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Effects of iron supplementation rates and time of application on iron biofortification and macronutrient uptake in radish (*Raphanus sativus*) microgreens

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Abstract. *Leal JS, Pabiona MG, Cristobal JU, Melencion AB, Labajo JRN. 2025. Effects of iron supplementation rates and time of application on iron biofortification and macronutrient uptake in radish (*Raphanus sativus*) microgreens. Asian J Agric 9: 94-102.* This study investigates strategies to enhance iron biofortification in radish (*Raphanus sativus*) microgreens through various iron supplementation rates and application timings. The primary objectives were to evaluate fresh weight at harvest and iron absorption efficiency under different treatment conditions. Results demonstrated significant variations in fresh weight, with microgreens treated with SNAP + 10 ppm iron chelate six days post-blackout (A1B3) yielding the highest fresh weight (149.38 g), while those treated four days post-blackout showed the lowest weight (113.54 g). In terms of iron content, treatment A2B1 exhibited the highest concentration (247.78 ppm), whereas A1B2 recorded the lowest (185.03 ppm). The study highlights the intricate relationship between iron supplementation levels (Factor A) and application timing (Factor B) concerning growth and nutrient uptake dynamics in radish microgreens. Overall fresh weight did not differ significantly across iron levels, based on iron concentration x application timing. Moderate iron concentrations initially supported fresh weight, but excess levels hindered growth, emphasizing the delicate balance required in iron supplementation strategies. Nitrogen uptake benefited from moderate iron levels and delayed application, while phosphorus and potassium assimilation were optimized with timely nutrient supply (p<0.05). Notably, total potassium content varied significantly (p<0.05), peaking at 20 ppm iron, suggesting iron's facilitative role in potassium uptake up to a threshold. Moreover, iron content in microgreens remained stable across tested concentrations but significantly increased with specific application timings, underscoring the importance of precise nutrient management for effective iron biofortification. These findings contribute to advancing tailored nutrient management practices to optimize growth, nutrient uptake dynamics, and iron biofortification in radish microgreens. Future research directions should explore underlying physiological mechanisms to refine strategies for sustainable crop production and enhance nutritional quality.

Keywords: Iron, EDTA, nutrient uptake, radish microgreens, *Raphanus sativus*

Abbreviations: DAB: Days After Blackout, DMY: Dry Matter Yield, SNAP: Simple Nutrient Addition Program

INTRODUCTION

Although required in minute quantities, micronutrients are indispensable for plants and humans, and their deficiency can lead to severe health problems. Currently, one-third of the global population suffers from micronutrient deficiency, with pregnant women and children being the most affected groups Thompson and Amoroso (2014) and Harding et al. (2017). Iron deficiency, a significant cause of hidden hunger, leads to conditions such as anemia and impaired mental development (Yoneyama et al. 2015). This global health issue has persisted for years (Zielińska-Dawidziak et al. 2016). Iron is essential for producing hemoglobin, which plays a vital role in oxygen transport and respiration in humans (Morrissey et al. 2015). In plants, iron is crucial for photosynthesis, redox reactions, electron transport, leaf pigmentation, and respiration (Yoneyama et al. 2015; Morrissey et al. 2015). Furthermore, iron is believed to modulate defense mechanisms against invaders in plants and animals (Aznar et al. 2015).

Radish (*Raphanus sativus* L.) microgreens have been identified as a suitable candidate for this process. Microgreens, defined as young and tender vegetables (Di Bella et al. 2020), are emerging as a functional food with the potential to address human nutritional deficiencies (Pannico et al. 2020). These microgreens contain higher concentrations of nutrients compared to their mature counterparts (Ebert 2022) and possess properties that promote health and prevent diseases (Kowitcharoen et al. 2021).

Microgreens, often referred to as superfoods or functional foods, are young and tender vegetables from families such as Brassicaceae, Asteraceae, and Fabaceae, among others (Di Bella et al. 2020; Li et al. 2021a). Their popularity is due to their concentrated bioactive compounds and higher nutrient content than mature vegetables (Chandra et al. 2012; Ebert 2022). This makes microgreens particularly suitable for biofortification efforts aimed at enhancing human health.

Iron's critical functions in human and plant biology underscore the importance of ensuring adequate iron intake. Vegetables, (which can be grown locally and come in various sizes, textures, shapes, and flavors), can significantly combat hunger and malnutrition (Fan 2016). One promising approach to addressing iron deficiency is the biofortification of vegetables, particularly through agronomic biofortification (Dias et al. 2015). Biofortification involves increasing the concentration of specific nutrients in the edible parts of plants (Giordano et al. 2019).

