

## Nutritional and biomass aspects of *Helianthus annuus* according to boron application in the soil

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### Abstract

Sunflower is considered highly demanding in boron (B); however, it has low efficiency in using this micronutrient. An experiment under protected cultivation was carried out to evaluate the effects of boron fertilization on nutrition and sunflower biomass production (*Helianthus annuus*). A completely randomized design with four replications was used. Five boron doses: 0 (Control), 1, 2, 3, and 4 kg-ha<sup>-1</sup>, were applied to the soil using boric acid as the source. Biometric assessments, measurements of the relative chlorophyll index (RCI), production of dry biomass and the boron use efficiency by plants were carried out. The data were subjected to analysis of variance and F test (p<0.05), with polynomial regression analysis when significant. Leaf area, RCI evaluated at 15 and 60 days, and biomass increase linearly according to the boron application up to the dose of 4 kg-ha<sup>-1</sup>, which corresponds to an increase of 31, 12, 14, and 61%, respectively, compared to control treatment. However, for plant height and nutritional efficiency, the best results were obtained with the dose of 2.64 kg-ha<sup>-1</sup> of B, with a decrease in higher doses. Boron fertilization in sunflower crop positively influences the nutritional and growth aspects of plants. In general, the application of up to 3 kg-ha<sup>-1</sup> of B in soils with low natural content increases the crop yield, without phytotoxicity aspects caused by the nutrient.

**Keywords:** Micronutrients; Nutritional efficiency; Plant nutrition; Soil fertility; Sunflower.

**Abbreviation** ABef\_absorption efficiency; B\_Boron; BAS\_Boron accumulation in the shoot; BAP\_Boron accumulation in the whole plant; CEC\_Cation exchange capacity; DAE\_Days after emergence; IAA\_Indole acetic acid; KCl\_Potassium chloride; PN\_Neutralizing power; PRNT\_Total neutralizing relative power; RCI\_Relative chlorophyll index; RNA\_Ribose nucleic acid; TRef\_Transport efficiency; UTef\_Use efficiency for conversion to dry matter.

### Introduction

Sunflower (*Helianthus annuus* L.) is an annual crop, originally from North America, being one of the four most important oilseeds in the world. Despite being native to a temperate climate region, due to its high drought tolerance and adaptation to a wide variety of soils, latitude, longitude, and photoperiod, it has been cultivated in several regions of the world (Vilvert et al., 2018). The sunflower crop occupies around 26 million hectares worldwide, with approximately 47 million tons (Khalid et al., 2020). In Brazil, the production estimate for the 2019/20 crop is 98.1 thousand tons, with an average yield of 1,581 kg ha<sup>-1</sup> (Conab, 2019), higher than the world average yield (1,300 kg ha<sup>-1</sup>). Nevertheless, according to Gomes et al. (2012), Brazilian sunflower production is still below its potential with nutritional management (Kaleri et al., 2019) associated with supplementary irrigation, the main factors involved that can increase the productive capacity of the crop.

The sunflower is considered a highly demanding plant in nutrients, among them boron (B). However, it has low efficiency regarding its use, and it is common to observe symptoms of nutritional disorders due to deficiency in the main grain-producing regions of Brazil (Souza et al., 2004; Euba Neto et al., 2014). In the Brazilian Cerrado region, where the largest sunflower planted area is concentrated, B deficiency in the soil occurs more frequently (Capone et al., 2016). This region is characterized by highly weathered soils (let al., 2019), with a low natural content of exchangeable bases, high acidity, concentrated rainy season, followed by the dry season, both with high temperatures and an average rainfall of 1,486 mm (± 146.8 mm) (Campos and Chaves, 2020; Penereiro et al., 2020). All these factors are detrimental to the maintenance of organic matter contents in the soil, the main source of boron availability for plants (Das et al., 2019).

The low organic matter content in the soil usually favors a lower B concentration at levels below those considered adequate ( $<0.20 \text{ mg dm}^{-3}$ ) for crop nutrition. According to Prado (2008), whenever the B concentration in the soil is lower than adequate, there is potential for the plants to respond to its supply. Alkaline soils, with a sandy texture and low organic matter content ( $<1.5\%$ ), negatively affect crop yield (Sobral et al., 2015; Jegadeeswari and Muthumanickam, 2017; ). On the other hand, in clayey soils ( $> 35\%$  clay), applications must be judicious, as high concentrations of B cause toxic effects on plants, affecting crop production (Camacho-Cristóbal et al., 2008). However, there is no consensus in the recent literature on which dose of boron is considered toxic for sunflower production, which varies between  $1.0$  and  $3.0 \text{ kg ha}^{-1}$ , with reduced development and grain yield at higher doses (Bhattacharyya et al., 2015; Capone et al., 2016; Dhassi et al., 2019; Khalid et al., 2020).

In the literature, it is observed that B is essential for sunflower growth, which affects the dry biomass and grain yield (Souza et al., 2004; Öztürk et al., 2010; Euba Neto et al., 2014; Tahir et al., 2014; Silva et al., 2016; Alves et al., 2017). This behavior of plants is directly related to the functions that B plays in the metabolism and integrity of the cell wall, besides affecting cell division, development of fruits and seeds, transport of sugars, development of hormones, and metabolism of N, Ca, P, and K in plants (Mehmood et al., 2018). Also, the formation of leaf buds, sugar and hydrocarbon metabolism and its transport, ribose nucleic acid (RNA) and indole acetic acid (IAA) metabolism, respiration, cytokinin production and transfer, and phenol metabolism are other functions influenced by B in plants (Immanuel et al., 2019).

However, the proper micronutrient management, especially B, becomes essential for increases in crop yields, avoiding waste and possible toxicity. However, knowledge of the critical interval between the lower and upper limits for sunflower production is of fundamental importance in ensuring high and sustainable production (Flores et al., 2018; Viçosi et al., 2020). Therefore, this research aimed to evaluate the effect of boron application via soil on nutritional aspects and the biomass yield of *Helianthus annuus*.

