

Macro-and micronutrient content in native varieties of *Phaseolus lunatus* L. fertilized with rock dust

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Abstract

The objective of this study was to evaluate the macro- and micronutrient content in the leaves of *Phaseolus lunatus* L. fertilized with MB-4[®]. The research was carried out in two consecutive experiments, with a 2x5 randomized factorial block design and four replicates, in which two native varieties of *Phaseolus lunatus* L. were evaluated (Lima bean 'Cara Larga' and Lima bean 'Branca') based on responses to five varying doses of MB-4[®] (0, 100, 200, 400, and 800 g hole⁻¹). Leaf analyses were performed to quantify nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, copper, iron, manganese, and zinc. Leaf analysis indicated that N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn levels increased significantly in the second year of cultivation, and that only B content decreased. The contents of N, P, Ca, B, Fe, Mn, and Zn were within the recommended range, whereas only K and Mg were outside the recommended range for the species. The values found between 200 and 400 g hole⁻¹, corresponding to 4 and 8 t ha⁻¹ of MB-4[®], respectively, were the ones that contributed the most to the nutrient content increase in Lima bean varieties.

Keywords: Lima bean 'Cara larga'; Lima bean 'Branca'; Fertility; Mineral nutrition of plants.

Introduction

The broad bean (*Phaseolus lunatus* L.), commonly known as "lima bean," is one of the four cultivated species of the genus *Phaseolus*. It is the second most important legume of the genus and is widely distributed and cultivated in the tropical region (Martínez-Nieto et al., 2020). Its cultivation takes place through creole seeds, which are extremely important for the culture. However, it may make it difficult to follow the recommended process of fertilization (Assunção Filho et al., 2022).

The cultivation of broad beans in northeast Brazil, the main producing region, is carried out by intercropping with corn (*Zeamays spp.*), pumpkin (*Cucurbita moschata* Duch.), common bean (*Phaseolus vulgaris* L.), macassar bean [*Vigna unguiculata* L. (Walp)], yam (*Dioscorea cayennensis* Lam.), and cassava (*Manihot esculenta* Crantz) (De Jesus et al., 2018).

To ensure the success of these intercropping systems, proper management must be used, especially regarding balanced fertilization. However, there are few studies concerning fertilization, and there is little information about nutrition and adequate fertilization in Brazil.

In search of solutions to reduce external dependence on fertilizers in Brazil, research on alternative nutrient sources has been conducted. Such sources include materials such as the practice of rocking, a fertilization technique based on the addition of powder of certain types of rock or minerals regulated by Law 12,890 on December 10, 2013, Normative

Instruction No. 5 on March 10, 2016, and the Federal Official Gazette, Federative Republic of Brazil.

The use of rock dust has numerous advantages over soluble fertilizers, such as labor savings due to its low solubility; thus, there is no need to fertilize frequently because of its slow and gradual residual effect. Additionally, it does not acidify the soil and can correct its acidity. Lastly, it does not salinize the soil and can improve soil chemical characteristics. However, owing to the low solubility of basalt powder, studies with successive evaluations of the chemical attributes of the soil are recommended to evaluate the nutrient availability in the long term (Alovisi et al., 2021; Duarte et al., 2021; Alovisi et al., 2020; Almeida Júnior et al., 2020; Writzl et al., 2019).

Some research works were carried out with rock dust in some crops such as bean (Duarte et al., 2021; Bertoldo et al., 2015), corn (Writzl et al., 2019; Almeida Júnior et al., 2020), and soy (Alovisi et al., 2020; Alovisi et al., 2021). However, information regarding its effects on plant nutrition is poorly understood. Therefore, the present study aimed to evaluate the macro-and micronutrient content in the leaves of two strains of *Phaseolus lunatus* L., fertilized with rock powder (MB-4[®]).

Results

Analysis of variance was performed separately between the first and second experiments, referred to as the first and

second cultures, respectively. It was not verified whether there was an interaction and significant difference between the broad beans for the foliar contents and doses of N, P, S, B, Cu, Fe, Mn, and Zn in the first culture, and K, S, and Zn in the second culture. However, there was an increase in leaf content for N, P, Mn, and Fe when compared to the crops in the second culture (Table 1).

