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Foliar Fertilization of Zinc, Iron and Manganese in Safflower (*Carthamus tinctorius* L.) under Rainfed Conditions

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ABSTRACT

Safflower (*Carthamus tinctorius* L.) is an important oilseed crop cultivated in semi-arid regions, where micronutrient deficiencies, especially zinc, iron, and manganese, often limit its growth and yield. For investigating the effects of foliar fertilization of zinc, iron and manganese in safflower under rainfed conditions, this project was conducted in Ilam. The studied soil had low concentrations of available zinc, iron, and manganese, measured at 0.61, 4.15, and 4.13 mg kg^{-1}, respectively. The research was conducted with the following treatments: 1- control, 2- water spray 3- foliar spraying with zinc sulfate 4- foliar spraying with manganese sulfate 5- foliar spraying with iron sulfate 6- foliar spraying with zinc and manganese 7- foliar spraying with zinc and iron 8- foliar spraying with iron and manganese 9- foliar spraying with zinc, iron and manganese in a randomized complete block design with three replications. The results showed that the simultaneous foliar spray with zinc, iron and manganese, with a yield of 1112 kg ha^{-1} was in the superior statistical group and the increase in yield compared to the control, and water spraying treatments was 13.4 and 12.5 percent, respectively. Regarding oil yield, it was also found that the combined foliar spraying of zinc, iron and manganese and combined foliar spraying of zinc-manganese were in the superior statistical group with 397 and 381 kg ha^{-1}, respectively. The increasing effect of foliar spraying on the concentration of zinc and iron in the grain was 24 and 19 percent, respectively, compared to the control. Also, the highest photosynthesis rate, SPAD and stomatal conductance were observed with the application of Zn + Fe + Mn compared to the other treatments. In general, the best results for grain yield, oil yield, improvement of nutrient concentration and photosynthesis rate were obtained with combined foliar spraying of zinc, iron and manganese and zinc-iron foliar spraying, respectively.

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1. Introduction

Safflower (*Carthamus tinctorius* L.), is an oilseed crop belonging to the Asteraceae (Compositae) family. Safflower is native to arid and semi-arid regions with seasonal precipitation. Safflower grains are suitable sources of polyunsaturated fatty acids and their florets can be used for dyeing (Hatim *et al.*, 2023). Using suitable plants in crop rotation systems has always been one of the challenges facing sustainable agriculture, and this issue is also evident in repeated crops in Ilam. Failure to observe agronomic issues has caused this crop to be unable to compete economically with other crops (Boostanian and Ehsanzadeh, 2025). The elements zinc, manganese and iron participate in most of the vital enzymatic activities of the plant, and although their need is small in terms of quantity, they play a very important role in plant growth and development. Zinc is one of the essential elements for plant growth, which is involved in the formation of indole acetic acid and regulates plant growth, and also activates many enzymes, and is necessary for the synthesis of chlorophyll and the formation of carbohydrates (Stanton *et al.*, 2022). Zinc deficiency is one of the most common micronutrient deficiencies in calcareous soils around the world, resulting in decreased yield and quality of safflower. Zinc, main building part of several enzymes, is taken up by roots from the soil solution as the Zn^{2+} ion and plays many

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important roles in protein synthesis and hormone metabolism (Korkmaz *et al*., 2024).

The fertilizer requirement of safflower varies according to the expected yield and in the case of rainfed safflower, it is between 40 and 60 percent less than that of rainfed safflower (Omidi *et al.*, 2021). Research by Ravi and Channal (2010) in India on soil with zinc and iron concentrations of 0.63 and 4.45 mg kg^{-1}, respectively, showed that safflower grain yield increased by 11, 9, and 18 percent, respectively, compared to the control, with foliar application of zinc sulfate, iron sulfate, and their combination. Manvelian et al. (2021) reported that zinc application improved the growth characteristics of safflower. Abbasi et al. (2021) observed that safflower absorbed higher amounts of zinc and iron compared to most plants. Saudy et al. (2022) concluded that there was a need for manganese application in oilseeds. Ziaei et al. (2023) reported that foliar application of zinc and manganese, along with soil application of phosphorus, increased safflower yield in Egypt. Ghassemi-Golezani and Farhangi-Abriz (2021) emphasized the role of manganese in the growth and development characteristics of plants, including safflower and reported that the chemical composition of the oil is affected by manganese application. Due to the low mobility of these elements in the plant, there is a strong need for these elements in the stages of safflower emergence from the rosette and early flowering. The low availability of these elements in stages of safflower emergence from the rosette and early flowering necessitates the need for foliar spraying.

