

Chia Biofortification With Lithium Sources and Doses Applied by Foliar Fertilization

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Abstract

Lithium (Li) is an element considered essential for humans, however, low concentrations in soil, water and food have caused low consumption by the world population.

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Consequently, fertilization via lithium leaf to increase its concentration in food employing biofortification is an alternative, given the growing health problems in the population caused by low intakes of this trace element. Thus, the objective was to evaluate doses and sources of lithium, applied by foliar fertilization in the development and productivity of chia, in the south of the state of Tocantins. The experiment was carried out at the Federal University of Tocantins, Campus de Gurupi, in the agricultural year 2016/17 in pots with 8dm³ containing a dystrophic red-yellow Latosol, with a clay texture, in a randomized block design, in a factorial scheme 5x2, with four replicates. The first factor was constituted by five doses (0, 10, 20, 30 and 40 g ha⁻¹) and the second factor by two sources of Li (lithium hydroxide - LiOH and lithium sulfate - Li₂SO₄). Two applications were carried out by foliar fertilization, the first at 75 and the second at 95 days after transplanting. At 120 days, the characteristics of plant height, the height of the upper stem, stem diameter, bunch length, number of bunches and after harvest (145 days), the weight of a thousand grains, grain yield and Li content were evaluated in the grains. The supply via Li leaf through LiOH and Li₂SO₄ sources promoted the biofortification of chia grains. The highest concentrations of Li in the grains were obtained with the application of 29.2 and 31.8 g ha⁻¹ of LiOH and Li₂SO₄, respectively. The best responses in cluster length, thousand-grain mass and pH were obtained using LiOH. Regardless of the source, doses of lithium above 35 g ha⁻¹ promote a reduction in the morphological and agronomic characteristics evaluated in the culture of chia.

Keywords: *Salvia hispanica*; lithium hydroxide; lithium sulfate; dosages.

1. Introduction

The increase in the world population has generated great demand for food in recent years. In 1800, there were one billion inhabitants on the planet, currently, we have a population of more than 7.6 billion inhabitants and projections indicate that in 2050 the global population will be 9.9 billion people [1,2]. Several studies estimate that global agricultural production had doubled by 2050 to meet the projected demands of population growth [3]. In the face of this significant increase in the world population, concerns about caring for a healthy life have become a priority [4,5], as interest in determining some nutrients in foods that meet all needs, in addition to trying to avoid diseases caused by the lack of certain essential elements [6,7]. Lithium is a micronutrient that deserves special attention because little importance has been given to the concentration in food and there are few studies on the influence of this metal on higher plants, as well as the tolerance to its presence. Among the various functions of lithium, it is used as an ingredient in the pharmaceutical industry for the psychiatric treatment of bipolar disorders [8,9]. Some studies report that endogenous lithium plays a physiological role in mood stability [10,11]. However, low levels in the body can cause abnormalities in animals (for example, reduced weight and incomplete ossification) and serious pathophysiological problems in humans, in addition to leading to different behavioral responses [12,13]. There is little information on the ideal levels of lithium in food, but the recommended average daily intake should be 1 mg for an adult of 70 kg per day [14,15]. The largest source of lithium diets is cereals and vegetables, which provide an average lithium concentration of 4.4 and 2.3 mg kg⁻¹, respectively [16,17]. The amount of lithium present in plants depends on the abundance in the soil, type of plant, and pH [18,19]. Lithium is the lightest of the alkali metals with high chemical activity, the concentration of this element can normally vary from 1 to 50 mg kg⁻¹ in the soil [20]. Some studies have shown plants capable of absorbing large amounts of

lithium present in the soil [21,22,18]. However, there are few foods, found with ideal lithium levels. Lithium deficiencies have increased in the population in recent years. For this reason, it is necessary to search for new techniques to enrich crops intended for human consumption and agronomic biofortification is an alternative to reduce micronutrient deficiencies in the population, through enrichment. of essential nutrients in crops through soil or foliar fertilization [23,24]. Studies conducted recently have shown lithium biofortification [17,25,26]. The choice of chia for this work is interesting, mainly due to its high nutritional value with a high content of antioxidants, fiber, protein α -linolenic acid (omega-3) and linoleic acid (omega-6), substances capable of favoring weight loss, cholesterol control, and blood pressure regulation [27]. This seed is widely demanded by the population today, thus enabling greater success in biofortification programs, presenting enormous potential for studies aimed at reducing lithium deficiency in the population. Thus, this study aimed to evaluate the dynamics of doses and sources (lithium hydroxide-LiOH and lithium sulfate - Li_2SO_4), applied by foliar fertilization in the development and productivity of chia, grown in the south of the state of Tocantins.

2. Material and methods

The experiment was conducted in the experimental area of the Federal University of Tocantins (UFT), University Campus of Gurupi, located in the southern region of the state of Tocantins, at an altitude of 280 m, at $11^\circ 43'45''$ latitude and $49^\circ 04'07''$ longitude. According to the Köppen classification, the Tocantins climate is of the Aw type, since it has an annual average temperature of 24.9°C , an average annual rainfall of 1850 mm, with rain concentrations in summer and dry winter, with the climatic type humid (B1) [28]. The data related to precipitation, temperature, and relative humidity during the period of conduction of the experiment were collected in the meteorological station of the Campus of Gurupi and are presented in Figure 1.

