agronomy

Article

Application of Molybdenum Nanofertilizer on the Nitrogen Use Efficiency, Growth and Yield in Green Beans

Ezequiel Muñoz-Márquez [1], Juan Manuel Soto-Parra [2], Linda Citlalli Noperi-Mosqueda [2] and Esteban Sánchez [1,*]

1 Centro de Investigación en Alimentación y Desarrollo A. C., Chihuahua 33089, Mexico
2 Facultad de Ciencias Agrotecnológicas, Universidad Autónoma de Chihuahua, Chihuahua 31000, Mexico
* Correspondence: esteban@ciad.mx; Tel.: +55-6394748400

Abstract: The increase in the cost of fertilizers and their low efficiency has led, through nanotechnology, to the generation of new innovative products that are sustainable and improve the productivity of crops. Therefore, the objective of the present study was to evaluate the efficacy of a molybdenum nanofertilizer compared to two conventional fertilizers (chelate and sodium molybdate) applied via foliar combined with soil fertilization of NH_4NO_3 in relation to the Nitrogen Use Efficiency, growth and yield in green bean cv. Strike. Green bean plants cv. Strike were cultivated under controlled conditions in an experimental greenhouse and irrigated with nutrient solution. The treatments consisted of the foliar application of three Mo sources (Nano fertilizer, Mo Chelate and Sodium Molybdate) in four doses 0, 5, 10 and 20 ppm Mo, complemented with the edaphic application of four doses of NH_4NO_3 (0, 3, 6 and 12 mM of N). The results obtained indicate that the highest accumulation of biomass and yield were obtained with the application of NanoMo, with increases in biomass of 24.31% and 36.47% more in yield with respect to Chelate and Molybdate. Finally, it is concluded that the application of NanoMo improves the assimilation and efficiency of nitrogen use, reducing excessive applications of nitrogenous fertilizers without affecting the yield of the green bean crop.

Keywords: *Phaseolus vulgaris* L.; foliar molybdenum; nanofertilizer; micronutrients; nitrogen metabolism; efficiency parameters

check for updates

Citation: Muñoz-Márquez, E.; Soto-Parra, J.M.; Noperi-Mosqueda, L.C.; Sánchez, E. Application of Molybdenum Nanofertilizer on the Nitrogen Use Efficiency, Growth and Yield in Green Beans. *Agronomy* **2022**, *12*, 3163. https://doi.org/10.3390/agronomy12123163

Academic Editor: Katarzyna Turnau

Received: 6 November 2022
Accepted: 7 December 2022
Published: 13 December 2022

1. Introduction

The global fertilizer industry currently faces the challenge of creating more efficient products that have a minimal adverse impact on the environment, primarily with a focus on nitrogenous fertilizers. Another challenge is the high cost of these fertilizers needed to ensure crop productivity. This component of the agricultural production system generally consumes more than 50% of the annual budget needed for this activity [1]. In the same way, given the continuous increase in the population, the acquisition of fertilizers to satisfy the growing demand for food becomes increasingly expensive and unsustainable [2].

An alternative solution is the improvement of fertilizers that are already commonly used or through the development of new specific fertilizers. Of the latter, nanotechnology has provided agriculture with a viable alternative that provides a solution to the excessive and costly use of conventional fertilizers. Nanofertilizers are a key tool to improve the growth, productivity and quality of crops with greater efficiency in the use of nutrients. In addition to that, they manage to reduce the waste of fertilizers and production costs. Nanofertilizers provide a larger contact surface to increase metabolic reactions within the plant, which results in an increase in the photosynthetic rate and greater production of dry matter [3].

In this context, it is necessary to highlight the role of Mo in plant metabolism. This essential micronutrient has, as one of its specific functions, to be a structural component of the enzyme Nitrate Reductase, which plays an essential role in the assimilation of nitrogen [4]. Furthermore, it is a vital part of a complex organic pterin called molybdenum cofactor

(Moco); this cofactor binds to molybdoenzymes in most biological systems [5]. Various studies have shown that molybdenum deficiency reduces the activity of molybdoenzymes, which negatively affects primary nitrogen assimilation and activity in legume nodules [6]. Directly in legumes, molybdenum is present in the biosynthesis of abscisic acid and the conversion of sulfite to sulfate carried out by sulfite oxidase and aldehyde oxidase, in addition to being an important part of the metabolism of sulfur amino acids containing [7].

Based on scientific and technical knowledge of the use and application of nutrients, it has been shown that the use of nanotechnology has reduced nitrogenous fertilizer losses by up to 60%. This makes it promising to evaluate new formulations that allow for improving Nitrogen Use Efficiency and, at the same time, reducing emissions to the environment [8].

In general, there is still little literature on the use of nanofertilizers in agriculture, so the objective of this study was to evaluate the efficacy of a molybdenum nanofertilizer compared to two conventional fertilizers (chelate and sodium molybdate) applied via foliar combined with edaphic fertilization of ammonium nitrate (NH_4NO_3), in relation to the efficiency of nitrogen use, growth and yield in green bean cv. Strike.

2. Materials and Methods

2.1. Crop Location and Management

The crop was developed in a greenhouse covered with anti-aphid mesh located in Lázaro Cárdenas, Meoqui, Chihuahua, Mexico (Latitude: N 28°23′9.80232″, Longitude: W 105°36′58.09392″), starting on 2 September 2020 to harvest on 3 November 2020, with an average temperature of 28.6 °C. Bean seeds cv. Short cycle Strike (60 days until physiological maturity) were germinated in polystyrene trays with 200 cavities; 12 days after germination, two plants were transplanted into each 400-gauge, 10 kg polyethylene bag, which contained vermiculite and perlite as substrate in a 2:1 ratio. A complete nutrient solution pH 6.0 was applied for 20 days according to Hoagland and Arnon [9] as proposed by Sánchez et al. [10] from germinating plants, which carried the following composition: 6.0 mM NH_4NO_3, 1.6 mM K_2HPO_4, 0.3 mM K_2SO_4, 4.0 mM $CaCl_2 \bullet 2H_2O$, 1.4 mM $MgSO_4$, 5.0 μM Fe-EDDHA, 2.0 μM $MnSO_4 \bullet H_2O$, 1.0 μM of $ZnSO_4 \bullet 7H_2O$, 0.25 μM $CuSO_4 \bullet 5H_2O$, 0.3 μM Na_2MoO_4 and 0.5 μM H_3BO_3 (all reagents J.T. Baker, City of Mexico, State of Mexico, Mexico). With the aim that the plants were well nourished in their early stages of development, 500 mL of the nutrient solution was applied per bag every third day. After 20 days, differentiated nitrogen treatments were applied in the nutrient solution every third day until the end of the crop. The molybdenum treatments were foliar applied every seven days from the appearance of the true leaves. The entire experiment was carried out in a single time (2 September to 3 November 2020), and the application of all treatments (Splits, sub-splits and sub-sub-splits) was carried out simultaneously.

2.2. Experimental Design and Treatments

An experimental design was established with a split-plot arrangement in a completely randomized design with four replications. The sources of Mo (representing the Splits) BROADACRE® Zn Mo Nanofertilizer (Agrichem of Mexico, Mazatlán, Sinaloa, Mexico); GRO BoMo® Chelate (Fertilizantes Tepeyac, Delicias, Chihuahua, Mexico) and Sodium Molybdate (J.T. Baker, City of Mexico, State of Mexico, Mexico), N doses as ammonium nitrate (NH_4NO_3 as source): 0, 3, 6 and 12 mM (representing the Sub-splits) and Mo doses: 0, 5, 10 and 20 ppm (representing the Sub-sub-splits). There was a total of 48 treatments, with 384 experimental units (plants) (two plants bag represent a repetition, having four repetitions in total) (Figure 1). Five foliar applications of the three different sources of molybdenum were made from day 21 after germination, and 16 applications of the differentiated nutrient solution in nitrogen from day 22 after germination. The additive linear model was as follows:

$$\Upsilon ijkm = \mu + \theta i + \varepsilon im + \Omega j + (\theta\Omega)ij + \lambda ijm + \beta k + (\theta\beta)ik + (\Omega\beta)jk + (\theta\Omega\beta)ijk + \varepsilon ijkm$$

where:

i = Molybdenum Source (Split)
j = Nitrogen doses (Sub-split)
k = Molybdenum doses (Sub-sub-split)
m = Repetition

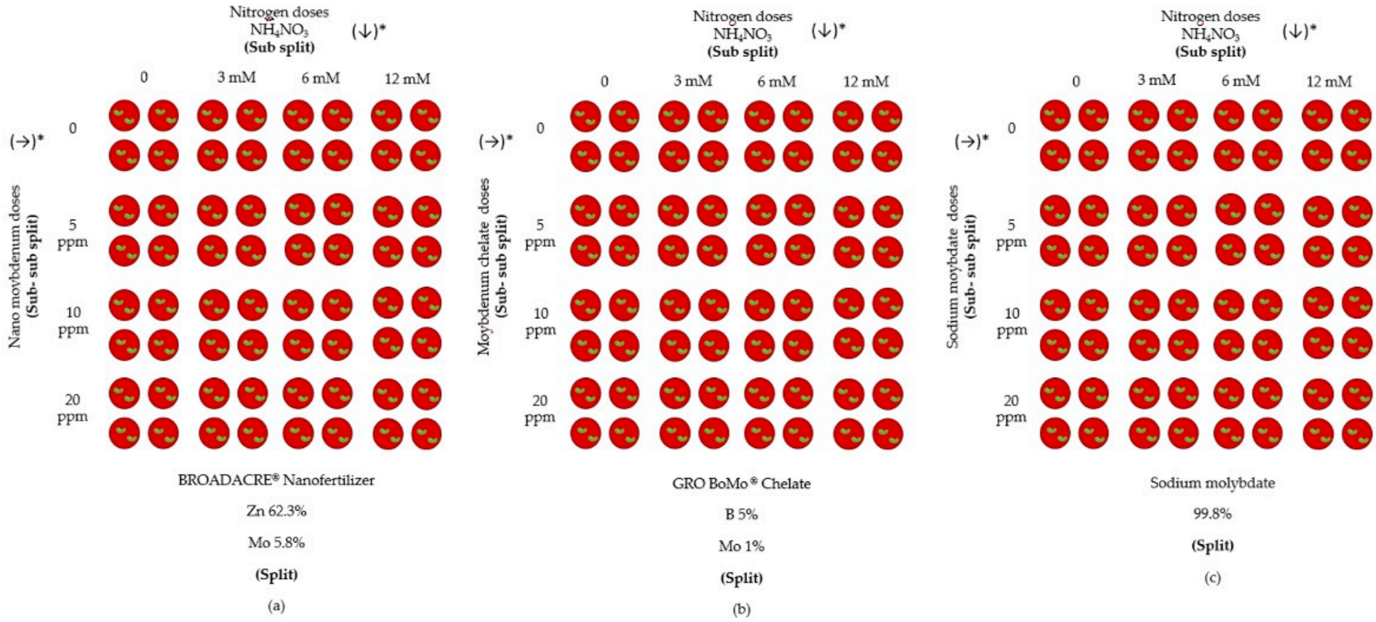

Figure 1. Experimental design. Nitrogen edaphic application supplemented with molybdenum foliar fertilization in green bean cv. Strike. The figure shows how the experiment was distributed inside the greenhouse. (**a**) Split where Nanofertilizer was applied, (**b**) split where Chelate was applied, (**c**) split where Sodium molybdate was applied. (↓)* Application direction in columns of nitrogen doses, (→)* application direction in rows of molybdenum doses.

