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Growth, nutrient accumulation and yield of onion as a function of micronutrient fertilization[1]

Crescimento, acúmulo de nutrientes e produtividade de cebola em função da adubação com micronutrientes

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HIGHLIGHTS:

With the exception of the yield characteristics, the doses of B, Cu and Zn studied influenced the development of onion.
There was a reduction in the non-commercial yield of onion in the experiment conducted in 2019.
Application of higher doses of B, Cu, and Zn is not recommended for the studied conditions.

ABSTRACT: Micronutrients structurally constitute several enzymes and act as a cofactor of essential proteins to maintain cell function, thereby contributing to crop growth and yield. The objective of this study was to evaluate the leaf content, growth, accumulation of micronutrients, classification and yield of onion as a function of fertilization with boron, cooper and zinc in two years of cultivation. The experiments were carried out from June to November in 2018 and 2019, in a soil classified as Ultisol, both at the Rafael Fernandes Experimental Farm, belonging to the Universidade Federal Rural do Semiárido, in the municipality of Mossoró, Rio Grande do Norte, Brazil. The experimental design was in randomized blocks with 15 treatments and four replicates. The treatments consisted of application of doses of B, Cu and Zn, in two experiments. Contents of B, Cu and Zn in the diagnostic leaf, growth, accumulation of B, Cu and Zn in the leaf, bulb and total, classification and commercial, non-commercial and total yields were evaluated. Application of B, Cu and Zn did not influence the number of leaves, relation of bulb shape, leaf, bulb, and total dry mass and yield of onion. Application of B, Cu and Zn, respectively at doses of 1-2-1 kg ha^{-1} favored a greater accumulation of B, Zn and Cu in the bulb. Higher number of leaves, leaf dry mass, bulb dry mass, total dry mass, class 1 bulbs and non-commercial yield were produced in Experiment 1.

Key words: *Allium cepa* L., plant nutrition, diagnostic leaf, doses of micronutrients

RESUMO: Os micronutrientes constituem estruturalmente diversas enzimas e atuam como cofatores de proteínas importantes para manter o funcionamento celular, com isso contribuem com o crescimento e aumento da produtividade das culturas. O objetivo deste estudo foi avaliar o teor foliar, o crescimento, o acúmulo de micronutrientes, a classificação e a produtividade da cebola em função da adubação com boro, cobre, zinco em dois anos de cultivo. Os experimentos foram realizados nos períodos de junho a novembro de 2018 e de 2019, em solo classificado como Argissolo, ambos na Fazenda Experimental Rafael Fernandes, pertencente à Universidade Federal Rural do Semiárido, no município de Mossoró, Rio Grande do Norte. O delineamento experimental foi em blocos casualizados com 15 tratamentos e quatro repetições. Os tratamentos foram constituídos pelas doses de B, Cu e Zn em dois experimentos. Foram avaliados os teores de B, Cu e Zn na folha diagnose, crescimento, acúmulo de B, Cu e Zn na folha, bulbo e total, classificação de bulbos e produtividade comercial, não comercial e total. A aplicação de B, Cu e Zn não influenciou o número de folhas, relação de formato de bulbo, massa seca de folha, bulbo, e total e a produtividade da cebola. A aplicação de B, Cu e Zn, respectivamente nas doses de 1-2-1 kg ha^{-1} favoreceu maior acúmulo de B, Zn e Cu no bulbo. Maior número de folhas, massa seca de folhas, massa seca de bulbo e massa seca total, classe 1 e produtividade não comercial foram produzidos no Experimento 1.

Palavras-chave: *Allium cepa* L., nutrição de plantas, diagnose foliar, doses de micronutrientes

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Introduction

The response of onion crop to the addition of micronutrients depends on the availability of micronutrients in the soil, pH, organic matter content, and interaction with other chemical elements. The application of Zn, Mn, B, Cu and Fe in onion crop increases the concentration of nutrients in the leaves, triggering enzymatic activation, increased carbohydrate metabolism, bulb diameter, bulb weight and commercial and total bulb yields (El-Tohamy et al., 2009; Acharya et al., 2015).

In an alkaline soil deficient in Zn and B, Acharya et al. (2015) studied application via soil and leaf of these nutrients in onion, observing an increase in the diameter of bulbs with the application of 10 kg ha^{-1} of borax and 60% increase in yield with foliar spraying of 0.5% zinc sulfate in comparison to the control.

Babaleshwar et al. (2017) observed increased commercial yield (30.35 t ha^{-1}) and total yield (36.04 t ha^{-1}) with soil application of 10 kg ha^{-1} of Zn. A study carried out in Santa Catarina, Brazil, in Inceptisols (Kurtz & Ernani, 2010), found that application via soil corresponding to 2.7 and 4.5 kg ha^{-1} of Zn in onion led to yields of 22.3 and 35.6 t ha^{-1} in comparison to the control, respectively, in two seasons. The authors observed no increase in yield with the application of B and Mn.

There are few studies in the literature on application of B, Cu and Zn via soil. In Brazil, there are few studies with micronutrients in onion, predominantly in the South and Southeast, where the conditions of climate, soil and crop management differ from those of the Brazilian semiarid region.

In this context, knowledge of the onion response to fertilization with micronutrients will be a fundamental tool in crop optimization, especially for producers in the Western region of Rio Grande do Norte, Brazil. The objective of this study was to evaluate the leaf content, growth, accumulation of micronutrients, classification and yield of onion as a function of fertilization with boron, copper, zinc in two years of cultivation.

Material and Methods

The experiments were conducted in the period from June to November 2018 (Experiment 1) and 2019 (Experiment 2), at the Rafael Fernandes Experimental Farm, belonging to the Universidade Federal Rural do Semi-Árido (UFERSA), located in the rural district of Alagoinha, in the municipality of Mossoró-RN (latitude 5° 3' 37" S, longitude 37° 23' 50" W and altitude of 72 m). Data of temperature, relative air humidity and precipitation during the conduction of the experiments are shown in Figure 1.

The soil was classified as Ultisol. Results of chemical and physical analysis of the soil (0-0.20 cm depth) of the experimental area are shown in Table 1.

