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EFFECT OF FOLIAR IRON RATES AND APPLICATION TIMING ON LETTUCE (*Lactuca sativa* L.) BIOFORTIFICATION IN AN NFT HYDROPONIC SYSTEM

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ABSTRACT

Iron (Fe) is an essential micronutrient for living organisms and plays a key role in numerous physiological processes. Agronomic biofortification with Fe can increase its concentration in edible plant tissues, representing a promising strategy to improve dietary Fe intake and help prevent iron-deficiency anemia, particularly in vulnerable populations. Two experiments were carried out in winter and summer to evaluate the effects of foliar iron application on hydroponically grown lettuce (*Lactuca sativa* L.) cv. 'Waldmann's Green' at three concentrations (2, 4, and 6 mg L^{-1}) and three application timings: 100% at 10 days after transplant (DAT); 100% at 20 DAT; and 50% at 10 DAT plus at 20 DAT. Production of leaf fresh and dry weight, root fresh and dry weight, and foliar N, P, K and Fe were evaluated. A randomized complete block design was used, with a 3x3+1 factorial arrangement, with five replications per treatment. Analysis of variance was performed for winter and summer experiments plus a combined analysis of both experiments. Fe rates or application timings did not affect fresh and dry matter contents of leaves or roots. However, the growing season significantly affected root fresh and dry matter content. N, P and K foliar concentrations were influenced by Fe application rates and timings, with significant variations between growing seasons. Efficient Fe biofortification in lettuce leaves was observed starting at 4 mg L^{-1} of Fe, being higher in summer.

Keywords: foliar application, micronutrient, mineral nutrition, nutrient film technique, soilless cultivation.

INTRODUCTION

Iron (Fe) is an essential micronutrient for both plants and humans, playing a key role in a wide range of physiological processes, including respiration, photosynthesis, and oxygen transport (Römheld & Nikolic, 2007; Giordano et al., 2019).

A widespread issue in many developing countries is micronutrient deficiency. Globally, two billion people suffer from anemia, and according to the World Health Organization (WHO), the primary cause of this condition is dietary Fe deficiency (Ramos-Padilla et al., 2020). In Peru, this problem persists, particularly in rural areas where access to high-quality food remains a challenge (Pillaca and Villanueva,

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2015). Addressing micronutrient deficiencies is also aligned with SDG 2 (Zero Hunger) and SDG 3 (Good Health and Well-being), which promote improved nutrition and human health as part of sustainable development.

Among the strategies employed to address nutrient deficiencies in populations, biofortification has emerged as an effective and sustainable alternative. This technique, whether achieved through genetic breeding, transgenic or agronomic management, enhances the concentration of essential nutrients in the edible parts of crops, improving their bioavailability and facilitating their integration into human diets (Vasconcelos et al., 2017; Garg et al., 2018; Bhardwaj et al., 2022; Ofori et al., 2022; Stangoulis and Knez, 2022; Monika et al., 2023). Within agronomic biofortification, foliar application represents a direct approach for supplying Fe to plant tissues; however, its efficiency may vary according to the applied rate, application timing, environmental conditions, and nutrient absorption through the leaf surface (Murillo-Castillo et al., 2013). Moreover, increasing Fe supply does not necessarily result in a proportional increase in its accumulation, since plant responses may depend on Fe availability in the growing medium and physiological nutrient requirements (Nayan and Fouzi, 2023).

Numerous studies have been conducted on Fe biofortification in economically important crops, such as maize, potato, rice, cassava, bean, and wheat (Monserrate et al., 2009; Ofori et al., 2022; Zulfiqar et al., 2024), reporting significant increases in Fe content. Furthermore, leafy vegetables like lettuce have been identified as promising candidates for biofortification due to their short production cycle, ease of cultivation, and high global demand (Giordano et al., 2019; Goto et al., 2000; Buturi et al., 2022; Burgess et al., 2024). In the Peruvian context, lettuce consumption has been estimated at 1.5 kg per capita (Instituto Nacional de Estadística e Informática, 2010), underscoring its potential as a viable vehicle for enhancing dietary Fe intake. However, information remains limited regarding the combined effects of foliar Fe rate and application timing on Fe accumulation and nutrient status in lettuce grown in Nutrient Film Technique (NFT) hydroponic systems, particularly under different growing seasons.

Therefore, this study aimed to evaluate the effects of different foliar Fe rates and application timings on biomass production, leaf nutrient status, and Fe biofortification of lettuce grown in an NFT hydroponic system across different growing seasons.

MATERIALS AND METHODS

Experimental site

Two experiments were conducted at EcoVerduras facilities in Vista Alegre Annex (13°03′09′′ S, 76°22′10′′ W, 130 m above sea level), Imperial District, Cañete Province, Lima, Perú. The first experiment was conducted in August 2021 during the winter season, whereas the second conducted in February 2022 during the summer season.

The experimental region is characterized by a type E(d)B′ climate, corresponding to arid conditions with year-round moisture deficiency (Köppen, 1936). During the experimental period, mean minimum and maximum temperatures were 16.7°C and 26.7°C in summer and 13.24°C and 19.62°C in winter. Mean minimum and maximum relative humidity values were 63.4% and 81.0% in summer and 79.4% and 88.6% in winter.

Treatments and experimental design

The experiments were conducted using a completely randomized block design with a 3x3+1 factorial arrangement and five replications. Fe rates (D_1 = 2 mg L^{-1}, D_2 = 4 mg L^{-1}, and D_3 = 6 mg L^{-1}) and application timings (AT_1 = 100% at 10 DAT, AT_2 = 100% at 20 DAT, and AT_3 = 50% at 10 and 20 DAT), resulting in the following treatments: T_1=D_1AT_1; T_2=D_1AT_2; T_3=D_1AT_3; T_4=D_2AT_1; T_5=D_2AT_2; T_6=D_2AT_3; T_7=D_3AT_1; T_8=D_3AT_2; T_9=D_3AT_3, plus a control treatment (T_{10}). Each experimental unit consisted of one NFT channel containing ten plants. The first and last plants in each channel were excluded from the evaluations to avoid potential border effects. Therefore, the eight central plants were used for data collection.

