









Zinc sources in enrichment and yield of lettuce cultivars

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Abstract

Zinc is an important element of plant metabolism, playing roles in several enzymatic reactions. Biofortifying lettuce with Zn is a strategy to meet the demand for this nutrient via food. This work aimed to evaluate the effect of foliar fertilization with different sources of zinc on the agronomic biofortification of special lettuce cultivars under low-altitude tropical conditions. The experiments were carried out in two seasons in a randomized block design, in a factorial scheme (4 x 3) + 1, composed of four lettuce cultivars (SVR 2005[®], Romanela[®], Crocantela[®], and Rubinela[®]), three sources of Zn (Zn sulfate, Zn oxide, and Zn chelate), and a control without zinc application, with four replications. The following characteristics were evaluated: head diameter (in cm); fresh leaf mass (in g plant⁻¹); head volume (in cm³); yield (in t ha⁻¹), and Zn content (in mg kg⁻¹). The special lettuce cultivars presented head diameters within the commercial requirement. 'Romanela' showed the best results for head diameter, head volume, number of commercial leaves, fresh leaf mass, and yield. The Zn oxide was the best source to biofortify the special lettuces with foliar content of 260 mg kg⁻¹ for 'Rubinela'.

Keywords: biofortification, *Lactuca sativa* L., micronutrient, Zn

Introduction

Lettuce (*Lactuca sativa* L.) is a leafy vegetable traditionally consumed by Brazilians, which is grown in all Brazilian municipalities and is an important source of nutrients for the population. This vegetable has low caloric content and is rich in vitamins, mineral salts, and fiber (Trentini & Hojo, 2019).

When evaluating new lettuce cultivars in the edaphoclimatic conditions of a region, climatic factors can favorably or unfavorably interfere with production. Under stress conditions, such as high temperatures, lettuce plants tend to reduce their cycle, compromising the quality of the leaves, which become more rigid and with a bitter taste as they pass from the vegetative to the reproductive phase prematurely, making the product unviable for commercialization (Milhomens et al., 2015).

In lettuce, zinc is an important element of plant metabolism, playing roles in various enzymatic reactions

and contributing to plant tolerance to environmental stress factors, being involved in protein synthesis, carbohydrate, and auxin metabolism (Angelini et al., 2020). In cerrado soils, zinc concentration is low, and this can be attributed to fertilization management practices that do not replace this element in the amount that is extracted by plants, low levels of organic matter, high phosphorus content in the soil, and high nitrogen fertilizers doses, which is very common in lettuce.

Soils with high concentrations of P can inhibit Zn absorption because the absorption rate of this element does not increase quickly enough to maintain the necessary concentration in the shoot, characterizing a dilution effect (Batista et al., 2018). Regarding absorption, according to Malavolta et al. (1997), there may be Zn insolubilization by phosphate on the root surface or non-competitive inhibition of Zn absorption by P.

The absence of zinc in lettuce promotes a

reduction in the leaf area and in the texture of the leaves, which become leathery and with necrosis on the edges (Prado & Cecílio Filho, 2016). Information regarding the foliar application of zinc aimed at biofortification of the lettuce plant is still restricted and producers often carry out these foliar applications containing zinc without the guarantee that this procedure is adequate and efficient to promote the accumulation of these nutrients in the plant (Lopes & Guilherme, 2016).

There are few studies related to agronomic biofortification with zinc in leafy vegetables. This study was proposed considering the zinc deficiency in soils, the importance of this element for plants and human beings, and due to the choice of special lettuce cultivars. In this sense, the objective was to evaluate the effect of foliar fertilization with different sources of zinc on the morphological characteristics and agronomic biofortification of special lettuce cultivars in tropical conditions of low altitude.

Material And Methods

Experiments were conducted in the Vegetable Science sector of the University Campus of Gurupi – CAUG, of the Federal University of Tocantins Foundation, located at south latitude 11°43'45" and west longitude 49°04'07" with an average altitude of 280 m. The region's climate is characterized by a semi-humid tropical climate, with a dry season of approximately 4 months. With these temperatures and rainfall rates, the climate receives the classification of AW – Tropical with humid summer and dry period in winter, according to the classification of Köppen & Geiger (1928). The climatic conditions of the period during which the experiments were carried out are shown in (Figure 1).

