Article

Application of nanomolybdenum in beans and its impact on nitrogen efficiency

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Abstract

The efficient use of nitrogen is a technique used to improve yields without the excessive addition of nitrogen fertilizers, in the same way, the use of nanofertilizers is an alternative to solve nutritional problems with greater efficiency and precision, both with the purpose of increasing crop productivity. Therefore, the objective of this study was to evaluate the foliar application of molybdenum (Mo) nanofertilizer combined with the edaphic fertilization of NH_4NO_3, on the total biomass, yield and efficiency in snap beans. The plants were germinated and grown under controlled conditions in an experimental greenhouse in Lázaro Cárdenas, Meoqui, Chihuahua, Mexico in September 2020 and irrigated with nutrient solution. The treatments consisted of the foliar application of four doses of the molybdenum nanofertilizer BROADACRE (0, 5, 10 and 20 ppm of Mo), complemented by the edaphic application of four doses of nitrogen in the form of NH_4NO_3 (0, 3, 6 and 12 mM of N). The results obtained indicate that the doses of 10 ppm of Mo and 6 mM of N favored the accumulation of biomass and the highest yield per plant; it is important to note that the highest efficiency was achieved with the doses of 5 ppm of Mo and 3 mM of N. Finally, it is concluded that the application of NanoMo increases the efficiency of nitrogen use, being able to reduce excessive applications of nitrogen fertilizers, without affecting the yield of snap beans.

Keywords: *Phaseolus vulgaris* L.; crop nutrition, micronutrients; nanofertilizers.

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Introduction

Nitrogen (N) is a mobile element that is always exposed to factors of loss by leaching, volatilization and denitrification (Bowles *et al*., 2018); its excessive and ineffective application, combined with the inefficient management of irrigation water, results in nitrate contamination of groundwater and surface streams (Landeros *et al*., 2016). By undergoing denitrification, it can be released into the atmosphere as nitrous oxide, which is a strong greenhouse gas (Bouwman *et al*., 2002).

Excessive applications of N and its negative effects on the environment have become a global concern, so female and male researchers from all over the world aim to develop strategies such as the efficient use of N to improve yields without the excessive addition of nitrogen fertilizers. Organic and inorganic N absorbed by the roots can be transported between different plant tissues to optimize nitrogen use efficiency (NUE) during its development and maintain its optimal growth and yield (Dong and Lin, 2020).

In addition, current studies focus on finding alternatives that directly impact crop production (Raliya *et al*., 2017), which is why nanotechnology positions itself as an alternative for agriculture with enormous potential to develop nanofertilizers that help and solve problems of biological demands, associated with crop nutrition with greater efficiency and precision and in this way, improve fertilizer efficiency to increase productivity, in order to migrate from conventional to more precision agriculture (Naderi and Danesh, 2013; Chhipa, 2017).

The use of nanofertilizers causes an increase in their efficiency, minimizes excessive application and their potential toxic effects; so, there is an opportunity for nanotechnology to have a significant influence on agriculture, the producer economy and the environment (Naderi and Danesh, 2013). One of the most outstanding characteristics of nanofertilizers is that they have a high contact surface of the nutrient ion, a slower and more precise release compared to traditional fertilizers (Subramanian *et al*., 2015).

On the other hand, despite the promising outlook presented by the use of nanofertilizers, it is necessary to evaluate them more deeply to determine that the use of this new technology is superior to the use of traditional fertilizers, in addition to the fact that studies should also focus on establishing the effects on plant development (Subramanian *et al*., 2015; Kah *et al*., 2018). Therefore, the objective of this study was to evaluate the foliar application of Mo nanofertilizers combined with the edaphic fertilization of NH_4NO_3 on the total biomass, yield and efficiency in snap beans.

Materials and methods

Crop management

The crop was developed in a greenhouse covered with anti-aphid mesh located in Lázaro Cárdenas, Meoqui, Chihuahua, Mexico (28° 23’ 9.80232” north latitude, 105°36’ 58.09392” west longitude) in September 2020. Seeds of beans *cv* Strike were germinated in polystyrene trays of 200 cavities, 12 days after germination they were transplanted into 400-gauge polyethylene bags with a capacity of 10 kg, which contained vermiculite and perlite as a substrate in a ratio of 2:1.

