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The Effect of Zinc and Iron Applications from Different Sources to Growth, Dry Matter, Zink and Ion Uptake by Lettuce (*Lactuca sativa*)

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ABSTRACT

Zinc (Zn) and iron (Fe) are among the micronutrients humans need. However, the main food sources in developing countries such as Malaysia have low micronutrients, making it insufficient to supply the minimum daily requirement. Foliar fertilization is one of the most effective and safe ways to enrich important micronutrients in plants. This study investigated variations in Zn and Fe sources to evaluate the effects of individual Zn and Fe foliar applications on growth, dry matter, and nutrient uptake by lettuce (*Lactuca sativa*). Based on the result, the application of Zn and Fe in the form of sulfate salt showed a lower toxicity effect in terms of growth and dry matter of plants than Fe and Zn in the form of ethylenediaminetetraacetic acid (EDTA). In terms of Zn uptake, it was found that there was a significant difference observed compared to the control, especially when 3 kg/ha Zn was applied, regardless of whether it was in the form of sulfate or EDTA. Furthermore, there was an increase in Fe uptake observed with increased Zn application. In contrast, the Fe application showed no difference in Fe intake compared to the control. It was found that there is a decrease in Zn uptake observed with increasing application of Fe rate. Sufficient Fe content is already available in the soil, and plants only take up what is needed for growth.

Keywords: Biofortification, foliar fertilizer, leaves number, plant height, toxicity

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INTRODUCTION

Humans require at least 22 nutritional elements to maintain their health, and one of the ways is by consuming food sources that are rich in nutrients (Rugeles-Reyes et al., 2019). Zinc (Zn) and iron (Fe) are among the micronutrients needed by humans, with the daily requirement for adults at 4.3–6.6 and 10–29 mg/day, respectively (Ministry of Health [MOH], 2017). Although it is important in human health, the main food sources in developing countries such as Malaysia have low micronutrients, making it insufficient to supply the minimum daily requirement.

Incidents of nutrient deficiency, especially Zn and Fe, are widely reported. It is estimated that 17.3% of the world population is at risk for inadequate Zn intake. The prevalence of insufficient Zn intake can range from 7.5% in high-income areas to 30% or more in areas of South and Southeast Asia, Sub-Saharan Africa, and Central America (Wessells et al., 2012). In Malaysia, iron deficiency or anemia was the main cause of years of living with disability among children and teenagers in 2013 (Kyu et al., 2016). Therefore, biofortification strategies have been developed to overcome this issue to enrich the food with essential nutrients for human nutritional needs.

In general, these beneficial elements are naturally present in the soil. Mineral elements found in the soil exist in many forms. Among them are free ions, ions adsorbed on mineral or organic surfaces, dissolved or precipitated compounds, or as part of the lattice structure contained in the soil biota. The paramount soil properties determining mineral accessibility are soil pH, redox conditions, cation exchange capacity, microbial activity, soil structure, organic matter, and water content. Although high concentrations of Fe and Zn occur in many soils, soil properties often limit the phytoavailability of these mineral elements (White et al., 2009).

Therefore, applying fertilizers to the leaves can enhance the uptake and transport of micronutrients to edible parts of the plant. Foliar fertilization is one of the most effective and safe ways to enrich important micronutrients in plants. Moreover, foliar spray with micronutrients is one of the ways to improve plant production, reduce the adverse effects on the environment, and need little infrastructure compared to other plant nutrient applications (Zahed et al., 2021).

Leaf application material can enter the inside of the leaf either through the penetration of the cuticle or through the stomatal pathway. However, the gap between beneficial and toxic concentrations is often quite close. Therefore, the right sources of raw materials aimed at the accumulation of micronutrients that contribute to human health must be obtained, and at the same time, do not negatively affect the plant's growth (Rouphael et al., 2018).

The concept of vegetable biofortification is very new in Malaysia. Currently, no health-beneficial nutrient-enriched products are available in the market, particularly for Zn and Fe. However, as time goes by, consumer awareness of health-based products is increasing. For consumers concerned about the need for these micronutrients, the easiest option available is buying synthetically processed supplement tablets. Therefore, producing vegetables enriched with these beneficial nutrients is very relevant nowadays. Apart from food safety issues, producing nutritious food that meets human dietary needs is also a challenge in the agricultural sector. Urgent action is needed to overcome this issue, and vegetable biofortification is seen as the strategic way to enhance micronutrients in the edible portion of crops.