Despite iron's importance, its concentration must be carefully managed. In both plants and humans, excessive iron can lead to the generation of harmful reactive oxygen species, resulting in cellular damage and impaired nutrient absorption (Przybysz et al. 2016; Buturi et al. 2021). Safe levels of iron intake for humans are as follows: 0.27 mg day^{-1} for infants 0-6 months, 11 mg day^{-1} for infants 7-12 months, 7 mg day^{-1} for children 1-3 years, 10 mg day^{-1} for children 4-8 years, 8 mg day^{-1} for adolescent 9-13 years both male and female, 11 mg day^{-1} (male) and 15 mg day^{-1} (females) for adolescent 14-18 years, 8 mg day^{-1} for adult males 19+ years, 18 mg day^{-1} for adult females 19-50 years, 8 mg day^{-1} for 51+ adult females, 27 mg day^{-1} for pregnant women, and 9-10 mg day^{-1} for lactating women (Turck et al. 2024). While for radish, the optimal iron concentration ranges from 5-20 mg kg^{-1} (Rout and Sahoo 2015). Potential mitigation strategies include the use of Biochar (Dad et al. 2020), plant natural detoxification mechanism, and soil pH management (Lapaz et al. 2021). In humans, toxic levels of iron can accumulate in vital organs, causing damage (Zhang et al. 2019), while in plants, high iron levels can interfere with the absorption of essential minerals (Zhang et al. 1999).

Conversely, iron deficiency is the most common form of malnutrition globally, leading to severe health consequences such as anemia, impaired mental development, cardiomyopathy, and heart failure in humans (Jankowska et al. 2010; Yoneyama et al. 2015; Lewis et al. 2016; Zhang et al. 2018). In plants, iron deficiency manifests as chlorosis in younger leaves, reduced crop growth, yield, and nutritional quality (Ghasemi et al. 2012).

Given the critical balance required for iron in biological systems, this study proposes the biofortification of radish microgreens as a solution to iron deficiency. The objectives of this study are (i) to determine the fresh weight of radish microgreens at harvest, (ii) to compare the effects of different treatments on iron absorption, and (iii) to compare the effects of the treatments on iron absorption. By focusing on microgreens, which are recognized for their dense nutrient content and health-promoting properties (Kowitcharoen et al. 2021; Ebert 2022), this research aims to find a feasible means to increase iron concentrations in these plants, thereby offering a potential strategy to alleviate iron deficiency in humans. The study will explore the potential of radish microgreens to serve as a nutrient-dense food source that can be easily integrated into diets to combat iron deficiency and improve overall health outcomes.

MATERIALS AND METHODS

Study area

The study was conducted as a pot (10”×20” trays) experiment, arranged according to the study lay-out in two five-tier shelves, inside a non-controlled-environment room with the light supplied by two 36W T8 LED lights per shelf with natural ventilation utilizing the existing wind at Purok 3A Kahaponan, Valencia City in the Province of Bukidnon Philippines (Figure 1). The area is situated at an elevation of 317.8 meters above sea level receiving an average of 2101 mm of rainfall annually, and temperatures ranging from 28°C to 30°C during the conduct of the study.