2.3. Plant Sampling

Once the physiological maturity of the plants was reached (60 days after germination), the samples were taken. Four plants of each treatment were separated into their different organs: leaf, stem, root and fruit. With fresh material stored at 4 °C (Forma Scientific Refrigerator, Marietta, OH, USA), the yield was determined; while the dry material was used to determine the total biomass, the total organic nitrogen concentration and the molybdenum concentration. All the material was previously washed with running water to eliminate surface environmental contamination, then two more rinses were carried out with distilled water and tri-distilled water (J.T. Baker, City of Mexico, State of Mexico, Mexico). Four repetitions per treatment were used for each variable analyzed.

2.4. Plant Analysis

2.4.1. Biomass

After environmental decontamination, the samples were placed in a forced air oven at 70 °C (Felisa® St. Livonia Oven, MI, USA) for 24 h until completely dry. Total biomass production was calculated based on the dry weight of plant material expressed in grams (g) [11].

2.4.2. Yield

The yield was obtained based on 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). The total yield was expressed in grams per plant (g/plant) [11].

2.4.3. Determination "In Vivo" of the Nitrate Reductase Activity (NR)

It was determined by the test proposed by Jaworski, 1961 [12,13]. Between 0.125 and 0.150 g of leaf blade segments were weighed and placed in a test tube containing 10 mL of infiltration medium, which was different depending on the determined NR activity: endogenous NR (potassium phosphate buffer 100 mM, pH 7.5 + 1% propanol (J.T. Baker, City of Mexico, State of Mexico, Mexico)); NR + NO_3^- (100 mM potassium phosphate buffer, pH 7.5 with 50 mM potassium nitrate (KNO3) + 1% propanol; NR + Mo (100 mM potassium phosphate buffer, pH 7.5 with 50 mM sodium molybdate (NaMoO4) + 1% propanol, and NR + NO_3^- + Mo (100 mM potassium phosphate buffer, pH 7.5 with 50 mM potassium nitrate (KNO_3) and 50 mM sodium molybdate ($NaMoO_4$) + 1% propanol). Next, the samples were subjected to a vacuum (0.8 bar) (Vacuum furnace, Felisa) for 10 min in the dark, after which time the vacuum was released and the samples were incubated for 60 min at 30 °C in darkness (Wise Cube® Incubator, Wise Laboratory Instrument, DAIHAN Scientific Co., Seoul, Korea). After one hour of incubation, the samples were placed in a water bath at 100 °C for 15 min to stop the NR activity. For the determination of the "in vivo" NR activity, 1 mL of the sample extract was taken and emptied into a test tube, 2 mL of 1% sulfanilamide in 1.5N HCL and 2 mL of d e NNEDA (0.02% N-1-naphthyl-ethylenediamide in 0.2N HCL); they were shaken in a Vortex (VWR® International, Thorofare, El Segundo, NJ, USA) and left to stand for 20 min at room temperature. Finally, the absorbance was read at 540 nm in a UV/Vis spectrophotometer (Genesys 10S, Thermo Scientific® Corporation, Cambridge, UK). The result was expressed in µmol of NO_2^- formed per mg of protein per hour (µmol $NO_2^- \cdot$ g.p.f.$^{-1} \cdot$ h^{-1}) [13].

2.4.4. Total Nitrogen Determination

The dried samples were ground in a small jar blender (Osterizer® Blender, Milwaukee, WI, USA) and placed in plastic bags (Nasco Whirl-Pak®, Cincinnati, OH, USA) for analysis. The total nitrogen concentration was determined using the Flash 2000 Organic Elemental Analyzer (Thermo Scientific® Corporation, Cambridge, UK), which bases its operation on the method initially described by Jean-Baptiste Dumas in 1826 [12]. A tin capsule was placed on a microbalance (Mettler Toledo®, Columbus, OH, USA), and 9 mg of vanadium pentoxide (JT Baker, City of Mexico, State of Mexico, Mexico) and 3 mg of the finely ground sample were weighed. Once the weight was taken, the capsule was closed. The samples were then placed in the Flash 2000 autosampler for analysis. Two certified standards of Methionine and Sulfanilamide (Thermo Scientific® Corporation, Cambridge, UK) were also analyzed in order to guarantee the accuracy of the results. The concentration of total organic N was expressed as a percentage (%).

2.5. Nitrogen Use Efficiency Parameters (NUE)

Nitrogen Use Efficiency (NUE) parameters were calculated as follows:

- The total nitrogen accumulation (TNA) was calculated with the nitrogen concentration multiplied by the total biomass of the plant [14];
- Nitrogen uptake efficiency (NUpE) was calculated as TNA divided by root dry weight (DW) (mg N g^{-1} RDW) [14];
- Nitrogen utilization efficiency (NUtE) was calculated as dry weight (DW) of leaf tissue divided by N concentration (g^2 LDW mg^{-1} N) [15].

Determination of the Photosynthetic Pigments Concentration

An amount of 0.125 g of foliar discs from various leaves of the plant, free of veins and with a diameter of 7 mm, were weighed, placed in test tubes, and 10 mL of 99.9% concentrated methanol (CH_3OH) was added (JT Baker, City of Mexico, State of Mexico, Mexico); they were shaken in a Vortex (VWR, Thorofare, El Segundo, NJ, USA) and left to stand for 24 h in the dark and at room temperature. After that time, the samples were read in a UV/Vis Spectrophotometer (Genesys 10S, Thermo Scientific® Corporation, Cambridge, UK) at wavelengths of 470 nm (carotenoids), 653 nm (chlorophyll b, Chl b) and 666 nm (chlorophyll a, Chl a). A blank containing exclusively methanol was used for reading. The calculation of the pigment concentration was carried out according to the following equations [10,16].

$$\text{Chl a}: [15.65(\text{A}_{666}) - (7.34\ (\ \text{A}_{653}))] \tag{1}$$

$$\frac{(\text{Chl a})(\text{V}_1)(\text{P}_1)}{(\text{P}_2)(2\pi\text{r}^2(\text{n})}$$

$$\text{Chl b}: [27.05(\text{A}_{653}) - (11.21\ (\ \text{A}_{666}))] \tag{2}$$

$$\frac{(\text{Chl b})(\text{V}_1)(\text{P}_1)}{(\text{P}_2)(2\pi\text{r}^2(\text{n})}$$

where V_1 is the extraction volume, P_1 is the weight in g per leaf disc (7 mm diameter), P_2 is the total weight in g, n is the number of leaf discs and r^2 is the radius of the leaf discs. The sum of the concentrations of chlorophyll a and chlorophyll b resulted in total chlorophyll, which was expressed in μg cm^{-2}.

2.6. Statistic Analysis

The data obtained were subject to a variance analysis based on the proposed additive linear model; the impact probabilities were $p > 0.05$ not significant, $0.05 \leq p \leq 0.01$ significant, $p < 0.01$ highly significant. The multiple range test was obtained. The Tukey test (α 0.05) was used to separate treatment means within each factor (split, sub-split and sub-sub-split). Subsequently, a response surface analysis of the plot $\times$ subplot interaction was performed for the plot-cell factor with the greatest statistical relevance [17].

The response surface analysis included the following steps: model fit and analysis of variance to estimate the parameters. The estimated surface will typically be curved, a hill whose peak occurs at the single estimated point of maximum response, a valley or a saddle-shaped surface without any maximum or minimum. It is determined (1) if the types of effects are linear, quadratic or cross products, how much of the residual error is due to the lack of adjustment and what is the contribution of each factor in the statistical adjustment; (2) canonical correlation is used to investigate the shape of the predicted response surface, calculating whether the fixed point is a maximum, minimum or a saddle point and which factor or factors are the most sensitive predicted responses; and (3) Ridge analysis is used for the search for the optimal response. The eigenvalues and eigenvectors of the canonical analysis characterize the shape of the response surface; the eigenvalues indicate the direction of the main orientation of the surface, and the signs and magnitudes of the associated eigenvectors give the shape of the surface in those directions. Positive eigenvalues indicate upward curvature directions, and negative eigenvalues indicate downward curvature directions. The eigenvector for the largest eigenvalue gives the direction of steep rise from the fixed point if positive or steep fall if negative. Eigenvectors corresponding to small or zero eigenvalues indicate directions of relative flattening. To determine if the solution is a maximum or a minimum, the sign of the eigenvalues is observed: if the eigenvalues are all negative, the solution is a maximum; if they are all positive, the solution is a minimum; if they have mixed signs, the solution is a saddle point; and if they contain zeros, the solution is a flattened area [18].

Once the statistical analysis was carried out, the SigmaPlot 14.0 program was used to obtain the graphs with the predicted results of the SAS program. The graphs are for those variables that were significant, either in linear, quadratic regression or interaction of factors.

3. Results

3.1. Effect of Edaphic Application of Nitrogen Supplemented with Foliar Fertilization of NanoMo on Biomass and Yield

Nitrogen assimilation is a limiting factor that determines the growth, development and productivity of plants [19]. Likewise, molybdenum is an essential micronutrient basic for molybdenum mononuclear enzymes, responsible for nitrogen assimilation and ascorbate–glutathione regulation within plant metabolism [3]. It should be noted that the fertilization of micronutrients such as Mo through foliar application and using novel technology allows its rapid absorption by the leaves and its transfer to different organs in short times due to the great mobility of this element [20]. In the present study, this characteristic of Mo was used, together with its application in the form of a nanofertilizer, to increase yield (Figure 2). The results showed that, with the foliar application of the Mo nanofertilizer, the highest biomass development and the highest fruit yield were obtained (Table 1). The application of foliar NanoMo increased biomass development by 21.37% and 24.31% compared to the application of sodium molybdate and molybdenum chelate, respectively. Similarly, the increase in yield compared to sodium molybdate was 21.76% and 36.47% compared to Mo chelate.

Figure 2. Effect of the edaphic application of nitrogen supplemented with foliar fertilization of NanoMo on the total biomass in green bean cv. Strike.

Table 1. Effect of edaphic application of nitrogen supplemented with foliar fertilization of NanoMo on biomass and yield.

	Growth	
	Biomass	**Yield**
Mo Source	0.0001 ^{U}	0.0001
NanoMo	2.92 a ^{V}	1.70 a
Mo Chelate	2.21 b	1.08 c
Na Molybdate	2.28 b	1.33 b
MSD	0.29 ^{W}	0.22
Nitrogen ^{X}	0.0002	0.0024
0	2.13 c	1.18 c
3	2.60 ab	1.30 bc
6	2.78 a	1.56 a
12	2.34 bc	1.44 ab
MSD	0.25	0.25
Molybdenum ^{Y}	<0.0001	<0.0001
0	2.26 b	2.27 b
5	2.36 b	1.36 b
10	2.65 a	2.65 a
20	2.60 a	2.60 a
MSD	0.23	0.23
SoMo × N	0.3968	0.0311
SoMo × Mo	<0.0001	<0.0001
N × Mo	0.0481	0.0599
SoMo × N × Mo	0.6020	0.7049
μ	2.47	1.37
C.V.	17.75	30.04
R^2	0.7724	0.7093

^{U} Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; ^{V} Means with the same letter are statistically equal (Tukey α 0.05); ^{W} Minimum significant difference; ^{X} Edaphic mM concentration of nitrogen; ^{Y} Leaf molybdenum ppm concentration, μ overall mean, CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction.