N and K content in broad bean leaves

Dosage had no significant effect on N content in the first culture. However, a significant interaction was verified by the 5% probability F test in the second culture (Figure 1A). In the regression analysis, a quadratic polynomial fit was observed for the 'Branca' and 'Cara larga' lima bean varieties.

It was verified that dosage had a significant effect only in the lima bean varieties for the P content in the first crop. A significant interaction was verified by the 5% probability F test in the second crop (Figure 1B). When performing the regression analysis for the second crop, a quadratic polynomial and a linear fit were observed for the 'Branca' and 'Cara larga' lima beans, respectively. A significant interaction was verified by the 5% probability F test for K content in the first culture (Figure 1C). When the regression analysis was carried out, it was observed that the 'Branca' and 'Cara Larga' lima bean varieties did not fit any model; thus, the average was used. In the second crop, the dosage effect on K content in the leaves (Figure 1D) was verified using quadratic polynomial adjustment by regression analysis.

Ca and Mg content in broad bean leaves

When performing regression analysis for the first crop, a quadratic polynomial fit was observed for the 'Branca' and 'Cara Larga' lima bean varieties. In the second crop, a regression analysis of Ca indicated a quadratic adjustment of the 'Branca' variety; there was no adjustment for the 'Cara Larga' lima bean variety. A significant interaction was verified by the F test at 5% probability for the Mg content of the first and second cultures (Figure 2C and 2D). When the regression analysis of Mg for the first and second crop was observed, a second degree polynomial fit was observed for the 'Branca' variety, while no adjustment was seen for the 'Cara larga' lima bean variety.

B and Cu content in broad bean leaves

It was verified that dosage had no significant effect on Cu and Fe content in the first culture. In the second culture, a significant interaction was verified by the 5% probability F test (Figures 3C and 3D). A second-degree polynomial fit was found for the 'Branca' variety, and a linear fit was observed for the 'Cara larga' variety when performing the regression analysis for Cu. The regression analysis of Fe did not indicate adjustment for the 'Branca' variety. However, a second-degree polynomial adjustment was found for the 'Cara larga' variety.

In the second crop, an increase in MB-4[®] dosage promoted an increase in the absorption and accumulation of B in the leaves of the 'Branca' lima bean variety (Figure 3B). Additionally, the highest dose applied was not sufficient to cause a decrease in the absorption or accumulation of B in the 'Branca' variety.

Mn and Zn content in broad bean leaves

It was verified that dosage had no significant effect on Mn and Zn content in the leaves of the first crop. In the second crop, a significant effect was verified by the 5% probability F test (Figure 4A and 4B). Regression analysis for Mn and Zn showed a linear adjustment for hole⁻¹.

Discussion

The dosage concentrations used in this study were 0, 2, 4, 8, and 16 t ha⁻¹. It was observed that there was an increase in N levels when the effect of these doses on the N content in leaves was verified, mainly after ten months of MB-4[®] application in the soil. The 'Branca' lima bean cultivar had the highest N content. The 'Branca' lima bean variety had the highest N content at 505 g of MB-4[®] hole⁻¹. Moreover, in the 'Cara larga' lima bean variety, the maximum levels were obtained with a dose of 155 g hole⁻¹ (Figure 1). A previous study by Silva (2007) showed that application of 0, 10, 20, 50, and 100 t ha⁻¹ of basalt powder on beans resulted in an increase in N content after one year of the experiment.

The two largest doses, 400 and 800 g hole⁻¹, did not contribute to the increase in N content in the leaves, mainly in the 'Cara larga' variety (Figure 1A). Notably, even with reduced dosage, the N values that were observed were in the appropriate range (between 30–50 g kg⁻¹) for *Phaseolus vulgaris*. This is in accordance with a previous study by Ambrosano et al. (1996) because there are no recommendations for lima bean culture in Brazil.

