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Scientific Article

Effects of Fe and Zn on growth, biofortification and quality of lettuce grown in hydroponics

Efeitos do Fe e Zn no crescimento, bioforticação e qualidade de alface cultivado em hidroponia

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ABSTRACT - Iron (Fe) and zinc (Zn) are essential elements for human health and their deficiencies cause reduced work capacity, physiological and immune system disorders, anemia and even death, being considered primary global public health problems. Agronomic biofortification aims to increase the concentration of these nutrients in the edible part of the plant and, consequently, increase human intake of these nutrients. The aim of this study was to evaluate the effects of Fe and Zn concentrations on the growth, biofortification and quality of lettuce grown in hydroponics. Six treatments corresponding to the combinations of Zn (0.06 and 0.24 mg L^{-1}) and Fe (2, 4 and 8 mg L^{-1}) concentrations were evaluated. Increase of Zn in the nutrient solution positively influenced only leaf Zn contents at 18 days after transplanting the seedlings and ascorbic acid at harvest. On the other hand, the increase in Fe concentration positively influenced the contents of photosynthetic pigments, ascorbic acid and Fe; however, it negatively affected the leaf Zn content, leaf area and leaf dry mass of lettuce. Greater biofortification of lettuce for Fe was observed with the Fe concentration of 8 mg L^{-1} in the solution.

Keywords: Biofortified food. Soilless cultivation. Nutrient deficiency. Hidden hunger. Lactuca sativa L.

RESUMO - O ferro (Fe) e o zinco (Zn) são elementos essenciais para a saúde humana e suas deficiências causam redução da capacidade de trabalho, distúrbios fisiológicos e no sistema imunológico, anemia e até a morte, sendo consideradas problemas primários de saúde pública global. A biofortificação agronômica visa aumentar a concentração destes nutrientes na parte comestível da planta e, consequentemente, aumentar a ingestão humana destes nutrientes. O objetivo do estudo foi avaliar concentrações de Fe e Zn no crescimento, biofortificação e qualidade da alface em hidroponia. Foram avaliados seis tratamentos correspondentes às combinações das concentrações de Zn (0,06 e 0,24 mg L^{-1}) e Fe (2, 4 e 8 mg L^{-1}). O aumento de Zn na solução nutritiva influenciou positivamente somente os teores foliares de Zn aos 18 dias após o transplante das mudas e ácido ascórbico na colheita. Por outro lado, o aumento da concentração de Fe influenciou positivamente os teores de pigmentos fotossintéticos, ácido ascórbico e Fe; entretanto, afetou negativamente o teor foliar de Zn, área foliar e massa seca de folhas da alface. Foi observada maior biofortificação da alface para Fe com a concentração de 8 mg L^{-1} de Fe na solução.

Palavras-chave: Alimento biofortificado. Cultivo sem solo. Deficiência de nutrientes. Fome oculta. Lactuca sativa L.

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INTRODUCTION

Hidden hunger is the phenomenon in which one or more micronutrients are deficient in the human body and there is an absence of symptoms, which can be fatal, affecting 2 billion people in the world (GÖDECKE; STEIN; QAIM, 2018). Among the nutritional deficiencies, iron (Fe) and zinc (Zn) are routine cases in the population (MAJUMDER; DATTA; DATTA, 2019), which are motivated by the low natural availability of micronutrients in the soil to plants, leading to the harvesting of products with low concentrations of Fe and Zn, low mobility of the element in the plant, and cultural practices such as liming necessary for acidic soils, which also contributes to reduced availability (BRIAT; DUBOS; GAYMARD, 2015). Additionally, a large part of the diet relies on cooking, industrial processing and storage, which degrade ascorbic acid, which has an intensifying effect on Fe absorption (TEUCHER; OLIVARES; CORI 2004). Other micronutrients are also important for humans, but their deficiencies do not generate pathologies as serious as those observed for Fe and Zn deficiencies, which can even be lethal (YOUNAS et al., 2023).

