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ORIGINAL ARTICLE

Biofortification of waterleaf (*Talinum triangulare*) seedlings with zinc and its benefits to growth and development

Biofortificação de mudas de cariru (Talinum triangulare) com zinco e seus benefícios ao crescimento e desenvolvimento

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ABSTRACT: Zinc (Zn) is an important micronutrient in the metabolic processes of plants and animals, and its deficiency in humans can cause several physiological disorders. One way to increase the availability of this nutrient is through the biofortification technique. The waterleaf (*Talinum triangulare* (Jacq.) Willd) is a vegetable that has great potential for biofortification, since it is consumed and cultivated in several countries. Thus, the aim of this work is to analyze the benefits of zinc biofortification to the growth and development of *T. triangulare* seedlings. The experiment was carried out during the months of February to May of 2019, at the Laboratory of Plant Physiology and Growth of the Federal University of Western Pará (UFOPA), in Santarém, state of Pará, Brazil. Six Zn concentrations were applied via soil: T1 - control; T2 - 12.5 mg kg^{-1}; T3 - 25 mg kg^{-1}; T4: - 50 mg kg^{-1}; T5 - 100 mg kg^{-1}; and T6 - 200 mg kg^{-1}. The variables analyzed were: length, number of mature and new leaves, buds, dry leaf mass, stem, root, aerial and total part, in addition to the root/aerial part ratio, relative growth rate and zinc and manganese contents. The T6 provided an increase of 284% in zinc and a reduction of 23.97% in manganese compared to T1. Growth and development variables were not significantly affected by different doses of Zn. The T5 and T6 proved to be the most suitable for the biofortification of the species, however additional studies are necessary.

RESUMO: *O zinco (Zn) é um micronutriente importante nos processos metabólicos de plantas e animais, e sua deficiência em humanos pode causar diversos distúrbios fisiológicos. Um modo de aumentar a disponibilidade desse nutriente é através da técnica de biofortificação. O cariru (Talinum triangulare (Jacq.) Willd) é uma hortaliça que apresenta grande potencial de biofortificação, pois é consumida e cultivada em diversos países. Assim, o objetivo deste trabalho é analisar os benefícios da biofortificação com zinco no crescimento e desenvolvimento de mudas de Talinum triangulare. O experimento foi realizado durante os meses de fevereiro a maio de 2019, no Laboratório de Fisiologia Vegetal e Crescimento de Plantas da Universidade Federal do Oeste do Pará (UFOPA), em Santarém, estado do Pará, Brasil. Foi aplicado via solo seis concentrações de Zn: T1 - controle; T2 - 12,5 mg* kg^{-1}*; T3 - 25 mg* kg^{-1}*; T4 - 50 mg* kg^{-1}*; T5 - 100 mg* kg^{-1} *e T6 - 200 mg* kg^{-1}*. As variáveis analisadas foram: comprimento, número de folhas maduras, novas e de brotos, massa seca das folhas, caule, raiz, parte aérea e total, além da relação raiz/parte aérea, taxa de crescimento relativa e teores de zinco e manganês. O T6 proporcionou incremento de 284% de zinco e redução de 23,97% de manganês em relação ao T1. As variáveis de crescimento e desenvolvimento não foram afetadas significativamente pelas diferentes doses de Zn. O T5 e T6 demonstraram-se os mais adequados para a biofortificação da espécie, entretanto estudos adicionais são necessários.*

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1 Introduction

Zinc (Zn) is an essential micronutrient for both plants and human beings. In vegetables, Zn is important for the synthesis of proteins related to the metabolism of carbohydrates, lipids, nucleic acids and auxin phytohormone (Das *et al*., 2018), participating in the control of cell differentiation and proliferation, chlorophyll biosynthesis and breathing and photosynthesis processes (Broadley *et al.*, 2012).

In humans, Zn is a component of approximately 300 enzymes related to structural stability, transcription factors and expression of genes under stress conditions (Jomova & Valko, 2011). Despite its importance, Zn deficiency in the human population affects approximately one third of the world population (Muthayya *et al*., 2013), and can cause several physiological disorders, such as affecting the growth of children, causing problems in brain development, decreasing performance and productivity in physical activities and greater susceptibility to diseases such as pneumonia and diarrhea (Cakmak & Kutman, 2018).

