Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Investigating the effect of foliar application of nano chelated iron on the amounts of metabolites and nutritional value of lettuce (*Lactuca sativa* Linn.)

Roghayeh Heydari[1], Elham Mohajel Kazemi[2,3], Houshang Nosrati[4], Maryam Kolahi*[5], Ali Movafeghi[6]

1-PhD Student of the Department of Plant, Cell and Molecular biology, Faculty of Natural Science, University of Tabriz, Tabriz, Iran

2- Associate Professor of the Department of Plant, Cell and Molecular biology, Faculty of Natural Science, University of Tabriz, Tabriz, Iran

3 -Nuclear Agriculture School, Nuclear Science and Technology Research Institute (NSTRI), Atomic Energy Organization of Iran (AEQI), Karaj, Iran

4- Professor of the Department of Plant, Cell and Molecular biology, Faculty of Natural Science, University of Tabriz, Tabriz, Iran

5- Associate Professor of Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran

6- Professor of the Department of Plant, Cell and Molecular biology, Faculty of Natural Science, University of Tabriz, Tabriz, Iran

ARTICLE INFO

Article History:

Received:2023/12/30

Accepted:2025/1/30

Keywords:

extraction,

pomegranate peel,

solvent,

effective compounds,

Antifungal.

DOI: 10.22034/FSCT.22.163.1.

*Corresponding Author E-

m.kolahi@scu.ac.ir

ABSTRACT

In recent years, many research studies have conducted to find ways to improve agricultural products and eliminate chemical fertilizer pollutants. To enhance crop yields and minimize the reliance on animal feed resources, it is essential to adapt innovative agricultural practices. Organic-based synthetic fertilizers, such as nano chelated iron can significantly enhance the nutritional system of plants while reducing the reliance on chemical fertilizers. Nano fertilizers are easily absorbed by plants and are more effective than traditional chemical fertilizers. Based on this information, this study was conducted to investigate the nutritional value of lettuce and its capacity to absorb micronutrient elements. The study was conducted at Tabriz University during the years 1400-1401, following a completely randomized design with three repetitions. Three levels of nano chelated iron (0, 0.5 and 1 g/L) were used to investigate the effect of nano chelated iron on the absorption of micronutrients by lettuce plants. Nano chelated iron significantly enhanced the levels of secondary metabolites, including total phenols and flavonoids, as well as the antioxidant capacity of lettuce compared to the control samples. Additionally, the results indicated that higher concentrations of nano chelated iron resulted in increased levels of total protein, soluble sugars, and free amino acids in lettuce plants compared to the control sample. It has been demonstrated that micronutrients such as Iron, Calcium, Zinc, Potassium, and Phosphorus accumulate in the roots and shoots of lettuce plants with the increased application of nano chelated iron. The increase was more pronounced in plants that received higher concentrations of nano chelated iron. This study demonstrated that nano chelated iron enhances plant primary and secondary metabolites, while also supplying iron and other micronutrients to the plants.

[DOI: 10.22034/FSCT.22.163.1]

1. Introduction

Leafy vegetables are regarded as one of the most essential components of a healthy diet for consumers due to their rich content of carbohydrates, proteins, vitamins, and minerals. Research studies indicate that the consumption of healthy and hygienic vegetables can help prevent heart disease and certain types of cancer, particularly digestive cancers. Lettuce (*Lactuca sativa* Linn.) is an annual plant belonging to the Asteraceae family. It is a rich source of vitamins, including A, B, and K, as well as essential minerals such as calcium, phosphorus, iron, potassium, and sodium. Additionally, it contains trace amounts of magnesium and sulfur [1]. The presence of secondary metabolites, such as terpenoids and flavonoids, suggests that lettuce possesses medicinal properties. According to statistics from the Food and Agriculture Organization of the United Nations (FAO), global lettuce production has increased by 118% over the past two decades. This significant growth has established lettuce as the fifth-largest crop worldwide, following corn, rice, potatoes, and tomatoes [2].

Today, the application of nanotechnology is expanding across various fields, including the food and agricultural industries. It is progressively transitioning from the laboratory stage to practical implementation, which will significantly enhance its presence and impact in the food industry. This advancement is expected to drive innovation and efficiency, marking a transformative shift in how nanotechnology is utilized in food production and processing [3]. Iron, as an essential micronutrient, plays a critical role in numerous physiological processes in plants. It is involved in key functions such as photosynthesis, respiration, nitrogen assimilation, the production and detoxification of reactive oxygen species (ROS), and osmotic regulation. Additionally, iron serves as a cofactor in the structure of various antioxidant enzymes, further underscoring its importance in maintaining plant health and stress responses [4]. The utilization of iron-based nano-fertilizers to precisely regulate nutrient release represents a promising advancement toward achieving sustainable and environmentally friendly agricultural practices. Nano-chelated iron-fertilizer possesses a stable and robust base or complex structure. This nano-complex contains 9% soluble iron, enabling it to effectively supply iron to plants in a water-soluble form across a broad pH range of 3 to 11. To enhance nutrient utilization efficiency and address current limitations, nano-iron fertilizers represent a promising and viable alternative. The utilization of nano-fertilizers represents an efficient and cost-effective approach that has the potential to replace conventional fertilizers. This innovative method facilitates the gradual and controlled release of essential nutrients into the soil, thereby enhancing nutrient availability and minimizing environmental impact. Moreover, fertilizers with these specific dimensions are more readily absorbed by plants and demonstrate significantly higher efficacy compared to conventional fertilizers [5]. Chelation of nutrients within a plant's biological system plays a crucial nutritional role by forming stable complexes with metal ions, thereby enhancing nutrient availability and preventing mineral leaching in the soil. They also facilitate the transport of nutrients within the plant by acting as carriers for essential metals and other bioactive compounds [6]. In this context, Sharafuddin Shirazi, in a study [7], examined the impact of nano-chelated iron on various parameters, including chlorophyll and carotenoid content, biomass yield, dry matter yield, and the uptake rates of essential elements (nitrogen, phosphorus, potassium, and iron) in the leaves of the medicinal plant *Thymus dena*. The analysis of variance results indicated that the application of nano-chelated iron fertilizer significantly enhanced several key traits, including chlorophyll content, carotenoid levels, biomass yield, dry matter yield, nitrogen uptake efficiency, iron content, leaf area index, and

[DOI: 10.22034/FSCT.22.163.1]

potassium absorption rate, at the 5% significance level. In an experiment conducted by Nasiri et al. [8], the impact of foliar application of iron and zinc fertilizers on the nutrient concentration in the aerial parts of *Matricaria chamomilla* was investigated. The study results indicated that the foliar application had a significant effect on the concentrations of phosphorus, magnesium, calcium, copper, zinc, and iron. In recent years, the growing awareness of the high nutritional value of leafy vegetables has led to an increasing consumption of these vegetables among urban communities. Lettuce, among other leafy vegetables, stands as one of the most significant and widely consumed broad-leaf vegetables globally. It is rich in essential nutrients, including a variety of vitamins, minerals, protein, dietary fiber, iron, and calcium, making it a vital component of a balanced diet. Due to its nutritional value and versatility, lettuce has the highest consumer base among leafy greens. As such, it plays an indispensable role in promoting human health and well-being, underscoring its importance as a staple in dietary practices worldwide. On the other hand, the application of nano-chelated iron solutions is particularly significant for revitalization purposes, owing to their high specific surface area. This characteristic results in an increased density of adsorption sites and enhances their capacity to mitigate stress factors effectively. For this purpose, the present study was conducted to assess the role of nano-chelated iron in enhancing the nutritional system of lettuce, a leafy vegetable cultivated for its edible vegetative parts. Additionally, the study aimed to investigate certain biochemical characteristics of this plant.