## Results

### **Relative chlorophyll index – RCI, height, and leaf area**

The biometric variables evaluated (leaf area and plant height) were influenced by the B doses in the soil, as shown in Table 1. However, after the polynomial regression analysis, a different behavior was observed for the two variables. In Fig 1a, it is possible to observe a linear increase in the leaf area of sunflower plants. With the highest dose,  $4.0 \text{ kg ha}^{-1}$  of B, the leaf area was around 31% higher than the treatment without the addition of boron. For the plant height, the best adjustment was quadratic behavior. The highest plant height ( $108.64 \text{ cm}$ ) was obtained with a dose of  $2.29 \text{ kg ha}^{-1}$  of B, about 8% higher than the control treatment (Fig 1b).

Regarding the assessments of the relative chlorophyll index (RCI), there were significant effects only for assessments performed at 15 and 60 days after emergence (DAE) (Table 1). After the polynomial regression study, it is observed that there were linear adjustments for the RCI evaluated at 15 and 60 DAE, which reached 34.7 and 39.7, respectively, with

the use of the highest dose. That is an increase of 12% in both evaluations concerning the control treatment (Fig 2).

### **Biomass production and boron content**

All variables relative to biomass yield were influenced by the B application in the soil, as shown in Table 2. After studies of polynomial regression, linear behavior was observed in all variables evaluated, which reached  $24.25 \text{ g plant}^{-1}$  of shoot dry matter,  $1.46 \text{ g plant}^{-1}$  of root dry matter,  $10.28 \text{ g plant}^{-1}$  of chapter dry matter, and  $35.99 \text{ g plant}^{-1}$  of total dry matter, with the use of the highest boron dose, which was around 51, 94, 90 and 61% higher than the treatment without B (Fig 3).

As an effect of the B application in the soil and, consequently, absorption by the plant increases nutrient content in the sunflower plant (Table 3). Without boron application, there was a higher boron content in the roots than the shoot, which was inverted with the boron application in the soil. The highest boron content in the shoot was observed with the use of  $3.34 \text{ kg ha}^{-1}$  of B, which reached  $108 \text{ mg plant}^{-1}$ , about a 398% increase when compared to the treatment without Boron. The content of B in the roots did not show the same increase. Its absorption was linear, reaching  $48.32 \text{ mg kg}^{-1}$  with the use of the highest dose applied to the soil, that is, an increase of approximately 78% concerning the treatment without B addition (Fig 4a).

In Fig 4b, it is possible to observe a similar behavior between the boron accumulation in the shoot (BAS) and the whole plant (BAP). The data were adjusted to the quadratic polynomial regression model. The dose of  $4.0 \text{ kg ha}^{-1}$  of boron provided the highest boron accumulation in the shoot ( $2.50 \text{ mg plant}^{-1}$ ) and the plant ( $2.59 \text{ mg plant}^{-1}$ ). The boron accumulation in the roots was adjusted to the linear model. The dose of  $4.0 \text{ kg ha}^{-1}$  of boron represents a 250% increase in the micronutrient accumulation in the root system of sunflower plants compared to the treatment without boron.

### **Absorption, transport, and use efficiency**

Table 4 shows that all efficiency parameters were influenced by the application of B to the soil. The transport and absorption efficiencies showed quadratic adjustments, which reached 98.55% and  $2.10 \text{ mg g}^{-1}$  of B, using  $2.46 \text{ kg ha}^{-1}$  (Fig 5a) and  $2.76 \text{ kg ha}^{-1}$  of B (Fig 5b), respectively. The boron use efficiency by sunflower plants (Fig 5c) showed the opposite behavior to the other calculated efficiencies. The dose of  $2.70 \text{ kg ha}^{-1}$  of boron results in the lowest micronutrient use efficiency of the plant ( $342.21 \text{ mg g}^{-1}$  of boron).

## Discussion

Fertilization with B was able to provide an increase in the biometric parameters of sunflower plants. However, the plant has a certain limit regarding the absorption, and consequently, the use by the plant. As can be seen at doses greater than  $2.5 \text{ kg ha}^{-1}$  of B, there are decreases in the plant height and their efficiency of absorption and transport of B. Similarly, in a study carried out by Al-Amery et al. (2011), it was observed that applications of B in the soil up to the dose of  $2.5 \text{ kg ha}^{-1}$  represented increases in leaf area and plant height of sunflower. Shehzad et al. (2016) found an increase in the sunflower leaf area at doses up to  $3 \text{ kg ha}^{-1}$ . In a study by Capone et al. (2016), with three sunflower cultivars, the authors verified that maximum crop response

was up to the application of 3 kg ha<sup>-1</sup> of B in the soil. In a recent study, Mehmood et al. (2018) observed that the B application in the soil linearly increases the plant height and stem diameter; however, the dose with the maximum efficiency did not exceed 2 kg ha<sup>-1</sup>.

Boron acts indirectly in the plant physiological activities; this function directly influences crop development (height, stem length, number of nodes, and leaf area), which favors greater interception and conversion of solar energy (Immanuel et al., 2019). This behavior is because B acts directly on the photosynthetic apparatus efficiency, thus increasing the net photosynthetic rate (Zahoor et al., 2011), and consequently, greater carbohydrates storage. Furthermore, boron has a major contribution to meristematic growth, cell division, stretching, cell wall stability, and binding of nitrogen synthesizers (Shehzad et al., 2016).

The relative chlorophyll index showed linear increments with the B application at 15 and 60 DAE; that is, the plants presented darker green leaves with the greatest B supply. This behavior is related to the genetic crop traits or the better efficiency regarding the N use by the plant.

According to Flores et al. (2018), there is evidence of a positive relationship between the N content and the amount of B absorbed by the plant. B acts directly on the synthesis of uracil, an RNA component. However, studies in the recent literature show a low correlation of B content in the plant with RCI. Dhassi et al. (2019) and Khalid et al. (2020) reported that even in soils with low natural B content, there was no significant response in RCI levels when evaluated in pre-flowering.

