



Fertigation with copper in beet crop in a semi-arid region¹

Fertirrigação com cobre na cultura da beterraba em região semiárida

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HIGHLIGHTS:

Beet response to copper fertigation depends on the initial concentration of this micronutrient in soil.

Necrotic spots on beet leaves are characteristic of copper toxicity.

Postharvest characteristics of tuberous root of beet are not influenced by copper fertigation.

ABSTRACT: Copper is an essential element for beet; however, it needs to be made available in adequate quantities since this nutrient, which is a heavy metal, can cause toxicity to plants and/or humans. This study aimed to evaluate the agronomic performance of beet fertigated with copper in a semi-arid region of Brazil. Two experiments were carried out in complete randomized blocks, with five treatments (0, 1.5, 3.0, 4.0, and 6.0 kg ha⁻¹ of copper) and four replicates. In 2019, the copper content was within the adequate range for beet. In 2021, fertigation with copper above 0.8 kg ha⁻¹ led to contents in the phytotoxicity range, which may explain the reduction in total and tuberous root dry mass accumulation. Fertilization with copper increased marketable yield in 2019 (17.32 t ha⁻¹ at the dose of 3.9 kg ha⁻¹ of copper), while, in 2021, there was no fit of the equations. Copper fertilization altered the tuberous root pH but did not influence the other tuberous root quality components. Plants fertilized with copper accumulated more copper in the leaves than in the tuberous root, with a maximum accumulation of 6.0 kg ha⁻¹ of copper in 2019 and 2021. Fertilization with 3.9 kg ha⁻¹ suits soils with low copper content.

Key words: *Beta vulgaris*, heavy metal, micronutrient, postharvest

RESUMO: O cobre é um elemento essencial à beterraba, no entanto, é necessário disponibilizá-lo em quantidade adequada uma vez que esse nutriente, que é um metal pesado, pode causar toxicidade às plantas e, ou, ao ser humano. Esta pesquisa teve como objetivo avaliar o desempenho agrônômico de beterraba fertirrigada com cobre em uma região semiárida do Brasil. Foram conduzidos dois experimentos em delineados em blocos casualizados ao acaso, com cinco tratamentos (0, 1.5, 3.0, 4.5 e 6.0 kg ha⁻¹ de cobre) e quatro repetições. Em 2019, o teor de cobre estava dentro da faixa adequada para a beterraba. Em 2021, a fertirrigação com cobre acima de 0.8 kg ha⁻¹ proporcionou teores na faixa de fitotoxicidade, o que pode explicar a redução no acúmulo de massa seca total e de raízes tuberosas. A adubação com cobre aumentou a produtividade comercial em 2019 (17.32 t ha⁻¹ na dose de 3.9 kg ha⁻¹ de cobre) enquanto que, em 2021, não houve modelo ajustado. A adubação alterou o pH da raiz tuberosa, mas não influenciou nos demais atributos de qualidade pós-colheita da raiz tuberosa. Plantas adubadas com cobre acumularam mais cobre nas folhas do que na raiz tuberosa, com máxima acumulada em 2019 e em 2021, na dose 6.0 kg ha⁻¹ de cobre. A adubação com 3.9 kg ha⁻¹ é adequada para solos com baixo teor de cobre.

Palavras-chave: *Beta vulgaris*, metal pesado, micronutriente, pós-colheita



INTRODUCTION

Copper (Cu) is nutrient for plants and participates in photosynthetic activity and photosynthetic electron transport, besides being an enzymatic cofactor (Morales et al., 2012; Mattos-Jr et al., 2023). The content of this micronutrient in semi-arid soils is usually classified as medium to low (Oliveira et al., 2018). Therefore, under these conditions, it may be necessary to use Cu fertilization to meet the nutritional demands of some crops.

Some crops, such as beet (*Beta vulgaris* L.), which is a vegetable of economic importance in Brazil, being part of the population's food routine due to its high nutritional value (Curvêlo et al., 2018; Guerra et al., 2022), do not have fertilizer recommendations established in the Brazilian semi-arid region for macronutrients and micronutrients. Therefore, it is necessary to use standard recommendations for regions with different edaphoclimatic characteristics (Trani et al., 2018). This aspect demonstrates the need to conduct research of this nature.