Foliar Fe applications were conducted in the morning using a manual sprayer without the addition of a surfactant, ensuring uniform wetting of the leaf surface. Applications were performed sequentially from the lowest (2 mg L^{-1}) to the highest concentration (6 mg L^{-1}) to minimize cross-contamination among treatments. A 6% Fe chelate with EDDHA was used as the Fe source and diluted in distilled water to avoid potential interference from the chemical composition of the solvent.

Experimental setup and management

Seeds of lettuce cv. ‘Waldmann’s Green’ were germinated in a sand substrate. At 15 days after sowing (DAS), seedlings were transferred to small plastic containers and maintained in a floating hydroponic system until final transplantation. Seedlings were transplanted at 35 DAS (winter cycle) and 31 DAS (summer cycle), when they had developed 3-4 true leaves, reached approximately 15 cm in height, and exhibited healthy white roots free from pathogen damage. Prior to transplanting into the definitive growing channels, all seedlings underwent washing, cleaning, and disinfection (4% sodium hypochlorite solution) of both internal and external surfaces.

For both experimental seasons, the NFT hydroponic system was used in a cultivation module. The system consisted of 23 cm wide polypropylene channels mounted on wooden support structures. The summer trial used 50% shade raschel mesh, while winter did not. A 1 HP centrifugal pump (Humboldt) circulated nutrient solution for 15-minute intervals at 6 scheduled times daily (7:00, 11:00, 12:00, 13:00, 15:00, and 18:00 h) through distribution and drainage piping.

The nutrient solution formulation used in the experiments is presented in Table 1. This formulation was adjusted based on water analysis results. Furthermore, Fe sources were deliberately excluded from the formulation, as Fe application constituted one of the experimental variables under investigation.

Parameters evaluated

At the end of each experimental period (35 DAT), the following variables were evaluated: a) Fresh and dry biomass (g plant^{-1}): after harvesting, shoots and roots were separated for fresh weight determination. Samples were then dried in a forced-air oven at 75°C until constant weight was achieved. Dry weights of each plant part were recorded after the drying process; b) Leaf nutrient analysis (N, P, K in %; Fe in ppm): all collected samples were properly labeled and submitted for nutritional analysis at the Agricultural Chemistry Laboratory of Valle Grande Institute, located in the San Vicente de Cañete District, Cañete Province, Lima Department, Perú.

Statistical analysis

The experimental data were subjected to individual analysis of variance using the F-test. Subsequently, when the ratio between the largest and smallest residual mean squares across experiments was less than fourfold, a combined analysis was performed using AgroEstat software (Barbosa and Maldonado Júnior, 2015). When the factors under study showed a significant effect, Tukey's test at a 5% probability level and regression analysis were conducted.

RESULTS AND DISCUSSION

Fresh and dry matter of leaves and roots

No significant effects of the biometric variable evaluated, either individually or in interaction, nor of the factorial comparison with the control treatment were observed during the winter experiment (Table 2). The overall mean values for leaf fresh weight (LFW), root fresh weight (RFW), leaf dry weight (LDW), and root dry weight (RDW) were 131.70, 12.00, 8.81, and 0.85 g plant^{-1}, respectively.

In summer, a significant effect of Fe application timing was observed only on RFW (Table 2). The overall mean values recorded for LFW, LDW, and RDW were 126.26, 9.40, and 1.07 g plant^{-1}, respectively.

The highest RFW (23.42 g plant^{-1}) was achieved with Fe application at 10 DAT, showing significant differences from the split application at 10 and 20 DAT, which showed the lowest value of 20.49 g plant^{-1}. Intermediate RFW (21.94 g plant^{-1}) was observed with application at 20 DAT, though this did not differ significantly from other treatments. While delayed Fe application showed positive effects, its impact was less pronounced

Table 1. Nutrient solution composition used in winter and summer experiments.

NS	Source	Amount (g)	Concentrated solution	DxL
A	Monoammonium Phosphate	550	5 L	5 mL
	Potassium Nitrate	350		
	Ammonium Nitrate	180		
	Magnesium Sulfate Heptahidratado	220		
B	This Zn 20% Chelate	25	2 L	2 mL
	This Mn 17% Chelate	20		
	This Cu 15% Chelate	10		
	Boric acid - INKABOR	1.2		
C	Calcium nitrate	450	1 L	1 mL

DxL: dose per liter of nutrient solution.

Table 2. Analysis of variance results for leaf fresh weight (LFW), root fresh weight (RFW), leaf dry weight (LDW), and root dry weight (RDW) of lettuce cv. 'Waldmann's Green' at 35 days after transplanting with different iron application rates and timings during summer and winter trials, and in the combined analysis.

Sources of variation	GL	LFW	RFW	LDW	RDW
Winter					
Iron rate (R)	2	0.50^{NS}	1.46^{NS}	0.32^{NS}	0.27^{NS}
Aplication timing (AT)	2	0.93^{NS}	1.41^{NS}	0.46^{NS}	1.09^{NS}
R x AT	4	0.44^{NS}	0.18^{NS}	0.56^{NS}	0.58^{NS}
Factorial x C	1	0.01^{NS}	1.31^{NS}	0.00^{NS}	1.65^{NS}
Error (MSE)	36	872.15	10.14	3.35	0.08
Coefficient of variation (%)	-	22.42	26.54	20.62	32.23
Summer					
Iron rate (R)	2	1.66^{NS}	0.08^{NS}	1.42^{NS}	1.72^{NS}
Application timing (AT)	2	1.01^{NS}	7.62**	2.21^{NS}	2.98^{NS}
R x AT	4	1.37^{NS}	1.69^{NS}	1.97^{NS}	0.71^{NS}
Factorial x C	1	0.31^{NS}	0.93^{NS}	2.69^{NS}	0.28^{NS}
Error (MSE)	36	167.53	4.22	1.04	0.0085
Coefficient of variation (%)	-	10.25	9.40	10.84	8.63
Combined seasons					
Treatments (Rate x Timing) (T)	9	0.69^{NS}	0.54^{NS}	0.64^{NS}	0.96^{NS}
Seasons (S)	1	1.90^{NS}	191.85**	3.17^{NS}	59.53**
T x S	9	0.75^{NS}	1.76^{NS}	0.97^{NS}	0.80^{NS}
Error (MSE)	72	519.84	7.18	2.20	0.03
Coefficient of variation (%)	-	17.68	15.83	16.21	18.74

GL: degrees of freedom; MSE: mean square error; C: control. Values correspond to the calculated Fisher–Snedecor F-statistic. *: Significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ^{NS}: not significant by the F-test.

than the early application (10 DAT). This effect may be related to the essential role of Fe in root metabolism, as it participates in key enzymatic processes of cellular respiration and adenosine triphosphate (ATP) production, facilitating the active uptake of water and nutrients (Briat et al., 2015).