In the present study, the biomass production in all evaluated parts increased with the greater B supply in the soil. Gormus and Barutcular (2016) state that sunflower plants respond to the addition of up to 3 kg ha<sup>-1</sup> of B in the soil. This behavior is because B increases metabolic and photosynthetic activities, which promote the growth of young plant parts and, consequently, biomass (Sheoran et al., 2018).

There is evidence in the literature that the application of doses above 3 kg ha<sup>-1</sup> negatively influences sunflower growth. Bhattacharyya et al. (2015) report that, under the conditions evaluated, the application of lower doses than these are sufficient to correct the deficiency of B in the soil and that the application of high doses can cause phytotoxic effects on the plant, among them the reduction of growth and yield. Gormus and Barutcular (2016) stated that B toxicity is little reported, not affecting sunflower growth at doses that do not exceed 3 kg ha<sup>-1</sup>. Dhassi et al. (2019) indicate that one of the symptoms of boron phytotoxicity in sunflower may be the decline of shoot dry matter. In the present study, there was only a reduction in plant height at doses greater than 3 kg ha<sup>-1</sup>, which may indicate the principle of disturbance in sunflower metabolism, which was not able to harm the biomass accumulation by the plant.

The increase in B content and accumulation in sunflower plants corroborates the results obtained in several studies carried out by Capone et al. (2016), Silva et al. (2016), Flores et al. (2018), Mehmood et al. (2018), and Khalid et al. (2020). The increase in boron levels in the soil increases its concentration in the leaves, even in high applied doses (4 kg ha<sup>-1</sup>), since the surplus can be accumulated in their vegetative structures, especially in the leaves (Capone et al., 2016). Ekmekci et al. (2020) also report that sunflowers can absorb and accumulate B, even at concentrations considered toxic in the soil (>0.9 mg dm<sup>-3</sup>). Prezotti and Guarçoni 2013,

demonstrated its high phytoextraction capacity, which can even be used to recover areas contaminated by micronutrients. Schiavon et al. (2018), when evaluating the rate of absorption of B by the sunflower, they observed that the leaves are the compartment with the highest accumulation of B, up to 60 days after emergence.

As in the present study, Ekmekci et al. (2020) observed a greater B accumulation in the leaves and shoots than the roots. This effect can be attributed to the greater transport efficiency of the nutrient reached with the dose close to 3 kg ha<sup>-1</sup>. With the decrease in transport efficiency, there was a greater increase in the B content in the roots due to the B dose increase in the soil. Immanuel et al. (2019) observed that the greater availability of B in the soil increased the sunflower root growth, promoting greater extraction of nutrients by the plant, which favored the greater shoot biomass production.

Boron participates in cell division and expansion processes, increasing the ability of the plant to penetrate deeper layers in search of water and nutrients (Trautmann et al., 2014). The reduction in B absorption efficiency with lower doses than a 2.76 kg ha<sup>-1</sup> dose possibly occurred due to B toxicity on the roots, as no visual symptoms were observed in the shoot, which interferes with the nutrient acquisition (Krudnak et al., 2013).

The initial concentration of B in the present study soil is considered low (0.14 mg dm<sup>-3</sup>) to produce sunflower in the soils of the Midwest region in Brazil. In this condition, the recommendation of fertilization of correction and production for the culture is of 2 kg ha<sup>-1</sup> (Sousa and Lobato, 2004). However, even with the application of 4.0 kg ha<sup>-1</sup>, in other words, twice the amount recommended for the crop, sunflower plants did not show symptoms of B toxicity, which can be explained by the potential leaching effect promoted by irrigation and the high mobility of B in the soil. However, in the present study, the soil particle-size is considered clayey (>350 g kg<sup>-1</sup> of clay), limiting mobility in the soil profile, reducing leaching, and increasing the concentration of B in the rhizosphere. Thus, because the ion-root contact is regulated by the mass flow (Prado, 2008), and the B concentration in the rhizosphere region increases, the absorption process is favored (Jahiruddin et al., 2001).

The decision of whether or not to apply B can also be based on leaf concentration, with sunflower contents varying between 35 and 100 mg kg<sup>-1</sup>, considered adequate by Sousa and Lobato (2004). Only the control treatment and the application of 4.0 kg ha<sup>-1</sup> of boron showed leaf contents outside the range considered appropriate for the crop. (21.70 and 103.50 mg kg<sup>-1</sup>, respectively).