From an ecological point of view, foliar fertilization is more acceptable because small amounts of nutrients are provided for rapid consumption by the plant. On the other hand, in calcareous soils, the percentage of immobilization of zinc, iron and manganese elements is high (Bolan *et al.*, 2023). According to the experiments conducted, it was determined that one of the most important reasons for the low yield of safflower is the lack of a suitable nutritional program for this crop and always one or more elements, based on the law of minimum, cause the failure to achieve maximum yield.

Due to the uneven growth rate in different vegetative stages, the nutritional needs of safflower are different in different stages of growth, so there is a need to quickly deliver nutrients to the plant in different vegetative and reproductive stages. The concentrations of zinc, iron and manganese in more than 60% of the region's lands for crops such as wheat and safflower are below the critical level, and the severity of the deficiency is related to zinc, manganese and iron, respectively (Soleimani *et al*., 2017). With the optimal application of fertilizer, in addition to achieving sustainable yield, it is also possible to reduce the application of chemical fertilizers. This research was conducted to answer questions about variability of grain and oil yield, yield components and concentrations of zinc, iron and manganese elements as affected by foliar spraying of zinc, iron and manganese of safflower under rainfed conditions.

2. Materials and methods

2.1. Characteristics of experimental location

The research was conducted in Sarabeleh, Ilam, located at 33°45'36" N latitude and 46°35'59" E longitude. The soil at the experimental site was classified as Fine mixed mesic *Calcixerollic Xerochrepts* according to the American soil taxonomy system. The results of the soil analysis at the experimental site are given in Table 1.

Table 1. Physical and chemical properties of 0-30 cm soil depth at the project site

Year	pH	EC (dS m^{-1})	Ava. P	Ava. K	Zn	Fe	Mn	O.C. (%)	Texture
			(mg kg^{-1})						
2010-2011	7.31	0.38	15	305	0.52	3.85	4.60	1.20	Silty Clay Loam
2011-2012	7.37	0.35	14	295	0.50	3.50	4.10	1.10	Silty Clay Loam
2012-2013	7.22	0.37	12	290	0.55	4.01	4.25	1.20	Silty Clay Loam
Mean	7.34	0.37	13.7	297	0.52	3.78	4.32	1.17	Silty Clay Loam

2.2. Experimental design and treatments

The experiment was conducted in a randomized complete block design with three replications from 2010 to 2012. The experimental treatments included 1- Control (without zinc, iron and manganese) 2- Foliar spraying with tap water 3- Foliar spraying of zinc sulfate (concentration of 3:1000) 4- Foliar spraying of iron sulfate (concentration of 3:1000) 5- Foliar spraying of manganese sulfate (concentration of 3:1000) 6- Foliar spraying of zinc and manganese 7- Foliar spraying of zinc and iron 8- Foliar spraying of iron and manganese 9- Foliar spraying of zinc, iron and manganese in a randomized complete block design with three replications.

Due to the row cultivation, foliar spraying was carried out with a motorized backpack sprayer model WJR2515 at the rapid growth rosette emergence stages (23: Decimal Code) and before flowering (62: Decimal Code) at a concentration of 3:1000. Due to its high solubility in water, availability and lower cost, the source of elemental sulfate was used. Nitrogen was applied at a rate of 100 kg ha^{-1} pure from urea source in three installments at the time of planting, rosette emergence and before flowering. Phosphorus (P_2O_5) and potassium (K_2O) were applied from superphosphate and potassium sulfate sources at a rate of 25 kg ha^{-1} each as a starter in the pre-planting stage.

