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Rock dust and humic substance promote *chloroleucon dumosum* seedling growth

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ABSTRACT

The effects of humic substances as well as silicate rocks as a source of nutrients in agriculture is a common strategy, but the specific effects of these substances on native trees are still poorly known. The application of these inputs in the production of seedlings for restoration depends on the analysis of their effects on the various parts of the plants, mainly on the root system, which plays an important role in the survival of the seedlings after planting. In this study, we used morphological, anatomical, and nutrient uptake data to report the effects of rock dust and humic substance in the growth of the slow-growing *Chloroleucon dumosum*. The experiment consisted of four treatments in scenarios with presence and absence of rock dust and peat humic substances in the best concentration obtained in a previous experiment. The treatments were repeated nine times in a randomized block design (DBC) arrangement. Seeds were sown in pots (1.7 L) with soil and sand substrate (2:1). SH promoted root growth of *C. dumosum* and the response was dependent on the source and its concentration. The rock powder promoted the dry mass of *C. dumosum* shoot at 60 days after the treatments. In treatments containing rock dust added to the substrate there was a reduction in nodulation. The growth variables were all significant in the treatments with addition of rock dust, with the presence of the highest averages appearing at 90 days after the treatment’s application. Rock dust promoted the growth of *C. dumosum* and when associated with SHs this growth was more pronounced. There was no significant effect for N, K and Si. The treatments with rock dust presented the lowest phosphorus content in the shoot tissue.

Keywords: Fabaceae. legume. silicate. root growth.

1 INTRODUCTION

Humic substances (HS) are the main components of soil organic matter (85 to 90%), resulting from chemical and biological transformations of plant residues and microbial activity. Beneficial effects of humic substances on plant growth and the root system have been recognized in many studies, but specific effects of these substances on different parts of plants and different growth stages still need to be further investigated (ROSE et al. 2014).

Rocks are the source of almost all chemical elements that occur naturally on the Earth's surface. The use of humic substances and silicate rocks as a source of nutrients in agriculture is an ancient practice, which has been reduced in the last century due to the increased use of high solubility fertilizers. In addition, so far, there is no consensus on the efficiency of these inputs in providing nutrients and promoting plant growth, mainly due to the slow solubilization of minerals present in rocks (SAMPAIO; FREITAS, 2021).

The low survival of caatinga seedlings has been an important bottleneck in the restoration of this ecosystem. Thus, in the search for alternatives to produce seedlings with better quality, this study analyzes the effect of humic substances and rock dust on the growth of *Chlroleucon dumosum* and the capacity of the plant, submitted to the treatments, to carry out symbiosis with microorganisms.

C. dumosum is a species present in several regions of Brazil, used in ecological restoration projects in the caatinga. The hypothesis of this research is that humic substances and silicate rock powder improve the growth and nodulation of *Chloroleucon dumosum,* with special action on root increment. The increase in root dry mass may be an indicator of greater tolerance to water stress, one of the main environmental filters in caatinga restoration.

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2 OBJECTIVES

The objective of this work was to analyze the effect of humic substances and rock dust on the growth of *Chlroleucon dumosum* and on its ability to carry out symbiosis with microorganisms.

3 METHODOLOGY

3.1 Effect of humic substances from peat and rock dust on the growth and nodulation of *Chloroleucon dumosum*

The experiment was conducted in a greenhouse at the Federal University of Alagoas, UFAL – Campus Arapiraca. The effect of fertilization with humic substances (HS) at a concentration of 100 mg/L-1 C and rock dust (RD) on the growth and nodulation of *C. dumosum* seedlings in a greenhouse was evaluated. The experiment consisted of four treatments: irrigated with SH, with rock powder (RD) added to the substrate, with rock powder (RD) added to the substrate plus irrigation with SH and control. Treatments were repeated nine times in a randomized block arrangement (RBD). *C. dumosum* seeds were subjected to mechanical scarification with sandpaper (FILHO et al., 2007) and subsequent surface disinfestation with alcohol (70%) for 1 minute, Sodium Hypochlorite (2%) for 3 minutes and 5 successive washes in distilled water. They were then submitted to hydroconditioning, a pre-germination treatment in which the seeds are submerged in water for 24 hours. Four seeds were sown in pots (1.7 L), with a substrate composed of earth and sand in a 2:1 ratio.

The soil used in the substrate was collected in a fallow area at the Federal University of Alagoas, being the same soil for all treatments, so that the only variable to be considered was the treatment itself. In preparing the substrate for the experiment, 10 gL-1 of rock dust were added to the soil in treatments with rock dust. SH was applied 8 days after plant emergence, when thinning was performed, leaving one plant per pot. Five mL of peat SH concentration (100 mg L-1) were applied every 15 days, until the end of the experiment (90 days), totaling seven applications. The effects of treatments were evaluated at 60 and 90 days after application of treatments based on the variables: number of nodules (NN), nodule dry mass (M), root dry mass (DRM), shoot dry mass (MSD), stem diameter (CD), plant height (HG) and number of leaves (LN). Os dados foram submetidos à análise de variância (ANOVA) pelo teste F e as médias comparadas pelo teste t (P≤0,05), com auxílio do software estatístico SISVAR v.5.6 (FERREIRA, 2011).