In the second crop, the increase in the dose of MB-4[®] promoted a progressive reduction in the absorption and accumulation of P by the 'Cara larga' lima bean variety (Figure 1B), following a linear relationship. The reductions were given constant rates from dose up to the highest dose applied. This shows that the application of MB-4 to the soil caused a decrease in the nutrient absorption or accumulation by the plants. In the 'Branca' lima bean, this decrease was perceived by the plant only when the MB-4[®] dose was 450 g hole⁻¹.

The overall mean revealed that P levels increased from the first to the second year of cultivation, ranging from 3.0 to 4.0 g kg⁻¹. This is supported by a previous study, where the P levels ranged from 2.0 to 3.0 g kg⁻¹ for *P. vulgaris* (Malavolta et al., 1997).

Phosphorus is recognized as one of the most important elements in plant metabolism and is essential for the establishment and development of plants (Gonçalves et al., 2000). This nutrient is crucial for plant metabolism and plays an important role in cell energy transfer, respiration, and photosynthesis. It is also a structural part of nucleic acids of genes and chromosomes, as well as of many coenzymes, phosphoproteins, and phospholipids (Marschner, 2012)

The overall K mean showed an increase in the content of this element from the first to the second crop (Figures 1C and 1D). However, the values remained well below the appropriate range (20–25 g kg⁻¹) for *P. vulgaris* (Malavolta et al., 1997).

The verified K deficiency can probably be explained by the high Mg content in rock dust. However, according to Marschner (2012), K deficiency causes an increase in N, P, Ca, and Mg levels, and accumulation of B. For Ca content, a significant interaction was verified by the 5% probability F test in the first and second cultures (Figure 2A and 2B).

In this study, the effect of fertilization on leaf Ca content was observed. This effect was observed in the increased Ca

Table 1. Lima bean leaf analysis of N, S, P, B, Cu, Fe, Mn, and Zn content in the first crop and K, S, and Zn content in the second crop. There was no significant interaction according to the F test ($p < 0.05$).

	N	P	S	B	Cu	Fe	Mn	Zn	K	S	Zn	
	-----Firstcrop-----						-----Secondcrop-----					
Varieties	-----g kg ⁻¹ -----						-----mg kg ⁻¹ -----			-----g kg ⁻¹ -----		-----mg kg ⁻¹ -----
'Cara larga'	34.76a	2.63a	2.47a	34.73a	3.09a	89.84a	40.10a	29.32a	6.42a	2.33a	26.61a	
'Branca'	36.57a	2.24a	2.46a	49.38a	3.61a	85.67a	45.50a	24.34a	7.23a	2.58a	25.52a	
Mean	35.66	2.43	2.46	42.05	3.35	87.75	42.80	26.83	6.83	2.45	26.06	
CV%	9.41	16.27	10.68	10.04	16.25	7.14	12.29	10.04	8.79	7.37	5.96	

Means followed by the same letter do not differ according to the Tukey's test ($p < 0.05$).