In terms of micronutrients, beetroot is less demanding in Cu than in iron, manganese, zinc, and boron (Silva & Klar 2014). However, according to Schmitt et al. (2022), high concentrations of Cu alter root growth and cause significant morphological changes in the tuberous root (Schmitt et al., 2022), in addition to reducing plant growth and increasing Cu content and accumulation in beet organs (Schmitt et al., 2020).

The reduction in beet growth due to excess Cu is attributed to the activation of the plant's antioxidant systems (Schmitt et al., 2022). Under these conditions, betacyanin accumulation and peroxide activity in active growth organs are the defense mechanisms activated by the plant to minimize the deleterious effects caused by excess Cu (Morales et al., 2012).

Another critical aspect of the study with Cu is the concern with food safety (Adress et al., 2015) since the excessive accumulation of Cu in food can harm human health (Schmitt et al., 2020). Considering that the literature fails to recommend Cu fertilization for beet established in semi-arid regions, this study aimed to evaluate the agronomic performance of beet fertigated with copper in a semi-arid region of Brazil.

MATERIAL AND METHODS

Two experiments were carried out at the Fazenda Experimental Rafael Fernandes, at the Universidade Federal Rural do Semi-árido - UFERSA, in Mossoró, RN, Brazil (5° 6' 37" S and 37° 23' 50" W, altitude 72 m). Field experiments were conducted between June and September in 2019 and between July and October in 2021. The experimental area is located in a semi-arid region, with an average temperature, between the months in which the experiments were carried out, of 26.6 °C in 2019 and 28.2 °C in 2021. In the respective years, the average values for relative humidity were 73.8 and 66.6%. The accumulated precipitation during the experimental period was 31.4 mm in 2019 and 1.0 mm in 2021.

The experimental farm has soil classified as Ultisol (United States, 2014), which corresponds to *Argissolo* in the Brazilian Soil Classification System (EMBRAPA, 2018). The chemical characterization of the soils in the areas was based on soil

samples collected at a depth of 0 to 20 cm and laboratory analysis (Table 1). According to Trani et al. (2018), Cu concentrations in the soil of the experimental area, in 2019 and 2021, are classified as low and medium, respectively. The 2019 soil had already been cultivated, while the 2021 soil was virgin. Both soils had a sandy texture.

The experiments were designed in complete randomized blocks, containing five treatments (0, 1.5, 3.0, 4.5 and 6.0 kg ha⁻¹ of Cu) and four replicates. The experimental unit was constituted by 72 plants each. The doses were established based on the recommendations of Trani et al. (2018). The copper source used was copper sulfate. A spacing of 0.25 x 0.10 m was used.

The plots had a total area of 4.5 m² (3.0 x 1.5 m), with a usable area of 2.8 m². Each plot, in both experiments, had six rows of plants, but only the four central rows were considered as usable area, also disregarding a row of plants at each end of the plot.

Plowing and harrowing of the soil were carried out before preparing the beds. 190 kg ha⁻¹ of P₂O₅ was applied in the form of single superphosphate (18% P₂O₅, 16% Ca and 10% S) (Silva et al., 2019), seven days before planting. Topdressing fertilization was carried out via fertigation with split applications of 120 kg ha⁻¹ of nitrogen and 180 kg ha⁻¹ of K₂O, 13.7 kg ha⁻¹ of magnesium, 47.25 kg ha⁻¹ of calcium and, only in 2021, 18.26 kg ha⁻¹ of sulfur. The nutritional sources were urea (45% N), magnesium sulfate (9% Mg and 12% S), calcium nitrate (14% N and 16% Ca), potassium chloride (60% K₂O) and potassium nitrate (13% N and 44% K₂O). Fertigation with Cu doses was also performed, split and applied weekly, starting at 23 and ending at 70 days after sowing (DAS), in the 2019 experiment, and starting at 29 DAS and ending at 76 DAS in 2021, according to the experimental conditions for soil and climate.