Many factors need to be considered to develop a vegetable biofortification program. Biofortification strategies depend on a number of factors, such as the chemical form, application rate, environmental conditions, growth stage, and plant types. Loose leaf lettuce (*Lactuca sativa*) was chosen in this study because lettuce is among the most consumed leafy vegetables in Malaysia. Based on the planted area, lettuce is among the major vegetables produced in Malaysia. There is an area of 4,728.23 ha of lettuce planted in Malaysia, producing 75,546.04 metric tons in 2021 (Department of Agriculture [DOA], 2021).

Many biofortification studies on lettuce have been conducted abroad and indirectly show its potential to be developed as a functional food. However, no study has been done on the effect of using different Zn and Fe raw materials on the biofortification of lettuce in Malaysia. Identifying raw materials for vegetable biofortification is the first thing that needs to be studied. Hence, variations in the source of Zn and Fe raw materials were investigated in this study to evaluate the effects of individual Zn and Fe foliar application on plant growth, i.e., plant height and leaves number of loose-leaf lettuce (*L. sativa*). In addition, the effect on plant dry weight and plant uptake of Zn and Fe was also investigated. The findings of this study are the identification of suitable raw materials for salad biofortification with Zn and Fe without causing a reduction in yield and crop quality.

MATERIALS AND METHODS

Glasshouse Experiment

A glasshouse experiment was conducted in Serdang, Selangor, Malaysia. The soil used for the glasshouse experiments had not been fertilized before; therefore, residual effects from previous fertilizer applications in the soil are assumed to be absent. The initial properties of the soil used for the glasshouse experiment are shown in Table 1.

Table 1
Initial chemical properties of the soil media used in the glasshouse experiment

Parameters		
pH (H_2O)		5.6 ± 0.04
Electrical conductivity (µS/cm)		219 ± 13.04
Cation exchange capacity ($cmol_{(+)}$/kg)		6.08 ± 0.26
Exchangeable bases ($cmol_{(+)}$/kg)		
	K	0.44 ± 0.03
	Ca	3.22 ± 0.20
	Mg	0.06 ± 0.04
	Na	1.02 ± 0.05

Table 1 *(Continue)*

Parameters		
Carbon (%)		1.05 ± 0.05
Nutrient concentration (%)		
	N (%)	0.11 ± 0.01
	P (%)	0.05 ± 0.002
	K (%)	0.40 ± 0.02
	Fe (%)	1.92 ± 0.24
	Zn (mg/kg)	27.06 ± 1.65 (Zarcinas et al., 2004—Mean Zn concentration: 38 mg/kg)

Note. Means (± standard error)

Experimental setups for Zn and Fe were done separately, as Zn and Fe were applied individually. For each setup, the experimental layout was in factorial (2 x 4) randomized complete block design (RCBD). Eight treatments with three replications totaled 24 planting boxes (50 cm x 20 cm). Each treatment received the same amount of 15–15–15 fertilizer (YaraMila™, Malaysia), which was applied to the soil equivalent to a rate of 500 kg/ha. In each setup, two different raw materials were tested: zinc sulfate ($ZnSO_4$) and Zn-EDTA at 0, 0.5, 1.5, and 3.0 kg/ha Zn, respectively. Meanwhile, iron sulfate ($FeSO_4$) and Fe-EDTA were applied at rates 0, 0.5, 1.0, and 2.0 kg/ha Fe, respectively.

Lettuce seeds are sown in seedling trays and transferred to planting boxes 10 days after sowing. The planting distance between plants is 10 cm, with five plants per plant box. Zinc and iron solutions were manually sprayed on the leaf surface according to treatment 20 days after planting. The amount of Zn and Fe sprayed is calculated based on the pot area for 5 plants. Due to the very small amounts of Zn and Fe, a solution with a concentration of 1,000 mg/L Zn and 1,000 mg/L Fe was prepared. For the Zn setup, the amount sprayed was 8, 24, and 48 ml/pot. Meanwhile, the amount sprayed for Fe was 8, 16, and 32 ml/pot.

The effect of Zn and Fe application on growth, i.e., plant height and the number of leaves, was recorded 30 days after planting. Plant height measurement begins from the base of the stem (at the soil surface) to the highest part of the plant by supporting the stem if needed without lifting or extending the leaves. Visible leaves number was counted, including the new leaves. Each plant's height and leaf number were measured and recorded as an average for each pot.

Dry Matter and Nutrient Analysis

At 45 days after sowing, five plant samples from each replicate of each treatment were harvested. Due to the small plant size and insufficient samples for analysis, five plants from each pot were composited to become one sample. Plant samples were washed in the laboratory and divided into roots and upper parts. The plant samples were dried at 60°C until a constant weight was obtained. Then, the dried sample was weighed to determine its dry mass before being ground into a fine powder.