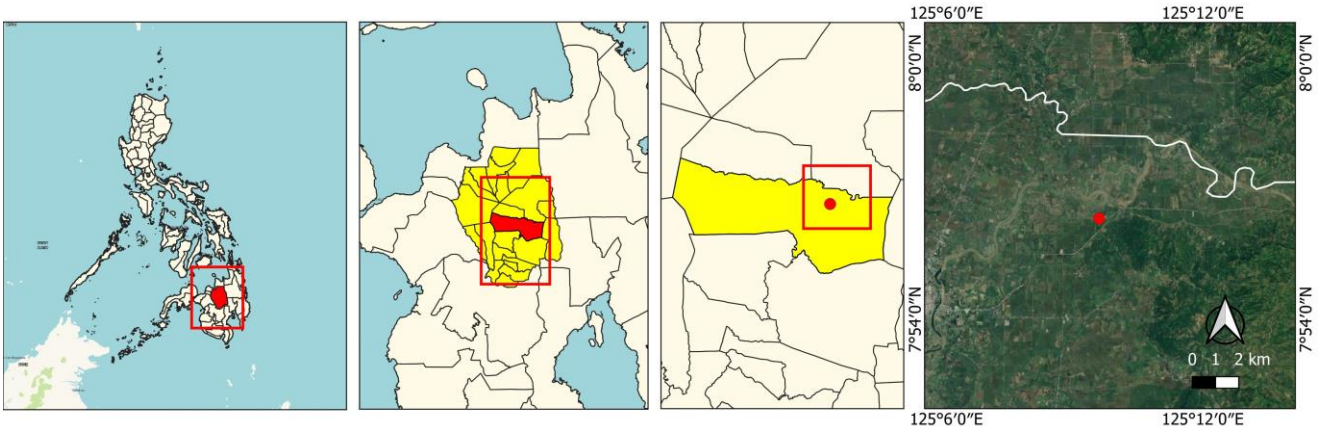

Figure 1. Location of Purok 3A, Kahaponan, Valencia City, Bukidnon, Philippines (7°56′22.01″N, 125°9′34.63″E)

Table 1. Treatments utilized during this study

Treatment	Description
Factor A	
A1	SNAP + 10 ppm iron chelate (Fe^{3+} EDTA)
A2	SNAP + 20 ppm iron chelate (Fe^{3+} EDTA)
A3	SNAP + 30 ppm iron chelate (Fe^{3+} EDTA)
Factor B	
B1	Nutrient solution applied at 2 DAB period
B2	Nutrient solution applied at 4 DAB period
B3	Nutrient solution applied at 6 DAB period
Combination	
A1B1	SNAP + 10 ppm iron chelate applied at 2 DAB period
A1B2	SNAP + 10 ppm iron chelate applied at 4 DAB period
A1B3	SNAP + 10 ppm iron chelate applied at 6 DAB period
A2B1	SNAP + 20 ppm iron chelate applied at 2 DAB period
A2B2	SNAP + 20 ppm iron chelate applied at 4 DAB period
A2B3	SNAP + 20 ppm iron chelate applied at 6 DAB period
A3B1	SNAP + 30 ppm iron chelate applied at 2 DAB period
A3B2	SNAP + 30 ppm iron chelate applied at 4 DAB period
A3B3	SNAP + 30 ppm iron chelate applied at 6 DAB period

Note: *SNAP: Simple Nutrient Addition Program; **DAB: Days After Blackout Period

Experimental design and treatments

The study was conducted using the factorial concept in a Completely Randomized Design (CRD) with two factors as follows: (A) the rate of iron supplementation and (B) the time of iron supplementation. Factor A had three levels: SNAP* + 10 ppm iron, SNAP + 20 ppm iron, and SNAP + 30 ppm iron, with Iron coming from an iron chelate source. On the other hand, Factor B also had three levels: two Days After the Blackout (DAB)** period, four days after the blackout period, and six days after the blackout period. The study had nine treatments replicated three times, thus having 27 samples. The treatments and their descriptions are presented in Table 1.

Procedures

Preparation of the nutrient solution

According to Rajan et al. (2019), seeds do not need nutrient supplementation to germinate. However, seeds require nutrients for better growth and development. Thus, we adopted the utilization of 25% commercial c, as also adopted by Kyriacou et al. (2019) and Corrado et al. (2021), with the formulation of the nutrient solution as follows: 2.0 mM NO_3- N, 0.25 mM S, 0.20 mM P, 0.62 mM K, 0.75 mM Ca, 0.17 mM Mg, 0.25 mM NH_4- N, 9 µM Mn, 0.3 µM Cu, 1.6 µM Zn, 20 µM B, and 0.3 µM Mo. For the iron treatment, 10 ppm, 20 ppm, and 30 ppm Fe from an iron chelate source (i.e. specifically Fe (III)-EDTA), were added to the treatments according to the factorial layout.

Care, maintenance, and harvest

After the microgreens emerged, the 10”×20” trays that were used to cover each microgreen tray for blackout were detached, allowing the microgreens to be exposed to light for 12 hours each day. The illumination was supplied by two 36-watt T8 LED lights per shelf. The microgreens were monitored daily, specifically at 6 am and 4 pm. Irrigation of the microgreens was carried out following the bottom watering technique, administering 250 mL of distilled water every day, at 6 o’clock in the morning till harvest, excluding a day scheduled for application of treatments. Thus, the biofortification of each treatment was implemented through fertigation following the designated biofortification schedule i.e., 2 DAB period, 4 DAB period, and 6 DAB period.