In both years of cultivation, the experimental design was in randomized blocks with 15 treatments, four replicates and two experiments. The treatments consisted of the isolated or combined application of the micronutrients B, Cu and Zn. For B and Zn, doses of 1 and 2 kg ha^{-1} were considered, respectively. For Cu, doses of 2 and 4 kg ha^{-1} were considered, according to Trani et al. (2014). The treatments were as follows: T1 ($B_0Cu_0Zn_0$), T2 ($B_1Cu_0Zn_0$), T3 ($B_2Cu_0Zn_0$), T4 ($B_0Cu_2Zn_0$), T5 ($B_0Cu_4Zn_0$), T6 ($B_0Cu_0Zn_1$), T7 ($B_0Cu_0Zn_2$), T8 ($B_1Cu_2Zn_1$), T9

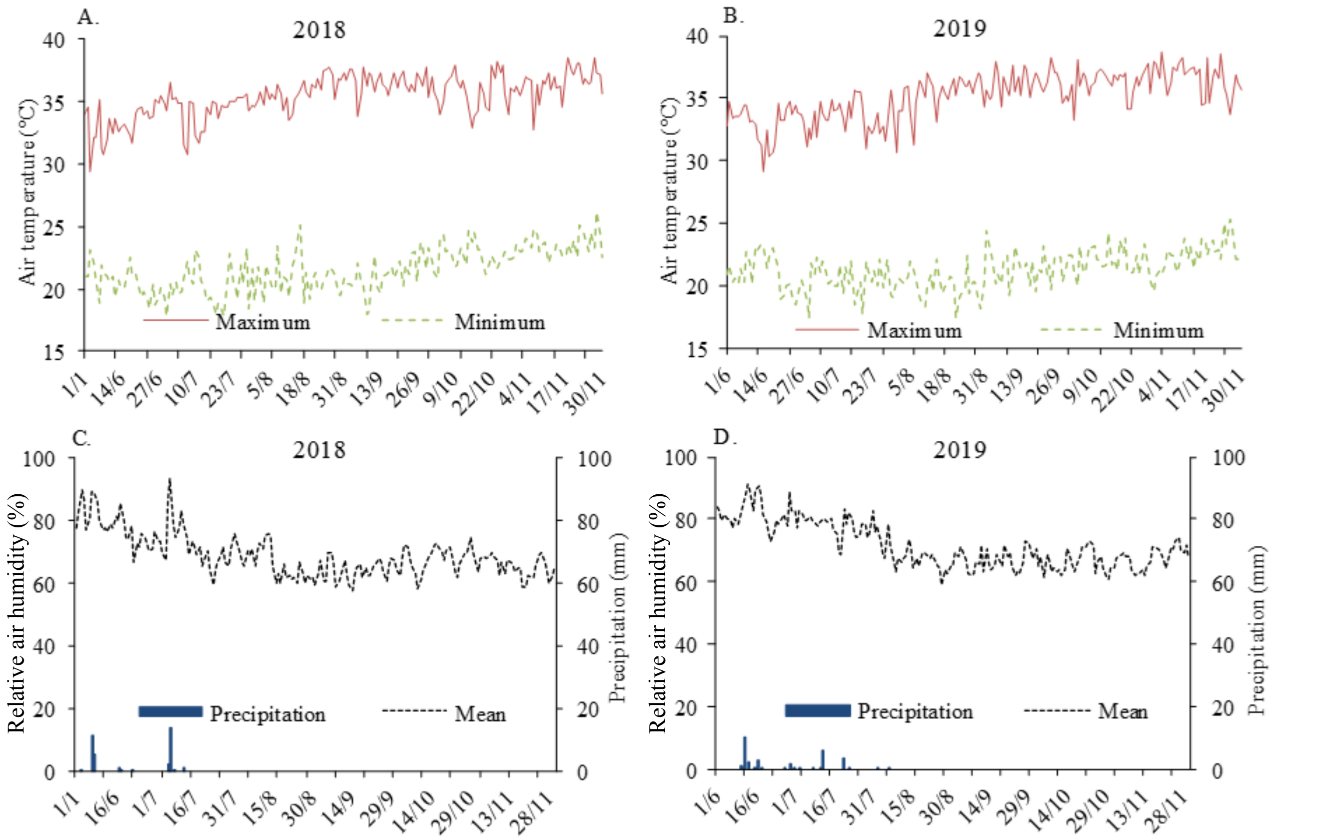

Figure 1. Maximum and minimum air temperatures (A, B), relative air humidity and precipitation (C, D), during the conduction of experiments I (2018) and II (2019), in the experimental area

Table 1. Chemical and physical characterization of the soil of the experimental area, at the Rafael Fernandes Experimental Farm, Mossoró, RN

Chemical attributes													
Experiment	pH H_2O	P (mg dm^{-3})	K	Ca (cmol$_c$ dm^{-3})	Mg	H + Al	SB	CEC	V (%)	OM (g kg^{-1})	B (mg dm^{-3})	Cu	Zn
1 (2018)	4.80	5.00	29.64	0.41	0.13	1.57	0.63	2.20	29	5.59	0.18	0.20	0.70
2 (2019)	6.30	3.20	51.00	0.55	0.25	0.33	0.97	1.30	75	4.14	0.19	0.10	0.50

Physical attributes					
Experiment	CS (g kg^{-1})	FS	Total Sand	Silt	Clay
1 (2018)	618.00	288.00	906.00	24.00	70.00
2 (2019)	620.00	280.00	900.00	30.00	70.00

H + Al - Potential acidity; SB - Sum of bases; CEC - Cation exchange capacity; V - Base saturation; OM - Organic matter; CS - Coarse sand; and, FS - Fine sand

($B_1Cu_2Zn_2$), T10 ($B_2Cu_2Zn_1$), T11 ($B_2Cu_2Zn_2$), T12 ($B_1Cu_4Zn_1$), T13 ($B_1Cu_4Zn_2$), T14 ($B_2Cu_4Zn_1$) and T15 ($B_2Cu_4Zn_2$).

Each plot was constituted by 3.5 m of bed with width of 1.0 m, containing eight rows of plants spaced by 0.10 × 0.06 m. The six central rows of plants consituted the useful area, disregarding two plants in each row. The soil preparation consisted of plowing, harrowing, and making of the beds. Basal fertilization was performed based on soil analysis, using 210 kg ha^{-1} of P_2O_5 (Silva, 2018) in Experiments 1 and 2, in the form of single superphosphate. Sowing was carried out manually, placing two to three seeds in 2.0 cm deep hole. Thinning was carried out 20 days after sowing (DAS), leaving one plant per hole. The cultivar used was the hybrid 'Rio das Antas'.