2- Material and Methods

2-1- Cultivation and treatment

This study was conducted using a completely randomized design with three replications in the cytochemistry laboratory at the University of Tabriz during the academic years 1400-1401. Lettuce (*Lactuca sativa* Linn.) seeds were prepared at Tabriz University, after which the seeds were subjected to a sterilization process. Ten seeds were planted in pots measuring 12 cm in diameter and 15 cm in height, filled with a sterilized substrate mixture of perlite and cocopeat at a 2:1 ratio. The pots were autoclaved prior to cultivation to ensure sterility. The pots were subsequently placed in a greenhouse under controlled environmental conditions, with day and night temperatures maintained at 25±1˚C and 20±1˚C, respectively, and a photoperiod of 16 hours of light followed by 8 hours of darkness. Following seed germination, the seedlings were irrigated twice weekly with a 25% Hoagland solution (pH=6–8.5). Subsequently, the nutrient concentration was gradually increased to 50% of the Hoagland solution, and ultimately, the seedlings were irrigated with a full-strength Hoagland solution. Approximately three weeks after the plants reached the three-leaf stage, the seedlings were subjected to foliar spray treatments with nano-chelated iron. The nano-chelated iron, obtained from Ahrar Sharq Publishing Knowledge Fund Company (Khazar), was applied in three concentrations (0, 0.5, and 1 g/L) with three replicates for each concentration. The treatments were administered every three days. Following five treatment stages and a 28-day cultivation period, the third leaf of the plants was utilized for the intended experimental analyses. Fresh samples were utilized for biochemical analyses, while root and leaf samples were transferred to an oven for drying to determine mineral nutrient concentration.

2-2- Measurement of secondary metabolite content (phenol, flavonoid) and antioxidant capacity using the DPPH method

The Folin-Ciocalteu reagent was employed to quantify the total phenolic content in the extracts [9]. A 0.1 g sample of leaf tissue was homogenized in 2 mL of 80% methanol. Subsequently, 100 µL of the resulting plant extract was transferred into test tubes for further analysis. To each tube, 2.8 mL of deionized water, 2 mL of 2% sodium carbonate solution, and 100 µL of Folin- Ciocalteu reagent were sequentially added. The resulting mixture was vortexed thoroughly and allowed to equilibrate

[DOI: 10.22034/FSCT.22.163.1]

at room temperature for 30 minutes. The absorbance of the solutions was subsequently measured using a spectrophotometer at a wavelength of 720 nm, with measurements being compared to those of the control sample. The data were ultimately expressed as milligrams of gallic acid equivalent per gram of fresh plant weight (mg GAE/g FW).

The total flavonoid content was determined using the aluminum chloride colorimetric method, with certain modifications implemented to enhance the procedure [10]. A volume of 500 µL of plant extracts was transferred into test tubes containing 80% methanol. Subsequently, 1.5 mL of 80% methanol, 100 µL of 10% aluminum chloride, 100 µL of 1 M potassium acetate, and 2.8 mL of distilled water were sequentially added to each test tube. The resulting mixture was vortexed thoroughly and subsequently allowed to incubate at room temperature for 40 minutes. The absorbance of the solutions was measured using a spectrophotometer at a wavelength of 415 nm. The total flavonoid content of the extracts was ultimately quantified and expressed as milligrams of quercetin equivalent per gram of fresh plant weight (mg QE/g FW).

To measure DPPH (2,2-diphenyl-1-picrylhydrazyl) activity, 0.1 g of leaf tissue was homogenized in 5 mL of methanol and subsequently centrifuged at 10,000 g for 5 minutes. Then, 1000 µL of the supernatant was diluted to a final volume of 2 mL using methanol. Subsequently, 2 mL of a 0.004% (w/v) methanolic solution of DPPH was added to the mixture. The resulting mixture was thoroughly vortexed and then allowed to stand at room temperature in the dark for 30 minutes. Subsequently, the absorbance of the samples was measured at a wavelength of 517 nm. The percentage of free radical inhibition was calculated using the following formula: [11].

$$\%I = (A_{control} - A_{sample}) / A_{control} \times 100$$

A control: Absorbance of control solution at 517 nm

A Sample: Absorbance of samples at a wavelength of 517 nm

2-3- Measuring the content of primary metabolites (soluble sugar, total protein and free amino acids)

The phenol-sulfuric acid method was employed to quantify soluble sugars [4]. In this procedure, 0.05 g of dried plant tissue powder was measured and transferred into a test tube. Subsequently, 5 mL of 70% ethanol was added to the tube, which was then stored in a refrigerator for one week. Then, the samples were filtered through filter paper to separate the solution from the sediment. The resulting filtrate was subsequently utilized for the quantification of soluble sugarsTo quantify soluble sugar content, 500 µL of the sample solution was combined with 2 mL of distilled water, 5 mL of concentrated sulfuric acid, and 1 mL of 5% phenol. The mixture was allowed to react for 30 minutes, after which the absorbance of the samples was measured at a wavelength of 485 nm using a spectrophotometer.

To conduct the final enzyme activity calculations, it is essential to quantify the protein concentration. For this purpose, the Bradford assay [12] was employed. A volume of 100 µL of the extract was pipetted into a fresh microtube, followed by the addition of 100 µL of distilled water. Next, 1 mL of Bradford reagent was added to each tube, followed by thorough vortexing. The tubes were then incubated at room temperature for 10 to 15 minutes. The absorbance of the samples was measured at a wavelength of 595 nm. Protein content was expressed as mg/g FW.

