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CROP PRODUCTION

Agronomic biofortification of *Allium schoenoprasum* as an alternative to combat zinc deficiency

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ABSTRACT. Zinc is an essential micronutrient for both humans and plants. In humans, it plays a vital role in various biological functions, as a component of approximately 300 proteins, with a recommended daily intake of 15 mg. About one-third of the global population faces zinc deficiency. In plants, zinc is crucial for phytohormone and carbohydrate pathways. Biofortification of staple crops is an efficient and sustainable method for addressing zinc deficiency. *Allium schoenoprasum* L., commonly known as chives, is a promising candidate for zinc biofortification due to its widespread use in Brazil and ease of cultivation. This study determined the responsiveness of chives to agronomic biofortification with zinc and the impact of biofortification on biochemical and nutritional parameters. Zinc was applied via foliar fertilization at rates of 0.0, 0.25, 0.5, 1, 1.5, 3, 6, and 12 kg ha^{-1}. The following parameters were assessed: fresh weight, shoot height, collar diameter, root and aerial part dry weight, and contents of selected sugars, free amino acids, macronutrients, micronutrients, chlorophylls, carotenoids, flavonoids, and phenolics. Linear regression and analysis of variance were performed using SigmaPlot software. The application of 6 kg ha^{-1} resulted in a 248% increase in the zinc content and an average increase of 34% in nitrogen, phosphorus, potassium, calcium, magnesium, manganese, sulfur, copper, and boron compared to the control. An application rate of 0.5 kg ha^{-1} led to a 26% average increase in plant growth. Thus, 6 kg ha^{-1} of zinc is recommended for biofortifying *Allium schoenoprasum* L., as this concentration significantly increased the zinc content without severely impacting plant growth.

Keywords: functional foods; chives; micronutrients; hidden hunger; Zn deficiency.

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Introduction

Zinc (Zn) is an essential element for human health, performing structural and catalytic functions in approximately 10% of proteins (Moraes et al., 2022). However, at least one-third of the world's population suffers from Zn deficiency, which impairs biochemical and physiological functions (Das & Green, 2016). It is estimated that 450 children under the age of 5 die from Zn deficiency annually (Black et al., 2010), and it is the fifth leading cause of disease in developing countries (World Health Organization [WHO], 2002). Zn is also an essential micronutrient in plants that plays a crucial role in growth, development, and reproduction. Zn, which is found in small amounts in the soil, usually in the form of Zn^{2+} ions, strongly interacts with organic and biological molecules, such as enzymes and proteins, due to its high polarization. This interaction is vital for many biological processes (Roat-Malone, 2007).

Zn deficiency in soil is a common problem in many agricultural regions worldwide, including Brazil (Moraes et al., 2022). This deficiency significantly affects crop growth and yield as well as crop nutritional quality. Several factors can cause Zn deficiency, including low Zn availability in the soil, soil pH, competition with other nutrients, and inappropriate agricultural practices (Kabata-Pendias & Szteke, 2015). Several agricultural practices can be adopted to increase Zn availability in plants, such as selecting cultivars that are more efficient in Zn absorption and the foliar application of Zn-containing fertilizers. In this context, biofortification is a promising strategy for improving the nutritional quality of food, thereby enhancing the health of the population (Low et al., 2007).

Chives (*Allium schoenoprasum* L.), both fresh and dehydrated, is one of the most consumed condiments by the Brazilian population. However, there are few studies on this species. The present work on biofortification can enhance cultural practices and combat Zn deficiency in the population. Therefore, the objectives of this study were to evaluate the responsiveness of *Allium schoenoprasum* L. to agronomic biofortification with Zn and determine the influence of biofortification on biochemical and nutritional parameters.

Material and methods

Experimental setup

The experiment was conducted in a greenhouse covered with a 50% shade cloth at the Biodiversity and Forests Institute (IBEF) of the Federal University of Western Pará, in the municipality of Santarém, Pará State, Brazil. The experimental site is located at 2°25'9.50" S and 54°44'30.94" W, with an altitude of 16 m.

Soil preparation

The soil used for the experiment was classified as a typical Dystrophic Yellow Latosol (Almada et al., 2021). The granulometric and chemical analysis of the soil presented the following results: very clayey texture, with 80% clay; pH in CaCl2 = 4.3; P extracted by Mehlich 1 = 6.70 mg dm^{-3}; K = 0.08 cmolc dm^{-3}; Ca = 0.95 cmolc dm^{-3}; Mg = 0.51 cmolc dm^{-3}; Zn = 0.43 mg dm^{-3}; B = 0.31 mg dm^{-3}; Fe = 154.00 mg dm^{-3}; Cu = 1.00 mg dm^{-3}; Mn = 4.34 mg dm^{-3}; H+Al = 4.13 cmolc dm^{-3}; sum of bases (SB) = 1.54 cmolc dm^{-3}; cation exchange capacity (T) = 5.67 cmolc dm^{-3}, and base saturation (V) = 27%.

The soil was air-dried and sieved through a 4-mm mesh, and the acidity was corrected using the base saturation elevation method, with a desired saturation of 80%. The soil was then incubated for 15 days and maintained at 80% field capacity in 5 dm^3 pots. The soil was fertilized with nitrogen (N), phosphorus (P), and potassium (K) (using urea, potassium chloride, and single superphosphate as sources) at rates equivalent to 90 kg ha^{-1} of N, 300 kg ha^{-1} of P_2O_5, and 120 kg ha^{-1} of K_2O. Additionally, 200 g of commercial substrate was added. Irrigation was carried out daily to maintain 80% field capacity.

Experimental design

The experiment consisted of 7 treatments with 4 replications, resulting in a total of 32 pots. Each pot contained 10 plants. The treatments were 0.25, 0.5, 1, 1.5, 3, 6, and 12 kg ha^{-1} of Zn applied as a foliar spray using $ZnSO_4.7H_2O$e as the source. A control treatment was also included, which was only irrigated with water.

Agronomic biofortification procedure with Zn and plant material collection

Chives were planted from seedlings in 5-L pots. Thinning was carried out at 30 days after planting, leaving only 10 plants per pot. The amount of Zn used for foliar application was calculated based on the plant population per hectare. The chive population per hectare was 333,334 plants (Brasil et al., 2020). Therefore, the following calculation was used:

$$GramsofZnperplot = \left(\frac{Doseperhectare(g)}{333{,}334}\right)x10$$

Zn was diluted in 40 mL of water, and the solution was applied via foliar spray using plastic sprayers. The chive plants were harvested after 60 days, wrapped in aluminum foil, and placed on ice for transport to the laboratory. Soil was collected, and the Zn content was analyzed in the laboratory.

Growth analysis

Three plants were randomly selected from each pot and cut at the collar region to determine the fresh weight of the aerial part (FWAP) of the plants. The aerial part was then wrapped in aluminum foil and properly identified. The collected material was taken to the Plant Physiology and Growth Laboratory of the Federal University of Western Pará, where it was washed with water. The material was superficially dried with paper towels to remove excess water, and the FWAP was quantified using a precision balance.