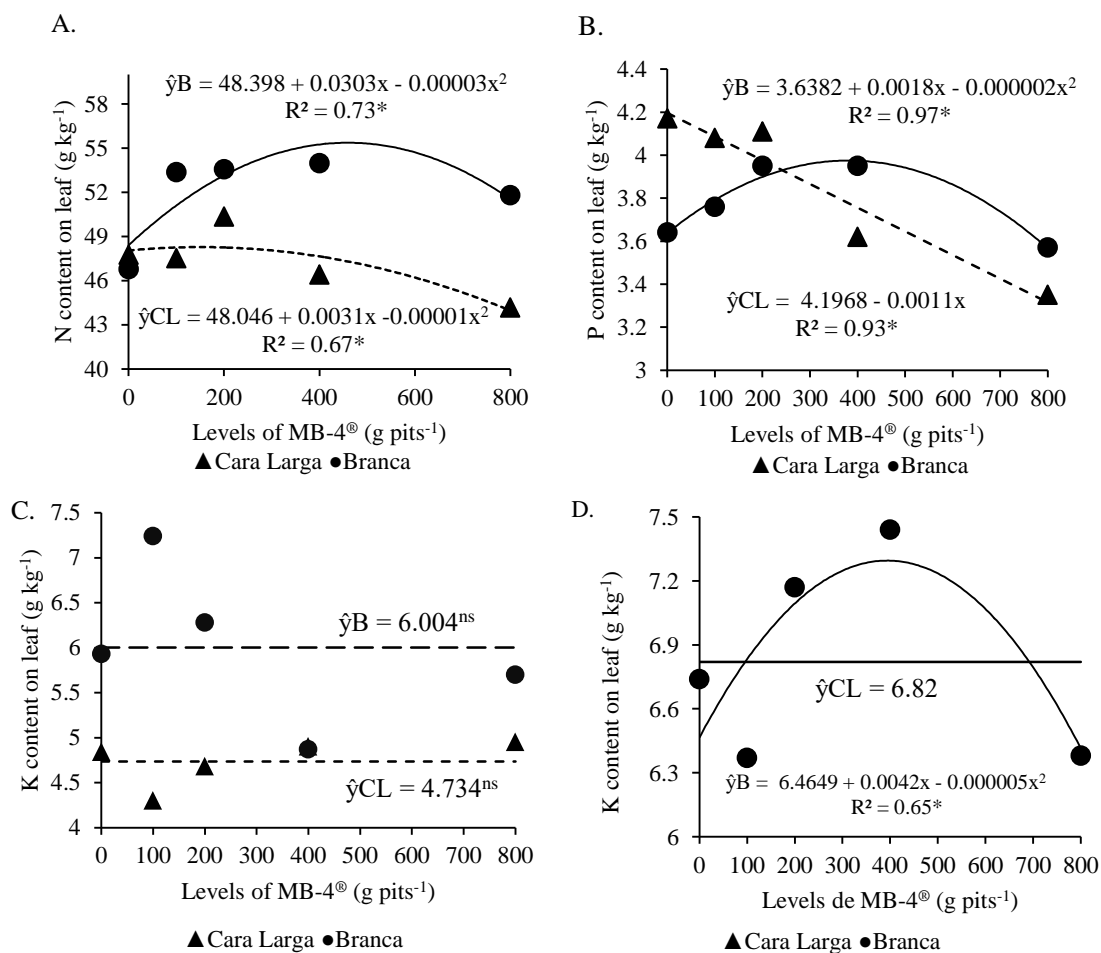


Figure 1. Nitrogen values in leaves, mean = 49.56 and CV (%) = 6.16 (A); phosphorus values in leaves, mean = 3.85 g and CV (%) = 7.02 (B); potassium values in leaves in the second planting, mean = 5.37 g and CV (%) = 16.89 in the first planting (C) and mean = 6.79 g and CV (%) = 8.79 in the second planting (D) as a function of MB-4[®] dosage.

Table 2. Chemical analysis of soil and MB-4[®] dosage.

	pH H ₂ O (1:2,5)	P**	K ⁺	Na ⁺	H ⁺ +Al ⁺ ₃	Al ⁺³	Ca ⁺²	Mg ⁺²	SB	CTC	V	m	M.O.
Soil	5.6	34.69	30.00	0.22	3.38	0.00	1.35	1.00	2.64	6.03	44	0.00	30.90
MB-4	8.4	18.63	40.00	0.48	0.83	0.00	8.30	2.65	11.53	12.36	93	0.00	49.41

pH determined in water; P and K with the Mehlich⁻¹ extractor; and Al⁺³, Ca⁺², Mg⁺² with the 1 mol L⁻¹ KCl extractor.