The irrigation system used was micro-sprinkler up to 22 DAS, and in the rest of the cycle, the drip system, with four tubes per bed, spaced by 0.20 m, with pressure-compensating drippers and an average flow rate of 1.5 L h^{-1}, spaced at 0.30 m. Irrigations were performed daily and the depths were determined based on the evapotranspiration of the crop (Allen et al., 2006), applying total depths of 904.51 and 1,102.62 mm in Experiments 1 and 2, respectively.

The water used in irrigation came from a deep tubular well of the Arenito Açu aquifer, with the following characteristics: pH 7.1, EC = 0.61 dS m^{-1}, 0.65, 1.73, 2.50, 1.90, 1.60, 0 and 4.00 mmol$_c$ L^{-1} of K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, CO_3^{2-} and HCO_3^- and SAR of 1.2 (mmol L^{-1})$^{0.5}$, respectively.

The fertilizer applications were carried out weekly via fertigation, starting at 22 DAS and ending at 84 DAS. In Experiment 1, 99.04 kg ha^{-1} of N, 40.51 kg ha^{-1} of P_2O_5, 213.94 kg ha^{-1} of K_2O, 13.77 kg ha^{-1} of Mg and 47.5 kg ha^{-1} of Ca were applied. In Experiment 2, 86.53 kg ha^{-1} of N, 40.51 kg ha^{-1} of P_2O_5, 213.94 kg ha^{-1} of K_2O, 13.77 kg ha^{-1} of Mg and 47.5 kg ha^{-1} of Ca were applied. The doses of N and K were established according to the recommendation for the crop, according to Gonçalves et al. (2019a, 2019b). The application of the other macronutrients was according to recommendation of Aguiar Neto et al. (2014). The fertilizer sources were purified MAP, urea, potassium nitrate, calcium nitrate, potassium chloride and magnesium sulphate. The doses of B, Cu and Zn were based of the soil analysis and fertilization recommendation for onion crops (Trani et al., 2014). The sources of micronutrients used were: boric acid, copper sulfate and zinc sulfate.

During the conduction of the experiment, manual weeding and phytosanitary control were performed according to the crop's need. Irrigation was suspended at 114 DAS (Experiment 1) and 130 DAS (Experiment 2) when 70% of the plants were lodged, and the curing process began. After 22 and 24 days of irrigation suspension, respectively, in Experiments 1 and 2, the bulbs were harvested and cleaned.

The following variables were evaluated: B, Cu and Zn contents in the diagnostic leaf (mg kg^{-1}). The tallest leaf was collected from 20 plants in the plot's observation area, at 60 DAS (Malavolta et al., 1997).

Growth analyses were carried out on eight plants in the plot's observation area at 120 days after sowing: plant height (PH), number of leaves (NL) and relation of bulb shape (RBS) obtained by calculating the ratio between the longitudinal diameter and the transverse diameter of the bulb. At harvest, eight plants were collected from the plot's useful area, separated into leaf and bulb and washed. The leaves were placed in a paper bag and the bulbs in an aluminum tray; and placed in a forced-air circulation oven, at a temperature of 65 °C, until reaching a constant mass. The total dry mass of the plant was calculated by summing the dry masses of leaf and bulb. The results were expressed in g plant^{-1}.

For the accumulation of B, Cu and Zn in leaf, bulb and total, eight plants were collected in the plot's observation area, at 140 DAS. Chemical analysis to determine the micronutrient contents present in each fraction of the plant (leaf and bulb) was performed in the extracts obtained by wet digestion in a microwave oven (EMBRAPA, 2009). The B content was determined by digital UV/VIS spectrophotometry using Azomethine H. Cu and Zn were determined by atomic absorption spectrophotometry according to the methodology proposed by EMBRAPA (2009). Based on the contents of B, Cu and Zn and the dry masses of leaf and bulb, accumulation of micronutrients was calculated. To determine the amount of these accumulated in each fraction of the plant, the micronutrient content was multiplied by the dry mass of that fraction. The total accumulations of each micronutrient was calculated by summing the accumulations in the leaf and bulb, and the results were expressed in mg plant^{-1}.

The classification of the bulbs was carried out according to the standards of the Ministry of Agriculture and Supply, being classified in: Class 1: Bulbs with diameter < 35 mm and double bulbs; Class 2: Bulbs with diameter within the range of 35-50 mm; Class 3: Bulbs with diameter within the range of 50-75 mm and Class 4: Bulbs with diameter within the range of 75-90 mm. The quantities of bulbs in each class were expressed as percentages of total bulbs obtained in each experimental unit. The yield of commercial bulbs (t ha^{-1}) was determined by the total weight of bulbs with diameter > 35 mm. Non-commercial bulbs corresponded to bulbs with diameter < 35 mm (class 1) and double bulbs, and total yield was expressed as the sum of commercial and non-commercial yields.

The obtained data were subjected to joint analysis of variance. When there was a significant effect for the treatments, the means were compared by the test of Scott & Knott (1974), at 0.05 probability of error, and for the experiments the means were compared by the t-test at 0.05 probability of error, using SISVAR 5.3 software (Ferreira, 2019).

Results and Discussion

The contents of B in the diagnostic leaf of onion in Experiment 1 varied from 30.5 to 115.5 mg kg^{-1}, the highest values being associated with the application of 1 and 2 kg ha^{-1} of B. In Experiment 2, the contents varied from 48.50 to 83.50 mg kg^{-1} of B, and the highest contents were related to the application of T9, T4, T12 and T13 (Table 2).

Boron is a micronutrient that may have its availability reduced, among other characteristics, due to the amount of $CaCO_3$ in the soil or in the irrigation water. According to Maia et al. (2001), Niaz et al. (2016) and Dridi et al. (2018), B can be precipitated with $CaCO_3$, making it unavailable to plants and/or reducing its availability.

In this context, the amount of bicarbonate in the applied irrigation water was 4.00 $mmol_c$ L^{-1}. Thus, the amount of $CaCO_3$ supplied via irrigation water until the period of collection of the onion diagnostic leaf, in Experiment 2, was 480 kg ha^{-1}; with this, it can be explained why the B content was higher in the treatment that did not have boron (T4), because the amount of $CaCO_3$ supplied via irrigation water may have influenced the response to the isolated application of B (T2 and T3) in the experiment 2 (Table 2).

All treatments of the Experiments 1 (30.50 to 115.75 mg kg^{-1}) and 2 (52.25 to 83.50 mg kg^{-1}) showed contents of boron within or above the range considered suitable (30 to 50 mg kg^{-1}), according to Trani et al. (2014), and no symptoms of toxicity were found in any of the treatments.