In the combined analysis, a significant individual effect of the growing seasons on RFW and RDW was observed (Table 2). The mean values recorded for LFW and LDW were 128.98 and 9.14 g plant^{-1}, respectively.

Higher values of RFW (21.86 g plant^{-1}) and RDW (1.07 g plant^{-1}) were obtained in summer, which were 45.11% and 20.30% higher, respectively, than those observed in winter. This effect may be related to variations in temperature and water availability, factors that influence root morphology and metabolism (Balliu et al., 2021). Lettuce may have developed a more robust root system to enhance water and nutrient uptake under warmer conditions, which would explain the higher values of RFW and RDW observed in summer.

Preciado-Rangel et al. (2022) did not find a significant effect of foliar Fe application on lettuce fresh tissue, similar to what was observed in the present study. However, other authors have reported both positive and negative effects in different lettuce cultivars. Suliburska et al. (2024) observed an increase in dry mass with 4.5 mg L^{-1} Fe, while Silva et al. (2024) recorded a reduction when applying 4 and 8 mg L^{-1} Fe in the nutrient solution. Similarly, Nayan & Fouzi (2023) reported decreases with 1.0 and 2.0 kg ha^{-1} Fe-EDTA, and Giordano et al. (2019) observed reductions in both fresh and dry mass with doses above 1.0 and 2.0 mM Fe. Buturi et al. (2022) also noted an 18% decrease in fresh mass with 1.02 and 2.02 mmol L^{-1} Fe, although with the higher dose, dry mass increased by 16%.

Leaf N, P, K, and Fe concentrations

During winter cultivation, significant effects were observed only for the interaction between the factors, as well as between the factorial treatments and the control, regarding foliar N and P concentrations (Table 3).

Interaction analysis revealed a significant quadratic response in foliar N concentration

Table 3. Analysis of variance results for leaf nitrogen (N), phosphorus (P), potassium (K), and iron (Fe) concentrations in lettuce cv. 'Waldmann's Green' at 35 days after transplanting, with different iron application rates and timings during summer and winter trials, and in the combined analysis.

Sources of variation	GL	N	P	K	Fe
Winter					
Iron rate (R)	2	2.38^{NS}	1.97^{NS}	1330.03**	418.21**
Application timing (AT)	2	1.87^{NS}	0.87^{NS}	377.10**	79.90**
R x AT	4	6.71**	16.79**	1074.23**	720.93**
Factorial x C	1	13.31**	5.67*	217.91**	1667.30**
Error (MSE)	36	0.0839	0.0014	0.0115	1.1631
Coefficient of variation (%)		7.35	6.51	4.85	0.88
Summer					
Iron rate (R)	2	3.00^{NS}	0.28^{NS}	9.61**	2.79.52**
Application timing (AT)	2	4.36*	0.94^{NS}	16.54**	152.64**
R x AT	4	0.38^{NS}	2.44^{NS}	8.23**	75.82**
Factorial x C	1	3.80^{NS}	3.63^{NS}	0.40^{NS}	233.57**
Error (MSE)	36	0.0871	0.0022	0.1911	132.3047
Coefficient of variation (%)		7.45	7.90	9.14	5.09
Combined seasons					
Treatments (Rate x Timing) (T)	9	1.77^{NS}	0.31^{NS}	1.53^{NS}	0.92^{NS}
Seasons (S)	1	0.04^{NS}	0.03^{NS}	68.74**	23.79**
T x S	9	2.73**	6.97**	8.54**	166.27**
Error (MSE)	72	0.0855	0.0018	0.1024	66.7339
Coefficient of variation (%)	-	7.40	7.24	7.99	4.68

GL: degrees of freedom; MSE: mean square error; C: control. Values correspond to the calculated Fisher–Snedecor F-statistic. *: Significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ^{NS}: not significant by the F-test.

exclusively under split Fe application at 10 and 20 DAT (Fig. 1). Foliar N increased progressively with Fe rate, with a peak at 4.48% (4.2 mg L^{-1} Fe) before declining at higher rates, suggesting potential uptake saturation or adverse physiological effects. Preciado-Rangel et al. (2022) reported similar trends in lettuce, with a highest foliar N (3.48%) at 5.56 mg L^{-1} Fe applied biweekly post-transplant, followed by reductions at elevated rates. In contrast, Buturi et al. (2022) observed linear increases in foliar N up to the highest tested Fe rate (2.02 mmol L^{-1}), highlighting context-dependent nutrient interactions.

The control plants showed significantly lower foliar N concentration (3.49%) compared to Fe-treated lettuce (3.99%), demonstrating that Fe application enhances N uptake. Our results indicate that split Fe applications are more effective than single applications, but only up to an optimal rate, beyond which benefits decrease. All observed foliar N concentrations, including the control, were within the recommended range of 2.1-5.6% for lettuce reported by Maynard and Hochmuth (2007). However, these values are used only as a nutritional reference, since the sampling methodology and plant material analyzed differ from those used in the present study.

Significant quadratic regression was observed in leaf P concentration when Fe was applied at 10 DAT or split between 10 and 20 DAT (Fig. 2). Under split Fe application, the maximum leaf P concentration (0.65%) was achieved at 4.3 mg L^{-1} Fe, followed by a decline at higher rates. A similar trend was reported by Preciado-Rangel et al. (2022), who found the highest leaf P concentration (0.40%) at 5.56 mg L^{-1} Fe, decreasing at higher rates. Conversely, Fe application at 10 DAT reduced leaf P concentration up to 5.1 mg L^{-1} Fe, reaching the lowest value (0.51%), with a slight subsequent increase (Fig. 2). In contrast, Giordano et al. (2019) and Buturi et al. (2022) reported increased leaf P content with increasing Fe rates in different lettuce cultivars.