The quality of the seedlings was evaluated using the Dikson Quality Index (DQI), calculated by the formula IQD = [total dry matter /(RAD + RPAR)], where RAD: is the ratio between the height of the aerial part and the stem diameter and RPAR: is the ratio of shoot dry matter to root dry matter (DICKSON et al., 1960).

3.2 Analysis of chlorophylls and carotenoids in *C. dumosum* seedlings at 90 DAT

Chlorophyll and carotenoid analyzes were performed according to the methodology of Lichtenthaler (1987). The ninth and tenth leaves were removed from a repetition of each treatment, these were kept in an ice box. Then, 20 mg of fresh mass were weighed under green

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light to avoid photodegradation, in a glass tube covered with aluminum foil, 5 mL of 80% acetone were added, the weighed mass was immersed in the acetone solution where it remained at rest for 48 h. The samples were read in a spectrophotometer at 470, 647 e 663 nm. After the readings, the chlorophyll content was calculated according to the following equations: chlorophyll a (ug.gMF-1) = [(12,25.A663) – (2,79.A647)] . V, chlorophyll b (ug.gMF-1) = [(21,50.A647) – (5,10.A663.).V, chlorophyll a+b (ug.gMF-1) = (7,15.A663) + (18,71.A647).V. The data were submitted to analysis of variance (ANOVA) by the F test and the averages compared by the t test (P≤0.05%), with the aid of the statistical software SISVAR v.5.6 (FERREIRA, 2011).

3.3 Quantification of Nitrogen, Phosphorus, Potassium and Silicon in the aerial part of *C. dumosum* via dry matter (DM)

To quantify the K content present in the dry matter, the methodology of Carmo et al. (2000) was used, with some adaptations. The material was dried in an oven with forced air circulation at 65 ºC for 72 h, ground in a Willys Knife Mill and packed in identified plastic bags. 500 mg of shoot dry mass were weighed on an analytical balance, transferred to porcelain crucibles, placed in an electric muffle with controlled temperature. The temperature was gradually increased to 550 º C and the material remained for 4 h until white ash was obtained. The ashes were dissolved in 25 ml of hydrochloric acid (0,1 mol L-1) HCL. Readings were taken with a Quimis flame photometer (model Q398M2P).

The levels of N, P and Si present in the dry matter of the aerial part were obtained by the methodology of Malavolta (1997). The data were submitted to analysis of variance (ANOVA) by the F test and the averages compared by the t test (P≤0,05%), with the aid of the SISVAR statistical software v.5.6 (FERREIRA, 2011).

4 RESULTS

4.1 Effect of humic substances and rock dust on nodulation and growth of *Chloroleucon dumosum* seedlings

4.1.1 Period 60 days after treatment

It was observed that, 60 days after planting, there was a stimulus in the growth of *C. dumosum* seedlings caused by the addition of rock dust (Figure 1). Treatments with the presence of SH had a positive effect on shoot dry mass, and when associated with rock dust, this effect became more pronounced (Figure 1A). It is proven that HS promote plant growth, several studies have proven the positive effect of the humified fraction of organic matter on the physiology and growth of plants (CANELLAS; OLIVARES, 2014; ROSE et al. 2014).

There was a significant effect for the aerial part dry mass variable, where the best averages were for the treatments with the presence of rock dust. When rock dust was associated with SH, there was an additive effect on dry mass production, but without promoting a significant increase. Although SH has been shown to promote plant growth, especially root growth, application of peat SH alone did not promote growth in *C. dumosum* plants 60 days after the start of SH application (Figure 1A).

Humic substances have activity like plant hormones and increase nutrient absorption and plant growth (NARDI et al. 2021). In addition to their bioactive effects, humic acids are

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considered additives for the introduction of microorganisms in the form of inoculants in the soil-plant system. The effect of humic materials depends on the origin and quality of organic matter, important in the final relationship between organic acids and mineral, chemical and biological components of the soil, promoting better and more stable interactions (CANELLAS; OLIVARES, 2014).

Studies have shown that silicon can stimulate plant growth and production. For example, the accumulation of silicon makes the leaves more erect, due to the greater structural rigidity of the tissues and this reduces self-shading and protects the plant against unfavorable abiotic factors, such as water stress, aluminum and iron toxicity (EPSTEIN, 1994; MIR et al. 2022).

The effect of rock dust on plant growth was proven when compared to the control, where the averages were lower. This result may represent a possible alternative to produce more resistant seedlings. At 60 days of treatment, it was still not possible to observe statistically significant differences for the variable's height, number of leaves and stem diameter (Figures 1B, 1C, e 1D).