The height was determined based on the size of the leaves, as it is a species without a true stem above the soil. To determine the height, all leaves were measured with a graduated ruler. The height of the first branch was measured from the collar to the apex of the leaf. The heights of the subsequent branches were measured in the same way, recording the height of each branch in centimeters.

The collar diameter (CD) was estimated 2 cm above the soil, and the measurements were recorded in millimeters. After growth analyses and removal of the leaves for chlorophyll determination, the dry weight was determined. Initially, the aerial part and root were separated. The samples were then placed in an oven at 70°C for 48h. After drying, the dry weight of the aerial part (DWAP) and roots (DWR) were determined using a precision balance.

Pigment quantification

The method proposed by (Lichtenthaler, 1987) was used. For each dose, readings were performed in triplicate at three wavelengths: 663.2 (chlorophyll a), 646.2 (chlorophyll b), and 480 nm (carotenoids). The results were expressed in mg per fresh weight (g). The dry weight of the leaves was used to quantify the total flavonoid and total phenol contents according to the methods previously described by Salgueiro et al. (2014).

Total phenolic contents

The total phenolic content was determined in the ethanolic extract of *Allium schoenoprasum* leaves according to the method described by Santos et al. (2016) with some modifications. In test tubes, 100 μL of the ethanolic extract of *Allium schoenoprasum* leaves (1:10, w:v) was increased to 500 μL of 10% Folin–Ciocalteu reagent (Sigma-Aldrich, Saint Louis, MO, USA) and 6.0 mL of distilled water. The mixture was left to react for 10 min., and 2.0 mL of 15% aqueous Na_2CO_3 solution was added. The solution was homogenized, and the tubes were wrapped in aluminum foil and left to rest for 2h. The absorbances of the samples were then measured using a UV/vis spectrophotometer (NOVA 3300, Piracicaba, São Paulo State, Brazil) at 760 nm, with ethanol as the blank. All findings were performed in triplicate. The total phenolic content was determined using a deficiency curve with gallic acid as the standard and expressed in mg of gallic acid equivalents per gram of plant (mg GAE g^{-1}).

Total flavonoid content

The total flavonoid content was determined following the methods described by Chang et al. (2002) and Salgueiro et al. (2014) with some modifications. In a test tube, 2 mL of 2.0% ethanolic $AlCl_3$ solution (m:v) was added to 1 mL of the ethanolic extract of *Allium schoenoprasum* leaves (1:10, m:v) and 1 mL of distilled water. After 30 min., the absorbances of the samples were measured using a UV/vis spectrophotometer (NOVA 3300, Piracicaba, São Paulo State, Brazil) at 415 nm, with ethanol as the blank. All measurements were performed in triplicate. The total flavonoid content was determined using a calibration curve with quercetin as the standard and expressed as mg of quercetin equivalents per gram of leaf (mg QE g^{-1}).

Quantification of sugars and free amino acids

Dry leaf and root matter samples were used to quantify reducing sugars (RS) following the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). The total soluble sugar (TSS) content was determined using the anthrone method (Yemm & Willis, 1954). The free total amino acid content was quantified using the method described by Yemm et al. (1955).

Macro- and micronutrients

The macro- and micronutrients (N, P, K, calcium (Ca), magnesium (Mg), sulfur (S), zinc (Zn), iron (Fe), manganese (Mn), boron (B), and copper (Cu)) were determined using the methods described by Malavolta et al. (1989) and Harris (2012).

Statistical analysis

To ensure the reliability of the analysis, outliers for each parameter were removed using the standardized Euclidean distance (Z-score). Standardization based on 4 samples enabled comparability between variables and avoided bias in the results. SigmaPlot 14.0 (trial version) was used for data analysis. Depending on the parameter, linear and nonlinear regression were applied, with the sample means of the parameters as predictors and their standard deviations as a measure of error. Analysis of variance (ANOVA) was performed to evaluate the statistical significance of the regression and individual regression coefficients. The results showed that the regression was significant ($p < 0.05$), with R^2 indicating that the model explained a percentage of variation in Y. Scatter plots with errors were used to evaluate the distribution of the data and to check linearity between the variables.

Results

Growth

Zn application increased the mean height, CD, FWAP, and DWR. FWAP (0.25, 0.5, 1.5, 3, and 12 kg ha^{-1}), DWR (0.25, 1, and 6 kg ha^{-1}), and CD (0.25, 0.5, 1, 3, and 12 kg ha^{-1}) increased compared to the control. A third-order inverse regression model was used for height, where there was an increase up to the 3 kg ha^{-1} dose and then an increase up to the 12 kg ha^{-1} dose. A quadratic model was used for CD, FWAP, and DWR, as all three parameters showed a decrease up to the 6 kg ha^{-1} dose and an increase up to the 12 kg ha^{-1} dose. A quadratic model was also used for DWR, but in this case, there was an increase up to the 6 kg ha^{-1} dose and a decrease up to the 12 kg ha^{-1} dose.

The 1 kg ha^{-1} dose led to a 10.47% decrease in FWAP compared to the control, while the 1.5 kg ha^{-1} dose led to a decrease of −39.98 and −15.32% in FWAP and DC, respectively. For height, the lowest mean was observed at 6 kg ha^{-1} (6.32%), while the lowest DWR was observed at 12 kg ha^{-1} with a decrease of −31.65%. The highest mean FWAP and collar was observed at the 0.25 kg ha^{-1} dose, with an increase of −8.85 and 36.90%, respectively. The highest FWAP was observed at the 0.5 kg ha^{-1} dose, with an increase of 34.88%. The greatest increase in height compared to the control was observed at the 1.5 kg ha^{-1} dose (17.72%), and for DWR, the highest mean was observed at the 6 kg ha^{-1} dose, with an increase of 8.58% (Figure 1).