Fe deficiency in humans is associated with damage to growth, immune system, cognitive and language development, loss of appetite, and reduced mood and productivity (LOUREIRO et al., 2018). The recommended daily intake for both men and women is around 8 and 18 mg day^{-1} (WISHART, 2017). Zn deficiency is associated with difficulties in immune system function, sensory function, neurobehavioral development, reproductive health, and physical growth and development (GRACIANO et al., 2020).

One of the strategies to combat hidden hunger is agronomic biofortification of food, a technique that aims to increase the concentration of nutrients in the edible parts of plants (ALMEIDA et al., 2020). Biofortifying lettuce proves to be an excellent strategy, since it is widely consumed in the economic classes of the population. Lettuce has a high content of antioxidants, such as vitamin C and E, and phytochemicals, such as vitamins B9, C, E, carotenoids and polyphenols (LAFARGA et al., 2020), mineral salts, terpenoids, vitamins A, B1, B2 and K, in addition to a low caloric value (CAMEJO et al., 2020).

Studies on agronomic biofortification of plants in hydroponic systems have grown because of their advantages, as they allow managing the nutritional status of the plant during its growth, due to greater efficiency in the use of water and nutrients, which promotes higher yield and better quality of vegetables (MERCÊS; MEDELO; CECILIO FILHO, 2022). Biofortification via hydroponic cultivation may be more efficient than the soil biofortification method, since the constant exposure of the root system to the fortified nutrient solution and the absence of interaction of the element with the physical, chemical and biological attributes of the soil maximize the absorption, translocation and accumulation of the nutrient in the edible parts of the plants (WIESNER-REINHOLD et al., 2017; ROUPHAEL; KYRIACOU, 2018). Lettuce is the main species cultivated in Brazil in hydroponics, in the Nutrient Film Technique (NFT) system.

Thus, given the importance of both nutrients and the importance of lettuce in the world diet, studies that evaluate the simultaneous biofortification with these elements in the crop, to combat hidden hunger, should be carried out. The aim of this study was to evaluate the biofortification of lettuce with Fe and Zn by increasing the concentrations of these micronutrients in the nutrient solution in hydroponic cultivation.

MATERIAL AND METHODS

The experiment was conducted between September 13 and November 4, 2022, in an NFT hydroponic system, with recirculation of the nutrient solution, in a greenhouse, with 51 m length, 12.80 m width and ceiling height of 3.0 m, at UNESP - Campus of Jaboticabal, SP, Brazil (21°15’22” S, 48°18’58” W and 575 m altitude).

The meteorological data during the experimental period were 23.2 °C, 30.9 °C, 17.3 °C and 62.8% for average, mean maximum and mean minimum temperatures and relative humidity, respectively. The data were extracted from the collection of the Agrometeorology area of the Department of Exact Sciences of the Agroclimatological Station of the Campus of Jaboticabal, SP.

Six treatments were evaluated in a randomized block design, in a 2 x 3 factorial scheme, with four replicates, corresponding to the combinations of Zn (0.06 and 0.24 mg L^{-1}) and Fe (2.0, 4.0 and 8.0 mg L^{-1}) concentrations. The lowest concentrations are those recommended by Furlani et al. (1999) for each micronutrient. The sources Rexene® Ferro Q48 (Fe-EDDHA 6%) and zinc sulfate were used.

The experimental unit had 20 plants, with a spacing of 0.2 x 0.2 m, cultivated in channels of 1.2 m in length, 0.1 m in diameter and 5% slope.