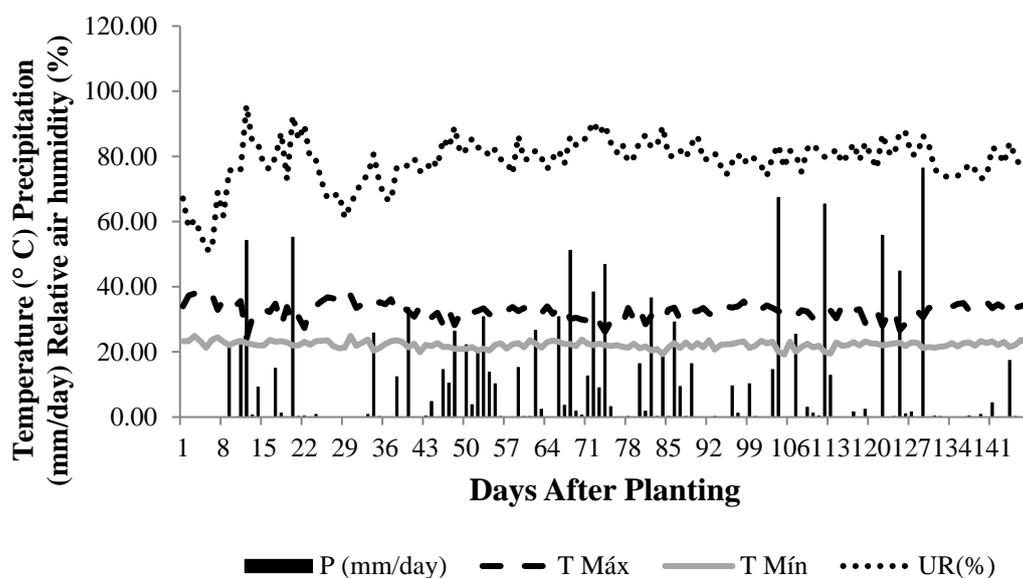


Figure 1: Daily variation in relative humidity (%), temperatures ($^\circ\text{C}$) and rainfall (mm/day) in the period from December 2016 to April 2017, UFT, Gurupi - TO (Source: Meteorological Station of Gurupi - TO).

The experiment was installed in a randomized block design in a 5x2 factorial arrangement, with four replications. The first factor was constituted by five doses (0, 10, 20, 30 and 40 g ha⁻¹) and the second factor by two sources of Li (lithium hydroxide - LiOH and lithium sulfate - Li₂SO₄). To evaluate the biofortification potential in the chia crop, seeds from producers in the Katueté region - Paraguay were used. For better germination uniformity, chia seedlings were produced in polystyrene trays with 128 cells, using the commercial substrate Nutrimax®. During the period between sowing and transplanting, the trays remained inside the greenhouse with an arc roof, being 20 m long, 6 wide, 1.75 m high. The side and front walls are made of 50% dark canvas, the ceiling covered with 150-micron polyethylene plastic film, without climate control, and the trays are placed on the benches. The water was supplied to the seedlings through irrigations done twice a day manually, to keep the substrate moisture content high. After 15 days the seedlings were transferred to pots with a volume of 8L in the open field, gluing 4 seedlings per pot. At 20 days after transplanting, thinning was carried out, keeping one plant per pot spaced 0.50 m between blocks and 0.40 m within the blocks. The soil used was collected in the 0-20 cm layer on the Baobá farm in São Desidério (S 12° 34.555 'W 46° 08, 405'), the extreme west of Bahia, classified with a deep and textured red-yellow Latosol clay [29]. To avoid the presence of harmful pathogens, the soil was placed in raw cotton bags measuring 50 x 70 cm (width and length) and inserted in a 300-liter vertical analog autoclave (CS / Prismatec), with a pedal, safety valve, and pressure regulation system at 120 °C for 2 hours for soil sterilization by moist heat. The result of the physical-chemical analysis of the soil was: pH in CaCl₂ = 5.9; M.O (%) = 1.5; P (Honey) = 92.1 mg dm⁻³; K = 90 mg dm⁻³; Ca + Mg = 3.4 cmolc dm⁻³; H + Al = 1.6 cmolc dm⁻³; Al = 0.0 cmolc dm⁻³; SB = 3.63 cmolc dm⁻³; V = 69%; 450 g kg⁻¹ of sand; 100 g kg⁻¹ of silt and 450 g kg⁻¹ of clay. Fertilization was carried out according to the soil analysis, applying 60 kg ha⁻¹ of P₂O₅ in the form of superphosphate triple (36%) and 60 kg ha⁻¹ of K₂O in the form of potassium chloride (60%), mixed in the soil preparation in a homogeneous way. 125 kg ha⁻¹ of nitrogen (46%) was applied in coverage at 30, 60 and 90 after transplant. During the conduct of the experiment, the soil was kept moist through individual daily irrigations in each experimental unit. The culture was sensitive to common pests of other species, with three applications of insecticide at 60, 70 and 80 days after transplanting for the control of cow (*Macrodactylus pumilio*) and whitefly (*Bemisia tabaci*) using Alfacipermethrin (0.5 g i.a. ha⁻¹) and Diflubenzuron - Phenyl-urea (3.6 g i.a. ha⁻¹), for the control of cow, and the control of whitefly Acetamiprid (75 g i.a. ha⁻¹). No fungi or viruses were observed in any of the plant's phenological phases. The concentrations of lithium were provided twice via leaf, in the morning (between 8:00 am and 9:00 am), half of which was applied 75 days before flowering, in the initial phase of cluster emission and the second at 95 days in the start of grain filling. To facilitate the application, a stock solution weighing 0.5 g (500 mg) of lithium diluted in 2 liters (2000 ml) was prepared, obtaining standard LiOH solution (0.25 mg ml⁻¹). For example, 1.65 ml of the stock solution was used to treat 10 g ha⁻¹ of Li. These doses of the standard solution were made up to 700 mL of water and applied uniformly to the plants of each experimental unit. The doses were converted to hectare considering the population of 200.000,00 plants, exporting the doses per pot per hectare. For the application, a high-pressure manual sprayer (Guaranyind) was used with a maximum pressure of 2070 KPa (300 PSI), a flow rate of 1.1 L/min and an adjustable tip. To prevent drift, a 1.0 m high plastic tarpaulin was installed around the experimental unit. The harvests were carried out manually at 145 days after transplanting when the plants reached 80% of yellow leaves, or with dark color, dried or dead [30].