Water depths for irrigation were established based on crop evapotranspiration (ETc). In the interval between basal fertilization and thinning (20 DAS), microsprinkler irrigation was used. Subsequently, the drip irrigation system

Table 1. Chemical attributes of soils (0 – 20 cm) in experimental areas at the Rafael Fernandes Experimental Farm, in 2019 and 2021

Chemical attributes		2019	2021
pH	H ₂ O	6.3	5.2
O.M.	g kg ⁻¹	4.1	14.9
B	mg dm ⁻³	0.19	0.77
Cu	mg dm ⁻³	0.10	0.33
Fe	mg dm ⁻³	6.61	163.03
Mn	mg dm ⁻³	2.36	15.8
Zn	mg dm ⁻³	0.50	0.64
P	mg dm ⁻³	3.2	1.9
K	mg dm ⁻³	51.0	39.1
Na	mg dm ⁻³	8.1	2.3
Ca	cmol _c dm ⁻³	0.55	0.66
Mg	cmol _c dm ⁻³	0.25	0.08
H+Al	cmol _c dm ⁻³	0.33	0.20
SB	cmol _c dm ⁻³	0.97	0.84
CEC	cmol _c dm ⁻³	1.3	3.69
V	%	75	23.04

O.M- Organic matter; H+Al- Potential acidity; SB- Sum of bases; CEC- Cation exchange capacity; V- Base saturation

was installed, with pressure-compensating drippers with an average flow of 1.5 L h⁻¹, spaced at 0.30 m. Three drip lines were distributed per plot, 0.50 m apart.

Direct sowing of three fruit of beet, cultivar Fortuna, was carried out per hole. At 20 DAS, thinning was performed, leaving only one plant per hole. Manual weeding was carried out during both experiments. Chemical control was used for the phytosanitary management of *Cercospora beticola*, *Conoderus scalaris*, *Liriomyza* spp., *Fusarium* spp. and *Macrophomina* spp.

The contents of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), after sulfuric digestion, and Cu, after dry digestion, were determined (EMBRAPA, 2009) in the nutritional status diagnosis leaf (NSDL) of beet in 20 fully expanded leaves per plot at 55 DAS selected for analysis (Malavolta et al., 1997).

One day before harvest, five plants were collected per plot to measure plant height (cm) and number of leaves per plant. In the laboratory, the plants were cleaned, separated into shoots and tuberous roots, and dried in an oven with forced air circulation at 65 °C. After reaching constant mass, shoot dry mass (SDM), tuberous root dry mass (TRDM) and total dry mass (TDM), from the sum of SDM and TRDM, were obtained. Dry mass was determined in g per plant.

Dry digestion was carried out to determine the copper content in the leaves, in the tuberous root, following the methodology of EMBRAPA (2009), and the total content in the beet was calculated. From the dry mass and the Cu content, the accumulation in beet was determined, in mg per plant.

At 79 DAS, the two experiments were harvested. Marketable yield (MY) included beetroots with diameter from 50 mm (HORTBRASIL, 2006) and that did not show defects, such as cracks, or phytosanitary problems. Those that did not include these characteristics were considered non-marketable (NMY). Total yield (TY) was defined from the sum of CY and NMY, all measured in tons per hectare (t ha⁻¹).