3.2. Biomass

N is the most critical nutrient in a fertilization program because it is essential for optimal crop growth; the vegetative development of the plant depends largely on the amount of N applied [21]. In addition, in the N fixation process, Mo is the cofactor of Nitrogenase and Nitrate reductase so that they can catalyze the redox reaction and convert elemental N into ammonium ions (NH_4^+) to be assimilated [22]; in this way, Mo influences the increase in biomass and crop yield.

In the case of the effect of the doses of nitrogen and Mo on the total biomass, it can be seen that the doses with which the greatest growth of the plant was obtained were 6 mM of N and 10 ppm of Mo (Figure 3). In both graphs, it can also be seen that with these doses the maximum development and production of biomass was reached. In addition, applying higher doses of nitrogen and Molybdenum has a negative effect and biomass production falls.

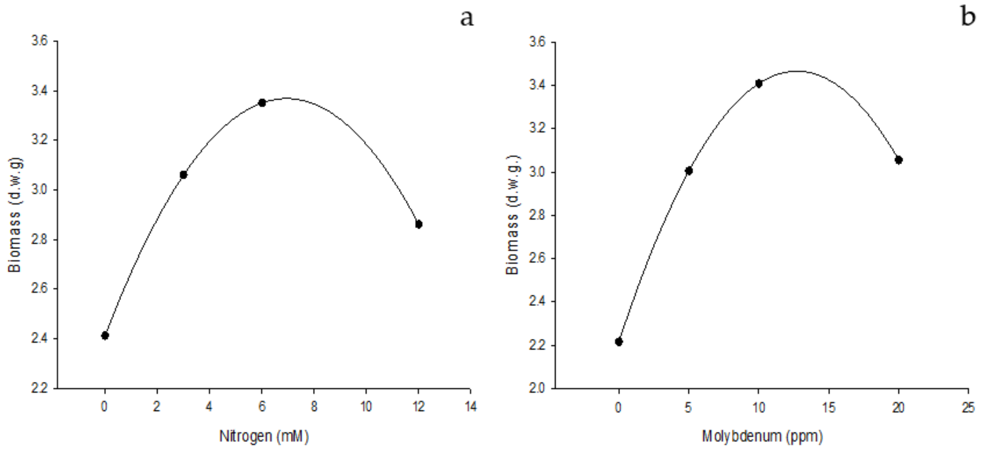

Figure 3. Effect of the edaphic application of nitrogen supplemented with foliar fertilization of NanoMo on the total biomass in dry weight per plant of green bean cv. Strike. (**a**) Effect of nitrogen on total biomass. (**b**) NanoMolybdenum effect on total biomass.

The data from the response surface analysis are shown below to provide greater clarity and statistical support to the graphs (Table 2).

Table 2. Response surface analysis for molybdenum sources in biomass.

Biomass											
NanoMo				**Mo Chelate**				**Na Molybdate**			
CV	11.50	R^2	0.7520	CV	23.61	R^2	0.1668	CV	26.92	R^2	0.1654
Regression		Factors		Regression		Factors		Regression		Factors	
L	<0.0001	N	Mo	L	0.9424	N	Mo	L	0.1266	N	Mo
C	<0.0001	<0.0001	<0.0001	C	0.0066	0.0347	0.4120	C	0.0349	0.0653	0.2754
P	0.1367	L, C	L, C	P	0.4723	L, C		P	0.7615	L, C	
Model	<0.0001			Model	0.0544			Model	0.0565		
Source	Es	SE	$p > t$	Source	Es	SE	$p > t$	Source	Es	SE	$p > t$
Int	1.7961	0.1210	<0.0001	Int	2.0772	0.1881	<0.0001	Int	1.8780	0.2211	<0.0001
N	0.2583	0.0358	<0.0001	N	0.1594	0.0557	0.0059	N	0.1644	0.0654	0.0149
Mo	0.1864	0.0215	<0.0001	Mo	−0.0413	0.0334	0.2209	Mo	0.0005	0.0392	0.9885
N × N	−0.0198	0.0026	<0.0001	N × N	−0.0120	0.0040	0.0047	N × N	−0.0126	0.0048	0.0109
Mo × N	0.0019	0.0012	0.1367	Mo × N	−0.0014	0.0019	0.4723	Mo × N	0.0007	0.0023	0.7615
Mo × Mo	−0.0077	0.0009	<0.0001	Mo × Mo	0.0022	0.0014	0.1339	Mo × Mo	0.0007	0.0017	0.6649
		Eigenvectors				Eigenvectors				Eigenvectors	
Eigenva	−0.6780	0.8535	0.5210	Eigenva	0.2267	−0.0652	0.9978	Eigenva	0.0762	0.0402	0.9999
	−0.8084	−0.5210	0.8535		−0.4362	0.9978	0.0652		−0.4564	0.9999	−0.0402

Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction; SE standard error; Int intercept; Es estimation; Eigenva: Eigenvalues; Eigenvectors.

3.3. Yield

Nitrogen increases the levels of compounds that are synthesized by increasing the photosynthetic rate; these assimilations are translocated to the edible parts of the plants. Recent research has shown that the supply of nitrogenous mineral fertilizers increased the weight and number of seeds per plant, in addition to the total yield [23]. In addition to the above, molybdenum is essential and indispensable for nitrogen fixation and consequently for plant performance [5].

Like the biomass, the yield had similar behavior, especially because these two variables have a great relationship. The highest fruit production occurred with the doses of 6 mM of N and 10 ppm of Mo (Figure 4). In the graphs, it can also be seen how applying the highest doses of nitrogen (12 mM) and molybdenum (20 ppm) affects the production of fruits per plant by 16.58%.

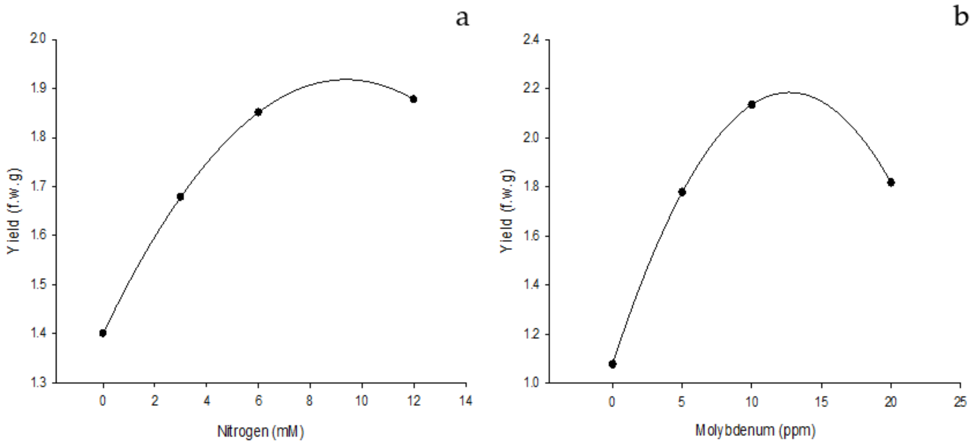

Figure 4. Effect of the edaphic application of nitrogen supplemented with foliar fertilization of NanoMo on the yield per plant of green bean cv. Strike. (**a**) Effect of nitrogen on yield. (**b**) Effect of NanoMolybdenum on yield.

The data from the response surface analysis are shown below to provide greater clarity and statistical support to the graphs (Table 3).

Table 3. Response surface analysis for molybdenum sources in yield.

Yield											
NanoMo				**Mo Chelate**				**Na Molybdate**			
CV	18.83	R^2	0.6661	CV	23.61	R^2	0.1668	CV	33.18	R^2	0.3181
Regression		Factors		Regression		Factors		Regression		Factors	
L	<0.0001	N	Mo	L	0.9424	N	Mo	L	<0.0001	N	Mo
C	<0.0001	0.0002	<0.0001	C	0.0066	0.0347	0.4120	C	0.1016	0.0016	0.0272
P	0.5980	L, C	L, C	P	0.4723	C	C	P	0.7316	L, C	L, C
Model	<0.0001			Model	0.5403			Model	0.0004		
Source	Es	SE	$p > t$	Source	Est	SE	$p > t$	Source	Est	SE	$p > t$
Int	0.7459	0.1154	<0.0001	Int	1.1932	0.1842	<0.0001	Int	0.8428	0.1593	<0.0001
N	0.1161	0.0342	0.0012	N	0.0215	0.0545	0.6947	N	0.1283	0.0472	<0.0001
Mo	0.1779	0.0205	<0.0001	Mo	−0.0001	0.0327	0.9967	Mo	−0.0083	0.0283	0.7683
N × N	−0.0058	0.0025	0.0224	N × N	−0.0042	0.0040	0.2931	N × N	−0.0061	0.0034	0.0809
Mo × N	−0.0006	0.0012	0.5980	Mo × N	0.0016	0.0019	0.3964	Mo × N	−0.0005	0.0016	0.7316
Mo × Mo	−0.0068	0.0009	<0.0001	Mo × Mo	−0.0007	0.0014	0.6069	Mo × Mo	0.0015	0.0012	0.2104
		Eigenvectors				Eigenvectors				Eigenvectors	
Eigenva	−0.2113	0.9991	−0.0407	Eigenva	0.2267	−0.0652	0.9978	Eigenva	0.1588	−0.0457	0.9989
	−0.6883	0.0407	0.9991		−0.4362	0.9978	0.0652		−0.2224	0.9989	0.0457

Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction; SE standard error; Int intercept; Es estimation; Eigenva: Eigenvalues; Eigenvectors.

3.4. Nitrate Reductase Activity (NR)

The nitrate reductase enzyme is a molybdoenzyme that acts in nitrogen metabolism and is responsible for catalyzing the reduction of nitrate (NO_3^-) to nitrite (NO_2^-) [24]; it is the limiting enzyme in the rate of N assimilation and, therefore, it plays a fundamental role in the growth and development of plants [25]. In the present study, the enzyme had higher activity in the Mo Chelate and Na Molybdate treatments (Table 4). When analyzing these results more closely and in comparison with the biomass and the yield, it can be seen that the Chelate and Molybdate had lower production, which may lead to an overaccumulation of nitrate due to a lower assimilation efficiency. On the contrary, in the NanoMo treatment, the activity of the enzyme was lower, assuming a higher translocation efficiency, which led to higher biomass yields and fruit production. Therefore, the application of nitrogen and NanoMo had a direct effect on the activity of the enzyme. The dose of N with which the highest endogenous activity was shown and induced with NO_3 was 6 mM (Figure 5).

Table 4. Effect of edaphic application of nitrogen supplemented with foliar fertilization of NanoMo on Nitrate reductase activity.

	Nitrate Reductase			
	Endogenous	**NO_3**	**Mo**	**NO_3 + Mo**
Mo Source	<0.0001 ^{U}	0.0005	<0.0001	0.0003
NanoMo	0.62 c ^{V}	2.88 b	1.02 c	2.96 b
Na Molybdate	1.41 b	3.40 a	1.42 b	3.13 b
Mo Chelate	1.98 a	3.56 a	2.13 a	3.50 a
MSD	0.37 ^{W}	0.31	0.38	0.23
Nitrogen ^{X}	<0.0001	<0.0001	<0.0001	<0.0001
0	0.47 c	2.78 b	0.66 c	2.76 b
3	1.42 b	3.45 a	1.62 b	3.43 a
6	1.63 ab	3.42 a	1.78 ab	3.17 a
12	1.83 a	3.46 a	2.03 a	3.42 a
MSD	0.40	0.36	0.32	0.29
Molybdenum ^{Y}	0.0090	<0.0001	0.1310	<0.0001
0	1.60 a	2.68 c	1.69 a	2.66 c
5	1.14 b	3.49 ab	1.32 a	3.29 b
10	1.16 b	3.26 b	1.54 a	3.20 b
20	1.46 ab	3.69 a	1.54 a	3.63 a
MSD	0.42	0.29	0.40	0.23
SoMo × N	<0.0001	<0.0001	<0.0001	<0.0001
SoMo × Mo	0.1634	<0.0001	0.9616	<0.0001
N × Mo	0.0048	<0.0001	0.0048	<0.0001
SoMo × N × Mo	<0.0001	<0.0001	<0.0001	<0.0001
μ	1.34	3.28	1.52	3.20
C.V.	59.00	16.82	49.38	13.70
R^2	0.7750	0.8851	0.7574	0.9286

^{U} Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; ^{V} Means with the same letter are statistically equal (Tukey α 0.05); ^{W} Minimum significant difference; ^{X} Edaphic mM concentration of nitrogen; ^{Y} Leaf molybdenum ppm concentration, μ overall mean, CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction.