The dried plant sample was digested with nitric acid at 110°C for 2 hr or until the yellow fumes disappeared and the sample solution became clear. After cooling, hydrochloric acid was added, and digestion was continued for half an hour or so until a clear solution was obtained. The sample was then diluted to 100 ml with distilled water before determining Zn and Fe was performed using inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Fisher Scientific, USA). Nutrient uptake by plants is calculated by multiplying the nutrient concentration by the dry weight of the plant part.

Statistical Analysis

Data on plant height, number of leaves, dry matter, and nutrient uptake of lettuce were analyzed by two-factor analysis of variance (ANOVA), i.e., by making raw material and application rate as fixed factors affecting all the parameters mentioned above. Differences between treatment means were tested using Tukey's honestly significant difference (HSD) at $p \leq 0.05$ significance level. All data were analyzed using the SAS Statistical Analysis System (SAS version 9.4).

RESULTS AND DISCUSSION

Effect of Zinc Fertilization on Plant Height and Leaves Number of Lettuces

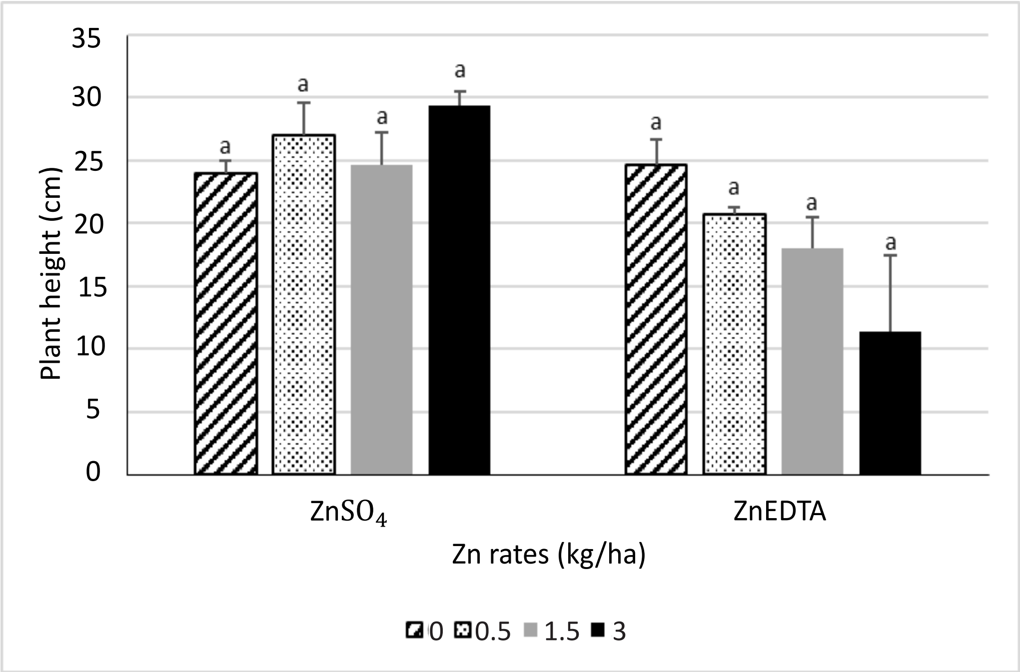
Foliar Zn application is an effective way to increase Zn concentration in food crops. Zinc sulfate is the inorganic form often used as a soil-applied Zn fertilizer, while the chelating source is Zn-EDTA (Doolette et al., 2018). However, to develop a more efficient foliar Zn fertilizer, information on the appropriate raw material and its effect on plant growth needs to be known in depth. The effect of Zn fertilization on plant height and leaves number of lettuces is shown in Table 2. Based on the table, there is an interaction between the raw material of Zn used and its rates on plant height and leaves number of lettuces. Hence, the plant height and leaves number of lettuces sorted by raw material of Zn used are presented in Figure 1.

Based on Figure 1, no significant difference was observed in plant height and the number of lettuces when the $ZnSO_4$-treated and untreated plants were compared. This result shows that $ZnSO_4$ might not affect plant height and the number of lettuce leaves. Zinc is an essential plant nutrient that has a vast effect if it is deficient in plants. However, this experiment was conducted in non-problematic conditions where the plant might have accumulated a somewhat satisfactory concentration of Zn from the soil; therefore, the external application of $ZnSO_4$ through foliar might not affect plant height and leaves number of lettuces significantly. Similar findings were also observed in the study by Munirah et al. (2015).