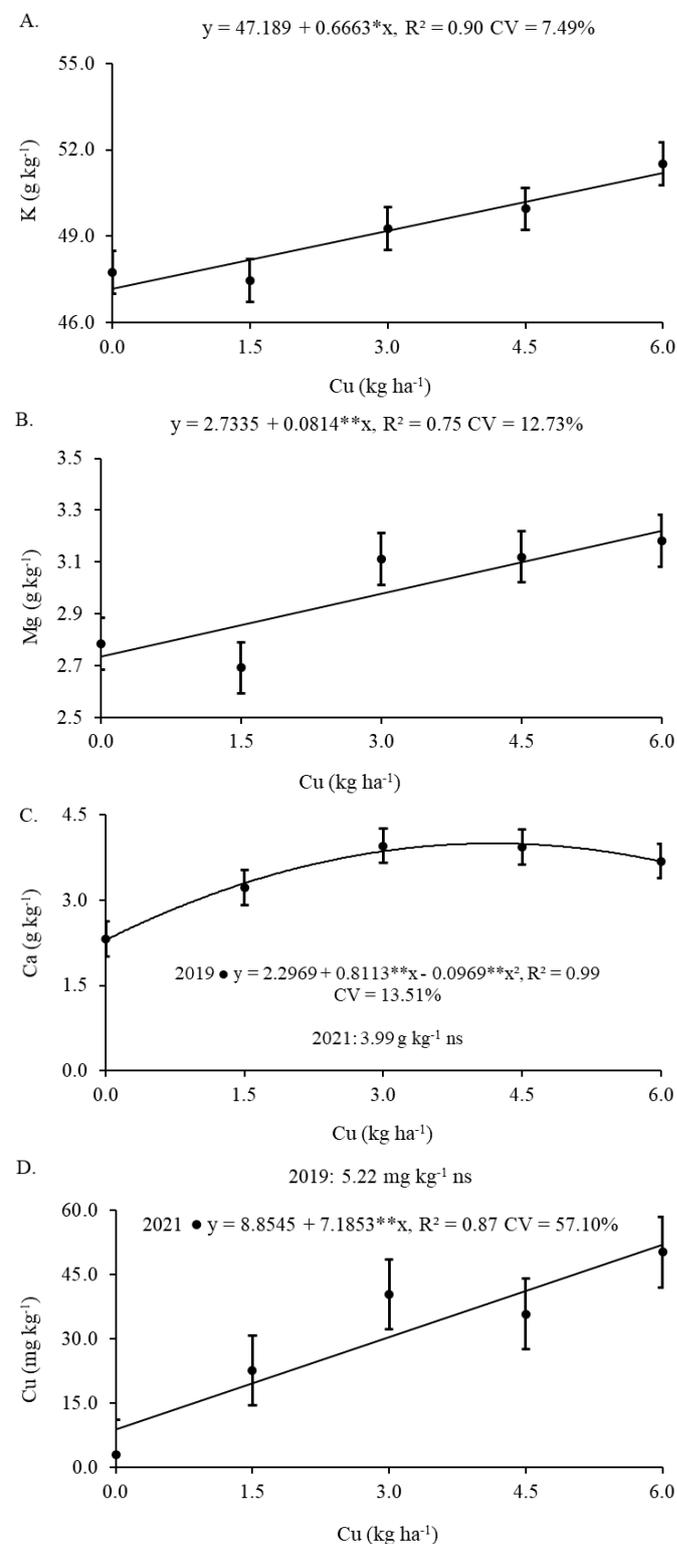
Quality attributes were determined after obtaining the extract of 10 randomly selected marketable tuberous roots. The pH was evaluated using a digital pH meter, soluble solids (SS) (%), by means of a digital refractometer and titratable acidity (mEq. 100 g⁻¹) (TA) (Zenebon et al., 2008). SS/TA ratio was defined from the SS and TA data. The anthrone method was used in the analysis of total soluble sugars (%) (TSS) (Yemm & Willis, 1954).

Data from the 2019 and 2021 experiments were separately subjected to analysis of variance using the F test. When the ratio of the highest residual mean square value was not greater than four times the lowest value, the data was jointly analyzed (Ferreira, 2018). When significant for the Cu dose factor, regression analysis was applied. SISVAR v.5.3 software (Ferreira, 2019) was used.

RESULTS AND DISCUSSION

The increase in copper doses increased linearly K and Mg content in NSDL. The maximum K content was 51.2 g kg⁻¹ (Figure 1A) and the maximum Mg content was 3.2 g kg⁻¹ (Figure 1B). These contents, for both nutrients, were obtained with fertilization of 6.0 kg ha⁻¹ of Cu. There was an interaction

between the factors Cu doses and years for Ca and Cu contents in the leaf. In 2019, fertilization with up to 4.2 kg ha⁻¹ of Cu increased the Ca content (3.4 g kg⁻¹) compared to the control treatment (2.3 g kg⁻¹). In the year 2021, there was no fit of the equations, with 3.4 g kg⁻¹ of Ca being the average content (Figure 1C). In the 2019 experiment, there was no significant fit of any equation studied for Cu content in the NSDL, with 5.2



** - Significant at $p \leq 0.01$ by the F test; * - Significant at $p \leq 0.05$ by the F test; ns - non-significant at $p < 0.05$. Vertical bars represent the standard error of the mean

Figure 1. Potassium (A), magnesium (B), calcium (C) and copper (D) contents in the nutritional status diagnosis leaf (NSDL) of beet in function of Cu doses

mg kg⁻¹ being the average Cu content between treatments. In 2021, the increase in Cu content was linear, with a maximum of 52.0 mg kg⁻¹, at the maximum dose applied (Figure 1D).