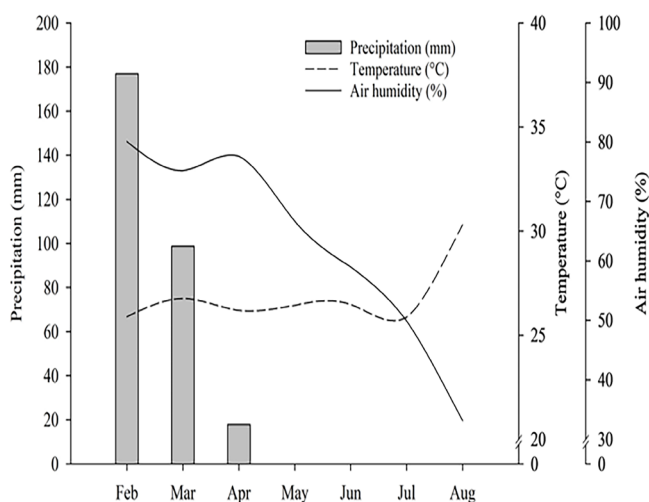


Figure 1. Temperature (in °C), relative air humidity (in %), and precipitation (in mm) during the experiment period. Gurupi - TO, 2021.

Two experiments were conducted. The first was in summer, between February and April; the second was from June to August. The experimental design adopted for each growing season was randomized blocks, in a factorial scheme (4 x 3) + 1, with four replications. In each season, four lettuce cultivars were evaluated: SVR 2005® (Seminis), Romanela®, Crocantela®, and Rubinela® (Feltrin Sementes), submitted to foliar application of three sources of Zn: Zn sulfate, Zn oxide, Zn chelate, and a control treatment without application of Zn, in the stages of full vegetative development (twenty-seven days after transplanting) with costal pump pressurized by CO₂ 183 L ha⁻¹.

The seedlings were produced in expanded polystyrene trays with 200 cells, filled with commercial substrate, in a seedling production nursery. The seedlings were transplanted when they presented the stage of 4 to 5 non-cotyledonary leaves.

The experiments were carried out in beds, with plots measuring 1.0 m in length and 1.0 m in width. Each plot consisted of 16 plants; the 4 central plants being considered as a useful plot. The spacing used was 0.25 cm between plants and 0.25 cm between rows. The doses established for foliar fertilization of each source of Zn were defined from the results of tests before the experiments, using 200; 72.50, and 562 g ha⁻¹ of Zn in the form of sulfate, chelate, and zinc oxide, respectively.

The base fertilization was carried out according to the nutritional requirement of the crop for cultivation in beds, using 150 g m⁻² of the 05-25-15 formulation, totaling 75 kg of nitrogen (N), 375 kg of phosphorus (P), and 225 kg ha⁻¹ of potassium (K). Tanned bovine manure in the amount of 5 kg m⁻² was also incorporated. As top-dressing, K₂O was used in the form of potassium chloride (KCl) and N and P in the form of MAP at 15 and 30 days after transplanting (DAT), via fertirrigation.

Irrigation was carried out daily by a drip system for 2 hours per day (one hour in the morning and one hour in the afternoon). Phytosanitary treatments were not necessary. Coverage with rice straw was carried out to maintain soil moisture and control weeds.

The harvest was carried out when the plants showed their maximum development, evaluating the following characteristics:

- Diameter of the head (DC, in cm): it was measured using a millimeter ruler in cm, where the plant was measured transversely;

- Number of commercial leaves (NCL): manually counted from the base to the apex, disregarding leaves smaller than three cm and senescent leaves;

- Stem length (SL, in cm): it was measured using a millimeter ruler;

- Fresh mass of the leaves (FML, in g plant⁻¹): obtained on a digital scale by weighing all the leaves of the plants in the useful plot;

- Head volume (HV, in cm³): it was obtained using a graduated container with a known volume with a capacity of 15 liters, where only 12 liters of water were placed inside the container. By the volume of water displaced by the plant in the container, the volume of the lettuce head was estimated;

- Yield (in ton ha⁻¹): it was obtained from weighing the plants in the useful plot and the value was converted to ton ha⁻¹.