‘Vanda’ lettuce, from the group of leaf lettuces (Sakata Seed Sudamerica®) that do not form heads, was sown in phenolic foam, with cells of 2 x 2 x 2 cm (Green up®), previously washed in running water (Phase 1). Eight days after sowing, when the plants showed expanded cotyledons, the seedlings were transferred to PVC channels (5 cm wide, and 5% slope), in an NFT hydroponic system (Phase 2). The nutrient solution used was that proposed by Furlani et al. (1999) for lettuce. Nutrient concentrations were: 24 (NH_4^+-N); 174 (NO_3^--N); 39 (P); 183 (K); 142 (Ca); 38 (Mg); 52(S); 0.3 (B); 0.02 (Cu); 2.0 (Fe); 0.40 (Mn); 0.06 (Mo) and 0.06 (Zn) mg L^{-1}. The nutrient solution was supplied continuously between 6:00 a.m. and 6:30 p.m.. Transplantation to the definitive channel was carried out when the plants had four leaves (Phase 3). In this phase, also between 6 a.m. and 6:30 p.m., there was continued circulation of the same solution used in Phase II, except for Fe and Zn, which had their concentrations corresponding to the treatments. In the reservoir, a proportion of 3.0 L of nutrient solution per plant was adopted. The nutrient solutions were constantly aerated. The pH was maintained between 5.5 and 6.5, and the solution was changed when the EC reached 0.8 mS cm^{-1}, which represents 40% of the initial EC.

At 13 days after transplantation (DAT) for Phase III, the contents of the pigments chlorophyll $a + b$ and carotenoids were evaluated as proposed by Lichtentaler (1987). At 18 DAT, the nutritional status of lettuce for Fe and Zn (mg kg^{-1}) was evaluated as proposed by Miyazawa et al. (2009). At 28 DAT for Phase III (52 days after sowing), harvest was performed and the following parameters were evaluated: a) Visual symptoms of Fe and Zn toxicity; b) Leaf area (cm^2 $plant^{-1}$): determined using the LICOR® LI 3100 instrument; c) Leaf dry mass (g $plant^{-1}$); d) biofortification of lettuce with Fe and Zn (mg kg^{-1}): leaf tissue contents were determined according to methods described by Miyazawa et al. (2009); and e) Ascorbic acid content as described by Strohecker and Henning (1967).

The data obtained were subjected to analysis of variance by the F test at 5% probability level, using the AgroEstat program (BARBOSA; MALDONADO JÚNIOR, 2015). For all traits, Tukey test was performed for Zn concentrations and polynomial regression study was carried out for Fe concentrations in the nutrient solution, and the equations with significant fit ($p < 0.05$) and highest coefficient of determination were chosen.

RESULTS AND DISCUSSION

According to the results of the analysis of variance presented in Table 1, no interaction of nutrients was observed in the characteristics evaluated. Leaf area and leaf dry mass were not influenced by the two micronutrients. Fe concentrations influenced the other traits, except for leaf Zn content at 18 days and ascorbic acid, which were influenced only by Zn (Table 1).

Table 1. Summary of the analysis of variance, polynomial regression study for Fe concentrations and Tukey test for Zn concentrations to assess nutritional status for iron (NS-Fe) and zinc (NS-Zn), leaf area (LA), leaf dry mass (LDM), biofortification for iron (B-Fe) and zinc (B-Zn) and ascorbic acid (AA) content.

Variation source	NS-Fe	NS-Zn	LA	LDM	B-Fe	B-Zn	AA
	F values						
Fe	11.23^{**}	2.27^{ns}	3.38^{ns}	2.80^{ns}	9.76^{**}	8.23^{**}	19.41^{**}
Zn	3.10^{ns}	7.28^{*}	0.00^{ns}	0.18^{ns}	4.11^{ns}	2.10^{ns}	6.08^{*}
Fe x Zn	1.66^{ns}	1.92^{ns}	0.12^{ns}	0.11^{ns}	1.41^{ns}	2.07^{ns}	0.22^{ns}
CV (%)	21.29	9.86	15.43	12.34	11.82	13.36	20.73
Equation	Polynomial regression for Fe						
1st degree	**	ns	*	*	**	**	**
2nd degree	ns	ns	ns	ns	ns	*	*
mg L^{-1}	Tukey test for Zn						
0.06	133.4 a	79.7 b	1667.8 a	20.6 a	147.9 a	87.4 a	5.9 b
0.24	114.4 a	88.8 a	1669.9 a	20.1 a	134.2 a	94.6 a	7.3 a

CV = Coefficient of variation; *, **, ns = significant at 5%, 1% and not significant by F teste, respectively. Means in the collumn followed by the equal letters do not differ by the Tukey test ($p > 0.05$).