The average leaf P concentration (0.59%) in Fe-treated plants was slightly higher than the control (0.55%), suggesting a positive effect of Fe on P uptake, though dependent on application rate and application timing. All leaf P concentrations

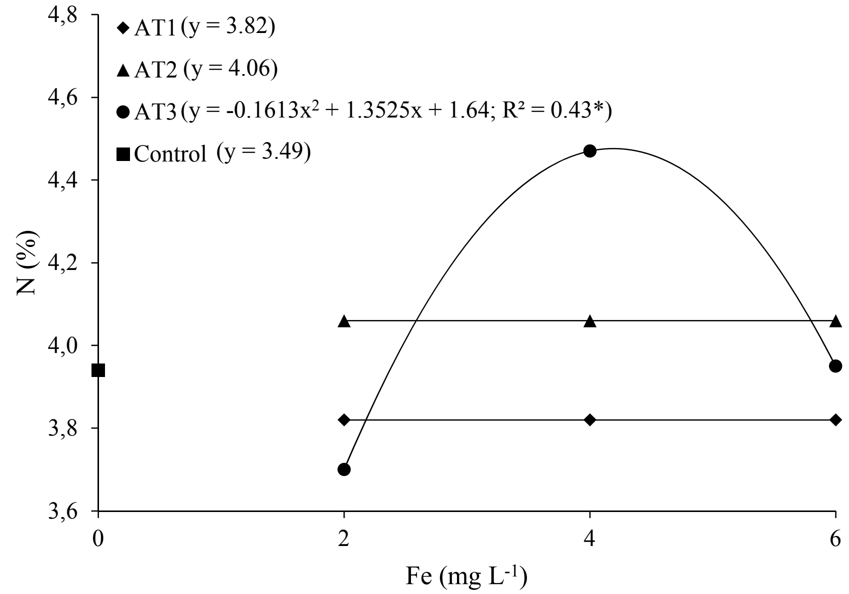

Fig. 1. Foliar N concentration (%) as influenced by iron application rates and timings (AT1: 10 DAT; AT2: 20 DAT; and AT3: split application at 10 and 20 DAT) during winter cultivation.

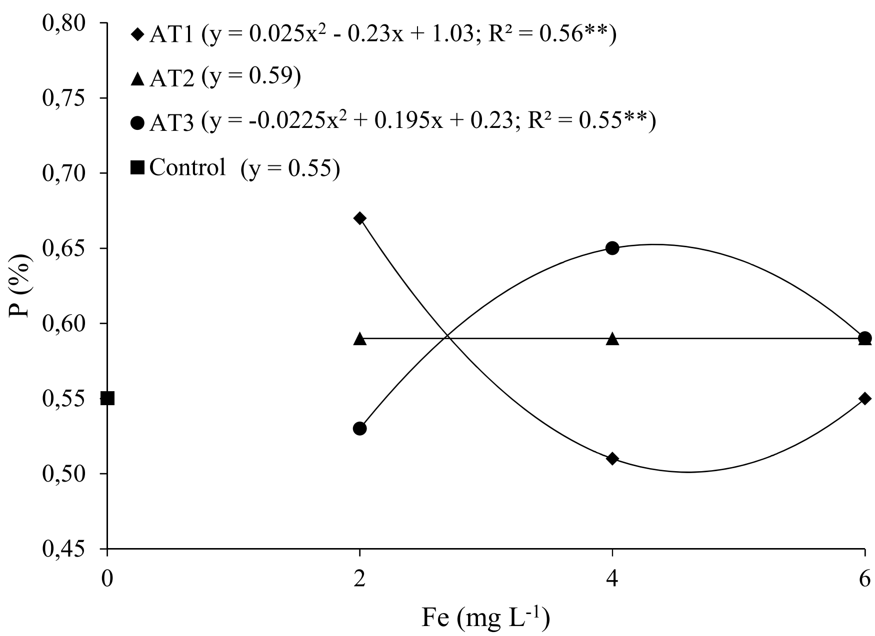

Fig. 2. Foliar P concentration (%) as influenced by iron application rates and timings (AT1: 10 DAT; AT2: 20 DAT; and AT3: split application at 10 and 20 DAT) during winter cultivation.

observed in this study, including the control, were within the optimal range for lettuce (0.5 - 0.9%) as reported by Maynard and Hochmuth (2007).

Unlike the trends observed for other foliar nutrients, winter leaf concentrations of K and Fe were significantly influenced by individual factors, interactions, and factorial comparisons with the control (Table 3).

The interaction analysis revealed a significant quadratic relationship between leaf K concentration and Fe application rates/timings (Fig. 3). No significant differences in leaf K

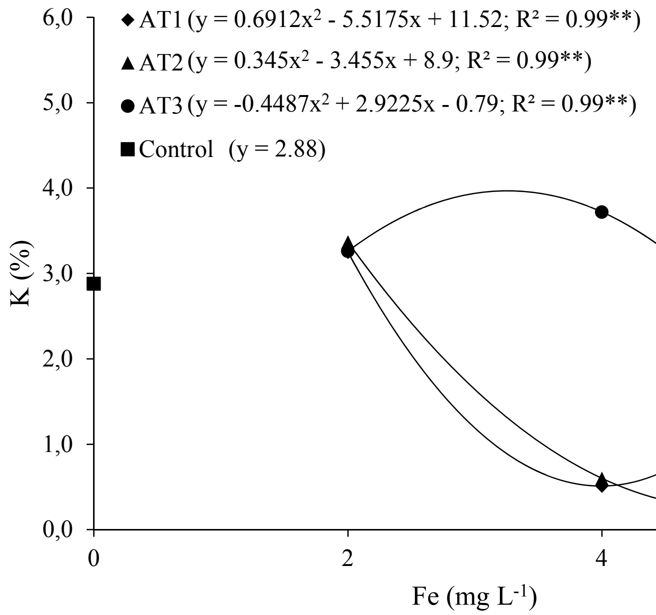

Fig. 3. Foliar K concentration (%) as influenced by iron application rates and timings (AT1: 10 DAT; AT2: 20 DAT; and AT3: split application at 10 and 20 DAT) during winter cultivation.

concentration were observed at the lowest Fe rate (2 mg L^{-1}) across application timings at 10 DAT, 20 DAT, or split 10+20 DAT, with values of 3.25%, 3.37%, and 3.26% respectively; all values were higher than the control (2.88%). However, Fe applications >2 mg L^{-1} at 10 or 20 DAT initially reduced leaf K to 0.51% (4 mg L^{-1} Fe) and 0.25% (5 mg L^{-1} Fe), followed by a rate - dependent recovery that was more pronounced at 10 DAT. Split Fe applications (10+20 DAT) produced different dynamics: leaf K highest record at 3.97% (3.3 mg L^{-1} Fe) before declining to 0.59% (6 mg L^{-1} Fe), ultimately falling below control levels (Fig. 3).