The evaluated characteristics were:

Height of plants (AP, cm) - obtained by measuring the length between the plant's collar to the highest end of the bunches, using a measuring tape graduated in cm; Height of the upper stem (HS, cm) - obtained by measuring the length between the stem of the plants to the highest end of the last stem, using a measuring tape graduated in cm; Stem diameter (DC, mm) - obtained by measuring the central part of the plant, using a digital caliper (MTX) 150mm / 6 "of stainless-steel metal; Bunch length (CC, cm) - obtained by measuring the main bunch, using a ruler graduated in cm; Number of clusters (NC) - determined by direct counting on the sampled plants; Productivity (PD, g vase⁻¹) - after threshing the bunches the grains were placed in paper bags measuring 7 x 11 cm (length x width) and placed in an oven with forced air circulation (Solab, model SL-102) at 60 °C until they reach constant weight. Subsequently, the samples are weighed on a precision scale of 0.01 g (Gehaka BK4000); Mass of one thousand grains (MMG, g) - determined after the drying process, by counting one thousand grains with the help of a thin-tipped histological forceps (10 cm), weighed later on a precision scale of 0.01 g (Gehaka BK4000); Li content in the grains (TLG mg kg⁻¹) - after drying in an oven with forced air circulation (Solab, model SL-102) at 60 °C, the grain samples were crushed separately with the aid of a pestle with kneading tamper, according to the methodology described by [31]. Then the material was subjected to digestion by wet route using sulfuric acid, perchloric acid, and catalytic mixture, composed of anhydrous sodium sulfate (Na₂SO₄) and copper sulfate pentahydrate (CuSO₄.5H₂O). In the acquired strata, the Li content was determined, according to the methodology described by [32]. The reading was performed on a Quimis flame photometer (Q398M2), using a standard solution diluted to 10% (v / v) to calibrate the device, with a measurement range in clinical analyzes for lithium (Li) from 0.0 to 1.5 mmol / L. To facilitate the measurement, the digest was diluted in the proportion of one part to five of deionized water (5 ml + 25 ml) and transferred to a 50 ml container before reading; pH (-log₁₀ [H⁺]) - after the preparation of the LiOH and Li₂SO₄ solutions, the reading was done in a pH meter of the Quimis brand (Q400MT). To facilitate the measurement, a sample of each dose was transferred to a container containing 40 ml of the total solution. The data obtained were subjected to analysis of variance using the F test, adopting 1 and 5% probability. Then they were submitted to regression analysis, evaluating the significance of the betas and the determination coefficients using the Sisvar version 5.6 program [33]. The regression graphs were plotted using the statistical program SigmaPlot version 12.0 ®.

3. Result

The summary of the respective average values of the analysis of variance for all agronomic characteristics and lithium content in the grain is shown in Table 1. The source factor shows a significant effect for the number of bunches, the mass of a thousand grains, lithium content in the grain and pH. For the dose factor, there is a significant effect for a thousand-grain mass, lithium content in the grain and pH. It is also verified, the significance of the interaction for bunch length, lithium content in the grain and pH. Thus, for the characteristics cluster length, a mass of a thousand grains, lithium content in the grain, and pH, a linear or quadratic response was calculated providing the regression equations and the determination coefficient (R²) when necessary.