- Zn content (in mg kg⁻¹ of dry mass) according to the Embrapa methodology (2009).

With the values of each plot, an average of the growing seasons was made and an analysis of variance was performed and the post-analysis, when significant, were compared using the Scott & Knott test (1974), with the Sisvar program version 5.6 (Ferreira, 2011). The mean contrast of the treatments without Zn application was performed with the treatments that received Zn applications, at the 5% probability level, with the Sisvar program version 5.6 (Ferreira, 2011).

Results And Discussion

No significant difference between the growing seasons was observed. Thus, the average of the growing seasons was considered to discuss the results concerning the sources of zinc and tropicalized cultivars.

The zinc sources significantly influenced the stem length of the cultivars (**Figure 2**). The cultivars 'Rubinela' and 'SVR 2005' differed from other cultivars when Zn sulfate was applied, resulting in lower averages of 3.51 and 3.45 cm, respectively, for this characteristic. Zn sulfate is one of the most used sources for soil or foliar applications in crops, due to its greater solubility in water (De Moraes et al., 2022).

Cultivares de alface tem o seu desenvolvimento ideal em temperaturas próximas de 22 °C, sendo a temperatura o fator decisivo para o bom desenvolvimento da cultura em uma região (Ferreira et al., 2018).

A stem with a length above the ideal is considered an undesirable characteristic, since the shorter the length, the greater the cultivar tolerance level to early bolting (Diamante et al., 2013), which, occurring early, makes the lettuce unfit for consumption, as there is production of latex that imparts a bitter taste to the leaves. Temperature is a decisive factor for adequate development of lettuce, which ideally should be close to

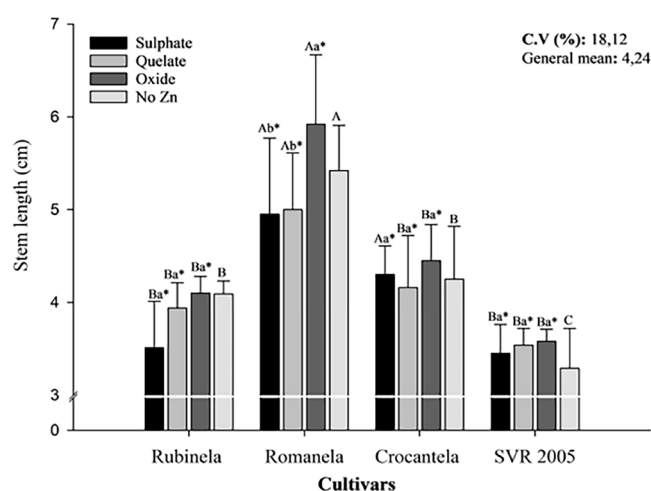


Figure 2. Mean of two planting times for stem length (SL, in cm) of four lettuce cultivars, as a function of foliar application of three sources of zinc. Gurupi – TO, 2021.

Capital letters in the bars indicate a difference between cultivars within each Zn source and lowercase letters indicate a difference between Zn sources within each cultivar, by the Scott-Knott test ($p = 0.05$). In the bars (*) indicates that there was no significant effect and (**) indicates a significant difference, of the control without Zn in relation to each source, employing of contrast ($p = 0.05$).

22 °C (Ferreira et al., 2018).

'Romanela' stem length differed from other cultivars when submitted to the application of chelate and oxide sources (Figure 2), which resulted in higher averages with results lower than 6.0 cm. Possibly, this occurred due the cultivar is not adapted to the environmental or temperature conditions of the region (Queiroz et al., 2014).

According to Müller et al. (2015), stem values up to 6.0 cm would be considered the most suitable for marketing lettuce, being acceptable up to 9.0 cm in length and considered unacceptable or not recommended above this value. In this sense, despite the statistical differences, none of the cultivars in this study had a stem length equal to or greater than the acceptable mark.

Brzezinski et al. (2017) evaluated four groups of lettuce cultivars and obtained an average stem length of 3.07 to 4.94 cm plant⁻¹, which are similar results to those obtained in this work.