The concentration of free amino acids was determined using a ninhydrin-based assay [13]. The ninhydrin reagent was prepared by dissolving 0.55 g of ninhydrin in 100 mL of ethanol. For the assay, a mixture of the sample extract and ninhydrin reagent was prepared at a ratio of 1:5 (v/v) and used as the working solution for subsequent analysis. The resulting solution was incubated in a water bath maintained at a temperature range of 70–80°C for 4–7 minutes. Following incubation, the test tubes were allowed to cool to room temperature, after which the absorbance of the

[DOI: 10.22034/FSCT.22.163.1]

solutions was measured at a wavelength of 570 nm. A standard curve was constructed using glycine solutions with concentrations ranging from 0 to 1 mM.

2-4- Measuring mineral elements

Nitric acid (10 mL, 65%) was added to the dried sample powder (0.5 g) for acid digestion, and the mixture was allowed to stand under a fume hood for 24 hours. Subsequently, the samples were heated at 90°C to complete the digestion process. After cooling, 1 mL of 30% H_2O_2 was added to the digested samples, followed by heating. Subsequently, the final volume of each sample was adjusted to 25 mL using distilled water. The concentrations of mineral elements in the samples were determined using atomic absorption spectrometry (Shimadzu AA630 model) and expressed as mg/g DW [14].

Statistical analysis

This study was conducted using a completely randomized block design with three replications. All data were subjected to analysis of variance (ANOVA) and processed using the SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). All experimental data are presented as mean ± standard deviation, with a significance level of $p < 0.05$ applied to all statistical tests. Graphical representations were generated using Microsoft Excel 2013 software.

3-Results and Discussion

3-1- Investigation of the content of secondary metabolites (phenols, flavonoids) and antioxidant capacity of lettuce plants under the influence of nano-chelated iron

The results of the mean comparison revealed a statistically significant difference in phenol and flavonoid content across the various treatments when compared to the control sample. In plants treated with varying concentrations of nano-chelated iron, the levels of phenol and flavonoids exhibited a significant increase compared to the control group. However, no statistically significant difference in phenol content was observed between the control and the 0.5 g/L nano-chelated iron treatment. The application of 1 g/L nano-chelated iron resulted in an increase of 18.91% in phenol content and 41.90% in flavonoid content compared to the control sample. The highest concentrations of phenol and flavonoids were recorded in the 1 g/L nano-chelated iron treatment, with values of 1.729 mg GAE/g FW and 0.552 mg QE/g FW, respectively. In contrast, the lowest phenol concentration was observed in the 0.5 g/L treatment, measuring 1.447 mg GAE/g FW, while the lowest flavonoid concentration was detected in the control sample, with a value of 0.389 mg QE/g FW (Figure 1A-B) ($p < 0.05$).

As illustrated in Figure 2, the antioxidant capacity of lettuce was significantly influenced by varying concentrations of nano-chelated iron. A comparative analysis of the mean effects of different levels of nano-chelated iron on lettuce's antioxidant capacity revealed statistically significant differences among the treatments. In lettuce plants treated with varying concentrations of nano-chelated iron, a significant enhancement in antioxidant capacity was observed compared to the control group. The antioxidant capacity in the 1 g/L nano-chelated iron treatment was measured at 13.58%, while the 0.5 g/L nano-chelated iron treatment exhibited an antioxidant capacity of 8.61%, both relative to the control sample. The highest antioxidant capacity was observed in the 1 g/L nano-chelated iron treatment, reaching 65.734%, whereas the lowest value was recorded in the control sample at 57.872% (Figure 1C) ($p < 0.05$). Leafy greens are recognized as an essential component of modern nutrition owing to their low caloric content and well-balanced nutrient profile. Given that minerals play a critical role in maintaining human health, any disruption in the body's mineral balance can pose significant risks to an individual's well-being. Iron is one of the essential minerals and electrolytes that has garnered significant attention due to its critical physiological and metabolic roles in the human body. During the growth phase of plants, iron serves as a cofactor or structural component for numerous enzymes involved in cellular metabolism. Increased iron availability can significantly enhance the activity of the phenylalanine ammonia-lyase (PAL) enzyme, thereby promoting the biosynthesis and

[DOI: 10.22034/FSCT.22.163.1]

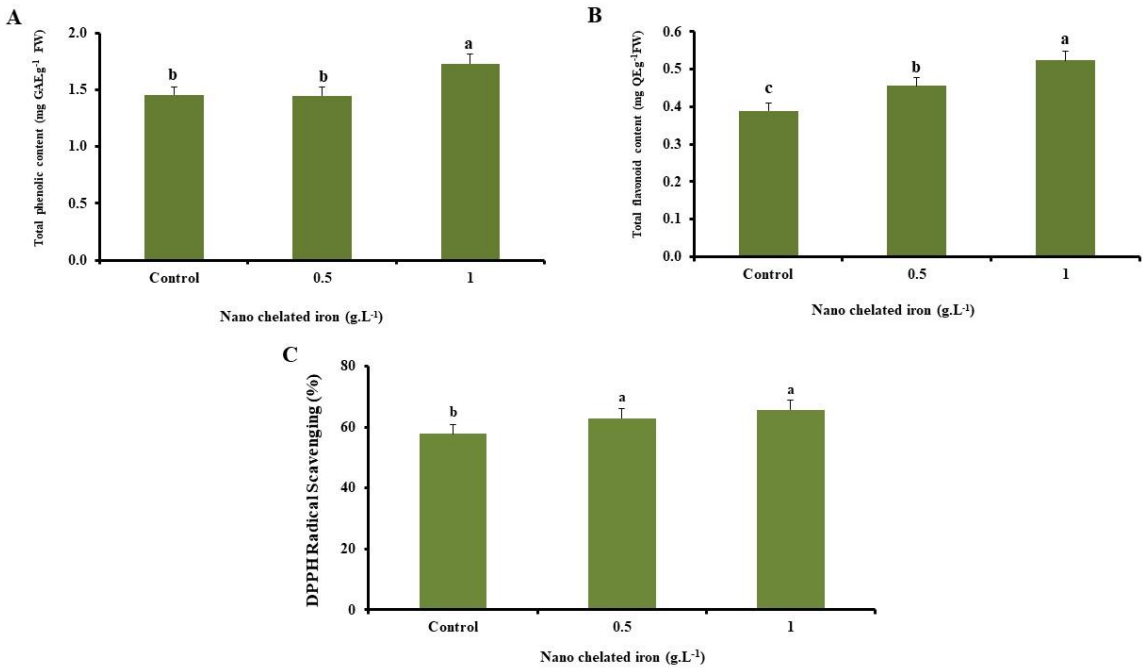

Figure 1- Effects of different concentrations of nano chelated iron on A) Total phenolic content, B) Total flavonoid content, C) DPPH Radical Scavenging

accumulation of secondary metabolites such as flavonoids and phenolic compounds [15]. According to various studies, the application of iron chelate as a foliar spray has been shown to significantly increase the levels of phenols and flavonoids in strawberry and orange fruits compared to control groups [16]. The use of micronutrients enhances antioxidant potential through mechanisms such as maintaining the integrity of cell membranes, increasing the production of metallothionein proteins, and protecting structural molecules [17]. Studies by Scaffier et al. [18] demonstrated that the application of micronutrients enhances carbohydrate levels by influencing the activity of enzymes involved in the carbohydrate biosynthetic pathway. This, in turn, may contribute to an increase in the synthesis of phenolic compounds.