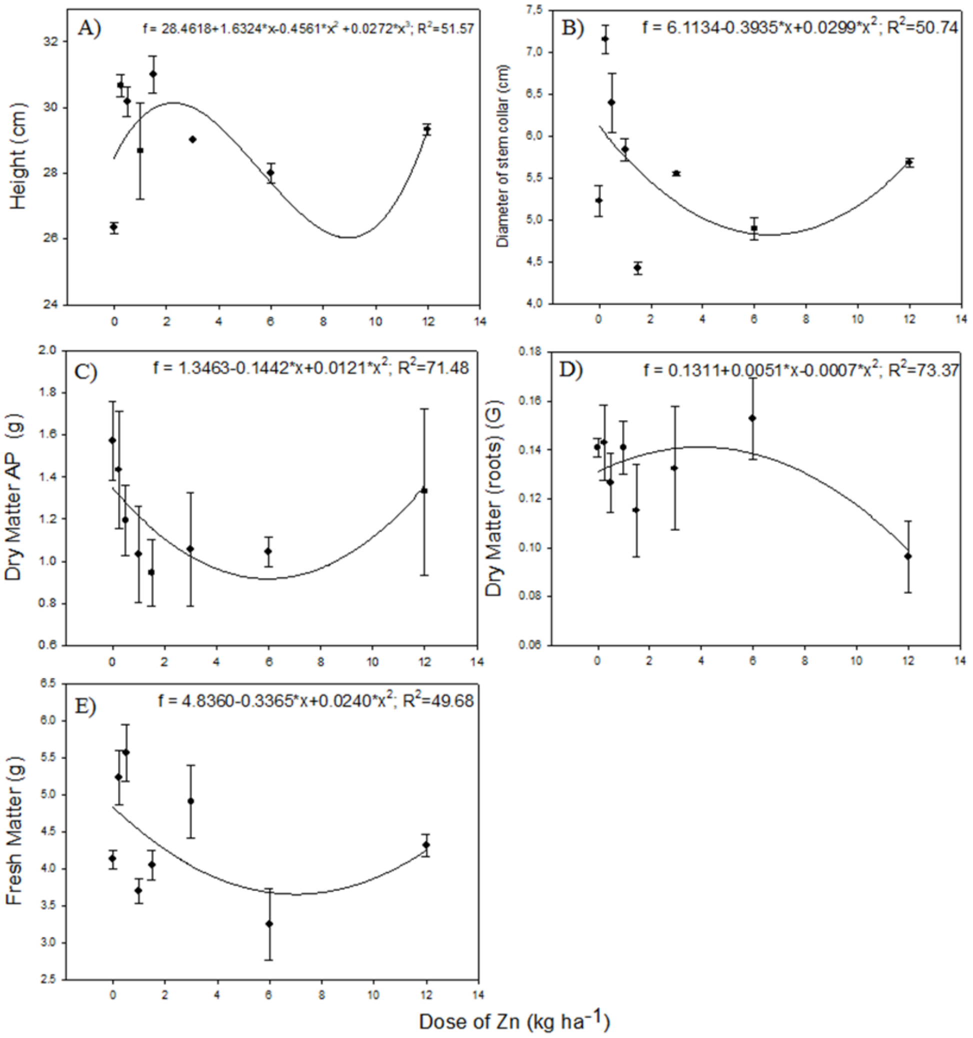

Figure 1. Regression model showing the effect of zinc (Zn) doses on plant properties: A) height (cm), B) stem collar diameter (cm), C) dry matter of aerial parts (AP) (g), D) dry matter of roots (g), and E) fresh matter (g).

Chlorophylls and carotenoids

Zn doses increased the mean contents of photosynthetic pigments, chlorophyll A, chlorophyll B, chlorophyll AB, and carotenoids, compared to the control. Chlorophyll A showed an increase at the 0.25 kg ha^{-1} dose, decreased to the 0.5 kg ha^{-1} dose, and reached stability after the 1.5 kg ha^{-1} dose. The models for chlorophyll B, chlorophyll AB, and carotenoids followed the same trend, with a decrease from the 0.25 kg ha^{-1} to the 6 kg ha^{-1} dose, followed by an increase to the 12 kg ha^{-1} dose.

The lowest mean for chlorophyll AB was observed at the 0.25 kg ha^{-1} dose, which was 28.16% of the control, while the 6 kg ha^{-1} dose led to a decrease of −7.79, −9.36, and −10.62% for chlorophyll A, chlorophyll B, and carotenoids, respectively. The highest means were observed at the 0.25 kg ha^{-1} dose, with an increase of 28% for chlorophyll A, 30% for chlorophyll B, 28% for chlorophyll AB, and 23% for carotenoids (Figure 2).

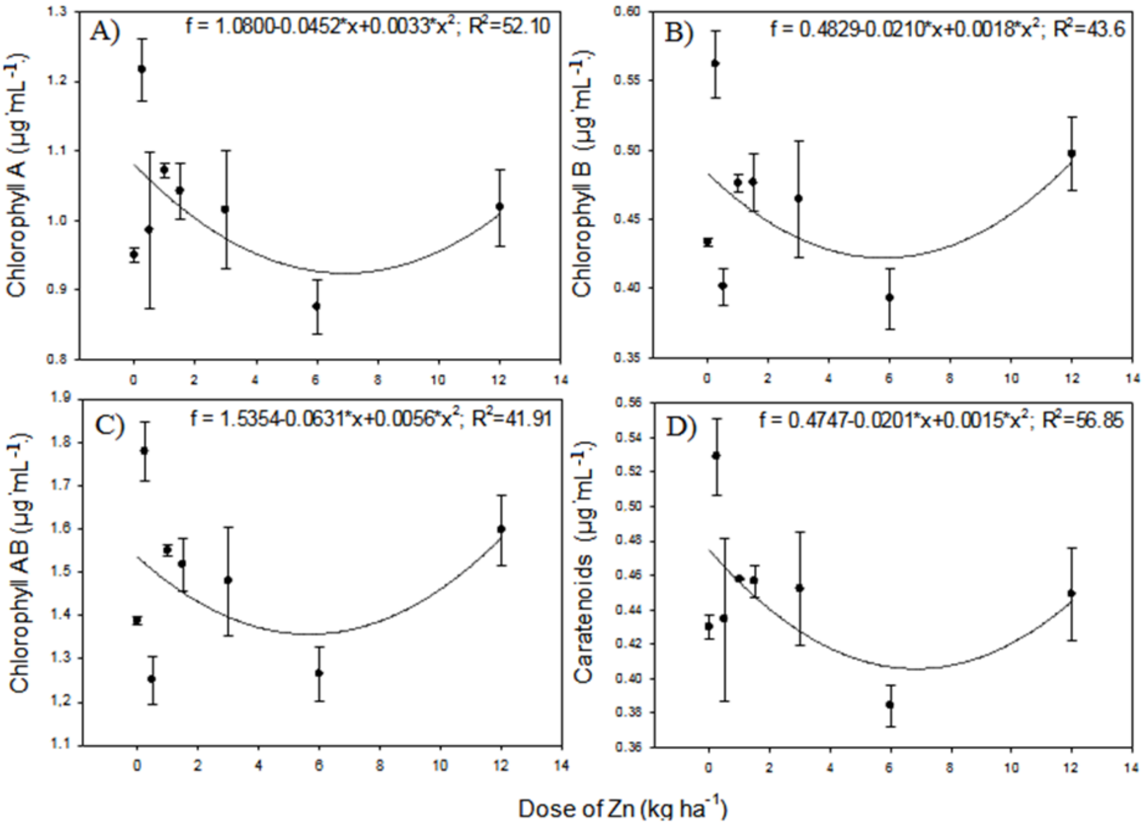

Figure 2. Regression model showing the effects of zinc (Zn) doses on plant properties: A) chlorophyll A, B) chlorophyll B, C) chlorophyll AB, and D) carotenoids.

Total phenol and total flavonoid content

Zn doses increased the total flavonoid content (except for the 1.5 kg ha^{-1} dose) in the aerial parts compared to the control, and the total phenol and total flavonoid contents in the roots decreased. The regression model for the total phenol and total flavonoid contents in the aerial parts was cubic, showing a decrease up to the 3 kg ha^{-1} dose, reaching a peak after the 6 kg ha^{-1} dose, and decreasing again at the 12 kg ha^{-1} dose. The regression model for the total flavonoid content in the roots was quadratic, indicating a decline in the total flavonoid content up to the 6 kg ha^{-1} dose, followed by an increase up to the 12 kg ha^{-1} dose.