The head diameter (HD) of cultivars varied according to the zinc source (**Figure 3**). When the sulfate source was applied, 'Rubinela' presented the lowest value for HD, with an average of 23.77 cm, which differs statistically from the other cultivars). For this trait, 'Romanela' stood out, as regardless of the treatment (with or without zinc application), this cultivar tended to produce heads with averages grouped in the group with the greatest diameters (Figure 3). This result can be

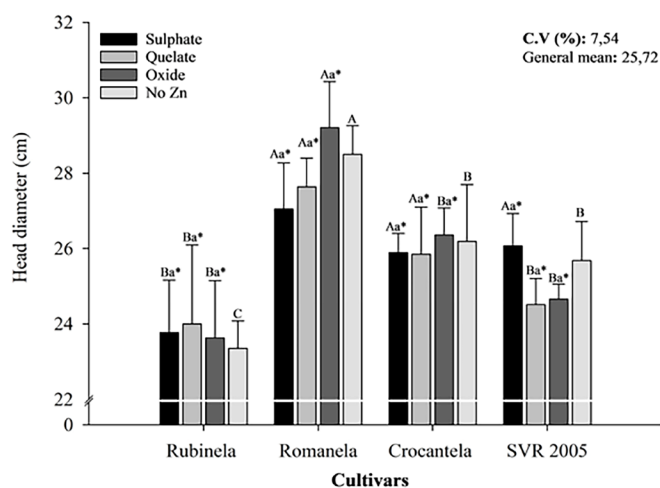


Figure 3. Mean of two planting times for head diameter (HD, in cm) of four lettuce cultivars, as a function of foliar application of three sources of zinc. Gurupi – TO, 2021.

Capital letters in the bars indicate a difference between cultivars within each Zn source and lowercase letters indicate a difference between Zn sources within each cultivar, by the Scott-Knott test ($p = 0.05$). In the bars (*) indicates that there was no significant effect and (**) indicates a significant difference, of the control without Zn in relation to each source, employing of contrast ($p = 0.05$).

justified by the genotype x environment interaction of each cultivar.

Special lettuce cultivars have not yet been tested in the edaphoclimatic conditions of the Gurupi, Tocantins, Brazil region, however, the results demonstrate that for the characteristic head diameter, the values are within acceptable standards by the market and that these cultivars have a genetic potential under high temperature and low altitude. 'SVR 2005' belongs to the curly lettuce group, being considered the preferred cultivar for cultivation by producers in the region, due to ease of transport and the liking of local consumers.

The head diameter is greatly affected by cultural practices and the inadequate recommendation of cultivars. Regarding this aspect, the results obtained in this work are similar to those of Mendes et al. (2020) who reported head diameters varying from 17.94 to 32.11 cm in semi-humid tropical conditions. Comparable results were also obtained by Costa Júnior et al. (2021), who evaluated the performance of cultivars in open field during the dry season of central Amazonia and observed a variation in head diameter from 16.00 to 25.50 cm.

The number of commercial leaves (NCL) is of great importance for the grower, as it indicates the adaptation of the genotype to the environment, as well as for commercialization. In this work, the number of commercial leaves varied between 15.41 and 23.10 leaves plant⁻¹, in which the highest average was observed for 'Romanela', followed by 'Crocantela' (Figure 4).

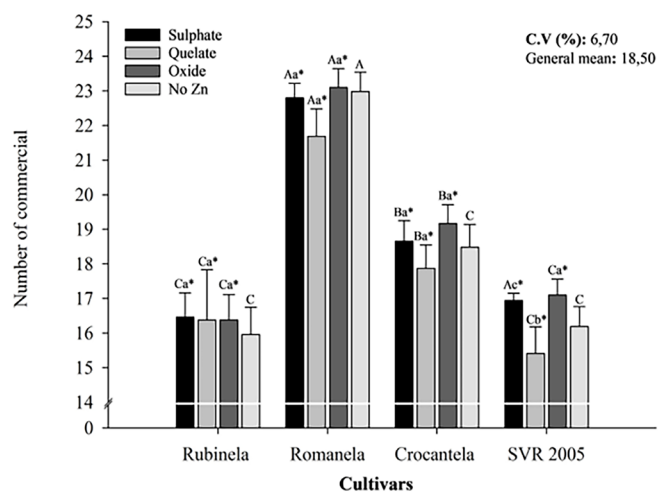


Figure 4. Mean of two planting times for the number of commercial leaves (NCL) of four lettuce cultivars, as a function of foliar application of three sources of zinc. Gurupi – TO, 2021.