Contrary to what we initially expected, treatments with rock dust caused a reduction in nodule formation (Figure 1E) and nodule dry mass (Figure 1F). One hypothesis for the low nodulation would be that structural and chemical barriers caused by silicon enrichment may have hindered colonization by bacteria and this reduced nodulation. Several studies show that Si enrichment in the plant promotes the formation of these barriers that end up preventing microbial colonization, mainly fungi and nematodes (MIR et al. 2022). The beneficial effects of Si have been demonstrated in several plant species, and, in the case of phytosanitary problems, it is capable of increasing plant resistance to attack by insects and pathogens. Silicon can confer resistance to plants through its deposition, forming a mechanical barrier (EPSTEIN, 1994). Mechanical barriers include changes in anatomy, such as thicker epidermal cells and a greater degree of lignification and silicification (MENDES, 2011). This may have negatively affected the symbiosis process of the plant with nitrogen-fixing bacteria, inhibiting nodulation. Although rock dust reduces the nodulation rate, this treatment was quite efficient in plant growth, proving to be an alternative to improve the quality of seedling production, as already reported by other authors for forest species (ARNOTT et al. 2021).

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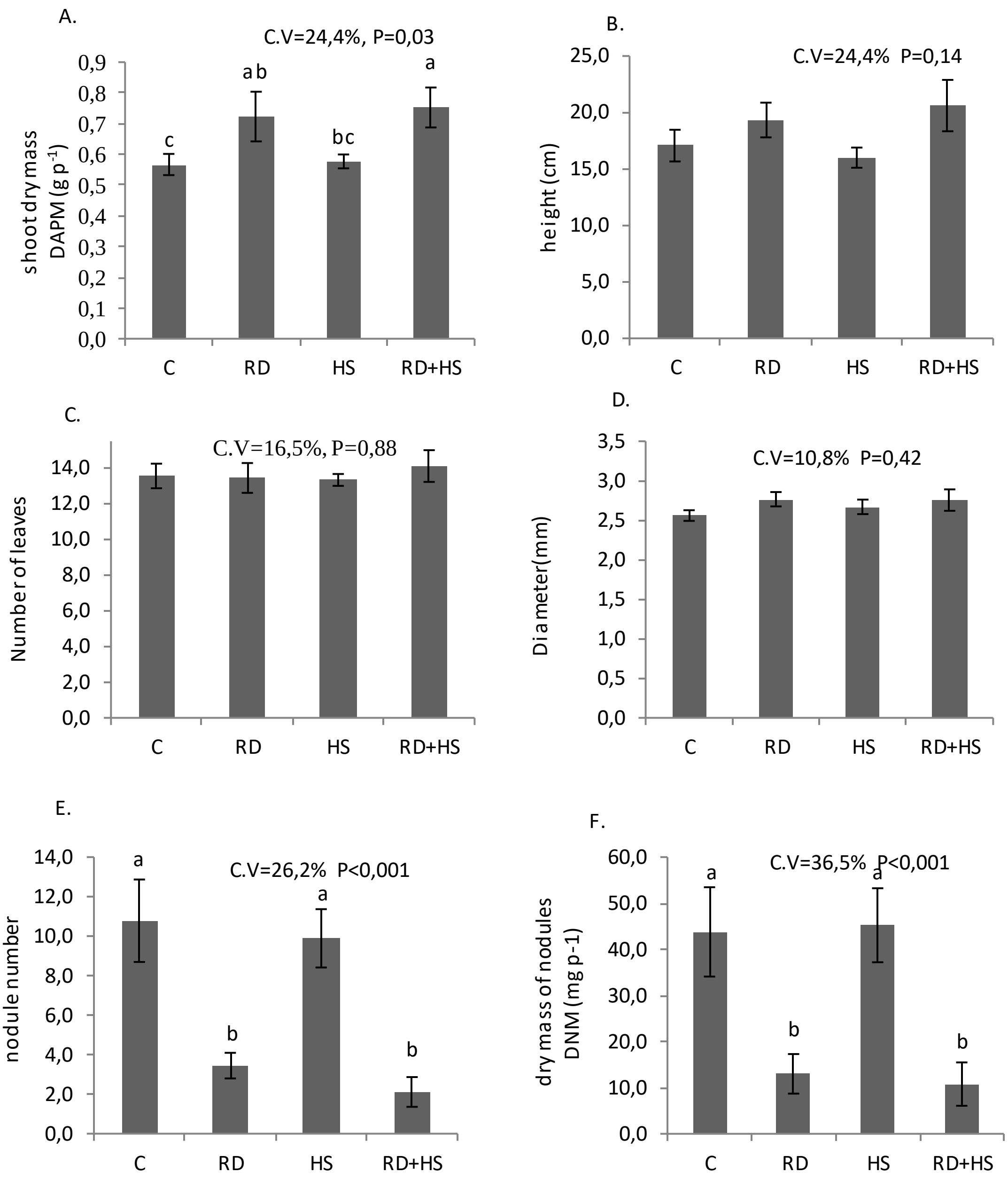

Figure 1. (A) dry mass of the aerial part of *C. dumosum* at 60 DAT (DAPM), (B) aerial part height, (C) number of leaves (NL), (D) collar diameter (DC), (E) number of nodules (NN), (F) dry mass of *C. dumosum* nodules at 60 DAT (DNM). C=Control; RD=rock dust; SH=humic substances; RD+SH=rock powder +humic substances.