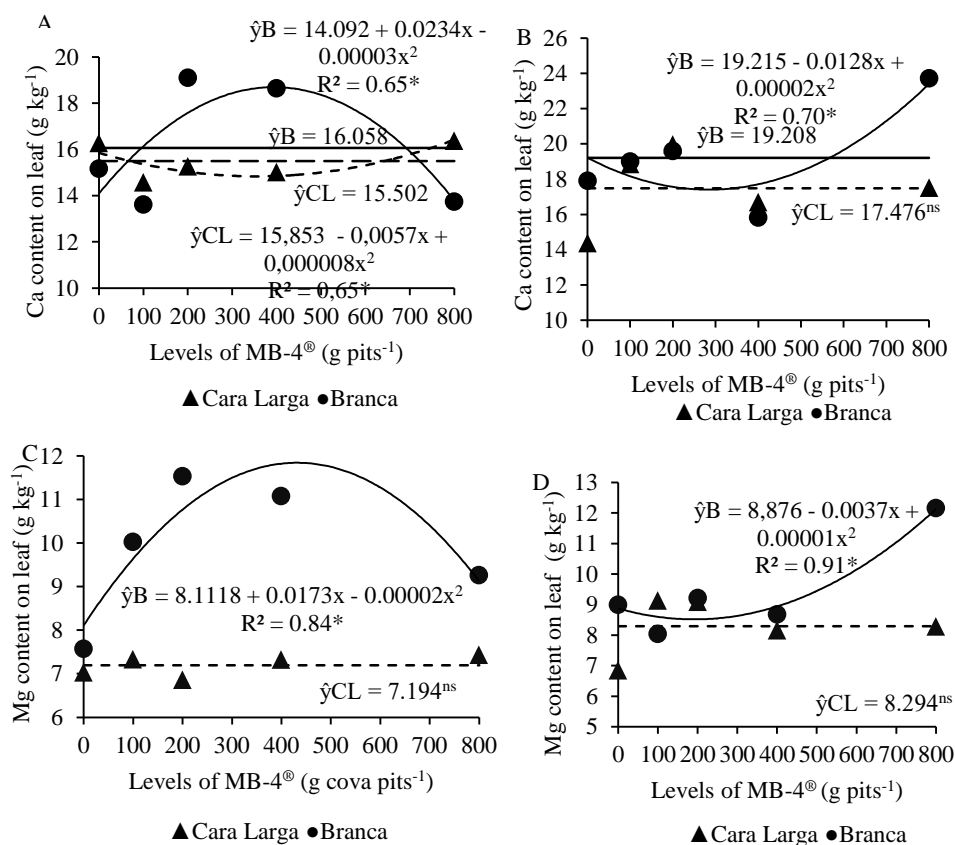


Figure 2. The mean values of calcium in leaves, mean = 15.18 and CV (%) = 7.09 in the first crop (A) and mean = 18.34 g and CV (%) = 8.49 in the second crop (B), the mean values of magnesium in leaves, mean = 8.54 g and CV (%) = 10.31 in the first crop (C) and mean = 8.85 g and CV (%) = 11.53 in the second crop (D) as a function of MB-4[®] dosage.