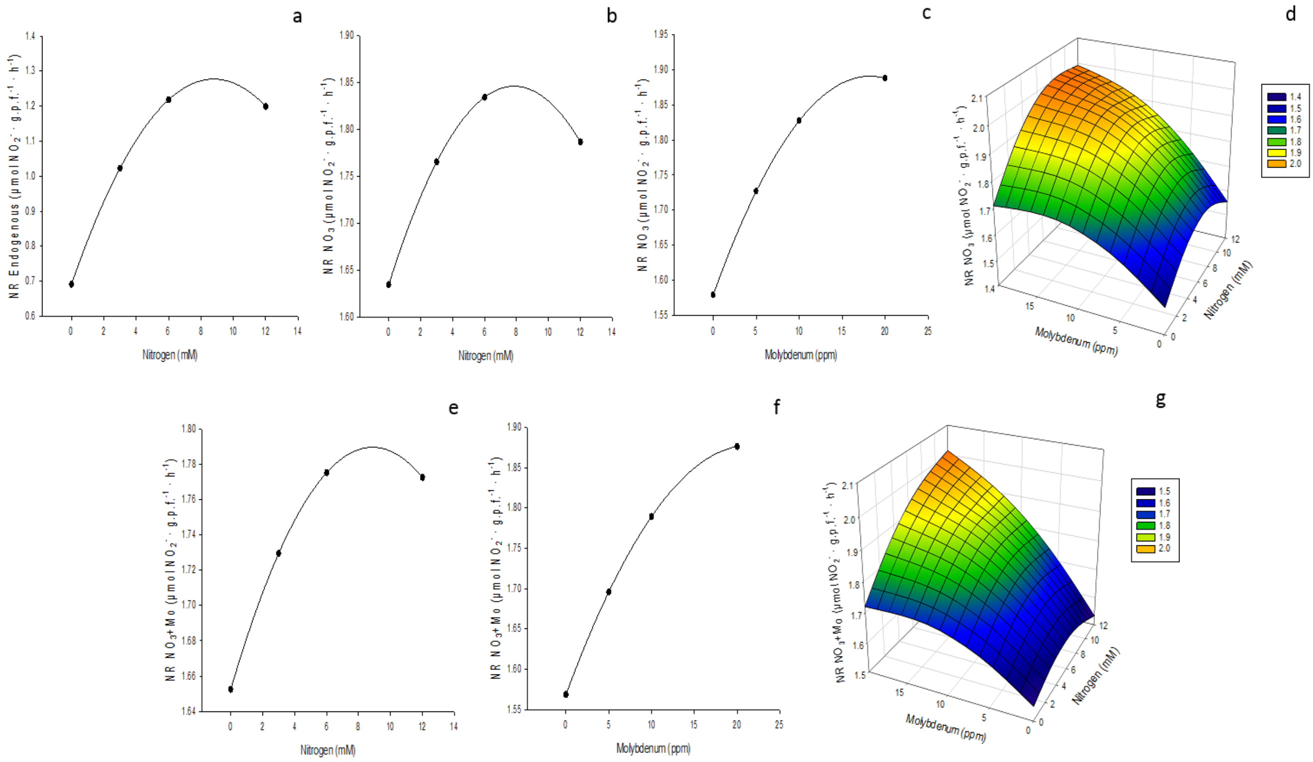

Figure 5. Effect of the edaphic application of nitrogen complemented with foliar fertilization of NanoMo on the activity of the enzyme Nitrate reductase (NR) in green bean plants cv. Strike. (**a**) Endogenous NR activity. (**b**) NR activity induced with NO_3. (**c**) Effect of Molybdenum on NR activity induced with NO_3. (**d**) Effect of nitrogen and Molybdenum on the NR Activity induced with NO_3. (**e**) NR activity induced with NO_3 + Mo. (**f**) Effect of molybdenum on the NR activity induced with NO_3 + Mo. (**g**) Effect of nitrogen and molybdenum on the NR activity induced with NO_3 + Mo.

The data from the response surface analysis are shown below to provide greater clarity and statistical support to the graphs (Tables 5–7).

Table 5. Response surface analysis for molybdenum sources in NR endogenous.

NR Endogenous											
NanoMo				Mo Chelate				Na Molybdate			
CV	18.83	R^2	0.6661	CV	23.61	R^2	0.1668	CV	33.18	R^2	0.3181
Regression		Factors		Regression		Factors		Regression		Factors	
L	0.0003	N	Mo	L	<0.0001	N	Mo	L	0.0041	N	Mo
C	0.0226	0.0010	0.0003	C	0.0015	<0.0001	0.6770	C	0.0007	0.0016	0.0272
P	0.0105	C	L, C	P	0.9649	L, C		P	0.0012	L, C	L, C
Model	<0.0001			Model	<0.0001			Model	<0.0001		
Source	Es	SE	$p > t$	Source	Est	SE	$p > t$	Source	Est	SE	$p > t$
Int	0.7115	0.2148	0.0016	Int	0.6672	0.3872	0.0902	Int	1.1540	0.3376	0.0012
N	−0.0022	0.0636	0.9717	N	0.5507	0.1146	<0.0001	N	0.2961	0.1000	0.0044
Mo	−0.0781	0.0381	0.0451	Mo	−0.0686	0.0688	0.3227	Mo	−0.1568	0.0600	0.0114
N × N	0.0083	0.0046	0.0792	N × N	−0.0305	0.0084	0.0006	N × N	−0.0255	0.0073	0.0010
Mo × N	−0.0060	0.0022	0.0105	Mo × N	−0.0001	0.0040	0.9649	Mo × N	0.0121	0.0035	0.0012
Mo × Mo	0.0037	0.0016	0.0307	Mo × Mo	0.0036	0.0030	0.2361	Mo × Mo	0.0054	0.0026	0.0427
		Eigenvectors				Eigenvectors				Eigenvectors	
Eigenva	0.5205	−0.6340	0.7733	Eigenva	0.3629	−0.0037	0.9999	Eigenva	0.6337	0.2288	0.9734
	0.1526	0.7733	0.6340		−1.0995	0.9999	0.0037		−1.0052	0.9734	−0.2288

Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction; SE standard error; Int intercept; Es estimation; Eigenva: Eigenvalues; Eigenvectors.

Table 6. Response surface analysis for molybdenum sources in NR NO_3.

NR Induced with NO_3											
NanoMo				**Mo Chelate**				**Na Molybdate**			
CV	55.26	R^2	0.3297	CV	10.79	R^2	0.0676	CV	18.08	R^2	0.4734
Regression		Factors		Regression		Factors		Regression		Factors	
L	<0.0001	N	Mo	L	0.4921	N	Mo	L	0.0013	N	Mo
C	0.1788	0.0017	0.0102	C	0.2748	0.6912	0.4204	C	0.0002	<0.0001	<0.0001
P	0.3384	L		P	0.7228			P	0.0001	C	L, C, Mo
Model	0.0002			Model	0.5263			Model	<0.0001		
Source	Es	SE	$p > t$	Source	Est	SE	$p > t$	Source	Est	SE	$p > t$
Int	0.4347	0.5731	0.4512	Int	3.5921	0.1383	<0.0001	Int	3.2165	0.2212	<0.0001
N	0.4861	0.1697	0.0058	N	0.0011	0.0409	0.9772	N	0.0261	0.0655	0.6917
Mo	0.1817	0.1018	0.0797	Mo	−0.0366	0.0245	0.1412	Mo	0.1173	0.0393	0.0041
N × N	−0.0219	0.0124	0.0833	N × N	0.0005	0.0030	0.8597	N × N	−0.0120	0.0048	0.0454
Mo × N	−0.0058	0.0060	0.3384	Mo × N	0.0005	0.0014	0.7228	Mo × N	0.0095	0.0023	0.0001
Mo × Mo	−0.0029	0.0044	0.5088	Mo × Mo	0.0017	0.0010	0.1116	Mo × Mo	−0.0065	0.0017	0.0004
		Eigenvectors				Eigenvectors				Eigenvectors	
Eigenva	−0.2422	−0.3048	0.9524	Eigenva	0.1765	0.0989	0.9950	Eigenva	−0.2350	0.8240	0.5665
	−0.8469	0.9524	0.3048		0.0176	0.9950	−0.0989		−0.8503	−0.5665	0.8240

Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction; SE standard error; Int intercept; Es estimation; Eigenva: Eigenvalues; Eigenvectors.

Table 7. Response surface analysis for molybdenum sources in NR induced with NO_3 and infiltered with Mo.

NR Induced with NO_3 and Infiltered with Mo											
NanoMo				**Mo Chelate**				**Na Molybdate**			
CV	54.30	R^2	0.2852	CV	10.55	R^2	0.1197	CV	27.28	R^2	0.3123
Regression		Factors		Regression		Factors		Regression		Factors	
L	0.0002	N	Mo	L	0.0440	N	Mo	L	0.0175	N	Mo
C	0.2043	0.0072	0.0212	C	0.7019	0.1259	0.4811	C	0.2818	0.0013	0.0002
P	0.4459	L		P	0.4513			P	0.0003		Mo
Model	0.0013			Model	0.1809			Model	0.0005		
Source	Es	SE	$p > t$	Source	Est	SE	$p > t$	Source	Est	SE	$p > t$
Int	0.7522	0.5797	0.1996	Int	3.2976	0.1329	<0.0001	Int	3.4539	0.3075	<0.0001
N	0.4518	0.1717	0.0109	N	0.0371	0.0393	0.3492	N	−0.1721	0.0910	0.0638
Mo	0.1612	0.1030	0.1230	Mo	−0.0055	0.0236	0.8146	Mo	0.0479	0.0546	0.3842
N × N	−0.0216	0.0126	0.0910	N × N	−0.0003	0.0028	0.9176	N × N	0.0017	0.0066	0.7975
Mo × N	−0.0047	0.0061	0.4459	Mo × N	−0.0010	0.0014	0.4513	Mo × N	0.0126	0.0032	0.0003
Mo × Mo	−0.0025	0.0045	0.5794	Mo × Mo	0.0008	0.0010	0.4057	Mo × Mo	−0.0038	0.0024	0.1177
		Eigenvectors				Eigenvectors				Eigenvectors	
Eigenva	−0.2176	−0.2434	0.9699	Eigenva	0.0966	−0.2851	0.9584	Eigenva	0.2789	0.8677	0.4969
	−0.8156	0.9699	0.2434		−0.0203	0.9584	0.2851		−0.5993	−0.4969	0.8677

Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction; SE standard error; Int intercept; Es estimation; Eigenva: Eigenvalues; Eigenvectors.