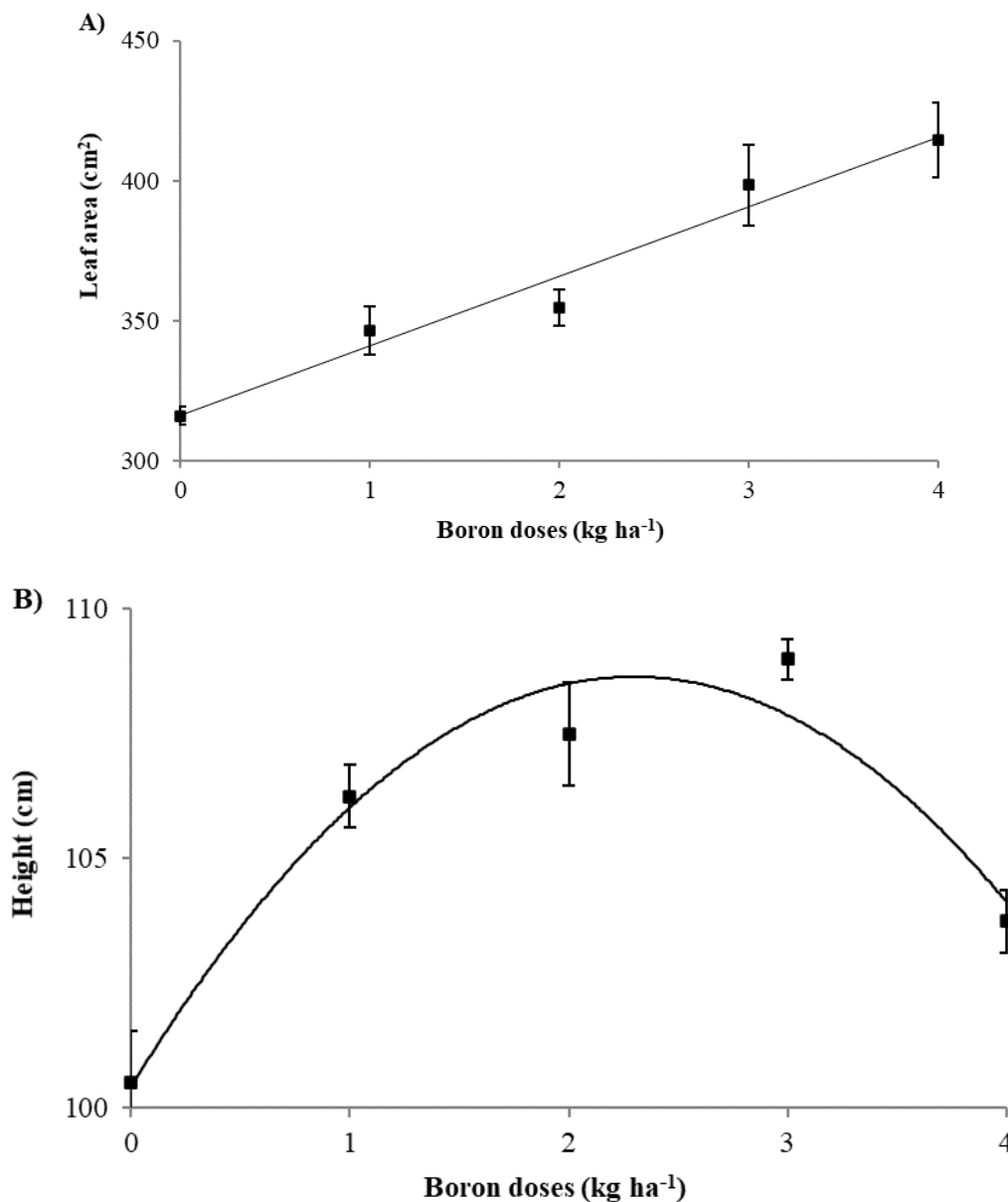
The boron application to the soil affected its transport efficiency (TRef) in sunflower plants, reaching 98.55% with the application of 2.46 kg ha<sup>-1</sup> of boron, occurring decrease with doses higher than 2.46 kg ha<sup>-1</sup>. According to Uluisik et al. (2018), boron is absorbed by roots in the form of boric acid and sent to the xylem for transport to the shoot. This indicates that the transpiration flow controls its transport in plants, giving the element mobility in the xylem cells. This flow allows boron to be transported in the sunflower without any energy consumption occurring (Ekmekci et al., 2020). However, the micronutrient has a very narrow range as to its ideal level for plants, which may have a toxic effect and, consequently, reduced transport and accumulation in plant tissues (Bañón et al., 2012; Landi et al., 2019).

The maximum efficiency of absorption and transport of boron by sunflower plants was estimated with around 2.5 kg

**Table 1.** Leaf area, plant height, and relative chlorophyll index (RCI) of *Helianthus annuus* plants according to the boron application in the soil.

Boron doses	Leaf area	Height	RCI <sup>1</sup>	RCI <sup>2</sup>	RCI <sup>3</sup>	RCI <sup>4</sup>
kg ha <sup>-1</sup>	cm <sup>2</sup>	cm	-----µg cm <sup>-2</sup> -----			
0	316.05 ± 3.22	100.50 ± 1.04	30.46 ± 0.78	31.52 ± 0.20	33.42 ± 0.30	34.96 ± 0.48
1.0	346.44 ± 8.73	106.25 ± 0.63	32.30 ± 0.45	31.60 ± 0.36	34.67 ± 0.64	38.01 ± 0.51
2.0	354.79 ± 6.37	107.50 ± 1.04	33.35 ± 0.44	32.41 ± 0.32	33.39 ± 0.13	37.05 ± 0.96
3.0	398.42 ± 14.38	109.00 ± 0.41	34.13 ± 0.89	32.89 ± 0.67	33.69 ± 0.55	38.15 ± 0.24
4.0	414.39 ± 13.32	103.75 ± 0.63	34.10 ± 0.48	32.34 ± 0.56	33.44 ± 0.09	39.98 ± 1.09
F-test	15.63**	17.93**	5.85**	1.63 <sup>ns</sup>	1.78 <sup>ns</sup>	6.31**
C.V. (%)	5.53	1.50	3.87	2.82	2.42	3.88

<sup>1</sup>, <sup>2</sup>, <sup>3</sup>, and <sup>4</sup> – evaluation at 15, 30, 45, and 60 days after emergence, respectively. \*\* significant at 1% probability by the F test. <sup>ns</sup> non-significant by the F-test.



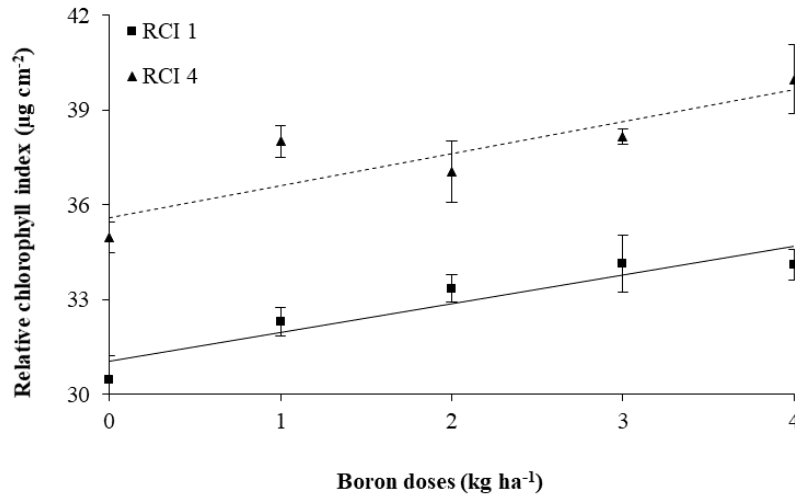
**Fig 1.** Leaf area (A) and plant height (B) of *Helianthus annuus* plants according to the boron application in the soil. Leaf area:  $y = 24.866x + 316.29$ ;  $R^2 = 0.97$ ;  $F = 60.45^*$ ; Height:  $y = -1.5536x^2 + 7.1393x + 100.44$ ;  $R^2 = 0.94$ ;  $F = 54.06^*$ .