At 18 DAT for Phase III, leaf Fe content increased linearly with the increase of Fe concentrations in the nutrient solution (Figure 1) and was not influenced by Zn concentrations in the nutrient solution. As for Zn, its leaf content increased from 79.7 to 88.8 mg kg^{-1} when the concentration changed from 0.06 to 0.24 mg L^{-1} (Table 1). These contents were within the range of 30 to 100 mg kg^{-1}, considered adequate by Trani et al. (2018).

Leaf Fe contents at 18 DAT were within the range of 50 to 150 mg kg^{-1}, considered adequate by Trani et al. (2018), only in the treatments 2 and 4 mg L^{-1} of Fe in the nutrient solution, which led to contents of 96.6 and 117.1 mg kg^{-1}, respectively. With 8 mg L^{-1} of Fe, a leaf content of 157.9 mg kg^{-1} was found. Regardless of the treatment, leaf Fe content was higher than that reported by Giordano et al. (2019), who observed leaf Fe contents of 75.5 and 93.0 mg kg^{-1} for green and red lettuce, respectively, when the Fe supply in the nutrient solution was 112 mg L^{-1}.

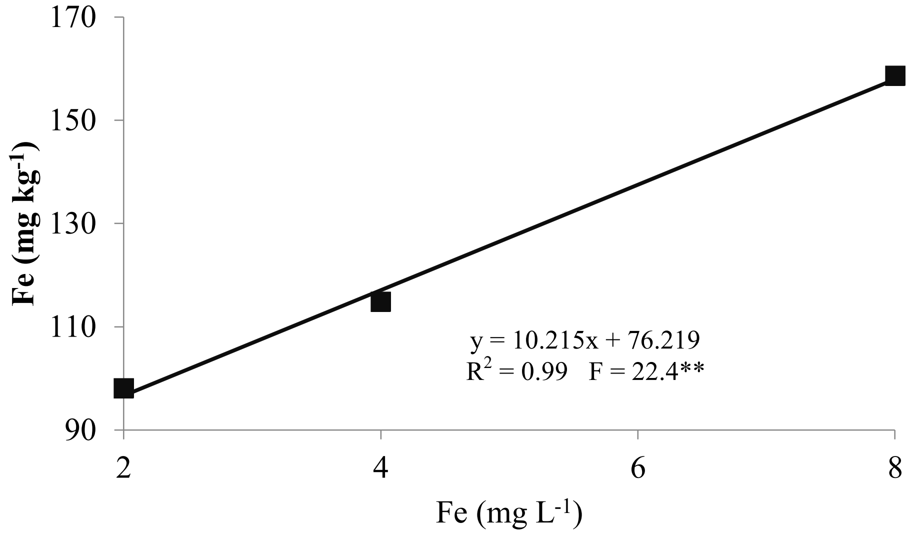

Figure 1. Foliar iron (Fe) content in lettuce, 18 days after transplanting to phase III of hydroponic cultivation, as a function of the concentration of iron in the nutrient solution. ** $p < 0.01$.

Although no significant effects of the factors were found in the analysis of variance for some traits, there were fits of the polynomial equation for all of them, except for Zn content in the leaf at 18 DAT for Phase III. When the Fe concentration increased to 4 and 8 mg L^{-1}, there were reductions of 4.4 and 13.3% compared to the leaf dry mass obtained with 2 mg L^{-1} (Figure 2). Although there were negative effects of Fe on traits that express growth, no symptoms of Fe toxicity were observed in lettuce plants.