The lowest increase in the total phenol content was observed at the 1 kg ha^{-1} dose, showing a decrease of −29.28% compared to the control. The total flavonoid content in the aerial parts and total flavonoid content in the roots showed decreases of −81.79 and −8%, respectively, at the 1.5 kg ha^{-1} dose. The highest mean total flavonoid content in the aerial parts was observed at the 0.25 kg ha^{-1} dose, with an increase of 39.32% compared to the control. The total flavonoid content in the roots was highest at the 1 kg ha^{-1} dose, with an increase of 0.85%, and for phenols, the highest content was observed at the 6 kg ha^{-1} dose, with a decrease of −4.01% compared to the control (Figure 3).

Sugars

At least one Zn dose led to an increase compared to the control. The regression model for AR in the aerial part was cubic, with an increase up to the 3 kg ha^{-1} dose, followed by a decrease up to the 6 kg ha^{-1} dose and an increase up to the 12 kg ha^{-1} dose. For AR in the roots, the model was quadratic, with an increase up to the 6 kg ha^{-1} dose and a decrease up to the 12 kg ha^{-1} dose. An increase in the AR content was observed in the roots (except at 12 kg ha^{-1}). Additionally, a decrease in the AR content in the roots of −4.76% was observed at the 12 kg ha^{-1} dose, and the lowest mean AR in the aerial and root parts was observed at the 12 kg ha^{-1} dose, with decreases of −15.30 and −4.76%, respectively, compared to the control. The highest AR contents were observed at the 1 and 3 kg ha^{-1} doses in the aerial part and roots, with increases of 50.48 and 66.33%, respectively, compared to the control.

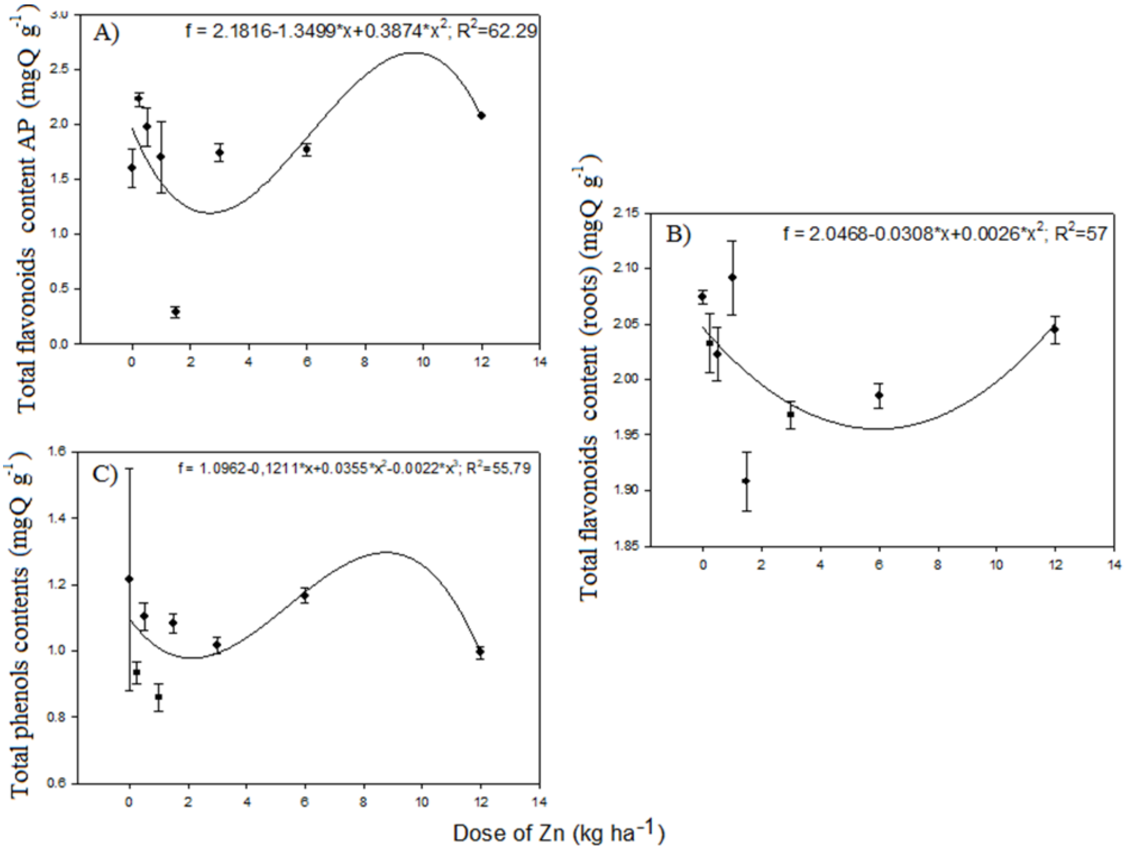

Figure 3. Regression model showing the effect of zinc (Zn) doses on plant properties: A) total flavonoid content of aerial parts (AP) (mg g^{-1}), B) total flavonoid content of roots (mg g^{-1}), and C) total phenol content (mg g^{-1}).

A quadratic model was also proposed for AST in the roots, with an increase up to the 6 kg ha^{-1} dose and a drastic decrease up to the 12 kg ha^{-1} dose. For AST AP, a third-degree inverse model was used, as there was a decrease from the 0.25 kg ha^{-1} dose to the 0.5 kg ha^{-1} dose and a subsequent increase up to the 1 kg ha^{-1} dose, with a slight decrease up to the 12 kg ha^{-1} dose. An increase in the AST content was observed in the aerial parts at the 0.25, 1, 1.5, 3, and 6 kg ha^{-1} doses and in the root at the 0.25 and 3 kg ha^{-1} doses. Additionally, a decrease in the AST AP content of −55.99% was observed at the 0.5 kg ha^{-1} dose, and the lowest mean AST in the roots was observed at the 12 kg ha^{-1} dose, with a decrease of −40.68% compared to the control. The highest AST contents were observed at the 0.25 and 0.5 kg ha^{-1} doses for the roots and aerial parts, with increases of 2.13 and 12.99%, respectively, compared to the control (Figure 4).

Amino acids

The quadratic regression model was used for the amino acid content in the roots and aerial parts, but they behaved in opposite ways. Although the amino acid content in the aerial parts decreased up to the 6 kg ha^{-1} dose and increased up to the 12 kg ha^{-1} dose, the amino acid content in the roots increased up to the 6 kg ha^{-1} dose and decreased up to the 12 kg ha^{-1} dose.

The lowest increase in the amino acid content in the roots and aerial parts was at the 1 kg ha^{-1} dose, at 8.81 and −8.67%, respectively, compared to the control. The highest increase occurred at the 3 kg ha^{-1} dose in the roots (317.32%) and at the 0.5 kg ha^{-1} dose for the aerial parts (60.05%) (Figure 5).