3.5. Photosynthetic Pigments

The importance of photosynthetic pigments lies in the capture of light and that the process of photosynthesis can be carried out. A low concentration of photosynthetic pigments is indicative of inadequate fertilization, especially nitrogen [26]. In the present study, nitrogen fertilization, coupled with foliar fertilization of the Mo nanofertilizer, had a highly significant influence on the concentration of photosynthetic pigments (Table 8), where the highest concentration was obtained by the plants fertilized with the nanofertilizer. The difference in concentration of Chlorophyll "a" increased by 16.78% compared to Mo Chelate and Sodium Molybdate. In Chlorophyll "b", the increase varied from 14.16% to 18.35% with respect to Molybdate of sodium and Chelate of Mo. Regarding the degradation compounds Carotenoids, the increases compared to Molybdate of sodium and Chelate of Mo were of 23.18% and 18.98%, respectively.

The data from the response surface analysis are shown below to provide greater clarity and statistical support to the graphs (Tables 9–13).

Table 8. Effect of edaphic application of nitrogen supplemented with foliar fertilization of NanoMo on photosynthetic pigments.

	Photosynthetic Pigments				
	Chl A	**Chl B**	**Carotenoids**	**Chl A + B**	**Chl A + B/C**
Mo Source	0.0001 ^{U}	0.0001	<0.0001	0.0017	0.0032
NanoMo	2.92 a ^{v}	1.58 a	6.53 a	2.02 a	0.74 b
Na Molybdate	2.43 b	1.35 b	5.02 b	1.73 b	0.73 b
Mo Chelate	2.43 b	1.29 b	5.29 b	1.99 a	0.77 a
MSD	0.15 ^{w}	0.10	0.47	0.16	0.02
Nitrogen ^{X}	0.0002	0.0024	<0.0001	0.0023	0.0019
0	2.20 c	1.13 c	4.99 c	2.09 a	0.71 b
3	2.46 b	1.44 b	5.55 b	1.83 b	0.77 a
6	2.56 b	1.38 b	5.65 b	1.97 ab	0.74 ab
12	2.81 a	1.66 a	6.28 a	1.76 b	0.74 ab
MSD	0.21	0.21	0.54	0.22	0.03
Molybdenum ^{Y}	<0.0001	<0.0001	<0.0001	0.0360	<0.0001
0	2.43 bc	1.26 b	1.62 c	2.02 a	1.70 a
5	2.58 ab	1.48 a	6.99 a	1.89 ab	0.43 b
10	2.41 c	1.34 b	6.54 b	1.92 ab	0.42 b
20	2.60 a	1.53 a	7.32 a	1.82 b	0.41 b
MSD	0.17	0.12	0.43	0.18	0.02
SoMo × N	0.3968	0.0311	<0.0001	<0.0001	0.0549
SoMo × Mo	<0.0001	<0.0001	<0.0001	0.1719	<0.0001
N × Mo	0.0481	0.0599	0.0026	<0.0001	<0.0001
SoMo × N × Mo	0.6020	0.7049	<0.0001	<0.0001	<0.0001
μ	2.51	1.4	5.62	1.91	0.74
C.V.	13.07	16.63	14.62	17.73	6.82
R^2	0.8215	0.8344	0.9476	0.7029	0.9953

^{U} Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; ^{V} Means with the same letter are statistically equal (Tukey ∝ 0.05); ^{W} Minimum significant difference; ^{X} Edaphic mM concentration of nitrogen; ^{Y} Leaf molybdenum ppm concentration, μ overall mean, CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction.

Table 9. Response surface analysis for molybdenum sources in photosynthetic pigments Chlorophyll a.

Chlorophyll a											
NanoMo				**Mo Chelate**				**Na Molybdate**			
CV	14.05	R^2	0.4898	CV	18.58	R^2	0.3153	CV	18.09	R^2	0.1051
Regression		Factors		Regression		Factors		Regression		Factors	
L	<0.0001	N	Mo	L	<0.0001	N	Mo	L	0.2100	N	Mo
C	0.3434	<0.0001	0.0111	C	0.8392	<0.0001	0.2786	C	0.2266	0.7094	0.1246
P	0.4455	L		P	0.0886			P	0.4571		L
Model	<0.0001			Model	0.0004			Model	0.2516		
Source	Es	SE	$p > t$	Source	Est	SE	$p > t$	Source	Est	SE	$p > t$
Int	2.1908	0.1481	<0.0001	Int	1.8870	0.1627	<0.0001	Int	2.3422	0.1417	<0.0001
N	0.1440	0.0438	0.0017	N	0.0863	0.0482	0.0786	N	0.0156	0.0419	0.6946
Mo	0.0283	0.0263	0.2863	Mo	0.0371	0.0289	0.2039	Mo	−0.0552	0.0251	0.0322
N × N	−0.0047	0.0032	0.1458	N × N	0.00004	0.0035	0.9909	N × N	−0.0014	0.0030	0.6457
Mo × N	−0.0012	0.0015	0.4455	Mo × N	−0.0029	0.0017	0.0886	Mo × N	0.0011	0.0014	0.4571
Mo × Mo	0.0001	0.0011	0.9472	Mo × Mo	−0.0007	0.0012	0.5556	Mo × Mo	0.0018	0.0011	0.0977
		Eigenvectors				Eigenvectors				Eigenvectors	
Eigenva	0.0147	−0.1908	0.9816	Eigenva	0.0603	0.8353	−0.5497	Eigenva	0.1914	0.1337	0.9905
	−0.1780	0.9816	0.1908		−0.1344	0.5497	0.8353		−0.0559	0.9905	−0.1373

Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction; SE standard error; Int intercept; Es estimation; Eigenva: Eigenvalues; Eigenvectors.

Table 10. Response surface analysis for molybdenum sources in photosynthetic pigments Chlorophyll b.

Chlorophyll b											
NanoMo				**Mo Chelate**				**Na Molybdate**			
CV	23.11	R^2	0.5943	CV	19.04	R^2	0.3815	CV	25.75	R^2	0.1230
Regression		Factors		Regression		Factors		Regression		Factors	
L	<0.0001	N	Mo	L	0.0002	N	Mo	L	0.2986	N	Mo
C	0.0117	<0.0001	0.0014	C	0.3009	<0.0001	0.0008	C	0.5398	0.1163	0.1060
P	0.0074	L	C, Mo	P	0.0005		Mo	P	0.0399		Mo
Model	<0.0001			Model	<0.0001			Model	0.1672		

Table 10. *Cont.*

Chlorophyll b											
NanoMo				Mo Chelate				Na Molybdate			
Source	Es	SE	$p > t$	Source	Est	SE	$p > t$	Source	Est	SE	$p > t$
Int	0.9718	0.1316	<0.0001	Int	0.9669	0.0885	<0.0001	Int	1.4313	0.1253	<0.0001
N	0.1424	0.0389	0.0006	N	0.0190	0.0262	0.4711	N	−0.0193	0.0371	0.6044
Mo	0.0289	0.0233	0.2204	Mo	0.0264	0.0157	0.0982	Mo	−0.0316	0.0222	0.1606
N × N	−0.0077	0.0028	0.0087	N × N	0.0030	0.0019	0.1236	N × N	0.0006	0.0027	0.8210
Mo × N	0.0038	0.0013	0.0074	Mo × N	−0.0034	0.0009	0.0005	Mo × N	0.0027	0.0013	0.0399
Mo × Mo	−0.0015	0.0010	0.1394	Mo × Mo	0.0001	0.0006	0.9168	Mo × Mo	0.0010	0.0009	0.2789
		Eigenvectors				Eigenvectors				Eigenvectors	
Eigenva	−0.0853	0.5119	0.8589	Eigenva	0.1736	0.8472	−0.5311	Eigenva	0.1586	0.5229	0.8523
	−0.3489	0.8589	−0.5119		−0.0581	0.5311	0.8472		−0.0290	0.8523	−0.5229

Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction; SE standard error; Int intercept; Es estimation; Eigenva: Eigenvalues; Eigenvectors.

Table 11. Response surface analysis for molybdenum sources in photosynthetic pigments Carotenoids.

Carotenoids											
NanoMo				Mo Chelate				Na Molybdate			
CV	21.52	R^2	0.8052	CV	21.14	R^2	0.8011	CV	32.31	R^2	0.5719
Regression		Factors		Regression		Factors		Regression		Factors	
L	<0.0001	N	Mo	L	<0.0001	N	Mo	L	<0.0001	N	Mo
C	<0.0001	<0.0001	<0.0001	C	<0.0001	0.0166	<0.0001	C	<0.0001	0.5816	<0.0001
P	0.4239	L	L, C	P	0.0146	L	C	P	0.3336		L, C
Model	<0.0001			Model	<0.0001			Model	<0.0001		
Source	Es	SE	$p > t$	Source	Est	SE	$p > t$	Source	Est	SE	$p > t$
Int	1.2719	0.5068	0.0149	Int	1.2302	0.4036	0.0035	Int	2.1923	0.5853	0.0004
N	0.3345	0.1501	0.0297	N	0.0476	0.1195	0.6915	N	0.0168	0.1733	0.9228
Mo	0.9949	0.0900	<0.0001	Mo	0.8986	0.0717	<0.0001	Mo	0.6209	0.1040	<0.0001
N × N	−0.0142	0.0110	0.2017	N × N	0.0086	0.0087	0.3273	N × N	−0.0019	0.0127	0.8802
Mo × N	0.0043	00.53	0.4239	Mo × N	−0.0107	0.0042	0.0146	Mo × N	0.0060	0.0061	0.3336
Mo × Mo	−0.0359	0.0039	<0.0001	Mo × Mo	−0.0301	0.0031	<0.0001	Mo × Mo	−0.0218	0.0045	<0.0001
		Eigenvectors				Eigenvectors				Eigenvectors	
Eigenva	−0.5070	0.9991	0.0418	Eigenva	0.3431	0.9954	−0.0955	Eigenva	−0.0540	0.9964	0.0845
	−3.6045	−0.0418	0.9991		−3.0491	0.0955	0.9954		−2.2035	−0.0845	0.9964

Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction; SE standard error; Int intercept; Es estimation; Eigenva: Eigenvalues; Eigenvectors.

Table 12. Response surface analysis for molybdenum sources in photosynthetic pigments Chlorophyll a + b.

Chlorophyll a + b											
NanoMo				Mo Chelate				Na Molybdate			
CV	19.05	R^2	0.4640	CV	21.42	R^2	0.0662	CV	32.31	R^2	0.5719
Regression		Factors		Regression		Factors		Regression		Factors	
L	<0.0001	N	Mo	L	0.6485	N	Mo	L	0.1029	N	Mo
C	0.0044	<0.0001	0.0030	C	0.3515	0.5554	0.3818	C	0.5091	0.2737	0.2798
P	0.0013	L, C	Mo	P	0.2965			P	0.3440		
Model	<0.0001			Model	0.5389			Model	0.2372		
Source	Es	SE	$p > t$	Source	Est	SE	$p > t$	Source	Est	SE	$p > t$
Int	2.4100	0.1388	<0.0001	Int	1.9758	0.1537	<0.0001	Int	1.8195	0.1508	<0.0001
N	−0.1267	0.0411	0.0031	N	0.0277	0.0455	0.5449	N	0.0404	0.0446	0.3686
Mo	−0.0180	0.0246	0.4675	Mo	0.0133	0.0273	0.6280	Mo	−0.0154	0.0268	0.5674
N × N	0.0093	0.0030	0.0032	N × N	−0.0030	0.0033	0.3653	N × N	−0.0035	0.0032	0.2896
Mo × N	−0.0049	0.0014	0.0013	Mo × N	0.0017	0.0016	0.2965	Mo × N	−0.0015	0.0015	0.3440
Mo × Mo	0.0017	0.0018	0.1225	Mo × Mo	−0.0013	0.0012	0.2595	Mo × Mo	0.0005	0.0011	0.6379
		Eigenvectors				Eigenvectors				Eigenvectors	
Eigenva	0.4224	0.8616	−0.5075	Eigenva	−0.0702	0.7925	0.6098	Eigenva	0.0666	−0.2303	0.9731
	0.0829	0.5075	0.8616		−0.1766	−0.6098	0.7925		−0.1370	0.9731	0.2303

Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction; SE standard error; Int intercept; Es estimation; Eigenva: Eigenvalues; Eigenvectors.