There was a 15.4% reduction in the leaf area of plants cultivated with 8 mg L^{-1} of Fe in the nutrient solution, when compared to plants cultivated with 2 mg L^{-1} (Figure 2). This result is similar to that reported by Giordano et al. (2019), who observed a reduction in leaf area of lettuce grown in hydroponics with the increase of Fe in the nutrient solution. As for leaf dry mass, there was also a linear reduction with the increase in Fe concentration from 2 to 8 mg L^{-1}, reaching a reduction of 13.3% in this interval. Similar results have been observed in the literature, as several authors have found a negative influence of the increase in Fe on dry mass production in different crops (CECILIO FILHO et al., 2015; DI GIOIA et al., 2019). Giordano et al. (2019) observed reductions of 9.7 and 18.1% in leaf dry mass for green lettuce and red lettuce, respectively, when the Fe in the nutrient solution increased from 0.84 to 112 mg L^{-1}, while Buturi et al. (2022) observed a reduction of 18% when the supply of Fe increased from 1.12 to 57.12 and 113.12 mg L^{-1}, regardless of the variety of lettuce.

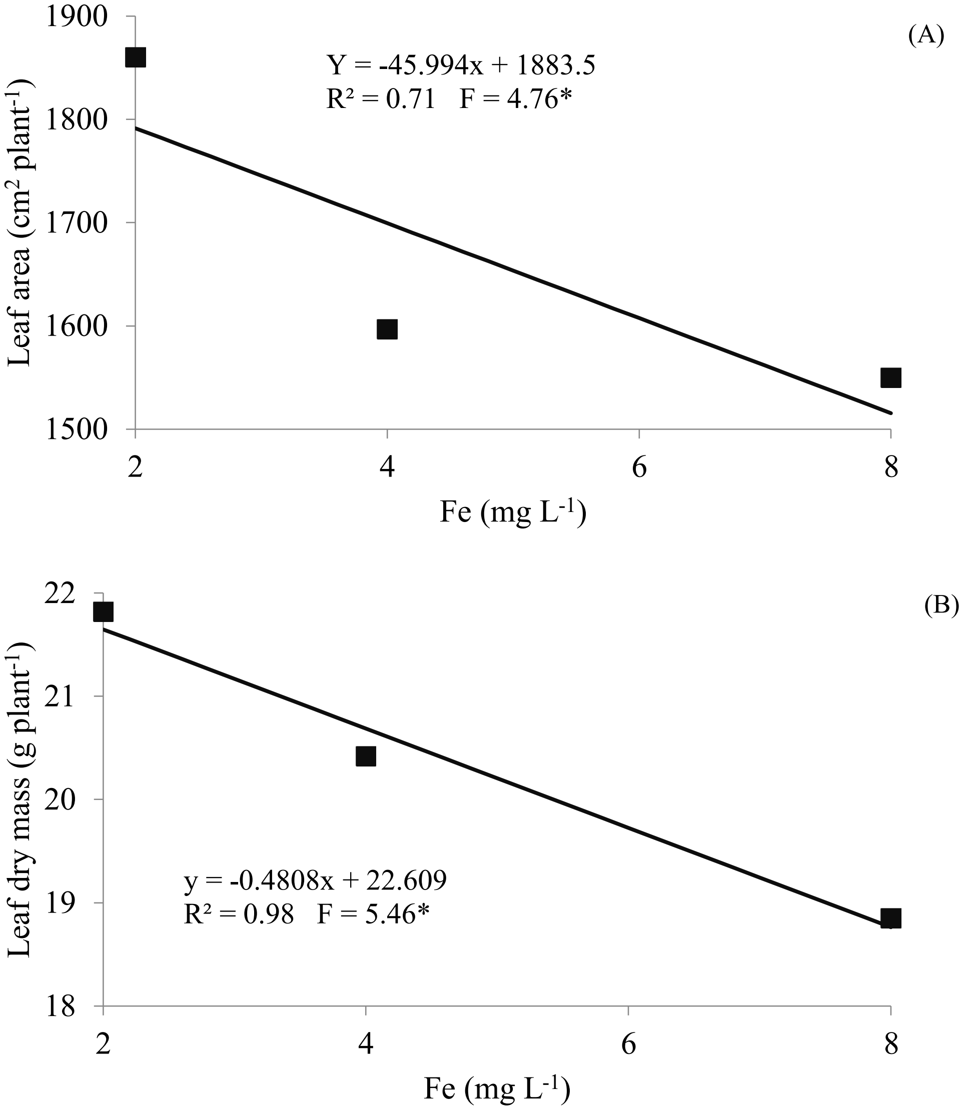

Figure 2. Leaf area (A) and leaf dry mass (B) of lettuce as a function of the concentration of iron (Fe) in the nutrient solution. * $p < 0.05$.

The reduction in plant growth with the increase in Fe supply may be related to the involvement of this micronutrient in the Fenton reaction, which leads to the overproduction of reactive oxygen species, which can lead to cell destruction, since they react with polyunsaturated fatty acids, proteins, and nucleic acids (PRZYBYSZ et al., 2016; GIORDANO et al., 2019). According to Zahra et al. (2021), its excessive accumulation can cause reduction in the number of leaves and leaf area and premature leaf senescence, reducing plant growth and production. Therefore, although no symptoms of Fe toxicity that could impair the commercial aspect of lettuce were observed, the reduction in growth is a sign of the damage caused by the nutrient.

At harvest, which was carried out at 28 DAT for Phase