However, for some crops, such as onions, which use polyols as primary photosynthetic metabolites, under limited supply of B, active absorption predominates, which is why there is no significant concentration gradient in the leaves (Brdar-Jokanović, 2020).

Shaaban et al. (2004) evaluated the ratio between Zn and B in wheat development, under low and high levels of calcium carbonate in the soil, and noted that the application of the appropriate dose of the combination of these two nutrients resulted in the ideal concentration of both in the tissues of the aerial part. According to these authors, this is due to B and Zn essentiality in the activity of ATPase in the plasma membrane and the role of B in the stability of this membrane.

The contents of Cu in Experiment 1 ranged from 4.50 to 10.75 mg kg^{-1}, the highest values being associated with application of 4 kg ha^{-1} of Cu, combined with B and Zn. In Experiment 2, the contents varied from 1.25 to 6.00 mg kg^{-1}; the highest contents were related to the isolated application of 2 and 4 kg ha^{-1} of Cu (Table 2).

The Cu concentration considered suitable for onion ranges from 10 to 30 mg kg^{-1} (Trani et al., 2014). The levels found are below the recommended for the crop, except for T12 (10.75 mg kg^{-1}), in Experiment 1. Low Cu content may be related to the form of application, absorption speed by plants, competition with Zn and soil pH (Malavolta et al., 1997).

The Cu content in the soil is low in both experiments compared to the literature, and in Experiment 2 it was lower than in Experiment 1. Thus, in addition to the factors already mentioned, the applied dose may not have been sufficient to provide the necessary Cu content in soil, which resulted in contents below the recommended for the crop, especially in Experiment 2. However, no visual symptoms of Cu deficiency were observed in plants of any of the experiments.

The contents of Zn, in Experiment 1, varied from 25.25 to 37.00 mg kg^{-1}, with the highest values being associated with the treatment 11. In Experiment 2, the levels varied from 19.75 to 26.00 mg kg^{-1}, and the highest levels were related to the treatment 8 (Table 2). The Zn content considered adequate,

Table 2. Contents of B, Cu and Zn in the diagnostic leaf of onion as a function of the application of B, Cu and Zn and experiments

Treatments	B		Cu		Zn	
	(mg kg^{-1})					
	Experiments					
	1	2	1	2	1	2
1 ($B_0Cu_0Zn_0$)	40.50 dB	66.00 bA	4.50 dA	2.25 cB	30.00 bA	21.00 bB
2 ($B_1Cu_0Zn_0$)	106.50 aA	53.25 cB	5.25 dA	1.75 cB	28.75 bA	21.50 bB
3 ($B_2Cu_0Zn_0$)	115.75 aA	48.50 cB	6.00 cA	1.25 cB	30.25 bA	19.75 bB
4 ($B_0Cu_2Zn_0$)	66.75 bB	81.00 aA	8.25 bA	5.75 aB	31.50 aA	21.25 bB
5 ($B_0Cu_4Zn_0$)	52.50 cA	61.75 cA	7.25 cA	6.00 aA	25.25 bA	23.75 aA
6 ($B_0Cu_0Zn_1$)	65.25 bA	53.25 cB	6.75 cA	5.00 aB	32.00 aA	25.75 aB
7 ($B_0Cu_0Zn_2$)	54.50 cA	66.00 bA	9.00 bA	4.75 aB	31.50 aA	24.50 aB
8 ($B_1Cu_2Zn_1$)	42.00 dB	66.25 bA	8.25 bA	2.75 bB	31.50 aA	26.00 aB
9 ($B_1Cu_2Zn_2$)	30.50 dB	83.50 aA	7.00 cA	3.00 bB	28.00 bA	22.50 bB
10 ($B_2Cu_2Zn_1$)	55.75 cA	66.50 bA	8.00 bA	2.50 bB	29.75 bA	22.00 bB
11 ($B_2Cu_2Zn_2$)	58.25 cA	52.25 cA	7.00 cA	3.00 bB	37.00 aA	19.75 bB
12 ($B_1Cu_4Zn_1$)	51.50 cB	79.50 aA	10.75 aA	3.50 bB	33.00 aA	22.25 bB
13 ($B_1Cu_4Zn_2$)	69.75 bA	78.75 aA	9.50 aA	3.75 bB	30.00 bA	20.50 bB
14 ($B_2Cu_4Zn_1$)	64.00 bA	68.50 bA	9.25 aA	3.25 bB	30.00 bA	20.25 bB
15 ($B_2Cu_4Zn_2$)	37.00 dB	72.75 bA	9.75 aA	1.25 cB	32.75 aA	23.50 aB
CV (%)	13.35		16.66		11.39	
Mean standard error	4.15	4.33	0.54	0.37	1.37	1.64

Means followed by the same lowercase letter, in the column by Scott-Knott test ($p > 0.05$) and means followed by the same uppercase letter, in the row by the t-test ($p > 0.05$), do not differ from each other according to Trani et al. (2014), for onion, varies from 30 to 100 mg kg^{-1}. The contents found were adequate in Experiment 1, except in treatments 2, 5, 9 and 10. In Experiment 2, the contents are below that reported as ideal for the crop. Similar to Cu, no visual symptoms of Zn deficiency were observed in plants.

In treatment 8 (combined application of 1-2-1 kg ha^{-1} of B, Cu and Zn), a content of 26 mg kg^{-1} of Zn was observed, while in treatments 6 and 7 (isolated application) the levels were 25.75 and 24.50 mg kg^{-1}. Although the result of T8 is numerically greater, it is statistically similar to that obtained with the isolated application in T6 and T7 (Table 2). The difference between T8 and T9 can be explained by the inhibition between Cu and Zn, resulting from the competition for the same exchange site. Like boron, it can also reduce the absorption of Zn, by non-competitive inhibition (Marschner, 1995; Malavolta et al., 1997).

The results of this study differ from those observed by Kurtz & Ernani (2010), who, studying the application of Zn via leaf and soil, did not observe an increase in Zn contents in the leaf (the contents varied from 14.1 to 15.1 mg kg^{-1}). The authors verified the difference between the application forms and claim that application via soil is more effective compared to foliar application.

There was an increase in plant growth with the application of B, Cu and Zn in comparison to the control in Experiment 1; the isolated applications of 2 kg ha^{-1} of Cu and 1 kg ha^{-1} of Zn contributed to greater plant height, 60.36 and 60.73 cm, followed by treatments 8, 10, 14 and 15, with the values ranging from 57.19 to 59.00 cm (Table 3).