Table 13. Response surface analysis for molybdenum sources in photosynthetic pigments Chlorophyll a + b/carotenoids.

Chlorophyll a + b/Carotenoids											
NanoMo				Mo Chelate				Na Molybdate			
CV	26.13	R^2	0.8858	CV	28.57	R^2	0.8752	CV	26.69	R^2	0.8827
Regression		Factors		Regression		Factors		Regression		Factors	
L	<0.0001	N	Mo	L	0.6485	N	Mo	L	<0.0001	N	Mo
C	<0.0001	0.9919	<0.0001	C	0.3515	0.7751	<0.0001	C	<0.0001	0.9380	<0.0001
P	0.7727		L, C	P	0.2965		L, C	P	0.7426		L, C
Model	<0.0001			Model	0.5389			Model	<0.0001		
Source	Es	SE	$p > t$	Source	Est	SE	$p > t$	Source	Est	SE	$p > t$
Int	1.5820	0.0694	<0.0001	Int	1.6486	0.0791	<0.0001	Int	1.5371	0.0703	<0.0001
N	−0.0024	0.0205	0.9063	N	0.0176	0.0234	0.4559	N	0.0128	0.0208	0.5391
Mo	−0.2166	0.0123	<0.0001	Mo	−0.2342	0.0140	<0.0001	Mo	−0.2100	0.0125	<0.0001
N × N	0.0001	0.0015	0.9520	N × N	−0.0014	0.0017	0.4124	N × N	−0.0008	0.0015	0.5894
Mo × N	0.0002	0.0007	0.7727	Mo × N	0.0003	0.0008	0.6707	Mo × N	−0.0002	0.0007	0.7426
Mo × Mo	0.0080	0.0005	<0.0001	Mo × Mo	0.0085	0.0006	0.8596	Mo × Mo	0.0077	0.0005	<0.0001
		Eigenvectors				Eigenvectors				Eigenvectors	
Eigenva	0.8002	0.0080	0.9999	Eigenva	0.8597	0.0117	0.9999	Eigenva	0.7740	−0.0091	0.9999
	0.0032	0.9999	−0.0080		−0.0513	0.9999	−0.0117		−0.0299	0.9999	0.0091

Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction; SE standard error; Int intercept; Es estimation; Eigenva: Eigenvalues; Eigenvectors.

3.6. Nitrogen Use Efficiency Parameters (NUE)

Nitrogen Use Efficiency (NUE) is defined as the biomass yield or yield per unit of available N [27]. This concept has many variants that can be divided into two main elements: N Uptake Efficiency (NUpE), which is defined as the ability of plant roots to take up N from the soil, and N Utilization Efficiency. N (NUtE) is defined as the fraction of N acquired by the plant that will be converted into total biomass or fruit yield [24].

In the present study it can be observed that, with the edaphic fertilization of ammonium nitrate together with the foliar fertilization of the Mo Nanofertilizer, it was possible to increase the Nitrogen Use Efficiency, in comparison with the foliar fertilization of Mo Chelate and sodium Molybdate. The increase in efficiency ranges from 34.56% over Mo Chelate to 44.16% more over Sodium Molybdate (Table 14).

Table 14. Effect of edaphic application of nitrogen supplemented with foliar fertilization of NanoMo on Nitrogen Use Efficiency.

Nitrogen Use Efficiency			
	TNA	**NUpE**	**NUtE**
Source Mo	0.0002 ^{U}	<0.0001	0.6624
NanoMo	238.92 a ^{V}	5.75 c	21.54 a
Na Molybdate	133.40 b	13.24 a	22.24 a
Mo Chelate	156.33 b	8.40 b	23.49 a
MSD	42.08	2.39	5.95
Nitrogen ^{X}	<0.0001	<0.0001	<0.0001
0	115.67 b	16.70 a	30.90 a
3	135.55 b	9.30 b	27.11 a
6	228.80 a	6.36 c	17.27 b
12	224.84 a	4.15 c	14.42 b
MSD	45.79	2.29	7.49
Molybdenum ^{Y}	0.3599	0.0318	0.0009
0	168.67 a	7.57 b	17.86 b
5	167.85 a	8.54 ab	22.47 ab
10	187.28 a	10.54 a	56.77 a
20	181.06 a	9.88 ab	22.64 ab
MSD	33.83	2.81	5.51

Table 14. *Cont.*

	Nitrogen Use Efficiency		
	TNA	**NUpE**	**NUtE**
SoMo × N	0.0322	<0.0001	<0.0001
SoMo × Mo	0.0002	0.4295	0.0378
N × Mo	0.0508	0.3090	0.0327
SoMo × N × Mo	0.2952	0.2646	<0.0001
μ	176.21	9.13	22.42
C.V.	36.04	57.94	46.18
R^2	0.7793	0.7653	0.7705

[U] Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; [V] Means with the same letter are statistically equal (Tukey α 0.05); [W] Minimum significant difference; [X] Edaphic mM concentration of nitrogen; [Y] Leaf molybdenum ppm concentration, μ overall mean, CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction.

For the parameter Total Nitrogen Accumulation (TNA), the application of the molybdenum nanofertilizer had a significant effect on the global concentration of N in the plant. Direct applications of 6 and 12 mM ammonium nitrate increased the N content within plant tissues (Figure 6). It should be noted that the 3.04% difference between the doses of 6 and 12 mM allows us to see that by applying the Mo nanofertilizer and a low dose of N (6 mM) the plant can assimilate enough N for its optimal growth and development. In the same way, it is shown that when applying higher amounts of N (12 mM) or higher, a negative response begins to have a direct impact on the accumulation of this nutrient. In Figure 6, it can also be verified that the interaction of these two essential elements (N and Mo) potentiates the accumulation of N for the benefit of the crop.

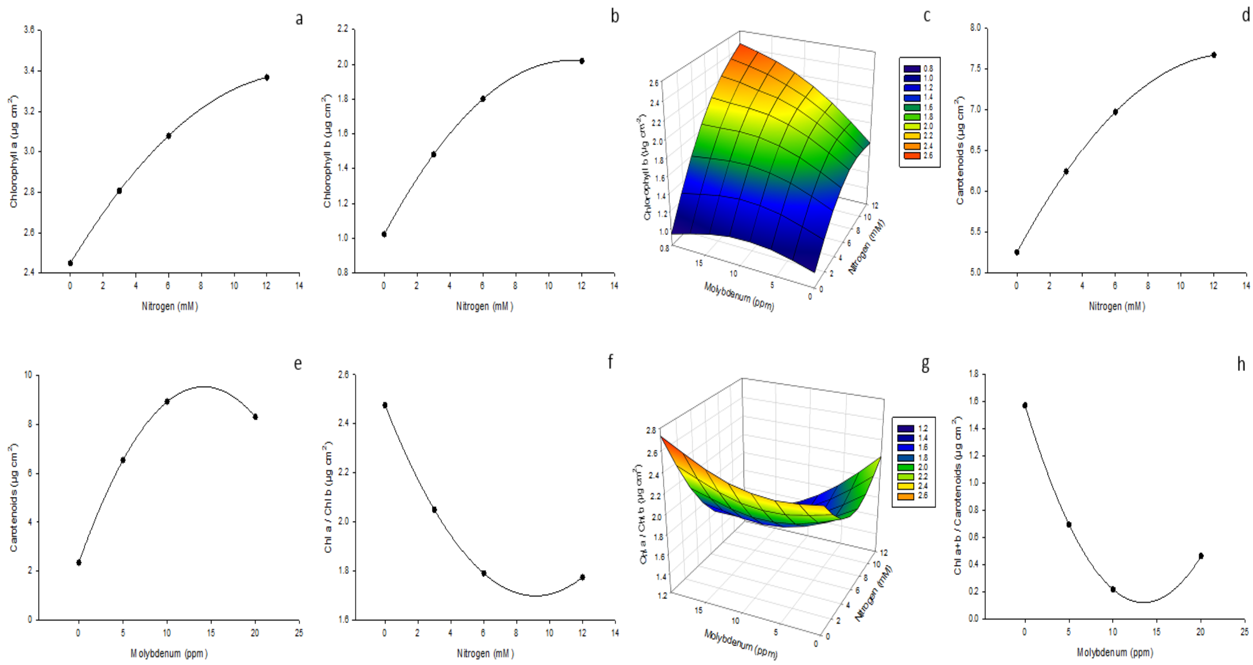

Figure 6. Effect of the edaphic application of nitrogen, complemented with foliar fertilization of NanoMo on the concentration of photosynthetic pigments. (**a**) Chlorophyll a (Chl a). (**b**) Chlorophyll b (Chl b). (**c**) Effect of nitrogen and molybdenum on Chlorophyll b (Chl b). (**d**) Carotenoids. (**e**) Effect of molybdenum on carotenoids. (**f**) Chlorophyll "a" plus Chlorophyll "b". (**g**) Effect of nitrogen and molybdenum on Chlorophyll "a" plus Chlorophyll "b". (**h**) Chlorophyll a + b among carotenoids.

In the case of Absorption Efficiency (NUpE) and N Utilization Efficiency (NUtE), the results show that N is better used if it is applied in smaller amounts and supplied more efficiently (Figure 7). This is where NanoMo plays a fundamental role, increasing the rate of N assimilation. It can also be seen how the efficiency decreases as the N concentration progressively increases, assuming a supersaturation of N that can reach toxicity levels for plants.