Numerous studies have established a correlation between the enhanced antioxidant capacity of strawberry fruit and elevated levels of vitamin C, flavonoids, and phenolic compounds [19]. Similarly, research on corn has demonstrated that iron deficiency leads to a reduction in antioxidant capacity compared to control samples, a finding that aligns with the results of the present study [20]. Studies by Manquián-Cerda [21] indicate that the antioxidant capacity of plants varies depending on the type and concentration of different treatments. The application of iron nano-chelate, which protects plants from nutrient deficiencies induced by stress, can enhance the plant's defense mechanisms, enabling it to better cope with stress conditions. The application of micronutrients, such as iron, enhances the activity of PAL enzyme, thereby increasing the synthesis of phenolic compounds and improving the antioxidant capacity in *Kandelia obovata* [22].

3-2- Investigating the content of primary metabolites (soluble sugar, total protein and free amino acids) of lettuce plants under the influence of nano-chelated iron

The results presented in Figure 2 illustrate the changes in soluble sugar content in lettuce plants treated with nano-chelated iron. Based on the mean comparison analysis, the soluble sugar content exhibited a significant increase

[DOI: 10.22034/FSCT.22.163.1]

with higher concentrations of nano-chelated iron compared to the control group. This suggests a positive correlation between the application of nano-chelated iron and the enhancement of soluble sugar levels in lettuce plants. A significant difference was observed among the different treatments. Compared to the control, the 1 g/L nano-chelated iron treatment resulted in an increase of approximately 47.71%, while the 0.5 g/L treatment showed an increase of about 28.75%. The highest soluble sugar content was recorded in the 1 g/L nano-chelated iron treatment, with a concentration of 2.944 mg/g DW (Figure 2A) ($p < 0.05$).

According to the results of the analysis of variance (Table 1), the total protein content of lettuce exhibited a significant increase with rising concentrations of nano-chelated iron compared to the control group. The highest total protein content in lettuce was observed in the 1 g/L nano-chelated iron treatment, with a value of 10.084 mg/g FW. In contrast, the lowest protein content was recorded in the control sample, with a value of 131.7 mg/g FW. Compared to the control, the total protein content in lettuce increased by 43.79% in the 1 g/L nano-chelated iron treatment and by 0.01% in the 0.5 g/L nano-chelated iron treatment (Figure 2B) ($p < 0.05$). The results of the analysis of variance presented in Table 1 indicate a statistically significant difference in free amino acid content among the various treatments compared to the control group in lettuce. As the concentration of nano-chelated iron increased, the free amino acid content exhibited a significant rise relative to the control sample. The control sample exhibited the lowest free amino acid content at 0.105 mg/g FW, whereas the highest content was recorded in the 1 g/L nano-chelated iron treatment, reaching 0.135 mg/g FW (Figure 2C) ($p < 0.05$).

Numerous researchers have highlighted the critical role of micronutrients, particularly iron, in regulating food balance, supporting cellular metabolic functions, and protecting cell membranes from oxidative damage caused by oxygen free radicals. Consequently, a deficiency in iron levels has been associated with impaired growth, depletion of energy reserves, and a reduction in sugar content. The findings from various studies indicate that the application of nano-iron oxide significantly enhances the soluble sugar content in *Moringa oleifera* compared to control samples [23]. The utilization of micronutrients, through the increase in soluble sugar content, improves the functionality of photosystems, stabilizes the transport pathways for soluble sugars, reduces ROS, and safeguards the structural integrity of macromolecules. Additionally, it maintains redox homeostasis and enhances the activity of various defense-related enzymes in plants [24]. Iron is a key structural component of RNA polymerase, and its availability directly influences protein synthesis. Consequently, the presence of micronutrients enhances the protein content by promoting improved growth conditions in plants. Micronutrients, including iron, have the ability to bind to biologically active molecules. These molecules, in turn, can directly interact with specific sites within proteins, nucleic acids, and subcellular structures, thereby safeguarding them from adverse environmental conditions [25]. The application of iron enhances plant growth conditions by increasing the concentration of soluble proteins, which are crucial for osmotic regulation. This process facilitates water transport across membranes and promotes the accumulation of osmolytes, ultimately supporting plant growth and stress tolerance. Various studies have demonstrated that the application of nano-iron oxide significantly enhances the content of soluble proteins and free amino acids in *Moringa oleifera* compared to control samples. This improvement is attributed to the application of micronutrients through thiol oxidation, as well as the interaction of metalloproteins with micronutrients, which protects the sulfhydryl groups of proteins and subsequently increases protein content [23]. Amino acid homeostasis in plants is maintained through several processes, including protein synthesis and

[DOI: 10.22034/FSCT.22.163.1]

degradation, the biosynthesis of new amino acids, and their absorption and transport. An increase in certain amino acids within plants can be attributed to the active proteolysis of glycoproteins present in the plant cell wall. Numerous studies have demonstrated that micronutrient deficiencies lead to a reduction in the levels of various amino acids in plants. This occurs due to the disruption of enzymatic and catalytic reactions, as well as alterations in the concentrations of specific reactants within the metabolic pathways. The application of micronutrients enhances crop production and increases plant dry matter content in rice by elevating amino acid levels and regulating the osmolality of ion transport [26].

Table 1- Variance analysis of biochemical traits of lettuce under the influence of nano-chelated iron

S.O.V	df	Phenol (mg g^{-1} FW)	Flavonoid (mg g^{-1} FW)	DPPH (%)	Soluble sugar (mg g^{-1} DW)	Total protein (mg g^{-1} FW)	Free amino acid (mg g^{-1} FW)
Nano-chelated iron	2	0.076**	0.006**	47.467**	0.688**	8.729**	**0.001****
Error	6	0.000054	0.000010	2.469	0.000024	0.000025	**0.000001**
C.V (%)	0.36	2.45	4.91	2.17	1.15	0.36	**8.54**

ns: not significant, *p < 0.05, **p < 0.01

Table 2- Variance analysis of mineral elements in roots and shoots of lettuce under the influence of nano-chelated iron