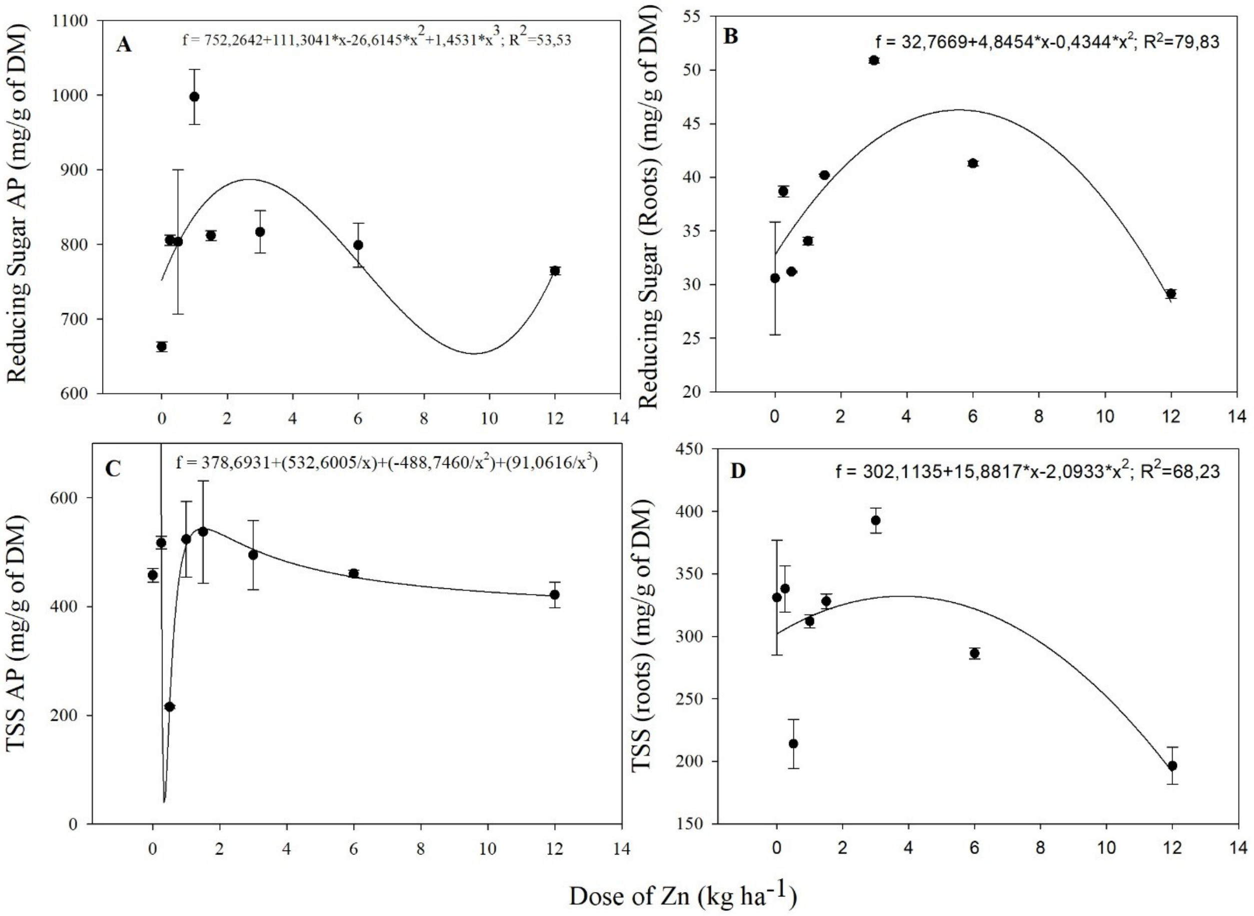

Figure 4. Regression model showing the effect of zinc (Zn) doses on plant properties: A) reducing sugar content in the aerial parts (AP) (mg g^{-1} of DM), B) reducing sugar content in the roots (mg g^{-1} of DM), C) total soluble sugar (TSS) content in AP (mg g^{-1} of DM), and D) TSS in the roots (mg g^{-1} of DM).

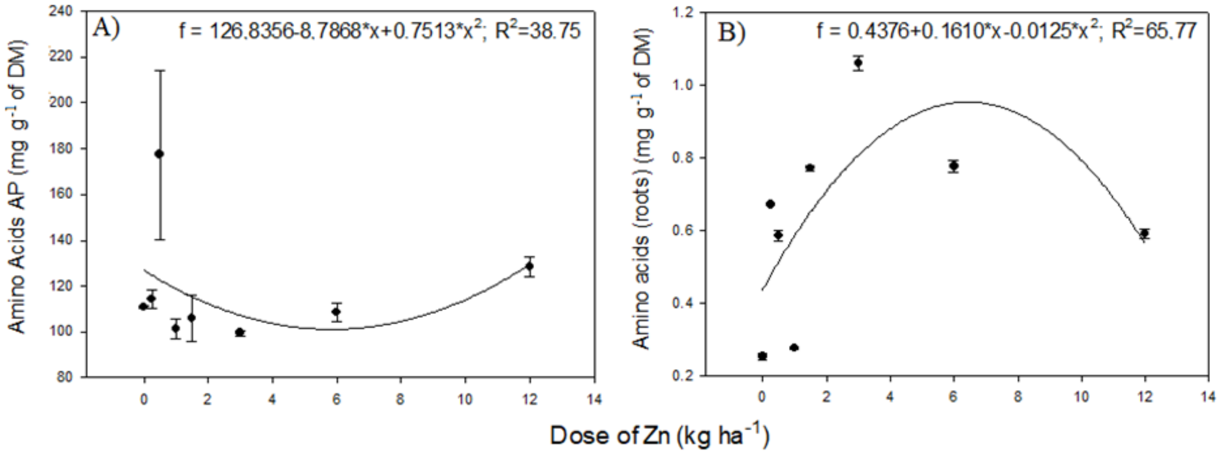

Figure 5. Regression model showing the effect of zinc (Zn) doses on plant properties: A) amino acid content in the aerial parts (AP) (mg g^{-1} of DM) and B) amino acid content in the roots (mg g^{-1} of DM).

Macronutrients

Zn doses influenced the nutrient contents compared to the control, with increases in N (except for 0.5 kg ha^{-1}), K (except for 3 kg ha^{-1}), Ca (except for 1 and 12 kg ha^{-1}), Mg (except for 12 kg ha^{-1}), P, and S (all). N, P, Ca, and Mg had a quadratic regression model, showing an increase up to the 6 kg ha^{-1} dose and a decrease up to the 12 kg ha^{-1} dose. For K, a cubic model was used, with a decrease up to the 3 kg ha^{-1} dose, an increase up to the 6 kg ha^{-1} dose, and a decrease up to the 12 kg ha^{-1} dose. For S, the model was linear, with an increase from the control to the 12 kg ha^{-1} dose.