Several studies report contrasting effects of increasing Fe doses on leaf K concentration in lettuce. Buturi et al. (2022) and Suliburska et al. (2024) observed a positive correlation, whereas Giordano et al. (2019) reported suppression, and Preciado-Rangel et al. (2022) found no significant influence across cultivars.

Unlike summer observations, all winter leaf K concentrations, including those of the control, fell below the recommended range of 4.0 to 8.0% for lettuce (Maynard and Hochmuth, 2007). Despite these suboptimal tissue K levels, no visual deficiency symptoms were observed in the lettuce plants.

Similar to K, leaf Fe concentration showed a significant quadratic response to Fe application rate and timing (Fig. 4). At 10 DAT, Fe concentration decreased to 112.9 ppm (4.7 mg L^{-1} Fe), followed by a slight recovery at intermediate rates. In contrast, applications at 20 DAT or split applications (10+20 DAT) achieved highest concentrations of 134.43 ppm (3.7 mg L^{-1} Fe) and 129.29 ppm (4.3 mg L^{-1} Fe), respectively, before declining at higher rates, with more pronounced reduction at 20 DAT. These results demonstrate that Fe uptake depends on both application rate and timing, with split applications showing most effective for maintaining balanced Fe absorption during winter production.

Multiple studies have reported increased leaf Fe concentrations in lettuce following elevated Fe applications (Giordano et al., 2019; Buturi et al., 2022; Preciado-Rangel et al., 2022; Suliburska et al., 2024; Silva et al., 2024). In contrast to our findings, Nayan and Fouzi (2023) observed no significant differences in Fe uptake regardless of application rate or source, attributing this to adequate native soil Fe levels.

All treatments resulted in leaf Fe concentrations exceeding control value (104.55 ppm), demonstrating the positive effect of Fe application under the evaluated conditions. This pattern suggests a dynamic interaction between micronutrient availability and plant physiological requirements based on application timing and rate. Fe content in the control treatment originated from the water source of the nutrient solution (0.06 ppm of Fe), which was

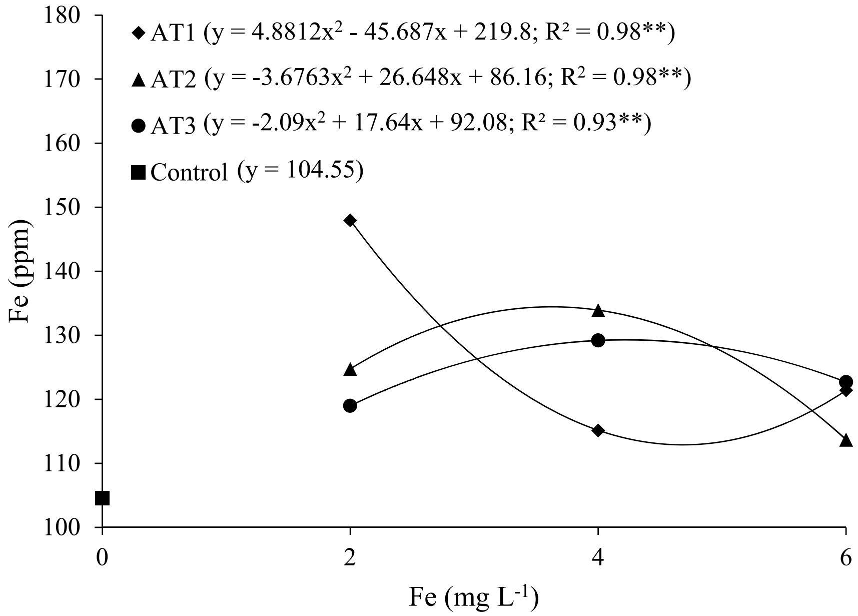

Fig. 4. Foliar Fe concentration (ppm) as influenced by iron application rates and timings (AT1: 10 DAT; AT2: 20 DAT; and AT3: split application at 10 and 20 DAT) during winter cultivation.

common to all treatments. All measured leaf Fe concentrations in winter, including those of the control (Fig. 4), fell within the optimal range for lettuce (50 - 200 ppm) as established by Maynard & Hochmuth (2007).

During the summer trial, only application timing significantly influenced leaf N concentration (Table 3). The highest leaf N content (4.17%) was achieved with Fe application at 20 DAT, which differed significantly from the earliest application at 10 DAT (3.87%). Split Fe application at both 10 and 20 DAT yielded intermediate N levels (3.92%), showing no significant differences from other application timings. These results indicate that late Fe application favors a higher foliar N concentration, while early application may limit its assimilation. Furthermore, the split application did not show additional benefits compared to the late application.

All observed foliar N concentrations across application timings (3.72 - 4.17%), the control (3.72%), and factorial treatments (3.99%), fell within the optimal range (2.1 - 5.6%) for lettuce cultivation as described by Maynard and Hochmuth (2007).

In contrast to our findings, Preciado-Rangel et al. (2022) reported that Fe application significantly influenced lettuce leaf N content, peaking at 3.478% with 5.56 mg L^{-1} Fe, while higher concentrations reduced N levels. Similarly, Buturi et al. (2022) observed a progressive increase in foliar N, reporting levels 13% higher than the control at 2.02 mmol L^{-1} Fe.

No significant effects of the individual factors, their interaction, or the factorial comparison with the control treatment were observed for leaf P concentration during summer (Table 3). The mean foliar P content was 0.59%, which falls within the optimal range of 0.5 - 0.9% reported for lettuce by Maynard and Hochmuth (2007). In contrast to the present study, Giordano et al. (2019), Buturi et al. (2022), and Preciado-Rangel et al. (2022) observed a significant effect of applying different Fe doses on foliar P content in various lettuce cultivars.