Among the ways of combating Zn deficiency, the technique known as agronomic biofortification stands out (Cakmak *et al*., 2017), which consists of providing the element to plants, especially Zn, via root or leaf fertilization, so that the plant can absorb and provide them to the consumer (Velu *et al*., 2014).

The biofortification has been used more frequently in cereals, due to their high consumption and low amounts of bioavailable Zn (Cakmak & Kutman, 2018). However, highly consumed leafy vegetables also have a great potential for biofortification with Zn, as they are a significant source of micronutrients for populations, despite the low contribution to the necessary amounts of nutrient compared to animal products (White *et al*., 2018).

Thus, it is necessary to focus biofortification studies on vegetables, such as waterleaf (*Talinum triangulare* (Jacq.) Willd), from Talinaceae family (formerly Portulacaceae), which is a widely cultivated plant in the North and Northeast regions of Brazil, especially by the rural populations, and in other countries, such as Nigeria and Cameroon, on the African continent (Fasuyi, 2007).

Waterleaf, an unconventional food plant (UFP), is used in the preparation of dishes such as salads, broths, soups and stews, and has good amounts of calcium, phosphorus, vitamin C and micronutrients (Araújo, 2018). This plant presents a great economic and agronomic potential of cultivation and management, mainly because it is planted and consumed by Amazonian and African rural populations (Alexandre *et al.*, 2018), despite is not yet very commercialized.

Nevertheless, although waterleaf is a strong candidate for Zn biofortification studies, studies are needed in order to evaluate the benefits of zinc in the growth and development of this plant. Furthermore, it is important to establish the beneficial threshold of Zn concentration for this plant and its consumers.

Thus, this work aimed to analyze the benefits of zinc biofortification to the growth and development of *Talinum triangulare* seedlings.

2 Material and Methods

The experiment was carried out during the months of February to May of 2019, at the Laboratory of Plant Physiology and Growth of the Federal University of Western Pará (UFOPA), in Santarém, state of Pará, Brazil. *Talinum triangulare* seedlings were produced by cutting. Each cutting was removed from the median portion of the matrix plant, with the sizes ranged from 10 to 12 cm in length. The cuttings were placed in 200 mL pots, containing a mixture of sandy soil and commercial substrate (1:1 v v^{-1}). After 30 days of growth, the seedlings were transplanted to 500 mL pots, containing the same mixture of soil. The seedlings remained in a controlled environment, with 12 h photoperiod, temperature of 27ºC and daily irrigation.

Physical and chemical characteristics of the commercial substrate are shown in Table 1.

After 15 days of transplantation, Zn was applied to T. triangulare seedlings, root pathway. Zinc Sulfate Heptahydrate ($ZnSO_4.7H_2O$) was used as a Zn source, with the following dosages: T1: control, without Zn application; T2: 12.5 mg kg^{-1}; T3: 25 mg kg^{-1}; T4: 50 mg kg^{-1}; T5: 100 mg kg^{-1} and T6: 200 mg kg^{-1}.

Table 1. Physical and chemical attributes of the commercial substrate used for planting waterleaf (*T. triangulare*) seedlings.

Tabela 1. Atributos físicos e químicos do substrato comercial utilizado para plantio das mudas de cariru (*T. triangulare*).

pH	K	P	Ca	Mg	Al	H+Al	SB	T	V	O.M	Clay	Silt	Sand
	mg dm $^{-3}$				cmol$_c$dm $^{-3}$				%	dag dm $^{-3}$		%	
5.9	354	49.8	6.6	1.43	0.01	3	8.9	8.9	75	15.22	14.15	10.9	74.95

Growth analysis was performed before and 30 days after the application of Zn. The number of mature, new and sprout leaves was evaluated, in addition to the measurement of the total length (cm) of the seedlings, using a millimeter ruler. Dry mass was weighed: seedlings were removed from the pots, and their leaves, stems and roots were separated and dried in a greenhouse, inside paper bags, at a temperature of 60ºC, up to constant weight. Weighing was performed in an analytical scale to obtain the leaf (LDM), stem (SDM) and root (RDM) dry mass. These were used to measure the aerial part of the plant dry mass (APDM) and the total dry mass (TDM). Subsequently, the root/aerial part ratio (R/AP) was measured, which is used to assess which of these two parts there was the largest growth investment by plants.