\* significant at 5% probability by the F test.

**Table 2.** Shoot dry matter (SDM), root dry matter (RDM), chapter dry matter (CDM), and entire plant dry matter (EPDM) of *Helianthus annuus* plants according to the boron application in the soil.

Boron doses	SDM	RDM	CDM	EPDM
kg ha <sup>-1</sup>	g plant <sup>-1</sup>			
0	16.00 ± 0.82	0.75 ± 0.03	5.41 ± 0.43	22.16 ± 0.70
1.0	18.00 ± 0.91	0.83 ± 0.10	6.85 ± 0.39	25.68 ± 1.18
2.0	20.50 ± 0.65	1.03 ± 0.07	8.65 ± 0.32	30.18 ± 0.46
3.0	21.75 ± 0.25	1.06 ± 0.03	9.32 ± 0.26	32.13 ± 0.37
4.0	24.25 ± 0.63	1.46 ± 0.14	10.28 ± 0.67	35.99 ± 1.28
F-test	21.75**	10.30**	19.84**	37.85**
C.V. (%)	6.86	16.66	10.84	6.03

\*\* significant at 1% probability by the F-test.



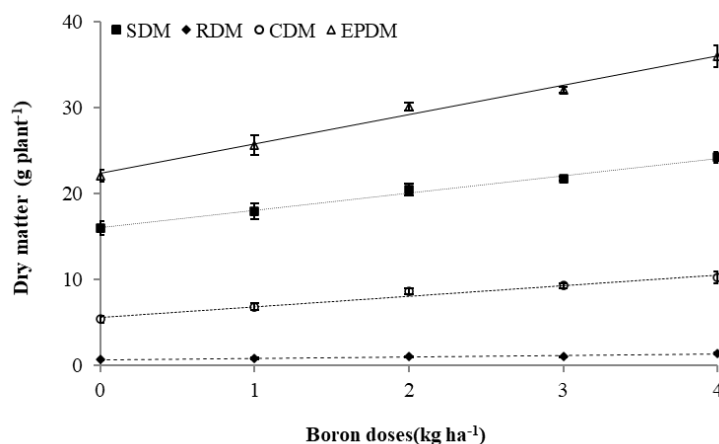
**Fig 2.** Relative chlorophyll index of *Helianthus annuus* at 15 (RCI 1) and 60 (RCI 4) days after emergence (DAE) plants according to the boron application in the soil. RCI 1:  $y = 0.911x + 31.046$ ;  $R^2 = 0.88$ ;  $F = 20.52^{**}$ ; RCI 2:  $y = 1.018x + 35.594$ ;  $R^2 = 0.77$ ;  $F = 19.50^{**}$ .

\*\* significant at 1% probability by the F test.

**Table 3.** Boron content in the shoot and roots and boron accumulation of boron in shoot, roots, and entire plant of *Helianthus annuus* according to the application of boron in the soil.

Boron doses	Content		Accumulation		
	Shoot	Root	Shoot	Root	Entire plant
kg ha <sup>-1</sup>	mg kg <sup>-1</sup>		mg plant <sup>-1</sup>		
0	21.70 ± 3.15	27.12 ± 0.26	0.35 ± 0.055	0.020 ± 0.001	0.37 ± 0.054
1.0	67.39 ± 2.49	29.05 ± 0.17	1.21 ± 0.066	0.024 ± 0.065	1.23 ± 0.065
2.0	92.85 ± 3.27	36.74 ± 0.88	1.90 ± 0.086	0.037 ± 0.002	1.94 ± 0.084
3.0	99.36 ± 1.94	39.68 ± 0.21	2.16 ± 0.033	0.042 ± 0.001	2.20 ± 0.034
4.0	103.50 ± 1.55	48.32 ± 0.89	2.51 ± 0.082	0.070 ± 0.007	2.58 ± 0.087
F-test	174.71**	214.52**	162.95**	31.05**	166.83**
C.V. (%)	6.67	3.23	8.27	18.26	8.14

\*\* significant at 1% probability by the F-test.