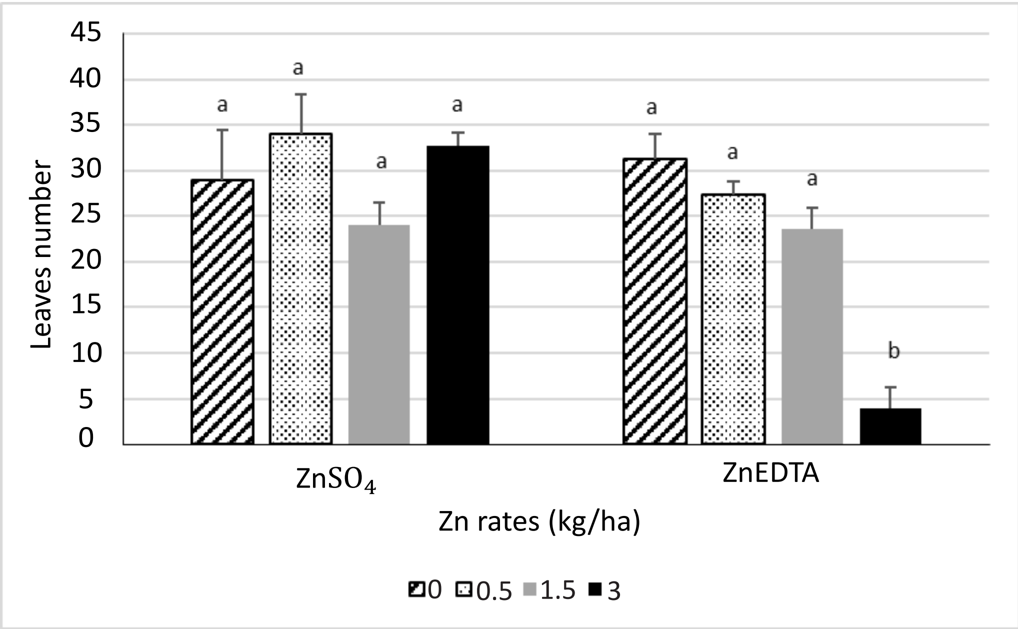
Table 2
The number of plant heights and leaves of biofortified lettuce with Zn

Treatment	Plant height	Leaves number
Raw material, n = 12		
$ZnSO_4$	26.25 ± 1.07 a	29.92 ± 1.86 a
Zn-EDTA	18.67 ± 2.08 b	21.58 ± 3.39 b
Zn rates (kg/ha); n = 6		
0	24.33 ± 1.03 a	30.17 ± 3.20 a
0.5	23.83 ± 1.87 a	30.67 ± 2.01 a
1.5	21.33 ± 2.20 a	23.83 ± 1.49 ab
3.0	20.33 ± 4.90 a	18.33 ± 6.53 b
Significance level		
Raw material	**	**
Zn rates	ns	**
Raw material x Zn rates	*	***
Mean	22.46	25.75
CV	21.83	21.21

Note. Means (± standard error) with different letters are significantly different ($p \leq 0.05$) using Tukey's honestly significant difference (HSD) test; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns = Not significant



(a)



(b)

Figure 1. Plant height (a) and leaves number (b) of lettuces sorted by Zn raw material
Note. Means with different letters for each raw material are significantly different ($p \leq 0.05$) using Tukey's honestly significant difference (HSD) test

No significant difference was observed in plant height applied with various rates of Zn-EDTA; however, leaf numbers had reduced significantly when 3 kg/ha Zn-EDTA was applied as compared to other treatments. According to Vadlamudi et al. (2020), Zn will become highly toxic to plants when it reaches a concentration of 200 ppm. Symptoms shown are smaller leaf size, green disease on new leaves, stunted growth of the entire plant, and reduced root growth. In addition, a high intake of Zn also affects the intake of other nutrients such as P, Fe, and Mn, resulting in plant structure deficiencies.