A significant effect of the factors, both individually and in interaction, was only observed on foliar K concentration during summer (Table 3). In the interaction analysis, a significant quadratic adjustment was observed for foliar K concentration based on the Fe doses and application timings (Fig. 5).

Application of the lowest Fe rate at 10 DAT reduced foliar K concentration, whereas application of 6 mg L^{-1} resulted in the highest concentration, reaching 5.16% (Fig. 5). This response may be associated with the well-developed root system at 10 DAT, which could have enhanced nutrient uptake efficiency. In contrast, at 20 DAT, 2 mg L^{-1} of Fe promoted a higher foliar K concentration (5.53%), which then decreased (4.70%) up to the 4.3 mg L^{-1} Fe rate and slightly increased at the highest rate. This

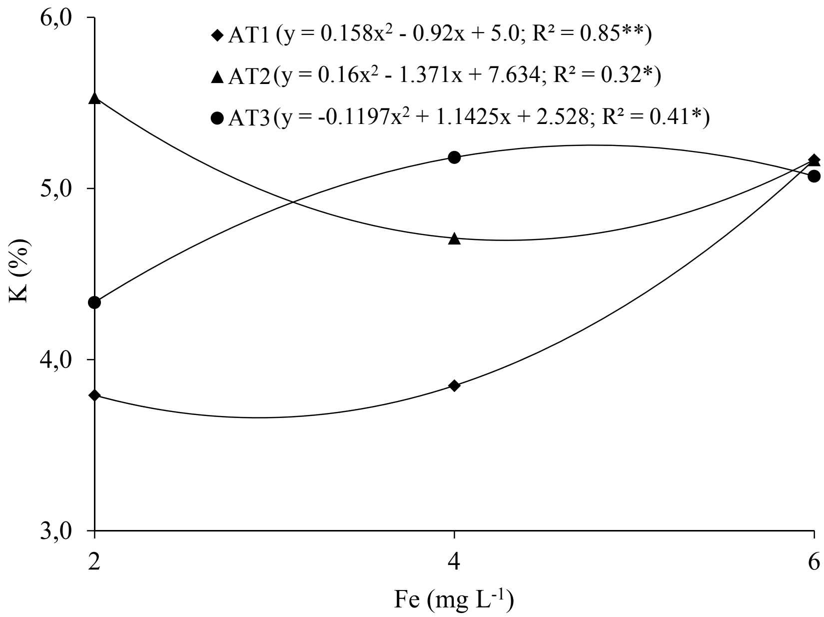

Fig. 5. Foliar K concentration (%) as influenced by iron application rates and timings (AT1: 10 DAT; AT2: 20 DAT; and AT3: split application at 10 and 20 DAT) during summer cultivation.

suggests that Fe application at this stage does not align with the crop's peak nutrient demand. Split application (at 10 and 20 DAT) showed an intermediate response, with the highest foliar K concentration (5.25%) achieved at 4.8 mg L^{-1} Fe, followed by a reduction at the highest rate. These results indicate that split Fe applications may help maintain stable K levels in later crop stages, thereby optimizing nutrition and potentially minimizing antagonistic effects.

Buturi et al. (2022) and Suliburska et al. (2024) observed an increase in foliar K concentration in lettuce varieties, whereas Giordano et al. (2019) reported a reduction with increasing Fe rates. In contrast, Preciado-Rangel et al. (2022) found no significant effect of Fe on foliar K content in lettuce.

All foliar K concentrations resulting from Fe application at 20 DAT or split application between 10 and 20 DAT fell within the optimal range (4.0-8.0%) for lettuce, as reported by Maynard and Hochmuth (2007). However, foliar K concentrations dropped below this optimal range when Fe was applied solely at 10 DAT at rates of 4.5 mg L^{-1} (Fig. 5). Similarly, the foliar K levels from the factorial treatment (4.77%) and the control (4.90%) also remained within the recommended range.

Regarding foliar Fe concentration during summer cultivation, significant effects were observed for all evaluated factors, both individually and through their interactions, as well as between the factorial treatments and the control (Table 3).

Interaction analysis revealed a significant quadratic response in foliar Fe concentration to both Fe application rates and timings (Fig. 6). Early Fe application at 10 DAT maximized foliar Fe accumulation (339.16 ppm at 5.3 mg L^{-1}), suggesting more efficient utilization during active root system development. In contrast, 20 DAT applications peaked at 276.12 ppm (4.8 mg L^{-1}), while split applications at 10 and 20 DAT showed an initial decline followed by gradual increase to 229.03 ppm at 6 mg L^{-1}, indicating either temporal redistribution inefficiency or reduced nutrient availability during critical growth stages. These findings demonstrate that early Fe applications (10 DAT) optimize absorption, whereas delayed (20 DAT) or split applications prove to be less effective, emphasizing the need for stage-specific fertilization strategies.

Recent research (Giordano et al., 2019; Buturi et al., 2022; Preciado-Rangel et al., 2022; Suliburska et al., 2024; Silva et al., 2024) demonstrates that foliar Fe concentrations increase with higher application rates of this micronutrient. In contrast, Nayan and Fouzi (2023) observed no significant differences in Fe uptake by lettuce regardless of source or application rate, suggesting sufficient native Fe availability in the growth medium.

In contrast, the control treatment showed

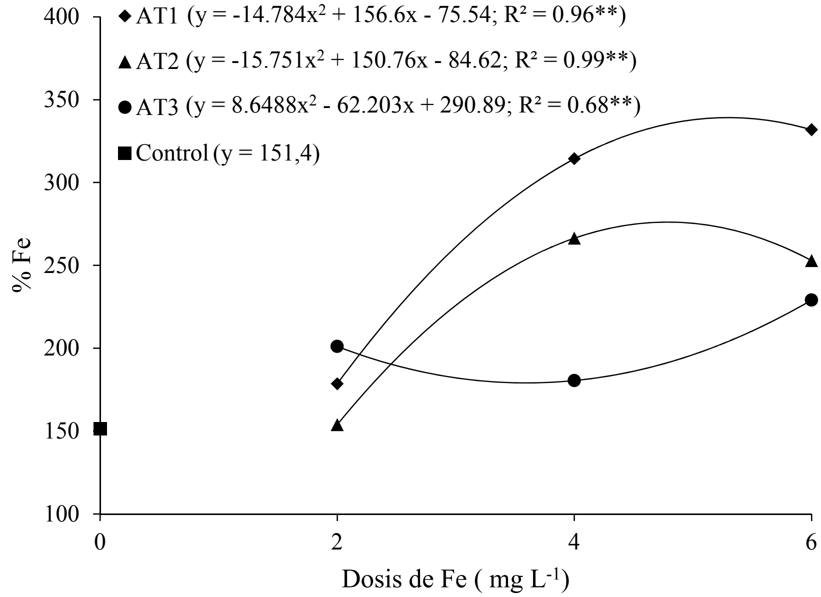

Fig. 6. Foliar Fe concentration (ppm) as influenced by iron application rates and timings (AT1: 10 DAT; AT2: 20 DAT; and AT3: split application at 10 and 20 DAT) during summer cultivation.