S.O.V	df	K shoot (mg g^{-1} DW)	K root (mg g^{-1} DW)	Ca shoot (mg g^{-1} DW)	Ca root (mg g^{-1} DW)	Zn shoot (mg g^{-1} DW)	Zn root (mg g^{-1} DW)	Fe shoot (mg g^{-1} DW)	Fe root (mg g^{-1} DW)	P root (mg g^{-1} DW)	P shoot (mg g^{-1} DW0
Nano-chelated iron	2	0.774**	0.136**	0.224**	0.359**	0.002**	0.010**	0.014**	0.008**	0.904**	**0.142****
Error	6	0.00017	0.00001	0.00029	0.00012	0.000001	0.000008	0.000002	0.000004	0.00019	**0.00010**
C.V (%)		1	1.89	1.05	1.04	8.06	8.81	4.92	7.02	1.16	**1.14**

ns: not significant, *p < 0.05, **p < 0.0

[DOI: 10.22034/FSCT.22.163.1]

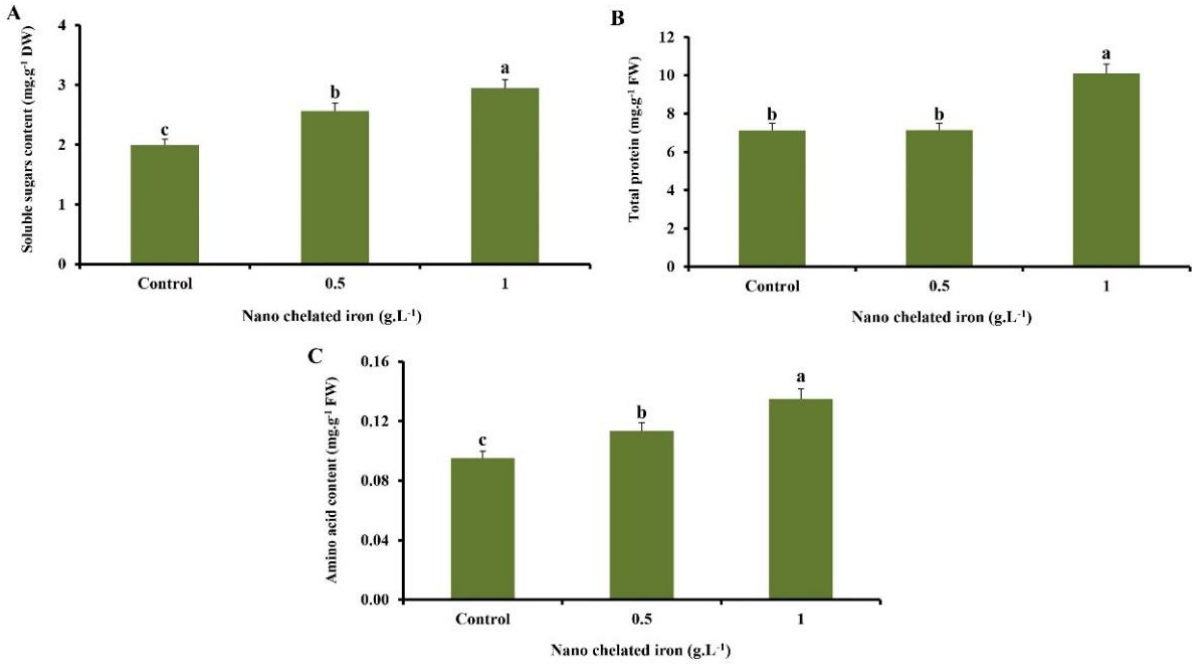

Figure 3- Effects of different concentrations of nano chelated iron on A) soluble sugar content, B) total protein content, C) free amino acid content

3-3- Investigating the concentration of zinc, iron, potassium, calcium and phosphorus elements in lettuce plants under the influence of nano-chelated iron

The analysis of variance results, as presented in Table 2, indicate that the application of nano-chelated iron significantly influenced the concentration of micronutrients in lettuce. With increasing concentrations of nano-chelated iron, the concentrations of zinc, iron, potassium, calcium, and phosphorus in both the roots and shoots of lettuce plants exhibited a significant rise compared to the control group. However, the concentration of phosphorus in the shoots displayed a distinct pattern: it initially increased with the application of nano-chelated iron but subsequently decreased at higher concentrations. The highest concentrations of iron (0.266 mg/g DW), zinc (0.278 mg/g DW), calcium (5.088 mg/g DW), potassium (1.382 mg/g DW), and phosphorus (5.46 mg/g DW) in the roots of lettuce plants were observed in the treatment with 1 g/L of nano-chelated iron. The highest concentration of elements in the lettuce shoot was observed in the 1 g/L nano-chelated iron treatment, with the following values: iron (0.303 mg/g DW), zinc (0.148 mg/g DW), calcium (6.57 mg/g DW), potassium (6.026 mg/g DW), and phosphorus (4.093 mg/g DW) (Figure 3A-J) ($p < 0.05$).

Hamzeh et al. (2022) reported that under iron complex treatment in plants, several iron transporters can facilitate the translocation of zinc from the root to the shoot, either as a divalent cation or in the form of a complex bound to siderophores [28]. Consequently, in the present study, the concentration of zinc in plants treated with nano-chelated iron was found to be higher in the shoot compared to the root [27]. The findings from studies on *Brassica napus* revealed that the application of iron significantly enhanced the concentrations of zinc, phosphorus, calcium, potassium, sodium, and iron in both the roots

[DOI: 10.22034/FSCT.22.163.1]

and shoots compared to the control samples [29]. Various studies have demonstrated that increasing the application of iron fertilizer significantly enhances the concentration of iron and phosphorus in the roots and shoots of wheat and lettuce plants compared to control samples. This is attributed to the critical role of iron complexes in facilitating electron transfer and actively promoting the absorption of essential elements such as phosphorus. Consequently, iron deficiency directly impairs phosphorus uptake in plants [30, 31]. Phosphorus is one of the essential elements absorbed by plants in significant quantities through the cation-anion exchange process in root cells with the soil. Iron deficiency can impair the absorption and translocation of phosphorus within the plant. By supplementing iron, the desired phosphorus concentration for optimal plant growth can be achieved, as the uptake of anions, such as phosphorus, plays a critical role in facilitating the absorption of cations by plants [32]. The application of iron complexes as elicitors enhances mineral uptake through several mechanisms, some of which are outlined below: 1: Iron complexes promote increased mineral uptake by facilitating deeper root penetration into the soil, 2: Iron improves soil properties, such as water retention capacity and cation exchange capacity, thereby enhancing the absorption and concentration of minerals, 3: Iron modifies transport mechanisms, reducing the rate of cation loss and optimizing mineral availability. The application of iron to plants, achieved by modulating the activity of transporters such as ZIPs (zinc/iron-regulated transporters) and IRT1 (iron-regulated transporter 1), enhances the uptake of micronutrients through divalent cation transporters. This results in an increased concentration of cations in both the roots and shoots of plants, such as rice [33].