4.1.2 Period 90 days after treatment

Rock dust effects on plant growth were significant at 90 DAT. The growth variables were significant in all treatments with the presence of rock dust (Figures 2A, 2B, 2C, 2D, 2E). There was a positive effect of the addition of rock dust on the variables: height, number of leaves, stem diameter, dry mass of the aerial part and dry mass of the root, which in relation to the control were statistically superior.

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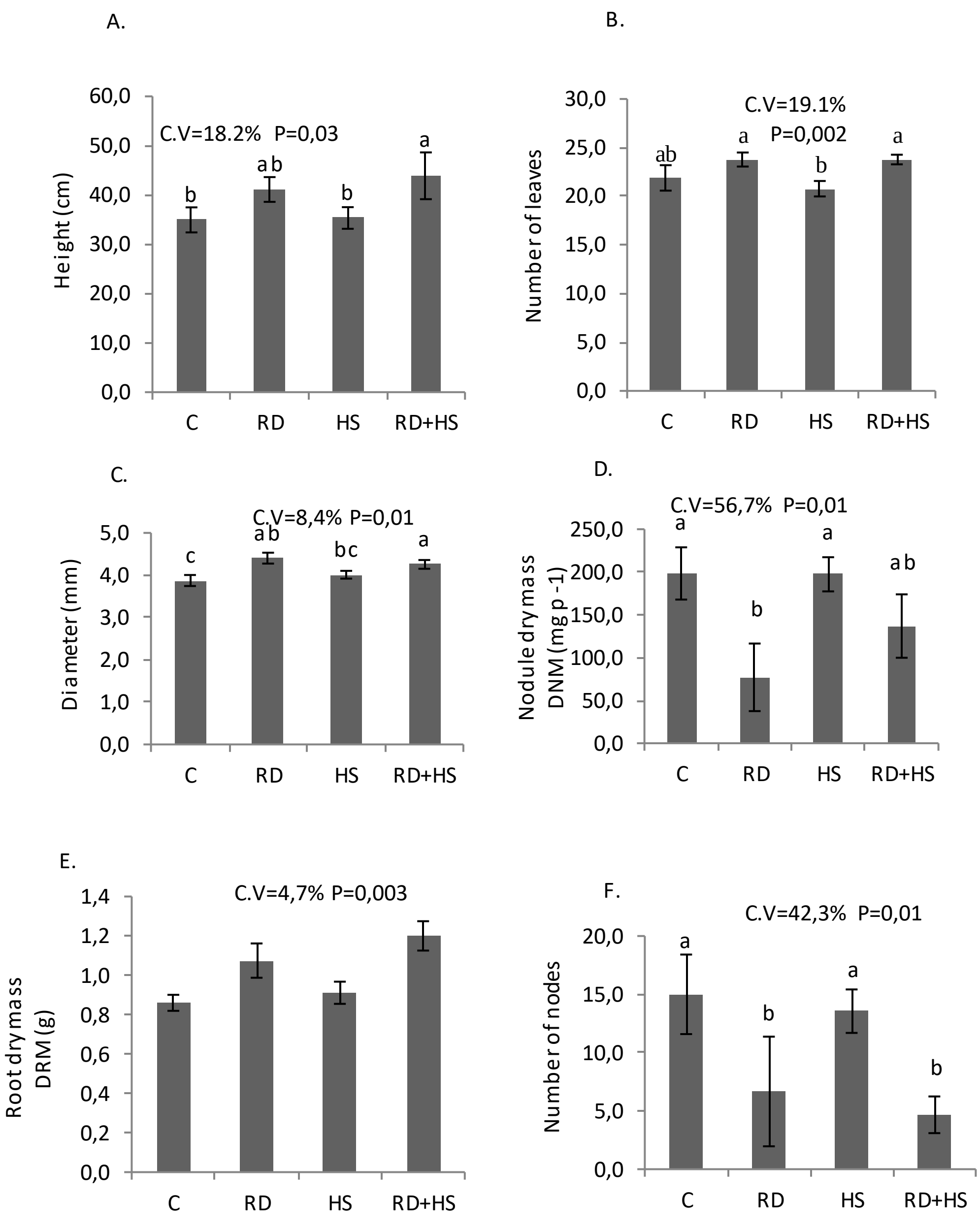
Silicon is the second most abundant element in the earth's crust and accumulates in the tissues of all plants and represents 0.1 to 10% of their dry matter (EPSTEIN,1994). The application of Silicon can correct soil acidity, increase phosphorus availability, increase base saturation and Calcium and Magnesium contents in soils, in addition to Silicon absorption bringing benefits to plants, such as greater cell wall resistance, greater photosynthetic rate, less water loss, greater absorption of Calcium and Magnesium and increased productivity (MIR et al. 2022).

The number of nodules and the nodule dry mass in the treatments with rock dust continued to be smaller, in relation to the control (Figures 2F; 2G).