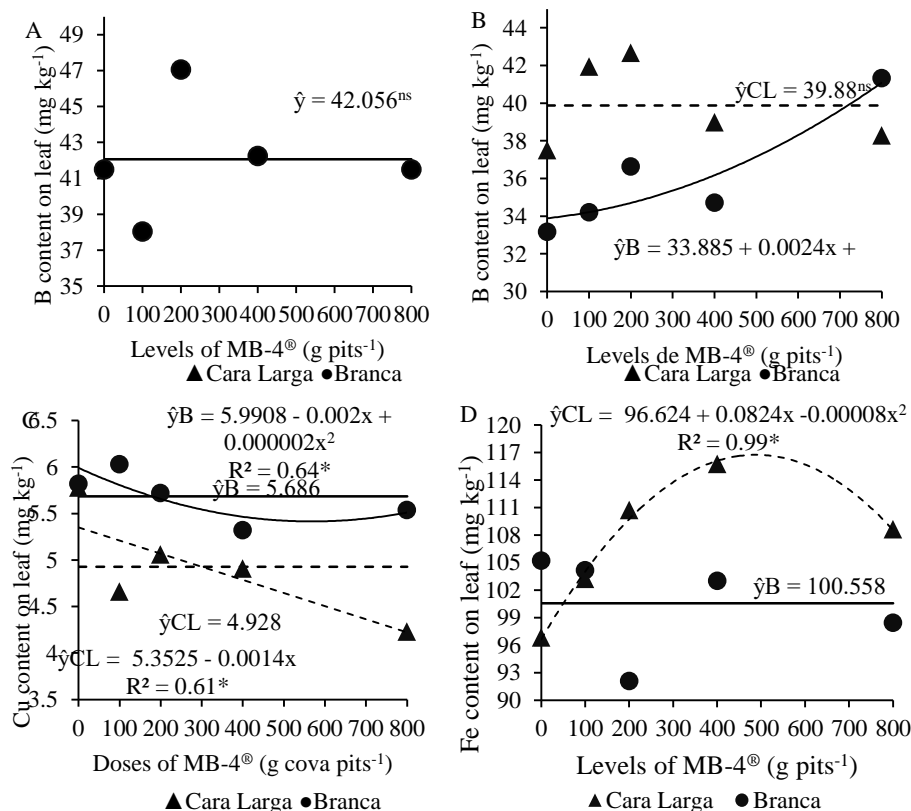


Figure 3. The mean values of boron in leaves, mean = 42.05 g and CV (%) = 10.04 in the first crop (A) and mean = 37.94 g and CV (%) = 6.42 in the second crop (B); the mean values of copper in leaves, mean = 5.30 and CV (%) = 8.90 in the first crop (C) and mean = 103.78 g and CV (%) = 8.55 in the second crop (D) as a function of MB-4[®] dosage.

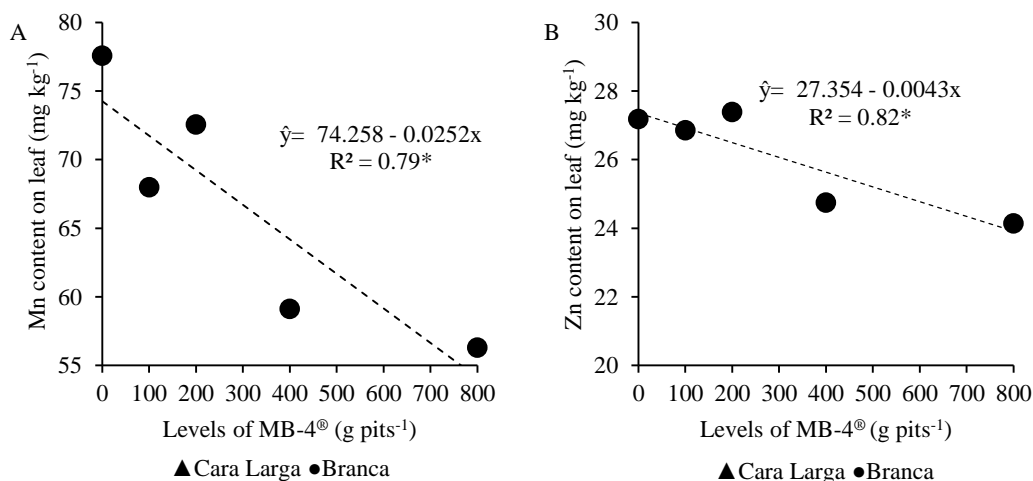


Figure 4. The mean values of manganese in leaves, mean = 66.71 g and CV (%) = 12.80 in the second crop (A), the mean values of zinc in leaves, mean = 26.06 g and CV (%) = 5.96 in the second crop (B) as a function of MB-4[®] dosage.