Capital letters in the bars indicate a difference between cultivars within each Zn source and lowercase letters indicate a difference between Zn sources within each cultivar, by the Scott-Knott test ($p = 0.05$). In the bars (*) indicates that there was no significant effect and (**) indicates a significant difference, of the control without Zn in relation to each source, employing of contrast ($p = 0.05$).

The greater number of leaves per plant generally results in greater head volume, leaf area, fresh mass, and, consequently, greater yield (Souza et al., 2018). Given what was observed for the NCL, regardless of the application or not of Zn, 'Romanela' showed a significant increase compared to the other cultivars, possibly indicating that this characteristic can be influenced by the environment of cultivation, in addition to the genetic component. 'Romanela' also stood out for stem length, possibly indicating that a greater stem length will support a greater number of leaves.

Diamante et al. (2013) reported values ranging from 27.99 to 37.75 leaves per plant for butterhead lettuce cultivars, demonstrating that in addition to the environment, the NCL variation will also depend on the group to which the cultivars belong. Magalhães et al. (2015) evaluated curly-type cultivars and recorded averages of 8.21 to 14.63 leaves per plant.

The characteristics related to the size of the plant, such as head volume, provide important information as the main way of packaging lettuce plants for transport is in plastic boxes. Thus, plants with larger head dimensions can be damaged during the transport and storage process, affecting the final quality of the product (ABCSEM, 2016).

Considering the interaction of Zn sources within each cultivar, 'Romanela' had the highest head volume, with an average of 482.30 cm³, as a function of the foliar application of zinc oxide (Figure 5).

'Rubinella' presented the greatest head volume when Zn chelate was applied, whereas for 'Romanela', the chelate and oxide sources were the ones that stood out. However, 'Crocantela' showed the highest averages when Zn sulfate or oxide was used. 'SVR 2005' showed similar results regardless of the Zn source (Figure 5).

The head volume is a relevant attribute to market lettuce. The application of zinc on the leaves did not interfere with the productive characteristics, which demonstrates that this technique can be a promising alternative for growers since, in addition to the benefits to the population, value can be added to the product (Graciano et al., 2020).

The cultivars presented different head volumes when Zn was not applied. The highest head volume value was observed for 'Romanela' with an average of 459.19 cm³ and the lowest value for 'Rubinella' with an average of 277.35 cm³ (Figure 5).

Similar results were obtained by Mendes et al. (2020), who analyzed the head volume characteristic of lettuce plants and observed that there was a significant difference between the cultivars tested in the spring/summer period, in the municipality of Gurupi - TO, where 'Mimosa' from the curly segment had the highest index with an average of 0.295 dm³, and 'Lucy Brown' the with an average of 0.220 dm³.

The larger the head volume, the greater the number of leaves, being visually highly appreciated by consumers (Costa Júnior et al., 2021) who prefer plants with greater volume and average mass, but without

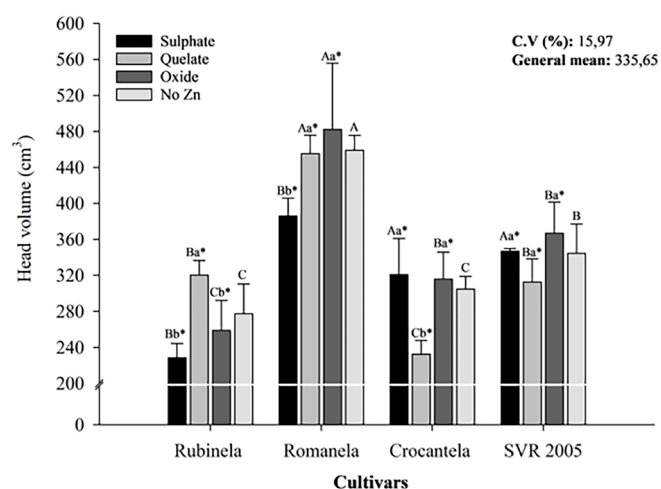


Figure 5. Mean of two planting times for head volume (HV, in cm³) of four lettuce cultivars, as a function of foliar application of three sources of zinc. Gurupi – TO, 2021.