To measure the mass gain of seedlings according to the initial mass and time, the Relative Growth Rate (RGR) was calculated, by the formula: RGR = ((ln P2 – ln P1)/T) (Hunt, 1982), considering the total dry mass (TDM), using plants before and after Zn application.

The analysis of Zn and Mn content was performed by the Soil Laboratory of the Brazilian Agricultural Research

Corporation (EMBRAPA) Eastern Amazon. Only three treatments 1 (control), 2 (12.5 mg kg^{-1}) and 6 (200 mg kg^{-1}) were evaluated, where 0.2 g of dry leaf tissue was used.

The experimental design used was entirely randomized, with six treatments and six replications. The data were submitted to the normality test of Shapiro Wilk and, when normality of the data ($p < 0.05$) was verified, analysis of variance was performed, with the Dunnet test.

3 Results and Discussion

Treatment 6 provided a 284% increase in zinc content in relation to the control (Figure 1). Significant increases in zinc contents due to the application of increasing doses of this nutrient have been observed in several studies with vegetables, such as mini lettuce and cabbage (Sago *et al.*, 2018; White *et al.*, 2018), since the presence of zinc in leaf tissue depends on the concentration of this element in the soil and its availability (White & Brown, 2010), thus, confirming the efficiency of cariru in zinc absorption, via soil application.

In the study of the chemical composition and energy content of the waterleaf by Manhães *et al.* (2008), verified the amount of 71 mg kg^{-1} of zinc in the dry mass of this plant, without fertilizing with Zn. In this study, the amount of 888.85 mg kg^{-1} of Zn was verified in the dry mass of *T. triangulare* seedlings submitted to a fertilizing of 200 mg kg^{-1} of zinc (T6), levels approximately 12.52 x higher than those found on the cited study before.

For the correct functioning of the body, the recommended daily intake is 10 mg day^{-1} of Zn for children aged 1 to 10 years, while for adults indications are 15 mg day^{-1} for men, 12 mg day^{-1} for women and 16 to 19 mg day^{-1} for lactating women (DHHS & DOF, 2005). To obtain the recommended ingestion of 15 mg day^{-1} of Znit would be necessary to intake 16.87 g of dry matter of this specie. However, as the waterleaf is consumed fresh, and has about 92.24% water (Manhães *et al.*, 2008), it is necessary to consume an amount of 217.39 g of its fresh matter to obtain the zinc daily concentration, which is equivalent to approximately a portion of the cariru sold at fairs.

As it is a large amount to be consumed and most of the brazilian population does not eat leafy vegetables regularly in the determined quantities (Silva & Claro, 2019), it's recommended to ingest other organic zinc forms to achieve the daily intakes of the micronutrient, because, according to Lima *et al*. (2019), minerals in their organic forms are more bioavailable to be absorbed by the body.

There is no statistical difference between the treatments for the Mn content. Although, the T6 plants showed a low decrease in the contents, in relation to the control (Figure 2). In the study of Adamczyk-Szabela *et al.* (2020), about the combined cadmium-zinc interactions altering the nutrients uptake by *Melissa officinalis*, this interaction significantly alters the manganese content in the plant, decreasing it. According to Ortega & Malavolta (2012) exist an antagonistic relationship between zinc and manganese, due to a cationic competition between these micronutrients, which can explain the tendency of T6 have a lower concentration of Mn than T1.