P content was significantly influenced ($p < 0.05$) by Cu fertilization, but there was no significant fit of any studied equation. The mean P content between treatments was 5.1 g kg⁻¹. For N content, fertilization with Cu did not statistically influence ($p < 0.05$). Comparing the averages between the 2019 and 2021 experiments, there was a statistical effect for N, P and K. For N, a higher content was observed in 2021, while higher P and K contents occurred in 2019. For Mg there was no significant difference between the averages of the years (Table 2). Cu fertilization had no significant effect ($p < 0.05$) on beet height. There was a significant difference between the years in which the experiments were conducted, with taller plants in 2021 (Table 2).

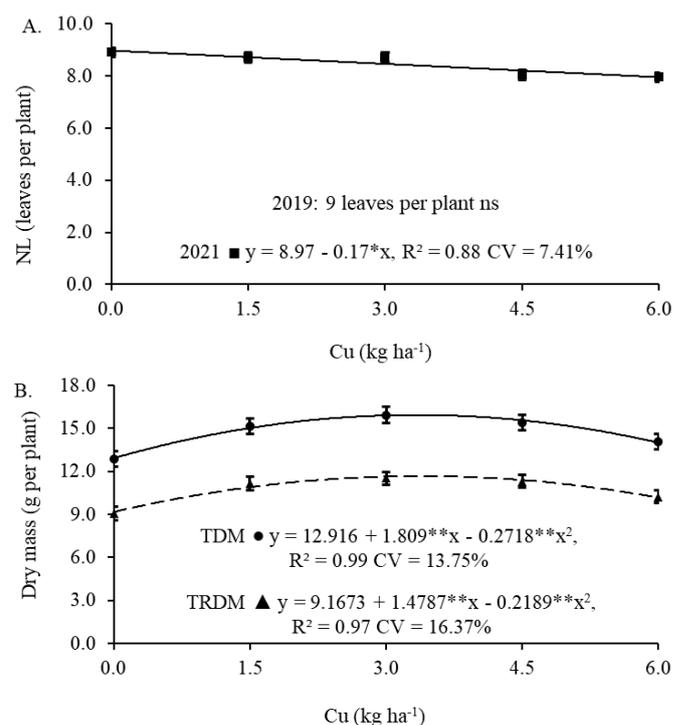
In 2019, the Cu content was within what is considered ideal for beet, while in 2021, doses greater than 0.8 kg ha⁻¹ (14.60 mg kg⁻¹) led to Cu contents higher than what is considered adequate, 5 – 15 mg kg⁻¹ (Trani et al., 2018). The difference in Cu contents in the NSDL in the 2019 and 2021 experiments can be explained by the Cu content in the soil of the 2021 experimental area (Table 1). Excess Cu in plants causes oxidative damage due to increased concentration of reactive oxygen species (Adress et al., 2015). With this, plants develop mechanisms that minimize the effect of toxicity by heavy metals, such as Cu. In beet, the defense mechanism against oxidative damage caused by excess copper is betacyanin accumulation and peroxidase activity in an active growth organ (Morales et al., 2012).

P and K contents were significantly higher in 2019, possibly due to the greater availability of these nutrients in the soil that year. According to the leaf content ranges considered adequate for beet as proposed by Trani et al. (2018), the N contents in 2019 and 2021 were within the range (30 – 50 g kg⁻¹) of adequate values for beet. The P and K contents in 2019 were above the maximum limit considered ideal for the crop, 4 and 40 g kg⁻¹, respectively, influenced by the P and K available in the soil of the experimental area in 2019. The K content in the NSDL was higher than the range established as ideal for beet, 20 – 40 g kg⁻¹, in all treatments. Leaf Mg contents up to the dose of 3.2 kg ha⁻¹ of Cu were lower than those reported by Trani et al. (2018), which suggests Mg deficiency. Despite the increased Mg content, the maximum value was close to the minimum limit, 3 g kg⁻¹ (Trani et al., 2018). In both experiments, the Ca content was below 25 g kg⁻¹, the minimum content defined for beet (Trani et al., 2018). Despite the indication of Ca deficiency, no visual symptoms were observed. In 2021, P and K in leaves

were within the recommended range. For Mg, the values for 2019 and 2021 were close to 3 g kg⁻¹, the ideal minimum value. The range was established by Trani et al. (2018) as a reference.