In Experiment 2, two groups of averages were formed, with plant height ranging from 50.56 to 58.09 cm (Table 3). The results of plant height in Experiment 1 were similar to those obtained by Abedin et al. (2012), who observed mean plant height of 61.30 cm with a combined application of 3 kg ha^{-1} of Zn + B. Manna & Maity (2016), who observed higher plant height of 63.93 and 67.25 cm, with foliar application of 0.5% B and Zn, attribute greater plant growth to the beneficial effect of

Table 3. Mean plant height of onion as a function of the application of B, Cu and Zn in experiments

Treatments	Plant height (cm)	
	Experiment 1	Experiment 2
1 ($B_0Cu_0Zn_0$)	49.48 cB	58.09 aA
2 ($B_1Cu_0Zn_0$)	55.36 bA	51.89 bA
3 ($B_2Cu_0Zn_0$)	53.60 bA	55.37 aA
4 ($B_0Cu_2Zn_0$)	60.36 aA	53.63 bB
5 ($B_0Cu_4Zn_0$)	55.20 bA	55.18 aA
6 ($B_0Cu_0Zn_1$)	60.73 aA	53.82 bB
7 ($B_0Cu_0Zn_2$)	53.14 bA	51.41 bA
8 ($B_1Cu_2Zn_1$)	57.19 aA	53.67 bA
9 ($B_1Cu_2Zn_2$)	49.95 cA	50.56 bA
10 ($B_2Cu_2Zn_1$)	59.00 aA	55.43 aA
11 ($B_2Cu_2Zn_2$)	54.85 bA	55.37 aA
12 ($B_1Cu_4Zn_1$)	54.47 bA	55.73 aA
13 ($B_1Cu_4Zn_2$)	55.55 bA	51.94 bA
14 ($B_2Cu_4Zn_1$)	57.55 aA	56.60 aA
15 ($B_2Cu_4Zn_2$)	58.09 aA	52.91 bB
CV (%)	5.41	
Mean standard error	1.40	1.56

Means followed by the same lowercase letter, in the column by Scott-Knott test ($p > 0.05$) and means followed by the same uppercase letter by the t-test ($p > 0.05$), in the row, do not differ from each other

application of these micronutrients, due to the role involved in physiological processes and cellular functions. In this context, these contribute with the increase in photosynthetic activity, chlorophyll formation, nitrogen metabolism and auxin content in plants, influencing higher plant height (Veer et al., 2018).

The variations in the number of leaves (NL) between treatments were slight, with the group of higher averages formed by treatments with isolated application of B and Zn and treatments 9, 10, 14 and 15. The number of leaves (NL) varied from 8.03 to 8.43 (Table 4).

For the relation of bulb shape (RBS), leaf dry mass (LDM), bulb dry mass (BDM) and total dry mass (TDM) variables, there was an isolated effect for experiments, observing that, except for the RBS, the highest averages were obtained in Experiment 1. The mean values for LDM, BDM and TDM were 2.65, 13.03 and 15.68 g plant^{-1}, respectively, as a function of the experiments (Table 5).

The observed results differ from those reported by Backes et al. (2018), who obtained a dry mass of onion bulbs of 27.4 g plant^{-1} and a total dry mass of 31.7 g plant^{-1} at 140 days after transplanting. The authors explain that the dry mass accumulation is influenced by the cultivar and the method of implementation of the crop.

The higher result observed in Experiment 1 may have been influenced by the soil's characteristics. In both experiments, the contents of B, Cu and Zn in the soil were low, with a low content of organic matter (OM) and a sandy texture; what differs the soils in the experiments is the pH, which in the first experiment is more acidic, and this difference, combined with other conditions, interfered with the availability of micronutrients for plants.

Table 4. Mean number of leaves (NL) of onion, as a function of the application of micronutrients B, Cu and Zn

Treatments	NL
1 ($B_0Cu_0Zn_0$)	7.65 b
2 ($B_1Cu_0Zn_0$)	8.13 a
3 ($B_2Cu_0Zn_0$)	8.43 a
4 ($B_0Cu_2Zn_0$)	7.81 b
5 ($B_0Cu_4Zn_0$)	7.67 b
6 ($B_0Cu_0Zn_1$)	8.39 a
7 ($B_0Cu_0Zn_2$)	8.03 a
8 ($B_1Cu_2Zn_1$)	7.53 b
9 ($B_1Cu_2Zn_2$)	8.17 a
10 ($B_2Cu_2Zn_1$)	8.30 a
11 ($B_2Cu_2Zn_2$)	7.81 b
12 ($B_1Cu_4Zn_1$)	7.70 b
13 ($B_1Cu_4Zn_2$)	7.81 b
14 ($B_2Cu_4Zn_1$)	8.22 a
15 ($B_2Cu_4Zn_2$)	8.21 a
CV (%)	8.69

Means followed by the same lowercase letter, in the column do not differ by Scott-Knott test ($p > 0.05$)

Table 5. Mean number of leaves (NL), relation of bulb shape (RBS), leaf dry mass (LDM), bulb dry mass (BDM) and total dry mass (TDM) of onion, as a function of the experiments

Experiment	NL	RBS	LDM	BDM	TBM
1 (2018)	8.14 a	1.01 b	2.65 a	13.03 a	15.68 a
2 (2019)	7.83 b	1.10 a	1.65 b	11.96 b	13.60 b
CV (%)	8.69	6.10	12.36	12.65	11.63
Mean standard error	0.09	0.008	0.03	0.20	0.22

Means followed by the same letter in the column do not differ statistically by t-test ($p > 0.05$)

The availability of B, Cu and Zn is reduced by pH higher than 6.0 (Malavolta et al., 1997; Niaz et al., 2016). As noted, the soil's pH in Experiment 1 was more acidic (4.8) in contrast to that in Experiment 2 (6.3), which may have influenced the availability of B, Cu and Zn in the soil solution and interfered in the crop response to fertilization with micronutrients, which resulted in lower plant height (PH), number of leaves (NL), leaf dry mass (LDM), bulb dry mass (BDM) and total dry mass (TDM) in Experiment 2. Maurya et al. (2018) state that, except for Mo, increase in the soil pH reduces micronutrients' availability for plants.