There was no difference between the treatments related to the number of buds ($p < 0.05$), but the T1 has a tendency of increase its quantities (Figure 3). In the study of Babajani *et al*. (2019), the treatments with zinc oxide and elemental selenium nanoparticles led to a great increase in growth and activation of lateral buds. This

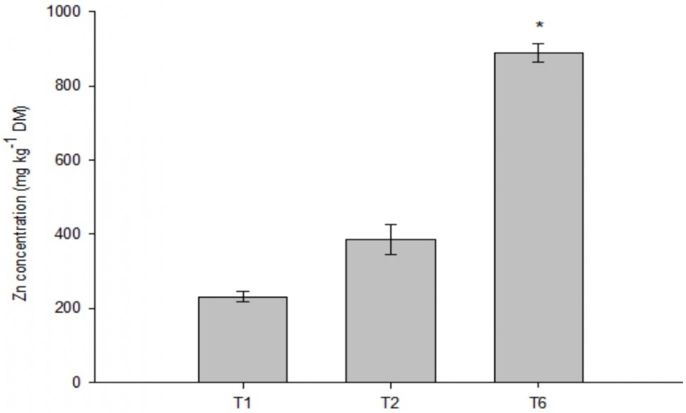

Figure 1. Zinc concentration (mg kg^{-1}) absorbed by waterleaf (*T. triangulare*) seedlings in three zinc application treatments (T1: control; T2: 12.5 mg kg^{-1} and T6: 200 mg kg^{-1}). The error bars represent the standard error of the mean (n= 3) and asterisk indicate significant difference in treatment compared to T1 plants (Dunnett $p < 0.05$).

Figura 1. Concentração de zinco (mg kg^{-1}) absorvido por mudas de cariru (*T. triangulare*) em três tratamentos de aplicação de zinco (T1: controle; T2: 12.5 mg kg^{-1} e T6: 200 mg kg^{-1}). As barras de erro representam o erro padrão das medias (n=3) e o asterisco indica diferença significativa no tratamento em comparação com as plantas do T1 (Dunnett $p < 0.05$).

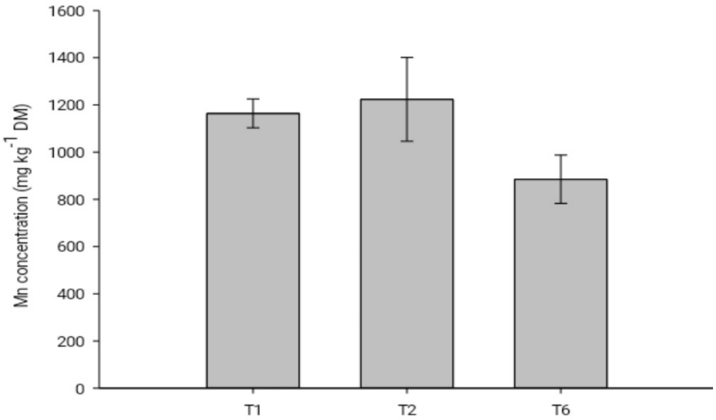

Figure 2. Manganese concentration (mg kg^{-1}) absorbed by waterleaf (*T. triangulare*) seedlings in three zinc application treatments (T1: control; T2: 12.5 mg kg^{-1} and T6: 200 mg kg^{-1}). The error bars represent the standard error of the mean (n = 3).

Figura 2. Concentração de manganês (mg kg^{-1}) absorvido por mudas de cariru (*T. triangulare*) em três tratamentos de aplicação de zinco (T1: controle; T2: 12.5 mg kg^{-1} e T6: 200 mg kg^{-1}). As barras de erro representam o erro padrão da média (n = 3).

result may be associated with the relationship of cytokinin and auxin phytohormones in the formation of lateral buds. The removal of the tip of the aerial part, a process carried out for the production of cuttings, breaks the apical dominance mediated by auxins, favoring the performance of cytokinins in the formation of lateral buds (Davies, 2004).

However, with the application of zinc, auxin levels tend to get higher again, because this mineral is involved in tryptophan synthesis, one of the precursors of indol-acetic acid (IAA), the main phytohormone of the auxin class, which will lead to inhibition or decrease in the formation of new lateral buds (Malavolta *et al*., 1997), which can be observed in the other treatments where Zn was applied.