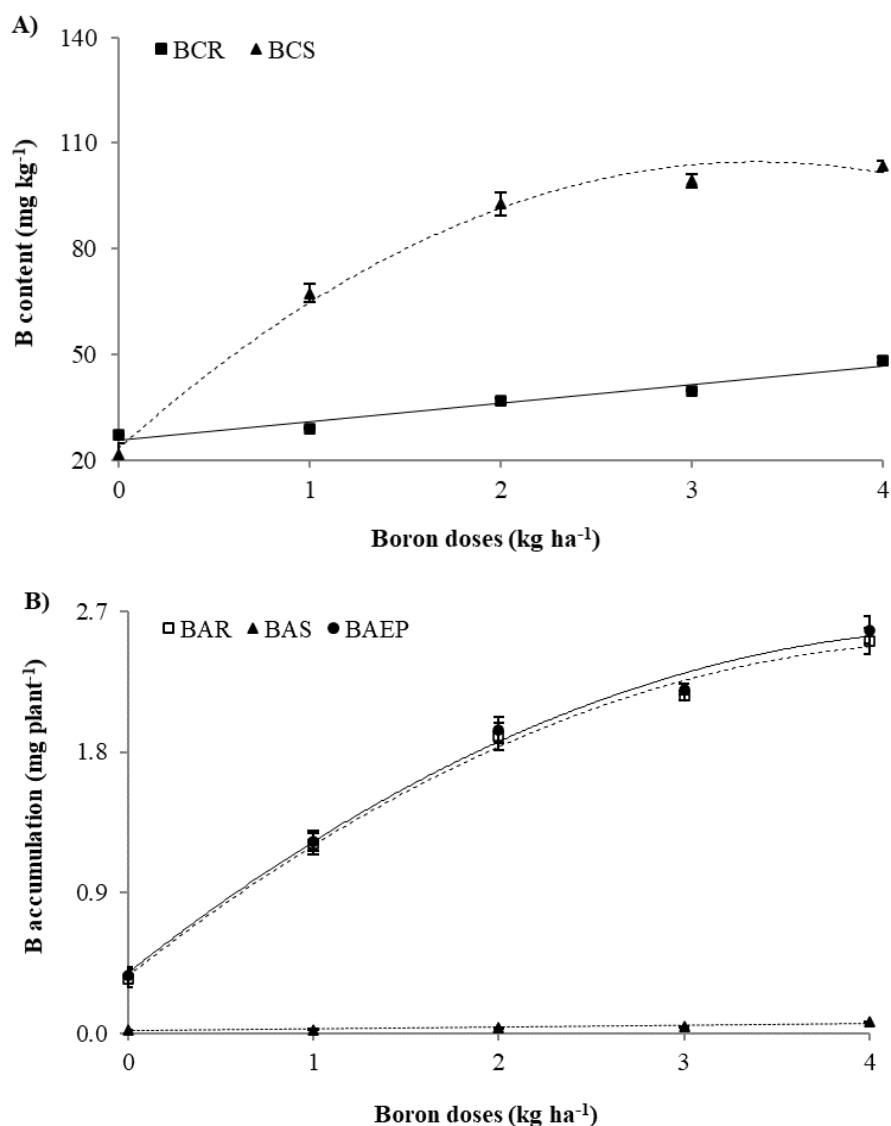
**Fig 3.** Shoot dry matter (SDM), root dry matter (RDM), chapter dry matter (CDM), and entire plant dry matter (EPDM) of *Helianthus annuus* plants according to the boron application in the soil. SDM:  $y = 2.025x + 16.05$ ;  $R^2 = 0.99$ ;  $F = 86.33^{**}$ . RDM:  $y = 1.221x + 5.66$ ;  $R^2 = 0.97$ ;  $F = 77.08^{**}$ . CDM:  $y = 0.165x + 0.696$ ;  $R^2 = 0.89$ ;  $F = 36.79^{**}$ . EPDM:  $y = 3.411x + 22.406$ ;  $R^2 = 0.98$ ;  $F = 149.82^{**}$ .

\*\* significant at 1% probability by the F test.

**Table 4.** Boron transport efficiency ( $BTR_{ef}$ ), boron absorption efficiency ( $AB_{ef}$ ), and boron use efficiency ( $U_{ef}$ ) of *Helianthus annuus* plants according to the boron application in the soil.

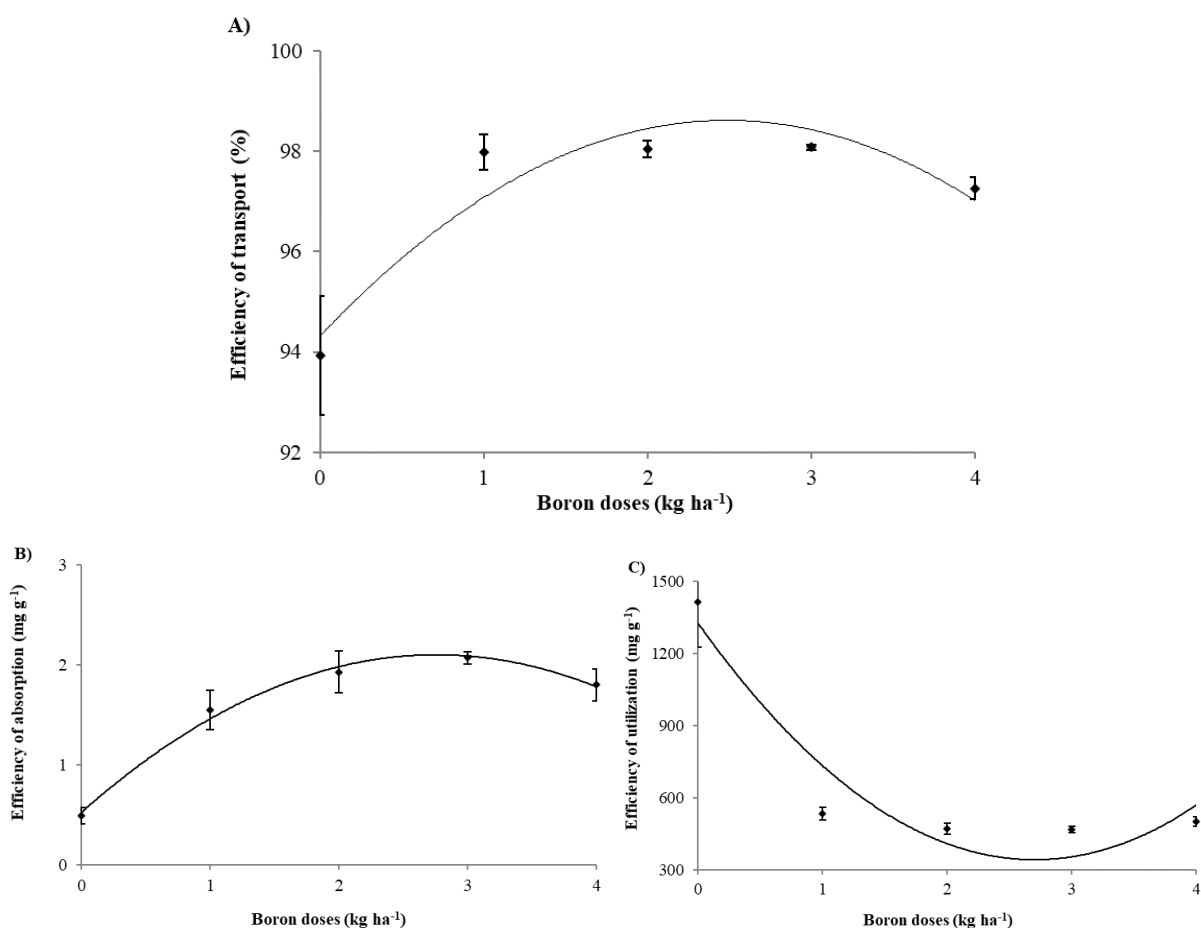
Boron doses kg ha <sup>-1</sup>	$TR_{ef}$ %	$AB_{ef}$		$U_{ef}$
		-----mg g <sup>-1</sup> -----		
0 (control)	93.93 ± 1.19	0.49 ± 0.082		1,416.40 ± 188.71
1.0	97.98 ± 0.35	1.55 ± 0.199		535.01 ± 27.16
2.0	98.04 ± 0.17	1.93 ± 0.206		472.09 ± 21.92
3.0	98.08 ± 0.05	2.07 ± 0.058		469.63 ± 13.27
4.0	97.26 ± 0.22	1.80 ± 0.218		501.97 ± 19.29
F-test	9.83**	17.02**		22.82**
C.V. (%)	1.17	19.52		25.47