the lowest foliar Fe concentration (151.4 ppm) (Fig. 6), underscoring the critical role of Fe supplementation in crop nutrition. This baseline Fe content reflects the concentration present in the irrigation water (0.06 ppm) used to prepare the nutrient solution, which was uniformly applied across all treatments.

Foliar Fe concentrations exceeded the optimal range for lettuce (50-200 ppm; Maynard and Hochmuth 2007) when Fe rates surpassed 2.3 mg L^{-1} (10 DAT), 2.6 mg L^{-1} (20 DAT), or 5.2 mg L^{-1} (split application at 10 and 20 DAT) (Fig. 6). In contrast, the control and treatments with lower Fe rates maintained foliar Fe levels within this optimal range.

The combined analysis revealed a significant treatment x season interaction effect solely on leaf N and P concentrations (Table 3).

The interaction analysis revealed distinct seasonal patterns in N accumulation: winter conditions produced maximum N content with T_6 (split application of 4 mg L^{-1} Fe at 10 and 20 DAT), showing a 21.7% increase over the control (T_{10}), while summer results peaked with T_8 (6 mg L^{-1} Fe at 20 DAT) at 12.9% above control levels (Table 4). Although winter N content averaged 4.4% higher than summer, this seasonal difference was not statistically significant.

In this study, winter and summer leaf N concentrations (2.1-5.6%) fell within the optimal range for lettuce (Maynard and Hochmuth 2007), indicating no N deficiency. At 35 DAT, average N content was 3.9% across both growing seasons, comparable to the 4.1% reported by Sosa-Baldivia et al. (2017) for soil-grown lettuce at the same growth stage.

The foliar P content results in winter showed a 23.8% difference between the highest (T_1: 2 mg L^{-1} Fe applied at 10 DAT) and lowest values (T_4: 4 mg L^{-1} Fe applied at 10 DAT). In summer, this difference was 14.5%, observed between T_{10} (control) and T_6 (4 mg L^{-1} Fe at 10 and 20 DAT). When comparing seasons, no significant differences were found in foliar P concentration, although winter values were 7.4% higher than those recorded in summer.

In both seasons, higher foliar P levels were observed at low Fe application rates. Although Guillén-Molina et al. (2016) reported that foliar Fe application increased leaf P content, while excessive Fe rates reduced it; this response was not observed in the present study. In summer, the highest leaf P levels occurred in the control treatment (T_{10}, Table 4), consistent with the findings of Wojciechowska et al. (2019), who reported that Fe nutrition through the substrate significantly affected macronutrient concentrations in lettuce, with a negative correlation between substrate Fe and leaf P/Ca contents.

Near harvest, lettuce leaf P content typically ranges from 0.5 - 0.9% (Maynard and Hochmuth, 2007). Sosa-Baldivia et al. (2017) and Preciado-Rangel et al. (2022) reported P levels of 0.4 - 0.6%

at 35 DAT, with slight variations depending on sampling time. In the present study, the average P content across seasons (summer and winter) was 0.58%, falling within Maynard and Hochmuth (2007) recommended range.

For foliar K and Fe concentrations, only the individual effect of seasons and the treatment x season interaction were significant in the combined analysis (Table 3).

In winter, treatment T_6 (4 mg L^{-1} Fe split-applied at 10 and 20 DAT) produced the highest foliar K concentration (3.71%), while T_{10} (control), T_4, and T_8 recorded the lowest values, with a 23.18% difference between T_6 and T_{10}. In contrast, summer results showed T_2 (2 mg L^{-1} Fe at 20 DAT) with the highest K levels, followed by T_8 and T_6, whereas T_1 and T_4 exhibited the lowest concentrations - a 31.46% difference between T_2 (maximum) and T_1 (minimum) (Table 4). Overall, foliar K was higher in summer, suggesting seasonal conditions enhance accumulation. The optimal Fe application rate should thus be adjusted by season to maximize uptake efficiency. Notably, adequate K levels (4.0 - 8.0%; Maynard and Hochmuth, 2007) were only achieved in summer, while winter values fell below this range.

Potassium ions distribute strategically within plant cells, maintaining high concentrations in both the cytosol and vacuoles, which serve as K storage organelles. This macronutrient plays a critical role in stomatal regulation - guard cells accumulate K to open stomata and lose it to close them (Römheld and Nikolic, 2007; Beltrán-Morales et al., 2019). The higher K concentrations observed in summer may reflect greater stomatal activity, while the lower concentrations recorded in winter may be associated with reduced stomatal activity.

Similar to K trends, Fe foliar content showed seasonal variations. In winter, applying 2 mg L^{-1} Fe at 10 DAT (T_1) yielded the highest concentration (147.95 ppm, Table 4), while higher rates (e.g., T_7: 6 mg L^{-1} at 10 DAT) did not consistently increase absorption, potentially due to nutrient saturation or antagonism. The control (T_{10}) showed the lowest values, confirming Fe supplementation necessity. Summer results differed significantly: T_7 (6 mg L^{-1} at 10 DAT) achieved peak concentration (331.83 ppm), followed by T_4 (4 mg L^{-1} at 10 DAT), indicating higher rates enhanced Fe accumulation. The lowest values occurred in control plants (T_{10}) and T_2 (2 mg L^{-1} at 20 DAT). Notably, split applications underperformed in both seasons, suggesting single early applications are optimal. Summer Fe levels were 55.4% higher than those recorded in winter (Table 4).