389.17 g hole⁻¹ when evaluating the weight of seedlings without the 'Branca' variety seeds. In this study, the 'Branca' lima bean variety reached its peak Ca absorption and accumulation at an MB-4[®] dose of 390 g hole⁻¹ in the first culture. In the second culture, it was observed that Ca uptake continued and increased linearly with increasing doses of MB-4[®]. There was also an increase in Ca in the second crop for the 'Cara Larga' variety, even if the equation was not adjusted. This result shows that in the 'Branca' variety, Ca absorption occurred more rapidly and at a greater intensity. Dantas et al. (1979) suggest that 17.9 g kg⁻¹ of Ca content in leaves is suitable for *P. vulgaris*. Foliar analysis of Mg content revealed a behavior very similar to that of Ca, especially in the first crop. The overall mean Mg content increased from the first crop to the second crop. However, the values were much higher than those recommended for the first crop for Mg.

According to Dantas et al. (1979), Mg content of 1.4 g kg⁻¹ in leaves is within the range suitable for *P. vulgaris*. However, Van Raij et al. (1996) cites that an Mg content of 2.5–5.0 g kg⁻¹ in the leaves as a critical range for assessing nutritional status. According to Marschner (2012), N and Mg are synergistic. Thus, it was observed that increased Mg uptake may have influenced the appropriate N values due to this favorable availability according to the analysis of the rock dust verifying Mg²⁺. Although the 'Branca' variety was responsible for higher absorption and accumulation of Mg in the first and second cultures, it was the second cultivation that showed higher absorption and accumulation of Mg, including the 'Cara larga' variety. This shows that the release of nutrients from rock dust to the soil solution is slow (Brandão, 2012). The 'Branca' variety responded more rapidly to Mg absorption and accumulation in relation to MB-4[®] dosage. The effect of dosage was verified using the F test at 5% probability for B content in the first culture (Figure 3A). When performing the regression analysis, we observed no adjustment; thus, the mean was used. In the second crop, a significant interaction was observed between dosage and the lima bean varieties with respect to B content (Figure 3B). A second-degree polynomial fit was observed for the 'Branca' variety when performing the regression analysis. No adjustment was found for the 'Cara larga' variety.

The means showed that there was a decrease in B micronutrient content in the second culture compared to in the first culture. Even with this reduction, the values were above those indicated by Van Raij et al. (1996), where a

B content of 15–26 mg kg⁻¹ was the critical range for *P. vulgaris*. There was a linear decrease in the Cu value of the 'Cara larga' variety in the second crop (Figure 3C). Despite this reduction, the means showed that there was a considerable increase when the two crop cultures were compared. This increase is within the Cu content range indicated by Van Raij et al. (1996), with 4–20 mg kg⁻¹ in *P. vulgaris*. There was also a reduction for the 'Branca' variety, reaching the minimum point at the dose of 500 g hole⁻¹. However, the Cu content remained within the range indicated for the cultivation of *P. vulgaris*. The Fe values in the first crop were already within the range indicated by Van Raij et al. (1996), with 40–140 mg kg⁻¹ for *P. vulgaris*. However, Fe levels increased in the foliar tissues of the bean varieties cultivated in the second crop. This increase was more accentuated in the 'Cara larga' variety at 515 g hole⁻¹. In the second crop, MB-4[®] dose increase promoted a progressive reduction in Mn and Zn absorption and accumulation in the leaves of the lima bean varieties, according to a linear relationship. The reductions occurred at constant rates from the dose up to the highest dose applied. This result demonstrates that an application of MB-4[®] in the soil was sufficient to cause a decrease in Mn and Zn absorption or accumulation in the lima bean varieties investigated in this study. According to Marschner (2012), high B content reduces the Mn content in leaves. Thus, the reduction in Mn content may be a function of another micronutrient. In the case of Zn, Van Steveninck et al. (1987) considered that their reduction in translocation in plants subjected to excessive doses of Zn is a possible mechanism for increasing tolerance to toxicity. According to the aforementioned authors, Zn accumulates in a greater proportion of vacuoles in the cells of the root cortex. The doses of Zn used in this study decreased the translocation of the root element to the leaves of lima bean plants. Even with the linear reduction in Mn and Zn leaf content in the native varieties of lima bean (Figures 4A and 4B), the values found in the study were within the range indicated by Van Raij et al. (1996), where Zn ranged from 18–50 mg kg⁻¹ and Mg ranged from 15–100 mg kg⁻¹ for *P. vulgaris*.