A complete nutrient solution was applied for 20 days according to Hoagland and Arnon (1950) and as proposed by Sánchez *et al*. (2006) for snap beans from the germination of the plants, which had the following composition: 6 mM NH_4NO_3, 1.6 mM K_2HPO_4, 0.3 mM K_2SO_4, 4 mM $CaCl_2$ $2H_2O$, 1.4 mM $MgSO_4$, 5 µM Fe-EDDHA, 2 µM $MnSO_4$ H_2O, 1 µM $ZnSO_4$ $7H_2O$, 0.25 µM $CuSO_4$ $5H_2O$, 0.3 µM Na_2MoO_4 and 0.5 µM H_3BO_3 (all J.T. Baker reagents, State of Mexico, Mexico), this with the objective that the plants were well nourished in their early stages of development and avoiding mortality from early malnutrition to ensure the life of the plants to harvest.

After 20 days, the differentiated treatments of N were applied every third day and until the end of the culture. Mo treatments were applied every week from the appearance of the true leaves, and five applications were made according to the specifications of the product.

Experimental design and treatments

A completely randomized design was used, with a combinatorial factorial treatment arrangement with four repetitions, the doses of Mo in the form of molybdenum nanofertilizer (NanoMo): 0, 5, 10 and 20 ppm with foliar application, and the doses of N in the form of NH_4NO_3: 0, 3, 6 and 12 mM with edaphic application, the dose of 6 mM of N was considered optimal according to Sánchez *et al*. (2016).

Plant sampling

Once the physiological maturity of the plants was reached (60 days after germination), the samples were taken. The plants were separated into their different organs: root, leaf, stem and fruit. The yield was determined with the fresh material, preserved at 4° C (Forma Scientific Refrigerator, Marietta, Ohio, USA), while the dry material was used to determine total biomass, total N concentration and Mo concentration. All the material was previously washed with running water to remove surface environmental pollution, then was rinsed twice more with distilled water and tri-distilled water (J.T. Baker, State of Mexico, Mexico). Four repetitions per treatment were used for each variable analyzed.

Plant analysis

Biomass

After environmental decontamination, the samples were placed in a forced-air furnace at 70 °C (Felisa® Furnace, St. Livonia, Michigan, USA) for 24 h and until their complete desiccation. Total biomass production was calculated based on the dry weight of the plant material expressed in grams per plant (g plant^{-1}) (Ponce *et al*., 2019).

Yield

The yield was obtained with the average of the repetitions of the fresh weight of the fruits per plant. Green beans were collected from each of the cultivated plants and weighed at the time of sampling (analytical balance, Precision Electronic Balance and Company Limited, Milpitas, CA, USA). Total yield was expressed in grams per plant (g plant^{-1}) (Ponce *et al*., 2019).

Determination of total N

The dried samples were ground in a blender (Osterizer® blender, Milwaukee, Wisconsin, USA) and placed in plastic bags (Nasco Whirl-Pak®, Cincinnati, Ohio, USA) for their analysis. The total N concentration was determined by means of the Flash 2000 organic elemental analyzer (Thermo Scientific® Corporation, Cambridge, UK), which bases its operation on the Dumas method (Armendáriz *et al*., 2019). A tin capsule was placed in a microbalance (Mettler Toledo®, Columbus, Ohio, USA), 9 mg of vanadium pentoxide (JT Baker, State of Mexico, Mexico) and 3 mg of the finely ground sample were weighed, once the weight was recorded, the capsule was closed. The samples were then placed in the Flash 2000's autosampler for their analysis; two certified standards of Methionine and Sulfanilamide (Thermo Scientific® Corporation, Cambridge, UK) were also analyzed to ensure the accuracy of the results. Finally, the analysis was run, and the total N concentration was expressed in percentage (%).

Agronomic efficiency of applied edaphic N and foliar Mo

The agronomic efficiency (AE) of N and Mo considers the amount in yield or total biomass of the crop per unit of applied fertilizer, estimated according to the following relationship: AE= Y/F. AE= B/F. Where: Y= yield in fruits per plant; B= total biomass per plant; and F= amount of applied fertilizer (Díaz *et al*., 2004).