The microgreens' harvest was carried out using a sterilized scissor, soaked for at least 10 minutes in 70% isopropyl alcohol, cutting the hypocotyl as close to the growing media as possible. After acquiring the fresh weight of every treatment, the microgreens were stored in a designated paper bag per treatment.

Sample preparation and analysis

After harvest, the microgreen samples were stored in paper bags and prepared in the Soil and Plant Analysis Laboratory (SPAL) as follows: They were washed with distilled water, air-dried, and placed in an a force draft oven to further remove excess moisture from the plant samples. Once moisture-free, the samples were ground and subjected to dry-ashing before chemical analysis. The microgreens were analyzed for total nitrogen (N), total phosphorus (P), total potassium (K), and total iron (Fe) content at the Soil and Plant Analysis Laboratory (SPAL), College of Agriculture, Central Mindanao University, Musuan, Maramag, Bukidnon, Philippines. The methods used were as follows: Total Nitrogen was determined using the Micro-Kjeldahl Method; Total Phosphorus was analyzed using the Dry Ashing/Vanado-Molybdate Method with a UV-Vis Spectrophotometer; Total Potassium was determined by the Dry Ashing/6N HCl Extraction Method with a Flame Photometer; and Total Iron was assessed through the Dry Ashing Method (6N HCl Extraction Method) with an Atomic Absorption Spectrophotometer (PCARRD 1991).

Data analysis

The data collected were analyzed using the Analysis of Variance (ANOVA) following a Factorial in Completely Randomized Design (CRD) procedure available in the Statistical Tool for Agricultural Research STAR IRRI software. If the F computed value is significant or highly significant, a post hoc test using the LSD test will follow.

RESULTS AND DISCUSSION

Fresh weight and nutrient concentrations

The fresh weight of radish microgreens at harvest showed different responses to varying iron concentrations and nutrient application timings (Table 2), there were no significant differences in the fresh weight among the different levels of Factor A (iron concentration) as well as Factor B (timing of nutrient application). Specifically, increasing iron concentration from A1 (131.34 g) to A2 (131.65 g) resulted in a slight increase in fresh weight, followed by a decline at the highest concentration (A3, 123.09 g). This suggests that moderate iron supplementation can be beneficial. However, excessive amounts may hinder

growth due to potential phytotoxicity by the production of Reactive Oxygen Species (ROS) that damage plant cells (Sharma et al. 2012).

The timing of nutrient application (Factor B) showed a clear pattern for the effects of nutrient concentration. Delaying the application from B1 (128.19 g) to B2 (121.93 g) led to a decrease in fresh weight, yet further delay to B3 (135.97 g) increased the fresh weight. This implies that the timing of nutrient availability is critical, potentially aligning with the plants' developmental stages and nutrient uptake efficiency. However, the interaction between Factors A and B did not produce statistically significant differences in the fresh weight, suggesting that while trends are present, they require more robust statistical power to confirm. Despite the lack of statistical significance, the interaction trends suggest that combinations of higher iron concentrations and delayed nutrient application may either mitigate or amplify the effects on fresh weight, depending on specific environmental conditions, plant growth stages, and other underlying factors.

The study found no significant differences in nitrogen (N), phosphorus (P), and potassium (K) levels across different treatments, suggesting that these nutrients were not substantially influenced by the iron concentrations or nutrient application timings under the conditions tested. However, the observed variations in fresh weight were predominantly influenced by the iron concentration and timing of nutrient application. Factor A2 demonstrated the highest iron concentration (230.62 ppm), indicating that moderate iron supplementation enhances iron uptake, which is crucial for processes such as nitrogen and potassium metabolism. Iron is essential for nitrate assimilation and ion transport, where moderate levels can enhance nitrate reductase activity, facilitating nitrogen assimilation. Conversely, excessive iron can induce oxidative stress, impairing nitrogen metabolism and root architecture (Mitra 2017). Iron also plays a critical role in potassium uptake by supporting energy production in the roots, which is essential for active potassium transport. However, excessive iron can disrupt this balance by altering redox reactions, impairing potassium transport (Duck and Connor 2016; Kobayashi et al. 2019). Interestingly, phosphorus uptake is less affected by iron levels, likely due to the involvement of different transporters and biochemical pathways (Verbon et al. 2017).