Material and methods

Research area

The experiments were conducted at coordinates of 6° 46' S and 35° 38' W, with an elevation of 617 m. The climate of the

region is rainy, tropical, hot, and humid, according to the classification of Köppen (year).

The soil in the experimental area was classified as yellow dystrophic ultisol (Embrapa, 2006). The experimental area was plowed and arrowed prior to experiment 1, corresponding to the first crop. At that time, soil was collected from the 0–20 cm layer for chemical analysis, as well as the chemical analysis of a sample of MB-4® rock dust used for fertilization (Table 2) following Embrapa's methodology (2009).

Experimental design

The experiment was set up in a randomized block design (DBC), with four replicates, in a 2 × 5 factorial scheme corresponding to two lima bean varieties ('Cara Larga' and 'Branca') and five dose concentrations of rock dust: 0, 100, 200, 400, and 800 g hole⁻¹, corresponding to 2, 4, 8, and 16 t ha⁻¹, respectively, intercropped with maize. Each experimental plot consisted of three lines with seven holes each, spaced 1.0 m between rows and 0.50 m between holes. To eliminate edge effects, the plants in the five holes in the central line were analyzed for plotting.

Plant materials, Conduction of study and experimental design

The maize (*Zea mays*) native 'Palha Roxa' and lima bean (*P. lunatus*) native 'Branca' and 'Cara larga' varieties were manually sowed in a hole. Four corn seeds and three Lima bean seeds of each variety were placed in each well. The doses of rock dust were applied and homogenized with the soil in the hole when sowing corn and Lima beans, only for the first crop (experiment 1). Thinning was performed on the fifteenth day post-germination, leaving two plants of each species per hole. Experiment 2, corresponding to these corn crops in the same area, was carried out forty days after the harvest to verify the residual effect of rock dust on subsequent cultivations. The average cycle time for both types of Lima beans was 165 days.

Traits measured

The methodology described for beans were used for nutritional analysis of the lima bean varieties. In this study, the first fully developed leaf was collected from the tip (Malavolta et al., 1997). One leaf per plant was taken from the centerline, totaling 10 leaves per plot.

The leaves were cleaned with cotton, moistened in distilled water, packed in kraft paper bags, dried in a forced-air oven at 72°C for 48 h, milled in a Wiley mill with a 30-mesh screen, and stored in hermetically sealed vials.

The nutrients analyzed were nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, copper, iron, manganese, and zinc. To determine the N content, the plant material was subjected to S digestion, using the method described by Nessler (Jackson, 1965). The other nutrients (P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn) were quantified via ICP-OES, after digestion with concentrated HNO₃ and H₂O₂ in an open digestion system. ICP conditions: plasma gas 8.0 L min⁻¹, 0.70 L min⁻¹ auxiliary gas and 0.55 L min⁻¹ carrier gas (Peters, 2005).

Statistical analysis

The data were submitted to analysis of variance (F Test). In significant cases ($p < 0.05$), a regression study was performed using the statistical software Assistat (v7.7) (Silva and Azevedo, 2009).

Conclusions

The leaf analysis indicated that the applied doses increased N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn content significantly in the second crop, and that only B content decreased. The N, P, Ca, B, Fe, Mn, and Zn contents were within the recommended range, while only K and Mg values were outside the recommended range. The values found between 200 and 400 g hole⁻¹, corresponding to 4 and 8 t ha⁻¹ of MB-4®, respectively, contributed the most to the increase in nutrient content in the lima bean varieties. Management with MB-4® is recommended for the species studied; however, it should not be used as the only source of fertilization.

Acknowledgments

The authors thank the Coordination of Improvement of Higher Education Personnel (Capes) for the financial assistance to carry out this work, the Laboratory of Mineral Nutrition of Plants of the Center of Sciences of Agricultural Technologies of the North Fluminense State University for performing the nutritional analysis of the plants, and the Nucleus of Extension in Territorial Development (NEDET/UFPB) for their support.

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