III, it was possible to observe that the treatments 4 and 8 mg L^{-1} of Fe in the nutrient solution showed increments in leaf Fe content of 9.7 and 28.9%, respectively, compared to the nutrient solution with 2 mg L^{-1}, indicating that the treatments promoted biofortification (Figure 3). Thus, a 50 g portion of lettuce biofortified with 4 and 8 mg L^{-1} of Fe in the nutrient solution would promote an intake of 6.85 and 8 mg of Fe, respectively. The highest leaf Fe content was above the range of 50 to 150 mg kg^{-1}, recommended by Trani et al. (2018) for the proper growth and development of lettuce.

Giordano et al. (2019), when evaluating the response of red lettuce and green lettuce to Fe concentrations in the nutrient solution (0.84, 28, 56 and 112 mg L^{-1}), found that, at harvest, there was an increase in leaf Fe content from 40.6 to 75.5 mg kg^{-1} for green lettuce and from 66.0 to 93.0 mg kg^{-1} for red lettuce. The same authors found that, regardless of the cultivar, the addition of 28 and 112 mg L^{-1} of Fe in the nutrient solution promoted increments of 20.5% and 53.7%, respectively, in leaf Fe content.

In the biofortification process, it is desirable that there is no reduction in plant growth, which was observed in the present study by the reduction in leaf dry mass. Treatments with 4 and 8 g L^{-1} of Fe in the nutrient solution showed reductions of 4.4 and 13.3% in leaf dry mass production. Aiming at a good relationship between production and biofortification, the concentration of 4 mg L^{-1} of Fe in the nutrient solution showed a better relationship when compared to the concentration of 8 mg L^{-1}. Thus, considering the average dry mass content of lettuce shoots (5%) and the Fe content of lettuce in the solution of 4 mg L^{-1} of Fe (137.07 mg kg^{-1}), there is 6.85 mg of Fe per kilogram of fresh lettuce. Thus, considering the recommended intake of 8 and 18 mg day^{-1} of Fe for men and women, respectively, a 50 g portion of lettuce per meal can meet 4.25 and 1.89% of the daily Fe requirement, respectively. To meet 20% of the recommended daily intake for men and women, portions of 233.6 and 525.55 grams of lettuce should be consumed per day.