Statistical analysis

The data obtained were subjected to an analysis of variance, to determine differences between the means of the treatments, the Tukey test (95%) was used, employing the statistical software SAS 9, 2007 (SAS Inst. Inc. Cary, NC). The data shown are average values ± standard error (se).

Results and discussion

Effect of the edaphic application of N complemented with foliar fertilization of Mo on the total biomass

One of the fundamental parameters for measuring nutrient efficiency is biomass accumulation (Szarka *et al*., 2012). The foliar applications of NanoMo and edaphic NH_4NO_3 directly influenced the total biomass of snap beans (Figure 1). The greatest accumulation of biomass was obtained with the doses of 10 ppm of Mo and 6 mM of N, which had an increase of 57.47% over the value of the control treatment; at this point, it is necessary to emphasize that, by itself, NanoMo played an important role in the activation of the metabolism of the assimilation of N, since the different doses evaluated had a positive impact on the accumulation of biomass.

The increase in plant biomass is due to the fact that the nanofertilizer is able to penetrate biological barriers and enter plant tissues where it can be translocated to different organs and assimilated into the metabolism of the plant more easily (Echeverría, 2019). In this case, it can be assumed that the Mo was mostly available to perform its work as an essential part in the enzymatic metabolism responsible for the assimilation of N, which allowed a greater development of the leaf area.

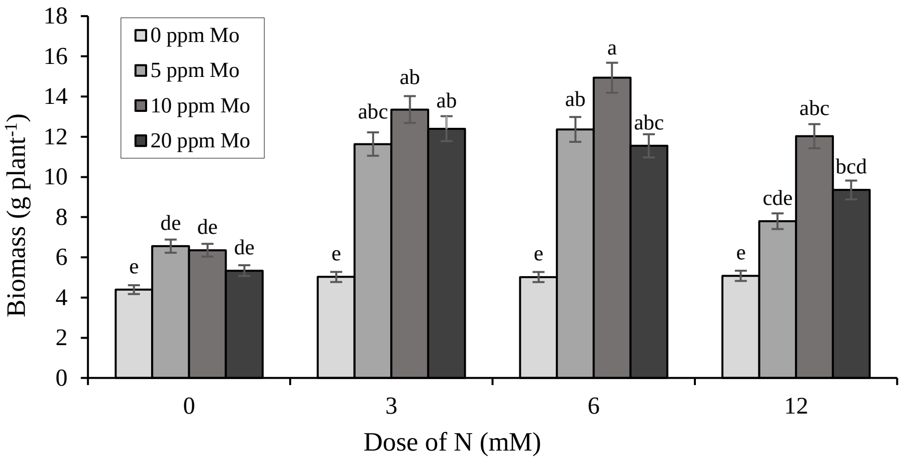

Figure 1. Effect of the edaphic application of N complemented with foliar fertilization of NanoMo on the total biomass of fruits in dry weight per plant in snap beans *cv* Strike. Columns with different letters differ statistically from each other (Tukey $p\leq 0.05$).

In studies conducted by Benzon *et al*. (2015), the application of nanofertilizers promoted growth and development in rice plants, increasing their dry matter content, attributing the results to the good conditions of nutrient availability promoted by nanofertilization for the absorption of N and other macronutrients essential for the growth and proper development of the crop. Due to the above, it is important to analyze in a particular way the effect of N and Mo on the efficiency focused on the production of total biomass per the amount of N and Mo applied, in order to observe the most efficient doses for the crop with the use of nanofertilizers more accurately.

Effect of the application of edaphic N on total biomass

N is the most critical nutrient in a fertilization program, by virtue of its essentiality for optimal crop growth, likewise, the vegetative development of the plant depends largely on the amount of N applied (Orozco *et al*., 2008). In the case of the effect of N on total biomass, which is closely related to yield, the treatments applied had a direct effect on the variable, with the doses of 6 mM N being those of higher biomass, with a difference of 48.38% over the control treatment, it is important to mention that there was no statistically significant difference between the doses of 6 mM of N and 3 mM of N, whose difference was only 3.32% (Figure 2A).