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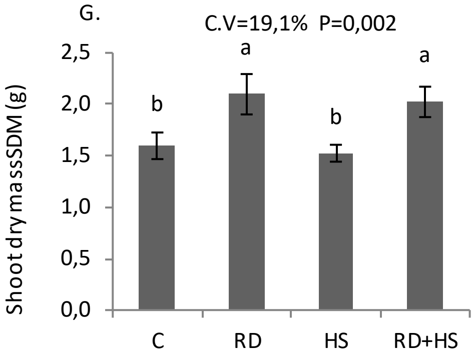

Figure 2. (A) Shoot height, (B) number of leaves (NF), (C) collar diameter (DC), (D) nodule dry mass (DNM), (E) root dry mass (DRM) of *C. dumosum* at 90 DAT, (F) number of nodules (NN), (G) shoot dry mass (DM). C=Control; RD=rock dust; SH=humic substances; RD+SH=rock powder +humic substances.

Humic substances can act to increase the population of endophytic diazotrophic bacteria, acting as a physical-chemical conditioner, in addition to promoting greater establishment of bacterial inoculum inside the plant Marques (2008). This can be explained in part by the effects of SH on increasing the number of lateral roots, which constitute the main site of host plant infection by endophytic bacteria. SH can have positive effects in increasing plant phytomass, as they have high surface reactivity and high molecular weight, acting as a reservoir of nutrients and contributing to the chemical stabilization of microaggregates, as already reported by Silva et al. (2016).

There was a significant effect for total dry mass and efficient use of water, where the treatments with the best performance were those with the addition of rock dust for both variables. There was no significant effect for the IQD (Table 1).

Table 1. Total dry mass (TDR), efficient water use (EUA) and Dickson quality index (DQI) in *C. dumosum* at 90 days after treatment application

TREATMENTS	TDR (g)	EUA H_20	IQD
C	(2.66±0.16) b	(0.49±0.03) b	(0.25±0.02)
RD	(3.25±0.25) a	(0.60±0.05) a	(0.29±0.02)
SH	(2.63±0.13) b	(0.49±0.02) b	(0.25±0.01)
RD+SH	(3.36±0.20) a	(0.62±0.04) a	(0.28±0.02)

Means followed by the same lowercase letter in the column do not differ statistically by Tukey's test (≤0.05).

Regarding the levels of photosynthetic pigments, including chlorophyll a, chlorophyll b, chlorophyll a+b and carotenoids, there was little variation on average between treatments (Figure 3), with no significant contrasts. The application of SH promoted a reduction in the production of chlorophyll b, although not significantly (Figure 3 A).

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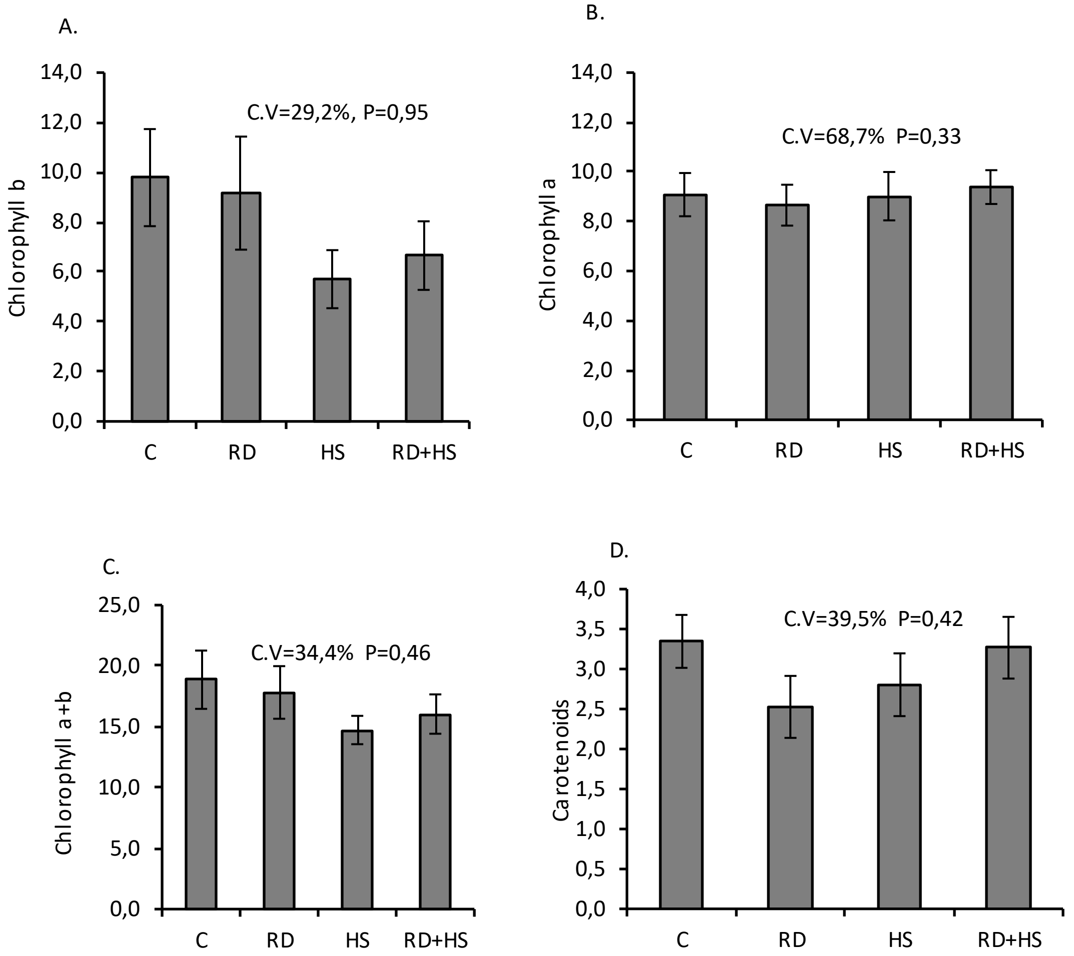