No statistical difference was observed about the number of mature and new leaves into the six treatments (Figure 3), despite the plants of T5 had an inclination of having a greater number of mature leaves. The increase in the number of leaves produced due to the application of zinc has also be related in the work of Babajani *et al.* (2019). This may be an interesting feature for the biofortification of this vegetable, since its mature leaves and tender stalks are used in feeding (Araújo *et al*., 2018). Moreover, the higher number of leaves is an important variable at the time of purchase by the customer, who opts for more vigorous vegetables and with a high abundance of this edible share (Graciano *et al*., 2020), which encourages its marketing and consumption.

There were no differences between the length (cm) of

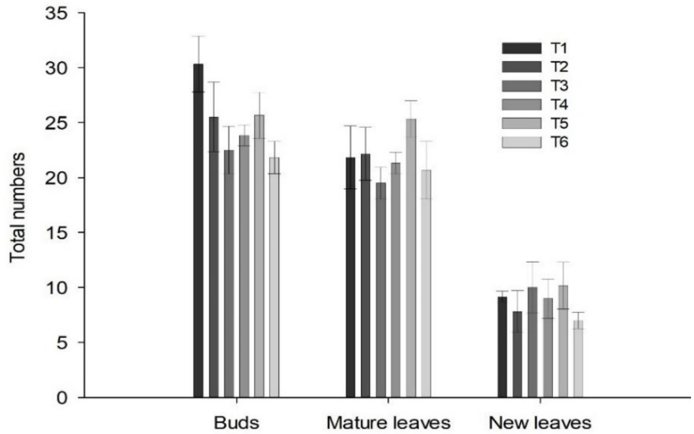

Figure 3. Number of buds, mature leaves, and new leaves of waterleaf (*T. triangulare*) seedlings in the six zinc application treatments (T1: control; T2: 12.5 mg kg^{-1}; T3: 25 mg kg^{-1}; T4: 50 mg kg^{-1}; T5: 100 mg kg^{-1}; and T6: 200 mg kg^{-1}). The error bars represent the standard error of the mean (n = 6).

Figura 3. Número de brotos, folhas maduras e folhas novas de mudas de cariru (*T. triangulare*) nos seis tratamentos de aplicação de zinco (T1: controle; T2: 12.5 mg kg^{-1}; T3: 25 mg kg^{-1}; T4: 50 mg kg^{-1}; T5: 100 mg kg^{-1}; and T6: 200 mg kg^{-1}). As barras de erro representam o erro padrão da média (n = 6).

the plants between treatments (Figure 4), instead in the presented in the work of Haider *et al.* (2018), where was increase in the height of mungbean with the zinc application. This can indicate that the different zinc concentrations did not have direct interference under the height of *T. triangulare* seedlings, which can be a favorable feature in terms of biofortification for this species, as it does not affect productive aspects even with high Zn concentrations in leaves.

No statistical difference between the treatments were

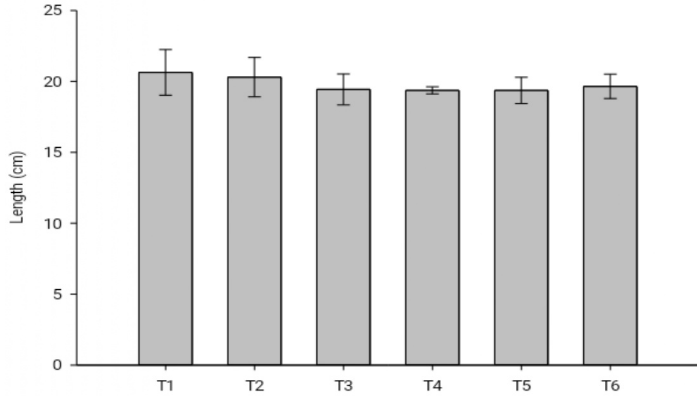

Figure 4. Length (cm) of waterleaf (*T. triangulare*) seedlings in six zinc application treatments (T1: control; T2: 12.5 mg kg^{-1}; T3: 25 mg kg^{-1}; T4: 50 mg kg^{-1}; T5: 100 mg kg^{-1} and T6: 200 mg kg^{-1}). The error bars represent the standard error of the mean (n = 6).