The lowest increases compared to the control were observed as follows: for N, at the 0.5 kg ha^{-1} dose, with a change of −2.05% and 14.95%; for K and S, at the 3 kg ha^{-1} dose, with a decrease of −1.82%; and for P, Ca, and Mg, at the 12 kg ha^{-1} dose, with changes of 1.67%, −15.06%, and −5.42%, respectively. On the other hand, the highest increase occurred at the 0.25 kg ha^{-1} dose for K, Ca, and Mg, with increases of 12.83, 21.39, and 26.01%, respectively, while the largest increase for P occurred when 1 kg ha^{-1} was applied, resulting in an increase of 23.60%. For N, an increase of 19.42% was observed when 3 kg ha^{-1} was applied. For S, the 12 kg ha^{-1} dose showed the highest increase, at 46.90% (Figure 6).

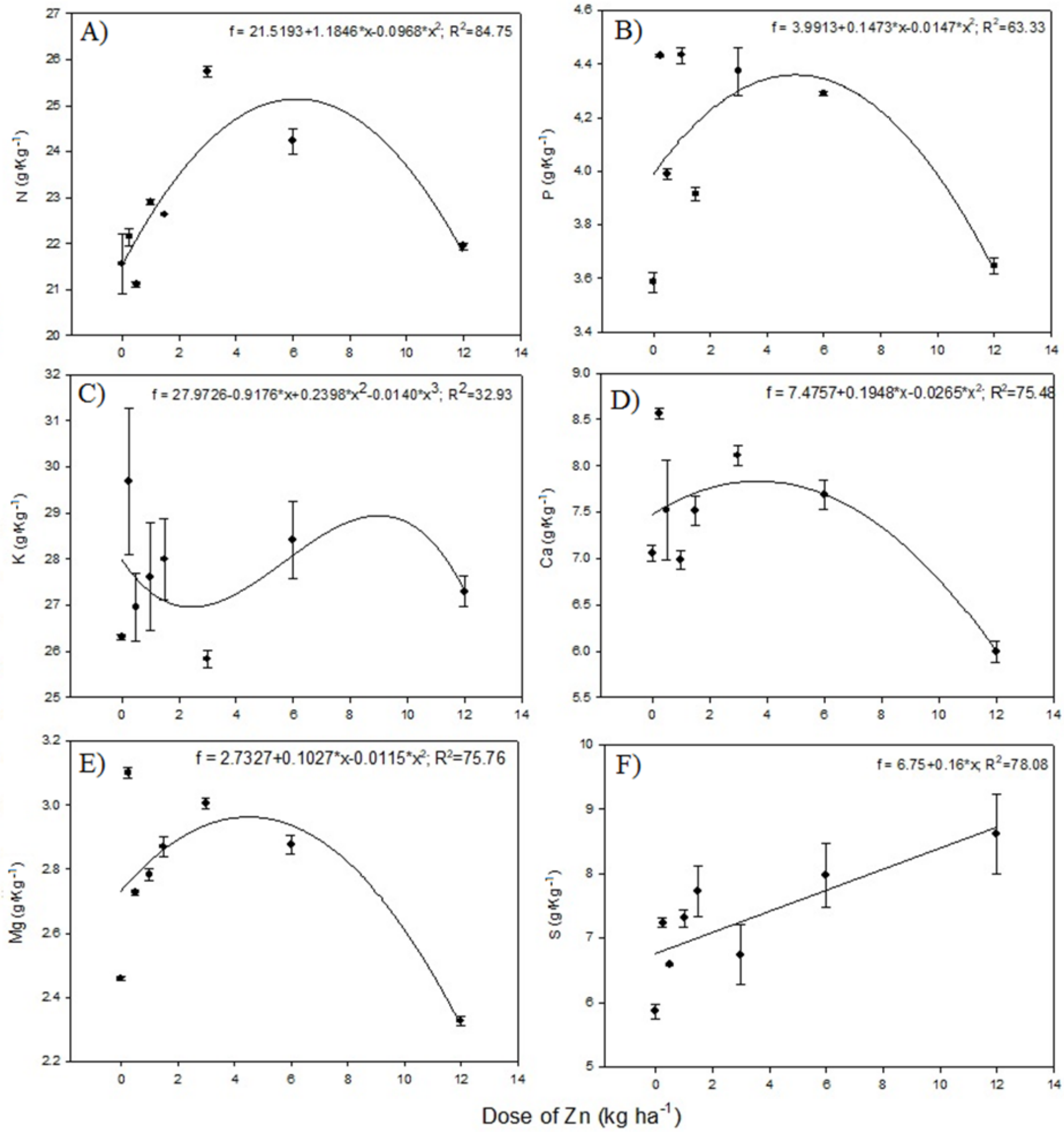

Figure 6. Regression model showing the effect of zinc (Zn) doses on plant properties: A) nitrogen (N) (g kg^{-1}), B) phosphorus (P) (g kg^{-1}), C) potassium (K) (g kg^{-1}), D) calcium (Ca) (g kg^{-1}), E) magnesium (Mg) (g kg^{-1}), and F) sulfur (S) (g kg^{-1}).

Micronutrients

Zn also influenced the micronutrient contents, providing an increase compared to the control for B (except for 1 and 12 kg ha^{-1}), Fe (0.25, 0.5, and 1 kg ha^{-1}), and Cu (except for 1.5 kg ha^{-1}). The regression model for Zn, Fe, and B was quadratic. For Zn, the behavior showed an increase up to the 6 kg ha^{-1} dose and then a decrease up to the 12 kg ha^{-1} dose; however, the 12 kg ha^{-1} dose still showed the highest Zn content. For Fe, the behavior was the opposite, with a decrease up to 6 kg ha^{-1} and an increase up to 12 kg ha^{-1}. For B, there was a slight increase up to 6 kg ha^{-1} and a decrease up to 12 kg ha^{-1}. For Cu, the model was cubic, showing a decrease up to the 3 kg ha^{-1} dose, an increase up to the 6 kg ha^{-1} dose, and a decrease up to the 12 kg ha^{-1} dose. For Mn, the regression model was Gaussian with 4 parameters, with a decrease from the control and an increase from 0.5 to 1 kg ha^{-1}, after which it remained stable up to the 12 kg ha^{-1} dose (Figure 7).

The lowest increase in Zn and Mn was observed at the 0.5 kg ha^{-1} dose, with decreases of −7.88 and −37.59%, respectively, compared to the control. For Fe and B, it occurred at the 1 kg ha^{-1} dose, with decreases of −23.95 and −4.27%, respectively, while for Cu, it was observed at the 1.5 kg ha^{-1} dose (−17.85%). The highest mean B, Cu, and Fe contents occurred at 0.5 kg ha^{-1}, with increase of 21.92, 65.87, and 50.65%, respectively, compared to the control. The Mn content was highest at the 3 kg ha^{-1} dose (−11.77%), while the Zn content was highest at the 12 kg ha^{-1} dose (218.28%).