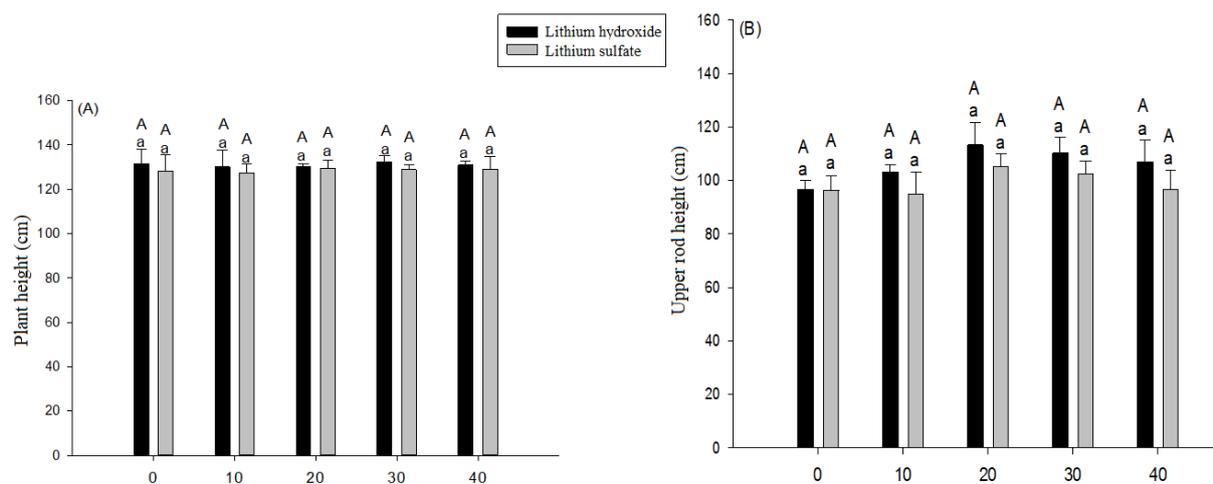
The application of sources and doses of lithium via foliar fertilization on chia plants did not result in changes in the characteristics of plant height (Figure 2A), the height of the upper stem (Figure 2B), stem diameter (Figure 2C) and grain yield (Figure 2D). It is noteworthy that no necrotic or chlorotic spots were observed on the plants,

regardless of their maturation stage, even at the maximum applied dose (40 g ha⁻¹).

Table 1: Summary of the analysis of variance of the characteristics plant height (AP), height of the upper stem (AHS), stem diameter (DMC), cluster length (CC), number of bunches per plant (NC), grain yield (PG), thousand grain mass (MMG), hydrogen water potential (pH), lithium content in grain (TLiG) of *Salvia hispanica* L, cultivated in the south of the state of Tocantins as a function of two sources (Li₂SO₄ and LiOH) and five doses (0, 10, 20, 30 and 40 g ha⁻¹) of lithium.

Variables	Variation source					Average General	CV. (%)
	Block	Source(F)	Dose(D)	FxD	Error		
	Degree of freedom						
	3	1	4	12	40		
AP	105.76	99.23 ^{ns}	457.06 ^{ns}	18.66 ^{ns}	143.33	129.63	9.24
AHS	62.87	152.10 ^{ns}	218.46 ^{ns}	121.41 ^{ns}	162.20	52.60	24.21
DM	1.58	0.63 ^{ns}	2.33 ^{ns}	3.45 ^{ns}	0.21	5.97	7.68
CC	7.49	7.23 ^{ns}	11.91 ^{ns}	25.41 [*]	8.55	12.83	22.80
PG	96.09	69.30 ^{ns}	67.04 ^{ns}	61.54 ^{ns}	95.89	47.90	20.44
MMG	0.010	0.021 [*]	0.013 ^{**}	0.007 ^{ns}	0.004	1.097	6.19
NC	38.03	216.23 [*]	80.79 ^{ns}	40.41 ^{ns}	36.13	25.68	23.41
TLiG	2.31	45.37 ^{**}	203.50 ^{**}	28.38 ^{**}	76.70	18.61	9.06
pH	0.0007	0.17 ^{**}	0.01 ^{**}	0.02 ^{**}	0.0008	7.62	0.39

CV: Coefficient of Variation. **: significant at the 1% probability level (p ≤ 0.01); *: significant at the level of 5% probability (p ≤ 0.05); ns: not significant (p > 0.05) by the F test and regression analysis.