2.3. Measurements nutrients and agronomic traits

Total nitrogen (N) (Bremner, 1965), Olsen P (De Silva *et al*., 2015), ammonium acetate (NH_4OAc-K) for potassium (K) (Rees *et al*., 2013) and diethylenetriaminepentaacetic acid (DTPA) extractable Zn (Lindsay and Norvell, 1978) were analyzed. The variety used was Safflower, local to Isfahan, planted in November and harvested in early July. Each experimental plot consisted of 6 lines, 5 meters long, with a line spacing of 50 cm and a distance of one meter between plots and two meters between repetitions, so that the planting density in each experimental plot was 20 plants per square meter. Harvesting was done after removing two side lines and half a meter from the top and bottom of each plot, at an area of 10 square meters and the yield obtained was converted to hectares. Before harvesting, the number of fertile bolls per square meter (in three one-square-meter squares of each plot), the number of full grains per boll (by counting full grains in 20 fertile bolls within each square), and after harvesting (when the grain moisture content reached about 8%), the weight of one thousand grains (by counting three times from three random groups of one thousand grains in each plot), the concentration of zinc, manganese, and iron in the grain, and the percentage of oil per hectare were measured. The content of phosphorus (P) and potassium (K) was measured using the Olsen and ammonium acetate methods, respectively (Normandin *et al*., 1998). Iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) were determined with method of diethylene tri-amine-penta-acetic acid (DTPA) and reading using atomic absorption spectrophotometry (Lindsay and Norvell, 1978). Oil yield was calculated from the product of grain yield and percentage of oil for each experimental treatment.

2.4. Statistical analysis

Since in composite analysis of data, homogeneity of variance must be established, this issue must first be examined for those agronomic characteristics that have become significant, so Bartlett's method, which is one of the most accurate methods for testing homogeneity of variance, was used. In this method, the natural logarithm of variance is used (Odoi *et al*., 2022). The M/C ratio of the test statistic is the null hypothesis of homogeneity of variances (Equation 1).

$$C = 1 + \left[(k+1)/3kE_{df}\right] = 1.028 \quad (1)$$

M must be calculated for each agronomic characteristic. Data analysis of variance and comparison of means were performed with the SAS 9.2 program.

3. Results and discussion

The results of test of homogeneity of variance are indicated in Tables 2 and 3. According to the chi-square table with 16 degrees of freedom, the table number at the one and five percent levels was 2.3 and 2.63, respectively. Therefore, the value obtained in this experiment was less than the table value, so the homogeneity of variances was proven.

Table 2. Statistical characteristics related to Bartlett's test for homogeneity of variance of quantitative traits

Year	Grain yield	Biological yield	1000 grain weight	Grains pod^{-1}	Pods m^{-2}
	Sum of error square				
First year	2009	9869	0.290	0.349	49.7
Second year	2511	10962	0.162	0.679	39.6
Third year	2715	12241	0.309	0.478	63.5
Mean	2413^{*}	10962^{**}	0.249^{**}	0.499^{ns}	49.9^{ns}
	The natural logarithm of the sum of error square				
First year	7.61	9.20	-1.24	-1.03	3.92
Second year	7.83	9.31	-1.81	-0.384	3.74
Third year	7.91	9.41	-1.15	-0.730	4.16
Mean	7.79^{**}	9.31^{**}	-1.36^{*}	-0.679^{ns}	3.95^{ns}

** and *: Significant at 1% and 5%, respectively, ns: nonsignificant.

Table 3. Statistical characteristics related to Bartlett's test for homogeneity of variance of oil yield and qualitative traits

Year	Oil percentage	Oil yield	Zn	Fe	Mn
	Sum of error square (Si_2)				
First year	0.589	328	0.799	6.21	2.19
Second year	0.336	318	0.897	6.77	2.85
Third year	0.314	293	0.739	7.05	2.09
Mean	0.416^{ns}	314*	0.829**	6.69**	2.29*
	The natural logarithm of the sum of error square (Si_2)				
First year	-0.498	5.77	-0.172	1.83	0.793
Second year	-1.11	5.76	-0.103	1.91	1.05
Third year	-1.16	5.68	-0.298	1.93	0.751
Mean	-0.875^{ns}	5.75*	-0.187*	1.90**	0.873*