Boron deficiency is predominant in soils of sandy texture and with low organic matter content (Niaz et al., 2016) and a high percentage of $CaCO_3$, and its availability is reduced with an increase in pH of the soil; it can also be complexed with organic matter and precipitated with $CaCO_3$ (Niaz et al., 2016; Dridi et al., 2018).

The greater B accumulation in the leaf was obtained with the application of T3, representing an increase of 31.08% in comparison to the control. Greater accumulations in the bulb and total were obtained with the application of T2, T4 and T8 treatments, with an increase in the accumulation of B in the bulb of 16.25, 13.32 and 28.89%, and in the total accumulation of 15.04, 8.72 and 21.80% in comparison to the control, in Experiment 1 (Table 6).

In Experiment 2, greater accumulation in the bulb and total were obtained in T1, T2, T3, T4, T6, T7 and T8 treatments. Although the treatments that formed the same group of means are similar to the control, there was an increase between 2.12 and 16.14% with application of micronutrients compared to the control (Table 6).

The accumulation of B varied in the vegetative parts of the plant, which can be explained by the difference in the contents absorbed, mass accumulation, availability of the nutrient in the soil solution and the mobility inside the plant (Moraes et al., 2016). According to the authors, a greater accumulation of B in the bulbs is important for the formation of cataphylls.

The positive effect for greater accumulation is due to the increase of dry mass in the bulbs and the redistribution of micronutrients from the leaves to the bulbs, which may have intensified during the curing process, with highter accumulation estimated at harvest period (Acharya et al., 2015; Trivedi & Dhumal, 2017).

The application of micronutrients contributed to the increase in Cu accumulation compared to the control in both experiments. In Experiment 1, treatments 13 and 15 showed higher accumulations, 0.137 and 0.142 mg plant^{-1} of Cu in the leaf. Higher accumulation in the bulb was obtained in T4, T9, T10, T11 and T13, with accumulations between 0.144 and 0.163 mg plant^{-1}. For the total accumulation per plant, the combined application of T13 favored greater accumulation, 0.285 g plant^{-1}, compared to the control (Table 7).

In Experiment 2, treatments 5, 12, 13, 14 and 15 contributed to a greater accumulation of Cu in the leaf. The accumulated quantities ranged from 0.021 to 0.030 mg plant^{-1}. For the accumulation in the bulb and in the plant, there was no significant effect between treatments. The accumulations varied between 0.52 and 0.97 mg plant^{-1} (Table 7).

In general, the combined application of the three micronutrients with the highest dose of Cu (4 kg ha^{-1}) favored the highest accumulation of Cu in onion. The concentration of this nutrient in the soil is low and the onion has a high response to Cu (Manna & Maity, 2016). In addition to that, fertilization with the highest dose of Cu may have contributed to this result.

Treatments 13 and 15 accumulated more Zn in the leaf, 0.065 and 0.061 mg plant^{-1}. In the bulb, greater accumulations were obtained with isolated B, Cu and Zn and treatments 8, 9, 10 and 12, ranging from 0.244 to 0.285 mg plant^{-1}, represented an increase of 11.44 to 41.79% compared to the control. Treatments 4, 6, 7, 8, 9, 10 and 12 contributed with a higher total accumulation of Zn, ranging from 0.254 to 0.315 mg plant^{-1}, with an increase of 23.30 to 52.91% in comparison to the control, in Experiment 1 (Table 8).

In experiment 2, a higher accumulation of Zn (0.037 and 0.034 mg plant^{-1}) in the leaf was represented by treatments

Table 6. Boron accumulation in the leaf, bulb and total in onion, as a function of the application of B, Cu and Zn and experiments

Treatments	B (mg plant^{-1})					
	Experiment 1			Experiment 2		
	Leaf	Bulb	Total	Leaf	Bulb	Total
1 ($B_0Cu_0Zn_0$)	0.222 bA	0.443 bA	0.665 bA	0.183 aA	0.378 aA	0.561 aB
2 ($B_1Cu_0Zn_0$)	0.225 bA	0.515 aA	0.765 aA	0.178 aB	0.424 aB	0.603 aB
3 ($B_2Cu_0Zn_0$)	0.291 aA	0.366 cA	0.657 bA	0.235 aB	0.407 aA	0.642 aA
4 ($B_0Cu_2Zn_0$)	0.222 bA	0.502 aA	0.723 aA	0.171 aB	0.321 aB	0.492 aB
5 ($B_0Cu_4Zn_0$)	0.152 cA	0.498 aA	0.651 bA	0.180 aA	0.277 bB	0.458 bB
6 ($B_0Cu_0Zn_1$)	0.147 cB	0.372 cA	0.519 cA	0.196 aA	0.413 aA	0.608 aA
7 ($B_0Cu_0Zn_2$)	0.150 cA	0.347 cB	0.497 cB	0.168 aA	0.439 aA	0.607 aA
8 ($B_1Cu_2Zn_1$)	0.239 bA	0.571 aA	0.810 aA	0.160 aB	0.386 aB	0.546 aB
9 ($B_1Cu_2Zn_2$)	0.156 cA	0.442 bA	0.598 cA	0.151 aA	0.245 bB	0.396 bB
10 ($B_2Cu_2Zn_1$)	0.194 cA	0.302 cA	0.496 cA	0.178 aA	0.194 bB	0.372 bB
11 ($B_2Cu_2Zn_2$)	0.164 cA	0.324 cA	0.488 cA	0.168 aA	0.144 cB	0.313 cB
12 ($B_1Cu_4Zn_1$)	0.250 bA	0.434 bA	0.684 bA	0.180 aB	0.117 cB	0.298 cB
13 ($B_1Cu_4Zn_2$)	0.178 cA	0.394 cA	0.572 cA	0.185 aA	0.193 bB	0.379 bB
14 ($B_2Cu_4Zn_1$)	0.171 cA	0.485 aA	0.656 bA	0.178 aA	0.210 bB	0.396 bB
15 ($B_2Cu_4Zn_2$)	0.217 bA	0.439 bA	0.655 bA	0.162 aB	0.237 bB	0.399 bB
CV (%)	15.78	16.37	12.49	15.78	16.37	12.49
Mean standard error	0.02	0.03	0.04	0.01	0.02	0.03

Means followed by the same lowercase letter, in the column by Scott-Knott test ($p > 0.05$) and means followed by the same uppercase letter by the t-test ($p > 0.05$), in the row, do not differ from each other

Table 7. Copper accumulation in the leaf, bulb and total in onion, as a function of the application of B, Cu and Zn and experiments