Winter foliar Fe concentrations remained within the optimal 50 - 200 ppm range for greenhouse lettuce (Maynard and Hochmuth 2007). However, elevated summer temperatures enhanced Fe absorption, causing treatments with higher Fe applications (T_7, T_4, T_5, T_8, T_9, T_3) to exceed recommended levels. The observed temperature-dependent enhancement of foliar Fe assimilation results from four concurrent physiological mechanisms: (1) thermal modification of cuticular wax architecture increases porosity, creating preferential pathways for apoplastic nutrient flux into epidermal cells; (2) temperature-mediated upregulation of metabolic activity enhances ATP-dependent membrane transporters, particularly those involved in ferrous iron (Fe^{2+}) uptake; (3) accelerated kinetics of redox reactions maintain Fe in bioavailable states, primarily as soluble Fe^{2+} or stable chelate complexes; and (4) evaporative concentration of foliar-applied solutions establishes steeper electrochemical potential gradients across the cuticle-epidermis interface, driving passive diffusion. These thermally modulated processes collectively account for the significant increase in foliar Fe bioavailability under elevated temperature regimes (Murillo-Castillo et al., 2013).

Our experimental results for Fe concentration align with recent studies (Buturi et al., 2022; Preciado-Rangel et al., 2022; Suliburska et al., 2024; Silva et al., 2024), which demonstrated that foliar or nutrient solution Fe applications can effectively increase Fe accumulation in lettuce leaves and enhance their nutritional quality. Collectively, these findings support Fe biofortification of soilless, greenhouse grown lettuce as an effective strategy to enhance dietary Fe intake while potentially increasing the concentration of health-promoting phytochemicals and, consequently, the market value of the crop.

CONCLUSIONS

Foliar Fe application at rates of 2, 4, and 6 mg L^{-1} at different timings did not significantly affect shoot or root biomass; however, the growing seasons had a significant effect on the root biomass of lettuce cv. ‘Waldmann’s Green’ grown in the NFT system.

Foliar N, P, and K concentrations were influenced by both Fe application rate and timing, with responses varying between seasons. During summer, late application of 6 mg L^{-1} Fe increased leaf N concentration, whereas 2 mg L^{-1} increased leaf K concentration. During winter, split application of 4 mg L^{-1} Fe increased N and

Table 4. Mean values and statistical significance of foliar N, P, K (%), and Fe concentrations (ppm) in lettuce cv. 'Waldman's Green' across iron application treatments (rate x timing), evaluated at 35 days after transplanting during winter and summer growing seasons.

N		P		K		Fe	
Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
4.46 a[#] (T_6)[$]	4.26 a (T_8)	0.67 a (T_1)	0.62 a (T_{10})	3.71 a (T_6)	5.53 a (T_2)	147.95 a (T_1)	331.83 a (T_7)
4.25 ab (T_5)	4.25 a (T_5)	0.65 ab (T_6)	0.61 a (T_8)	3.44 b (T_5)	5.27 a (T_8)	133.93 b (T_5)	314.30 a (T_4)
4.19 ab (T_1)	4.06 a (T_4)	0.60 abc (T_2)	0.61 a (T_5)	3.37 b (T_2)	5.18 ab (T_6)	129.20 c (T_6)	266.39 b (T5)
3.99 ab (T_2)	4.01 a (T_9)	0.59 abc (T_5)	0.60 a (T_4)	3.34 b (T_9)	5.16 ab (T_7)	124.74 d (T_2)	252.88 bc (T_8)
3.94 ab (T_9)	3.99 a (T_2)	0.59 abc (T_8)	0.60 a (T_3)	3.30 b (T_7)	5.07 ab (T_9)	122.68 de (T_9)	229.02 c (T_9)
3.94 ab (T_8)	3.94 a (T_6)	0.58 abc (T_9)	0.58 a (T_9)	3.26 b (T_3)	4.89 ab (T_{10})	121.39 ef (T_7)	201.08 d (T_3)
3.79 ab (T_7)	3.83 a (T_7)	0.55 bc (T_7)	0.58 a (T_7)	3.25 b (T_1)	4.71 abc (T_5)	119.00 f (T_3)	180.45 d (T_6)
3.70 ab (T_3)	3.80 a (T_3)	0.54 bc (T_{10})	0.57 a (T_1)	2.87 c (T_{10})	4.33 bcd (T_3)	115.15 g (T_4)	178.51 d (T_1)
3.63 b (T_4)	3.72 a (T_1)	0.53 bc (T_3)	0.56 a (T_2)	2.87 c (T_4)	3.84 cd (T_4)	113.70 g (T_8)	153.89 e (T_2)
3.49 b (T_{10})	3.71 a (T_{10})	0.51 c (T_4)	0.53 a (T_6)	2.85 c (T_8)	3.79 d (T_1)	104.54 h (T_{10})	151.39 e (T_{10})
3.94 A[&]	3.96 A	0.58 A	0.59 A	2.21 B	4.78 A	123.23 B	225.98 A

[#] Means followed by the same lowercase letter within a column are not statistically different ($p \leq 0.05$) according to Tukey's test.
[&] Means followed by the same uppercase letter within a row are not statistically different ($p \leq 0.05$) according to Tukey's test.
[$] T_1 = 2 mg L^{-1} Fe (100% at 10 DAT); T_2 = 2 mg L^{-1} Fe (100% at 20 DAT); T_3 = 2 mg L^{-1} Fe (50% at 10 DAT and 50% at 20 DAT); T_4 = 4 mg L^{-1} Fe (100% at 10 DAT); T_5 = 4 mg L^{-1} Fe (100% at 20 DAT); T_6 = 4 mg L^{-1} Fe (50% at 10 DAT and 50% at 20 DAT); T_7 = 6 mg L^{-1} Fe (100% at 10 DAT); T_8 = 6 mg L^{-1} Fe (100% at 20 DAT); T_9 = 6 mg L^{-1} Fe (50% at 10 DAT and 50% at 20 DAT); T_{10} = control

K concentrations, while early application of 2 mg L^{-1} enhanced leaf P concentration.

Iron biofortification was effective at Fe application rates of 4 mg L^{-1} or higher, significantly increasing leaf Fe levels in lettuce cv. 'Waldmann's Green' grown in NFT systems during summer. These findings demonstrate the potential of foliar Fe application as an agronomic biofortification practice for NFT lettuce production.

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