** and *: Significant at 1% and 5%, respectively, ns: nonsignificant

3.1. Yield and yield components

Analysis of variance of data showed that the effect of foliar spraying on grain yield, biological yield and thousand-grain weight was significant at the levels of one, one and five percent, respectively, but did not show significant differences on the number of seeds per boll and the number of bolls per square meter. According to the comparison of the mean data using the Duncan's multiple range test, it was determined that the combined foliar spraying treatment of zinc, iron and manganese with a yield of 1472 kg ha^{-1} was better than control treatment (Table 4). The zinc-manganese foliar spraying treatment was in the next group, so it produced an 11.5 percent increase in yield with 1454 kg ha^{-1}. The iron-manganese and zinc-iron and zinc foliar spraying treatments were also in the same statistical group and higher than the control. In these treatments, grain yield increased by 10.6, 9.97 and 9.66 percent compared to the control (Table 4). The highest biological yield was also obtained with the combined application of zinc, iron and manganese (with an increase of 11.5 and 10.5 percent compared to the control and water foliar application, respectively). The zinc-manganese foliar application treatment (with an increase of 10.2 percent compared to the control) was in the next statistical group and iron-manganese, iron-zinc and zinc treatments (with an increase of 8.85, 7.81 and 6.06 percent compared to the control) were jointly placed after it. In addition, manganese foliar spraying also produced a 22.3% increase in biological yield compared to the control, but the effect of iron foliar spraying was not significantly different from the control. (Table 5).

In the research of Ravi and Channal (2010), with zinc and iron foliar spraying, the increase in dry weight of safflower was 9% compared to the control. Also, regarding the weight of one thousand seeds, it was determined that the combined zinc, iron and manganese foliar spraying treatment with 30.7 grams (6.97% increase compared to the control) and zinc-manganese foliar spraying with 30.2 grams (5.23% increase compared to the control) were in the superior statistical group. Iron-manganese, zinc-iron and zinc foliar spraying were in the next group. Manganese foliar spraying and iron foliar spraying were in the lower groups. Regarding the number of seeds per boll and the number of bolls per square meter, although the differences were not significant, it was found that the aforementioned characteristics increased by 5.99 and 7.73 percent with combined foliar application of zinc, iron, and manganese compared to the control, respectively. However, oil yield, which is affected by changes in grain yield and oil percentage, had the highest production with combined foliar application of zinc, iron, and manganese, as well as zinc-manganese foliar application. So that the aforementioned treatments were in a higher statistical group with 16.6 and 14.7 percent increase compared to the control, respectively. After them, foliar application of iron-manganese, zinc-manganese, and zinc were in a higher statistical group with 13.3, 12.5, and 11.6 percent increase compared to the control, respectively. Also, foliar application of manganese (with 8.30 percent increase) showed a significant difference compared to the control, but iron foliar application was not effective.

Regarding zinc concentration, zinc sulfate foliar spraying achieved the highest zinc concentration, so that the zinc concentration increased from 28.6 mg kg^{-1} in the control (with a 55.9% increase) to 44.6 mg kg^{-1} with zinc foliar spraying. Although zinc foliar spraying achieved the highest zinc concentration, this result did not show a significant difference with zinc-manganese, zinc-iron-manganese and zinc-manganese foliar spraying. On the other hand, the lowest zinc concentration was achieved with iron-manganese foliar spraying. Changes in iron concentration with foliar spraying were not statistically significant. Also, grain manganese increased from 41.3 mg kg^{-1} in the control (with a 44.3% increase) to 59.6 mg kg^{-1} with manganese foliar spraying. Treatments containing manganese were placed in one group. Also, foliar spraying of other elements had no significant effect on manganese concentration (Table 5).

Table 4. Effect of foliar spray on quantitative traits of safflower in third year

Foliar application	Grain yield	Biological yield	1000 grain weight (g)	Grains pod^{-1}
	(kg ha^{-1})			
C	910^{d}	2199^{d}	28.7^{d}	26.7^{c}
W	912^{d}	2226^{cd}	28.9^{d}	26.6^{c}
Zn	1030^{b}	2292^{c}	29.8^{b}	27.8^{ab}
Fe	957^{cd}	2252^{cd}	29.4^{c}	27.2^{bc}
Mn	990^{c}	2202^{c}	29.5^{c}	27.3^{bc}
Zn + Fe	1034^{b}	2449^{b}	29.9^{b}	27.9^{ab}
Zn + Mn	1054^{ab}	2224^{ab}	30.2^{ab}	28.1^{a}
Fe + Mn	1042^{b}	2482^{ab}	30.0^{b}	27.9^{ab}
Zn + Fe + Mn	1072^{a}	2566^{a}	30.7^{a}	28.3^{a}

In each column, similar letters indicate no significant difference between the means.