Capital letters in the bars indicate a difference between cultivars within each Zn source and lowercase letters indicate a difference between Zn sources within each cultivar, by the Scott-Knott test ($p = 0.05$). In the bars (*) indicates that there was no significant effect and (**) indicates a significant difference, of the control without Zn in relation to each source, employing of contrast ($p = 0.05$).

visible or organoleptic signs of flowering. The superior values obtained by the special lettuce cultivars for head volume compared to 'SVR 2005' demonstrate the ability to express the genetic potential of each cultivar in the environment to which they were submitted.

'Romanela' and 'Crocantela' presented the highest leaf fresh mass values, with averages of 232.12 and 231.80 g plant⁻¹, respectively, as a function of Zn oxide foliar application, statistically differing from 'SVR 2005' (179.35 g plant⁻¹). As for the control without Zn application, the cultivars significantly differed as 'Romanela' presented an average of 200.00 g plant⁻¹, being superior to the other cultivars (Figure 6).

'Crocantela' and 'Romanela' are innovative cultivars, belonging to the crunchy segment and presenting advantageous characteristics for summer cropping in open field in conventional system of beds, being superior compared to the conventional curly cultivars (Rossi et al., 2020). The differences observed in the present work can be attributed to the genetic characteristics of each cultivar, since in addition to the treatments with zinc, the environmental factor may also have influenced the greater development of leaves for the special cultivars.

Superior results were found in work carried out by Rossi et al. (2020), who evaluated the physical characteristics of 'Romanela' in different production systems in the municipality of Araras – SP and observed an average of 413.30 g plant⁻¹ in conventional cropping.

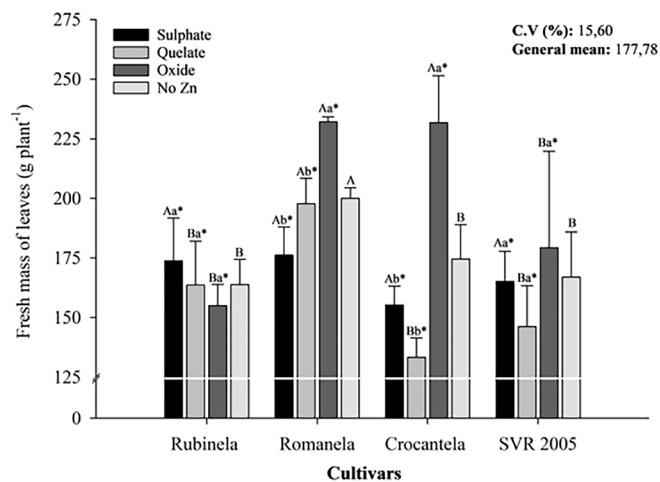


Figure 6. Mean of two planting times for fresh mass of leaves (FML, in g plant⁻¹) of four lettuce cultivars, as a function of foliar application of three sources of zinc. Gurupi – TO, 2021.

Capital letters in the bars indicate a difference between cultivars within each Zn source and lowercase letters indicate a difference between Zn sources within each cultivar, by the Scott-Knott test ($p = 0.05$). In the bars (*) indicates that there was no significant effect and (**) indicates a significant difference, of the control without Zn in relation to each source, employing of contrast ($p = 0.05$).

Lettuce leaves are the main component that characterizes plant development, therefore, the greater the leaves' fresh mass, the greater the environmental adaptability of the cultivar. As the main product of commercial interest is the leaves, the evaluation of this characteristic is extremely important because, at the time of purchase, the consumer considers the volume and appearance of the leaves (Souza et al., 2018).

Lettuce is one of the most efficient species in absorbing nutrients, however, the yield and final quality of the product are influenced by climatic conditions. Plants grown under high temperatures may experience premature bolting, a characteristic that is not desired by the market due to the high accumulation of latex that causes a bitter taste in the leaves (De Moraes et al., 2022).

The highest productive performance was observed for 'Romanela', with an average of 23.19 t ha⁻¹ with the Zn oxide source, whereas 'SVR 2005' showed a yield of 14.39 t ha⁻¹ with the Zn chelate source (Figure 7). Some studies suggest that the production and quality of the product for sale can be influenced by the genotype x environment interaction, management practices, and climatic factors (Rouphael et al., 2017), which possibly explains the behavior of the special lettuce cultivars despite the climatic conditions variations that will occur at different times of the year.