[DOI: 10.22034/FSCT.22.163.1]

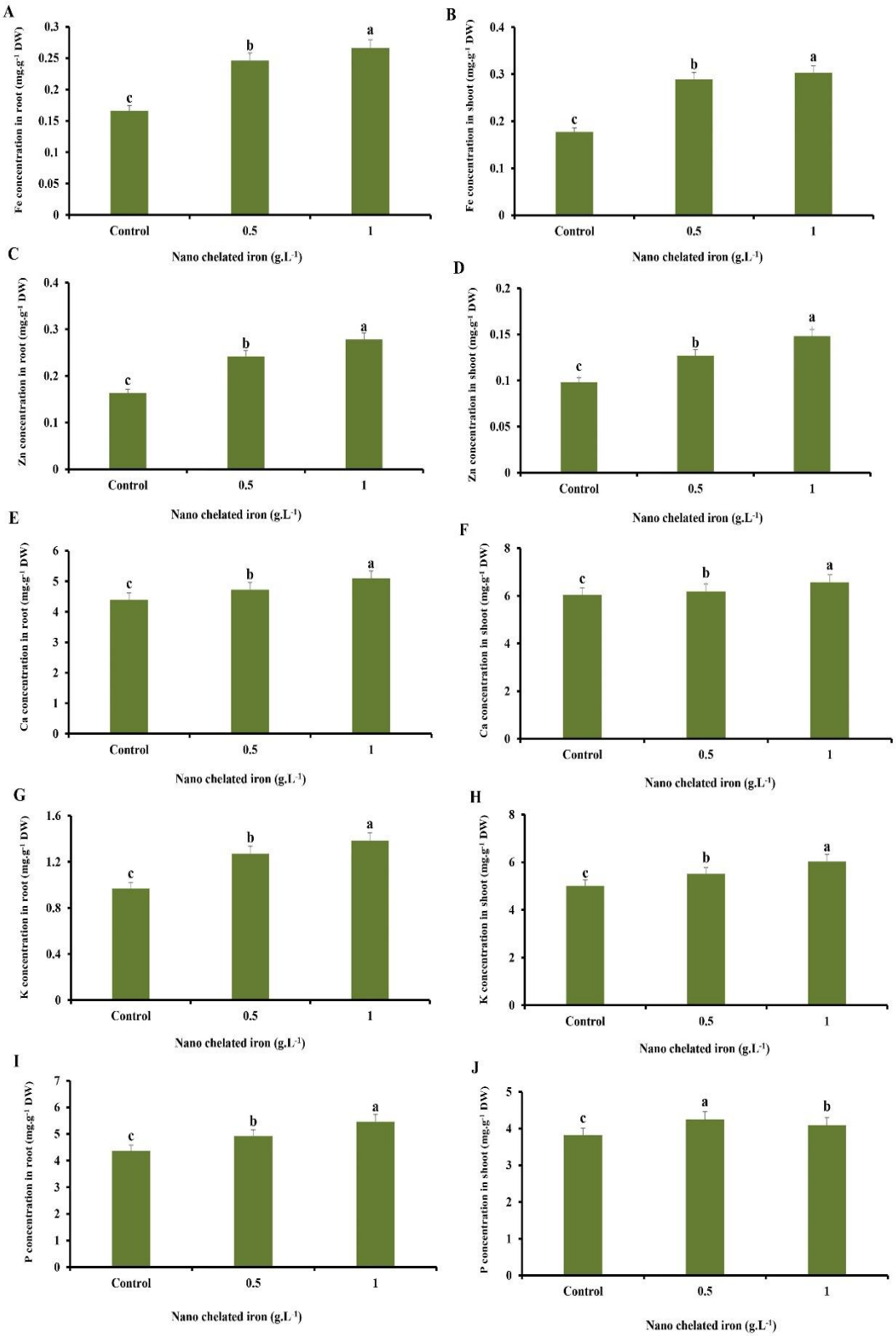

Figure 3- Effects of different concentrations of nano chelated iron A) Fe concentration in root, B) Fe concentration in shoot, C) Zn concentration in root, D) Zn concentration in shoot, E) Ca concentration in root, F) Ca concentration in shoot, G) K concentration in root, H) K concentration in shoot, I) P concentration in root, J) P concentration in shoot

4-Conclusion

In the current study, the impact of nano-chelated iron on the levels of primary and

[DOI: 10.22034/FSCT.22.163.1]

secondary metabolites, as well as the mineral concentration of lettuce, was investigated. Based on the obtained results, the application of increasing concentrations of nano-chelated iron significantly enhanced the levels of secondary metabolites, including phenols and flavonoids, as well as the antioxidant capacity of lettuce plants, compared to the control group. The levels of primary metabolites, including total protein, soluble sugars, and free amino acids, in lettuce plants exhibited a significant increase following the application of nano-chelated iron. Iron appears to have enhanced the nutritional value of lettuce plants by modulating the biosynthesis pathways of phenolic compounds, sugars, proteins, and amino acids. Furthermore, the application of nano-chelated iron significantly enhanced the concentrations of micronutrients, including zinc, iron, calcium, potassium, and phosphorus, in both the roots and shoots of lettuce plants. This treatment resulted in a statistically significant increase in micronutrient levels compared to the control group. The findings of this study demonstrate that nano-chelated iron is effective in supplying the iron necessary for lettuce growth under soilless cultivation conditions. Beyond fulfilling the plant's iron requirements, nano-iron chelate also exhibits the capacity to consistently deliver other essential micronutrients, including calcium, zinc, potassium, and phosphorus. This highlights its potential as a comprehensive nutrient source in soilless agricultural systems. Based on the findings of the current study, it can be concluded that nano-chelated iron serves as an effective and reliable fertilizer for supplying essential nutrients, including iron, zinc, potassium, calcium, and phosphorus, in the soilless cultivation of lettuce. In this context, other nano-fertilizers or nanoparticles should be evaluated for their potential to enhance the nutritional quality of vegetables.

Acknowledgements

We extend our gratitude to the University of Tabriz and Shahid Chamran University for their financial support, which made this research possible.

5- References

[1] Abdalla, M. A., Li, F., Wenzel-Storjohann, A., Sulieman, S., Tasdemir, D., & Mühling, K. H. 2021. Comparative metabolite profile, biological activity and overall quality of three lettuce (*Lactuca sativa* L., Asteraceae) cultivars in response to sulfur nutrition. Pharmaceutics, 13(5), 713. https://doi.org/10.3390/pharmaceutics13050713.

[2] Roa, J. 2023. Informal Food Markets in Quezon City and Pasay City, Philippines: A Rapid Assessment. Resilient Cities Initiative Research Report. https://doi.org/10.4160/9789290606642.

[3] Baruah, S., & Dutta, J. 2009. Nanotechnology applications in pollution sensing and degradation in agriculture: a review. Environmental Chemistry Letters, 7, 191-204. https://doi.org/10.1007/s10311-009-0228-8.