Table 5. Effect of foliar spray on oil yield and qualitative traits of safflower in third year

Foliar application	Oil percentage	Oil yield	Zn	Fe	Mn
		(kg ha^{-1})	(mg kg^{-1})		
C	27.7^{b}	339^{c}	27.9^{c}	109^{b}	43.9^{b}
W	27.7^{ab}	341^{c}	28.0^{c}	109^{b}	44.0^{b}
Zn	28.2^{a}	378^{ab}	42.9^{a}	112^{a}	44.2^{b}
Fe	27.9^{ab}	349^{bc}	28.2^{c}	119^{a}	43.0^{b}
Mn	28.1^{a}	367^{b}	29.1^{b}	113^{a}	59.8^{a}
Zn + Fe	28.3^{a}	379^{ab}	40.0^{ab}	113^{a}	42.2^{b}
Zn + Mn	28.5^{a}	384^{ab}	42.1^{a}	109^{b}	58.9^{a}
Fe + Mn	28.4^{a}	382^{ab}	28.1^{c}	110^{ab}	54.9^{a}
Zn + Fe + Mn	28.6^{a}	389^{a}	39.9^{a}	112^{a}	57.9^{a}

In each column, similar letters indicate no significant difference between the means.

3.2. The effects of micronutrients on photosynthesis, SPAD (chlorophyll index) and stomatal conductance

The results of the variance analysis of data showed that there is a statistically significant difference between different levels of treatments for these traits. The results of comparing the means with Duncan's multiple range test at 5% probability level indicated that in treatment of Zn + Fe + Mn, the highest amount of photosynthesis was observed to be 37.4 µmol CO_2 m^{-2} s^{-1} (Fig. 1a). Among the treatments, Zn + Fe + Mn and Zn had higher values of SPAD index and other treatments had lower values than the previous two treatments (Fig. 1b). The results of stomatal conductance showed a significant difference between different treatments. The treatment of Zn + Fe + Mn generated the highest rates for stomatal conductance (Fig. 1c).

In the studied soil, pH was 7.43 and TNV was 32% (Table 1). At high soil pH, the absorption of elements, especially micronutrients, is limited (Arbad and Ismail, 2011). The absorbable concentrations of zinc, manganese, and iron in the studied soil were low. Soil application of micronutrients, in addition to their low efficiency of absorption by the plant, is not very economical, and therefore, alternative methods can be used. Also, elements such as zinc cannot able to be re-transported inside the plant, so their foliar application is more appropriate. The greatest effect of foliar application of the studied elements on changes in element concentrations was foliar application of zinc sulfate resulted in the highest zinc concentration, and zinc concentration increased from 28.6 mg kg^{-1} in the control (an increase of 55.9%) to 44.6 mg kg^{-1} with zinc foliar application, and manganese concentration in the grain increased from 41.3 mg kg^{-1} in the control (an increase of 44.3%) to 59.6 mg kg^{-1} with manganese foliar application.