In terms of efficiency, the dose of 3 mM of N was more efficient, accumulating 3.53 g plant^{-1} against 1.82 in the dose of 6 mM of N (Figure 2B). These results of agronomic efficiency give information that allow observing that a smaller amount of nitrogen fertilizer can be applied without affecting the growth of the plant, and without falling into N deficiency, since a low availability of this macroelement negatively affects biomass and fruit production (Stefanelli *et al*., 2010).

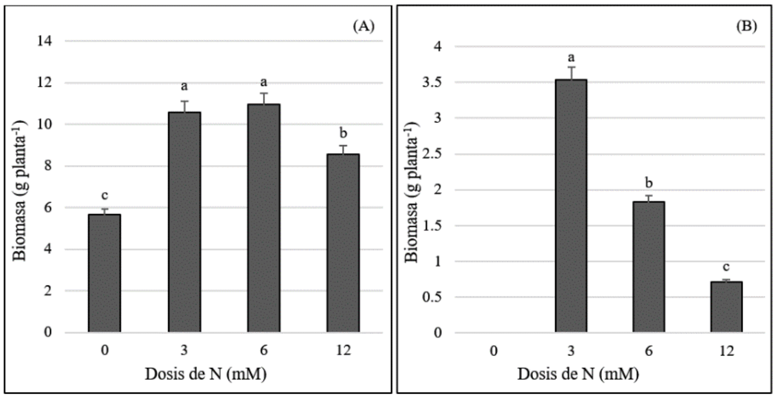

Figure 2. A) effect of the edaphic application of N on the total biomass in fresh weight per plant; and B) agronomic efficiency in total biomass produced per amount of edaphic N applied per plant in snap beans. Columns with different letters differ statistically from each other (Tukey $p \leq 0.05$).

Effect of foliar application of Mo on total biomass

In the process of N fixation, Mo is the cofactor of nitrate reductase and nitrogenase so that they can catalyze the redox reaction and convert elemental N into NH_4^+ ions to be assimilated (Mendel and Hänsch, 2002), in this way, Mo influences the increase in crop biomass and yield. In this study, it can be seen that the foliar applications of NanoMo significantly influenced the increase in biomass, where the dose of 10 ppm of Mo had the highest concentration (Figure 3A).

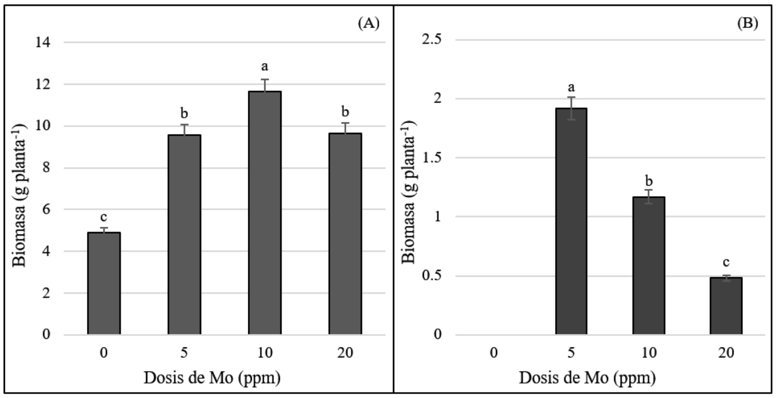

Figure 3. A) effect of the foliar application of NanoMo on total biomass in fresh weight per plant; and B) agronomic efficiency of biomass produced per amount of foliar Mo applied per plant in snap beans. Columns with different letters differ statistically from each other (Tukey $p \leq 0.05$).

It should be noted that, in terms of efficiency, the dose of 5 ppm was the most efficient with 1.91 against 1.16 with the dose of 10 ppm of Mo (Figure 3B), this means that with the lowest dose of Mo (5 ppm), the greatest accumulation of biomass produced per unit of fertilizer applied can be obtained. In this case, the nanofertilizer more efficiently placed Mo at active sites where it could be assimilated more easily and play its elemental role in the assimilation of N for the development of crop biomass.

In previous studies, Nasar and Shah (2017) showed that Mo applications significantly increased the biomass content in lentil crops. Similarly, Mo applications drastically improved the total biomass content in chickpeas in studies conducted by Sawires (2001). Under the experimental conditions, the doses of 5 ppm of Mo and 3 mM of N obtained the highest efficiency in biomass production and productivity of the crop per amount of N and Mo applied.