There was interaction between dose and year factors for the number of leaves per plant. In 2019, no fits were obtained for the studied equations. In the 2021 experiment, the number of leaves decreased linearly with increasing Cu doses (Figure 2A). The highest total (15.89 g per plant) and tuberous root (11.66 g per plant) dry mass of beet was achieved with 3.4 kg ha⁻¹ of Cu (Figure 2B). Cu did not cause significant effect on SDM. When comparing the average dry mass of the experiments, it was found that in 2021 the SDM, TRDM and TDM were significantly ($p \leq 0.05$) higher than the 2019 results (Table 2).

The decrease in the number of leaves in 2021 due to copper fertilization can be explained by the greater availability of this micronutrient in the soil that year. This result is also justified by the Cu content in the NSDL above the recommended levels for beet. Copper is a heavy metal that, depending on the amount available to be absorbed by the plant, can cause physiological, morphological, and molecular disorders at all stages of growth (Rahman et al., 2012; Adress et al., 2015; Htwe et al., 2020). In excess, Cu can be cytotoxic, inducing plant stress and



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Figure 2. Number of leaves (NL) (a) and total dry mass (TDM) and tuberous root dry mass (TRDM) (b) of beet in function of Cu doses

Table 2. Average content of N, P, K and Mg in the nutritional status diagnosis leaf, dry mass and yield of beet fertilized with Cu in the years 2019 and 2021

Year	N	P	K	Mg	HEI	SDM	TRDM	TDM	NMY (t ha ⁻¹)
	(g kg ⁻¹)				(cm)	(g plant ⁻¹)			
2019	31.5 b	6.6 a	58.9 a	2.9 a	28.38 b	3.34 b	9.29 b	14.10 b	1.19 a
2021	43.9 a	3.7 b	39.4 b	3.1 a	30.11 a	4.70 a	12.00 a	15.34 a	0.99 a
CV (%)	7.50	13.57	7.49	12.73	6.59	17.50	16.37	13.75	49.78

N - Nitrogen; P - Phosphorus; K - Potassium; Mg - Magnesium; HEI - Height; SDM - Shoot dry mass; TRDM - Dry mass tuberous root; TDM - Total dry mass; NMY - Non-marketable yield; CV - Coefficient of variation. Averages followed by the same letter in the column do not differ significantly ($p \leq 0.05$) from each other

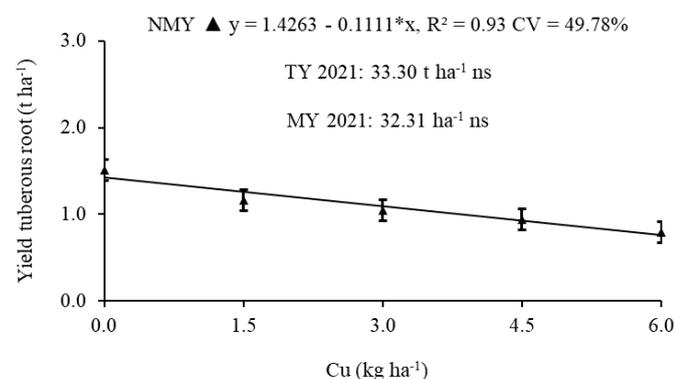
causing damage (Nagajyoti et al., 2010). Biomass reduction and leaf chlorosis are characteristic of plants exposed to high levels of Cu (Yruela, 2005). Furthermore, increasing the Cu content in the soil solution significantly reduces root growth, culminating in poor plant development (Schmitt et al., 2022). Levels of lipid peroxidation and hydrogen peroxide (H_2O_2) in beet leaves remained stable when plants were subjected to doses of Cu in the nutrient solution, indicating the efficiency of the plant's antioxidant system due to increased peroxidase activity (Schmitt et al., 2022). However, in order to maintain homeostasis under stress, the same authors state that, possibly, the energy required to maintain the antioxidant system culminated in the reduction of dry mass by the plant. This could also explain the reduction of TRDM and TDM at doses above 3.4 kg ha^{-1} of Cu.

Total and marketable yields were significantly influenced by the interaction between Cu doses and years. In 2019, the highest TY (18.46 t ha^{-1}) ($y = 11.348 + 3.748**x - 0.4933**x^2$, $R^2 = 0.58$, $CV = 7.89\%$) and CY (17.32 t ha^{-1}) ($y = 9.8955 + 3.7875**x - 0.4828**x^2$, $R^2 = 0.59$, $CV = 7.87\%$) were achieved with the application of 3.8 and 3.9 kg ha^{-1} of Cu, respectively. In 2021, fits were not obtained for the studied equations for TY and CY, which had averages of 33.30 and 32.31 t ha^{-1} , respectively (Figure 3).