Treatments	Cu (mg plant^{-1})					
	Experiment 1			Experiment 2		
	Leaf	Bulb	Total	Leaf	Bulb	Total
1 ($B_0Cu_0Zn_0$)	0.006 fA	0.050 dA	0.057 fA	0.003 bA	0.065 aA	0.068 aA
2 ($B_1Cu_0Zn_0$)	0.009 fA	0.107 bA	0.116 eA	0.002 bA	0.067 aB	0.069 aB
3 ($B_2Cu_0Zn_0$)	0.010 fA	0.113 bA	0.123 eA	0.004 bA	0.072 aB	0.076 aB
4 ($B_0Cu_2Zn_0$)	0.060 dA	0.144 aA	0.204 bA	0.009 bB	0.061 aB	0.070 aB
5 ($B_0Cu_4Zn_0$)	0.066 dA	0.082 cA	0.149 dA	0.024 aB	0.055 aB	0.079 aB
6 ($B_0Cu_0Zn_1$)	0.029 eA	0.115 bA	0.144 dA	0.002 bB	0.067 aB	0.069 aB
7 ($B_0Cu_0Zn_2$)	0.031 eA	0.072 cA	0.104 eA	0.002 bB	0.056 aA	0.058 aB
8 ($B_1Cu_2Zn_1$)	0.036 fA	0.108 bA	0.145 dA	0.009 bB	0.054 aB	0.063 aB
9 ($B_1Cu_2Zn_2$)	0.058 cA	0.163 aA	0.221 bA	0.009 bB	0.058 aB	0.067 aB
10 ($B_2Cu_2Zn_1$)	0.047 dA	0.146 aA	0.193 cA	0.010 bB	0.062 aB	0.072 aB
11 ($B_2Cu_2Zn_2$)	0.065 dA	0.160 aA	0.226 bA	0.012 bB	0.059 aB	0.071 aB
12 ($B_1Cu_4Zn_1$)	0.097 dA	0.078 cA	0.175 cA	0.030 aB	0.056 aA	0.086 aB
13 ($B_1Cu_4Zn_2$)	0.137 aA	0.147 aA	0.285 aA	0.021 aB	0.047 aB	0.068 aB
14 ($B_2Cu_4Zn_1$)	0.089 bA	0.080 cA	0.169 cA	0.025 aB	0.067 aA	0.092 aB
15 ($B_2Cu_4Zn_2$)	0.142 aA	0.075 cA	0.217 bA	0.026 aB	0.071 aA	0.097 aB
CV (%)	24.17	17.69	15.28	24.17	17.69	15.28
Mean standard error	0.006	0.009	0.012	0.001	0.005	0.006

Means followed by the same lowercase letter, in the column belonging to the same group, by Scott-Knott test ($p > 0.05$) and means followed by the same uppercase letter by the t-test ($p > 0.05$), in the row, do not differ from each other

Table 8. Zinc accumulation in the leaf, bulb and total in onion, as a function of the application of B, Cu and Zn and experiments

Treatments	Zn (mg plant^{-1})					
	Experiment 1			Experiment 2		
	Leaf	Bulb	Total	Leaf	Bulb	Total
1 ($B_0Cu_0Zn_0$)	0.004 eB	0.201 bB	0.206 bB	0.020 cA	0.410 cA	0.430 cA
2 ($B_1Cu_0Zn_0$)	0.004 eB	0.240 aB	0.243 bB	0.020 cA	0.530 bA	0.550 bA
3 ($B_2Cu_0Zn_0$)	0.002 eB	0.257 aB	0.259 aB	0.021 cA	0.494 bA	0.512 bA
4 ($B_0Cu_2Zn_0$)	0.002 eB	0.270 aB	0.272 aB	0.017 cA	0.611 aA	0.632 aA
5 ($B_0Cu_4Zn_0$)	0.011 dB	0.192 bA	0.204 bB	0.027 bA	0.254 dA	0.280 dA
6 ($B_0Cu_0Zn_1$)	0.030 cA	0.224 aB	0.254 aB	0.026 bA	0.414 cA	0.440 cA
7 ($B_0Cu_0Zn_2$)	0.037 bA	0.234 aB	0.271 aB	0.037 aA	0.381 cA	0.419 cA
8 ($B_1Cu_2Zn_1$)	0.041 bA	0.267 aB	0.308 aB	0.027 bB	0.358 cA	0.386 cA
9 ($B_1Cu_2Zn_2$)	0.040 bA	0.252 aA	0.292 aA	0.034 aA	0.311 dA	0.345 dA
10 ($B_2Cu_2Zn_1$)	0.025 cA	0.250 aB	0.275 aB	0.021 cA	0.348 cA	0.369 dA
11 ($B_2Cu_2Zn_2$)	0.038 bA	0.176 bB	0.214 bB	0.022 cB	0.346 cA	0.369 dA
12 ($B_1Cu_4Zn_1$)	0.030 cA	0.285 aA	0.315 aA	0.019 cB	0.322 dA	0.342 dA
13 ($B_1Cu_4Zn_2$)	0.065 aA	0.133 bB	0.199 bB	0.018 cB	0.304 dA	0.321 dA
14 ($B_2Cu_4Zn_1$)	0.031 cA	0.186 bB	0.217 bB	0.020 cB	0.349 cA	0.368 dA
15 ($B_2Cu_4Zn_2$)	0.061 aA	0.149 bB	0.209 bB	0.026 bB	0.373 cA	0.400 cA
CV (%)	20.54	15.99	15.04	20.54	15.99	15.04
Mean standard error	0.003	0.02	0.02	0.002	0.03	0.03

Means followed by the same lowercase letter, in the column by Scott-Knott test ($p > 0.05$) and means followed by the same uppercase letter by the t-test ($p > 0.05$), in the row, do not differ from each other

7 and 9, with increases of 85 and 70% compared to the control. Higher accumulations of Zn in the bulb and total were obtained at the dose of 4 kg ha^{-1} of Cu, 0.611 and 0.632 mg plant^{-1}, and the increases in comparison to the control were 49.02 and 46.98% in the bulb and total, respectively (Table 8).

Higher Zn accumulation in the bulb is due to the higher dry mass at the harvest and, although Zn is little mobile in the plant (Malavolta et al., 1997), onion is demanding and responsive to Zn (Kurtz & Ernani, 2010), which influences the nutritional content, dry mass and, as a consequence, higher accumulation of Zn by the crop.