\*\* significant at 1% probability by the F-test.



**Fig 4.** Boron content (A) in the roots (BCR) and shoot (BCS), and Boron accumulation (B) in the shoots (BAS), roots (BAR) e entire plant (BAEP) of *Helianthus annuus* plants according to the application of boron in the soil. A) BCR:  $y = 5.3013x + 25.582$ ;  $R^2 = 0.96$ ;  $F = 19.07^*$ ; BCS:  $y = -7.2896x^2 + 48.715x + 23.269$ ;  $R^2 = 0.99$ ;  $F = 48.79^*$ ; B) BAS:  $y = -0.1036x^2 + 0.9413x + 0.3649$ ;  $R^2 = 0.99$ ;  $F = 33.70^*$ ; BAR:  $y = 0.0118x + 0.015$ ;  $R^2 = 0.893$ ;  $F = 9.47^*$ ; BAEP:  $y = -0.1007x^2 + 0.9419x + 0.3846$ ;  $R^2 = 0.99$ ;  $F = 31.32^*$ .

\* significant at 5% probability by the F test.



**Fig 5.** Boron efficiency of transport ( $TR_{ef}$ ) (A), efficiency of absorption ( $AB_{ef}$ ) (B), and boron efficiency of utilization ( $U_{ef}$ ) (C) of *Helianthus annuus* plants according to the boron application in the soil.  $TR_{ef}$ :  $y = -0.6971x^2 + 3.446x + 94.312$ ;  $R^2 = 0.8967$ ;  $F = 21.07^*$ ;  $AB_{ef}$ :  $y = -0.2071x^2 + 1.1426x + 0.5257$ ;  $R^2 = 0.9921$ ;  $F = 25.37^*$ ;  $U_{ef}$ :  $y = 134.85x^2 - 728.83x + 1327.6$ ;  $R^2 = 0.90$ ;  $F = 34.06^*$ . \* significant at 5% probability by the F test.

ha<sup>-1</sup>, with a subsequent reduction in the efficiency. Efficiency in the use of nutrients is complex, as it is linked to the crop capacity to absorb nutrients from the soil, carry out transport, store, and mobilize when necessary (Immanuel et al., 2019).

The reduction in the boron use efficiency with the increase in the applied dose is reported by Flores et al. (2018), which states that the micronutrient ability to use and its conversion into biomass decreases with increasing plant absorption. Bhattacharyya et al. (2015) observed that increasing fertilization with B at doses greater than 2 kg ha<sup>-1</sup> via soil reduced the efficiency of using sunflower B. When the nutrient use efficiency is reduced; plants need larger amounts to produce one gram of dry matter; that is, the greater its absorption of the nutrient, the lower its conversion into biomass, and the lower its efficiency, which can result in higher production cost (Flores et al., 2017; Flores et al., 2019). Thus, there is a deleterious effect, both in the efficiency of absorption and transport, and in the efficiency of use, with an increase in the applied boron dose.

## Materials and Methods

### Location and characteristics of the experimental area

The study was carried out in a greenhouse at the School of Agronomy of the Federal University of Goiás, State of Goiás, Brazil, at 16°35'12" S, 49°21'14" W. The climate of the

region is Aw type according to the Köppen classification (Cardoso et al., 2014), with dry winter and rainy summers. The greenhouse temperature during the plant growth and the experiment was around 32 ± 3 °C during the day, and 26 ± 3 °C during the night.

The previous chemical analysis of the soil was carried out according to procedures proposed by Teixeira et al. (2017), and presented the following properties: pH: 4.4 (CaCl<sub>2</sub>); organic matter: 2.3 g dm<sup>-3</sup>; P: 0.8 mg dm<sup>-3</sup>; K<sup>+</sup>: 25 mg dm<sup>-3</sup>; Ca<sup>2+</sup>: 0.4 cmol<sub>c</sub> dm<sup>-3</sup>; Mg<sup>2+</sup>: 0.3 cmol<sub>c</sub> dm<sup>-3</sup>; SO<sub>4</sub><sup>2-</sup>: 5.6 mg dm<sup>-3</sup>; Zn: 1.4 mg dm<sup>-3</sup>; B: 0.14 mg dm<sup>-3</sup>; Al<sup>3+</sup>: 0.5 cmol<sub>c</sub> dm<sup>-3</sup>; H+Al<sup>3+</sup>: 1.27 cmol<sub>c</sub> dm<sup>-3</sup>; cation exchange capacity (CEC): 2.03 cmol<sub>c</sub> dm<sup>-3</sup>; base saturation (V%): 37.4%. The soil granulometric analysis showed 530, 180 and 135 g kg<sup>-1</sup> of clay, silt, and sand, respectively.