The cultivars yielded differently when Zn was not applied (Figure 7). 'Romanela' presented the highest yield, with an average of 23.00 t ha⁻¹, and 'Rubinela' was the lowest, with an average of 14.25 t ha⁻¹. The different

yields may be related to the genetic difference between cultivars, which may have different morphological and productive characteristics even under similar climatic conditions (Cardoso et al., 2018).

Although the Zn foliar application did not significantly influence the performance of the cultivars, it is important to emphasize the Zn role in the metabolism of plants, as Zn is an essential element in the synthesis of tryptophan, which is a precursor of the indole acetic acid, phytohormone that promotes plant growth (Angelini et al., 2020).

Lettuce is a vegetable with the possibility of successive crops in the same year, due to its short cycle and the availability of cultivars adapted to the different climatic conditions of the country (Costa Júnior et al., 2021). Because of this, the production of special lettuces associated with the management of foliar fertilization with zinc, in addition to increasing crop yield, can promote biofortified lettuce plants and meet the daily levels of this micronutrient required by the human body.

Graciano et al. (2020) aiming at the biofortification of lettuce reported that foliar application of Zn sulfate resulted in the highest yield (38 t ha⁻¹) for 'Isabela' at 694.44 g ha⁻¹ of Zn. Reyes et al., (2017) applied zinc sulfate via foliar in arugula with doses of up to 1.5 kg ha⁻¹ to promote biofortification and did not observe a significant reduction in yield.

The Zn foliar application significantly improved the accumulation of Zn in the special lettuce cultivars (Figure 8). 'Rubinela' showed the highest leaf Zn content

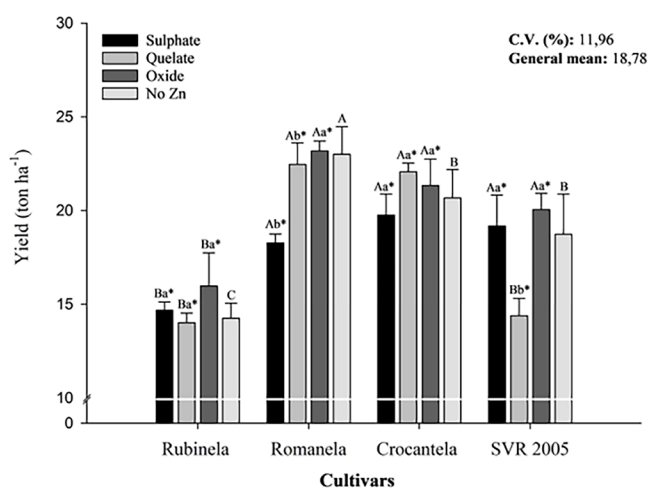


Figure 7. Mean of two planting times for yield (in t ha⁻¹) of four lettuce cultivars, as a function of foliar application of three sources of zinc. Gurupi – TO, 2021.

Capital letters in the bars indicate a difference between cultivars within each Zn source and lowercase letters indicate a difference between Zn sources within each cultivar, by the Scott-Knott test ($p = 0.05$). In the bars (*) indicates that there was no significant effect and (**) indicates a significant difference, of the control without Zn in relation to each source, employing of contrast ($p = 0.05$).

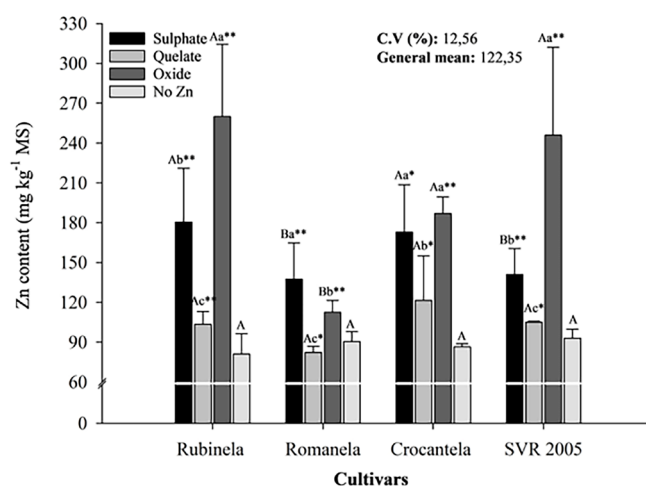


Figure 8. Mean of two planting times for Zn content (in mg kg⁻¹) of four lettuce cultivars, as a function of foliar application of three sources of zinc. Gurupi – TO, 2021.