Figura 4. Comprimento (cm) de mudas de cariru (*T. triangulare*) em seis tratamentos de aplicação de zinco (T1: controle; T2: 12.5 mg kg^{-1}; T3: 25 mg kg^{-1}; T4: 50 mg kg^{-1}; T5: 100 mg kg^{-1} e T6: 200 mg kg^{-1}). As barras de erro representam o erro padrão da média (n = 6).

observed related the leaf, stem, root, aerial part and total dry mass (Figura 5). However, the dry mass of the stem, aerial part and total dry mass presented an increase tendency in the T5. Increases in dry mass with the increase in Zn concentration were also found by Salimi *et al*.

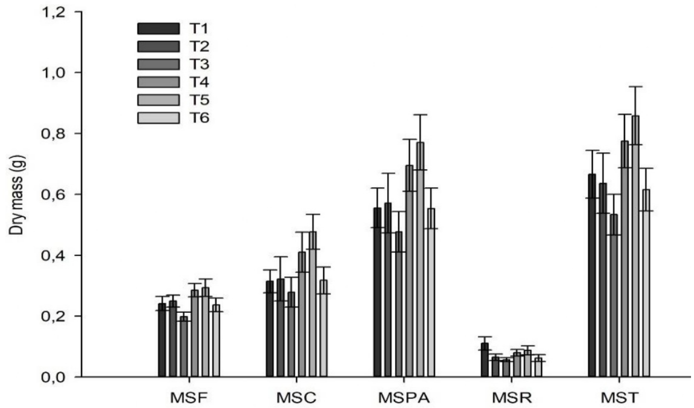

Figure 5. Leaf Dry Mass (LDM), Stem Dry Mass (SDM), Aerial Part Dry Mass (APDM), Root Dry Mass (RDM) and Total Dry Mass (TDM) of waterleaf (*T. triangulare*) seedlings in the six zinc application treatments (T1: control; T2: 12.5 mg kg^{-1}; T3: 25 mg kg^{-1}; T4: 50 mg kg^{-1}; T5: 100 mg kg^{-1}; and T6: 200 mg kg^{-1}). The error bars represent the standard error of the mean (n=6).

Figura 5. Massa seca da folha (MSF), massa seca do caule (MSC), massa seca da parte aérea (MSPA), massa seca da raiz (MSR) e massa seca total (MST) de mudas de cariru (*T. triangulare*) nas seis mudas de zinco tratamentos de aplicação (T1: controle; T2: 12.5 mg kg^{-1}; T3: 25 mg kg^{-1}; T4: 50 mg kg^{-1}; T5: 100 mg kg^{-1}; and T6: 200 mg kg^{-1}). As barras de erro representam o erro padrão da média (n = 6).

(2019), with leaf-fertilized tomato seedlings with 50 and 100 mg L^{-1} of zinc, indicating that the higher the availability of this nutrient in the environment, the greater its absorption and consequently its incorporation into the structures of the plant.

Despite there was no significant difference around the treatments about the root/aerial part ratio (R/AP) the T1 showed the largest value (Fig. 6), pointing that in this treatment there was greater investment on the plant roots than on its aerial parts. These results are possibly related to auxins, which in small amounts stimulate root growth, as seen in T1, while its presence in larger amounts inhibit root growth and promote aerial part growth (Davies, 2004), as observed in the other treatments.

There were no differences between the six treatments for the total dry mass (TDM) Relative Growth Rate (Figure 7). This can also be an interesting factor for the biofortification of the waterleaf, because fertilization with Zn, even in high doses, does not negatively affect its productivity, as it did not reach its toxicity threshold.