Effect of Fe Fertilization on Plant Height and Leaves Number of Lettuces

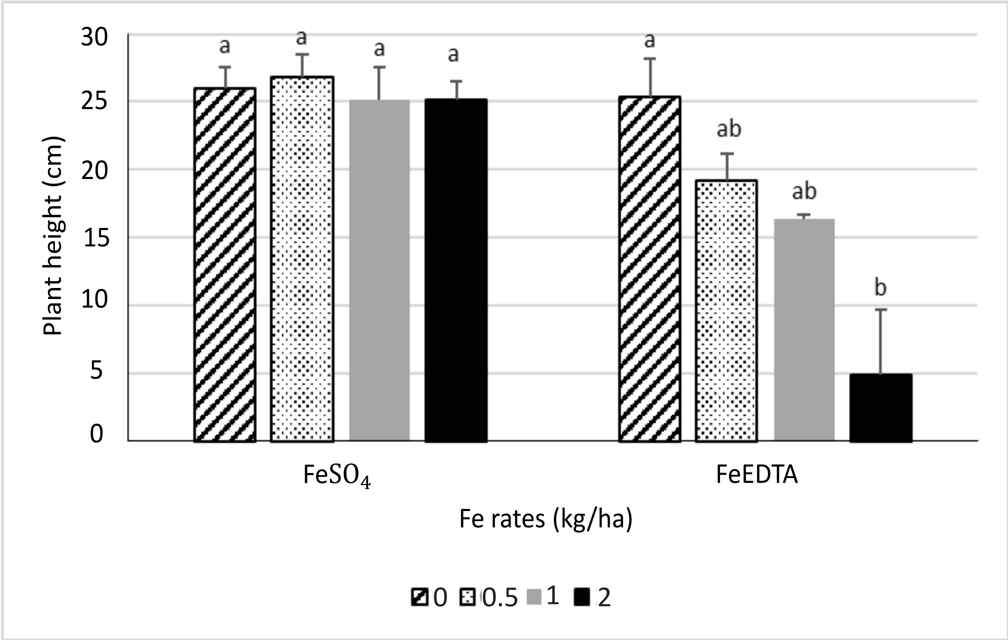
Iron is the third most limiting nutrient for plant uptake and plant metabolism, and this is due to its low solubility when in the oxidized ferric form and aerobic environments. Symptoms of Fe deficiency that can be seen in plants are interveinal chlorosis on young leaves and stunted root growth (Rout et al., 2015). The effect of Fe fertilization on plant height and leaf number is shown in Table 3. There is an interaction between the raw material of Fe used and its rates on plant height and leaves number of lettuces. Hence, the plant height and leaves number of lettuces sorted by raw material of Fe used are presented in Figure 2. No significant difference was observed in plant height and the number of lettuces applied with various rates of $FeSO_4$. This result shows that $FeSO_4$ fertilization might not affect plant height and leaves the number of lettuces.

Iron deficient symptoms do not happen to plants without Fe foliar application, which means its effect on growth cannot be seen significantly if sufficient Fe content is already available. According to El-Jendoubi et al. (2014), Fe fertilization on plants will re-green the leaves and increase biochemical and metabolic compounds in the leaves. Meanwhile, with Fe-EDTA application, plant height and leaves number reduced significantly when 2 kg/ha Fe was applied compared to the control (0 kg/ha Fe). Iron is an important nutrient for plants that functions to receive and donate electrons in the electron transport chain for photosynthesis and respiration. However, if iron accumulates at high levels, it becomes toxic and acts as a catalyst through the Fenton reaction to produce hydroxyl radicals, which can cause damage to lipids, proteins, and DNA (Connolly & Guerinot, 2002). It is found that tissue Fe concentration ranges between 500 and 5,000 mg/kg will cause yield losses between 40 to 100% (Zahra et al., 2021).

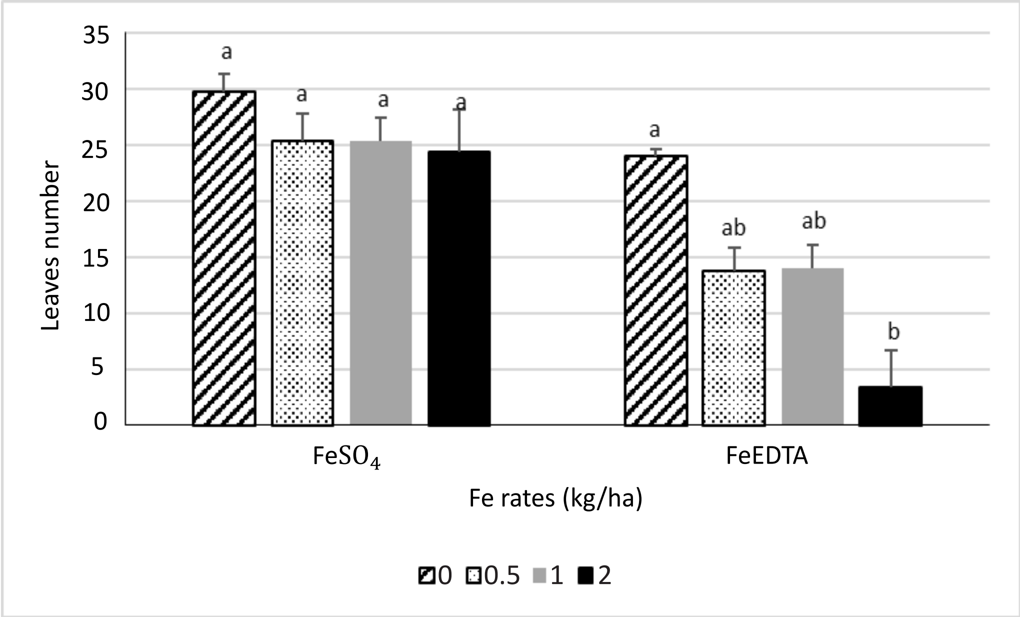
Table 3
The number of plant heights and leaves of biofortified lettuce with Fe