Leaf Zn content was influenced by the Fe concentrations in the nutrient solution, highlighting the influence of one micronutrient on the other. A plateau-type equation fitted to the data, and the maximum leaf Zn content was 105.08 mg kg^{-1} at the Fe concentration of 2 mg L^{-1} in the nutrient solution; higher Fe concentrations reduced Zn availability to the plants (Figure 3). However, there was no effect of Zn concentrations on leaf Fe content. The antagonistic interaction between Fe and Zn can be explained by three possible mechanisms: competition between Zn^{2+} and Fe^{2+} during absorption (KABATA-PENDIAS, 2010); interference in the chelation process during the absorption and translocation of Fe (KABATA-PENDIAS, 2010); and inhibitory competition between Fe and Zn during unloading in the xylem (ALLOWAY, 2008). Tewari, Kumar and Sharma (2008) reported that, due to the similarities in ionic radii, the transporters of Zn and Fe have affinity, indicating competition in absorption when Fe concentration increased. This interaction between Fe and Zn can negatively affect their uptake by plants when present in the same solution. This is due to the chemical similarity and the sharing of transporters, causing them to compete for absorption and transport (OLIVEIRA et al., 2023).

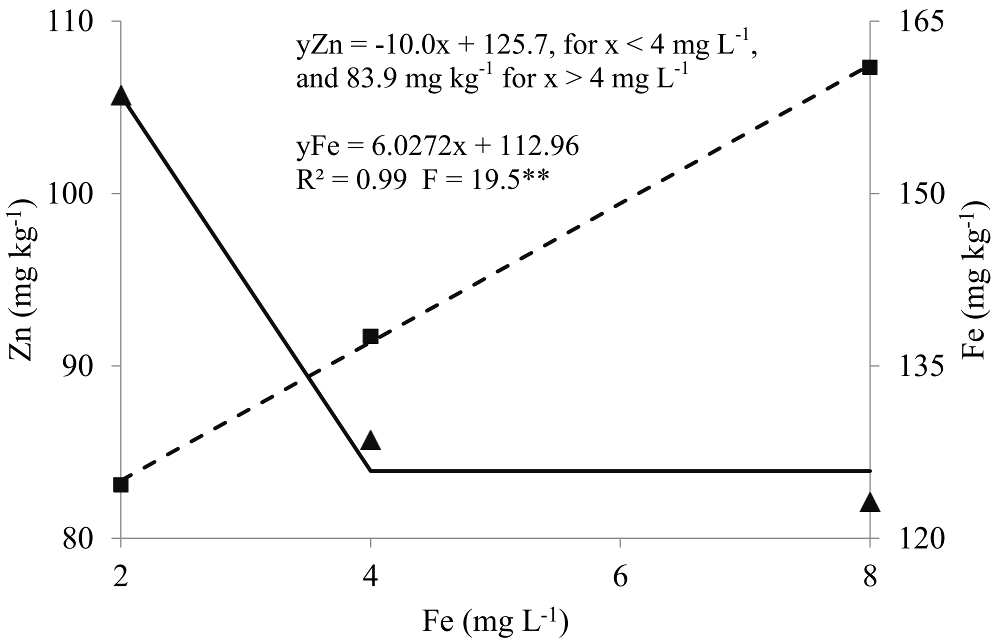

Figure 3. Iron (Fe) and zinc (Zn) content in the dry mass of lettuce leaves at harvest as a function of the concentration of iron (Fe) in the nutrient solution. ** $p < 0.01$.

Different results have been reported in the literature for the relationship between Fe and Zn. Di Gioia et al. (2019) found linear increase of leaf Zn content in arugula, red cabbage, and red mustard microgreens when there was an increase of Fe in the nutrient solution. Likewise, Buturi et al. (2022) found a similar result for lettuce in hydroponics. However, Przybysz et al. (2016) observed no response for broccoli and mung bean sprouts. Mercês, Medelo and Cecilio

Filho (2022) found similar results to those of the present study while evaluating the effects of Fe concentrations in the nutrient solution on cabbage. The same authors found that the leaf Zn content decreased from 250.8 to 195.2 mg kg^{-1}, when Fe concentration in the nutrient solution increased from 2 to 10 mg L^{-1}, representing a reduction of 22.16% in leaf Zn content.