[4] Kochert, G. 1978. Carbohydrate determination by the phenol-sulfuric acid method. Handbook of phycological methods, Physiological and biochemical methods., 95.

[5] Cui, H. X., Sun, C. J., Liu, Q., Jiang, J., & Gu, W. 2010. Applications of nanotechnology in agrochemical formulation, perspectives, challenges and strategies. In international conference on Nanoagri, Sao pedro, Brazil (pp. 28-33).

[6] Liu, X. M., Feng, Z. B., Zhang, F. D., Zhang, S. Q., & He, X. S. 2006. Preparation and testing of cementing and coating nano-subnanocomposites of slow/controlled-release fertilizer. Agricultural Sciences in China, 5(9), 700-706. https://doi.org/10.1016/S1671-2927(06)60113-2.

[7] Sharafaldin Shirazi, sh. and Fazli, F. 2012. The effect of microcalt and iron sulfate on yield and yield components of Thymus Celak daenesis. Bimonthly Scientific-Research Journal of Medicinal and Aromatic Plants of Iran, Vol.31, No.2, p. (In persian).

[8] Nasiri, Y. Zahtabsalmasi, S. Nasralehzadeh, p. Qasemigalazani, K. Najafi, N. A. and Javanmard, A. A. 2013. Evaluation of the effect of foliar spraying of iron and zinc sulfate on flower yield and concentration of nutrients in the aerial part of German chamomile, Journal of Agricultural Science and Sustainable Production. 23: 105-115. (In persian).

[9] Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of food and drug

[DOI: 10.22034/FSCT.22.163.1]

analysis, 10(3). https://doi.org/10.38212/2224-6614.2748.
[10] Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food chemistry, 91(3), 571-577. https://doi.org/10.1016/j.foodchem.2004.10.006.
[11] Miliauskas, G., Venskutonis, P. R., & Van Beek, T. A. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food chemistry, 85(2), 231-237. https://doi.org/10.1016/j.foodchem.2003.05.007.
[12] Mm, B. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 72, 248-254.
[13] Hwang, M. N., & Ederer, G. M. 1975. Rapid hippurate hydrolysis method for presumptive identification of group B streptococci. Journal of Clinical Microbiology, 1(1), 114-115. https://doi.org/10.1128/jcm.1.1.114-115.1975.
[14] Bichi, A. M., & Ibrahim, S. R. 2018. Plant diversity and profile distribution of some available Micronutrients in selected soils of Kano State, Nigeria. Bayero Journal of Pure and Applied Sciences, 11(2), 20-31. 10.4314/bajopas.v11i2.4.
[15] Pestana, M., Correia, P. J., Saavedra, T., Gama, F., Abadía, A., & de Varennes, A. 2012. Development and recovery of iron deficiency by iron resupply to roots or leaves of strawberry plants. Plant physiology and biochemistry, 53, 1-5. https://doi.org/10.1016/j.plaphy.2012.01.001.
[16] Chen, P., Cao, Y., Bao, B., Zhang, L., & Ding, A. 2017. Antioxidant capacity of Typha angustifolia extracts and two active flavonoids. Pharmaceutical biology, 55(1), 1283-1288. https://doi.org/10.1080/13880209.2017.1300818.
[17] Lee, K. H., Cha, M., & Lee, B. H. 2020. Neuroprotective effect of antioxidants in the brain. International journal of molecular sciences, 21(19), 7152. 10.3390/ijms21197152.
[18] Schefer, S., Oest, M., & Rohn, S. 2021. Interactions between phenolic acids, proteins, and carbohydrates—Influence on dough and bread properties. Foods, 10(11), 2798. https://doi.org/10.3390/foods10112798.
[19] de Silva, N. D. G., Cholewa, E., & Ryser, P. 2012. Effects of combined drought and heavy metal stresses on xylem structure and hydraulic conductivity in red maple (*Acer rubrum* L.). Journal of experimental botany, 63(16), 5957-5966. https://doi.org/10.1093/jxb/ers241.
[20] Jalali, M., Ghanati, F., & Modarres-Sanavi, A. M. 2016. Effect of Fe3O4 nanoparticles and iron chelate on the antioxidant capacity and nutritional value of soil-cultivated maize (*Zea mays*) plants. Crop and Pasture Science, 67(6), 621-628. https://doi.org/10.1071/CP15271.
[21] Manquián-Cerda, K., Cruces, E., Escudey, M., Zúñiga, G., & Calderón, R. 2018. Interactive effects of aluminum and cadmium on phenolic compounds, antioxidant enzyme activity and oxidative stress in blueberry (*Vaccinium corymbosum* L.) plantlets cultivated in vitro. Ecotoxicology and environmental safety, 150, 320-326. https://doi.org/10.1016/j.ecoenv.2017.12.050.
[22] Sun, B., Jing, Y., Chen, K., Song, L., Chen, F., & Zhang, L. 2007. Protective effect of nitric oxide on iron deficiency-induced oxidative stress in maize (*Zea mays*). Journal of plant physiology, 164(5), 536-543. https://doi.org/10.1016/j.jplph.2006.02.011.
[23] Tawfik, M. M., Mohamed, M. H., Sadak, M. S., & Thalooth, A. T. 2021. Iron oxide nanoparticles effect on growth, physiological traits and nutritional contents of *Moringa oleifera* grown in saline environment. Bulletin of the National Research Centre, 45(1), 1-9. https://doi.org/10.1186/s42269-021-00624-9.
[24] Ahanger, M. A., & Agarwal, R. M. 2017. Potassium up-regulates antioxidant metabolism and alleviates growth inhibition under water and osmotic stress in wheat (*Triticum aestivum* L). Protoplasma, 254, 1471-1486. https://doi.org/10.1007/s00709-016-1037-0.
[25] Khan, M. A., & Domashevskiy, A. V. 2021. Iron enhances the binding rates and translational efficiency of iron responsive elements (IREs) mRNA with initiation factor eIF4F. PLoS One, 16(4), e0250374. 10.1371/journal.pone.0250374.
[26] He, Y., Dai, S., Dufresne, C. P., Zhu, N., Pang, Q., & Chen, S. 2013. Integrated proteomics and metabolomics of *Arabidopsis* acclimation to gene-dosage dependent perturbation of isopropylmalate dehydrogenases. PLoS One, 8(3), e57118. https://doi.org/10.1371/journal.pone.0057118.
[27] Hamzah Saleem, M., Usman, K., Rizwan, M., Al Jabri, H., & Alsafran, M. 2022. Functions and strategies for enhancing zinc availability in plants for sustainable agriculture. Frontiers in Plant Science, 13, 1033092. 10.3389/fpls.2022.1033092.