In the end, the application of edaphic NH_4NO_3 in low doses, combined with the foliar application of a Mo nanofertilizer in low concentrations, is an alternative to reduce excessive nitrogen fertilization without affecting the productivity of the snap bean crop. Effect of the edaphic application of N complemented with foliar fertilization of Mo on yield. As mentioned above, Mo has a central role in the metabolism of N, although not directly, but as a compositional part of the enzymes responsible for the fixation of N. The effects of Mo on N fixation are carefully studied as it has a direct effect on plant yield (Li *et al*., 2007).

In the present experiment, the foliar applications of NanoMo and edaphic fertilization of NH_4NO_3 directly influenced the yield of snap beans, where the highest fruit production was with the doses of 10 ppm of Mo and 6 mM of N, with an increase of 75.92% in relation to the minimum value of the control and 43.17% to the treatment of 10 ppm of Mo and 3 mM of N (Figure 4). At this point it should be noted that the combination of NanoMo and NH_4NO_3 managed to raise the productivity of the crop.

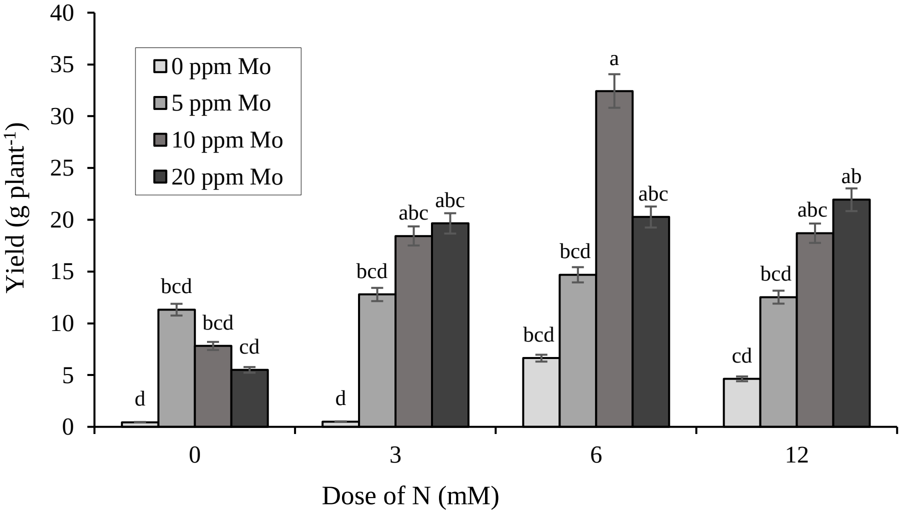

Figure 4. Effect of the edaphic application of N complemented with foliar fertilization of NanoMo on the total yield of fruits in fresh weight per plant in snap beans cv **Strike.** Columns with different letters differ statistically from each other (Tukey $p \leq 0.05$).

This can be explained by the proper functioning of the nitrogen metabolism of the plant, which, by having enough of these two essential elements, could assimilate the N and metabolize it to achieve the good development and production of the fruit. Snyder (2017) mentions that, to optimize crop production and minimize losses, the dose and type of mineral fertilizers applied, and the time and method of application are of vital importance, being critical practices for the crop.

Due to the above, it should be noted that the yield is closely linked to the biomass of the plant, it is for this reason that the metabolism of N affects these two variables in a similar way, it should be remembered that a large number of studies worldwide support this fact, in addition, an important indication regarding the efficient use of N is precisely the yield of the fruit and the production of dry matter of the crop (Arenas *et al*., 2021). In this context, it is important to see individually the effect of N and Mo on efficiency focused on the amount of fruit produced per the amount of M and Mo applied, in this way the most efficient dose for crop productivity is observed more accurately.

Effect of the edaphic N application on yield

N increases the levels of compounds produced and synthesized due to the increase in the photosynthetic rate, these assimilates are translocated to different parts of the plants, recent studies have shown that the supply of mineral nitrogen fertilizers increased the weight and number of seeds per plant, in addition to the total yield (Bekele *et al*., 2019).