Ascorbic acid content increased up to 7.76 mg $100g^{-1}$, maximized with the Fe concentration of 4 mg L^{-1} in the nutrient solution (Figure 4), which promoted an increase of 81% compared to that obtained with the Fe concentration of 2 mg L^{-1}. At the highest concentration of Zn, the ascorbic content acid was 7.32 mg 100 g^{-1} of fresh mass, 23.2% higher than at the lowest concentration of Zn in the nutrient solution (Table 1). Giordano et al. (2019) found similar results for red lettuce, with a quadratic fit of the equation and an increase of about four times. For green lettuce, the same authors found an increasing linear result, with increment of 62.7%, as observed by Buturi et al. (2022), who found a 60% increase in the ascorbic acid content in romaine lettuce. Ascorbic acid is the most efficient enhancer of Fe absorption, being able to overcome all possible effects of Fe absorption inhibitors in the diet (BUTURI et al., 2022).

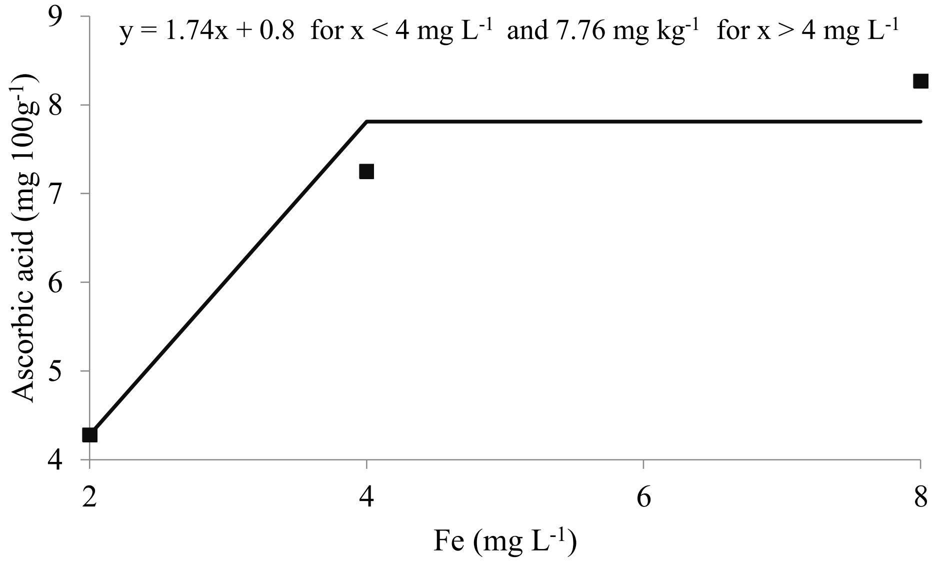

Figure 4. Ascorbic acid content in the fresh mass (MF) of lettuce leaves as a function of the concentration of iron (Fe) in the nutrient solution.

The concentration of Zn in the nutrient solution positively influenced ascorbic acid content, which increased by 23.2%. However, studies evaluating the concentration of Zn in ascorbic acid production are scarce and deserve further investigation.

CONCLUSION

In the joint evaluation of the increase in the concentrations of the two micronutrients in the nutrient solution for the biofortification of lettuce, it is concluded that the increase in Zn availability in the nutrient solution, in addition to increasing leaf Zn content, increases the content of ascorbic acid in lettuce leaves, the main enhancer of Fe absorption by the human body, thus representing an additional effect to agronomic biofortification. However, when the Fe concentration in the nutrient solution increased, there was a decrease in Zn absorption by the plant, making it possible to achieve partial biofortification, observed only for Fe, in addition to increasing the content of ascorbic acid. Despite the 4% reduction in the dry mass of lettuce leaves, the Fe and Zn concentrations of 4 and 0.06 mg L^{-1}, respectively, in the nutrient solution are recommended to biofortify lettuce with Fe and increase the concentration of ascorbic acid, enhancing the efficiency of agronomic biofortification in the treatment of anemia in humans.

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