Cu fertilization reduced the NCY of beet tuberous roots. The lowest NCY was obtained with fertilization of 6.0 kg ha^{-1} of Cu, 0.75 t ha^{-1} , which corresponds to a decrease of 53% compared to the control treatment (Figure 3). NCY did not differ statistically ($p < 0.05$) between the 2019 and 2021 experiments (Table 2).

The difference between the 2019 and 2021 yields was possibly influenced by phytosanitary problems that resulted in the loss of plants at the end of the cycle of the first experiment. Compared with results obtained by other authors, 25.40 t ha^{-1} (Silva et al., 2019) and 21.94 t ha^{-1} (Batista et al., 2016) in the same experimental area, lower yield was found in 2019, while in 2021 it was higher, even being close to the national average, which varies between 35 and 50 t ha^{-1} (Trani et al., 2018).

There was interaction between the years of the experiments and the doses of copper for the accumulation of Cu in the leaves, in the tuberous root and in the whole beet plant. The

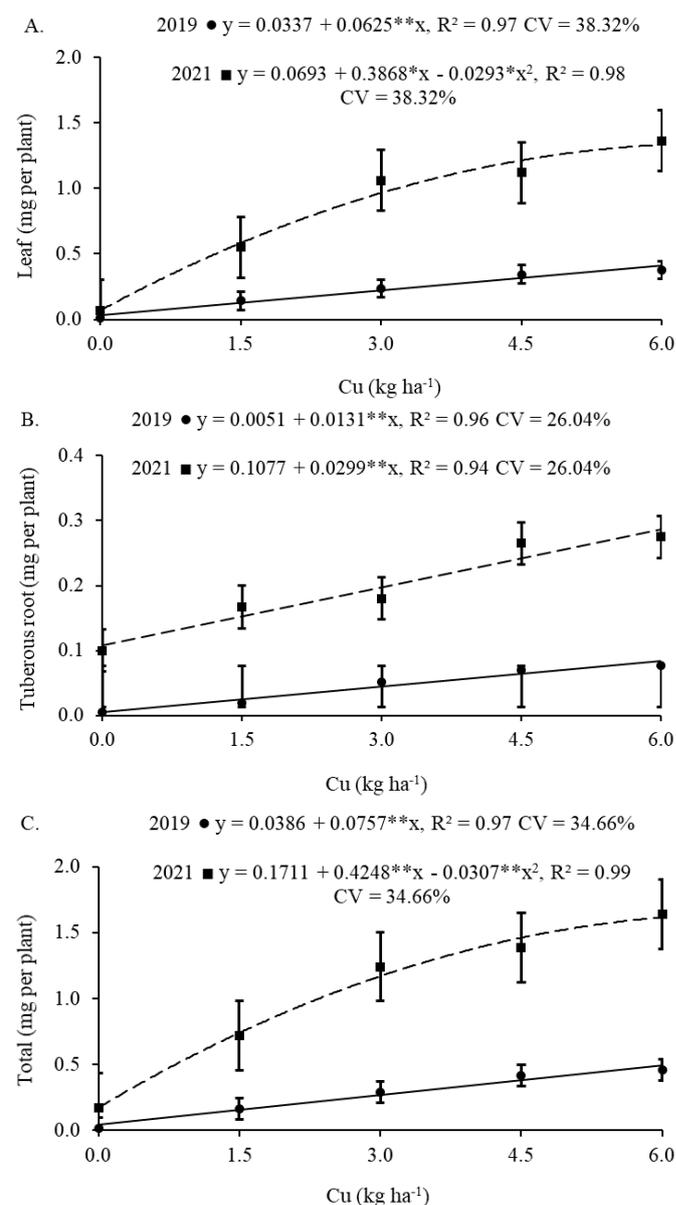


* - Significant at $p \leq 0.05$ by the F test; ns - non-significant at $p < 0.05$. CV - coefficient of variation. Vertical bars represent the standard error of the mean

Figure 3. Marketable tuberous root yield (MY), non-marketable tuberous root yield (NMY) e total tuberous root yield (TY) of the beet in function of Cu doses

largest accumulations in 2019 and 2021 occurred with 6.0 kg ha^{-1} of Cu. In 2019, leaves accumulated $0.40 \text{ mg per plant}$, tuberous root accumulated $0.09 \text{ mg per plant}$, and the total accumulation was $0.49 \text{ mg per plant}$. In the year 2021, Cu accumulations in the leaves, tuberous root and in the whole plant were 1.33 , 0.28 and $1.61 \text{ mg per plant}$, respectively (Figure 4).