It can still be observed that, although onion is more responsive to Zn and Cu, there was a reduction in the accumulation of Zn, when maximum combined doses of Cu and Zn with B were applied, which may be due to the inhibition between Cu and Zn, due to competition for the same exchange site (Marschner, 1995).

Less accumulation of nutrients in the leaves occurs because the leaves lose their position as a nutritional source and become the main sinnk for the bulbs (Moraes et al., 2016). This occurs because when the development of the bulb starts the redistribution of nutrients to this organ is greater, and at the end of the cycle there is a reduction in the mass of the vegetative part and an increase in the mass of the bulbs.

Higher dry mass in the bulb contributed to the greater accumulation of micronutrients in this organ during the harvest period. The decreasing sequence of micronutrient accumulation in onion in Experiment 1 was: B > Zn > Cu, and in Experiment 2 it was: Zn > B > Cu.

In this study, the chemical characteristics of the soil in Experiments 1 and 2 differed in terms of pH, organic matter (OM), base saturation and availability of micronutrients. This difference between the experiments may have interfered with lesser or greater absorption capacity and development of plants, affecting the accumulation of micronutrients in both

Table 9. Percentages of onion bulbs in Class 1, 2, 3, 4 and commercial (CY), non-commercial (NCY) and total (TY) yields of onion as a function of the experiments

Experiment	Class 1	Class 2	Class 3	Class 4	CY	NCY	TY
	(%)				(t ha^{-1})		
1 (2018)	4.92 a	7.37 b	70.55 a	17.28 a	95.80 a	4.80 a	100.50 a
2 (2019)	3.53 b	9.22 a	68.75 a	18.48 a	95.07 a	3.51 b	98.58 a
CV (%)	53.82	51.97	12.80	55.01	15.30	58.15	15.09
Mean standard error	0.29	0.56	1.15	2.27	1.88	0.85	1.94

Means followed by the same letter in the column do not differ statistically by t-test ($p > 0.05$)

experiments. In the area of Experiment 1, there was no history of prior cultivation, whereas in the area of Experiment 2, there was a history of cultivation in previous years with onion, beet, and carrot; consequently, pH and base saturation values were higher, with low contents of B, Cu, Zn and OM.

This information helps to explain the inferior results obtained in Experiment 2. According to Malavolta et al. (1997), pH values close to 7.0 reduce Cu and Zn availability in the soil solution. As the soil of Experiment 2 has a history of cultivation and has a greater pH than that of Experiment 1, this may be related to the addition of calcium via fertigation, carbonate and bicarbonate via irrigation water, which over time tends to contribute to an increase in pH (Maia et al., 2001), precipitating B with $CaCO_3$, and reducing its availability for plants.

Base saturation values of the soil in Experiments 1 and 2 were 29 and 75% and the soil pH was 4.8 and 6.3, respectively. These characteristics, mainly observed in Experiment 2, contributed to the reduction of Cu and Zn in the soil solution, interfering with absorption. As Resende (2005) explained, soils with pH above 6.0 and base saturation greater than 50% reduce the availability of Cu, Fe, Mn and Zn in the soil solution.

No significant differences were observed between treatments for the following variables: classification of bulbs, commercial and non-commercial yields (Table 9). However, between experiments, there were differences in the percentage of bulbs in the classes 1 and 2, with higher values of class 1 in the first experiment and class 2 in the second (Table 9).

Regarding the characteristics related to onion yield, the results obtained in this study are superior to those observed in the literature. Several studies confirm the positive influence of Zn (Kurtz & Ernani, 2010; Maurya et al., 2018; Rashid & Islam, 2019), B (Maurya et al., 2018; Rashid & Islam, 2019) and Cu (Rashid & Islam, 2019).

The positive influence of these micronutrients on onion yield found in the literature may be related to the conditions of the soil where the studies were carried out, as well as the applied doses. The studies are primarily carried out in regions of India, in the limestone soils, with alkaline pH, medium texture, low OM content, and high doses of B and Zn, as verified by Acharya et al. (2015); Manna & Maity (2016); Babaleshwar et al. (2017) and Maurya et al. (2018).

The lack of response to B, Cu and Zn applications in this experiment for yield may be related to the application of doses, which may not have been sufficient to influence yield, and factors such as pH, low content of organic matter and sandy soil, may also have influenced the response to fertilization with micronutrients

Although the treatments with micronutrients did not significantly influence onion yield, the results obtained are superior to those observed in the literature: Babaleshwar et al. (2017) obtained commercial yield of 30.35 t ha^{-1} and total yield of 36.04 t ha^{-1} with the application of 10 kg ha^{-1} of zinc sulfate, compared to the control. Manna & Maity (2016) obtained 25.89 and 30.74 t ha^{-1} of commercial and total yields of onion, respectively with foliar application of 0.5% B; Maurya et al. (2018) applied NPKS + 50 kg ha^{-1} of zinc sulfate, obtaining commercial and total yields of 24.10 and 24.6 t ha^{-1}, respectively. Acharya et al. (2015) performed foliar application of 0.5% zinc sulfate at 30 and 45 DAT and obtained a yield of 16 t ha^{-1} of onion.

Therefore, the yield above 80 t ha^{-1} obtained in this study was possibly influenced by favorable climatic conditions, such as little temperature variation and low relative air humidity, absence of rain, low incidence of pests and diseases, use of appropriate technologies, such as drip irrigation, fertigation, direct sowing, and dense cultivation.

In addition to these factors, the fertilization established according to soil analysis and recommendation for the crop contributed to increasing yield, which was higher than the national (31.95 t ha^{-1}) and the Northeast (28.00 t ha^{-1}) averages.

Several factors possibly interfered with the results obtained in this study. The chemical characteristics of the soil in Experiments 1 and 2 differed with respect to pH, OM, base saturation and consequently affected the availability of micronutrients in the soil solution. The micronutrients studied are more available under acidic pH conditions. Soil pH close to neutrality tends to reduce the availability to plants, mainly when associated with sandy soils and low organic matter content, influencing the plant's response to growth, B, Cu and Zn accumulation and yield.

Conclusions

1. Application of B, Cu and Zn did not influence the number of leaves, relation of bulb shape, leaf dry mass, bulb dry mass, total dry mass and yield of onion.

2. Application of B, Cu and Zn at doses of 1-2-1 kg ha^{-1} favored a greater accumulation of B, Zn and Cu in the bulb.

3. Higher number of leaves, leaf dry mass, bulb dry mass, total dry mass, class 1 bulbs and non-commercial yield were produced in Experiment 1.

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