Treatment	Plant height	Leaves number
Raw material, n = 12		
$FeSO_4$	25.79 ± 0.78 a	26.17 ± 1.27 a
Fe-EDTA	16.42 ± 2.58 b	13.75 ± 2.41 b
Fe rates (kg/ha); n = 6		
0	25.67 ± 1.47 a	26.83 ± 1.49 a
0.5	23.00 ± 2.07 a	19.50 ± 2.99 b
1.0	20.75 ± 2.24 ab	19.67 ± 2.85 b
2.0	15.00 ± 5.07 b	13.83 ± 5.21 b
Significance level		
Raw material	***	***
Fe rates	**	***
Raw material x Fe rates	**	*
Mean	21.10	19.96
CV	18.77	17.91

Note. Means (± standard error) with different letters are significantly different ($p \leq 0.05$) using Tukey's honestly significant difference (HSD) test; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns = Not significant



(a)



(b)

Figure 2. Plant height and leaves number of lettuces sorted by Fe raw material
Note. Means with different letters for each raw material are significantly different ($p \leq 0.05$) using Tukey's honestly significant difference (HSD) test

Dry Matter, Zn, and Fe Uptake in Leaves of Zn Biofortified Lettuces

Zinc is essential for plant metabolism, as it plays the main role in chloroplast development, protein synthesis, and metabolism of carbohydrates, lipids, and nucleic acids (Buturi et al., 2021). Results from the glasshouse study show that the raw materials at different levels of Zn rates affected the dry matter of lettuce. As shown in Table 4, there is an interaction between raw materials and Zn rates. Hence, the dry weights of lettuce sorted by raw material are presented in Figure 3.

Based on Figure 3, no significant difference was observed among the treatments when $ZnSO_4$ was applied at 0–3 kg/ha Zn. In contrast to Zn-EDTA, significant differences between treatments were observed. Particularly between control (without Zn fertilization) and Zn at 3 kg/ha. Increasing Zn-EDTA rates reduced the dry matter of lettuce. The Zn concentration in plant samples that received 3 kg/ha Zn as Zn-EDTA was between 503.2–831.5 ppm. Compared with the same rate as $ZnSO_4$, the recorded Zn concentration was only between 234.9–354.4 ppm. A high concentration of Zn has affected the growth of the plants, thus giving a lower yield. As stated in Rugeles-Reyes et al. (2019), the majority of plants showed reduced yield when foliar

Table 4
Dry matter, zinc, and iron uptake in Zn biofortified lettuce

Treatment	Dry weight (g/5 plants)	Zinc uptake (mg/5 plants)	Iron uptake (mg/5 plants)
Raw material, n = 12			
$ZnSO_4$	35.52 a	6.74 a	34.64 a
Zn-EDTA	26.39 b	5.98 a	36.00 a
Zn rates (kg/ha); n = 6			
0	33.15 a	2.22 c	24.80 a
0.5	34.38 a	4.60 bc	35.81 a
1.5	28.55 b	6.98 b	38.71 a
3.0	27.73 b	11.64 a	41.97 a
Significance level			
Raw material	***	ns	ns
Zn rates	**	***	ns
Raw material x Zn rates	***	ns	ns
Mean	30.95	6.36	35.32
CV	8.296	24.13	57.81

Note. Means (± standard error) with different letters are significantly different ($p \leq 0.05$) using Tukey's honestly significant difference (HSD) test; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns = Not significant