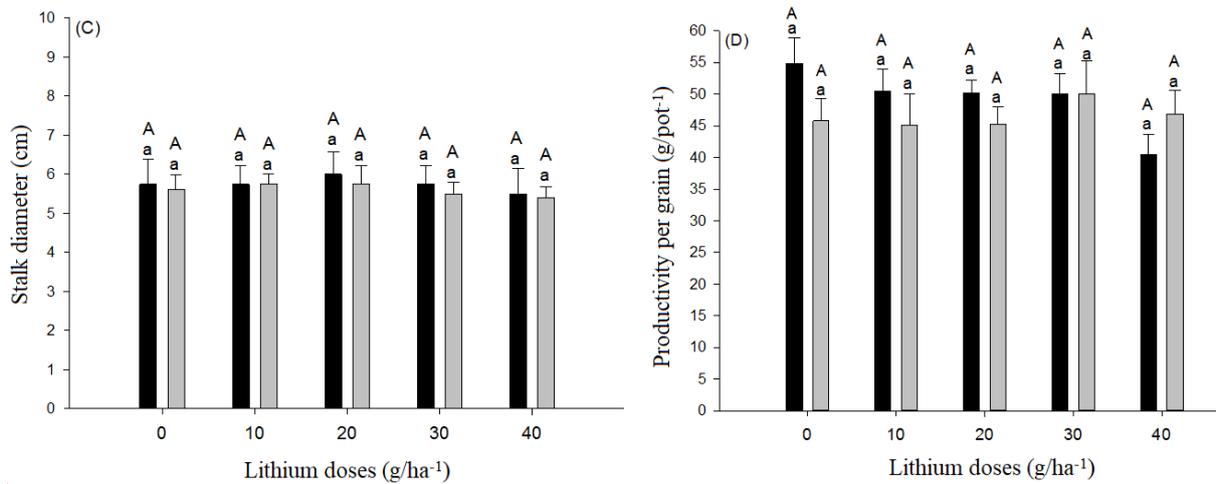


Figure 2: Plant height, the height of the upper stem, stem diameter, and grain yield of chia plants as a function of two sources (LiOH and Li₂SO₄) and five doses (0, 10, 20, 30 and 40 g ha⁻¹) of lithium. Averages followed by the same lowercase letter, comparing the sources within each dose and averages followed by the same capital letter comparing the doses of lithium within each Li source do not differ by the Tukey test ($p \leq 0.05$).

On the other hand, lithium application negatively influenced the length of the bunch (Figure 3), when the Li₂SO₄ source was used, being maximum at a dose of 40 g ha⁻¹ resulting in a reduction of 54.6% in relation to the control treatment. However, for the LiOH source, an increase was observed up to the dose of 20.7 g ha⁻¹, indicating a positive effect of the source used for this characteristic.

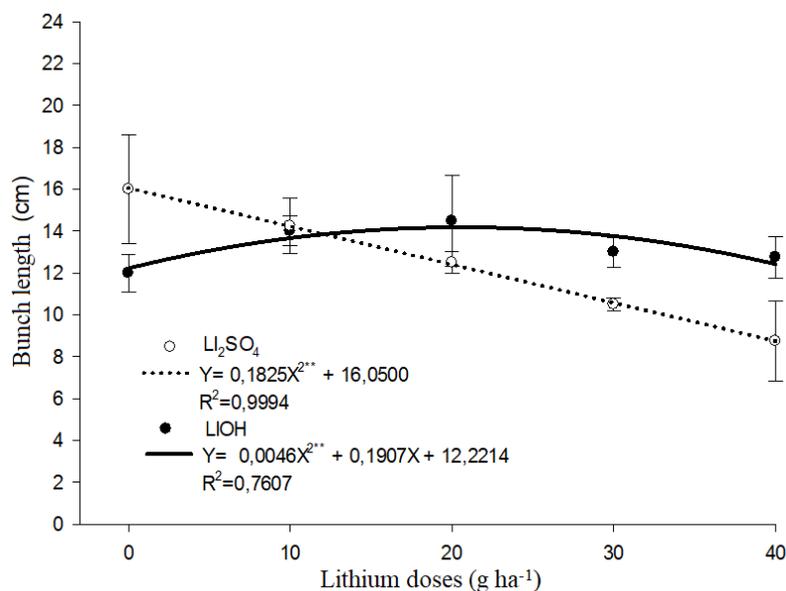


Figure 3: Bunch length of chia plants as a function of two sources (LiOH and Li₂SO₄) and five doses (0, 10, 20, 30 and 40 g ha⁻¹) of lithium.

The application of LiOH via foliar fertilization in the chia plants showed a greater response in the mass of a

thousand grains (Figure 4A) and some bunches (Figure 4B), showing superior age of approximately 4.4 and 16.6 %, respectively, in comparison with the source Li_2SO_4 .

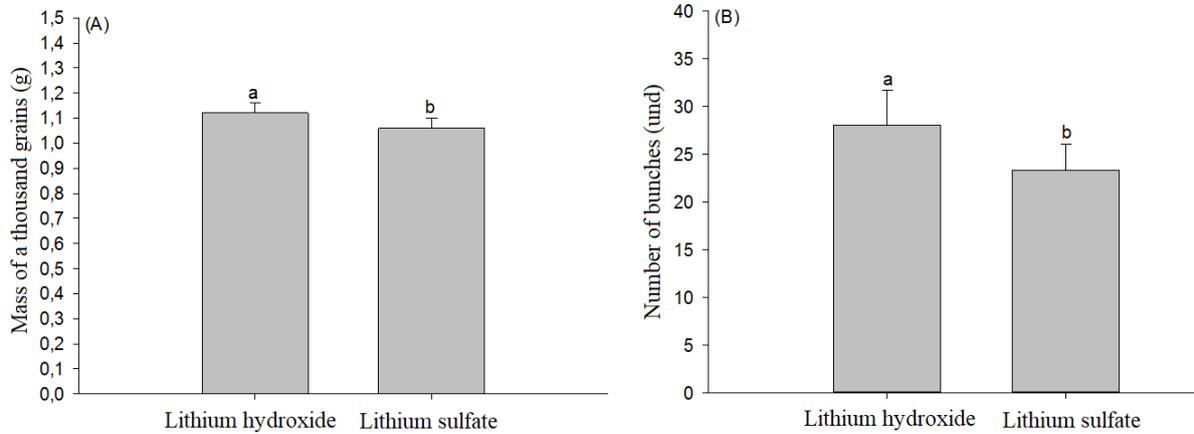


Figure 4: The number of bunches and mass of a thousand grains of chia plants as a function of two sources (LiOH and Li_2SO_4) and five doses (0, 10, 20, 30 and 40 g ha^{-1}) of lithium. Averages followed by the same lower-case letter, between the sources do not differ by the Tukey test ($p \leq 0.05$).