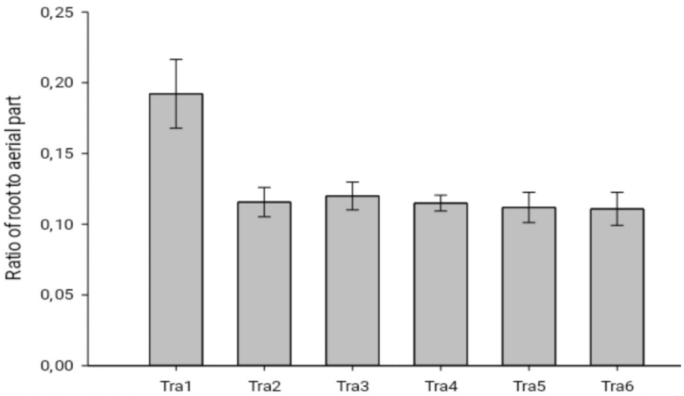

Figure 6. Root/Aerial Part Ratio (R/AP) of waterleaf (*T. triangulare*) seedlings in the six zinc application treatments (T1: control; T2: 12.5 mg kg^{-1}; T3: 25 mg kg^{-1}; T4: 50 mg kg^{-1}; T5: 100 mg kg^{-1} and T6: 200 mg kg^{-1}). The error bars represent the standard error of the mean (n=6).

Figura 6. Razão raiz/parte aérea (R/PA) de mudas de cariru (*T. triangulare*) nos seis tratamentos de aplicação de zinco (T1: controle; T2: 12.5 mg kg^{-1}; T3: 25 mg kg^{-1}; T4: 50 mg kg^{-1}; T5: 100 mg kg^{-1} e T6: 200 mg kg^{-1}). As barras de erro representam o erro padrão da média (n = 6).

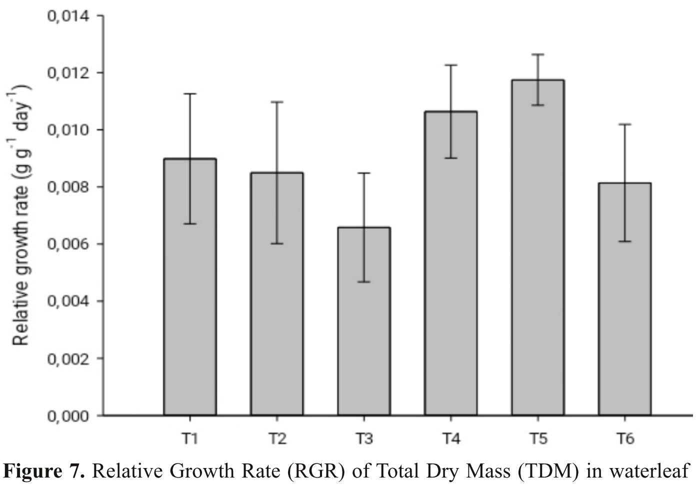

Figure 7. Relative Growth Rate (RGR) of Total Dry Mass (TDM) in waterleaf (*T. triangulare*) seedlings in six zinc application treatments (T1: control; T2: 12.5 mg kg^{-1}; T3: 25 mg kg^{-1}; T4: 50 mg kg^{-1}; T5: 100 mg kg^{-1} and T6: 200 mg kg^{-1}). The error bars represent the standard error of the mean (n = 6).

Figura 7. Taxa de crescimento relativo (TCR) da massa seca total (MST) em mudas de cariru (*T. triangulare*) em seis tratamentos de aplicação de zinco (T1: controle; T2: 12.5 mg kg^{-1}; T3: 25 mg kg^{-1}; T4: 50 mg kg^{-1}; T5: 100 mg kg^{-1} e T6: 200 mg kg^{-1}). As barras de erro representam o erro padrão da média (n = 6).

4 Conclusion

Growth and development variables were not significantly affected by different doses of Zn, who can be a favorable feature in terms of biofortification for this species. The T6 (200 mg kg^{-1} of Zn) provided the largest increase in zinc content, without impairing growth when compared to T1, and together with T5 (100 mg kg^{-1} of Zn), it presented the greatest potential for the biofortification of *T. triangulare*. However, additional studies are still necessary.

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