Figure 3 - Analysis of chlorophylls and carotenoids in C. dumosum. (A) Chlorophyll b, (B) Chlorophyll a, (C) Chlorophyll a+b, (D) Carotenoids. Values expressed in µg/ml of MF. C=Control;RD=rock powder; SH=humic substances; RD+SH=rock powder +humic substances.

Plants treated with rock dust had low phosphorus content (Figure 4A), which may have affected nodulation. P is an essential nutrient for plant development and its deficiency limits production and growth capacity (MORINIÈRE et al. 2016; PUEYO et al. 2021). In addition, P is a very important element for the nutrition of legumes and for N2 nodulation and fixation (WANG et al. 2020). Low soil fertility, due to its great deficiency of P and micronutrients, can affect nodulation, which are probably the most limiting factors for N2 fixation, consequently a plant with less phosphorus can also have lower N contents (Figure 4B).

There was no significant difference between treatments for accumulation of N, K and Si in the aerial part of the plant (Figures 4AB, 4C, 4D). Nitrogen is considered the most abundant element, constituting about 80% of the earth's atmosphere. It is the macronutrient that most commonly limits plant growth and production. This is because plants do not have mechanisms for the direct absorption of Nitrogen (N2) available in the atmosphere.

Plants treated with rock dust added to the substrate showed the highest levels of Magnesium (Mg) in the shoot tissue (Figure 4E). For the accumulation of Calcium (Ca) in shoot tissue, there was no significant difference between treatments (Figure 4F).

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Mg is essential for plants, being absorbed from the soil solution in the form of the Mg+2 ion, has a structural function and is involved as a cofactor in many enzymatic transfers in respiration, photosynthesis and DNA and RNA synthesis. Ca is an immobile element in plants, in the aerial part, the deficiency of the nutrient is characterized by the reduction of the growth of the meristematic tissues, causing damages in the extremities and young leaves, which are deformed and chlorotic. Ca deficiency is rare under field conditions, especially when soils have already been amended with lime (TAIZ; ZEIGER, 2004).