The increasing doses of LiOH and Li_2SO_4 showed to be able to promote positive and promising responses for the mass of a thousand grains, up to the dose of 23.5 g ha^{-1} , increasing the grain of approximately 8.6%, with the control treatment (Figure 5A).

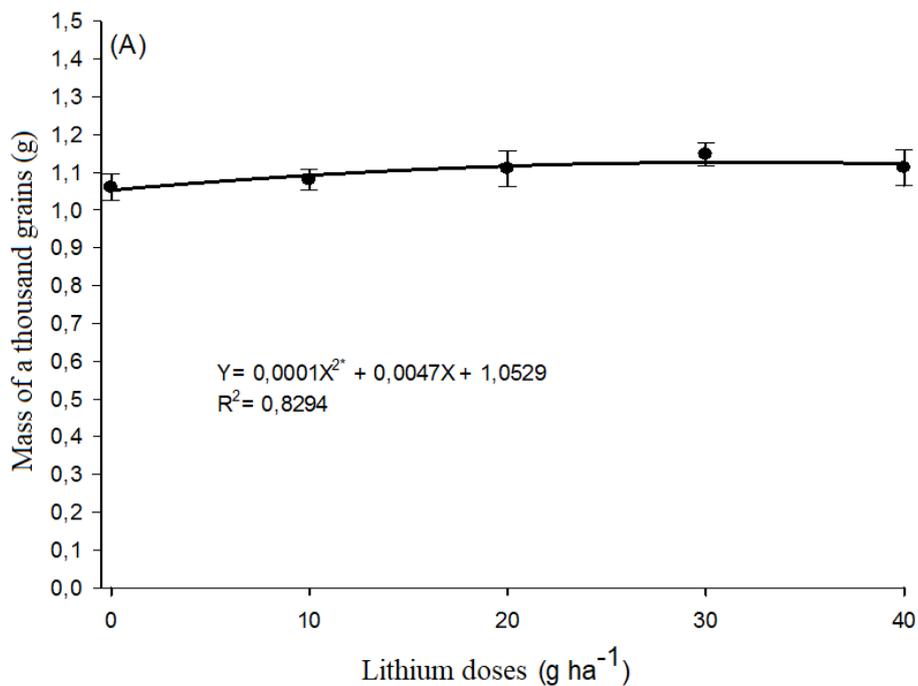


Figure 5: Mass of a thousand grains of chia plants as a function of five doses of lithium (0, 10, 20, 30 and 40 g ha^{-1}).

The lithium content found in the chia grain showed that the foliar application of LiOH and Li₂SO₄ results in the accumulation of the trace element in the grains (Figure 6A). For the LiOH source, the maximum estimated efficiency was 29.2 g ha⁻¹, providing a maximum content of 33.6 mg kg⁻¹ of Li in the grain. For the Li₂SO₄ source, the maximum estimated efficiency was 31.8 g ha⁻¹, providing a maximum content of 18.5 mg kg⁻¹ of Li in the grain. It is evident the superiority of the LiOH source when compared to the Li₂SO₄ source, as well as the success of biofortification of chia with the two lithium sources, with an increase of 56.5 and 21.1% in the grain for LiOH and Li₂SO₄, respectively, when compared to the control treatment.