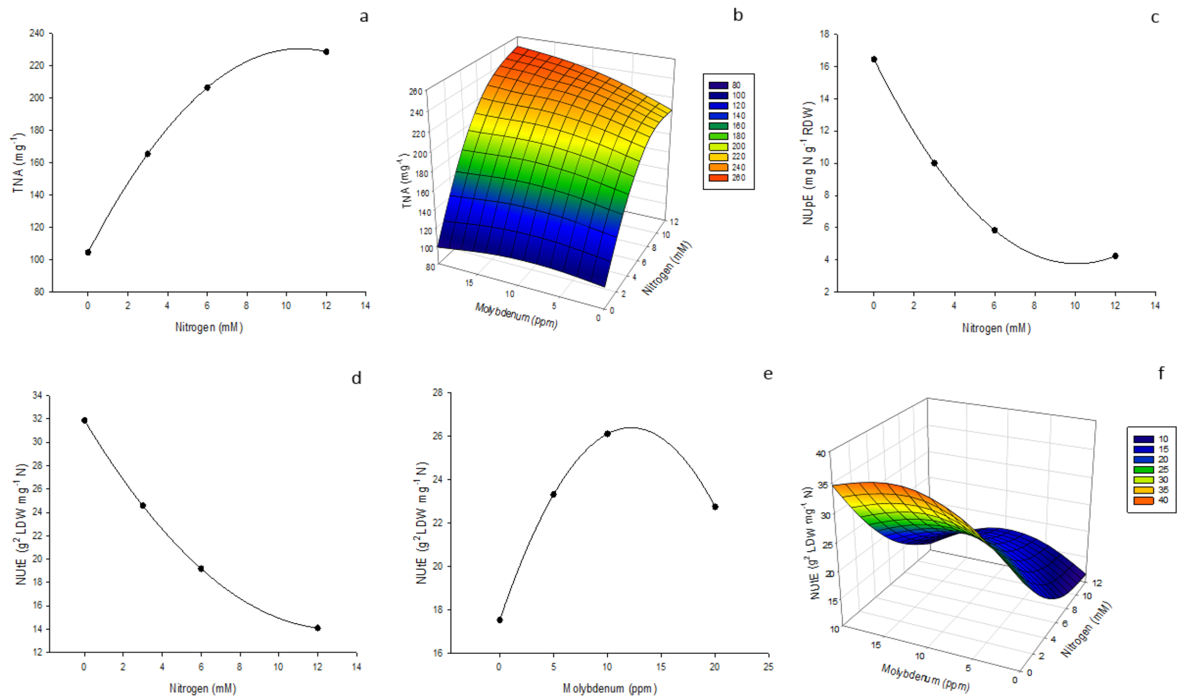

Figure 7. Effect of the edaphic application of nitrogen complemented with foliar fertilization of NanoMo on the parameters of Nitrogen Use Efficiency (NUE). (**a**) Total Nitrogen Accumulation (ATM). (**b**) Effect of nitrogen and molybdenum on ATN. (**c**) Nitrogen Uptake Efficiency (NupE). (**d**) Nitrogen Utilization Efficiency (NUE). (**e**) Effect of Molybdenum on Nitrogen Utilization Efficiency. (**f**) Effect of Nitrogen and Molybdenum on Nitrogen Utilization Efficiency.

The data from the response surface analysis are shown below to provide greater clarity and statistical support to the graphs (Tables 15–17).

Table 15. Response surface analysis for molybdenum sources in Total Nitrogen Accumulation (TNA).

Total Nitrogen Accumulation (TNA)											
NanoMo				**Mo Chelate**				**Na Molybdate**			
CV	30.38	R^2	0.3806	CV	46.71	R^2	0.4299	CV	57.82	R^2	0.3658
Regression		Factors		Regression		Factors		Regression		Factors	
L	0.0001	N	Mo	L	<0.0001	N	Mo	L	<0.0001	N	Mo
C	0.0024	0.0007	0.0017	C	0.0005	<0.0001	0.3656	C	0.0262	<0.0001	0.4915
P	0.2868		L, C	P	0.7742	L, C		P	0.4612	L, C	
Model	<0.0001			Model	<0.0001			Model	<0.0001		
Source	Es	SE	$p > t$	Source	Est	SE	$p > t$	Source	Est	SE	$p > t$
Int	160.6188	26.1540	<0.0001	Int	91.4985	26.3066	0.0010	Int	57.3632	27.7886	0.0435
N	−0.3029	7.7468	0.9689	N	37.5070	7.7921	<0.0001	N	28.4917	8.2307	0.0010
Mo	15.7424	4.6481	0.0013	Mo	−7.8593	4.6752	0.0981	Mo	−4.0110	4.9384	0.4200
N × N	0.5085	0.5687	0.3750	N × N	−2.2447	0.5721	0.0002	N × N	−1.5872	0.6043	0.0110
Mo × N	0.2973	0.2765	0.2868	Mo × N	0.0801	0.2781	0.7742	Mo × N	0.2179	0.2938	0.4612
Mo × Mo	−0.7279	0.2047	0.0008	Mo × Mo	0.2977	0.2059	0.1537	Mo × Mo	0.2021	0.2175	0.3566
		Eigenvectors				Eigenvectors				Eigenvectors	
Eigenva	19.1732	0.9953	0.0965	Eigenva	29.8228	0.0217	0.9997	Eigenva	20.7674	0.0836	0.9964
	−73.6638	−0.0965	0.9953		−80.8641	0.9997	−0.0217		−54.6915	0.9964	−0.0836

Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction; SE standard error; Int intercept; Es estimation; Eigenva: Eigenvalues; Eigenvectors.

Table 16. Response surface analysis for molybdenum sources in Nitrogen Absorption Efficiency (NUpE).

Nitrogen Absorption Efficiency (NUpE)											
NanoMo				Mo Chelate				Na Molybdate			
CV	48.05	R^2	0.3405	CV	64.13	R^2	0.5907	CV	48.76	R^2	0.6132
Regression		Factors		Regression		Factors		Regression		Factors	
L	<0.0001	N	Mo	L	<0.0001	N	Mo	L	<0.0001	N	Mo
C	0.1249	<0.0001	0.2208	C	0.0001	<0.0001	0.0015	C	<0.0001	<0.0001	0.1619
P	0.0803			P	0.0012		L, C	P	0.5020	L, C	L
Model	0.0002			Model	<0.0001			Model	<0.0001		
Source	Es	SE	$p > t$	Source	Est	SE	$p > t$	Source	Est	SE	$p > t$
Int	7.4187	0.9957	<0.0001	Int	12.0365	1.9422	<0.0001	Int	21.4271	2.3263	<0.0001
N	−0.0712	0.2949	0.8100	N	−2.8226	0.5752	<0.0001	N	41.3862	19.1956	0.0352
Mo	0.0913	0.1769	0.6078	Mo	0.6268	0.3451	0.0745	Mo	21.1314	11.5173	0.0717
N × N	−0.0371	0.0216	0.0915	N × N	0.1944	0.0422	<0.0001	N × N	−3.4424	1.4093	0.0177
Mo × N	0.0187	0.0105	0.0803	Mo × N	−0.0702	0.0205	0.0012	Mo × N	−0.0570	0.6853	0.9339
Mo × Mo	−0.0091	0.0077	0.2469	Mo × Mo	−0.0019	0.0152	0.8976	Mo × Mo	−0.6386	0.5073	0.2132
		Eigenvectors				Eigenvectors				Eigenvectors	
Eigenva	−0.5235	0.5683	0.8227	Eigenva	7.5713	0.9651	−0.2617	Eigenva	7.8851	0.9990	−0.0440
	−1.7260	0.8227	−0.5683		−0.7676	0.2617	0.9651		−3.3005	0.0446	0.9990

Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction; SE standard error; Int intercept; Es estimation; Eigenva: Eigenvalues; Eigenvectors.

Table 17. Response surface analysis for molybdenum sources in Nitrogen Utilization Efficiency (NUtE).

Nitrogen Utilization Efficiency (NUtE)											
NanoMo				Mo Chelate				Na Molybdate			
CV	50.26	R^2	0.3463	CV	78.08	R^2	0.3554	CV	38.33	R^2	0.5644
Regression		Factors		Regression		Factors		Regression		Factors	
L	0.1641	N	Mo	L	0.0001	N	Mo	L	<0.0001	N	Mo
C	<0.0001	0.0002	0.0559	C	0.0106	<0.0001	0.1565	C	<0.0001	<0.0001	0.7685
P	0.4688	L, C	L	P	0.2757	L, C	L	P	0.6558	L, C	
Model	0.0001			Model	<0.0001			Model	<0.0001		
Source	Es	SE	$p > t$	Source	Est	SE	$p > t$	Source	Est	SE	$p > t$
Int	8.8866	3.9011	0.0264	Int	31.6067	6.6101	<0.0001	Int	34.8012	3.0713	<0.0001
N	4.8201	1.1553	0.0001	N	−6.1431	1.9579	0.0027	N	−5.8532	0.9097	<0.0001
Mo	1.8566	0.6933	0.0096	Mo	2.6727	1.1747	0.0266	Mo	0.4775	0.5458	0.3852
N × N	−0.3935	0.0848	<0.0001	N × N	0.3612	0.1437	0.0148	N × N	0.3491	0.0667	<0.0001
Mo × N	−0.0300	0.0412	0.4688	Mo × N	−0.0769	0.0699	0.2757	Mo × N	−0.0145	0.0324	0.6558
Mo × Mo	−0.0679	0.0305	0.0300	Mo × Mo	−0.0973	0.0517	0.0650	Mo × Mo	−0.0139	0.0240	0.5629
		Eigenvectors				Eigenvectors				Eigenvectors	
Eigenva	−6.6862	−0.1197	0.9928	Eigenva	13.2354	0.9949	−0.0999	Eigenva	12.5825	0.9995	−0.0312
	−14.2760	0.9928	0.1197		−9.9666	0.0999	0.9949		−1.4127	0.0312	0.9995

Non-significant probability $p > 0.05$, significant $0.05 \leq p \leq 0.01$, highly significant $p < 0.0001$; CV coefficient of variation, R^2 regression coefficient; Regression Analysis: Linear L, quadratic C, P NxMo interaction; SE standard error; Int intercept; Es estimation; Eigenva: Eigenvalues; Eigenvectors.

4. Discussion

In the present investigation, the application of NanoMo and ammonium nitrate had a direct beneficial effect on the growth of green beans. The increase in the development and production of green bean plants is due not only to the excellent response to ammonium nitrate fertilization at low doses but also to the additional foliar fertilization of NanoMo at sufficiently low doses, which allowed the rapid absorption of Mo and consequently the adequate assimilation of N. Studies carried out by [28] obtained highly significant yields when foliarly fertilizing the spinach crop with Mo nanoparticles, reporting a high efficiency in the assimilation of nitrate (NO_3).

The effects of N and Mo applications on green beans are supported by already published research on important crops. Previous studies [3] showed that molybdenum applications significantly increased biomass content in lentil crops. Similarly, molybdenum applications drastically improved the total biomass content in chickpea in studies conducted by [29].

In yield, molybdenum can be considered to have a central role in nitrogen metabolism, although not directly, if as a compositional part of nitrate reductase and nitrogenase, enzymes are responsible for nitrogen fixation. At present, the effects of Mo on N fixation are being carefully studied since they have a direct effect on the yield of plants [30].

The interaction of N and NanoMo potentiated the NR activity. It can be assumed that with the nanofertilizer, Mo was in sufficient quantity within the active sites where it could be easily metabolized, and the NR enzyme could play its role in the N assimilation mechanism. It can also be seen that, with the addition of 6 mM of N, the activity of the enzyme reached its highest point of activity, beginning to decrease with the addition of greater amounts of N (12 mM), which indicates a clear overaccumulation of N with a negative effect on the plant (Figure 5). Studies carried out by Hachiya and Sakakibara, 2017 [31] mention that nitrate toxicity occurs with high doses of nitrogen and low enzymatic activity due to supersaturation and nitrate toxicity.

In the present study, it is important to highlight that the increase in the concentration of pigments in the leaves is of great relevance for the development of the plant. By increasing the development of the photosynthetic system, the assimilative capacity of the plant is increased, which leads to higher growth rates and yield [32]. The effect on the results obtained can be explained by the high efficiency in nitrogen assimilation derived from the foliar application of the nanofertilizer. In this case, the NanoMo was quickly absorbed by the leaves, from where it could be translocated and assimilated in the metabolism of the plant to form a structural part of the enzymes responsible for the assimilation of N, a key element in the formation of chlorophylls. This agrees with what was reported by [28], who obtained similar results when applying Mo nanoparticles in spinach crops.