In the present study, the edaphic application of NH_4NO_3 significantly influenced the yield of the plants, the dose of 6 mM of N had the highest production of fruits per plant, with a difference of 66.2% over the value of the control and 30.6% over the lowest dose of 3 mM of N (Figure 5A). It is necessary to bear in mind that the highest efficiency of fruits produced per unit of N applied is sought and that, in this research, it was obtained with the dose of 3 mM of N, which has an efficiency of 4.27 against 3.07 with the dose of 6 mM of N (Figure 5B).

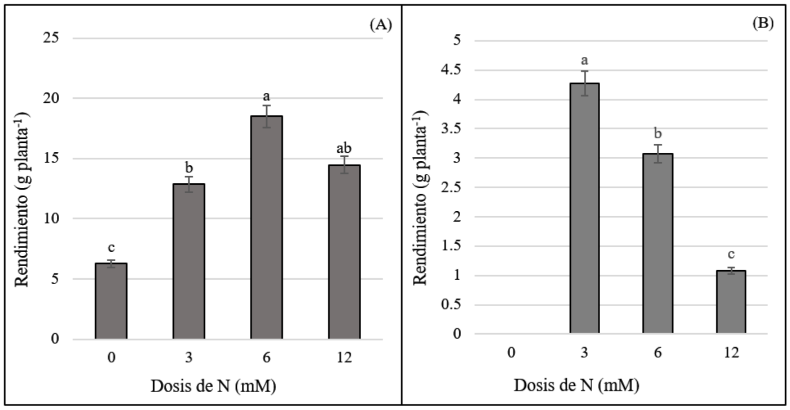

Figure 5. A) effect of the edaphic application of N on yield in fresh weight per plant; and B) efficiency in fruits produced per amount of edaphic N applied per plant in snap beans. Columns with different letters differ statistically from each other (Tukey $p \leq 0.05$).

Effect of foliar application of Mo on yield

Plants with Mo deficiency show stunted growth (Rana *et al*., 2020b), with less content of chlorophyll and ascorbic acid (Liu, 2002); that is, a low concentration of this essential micronutrient results in the deterioration of the development and yield of plants (Rana *et al*., 2020a). In this study, as in biomass, the direct effect of the foliar application of NanoMo shows that the highest fruit production was obtained with the dose of 10 ppm (Figure 6A). However, we have that the most efficient dose is that of 5 ppm with an efficiency of 2.56 against 1.93 with the dose of 10 ppm Mo (Figure 6B). With these results it can be indicated that the best dose of NanoMo is 5 ppm, confirming that with the application of the nanofertilizer in low concentrations the yield is not affected.

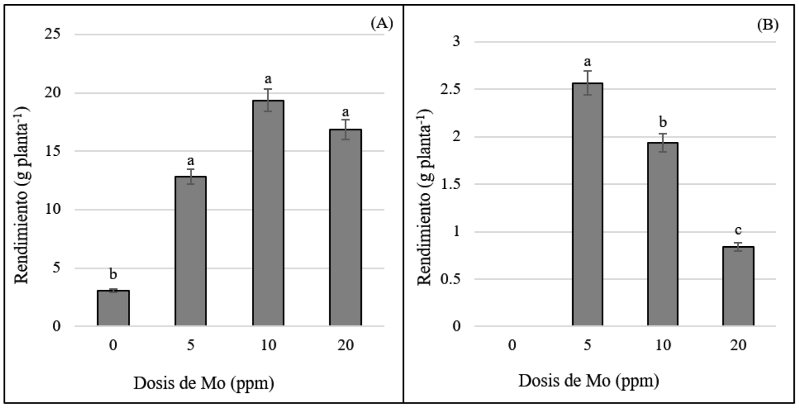

Figure 6. A) effect of the foliar application of NanoMo on the total yield in fresh weight per plant; and B) efficiency in fruits produced per amount of foliar Mo applied per plant in snap beans. Columns with different letters differ statistically from each other (Tukey $p \leq 0.05$).

Conclusions

The foliar application of Nanofertilizer of Mo increased the efficiency of nitrogen use, and the highest efficiency in productivity per unit of fertilizer applied was achieved with the dose of 5 ppm of Mo and 3 mM of N, the above suggests that excessive applications of nitrogen fertilizers can be reduced, and thereby minimize the toxic effects on the environment and the human being, without affecting the yield of the snap bean crop.

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