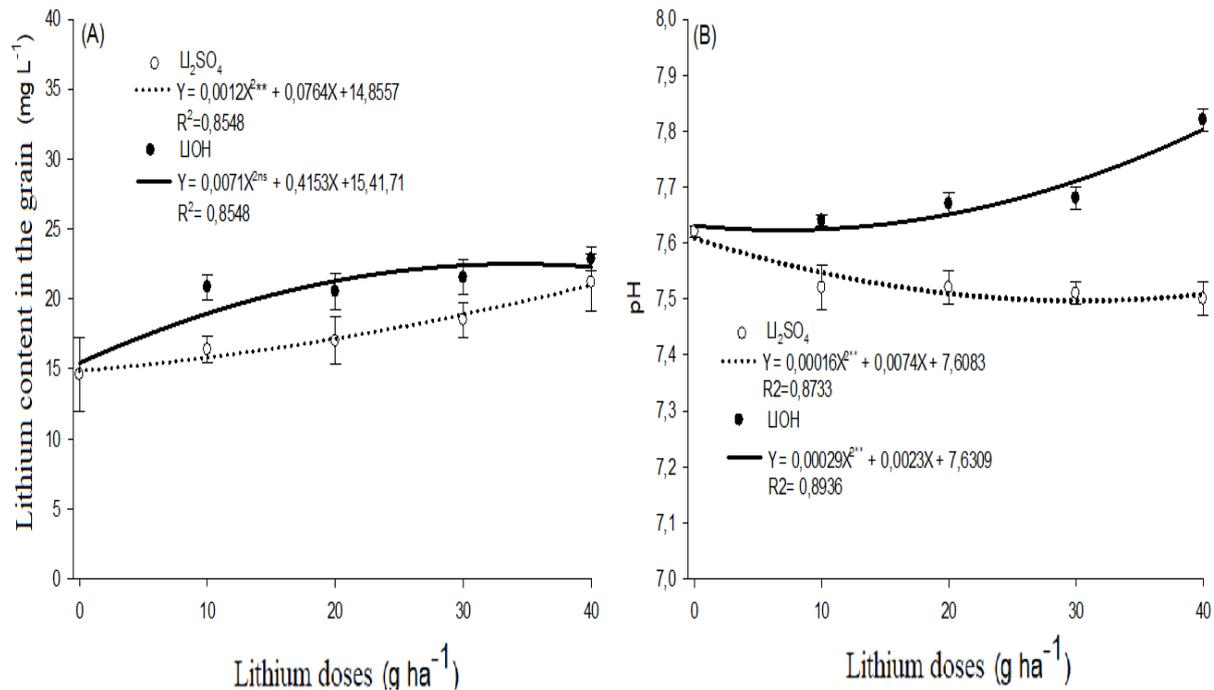


Figure 6: Lithium content in the grain and pH of the water of plants creaky due to two sources (LiOH and Li₂SO₄) and five doses (0, 10, 20, 30 and 40 g ha⁻¹) of lithium.

Evaluating the pH, it was observed that the increase of the doses of LiOH in the water solution applied via leaf resulted in an increase of the pH values up to the maximum dose (40g ha⁻¹), presenting a pH of 7.8. However, doses growing Li₂SO₄ showed a slight decrease in pH (Figure 6B).

4. Discussion

The concentration of lithium in plants is variable. According to [34,35], lithium ions are almost immobile in phloem tissues, however, plants subjected to high doses of lithium tend to store high amounts of this element in the vacuole and cell wall. Although lithium is not considered an essential micronutrient to promote plant development, the presence of this element is detected in most cultures, because lithium participates in the same route as potassium (K⁺) and can replace it by up to 50% facilitating its transport within the plants. However, this metal presents different absorption, distribution and tolerance levels for plants depending on its availability in the soil, nutrient solution, or hydroponic system [21,36,19,37]. The results obtained show that the application of

sources and increasing doses of lithium in the culture of chia utilizing foliar fertilization do not increase plant height and height of the upper stem. A probable explanation for the lack of response may be since the doses of foliar fertilization in the culture of chia were applied when these morphological characteristics were already fully developed. According to [38], chia is a rustic species that is adapting to the edaphoclimatic conditions of the Cerrado. However, little is known about the effect and requirements of macro and micronutrient fertilization in the different phenological phases of the crop. It is important to note that the plant height averages, the height of the upper stem, stem diameter, bunch length, number of bunches, and thousand-grain mass obtained in this study are similar to the averages obtained by [38] when phosphorus doses in chia culture were evaluated. They are also similar to the averages obtained by [39] evaluating nitrogen fertilization on chia and [40] who evaluated the initial growth of chia submitted to nitrogen, phosphate and potassium fertilization. Among the grain production characteristics of the chia crop, the length of the bunch, the number of bunches and the mass of a thousand grains are considered of great importance, since they directly correlate with the productivity of the crop. In this study, it was found that low doses of lithium caused a positive effect on the crop, resulting in an increase in the cluster length averages (LiOH source) and an increase in the mass of a thousand grains through foliar fertilization. A probable explanation for this increase in characteristics may be due to a stimulating effect caused by the lower doses applied. According to [41,42], small concentrations of lithium have a stimulating effect on processes such as growth, flowering, or germination called 'hormesis'. Some investigations show that the application of lithium, regardless of the source used, can promote beneficial stimulating effects on the development of plants [22,43,44]. According to [17] the growth of *Apocynum venetum*, was stimulated by lithium through the effects on liquid photosynthesis, which can be explained by its strong impact on chlorophyll and the photochemical apparatus. Reference [45] reported that in small amounts lithium has a positive effect on plant metabolism, increasing the photochemical activity of chloroplasts and resistance to diseases, favoring development. However, there are reports of studies showing that the application of high concentrations of lithium reduces the growth and dry mass of the aerial part in plants of *Zea mays* and *Brassica napus* [37,46]. Reference [47] reported that although the toxic effects and essential nature of lithium in plants are not clear enough, evidence from previous studies has shown that high concentrations of lithium decrease ATP production in leaf tissues, which can interrupt stretching and cell differentiation in plants slowing plant growth. It is important to mention that foliar fertilization with lithium was carried out at the initial stage of the cluster emission and in the grain filling, at which time these characteristics were not fully developed, therefore, depending on the source used, high doses caused a reduction in the cluster length and mass of a thousand grains and low doses increased the same characteristics. The lithium doses promoted an increase in lithium contents in the grain, according to the maximum efficiency point evaluated, this increase occurred positively until the doses 29.2 and 31.8 g ha⁻¹ using the sources LiOH and Li₂SO₄, respectively. In this study, lithium doses applied by foliar fertilization were absorbed by the plants, and the best averages were observed when using lithium in the form of LiOH. This fact probably results from greater absorption, better use by the plant, translocation and greater efficiency in redistributing LiOH to the grains, when compared to the Li₂SO₄ source. Reference [35] reported that the specific ability to accumulate different amounts of lithium in different parts of the plant depends on the adaptation or tolerance to this element. Regarding chia culture, there is no published information so far on adaptation, toxicity or distribution of lithium in plant tissues. However, the increase in lithium levels can also be explained by the fact that chia is a plant that belongs to the class of dicots [48,49,21] state that dicot plants have a greater capacity to absorb lithium and increase the content for plants. It is