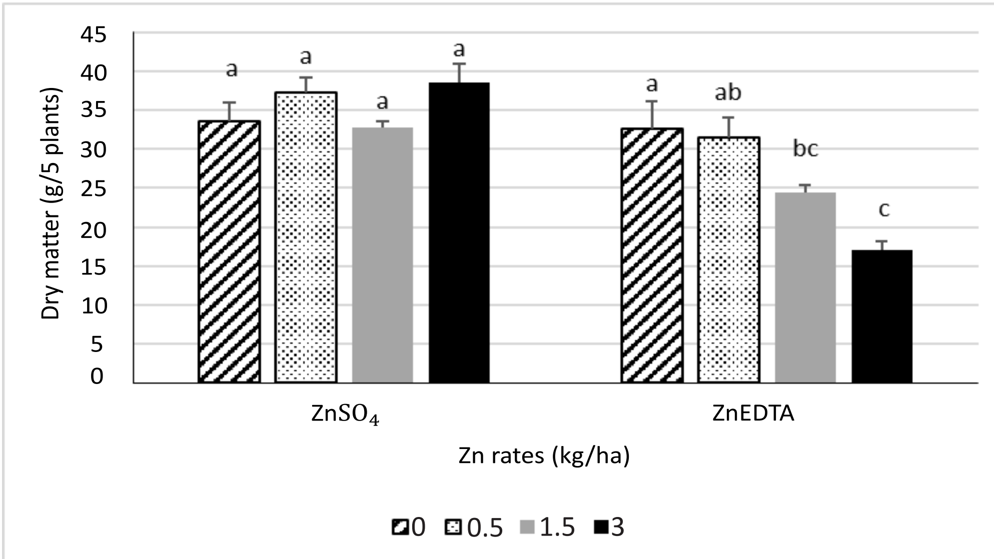


Figure 3. Dry matter of leaves of Zn biofortified lettuce sorted by Zn raw material
Note. Means with different letters for each raw material are significantly different ($p \leq 0.05$) using Tukey's honestly significant difference (HSD) test

Zn concentration was higher than 100 ppm. As well as what is stated by Buturi et al. (2021), phytotoxicity symptoms are usually apparent at leaf Zn concentrations higher than 100-700 ppm, where the symptoms shown are growth and yield reduction, leaf chlorosis and necrosis, restricted stomatal conductance, and carbon dioxide (CO_2) fixation, and also changes in chlorophyll structure and concentration. This study found that lettuce has a high Zn tolerance of more than 300 ppm, so it is more appropriate for studies of Zn biofortification.

Apart from dry weight, plants biofortified with Zn were also tested in terms of Zn and Fe uptake. Plants have different abilities to accumulate nutrients in their tissues, and the Zn hyperaccumulation characteristic is mostly seen in Brassicaceae members until many biofortification studies have been done on leafy brassicas. Meanwhile, for non-brassica, higher Zn susceptibility was also found in hydroponically grown lettuce by Barrameda-Medina et al. (2014), where Zn concentration in the leaves increased by 270% compared to the control; however, there was a decrease in biomass. Based on Table 4, there is no interaction between the raw material of Zn used and its rates on Zn and Fe uptake of lettuce. Lettuce applied with $ZnSO_4$ and Zn-EDTA shows no difference in Zn and Fe uptake. According to Buturi et al. (2021), biofortification of Zn, mainly in the form of sulfate, increases the content of Zn in vegetables; however, no differences were observed in this study.

In terms of rates, lettuce treated with 3 kg/ha Zn showed higher Zn uptake and differed significantly from the control. It is shown by the significant difference in Zn concentration between the two, which is 57.8–80.7 ppm for the control, while

234.9–831.5 ppm for those treated with 3 kg/ha Zn. In contrast to Fe uptake, no significant differences were observed at all Zn rates applied. According to Zou et al. (2019), foliar Zn application increased Fe concentrations in grain, and foliar Zn spray probably caused the formation of Zn binding compounds in grain, which most likely act as a sink for Fe transport and storage. Although Fe uptake in lettuce is seen to increase with the increasing Zn rates, no statistically significant difference was observed in this study, which shows that the application of Zn to plants does not affect the uptake of Fe in lettuce.

Dry Matter, Zn, and Fe Uptake in Leaves of Fe Biofortified Lettuces

The concentration of Fe in the soil often exceeds the plant's needs, which is between 20-40 mg/kg; however, not all amount is available for plant nutrition (Buturi et al., 2021). Iron deficiency symptoms in plants always occur in overly limed or alkaline soil, where the observed symptoms are interveinal chlorosis in younger leaves (Uchida, 2000). Results from the glasshouse study show that the dry matter of lettuce was not affected by the raw materials at different levels of Fe rates because there is no interaction between the raw material of Fe used and its rates on the dry matter of lettuce (Table 5). Lettuce with $FeSO_4$ generally shows higher dry matter than plants treated with Fe-EDTA. However, in terms of Fe rates, it was found that lower dry weights were obtained for all Fe rates compared to controls.