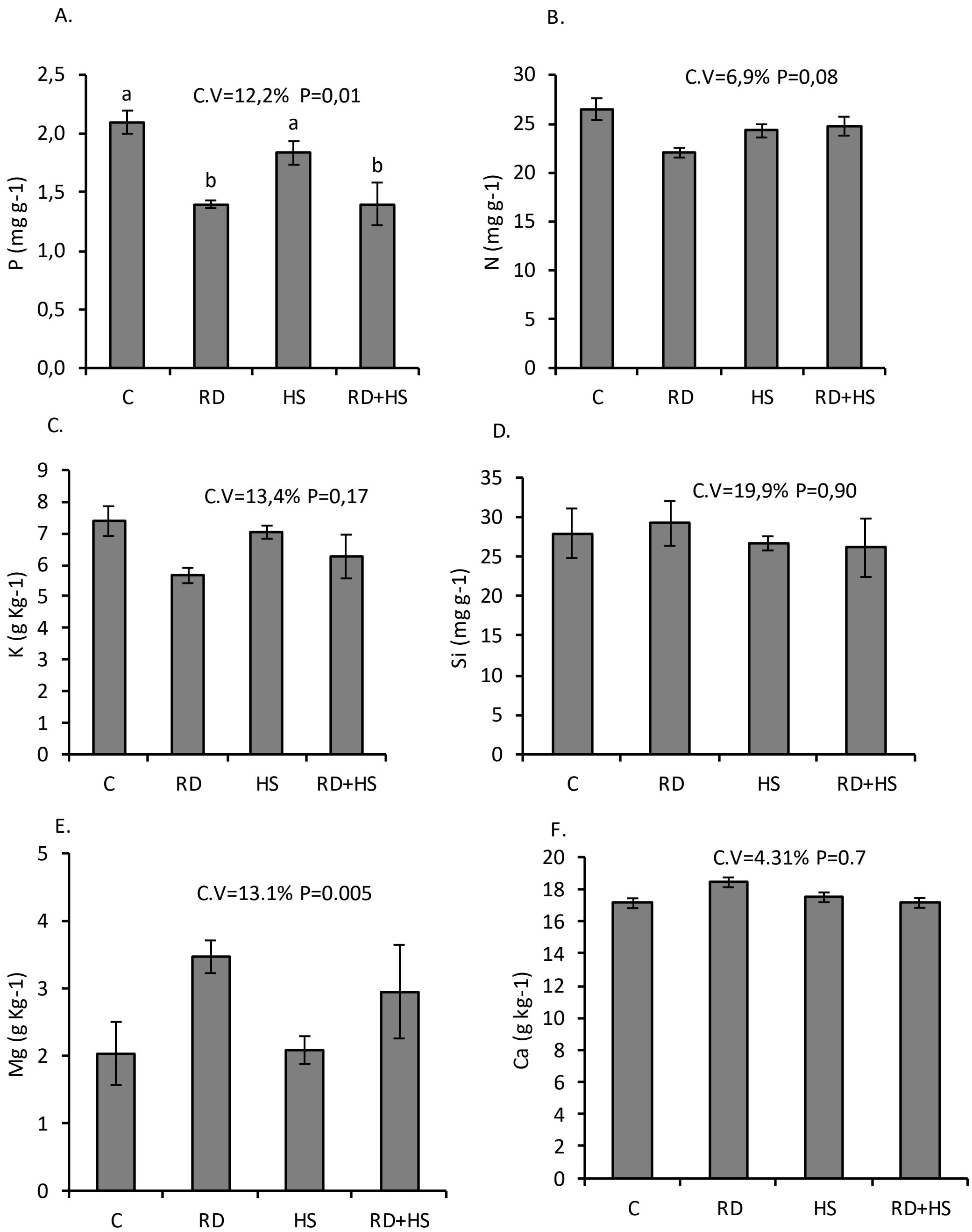

Figure 4. Content of P (A), N (B), K (C) and Si (D), Mg (E), Ca (F) in the aerial part of *C. dumosum*. C=Control; RD=rock powder; SH=humic substances; RD+SH=rock powder + humic substances.

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There was no statistical difference between treatments for potassium content (Figure 4C). For legumes, potassium deficiency, in addition to affecting plant growth, reduces nodulation (number and size of nodules), affecting nitrogen fixation (XU et al. 2020).

Plants from treatments with the presence of rock dust showed efficient use of nitrogen. Despite having low levels of P in the tissues, the plants treated with rock dust and humic substances in the substrate managed to make efficient use of phosphorus and this was enough to keep them healthy and growing (Figures 5A, 5B).

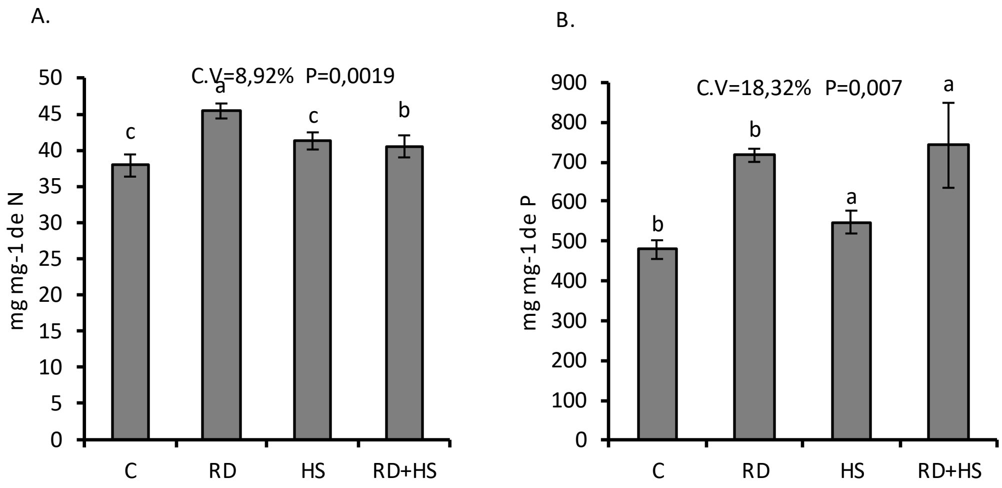

Figure 5. Efficient use of nitrogen (A) and phosphorus (B) by C. dumosum plants 90 days after treatment application

P deficiency has a negative impact on biological N2 fixation. The reduction in N2 fixation in legumes with limited supply of P is generally explained by a decrease in host growth and, consequently, in the fixed demand for N, in the growth and functioning of the nodules or in the growth of both (ROSE et al. 2014; WANG et al. 2020).

5 CONCLUSION

The treatment with rock dust was positive and can be recommended to produce *Chloroleucon dumosum* seedlings, causing a greater increase in the dry mass of the aerial part, in the measurement made after 60 days, mainly when combined with humic substances. This benefit was greater 90 days after planting, when in addition to the dry mass of the aerial part, there was an increase in height, stem diameter and number of leaves. The increase was also observed in the treatment with rock dust associated with humic substances, but not when humic substances were alone.

On the other hand, rock dust inhibited *Chloroleucon dumosum* nodulation, even when added to humic substances. The addition of humic substances did not change any of the analyzed parameters related to plant growth, except when associated with rock dust, when it promoted greater growth (but without statistical significance) in relation to rock dust alone.