In the 2021 experiment, the plants accumulated more copper than the 2019 ones. This result is possibly related to the higher content of Cu available in the soil in the 2021 experimental area. In both experiments, Cu had greater accumulation in the leaves when plants were subjected to Cu doses in the plant organs, indicating low redistribution of this micronutrient in the beet plant. In the 2019 and 2021 experiments, at the end of the cycle, necrotic spots were observed on the leaves of the plots corresponding to the doses 4.5 and 6.0 kg ha^{-1} of Cu. The spots are attributed to excess copper since the other treatments did not show these foliar



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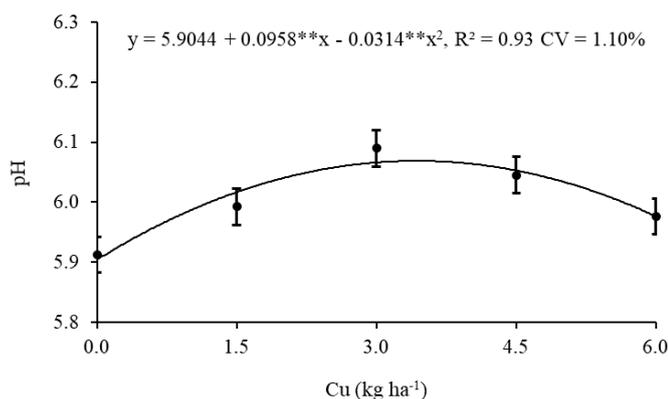
Figure 4. Accumulation of Cu in leaves (A), tuberous root (TR) (B) and total (C) of beet in function of Cu doses

symptoms. Symptoms of chlorosis and leaf necrosis caused by Cu stress are reported by Kumar et al. (2021).

For the quality variables, there was a significant effect of fertilization with Cu only for pH. There was an increase in pH with the application of Cu up to a dose of 3.2 kg ha⁻¹ (Figure 5). The average values of pH and TA were statistically higher in the 2019 experiment. In 2021, the average SS contents and SS/TA ratio were significantly ($p < 0.05$) higher. The TSS content did not differ statistically ($p < 0.05$) between years (Table 3).

Cu doses did not influence postharvest quality attributes, except for tuberous root pH. However, the chemical characteristics of the soils may have influenced the characteristics of pH, TA, SS, and SS/TA ratio since there was a difference between the means obtained in the 2019 and 2021 experiments. Influence of nutrient concentration in the soil on postharvest quality attributes of beet tuberous root has also been reported by Costa et al. (2023a) and Costa et al. (2023b).

As a result, the 2021 marketable tuberous root showed greater sweetness and a milder flavor since it had lower acidity and higher SS content, a result similar to that obtained by Costa et al. (2023b).



** - Significant at $p \leq 0.01$ by the F test; CV - coefficient of variation. Vertical bars represent standard error of the mean

Figure 5. pH of the tuberous root of beet in function of Cu doses

Table 3. Mean values of tuberous root quality attributes of beet fertilized with Cu, in 2019 and 2021, in Mossoró, RN

Year	pH	TA (mEq. 100 g ⁻¹)	SS (%)	SS/TA	TSS (%)
2019	6.03 a	3.11 a	10.60 b	3.62 b	8.22 a
2021	5.97 b	2.03 b	12.60 a	6.43 a	8.88 a
CV (%)	1.10	28.39	9.78	23.82	12.83

pH- Hydrogen potential; TA - Titratable acidity; SS - Soluble solids; TSS - Total soluble sugars. CV - Coefficient of variation. Averages followed by the same letter in the column do not differ significantly ($p \leq 0.05$) from each other

CONCLUSIONS

1. In soils with low copper content, fertilizing with 3.9 kg ha⁻¹ of Cu is recommended for the beet crop.
2. The deleterious effects of excess copper on beet occur on dry mass accumulation and on the number of leaves.
3. Copper does not alter the postharvest quality (titratable acidity, soluble solids, soluble solids to titratable acidity ratio, and total soluble sugars) of beet tuberous root.

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