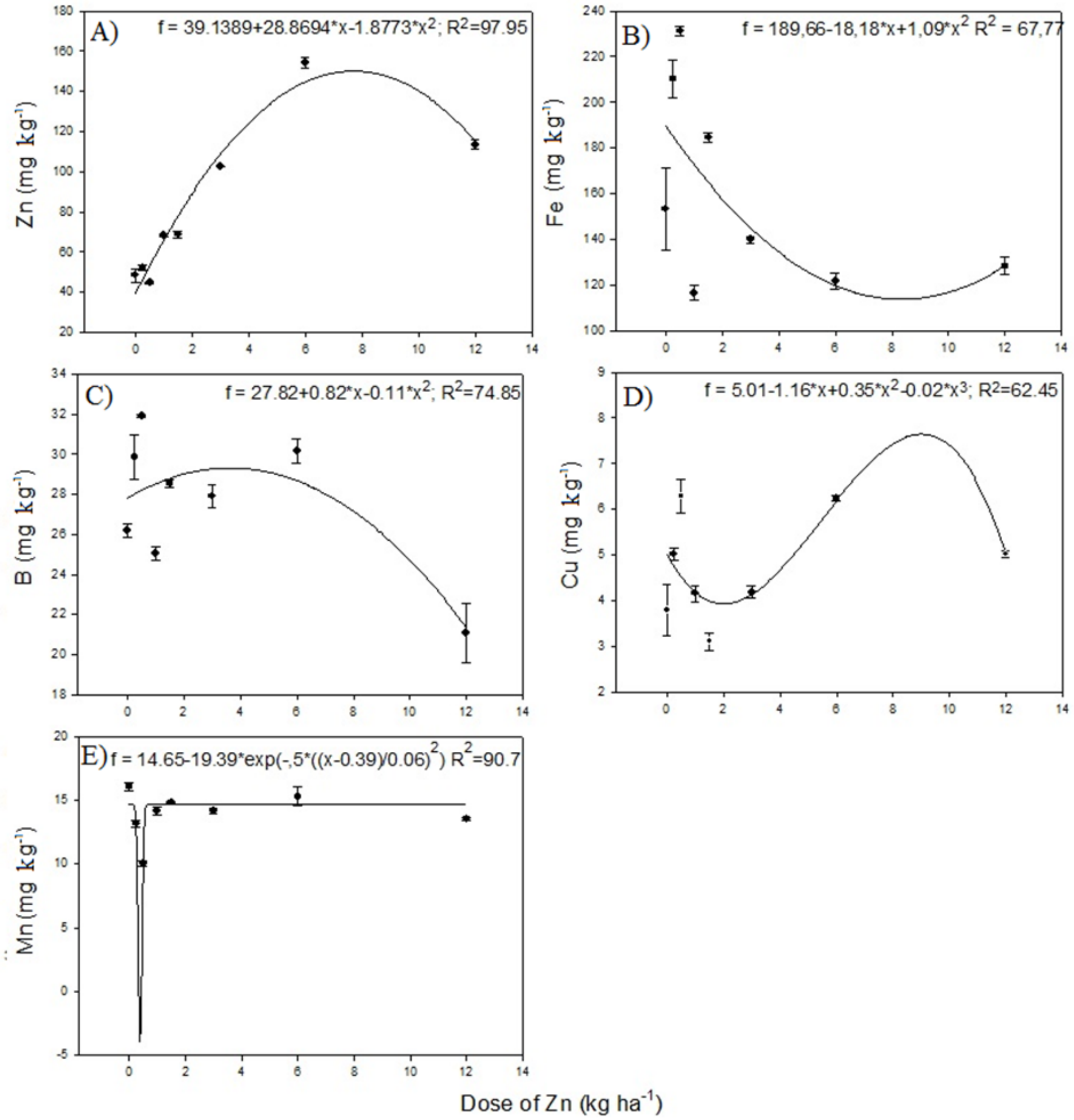

Figure 7. Regression model showing the effect of zinc (Zn) doses on plant properties: A) Zn (mg kg^{-1}), B) iron (Fe) (mg kg^{-1}), C) boron (B) (mg kg^{-1}), D) copper (Cu) (mg kg^{-1}), and E) manganese (Mn) (mg kg^{-1}).

Discussion

Growth

Zn promoted the growth of chives, but no pattern was observed between the dose and growth. Graciano et al. (2020) showed a 24% increase in lettuce growth with the application of 0.8 kg ha^{-1} of Zn, and Rugeles-Reyes et al. (2019) observed an increase in arugula growth. However, Ferreira Bertino et al. (2022) did not show significant onion growth at the 1 kg ha^{-1} dose, and peak onion growth was observed at the 1.5 kg ha^{-1} dose. Zn influences growth and production, even though it is not a direct component of the molecular structure of growth phytohormones, such as auxins produced via tryptophan formation, ethylene, and gibberellic acid (Singh et al., 2018). DC is fundamental to the sustainability of the plant. An increase of 36% was observed at the 0.25 kg ha^{-1} dose, followed by a progressive decrease with increasing doses, up to an 8% increase at the 12 kg ha^{-1} dose. No influence of Zn on DC was observed by Kume et al. (2021) in corn or Coelho et al. (2022) in tomato.

Biomass gain was more significant at lower Zn concentrations, according to de Souza et al. (2021), who obtained a linear decrease in fresh mass of *Vigna unguiculata* with increasing doses, starting from the 6 kg ha^{-1} dose. Costa et al. (2023) obtained a 20% increase in MSR at the 3 kg ha^{-1} dose, and Carmona et al. (2020) observed a 38% increase in biomass at the 30 mg L^{-1} dose in beetroot. In addition to managing growth through hormone formation, Zn also influences the N content (Costa et al., 2023), as observed in the present study for the 3 and 6 kg ha^{-1} doses, which showed increases of 19 and 12% in N, respectively, compared to the control. Zn and N interact and affect each other's availability. Hakoomat et al. (2014) demonstrated that Zn application increased the nitrate reductase activity in rice roots. This means that Zn acts as a catalyst, accelerating the rate of conversion of nitrate to ammonium, which is the assimilable form of N. In addition, Zn acts as an N stabilizing agent in the soil, reducing losses from volatilization and leaching. This occurs through the formation of complexes with N, making it less susceptible to conversion to gas or leaching by the soil. These results agree with those of Costa et al. (2023). In this study with chives, the 3rd dose resulted in an increase of 111% in the average Zn content and 19.48% in the N content.

However, in experiments with wheat (Jalal et al., 2023; Petković et al., 2019) and arugula (Rugeles-Reyes et al., 2019), no statistical differences were observed in biomass. Ciriello et al. (2022) observed a decrease in dry matter in basil. Thus, high Zn doses cause toxicity, as observed for the 12 kg ha^{-1} dose. According to Ahmad et al. (2020), the plant's development stage is also an important factor in the response to Zn.