Capital letters in the bars indicate a difference between cultivars within each Zn source and lowercase letters indicate a difference between Zn sources within each cultivar, by the Scott-Knott test ($p = 0.05$). In the bars (*) indicates that there was no significant effect and (**) indicates a significant difference, of the control without Zn in relation to each source, employing of contrast ($p = 0.05$).

in dry mass, with an average of 260.00 mg kg⁻¹ obtained with the application of the oxide source, which differs statistically from the values observed for the other sources.

According to the results shown in Figure 8, it is observed that there was a significant accumulation of Zn in the special lettuce cultivars, due to the foliar application with zinc. The Rubinela cultivar showed the highest leaf Zn content in dry mass, with an average of 260.00 mg kg⁻¹ obtained with the application of the oxide source, which differs statistically from the values presented in the other sources.

The lowest zinc foliar value observed in 'Romanela', as a function of the Zn sources, was 82.20 mg kg⁻¹, and in 'Crocantela' the lowest average was 121.50 mg kg⁻¹ using the zinc chelate source. The 'SVR 2005' showed 246.00 mg kg⁻¹ of Zn per dry leaf mass (Figure 8). Compared to the other cultivars, 'Romanela' proved to be more productive, however, it is the cultivar with the lowest zinc content in the leaves, demonstrating that the practice of foliar fertilization, even though it did not promote the accumulation of zinc in lettuce, also did not cause damage to the crop.

All Zn sources improved foliar Zn content for all cultivars (Figure 8). 'Rubinela' presented 260.00 mg of Zn kg⁻¹ of leaves when Zn oxide was applied, which is three times higher than the foliar Zn content obtained in the control (81.00 mg kg⁻¹). A similar result is observed for 'Crocantela' as the highest leaf Zn content was obtained with the application of Zn oxide, resulting in an average of 187.00 mg kg⁻¹, which is twice as high as the control (86.50 mg kg⁻¹).

The increase in Zn content in the leaves due to the application of a Zn source has been demonstrated in other studies. Meneses et al. (2016) evaluated Zn contents in two lettuce cultivars and the soil as a function of zinc doses applied to the soil in two growing seasons and observed that the limit Zn content in the leaves of 'Saladela' and 'Vanda' was 1,023.50 and 704.10 mg kg⁻¹, respectively, however, the highest doses of Zn in the soil caused symptoms of phytotoxicity in the leaves. According to White & Broadley (2011), there is a Zn concentration limit in leafy vegetables, which generally ranges from 100 to 700 mg kg⁻¹.

Graciano et al. (2020) assessed the effect of different doses of Zn in curly lettuce cultivars aiming at biofortification and observed that doses between 300 and 706 g ha⁻¹ of Zn via foliar provided biofortified plants and that 'Thais' stood out with higher leaf content with an average of 231.66 mg kg⁻¹ of Zn. In addition to the most diverse benefits of zinc to plants, this nutrient

performs several functions in humans, such as: helping in the intellectual development of children, strengthening the immune system, growth of adolescents and finally, its deficiency in pregnant women can affect the development of the brain of the fetus (Angelini et al., 2020).

The findings reinforce that lettuce is a species with the potential to promote biofortification with zinc, being considered profitable as this management with foliar fertilization did not affect the genetic potential of the cultivars and can result in several benefits to plants and human health.

The performance of the lettuce special cultivars was not affected by the local climatic conditions, showing the adaptability of the cultivars in the region for the special lettuce segment.

Conclusions

Zinc foliar application resulted in higher yield for 'Romanela' and 'Crocantela'.

The highest foliar accumulation of zinc was in 'Rubinela' and 'SVR 2005'.

Zinc oxide was the best source to biofortify special lettuce cultivars with leaf contents of 260 mg kg⁻¹.

Acknowledgment

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