important to mention that the doses of lithium were divided into two application periods, the second being applied to the filling of the grain. Although there is evidence in the literature that shows an increase in the concentration of lithium levels in plants, evaluating different doses and sources of lithium, the physiological functions of this trace element have not been fully clarified. Reference [50] reports that lithium can produce a stimulating effect on the development of plants, while [51] reports that lithium has a reducing or potentially toxic effect in high concentrations. Symptoms of lithium toxicity induce leaf limb chlorosis, necrotic leaf damage, cell death, and degradation of chlorophyll content [52]. According to [53,54], the adverse effects of lithium may be possible because the ion (Li^+) inhibits the cycle of inositol monophosphates, crosses cell membranes, and interferes with calcium signaling, affecting plant metabolism. Calcium plays a crucial role in the control of plant metabolism, mainly in the stabilization of the cell membrane and in osmoregulation [55,56]. Severe conditions under lithium stress can also result in the production of free radicals (known as reactive oxygen species-ROS) from Fenton-type reactions and can induce oxidative damage to different organelles, thus reducing plant growth and development [19,52]. Morphological, metabolic and physiological anomalies in plants are governed by the formation of ROS, which leads to cell membrane peroxidation, stem chlorosis, lipid peroxidation and protein degradation [57]. In this study, the doses of lithium applied to the chia culture did not show toxicity effects since there did not visual signs of progressive chlorosis or generalized necrotic spots on the leaves, stems or bunches of plants, showing that the doses used (divided into two applications) result in a toxic effect on plants, despite already compromising the productive components of chia. The hydrolysis of micronutrients depends on the pH and concentration in the solution [58]. It is known that pH can significantly influence the adsorption of metal ions. According to [59] anion absorption is increased with decreasing pH in the medium and cation absorption, with increasing pH in the medium. Reference [60] reported that a nutrient solution with pH 4.5 was the one that produced plants with a lower content of manganese in the leaves (508 mg kg^{-1}), the opposite, the nutrient solution with pH 6.0 produced plants with a higher content of this micronutrient in the leaf tissue (955 mg kg^{-1}). Reference [61] found that at pH values equal to 5.9 only 11% of the added zinc was adsorbed, against 61 % when the pH was raised to 7.2. Based on this, we can speculate that the greater response of LiOH applied via the leaf is related to the pH of the solution, which may have influenced the greater absorption of this trace element in the chia plants when compared to Li_2SO_4 , which presented a slight decrease in pH in the solution with increasing doses (Figure 6B). However, further research is needed to confirm these results. Depending on the species, doses, source, phenological phase of the application, it is possible to achieve biofortification with lithium, thus increasing the levels of this element in vegetables and cereals. Reference [62] reported that the fortification of cereals can be beneficial in stabilizing mood and in decreasing the rate of aggression in the population caused by low consumption of this element. Considering that the recommended minimum consumption of chia is 25 g day^{-1} [63,64] and according to [15] the recommendation of minimum daily lithium intake for adults is 1 mg per day, it is possible to estimate that the consumption of 25 g of chia grown with the application of 29.2 g ha^{-1} (31.8 mg kg^{-1}) of LiOH or 31.8 g ha^{-1} (18.8 mg kg^{-1}) of Li_2SO_4 , would represent 69.5 and 46.2% of the recommended level, respectively. However, these calculations do not include the loss of lithium content absorbed in the grain that can occur during processing; so, they also, do not include the availability of this metal in the body. It is also important to clarify that, to date, no biofortified food with lithium has been commercialized for this purpose, since it is a process that would need to be preceded by clinical trials, testing the responses in the body. Agronomic biofortification is being investigated to increase yields and nutrient concentrations, however, further studies are

needed to adjust the concentrations to be applied, aiming at greater food security [65,66].

5. Conclusion

In this study, it was possible to identify the promising source and dose of lithium for a greater enrichment of this trace element in the chia grains through biofortification via leaf. The foliar application of 31.83 g ha⁻¹ in the form of LiOH in the chia plants provides greater efficiency in the absorption of this element. Regarding the agronomic characteristics, the LiOH source provides better responses when compared to the Li₂SO₄ source. However, doses greater than 35 g ha⁻¹ of LiOH and Li₂SO₄ promote a reduction in cluster length and mass of a thousand grains in the chia culture. Regarding toxicity visually, there was no formation of necrotic or chlorotic spots on the leaves, regardless of the source of Li or the dose used. Future research on the effect of Li on chia genotypes is needed to obtain more information on the response of the LiOH source.

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