Table 5
Dry matter, zinc, and iron uptake in Fe-biofortified lettuce

Treatment	Dry weight (g/5 plants)	Zinc uptake (mg/5 plants)	Iron uptake (mg/5 plants)
Raw material, n = 12			
$FeSO_4$	32.63 a	2.08 a	39.30 a
Fe-EDTA	19.00 b	1.07 b	26.35 a
Fe rates (kg/ha); n = 6			
0	34.25 a	2.22 a	23.47 a
0.5	28.78 ab	1.48 ab	35.30 a
1.0	22.18 b	1.33 b	35.13 a
2.0	18.05 b	1.25 b	37.40 a
Significance level			
Raw material	***	***	ns
Fe rates	**	*	ns
Raw material x Fe	ns	ns	ns
Mean	25.82	1.57	32.83
CV	26.50	28.47	62.60

Note. Means (± standard error) with different letters are significantly different ($p \leq 0.05$) using Tukey's Honestly Significant Difference (HSD) test; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns = Not significant

Significant differences with control were observed when Fe was applied at 1 and 2 kg/ha Fe. Although Fe is an important nutrient for plants, excessive Fe uptake by plant cells will result in toxicity and a reduction in crop yield. Various symptoms can be associated with high Fe in plants, including stunted growth, reduced leaf size, brown or black spots or necrotic spots on leaves, blackened leaf tips and stem bases, hardening of stems, stunted roots, lack of root branching, formation precipitate on the roots (Rout et al., 2015).

In terms of Zn and Fe uptake, there was no interaction between the raw material of Fe used and the rates of Zn and Fe uptake by lettuce. Based on Table 5, Zn uptake by plants treated with $FeSO_4$ was significantly higher than plants treated with Fe-EDTA. It was expected because the higher plant weight when $FeSO_4$ was applied caused it to accumulate more nutrients, including Zn. However, it was found that the increasing rate of Fe fertilization resulted in lower Zn uptake significantly. It happened maybe due to the reduced plant size based on the dry weight data in Table 5, so the accumulation is also low. However, this is different when Zn is applied, where Fe uptake increases with increasing Zn application rate, although there is a decrease in plant size. The application of Fe to lettuce does not have a synergistic effect on Zn uptake.

For Fe intake, no significant difference was seen regardless of the Fe raw material used. Similarly, for Fe rates, no difference was seen in Fe uptake by lettuce on all Fe treatments applied. The same explanation for the effect of Fe use on plant height and the number of leaves also applies to this, where sufficient Fe content is already available in the soil; therefore, the plant only takes what it needs for growth. According to Hochmuth (2011), Fe uptake depends on the plant's ability to convert Fe^{3+} to Fe^{2+} and separate it from complex compounds or chelates. This reduction occurs on the cell surface, and the electrons in the cell are used. The same thing also happens at the root tip, where Fe absorption occurs a lot; that is, Fe chelated in the soil solution moves to the root through mass flow or diffusion. Fe is reduced and removed from chelating molecules in roots and moves through the cell membrane. However, Fe uptake can be disturbed if there are other cations in the soil solution, such as manganese (Mn) and calcium (Ca).

CONCLUSION

In the biofortification program, value over volume is preferred. However, the effect of biofortification on growth still needs to be emphasized so that the plant yield obtained is not lower than conventional cultivation methods. Applying Zn and Fe in the form of sulfate salt showed a lower toxicity effect in terms of growth and dry matter of plants than Fe and Zn in the form of EDTA. Although the beneficial effects of applying $ZnSO_4$ and $FeSO_4$ on lettuce plant height and leaf number are not seen, they do not adversely affect crop growth and yield. Perhaps the beneficial effects on plant growth and yield may be observed in plants grown in Zn and Fe-deficient conditions.

In terms of Zn uptake, it was found that there was a significant difference observed compared to the control, especially when 3 kg/ha Zn was applied, regardless of whether it was in the form of sulfate or EDTA. Furthermore, there was an increase in Fe uptake observed with increased Zn application compared to the control. In contrast to Fe, the Fe application did not show any difference in Fe intake compared to the control. It was found that there is a decrease in Zn uptake observed with increasing application of Fe rate. It is believed that sufficient Fe content is already available in the soil, and plants only take up what is needed for growth.

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