[DOI: 10.22034/FSCT.22.163.1]

[28] Benáková, M., Ahmadi, H., Dučaiová, Z., Tylová, E., Clemens, S., & Tůma, J. 2017. Effects of Cd and Zn on physiological and anatomical properties of hydroponically grown *Brassica napus* plants. Environmental Science and Pollution Research, 24, 20705-20716. https://doi.org/10.1007/s11356-017-9697-7.

[29] Nam HI, Shahzad Z, Dorone Y, Clowez S, Zhao K, Bouain N, Lay-Pruitt KS, Cho H, Rhee SY, Rouached H. 2021. Interdependent iron and phosphorus availability controls photosynthesis through retrograde signaling. Nat Commun, 12(1), 7211. 10.1038/s41467-021-27548-2.

[30] Johan, P. D., Ahmed, O. H., Omar, L., & Hasbullah, N. A. 2021. Phosphorus transformation in soils following co-application of charcoal and wood ash. Agronomy, 11(10), 2010. https://doi.org/10.3390/agronomy11102010.

[31] Heydari, R., Kazemi, E. M., Kolahi, M., Movafeghi, A., & Nosrati, H. (2024). Modulation of cadmium induced oxidative stress pathways in lettuce (*Lactuca sativa* L.) by nano-chelated iron. *Scientia Horticulturae*, *337*, 113530. https://doi.org/10.1016/j.scienta.2024.113530.

[32] Clemens, S., Deinlein, U., Ahmadi, H., Höreth, S., & Uraguchi, S. 2013. Nicotianamine is a major player in plant Zn homeostasis. Biometals, 26, 623-632. https://doi.org/10.1007/s10534-013-9643-1.

[33] Heydari, R., Kolahi, M., Mohajel Kazemi, E., Nosrati, H., & Movafeghi, A. (2024). The role of nano-chelated iron on anatomical and biochemical characteristics and concentration of mineral nutrients in lettuce (*Lactuca sativa* L.) under cadmium toxicity. *Physiology and Molecular Biology of Plants*, *30*(8), 1383-1400. doi: 10.1007/s12298-024-01490-1.

[DOI: 10.22034/FSCT.22.163.1]

مجله علوم و صنایع غذایی ایران

سایت مجله: www.fsct.modares.ac.ir

مقاله علمی_پژوهشی

بررسی اثرات محلول‌پاشی نانو کلات آهن بر میزان متابولیت‌ها و ارزش غذایی گیاه کاهو (*Lactuca sativa* Linn.)

رقیه حیدری[۱]، الهام محجل کاظمی[۲،۳]، هوشنگ نصرتی[۴]، مریم کلاهی[۵*]، علی موافقی[۶]

۱-دانشجوی دکتری گروه علوم گیاهی، زیست سلولی و مولکولی، دانشکده علوم طبیعی، دانشگاه تبریز، تبریز، ایران

۲-دانشیار گروه علوم گیاهی، زیست سلولی و مولکولی، دانشکده علوم طبیعی، دانشگاه تبریز، تبریز، ایران

۳-پژوهشکده کشاورزی هسته‌ای، پژوهشگاه علوم و فنون هسته‌ای، کرج، ایران

۴-استاد گروه علوم گیاهی، زیست سلولی و مولکولی، دانشکده علوم طبیعی، دانشگاه تبریز، تبریز، ایران

۵-دانشیار گروه زیست، دانشکده علوم، دانشگاه شهید چمران، اهواز، ایران

۶-استاد گروه علوم گیاهی، زیست سلولی و مولکولی، دانشکده علوم طبیعی، دانشگاه تبریز، تبریز، ایران

اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۲/۱۰/۹

تاریخ پذیرش: ۱۴۰۳/۱۱/۱۱

کلمات کلیدی:

سیستم تغذیه‌ای،

کاهو (*Lactuca sativa* Linn.)،

عناصر ریزمغذی،

نانو کلات آهن

DOI: 10.22034/FSCT.22.163.1.

* مسئول مکاتبات:

m.kolahi@scu.ac.ir

چکیده

در سال‌های اخیر بسیاری از پژوهش‌ها در پی یافتن راهکارهای مناسبی جهت بهبود ارزش غذایی گیاهان و افزایش تولید محصولات بودند. به منظور افزایش عملکرد گیاهان و کاهش مصرف منابع غذایی حیوانی نیاز به استفاده از تکنیک‌های نوین زراعی است. یکی از مهم‌ترین تکنیک‌ها جهت بهبود سیستم تغذیه‌ای گیاهان و کاهش کاربرد کودهای شیمیایی استفاده از کودهای سنتتیک با بنیان آلی مانند نانو کلات آهن است. کودهای نانو به‌راحتی توسط گیاه جذب می‌شوند و کارایی بیشتری نسبت به کودهای شیمیایی معمولی دارند. بر همین اساس، این پژوهش به‌منظور ارزیابی تأثیر نانو کلات آهن بر ارزش غذایی و میزان جذب عناصر ریزمغذی توسط گیاه کاهو انجام شد. این پژوهش به‌صورت طرح کاملاً تصادفی در ۳ تکرار در آزمایشگاه سیتوشیمی دانشگاه تبریز در سال ۱۴۰۱-۱۴۰۰ صورت گرفت. جهت بررسی اثرات نانوکلات آهن بر میزان پتانسیل گیاه کاهو به منظور جذب عناصر ریزمغذی از ۳ سطح نانو کلات آهن (۰، ۰/۵ و ۱ گرم در لیتر) استفاده شد. در حضور نانو کلات آهن محتوای متابولیت‌های ثانویه از جمله فنل کل، فلاونوئید و به‌علاوه ظرفیت آنتی-اکسیدانی کاهو نسبت به نمونه‌ی شاهد افزایش معنی‌داری داشت. همچنین با توجه به نتایج، با افزایش غلظت نانو کلات آهن در میزان متابولیت‌های اولیه مانند پروتئین کل، قند محلول، آمینواسیدهای آزاد گیاه کاهو نسبت به نمونه‌ی شاهد افزایش مشاهده شد. با افزایش اعمال نانو کلات آهن به گیاه کاهو تجمع عناصر ریزمغذی از جمله آهن، کلسیم، روی، پتاسیم و فسفر در ریشه و اندام هوایی سیر صعودی نشان داد که این افزایش در تیمارهای نانو کلات آهن با غلظت بالاتر بیشتر بود. نتایج به‌دست‌آمده از این پژوهش گویای آن بود که نانو کلات آهن علاوه بر افزایش تولید متابولیت‌های اولیه و ثانویه گیاهان می‌تواند به‌عنوان کود مطمئنی جهت تأمین آهن و عناصر ریزمغذی برای آن‌ها مورداستفاده قرار گیرد.

[DOI: 10.22034/FSCT.22.163.1]