend also seen in leaf Mg concentration, like that reported by Wang *et al*. (2020), where a significant positive linear correlation was seen between crop yield and leaf Mg concentration in vegetables, fruits and grasses. The foliar application of $MgSO_4$ on *Spinacia oleracea* also increased Mg concentration in its leaves (Borowski and Michałek, 2012; Setareh *et al*., 2021). In addition, Cai *et al*. (2018) evaluated different concentrations of NanoMg fertilizer in tobacco plants and found Mg concentration increased in the lower and middle leaves. They attribute this increase to Mg high mobility once absorbed by the roots and distributed throughout the vascular system, thus, increasing its concentration in leaves and sink organs (Cai *et al*., 2018; Buturi *et al*., 2021).

Table 2. Effect of foliar application of NanoMg® and $MgSO_4$ on Mg concentration in green bean cv. Strike

Foliar application of Mg (ppm)	Mg content (mg/100 g DW)									
	Leaf		Root		Pod		Stem		Total	
50 $MgSO_4$	1,130	c	1,732	ab	414	d	564	b	3,840	bc
100 $MgSO_4$	1,259	b	1,623	b	445	bc	580	b	3,907	abc
200 $MgSO_4$	1,331	a	1,282	c	455	b	643	a	3,711	c
50 NanoMg	1,154	c	1,798	a	427	cd	599	b	3,979	ab
100 NanoMg	1,108	c	1,708	ab	438	bc	508	c	3,762	c
200 NanoMg	1,320	ab	1,641	b	526	a	587	b	4,073	a

*Different letters show statistically significant differences (LSD test, $P \leq 0.05$).

In relation to the root, the highest concentration was found in the NanoMg® treatment at 50 ppm. In this treatment, as the dose increased, the concentration of Mg in the root decreased. This may be because Mg translocate to different plant tissues with the preferential distribution to developing tissues (Chaudhry *et al*., 2021). For instance, Mg was directed to leaves and pods in the present study.

In the stem, the highest Mg concentration was found in the 200 $MgSO_4$ treatment. This result is different to that reported by Delfani *et al*. (2014) where Mg nanoparticles enhanced the concentration of Mg in the stem of black-eyed pea (*Vigna unguiculata*), proposing that their higher availability and mobility led to greater concentrations of Mg.

With respect to total Mg concentration, the 200 NanoMg treatment increased this parameter by 9.75% when compared to the 200 $MgSO_4$ plants. However, this difference was not significant with the 50 NanoMg and 100 $MgSO_4$ treatments, suggesting that lower doses of either fertilizer are sufficient to improve Mg uptake.

Mg use efficiency parameters (MgUE)

Mg use efficiency (MgUE) is described as the biomass yield or yield per unit of available Mg. The efficiency of fertilizers is not only dependent of the known concentration of nutrients but also by the uptake and use efficacy done by the plant (Ponce-Garcia *et al*., 2022). For instance, Mg use efficiency (MgUE) and Mg utilization efficiency (MgUtE) are important parameters indicating the relative efficiency of agricultural fertilization (Wang *et al*., 2020). Hence, MgUpE and MgUtE are important terms that complement MgUE and that relate biomass and Mg accumulation within the plant (Congreve *et al*., 2022).

Table 3. Effect of foliar application of NanoMg® and $MgSO_4$ on parameters of magnesium use efficiency (UEMg), total accumulated magnesium (TMgA), magnesium utilization efficiency (MgUtE), magnesium uptake efficiency (MgUpE) in green bean cv. Strike

Treatment (ppm)	TMgA (mg)		MgUpE (mg Mg g^{-1} RDW)		MgUtE (g^2 LDW mg^{-1} Mg)	
50 $MgSO_4$	89.15	b	50.78	abc	0.28	abc
100 $MgSO_4$	96.73	ab	57.64	bc	0.27	cd
200 $MgSO_4$	151.20	a	116.35	a	0.21	d
50 NanoMg	84.37	b	45.86	abc	0.36	a
100 NanoMg	62.94	bc	36.08	bc	0.34	bc
200 NanoMg	112.31	ab	66.82	b	0.25	d

*Different letters show statistically significant differences ($P \leq 0.05$).

Both Mg fertilizers regardless of the dose increased Mg contents and TMgA. However, the 200 $MgSO_4$ treatment increased this parameter by 78% when compared to the 100 NanoMg group (Table 3). Nonetheless, this increase was not significantly different to the 100 $MgSO_4$ treatment, suggesting that high doses of $MgSO_4$ are not necessary to incorporate Mg in plant tissues. Concerning (MgUpE), the 200 $MgSO_4$ treatment attained the highest value increasing Mg uptake by 222% compared to the lowest value, the 100 NanoMg treatment. However, the lowest doses of both fertilizers achieved comparable results, indicating that high doses of these fertilizers are not necessary to boost Mg uptake. This may be because plants have developed highly efficient transport frameworks for Mg uptake to maintain a constant higher concentration in each tissue (Chaudhry *et al*., 2021).

In relation to MgUtE, the results suggest that high doses of $MgSO_4$ or NanoMg are not required to improve Mg use efficiency, as the lowest doses of both fertilizers indicate a higher utilization efficiency (Table 3). Ponce-Garcia *et al*. (2019) reported similar results when applying 50 mg kg^{-1} of Zn nanoparticles on green bean plants grown in acidic soils.

In summary, it is noticeable that at higher doses, greater absorption and accumulation of Mg is allowed, but the efficiency of utilization decreases. This is probably since the excess Mg is reserved in the vacuole in order to maintain the cytosolic and chloroplast Mg balance, and to be subsequently available for recovery, improving its load in the xylem in the roots and its distribution to the sink organs. Therefore, it is reflected in higher biomass and yield (Chaudhry *et al*., 2021).

The results indicate that NanoMg® at doses of 100-200 ppm allowed a higher total concentration and accumulation of Mg. This element is part of chlorophyll, whose levels were favored, thus increasing light energy uptake. Consequently, high yields were obtained. This corroborates that the application of nanofertilizers via foliar application produces favorable physiological results. For its part, $MgSO_4$, obtained positive results with the 200-ppm dose, also allowed the highest values of efficient use of Mg, increasing biomass, as well as resulting in yields comparable to those of NanoMg®.

Conclusions

The most efficient treatments for increasing biomass, yield and Mg use efficiency parameters were $MgSO_4$ and NanoMg at 200 ppm. The results obtained with both sources do not differ significantly from each other. Therefore, it is concluded that the application of Mg in the form of nanofertilizer is an efficient and innovative alternative, comparable to the application of $MgSO_4$, so that migrating from one source to the other does not represent a reduction in production while it can help mitigate the negative impacts of traditional fertilizers. Likewise, it is recommended to work with higher doses, in order to appreciate the trend and find the maximum point of Mg application, as well as the moment in which some doses would generate toxicity.

Authors' Contributions

Conceptualization: E.S. Methodology: A.S.M., C.A.R.E., and J.C.A.P. Formal analysis: C.A.R.E. and A.S.M. Investigation: A.S.M., C.A.R.E., and J.C.A.P.; writing-original draft preparation: A.S.M., and E.S. Writing-review and editing: E.S. 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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