### Treatments and experimental design

A completely randomized design with four replications was used. Five boron doses: 0 (control), 1, 2, 3, and 4 kg ha<sup>-1</sup>, applied to the soil using boric acid as the source (17% de B, solubility in water: 63 g L<sup>-1</sup>), were evaluated. Each experimental plot consisted of a pot (4 dm<sup>3</sup>) filled with 3.5 dm<sup>3</sup> of soil from the 0-0.20 m layer. The soil is classified as a Rhodic Hapludox (Soil-Survey-Staff, 2014).

### Experiment development

The soil correction for the neutralization of exchangeable aluminum and elevation of the calcium and magnesium contents was carried out considering the increase of base saturation to 60% (Sousa and Lobato, 2004). Dolomitic limestone was applied (CaO=36%; MgO=15%; PN=98%; PRNT=92.54%), keeping the soil mass moist (60% of the water

retention capacity of soil), incubated for 30 days, to occur the limestone reaction with the soil (Pádua et al., 2006).

For planting fertilization, 50 mL per pot of a nutrient solution containing 1.5 mg dm<sup>-3</sup> of Cu (CuSO<sub>4</sub>·5H<sub>2</sub>O p.a.), 0.15 mg dm<sup>-3</sup> of Mo (NaMoO<sub>4</sub>·2H<sub>2</sub>O p.a.), 4.0 mg dm<sup>-3</sup> of Fe [Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>·4H<sub>2</sub>O p.a.], and 5.0 mg dm<sup>-3</sup> of Zn (ZnSO<sub>4</sub> p.a.) was applied to the soil surface contained in the pots. Also, the following macronutrient doses were applied to the soil: 110 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> (simple superphosphate), 60 kg ha<sup>-1</sup> of K<sub>2</sub>O (KCl), and 120 kg ha<sup>-1</sup> of N (urea). Part of N fertilization was applied at sowing (20 kg ha<sup>-1</sup> de N). The rest was divided into two applications of 50 kg ha<sup>-1</sup> at 20 and 40 days after sowing, respectively, as indicated by Sousa and Lobato (2004). Boron doses were applied to the soil surface and incorporated at 10 cm depth immediately after the plant emergence.

Planting was performed on 27/06/2018, using the Altis 99 sunflower hybrid (*Helianthus annuus*) obtained in the winter season of 2017. Ten days after emergence (DAE), thinned was performed, leaving two viable and healthy plants per pot. Irrigation was performed with distilled and deionized water by weighing the pots, maintaining the humidity corresponding to 60% of the soil water retention capacity.

### Relative chlorophyll index – RCI, plant height, and leaf area

The evaluations of the relative chlorophyll index were performed in the third expanded leaf of each plant, according to procedures proposed by Castro et al. (2019), with the aid of the chlorophyll meter FALKER ClorofiLOG model CFL1030. The determination of the RCI was made in four moments: 1/3 of the vegetative stage (15 DAE), half of the vegetative stage (30 DAE), end of the vegetative stage, in pre-flowering (45 DAE), and at the time of harvest (60 DAE). At the harvest, the plant height (distance from the base to the last leaf insertion) was evaluated. The leaf area was also evaluated with the aid of the leaf area integrator model LI-3100 Area Meter.

### Biomass production and boron content determination

The harvest was then carried out, separating the aerial part, chapter, and roots of the sampled plants. The plant tissue samples were properly washed in a 0.1% detergent solution, 0.3% acid solution, and distilled water, to eliminate possible residues. Then they were placed in a forced-air circulation oven (65 °C, 48 hours) to stabilize its dry weight and determine the dry biomass of each part. The boron levels in each part were determined according to the method described by Silva et al. (2009). Then, Boron accumulation was calculated from the B content in each part of the plant and the dry biomass amount.

### Absorption, transport, and use efficiency

From the dry matter and content of nutrients in plants data, the calculation of the following nutritional indexes was estimated: boron absorption efficiency (AB<sub>ef</sub>), Equation 1 (Swiader et al., 1994), boron transport efficiency (TR<sub>ef</sub>), Equation 2 (Li et al., 1991), and boron use efficiency for

conversion to dry matter (UT<sub>ef</sub>), Equation 3 (Siddiqi and Glass, 1981). The estimation of these indexes was according to below:

Equation 1:

$$AB_{ef} = \frac{\text{total nutrient content in plant}}{\text{root dry matter}}$$

Equation 2:

$$TR_{ef} = \frac{(\text{total nutrient content in the shoot})}{(\text{total nutrient content in the plant})} \times 100$$

Equation 3:

$$UT_{ef} = \frac{(\text{total dry matter produced})^2}{\text{total nutrient content in the plant}}$$

### Statistical analysis

The results obtained were subjected to analysis of variance by the F-test, using the AgroEstat software (Barbosa and Maldonado Júnior, 2015), and then, polynomial regression analysis was applied. Linear and quadratic mathematical models were tested, applying the best fit to the data, adopting a criterion for choosing the model the magnitude of the significant regression coefficients at 5% probability by the t-test.

The maximum points were calculated by deriving the significant equations. For all variables studied, the standard error of the sample mean (s/√n) was calculated, which: s corresponds to the standard deviation, and n, the number of observations in the sample (Lunet et al., 2006).

### Conclusion

Boron fertilization in sunflower crop positively influences the nutritional and growth aspects of plants. In general, the application of up to 3 kg ha<sup>-1</sup> of B in soils with a low natural level (<0.20 mg dm<sup>-3</sup>) increases the sunflower yield, with non-expressing phytotoxicity aspects caused by the nutrient.

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