The treatments did not alter the chlorophyll and carotenoid content in the plants, but the phosphorus (P) concentration was lower in the two rock dust treatments (alone or combined with SH) and the MG concentration was higher in the treatments where there was addition of rock dust. Although treatments with rock dust conditioned low levels of phosphorus in the tissue, this fact was not important to reduce the performance of *C. dumosum* seedlings in this initial phase.

Since rock dust added to the substrate inhibited nodulation, we suggest adding this substrate in other phases of plant growth, only after nodulation, so that this process is not inhibited in the initial phase of growth.

6 REFERENCES

ARNOTT, A.; GALAGEDARA, L.;THOMAS, R.; CHEEMA, M.; SOBZE, J. M. The potential of rock dust nanoparticles to improve seed germination and seedling vigor of native species: A review. **Science of the Total Environment**, v. 775, p.145139, 2021.

CANELLAS, L. P.; OLIVARES, F. L. Physiological responses to humic substances as plant growt promoter. **Chemical and Biological Technologies in Agriculture**, v. 1, n. 3, p. 1-11, 2014.

DICKSON, A. et al. Quality appraisal of white spruce and white pine seedling stock in nurseries. **Forest Chronicle**, v. 36, p. 10-13, 1960.

EPSTEIN, E. The anomaly of silicon in plant biology. **Proceeding of the National Academy Science**, Washington, v. 91, n. 1, p. 11-17, 1994.

FERREIRA, D.F. Sisvar: A computer statistical analysis system. **Ciência e Agrotecnologia**; Lavras; v. 35, n.6, p.1039-1042, 2011.

LICHTENTHALER, H. K. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. **Methods in Enzymology**, p.350-382, 1987.

MALAVOLTA, E.; VITTI, G. C.; OLIVEIRA, S. A. **Avaliação do estado nutricional das plantas: princípios e aplicações**. 2. ed. Piracicaba: Associação Brasileira de Potássio e do Fósforo, 319 p., 1997.

MARQUES, J. R. B.; SILVA, L. G.; CANELLAS, L. P.; OLIVARES, F. L. Promoção do enraizamento de microtoletes de cana-de-açúcar pelo uso conjunto de substâncias húmicas e bactérias diazotróficas endofíticas. **Revista Brasileira Ciência do Solo**, v.32, p.1121-1128, 2008.

MENDES, L.; SOUZA, C. H. E. S.; MACHADO, V. J. Adubação com silício: influência sobre o solo, planta, pragas e patógenos. **Cerrado Agrociências**. UNIPAM, v.2, p. 51-63, 2011.

MIR, R. A.; BHAT, B. A.; YOUSUF, H., ISLAM, S. T.; RAZA, A.; RIZVI, M. A.; CHARAGH, S.; ALBAQAMI, M.; SOFI, P. A.; ZARGAR, S. M. Multidimensional Role of Silicon to Activate Resilient Plant Growth and to Mitigate Abiotic Stress. **Frontiers in Plant Science**, v.13, p. 1-26, 2022.

PUEYO, J. J.; QUIÑONES, M. A.; COBA DE LA PEÑA, T.; FEDOROVA, E. E.; LUCAS, M. M. Nitrogen and Phosphorus Interplay in Lupin Root Nodules and Cluster Roots. **Frontiers in Plant Science**, v.12, p. 1–9, 2021.

ROSE, M. T.; PATTI, A. F.; LITLLE, K. R.; BROWN, A. L.; JACKSON, W. R.; CAVAGNARO, T. R. A Meta-Analysis and Review of Plant-Growth Response to Humic Substances: Practical Implications for Agriculture, 2014.

ISSN 1980-0827 – Volume 19, Number 4, Year 2023

SAMPAIO, E.; FREITAS, A. D. Sampaio&Freitas.pdf. In **Restauração na Caatinga**, 2nd ed (Ed J.M.F. Silva), p. 10–26. Maceó: EDUFAL, 2021.

SILVA, R. J. et al. Humic substances, purified MAP and hydrogel in the development and survival of Eucalyptus urograndis. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v.20, n.7, p.625-629, 2016.

TAIZ, L.; ZEIGER, E. **Fisiologia vegetal**. 3.ed. 719p. Porto Alegre: Artmed, 2004.

WANG, Y., YANG, Z., KONG, Y., LI, W., Du, H., ZHANG, C. mPAP12 Is Required for Nodule Development and Nitrogen Fixation Under Phosphorus Starvation in Soybean. **Frontiers in Plant Science**, v.11, p. 1–12, 2020.

XU, X.; DU, X.; WANG, F.; SHA, J.; CHEN, Q.; TIAN, G.; ZHU, Z.; GE, S.; JIANG, Y. Effects of Potassium Levels on Plant Growth, Accumulation and Distribution of Carbon, and Nitrate Metabolism in Apple Dwarf Rootstock Seedlings. **Frontiers in Plant Science**, v.11, p. 1–13, 2020.