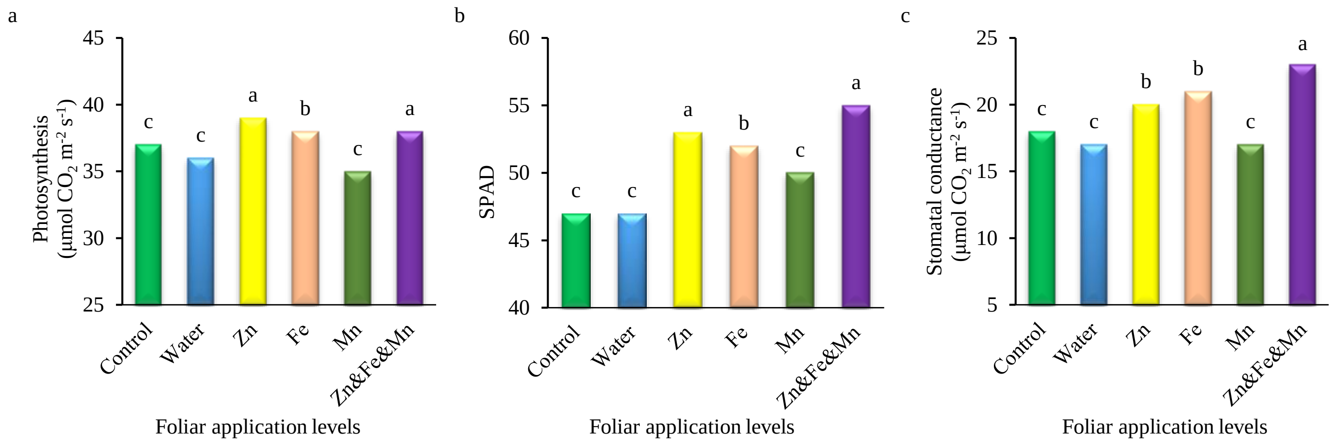

Figure 1. Comparison of average foliar application of experimental treatments on photosynthesis (a), SPAD (b), and stomatal conductance

Cakmak (2009) stated that foliar application is an effective method for increasing zinc concentration in the grain. Furthermore, according to the results obtained, it was observed that in addition to the effects of foliar application of the aforementioned elements on the concentration of these elements in safflower seeds, changes in yield components also had an increasing trend, which ultimately showed itself in grain yield. In this study, combined foliar application of iron, zinc, and manganese increased safflower yield by 12.9 and 10.2 percent compared to the control and water foliar application, respectively (Table 5). Kumara et al. (2020) also reported an increase in safflower yield affected by foliar application of micronutrients. Al-Doori (2023) also reported that foliar application of manganese increased safflower yield. Rapid plant growth and formation of yield components occur in the rosette and flowering stages, respectively, and the need for elements, including zinc, manganese, and iron, increases in each of these stages. Previous research has shown that the weight of a thousand seeds and the number of seeds per plant have the greatest effect on grain yield. In the present study, foliar application of Zn, Fe and Mn improves the growth characteristics of the safflower plant. The increase in the plant growth features under foliar spraying of Zn, Fe and Mn could be ascribed to the increase in the uptake of mineral sources of nutrients, which consequently created more favorable conditions for the better growth of safflower plant.

Hatim et al. (2023) showed the positive effects of foliar spraying of micronutrients in improving the quality of safflower at associative application of micronutrients, which was in line with the results of our research. Zn, as an essential element in suitable concentration, had positive and significant effect on plant growth. It has been reported that Zn improves activity of several enzymes in plants and then affects increasing photosynthesis and chlorophyll accumulation. Safflower plant height in our experiment was affected by micronutrient application. The results of our experiment showed that the use of Zn, Fe and Mn increases the growth indices in the plant, as similarly reported by other researchers. Also, in the experiment conducted by Magodia et al. (2025) on safflower plant, similar to our research results, it was found that the use of micronutrients can increase the plant height by 10.2%.

4. Conclusion

Soil analysis specified that the soil of the studied location had a very low amount of organic matter and essential nutrients. For this reason, the application of Zn + Fe + Mn could significantly improve most of the studied traits. In this study, among the yield components, the changes in 1000-seed weight were statistically significant, and the role of this component on the changes in grain yield affected by foliar spraying of the aforementioned elements can be considered more prominent. The highest and lowest average weight of one thousand seeds in the present study belonged to the combined foliar spraying treatments of zinc, iron and manganese and the control treatment, respectively.

Zn, by participating in the production of pollen tube protein, causes its storage in this organ and leads to increased pollination and grain formation. Manganese also increases 1000-seeds weight by affecting the reproductive growth process and helping to materialize and produce carbohydrates and proteins. Increased oil yield due to the increasing effect of oil percentage and also increasing grain yield, the greatest effect was obtained among the performance characteristics of safflower from the combined foliar spraying of zinc, iron and manganese and zinc-manganese. Given that these two treatments were in the same statistical group, if the purpose is to increase oil yield, iron foliar spraying can be omitted. The results of some studies indicate the effect of zinc and manganese on increasing the percentage of safflower oil. Foliar application of Zn + Fe + Mn proved to be most effective in optimizing nutrient availability and accumulation, thereby promoting overall crop productivity in micronutrient-deficient soils. Given the antagonistic effects, the need for closer attention to this issue is important and is recommended.

Conflict of interests

The author has stated that there is no conflict of interest.

Ethics approval and consent to participate

No humans or animals were used in the present research. The author has adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

The final article has been approved by the author.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

The first draft of this manuscript was written by the researcher and has been revised and modified.

Informed consent

The author declares not to use any patients in this research.

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