The Nitrogen Use Efficiency parameters are considered very important traits in agriculture to reduce the excessive use of nitrogen fertilizers or when nitrogen availability limits plant growth, with substantial benefits for farmers and the environment [33]. Crops with higher NUE promote higher yields with lower amounts of N and require less N to produce the same yield as those with lower NUE capacity and high N applications [34]. Therefore, when the NUE increases, both the costs of crop production and the harmful input of NO_3^- to ecosystems are reduced [35,36].

5. Conclusions

The foliar application of the Mo nanofertilizer favored the activity of the enzyme Nitrate reductase and photosynthetic pigments accumulation, which translated into a higher Nitrogen Use Efficiency. A higher yield was obtained with the reduced application of nitrogen and NanoMolybdenum, having a favorable direct impact on the environment and the economy of the producers.

Author Contributions: E.S., J.M.S.-P. and E.M.-M. designed the study. E.S., L.C.N.-M. and J.M.S.-P. analyzed the data, E.S. and E.M.-M. prepared the manuscript, while E.M.-M. and L.C.N.-M. conducted the experiments. E.S., J.M.S.-P. and E.M.-M. organized the data and performed the statistical analysis. All authors have read and agreed to the published version of the manuscript.

Funding: This research work was funded by the Consejo Nacional de Ciencia y Tecnología (CONACyT National Science and Technology Council of Mexico) and was duly approved in the Convocatoria Atención a Problemas Nacionales: Project #1529 "Biofortification of basic agricultural crops representing the key to combatting malnutrition and ensuring food security in Mexico".

Acknowledgments: We would like to thank the Consejo Nacional de Ciencia y Tecnología (CONACyT—Mexico) for the support provided by means of the Convocatoria Atención a Problemas Nacionales: Project #1529 "Biofortification of basic agricultural crops representing the key to combat malnutrition and ensure food security in Mexico".

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Trenkel, M.E. *Slow and Controlled-Release and Stabilized Fertilizers: An Option for Enhancing Nutrient Use Efficiency in Agriculture*, 2nd ed.; IFA: Paris, France, 2010; pp. 1–163.
2. Wang, X.; Fan, J.; Xing, Y.; Xu, G.; Wang, H.; Deng, J.; Wang, Y.; Zhang, F.; Li, P.; Li, Z. The Effects of Mulch and Nitrogen Fertilizer on the Soil Environment of Crop Plants. *Adv. Agron.* **2018**, *153*, 121–173. [CrossRef]
3. Nasar, J.; Shah, Z. Effect of iron and molybdenum on yield and nodulation of lentil. *ARPN J. Agric. Biol. Sci.* **2017**, *12*, 332–339.
4. Santos, R.L.; Freire, F.J.; Oliveira, E.C.A.; Freire, M.B.G.S.; West, J.B.; Barbosa, J.A.; Moura, M.J.; Bezerra, P.C. Nitrate reductase activity and nitrogen and biomass accumulation in sugarcane under molybdenum and nitrogen fertilization. *Rev. Bras. Cienc. Solo* **2019**, *43*, e0180171. [CrossRef]
5. Rana, M.S.; Bhantana, P.; Imran, M.; Saleem, M.H. Molybdenum potential vital role in plants metabolism for optimizing the growth and development. *Ann. Environ. Sci. Toxicol.* **2020**, *4*, 32–44. [CrossRef]
6. Carlim, E.L.; Meert, L.; Reis, B.; Alleman, L.E. Fertilization with nickel and molybdenum in soybean: Effect on agronomic characteristics and grain quality. *Terra Latinoam.* **2019**, *37*, 217–222. [CrossRef]
7. Tallkvist, J.; Oskarsson, A. Molybdenum. In *Handbook on the Toxicology of Metals*, 4th ed.; Academic Press: Cambridge, MA, USA, 2016; Chapter 47. [CrossRef]
8. Alfaro, V.M.; Mejía, B.J.; Salazar, S.F. Nanofertilizante de aplicación foliar para uso en praderas. *Campo Tecnol. INIA* **2020**, *17*, 14–15.
9. Hoagland, D.R.; Arnon, D.I. *The Water Culture Method for Growing Plants without Soil*; California Agricultural Experiment Station, University of California: Berkeley, CA, USA, 1950; p. 347.
10. Sánchez, E.; Romero, L.; Ruíz, J.M. *Caracterización del Estado Nutricional y Fisiológico en Plantas de Judía (Phaseolus vulgaris L. cv. Strike) Sometidas a un Estrés por Nitrógeno*; Editorial de la Universidad de Granada: Granada, Spain, 2006; ISBN 84-338-4168-8.
11. Dubos, B.; Snoeck, D.; Flori, A. Excessive use of fertilizer can increase leaching processes and modify soil reserves in two ecuadorian oil palm plantations. *Exp. Agric.* **2017**, *53*, 255–268. [CrossRef]
12. Jaworski, E.G. Nitrate reductase assay in intact plant tissues. *Biochem. Biophys. Res. Commun.* **1971**, *43*, 1274–1279. [CrossRef]
13. Ponce-García, C.O.; Soto-Parra, J.M.; Sánchez, E.; Muñoz-Márquez, E.; Piña-Ramírez, F.J.; Flores-Córdova, M.A.; Pérez-Leal, R.; Yáñez Muñoz, R.M. Efficiency of Nanoparticle, Sulfate, and Zinc-Chelate Use on Biomass, Yield, and Nitrogen Assimilation in Green Beans. *Agronomy* **2019**, *9*, 128. [CrossRef]
14. Chhipa, H. Nanofertilizers and nanopesticides for agriculture. *Environ. Chem. Lett.* **2017**, *15*, 15–22. [CrossRef]
15. Sidiqui, M.H.; Al-Whaibi, M.H.; Firoz, M.; Al-Khaishany, M.Y. Role of nanoparticles in plants. In *Nanotechnology and Plant Sciences*; Siddiqui, M.H., Al-Whaibi, M.H., Mohamed, F., Eds.; Springer: New York, NY, USA, 2015; pp. 19–35.
16. Wellburn, A.R. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant Physiol.* **1994**, *14*, 307–313. [CrossRef]
17. SAS Institute Inc. *SAS/STATR User's Guide, Version 6*, 4th ed.; SAS Institute Inc.: Cary, NC, USA, 1989; Volume 2, pp. 1457–1478.
18. Vargas, H.M.; Zarate, D.L.G.P.; Burguete, H.F. Factoriales fraccionados y superficie de respuesta, uso de paquetes estadísticos para microcomputadoras. *Monogr. Man. Estad. Cómput.* **1991**, *10*, 1.
19. Kiba, T.; Krapp, A. Plant Nitrogen Acquisition Under Low Availability: Regulation of Uptake and Root Architecture. *Plant Cell Physiol* **2016**, *57*, 707–714. [CrossRef]
20. Pacheco, R.S.; Brito, L.F.; Straliotto, R.; Pérez, D.V.; Araújo, A.P. Seeds enriched with phosphorus and molybdenum as a strategy for improving grain yield of common bean crop. *Field Crops Res.* **2012**, *136*, 97–106. [CrossRef]
21. Naderi, M.R.; Danesh-Shahraki, A. Nanofertilizers and their roles in sustainable agriculture. *Int. J. Agric. Crop Sci.* **2013**, *5*, 22–29.
22. Mendel, R.R.; Hänsch, R. Molybdoenzymes and molybdenum cofactor in plants. *J. Exp. Bot.* **2002**, *53*, 1689–1698. [CrossRef]
23. Bekele, G.; Dechassa, N.; Tana, T.; Sharma, J.J. Effects of nitrogen, phosphorus and vermicompost fertilizers on productivity of groundnut (*Arachis hypogaea* L.) in Babile, Eastern Ethiopia. *Agron. Res.* **2019**, *17*, 1532–1546.
24. Subramanian, K.S.; Manikandan, A.; Thirunavukkarasu, M.; Rahale, C.S. Nano-fertilizers for balanced crop nutrition. In *Nanotechnologies in Food and Agriculture*; Springer: Cham, Switzerland, 2015; pp. 69–80.
25. Kah, M.; Kookana, R.S.; Gogos, A.; Bucheli, T.D. A critical evaluation of nanopesticides and nanofertilizers against their conventional analogues. *Nat. Nanotechnol.* **2018**, *13*, 677. [CrossRef]
26. Chen, B.M.; Wang, Z.H.; Li, S.X.; Wang, G.X.; Song, H.X.; Wang, X.N. Effects of nitrate supply on plant growth, nitrate accumulation, metabolic nitrate concentration and nitrate reductase activity in three leafy vegetables. *Plant Sci.* **2004**, *167*, 635–643. [CrossRef]
27. Raliya, R.; Saharan, V.; Dimkpa, C.; Biswas, P. Nanofertilizer for precision and sustainable agriculture: Current state and future perspectives. *J. Agric. Food Chem.* **2017**, *66*, 6487–6503. [CrossRef]
28. Ahmadreza, A.; Babak, V.K.; Maryam, A.I. Effect of green synthesized molybdenum nanoparticles on nitrate accumulation and nitrate reductase activity in spinach. *J. Plant Nutr.* **2019**, *43*, 13–27. [CrossRef]
29. Sawires, E.S. Effect of phosphorus fertilization and micronutrients on yield and yield components of chickpea (*Cicer arietinum* L.). *Ann. Agric. Sci. Cairo* **2001**, *46*, 155–164.
30. Wei, L.-P.; Li, Y.-R.; Yang, L.-T. Effects of Molybdenum on Nitrogen Metabolism of Sugarcane. Academy of Agricultural Sciences, Sugarcane Research Center, Chinese Academy of Agricultural Sciences, China. *Sugar Tech* **2007**, *9*, 36–42. [CrossRef]

31. Hachiya, T.; Sakakibara, H. Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. *J. Exp. Bot.* **2017**, *68*, 2501–2512. [CrossRef] [PubMed]
32. Combatt, C.; Enrique, P.P.; Dairo, J.; Jarma-Orozco, A. Efecto del nitrógeno y el potasio sobre el intercambio gaseoso y la distribución de biomasa en albahaca (*Ocimum basillicum* L.). *Rev. Colomb. Cienc. Hortícolas* **2018**, *12*, 192–201. [CrossRef]
33. Armendáriz-Fernández, K.V.; Herrera-Hernández, I.M.; Muñoz-Márquez, E.; Sánchez, E. Characterization of Bioactive Compounds, Mineral Content, and Antioxidant Activity in Bean Varieties Grown with Traditional Methods in Oaxaca, Mexico. *Antioxidants* **2019**, *8*, 26. [CrossRef]
34. Rosales, M.A.; Franco-Navarro, J.D.; Peinado-Torrubia, P.; Díaz-Rueda, P.; Álvarez, R.; Colmenero-Flores, J.M. Chloride Improves Nitrate Utilization and NUE in Plants. *Front. Plant Sci.* **2020**, *11*, 442. [CrossRef]
35. Hua, L.; Bin, H.; Chengcai, C. Nitrogen use efficiency in crops: Lessons from Arabidopsis and rice. *J. Exp. Bot.* **2017**, *68*, 2477–2488. [CrossRef]
36. Hasaan, A.E.; Said, M.G.; Alaa, H.R.; Radi, S.K. Effects of Different Nitrogen Fertilization Rates and Foliar Application of Humic Acid, Fulvic Acid and Tryptophan on Growth, Productivity and Chemical Composition of Common Bean Plants (*Phaseolus vulgaris* L.). *Alex. Sci. Exch. J.* **2020**, *41*, 191–204. [CrossRef]