Chlorophylls and carotenoids

The pigment content increased most significantly at lower doses, with a decrease at the 6 kg ha^{-1} dose. The highest contents of carotenoids, chlorophyll A, and chlorophyll B were observed at the 0.25 kg ha^{-1} dose, with increases of 23, 29, and 28%, respectively. Studies in cauliflower (Raza et al., 2022) revealed a 48% increase in chlorophyll A, 31% in chlorophyll B, and 20% in carotenoids at the 100 μM (6.538 ppm Zn) dose. In contrast, Subbaiah et al. (2016) showed an increase of approximately 30% in the chlorophyll content of corn at the 400 ppm Zn dose, while Nekoukhou et al. (2022) observed a 181% increase in mint at the 160 mg L^{-1} dose. These authors suggest that Zn increases chlorophyll due to its role in photosynthetic pigments, acting as a cofactor for DNA polymerase and ALA synthase, which are crucial in chlorophyll biosynthesis. In addition, Zn influences the expression of genes related to chlorophyll synthesis, modulating the availability of proteins and photosynthetic pigments. It also binds directly to the chlorophyll molecule, stabilizing its three-dimensional structure and ensuring its active form as a cofactor for enzymes involved in the photochemical reactions of photosynthesis, such as water photolysis and electron transfer in photosystems. Furthermore, Zn has antioxidant properties that protect chlorophylls and other cellular components from damage due to free radicals.

Guerrero-Martin et al. (2023) observed a significant decrease of more than 70% in chlorophyll A and B contents at the 0.2 μmol dm^{-3} dose in red kidney beans. Fortis-Hernández et al. (2022) observed a decrease in carotenoids in lettuce, with the highest decrease at the 20 mg L^{-1} dose (25%) and the lowest decrease at the 25 mg L^{-1} dose. Excess Zn promotes metabolic disturbances that interfere with the electron flow generated in water photolysis in the thylakoid membrane and increases H_2O_2 generation and lipid peroxidation of the membrane (Souza et al., 2021; Ruiz-Torres et al., 2021). Toxic Zn doses can also decrease the chlorophyll content, as it can alter the Mg and Fe concentrations, consequently decreasing chlorophyll formation (García-Gómez et al., 2017). However, this was not observed in the present study (Figure 2), indicating that chives tolerate high Zn doses.

Total phenol and flavonoid contents

Plant phenolic compounds constitute a chemically heterogeneous group, with approximately 10,000 compounds, including flavonoids. In general, the total phenolic and flavonoid contents increase in response to biotic and abiotic stress (Rehman et al., 2023). Studies in cauliflower with Zn application have reported increases in the total phenolic and flavonoid contents (Tian et al, 2021; Ciriello et al., 2022; Raza et al., 2022; Malka et al., 2023; Sorahinobar et al., 2023). Similar results were observed by Fortis-Hernández et al. (2022) in lettuce, López-Morales et al. (2020) in beans, and Nekoukhou et al. (2022) in mint.

Among the functions of phenolics and flavonoids is the ability to eliminate reactive oxygen species produced upon exposure to various abiotic and biotic stressors, such as salinity, drought, temperature, UV light, disease progression, and metals (Sakihama et al., 2002; Tuladhar et al., 2021). In the present study, no significant increase in the total phenolic content in the leaves or flavonoids in the roots was observed; only a slight increase in flavonoids was observed in the leaves, confirming that Zn is not acting as a stress agent and that chives are a Zn-tolerant species.

Sugars

Chives showed a 17% increase in the AST content at the 3 kg ha^{-1} dose, which is consistent with the results of other studies, such as those by Raza et al. (2022) in cauliflower, Rehman et al. (2023) in rice, and Kachinski et al. (2022) and Silva et al. (2021) in beans. Zn is the only metal required by all six classes of enzymes (oxidoreductases, transferases, hydrolases, liases, isomerases, and ligases) and is thus involved in carbohydrate metabolism through its effects on photosynthesis and sugar transformation (Sadeghzadeh, 2013). Reduced photosynthesis under Zn deficiency may result from a decrease in carbonic anhydrase activity, photosynthetic activity of the chloroplasts, and the chlorophyll content, as well as changes in the chloroplast structure and decreased ribulose-1,5-bisphosphate carboxylase oxygenase activity (Römheld & Marschner, 1991). Thus, Zn generally favored the increase in carbohydrates (Figure 3), increasing plant growth (Figure 1).

Amino acids

In chives, the amino acid content peaked at 0.5 kg ha^{-1} in the aerial parts and at 3 kg ha^{-1} in the roots, with increases of 60 and 424%, respectively. Raza et al. (2022) observed a 40% increase at the 50 μM dose in cauliflower, and Rehman et al. (2023) found a 123% increase at the 400 mM dose in rice. The increase in the amino acid content may be related to metal defense mechanisms (Ghnaya et al., 2009) through the increase in protease activity, which enables osmoregulation and detoxification of reactive oxygen species (Boussama et al., 1999; Hsu & Kao, 2003; Pena et al., 2006).

Macro- and micronutrients

The Zn content showed a direct relationship with the applied dose up to 6 kg ha^{-1}, which resulted in the highest Zn content, a 318% increase compared to the control, totaling 154.15 mg kg^{-1}. Petković et al. (2019) showed a 220% increase in alfalfa at the 1 kg ha^{-1} Zn dose (49.9 mg kg^{-1}). Jalal et al. (2023) obtained the highest absorption rate in wheat at the 1.5 kg ha^{-1} dose, an increase of 23.76%, totaling 40.1 mg kg^{-1}, followed by a decrease in absorption. Kachinski et al. (2022) obtained a 150% increase at the 0.6 kg ha^{-1} dose in beans (28.5 mg kg^{-1}), with no significant difference up to the 1.5 kg ha^{-1} dose.

The 6 kg ha^{-1} dose provided an increase in the contents of nutrients N, K, P, Ca, Mg, S, Cu, and B compared to the control. Nandal and Solanki (2021) stated that a Zn supply can favor nutrient absorption, especially that of macronutrients, due to the better structure of the cell membrane. Similar results have been observed by Souza et al. (2021) in wheat, as they showed a decrease in the Fe, Cu, and P contents and an increase in the K and Zn contents at a dose above 6 kg ha^{-1} of Zn. However, Costa et al. (2023) obtained the highest Mg and N contents at the 4.5 kg ha^{-1} Zn dose, while in the present study, these contents were highest at doses below 4 kg ha^{-1} Zn. In alfalfa, Petković et al. (2019) did not observe a significant difference in the P, K, Ca, Mg, Cu, Fe, and S contents. In the present study, S showed a direct relationship with Zn. As an essential element, Zn supply is beneficial in restoring nutrient absorption, thus maintaining the structural and functional capacity of different organelles, such as chloroplasts and mitochondria (Ahmad et al., 2020).

Conclusion

In relation to the Zn content in chives, the 6 kg ha^{-1} dose was the most advantageous, as it increased the Zn content by more than 218%. The 6 kg ha^{-1} dose was also better for all nutrients (N, K, P, Ca, Mg, S, Cu, and B), with the elements having an average increase in value of 34%. This nutritional increase is a positive factor for health since these elements play essential roles in the body. For producers and consumers, the parameters of size and fresh mass are the most interesting, and the most advantageous dose was 0.5 kg ha^{-1}, as it increased height by 14.55% and fresh mass by 34.88%, increasing the productivity per hectare and improving plant quality, with more vigorous plants less susceptible to pests and diseases.

Data availability

The data will be made available to the authors upon request.

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