While, no significant correlations were found between nitrogen, phosphorus, potassium, and iron concentrations (iron vs. nitrogen: r=-0.0800, p=0.6915; iron vs. phosphorus: r=-0.1493, p=0.4574; iron vs. potassium: r=0.1008, p=0.6170), the correlation analysis did reveal a significant negative correlation between total nitrogen and potassium content (r=-0.4147, p=0.0315) presented in Table 3. This suggests that, although iron did not directly influence these nutrients, increased nitrogen concentrations might be associated with reduced potassium uptake. These results imply that while iron enrichment may not directly alter the uptake of these nutrients, its effects on plant growth and development are likely due to its role in supporting energy production, enzyme activity, and metabolic pathways rather than direct nutrient interactions. Therefore, iron's influence on microgreens may be independent of the levels of nitrogen, phosphorus, and potassium, highlighting the potential for targeted iron enrichment to enhance the nutritional content of crops without adversely affecting the uptake of other essential nutrients. This selective influence underscores the importance of iron in biofortification strategies, particularly in addressing iron deficiency in human diets.

Table 2. Fresh weight (g) of radish microgreens and their nutrient concentrations at harvest

Treatment	Fresh weight (g)	N (%)	P (%)	K (%)	Fe (ppm)
Factor A					
A1	131.34	3.44	0.63	2.07b	194.76
A2	131.65	3.28	0.60	2.86a	230.62
A3	123.09	3.31	0.65	2.50ab	201.41
F-test (p<0.01)	ns	ns	ns	*	ns
Factor B					
B1	128.19	3.37	0.58	2.50	214.93
B2	121.93	3.32	0.60	2.39	196.31
B3	135.97	3.34	0.69	2.55	215.56
F-test (p<0.01)	ns	ns	ns	ns	ns
Factor A x Factor B					
A1B1	131.10	3.33	0.59	2.48	209.68
A1B2	113.54	3.52	0.60	1.25	185.03
A1B3	149.38	3.47	0.69	2.44	189.58
A2B1	134.26	3.34	0.56	2.79	247.78
A2B2	128.32	2.96	0.57	3.12	205.37
A2B3	132.38	3.55	0.64	2.67	238.72
A3B1	119.20	3.44	0.58	2.22	187.32
A3B2	123.92	3.48	0.64	2.80	198.53
A3B3	126.16	3.00	0.72	2.49	213.37
F-test	ns	ns	ns	ns	ns

Note: **: Highly significant, *: Significant, ns: Not significant, means followed by the same letter are not significantly different at a 5% level of significance

Table 3. Pearson correlation analysis between nutrient concentration of NPK and Fe

Variable	Total nitrogen content	Total phosphorus content	Total potassium content
Total nitrogen content			
Total phosphorus content	r=-0.0232; p=0.9086		
Total potassium content	r=-0.4147; p=0.0315	r=-0.1198; p=0.5518	
Total iron content	r=-0.0800; p=0.6915	r=-0.1493; p=0.4574	r=0.1008; p=0.6170

Average height at harvest, dry matter yield, and nutrient uptake

Average height at harvest and dry matter yield

The height of radish microgreens at harvest varied significantly depending on the timing of nutrient application (Factor B) ($p<0.05$). Microgreens that received the nutrient solution during the B3 growth stage were the tallest, with an average height of 13.49 cm, which was notably higher than those treated at the B2 stage (11.82 cm) and slightly taller than those treated at B1 (12.46 cm). These results are consistent with findings by Li et al. (2021b), who observed increased height in radish, kale, broccoli, cabbage, and mustard microgreens when nutrient supplementation was provided during later growth stages. The greater height observed at B3 could be attributed to the plants' increased nutrient demand during this advanced vegetative stage, supporting Bodale et al. (2021), who highlighted the importance of timely nutrient application for maximizing growth during critical stages of development.

Considering Factor A (Fe concentration), no significant differences in plant height were observed among the treatments. Heights ranged from 12.35 cm at 10 ppm Fe (A1) to 12.98 cm at 20 ppm Fe (A2), and 12.45 cm at 30 ppm Fe (A3), indicating that within this concentration range, Fe did not significantly influence the height of radish microgreens. This finding contrasts with some literature, including the study by Mitra (2015), which suggested that higher concentrations of Fe could lead to toxicity and negatively affect plant growth. In this study, however, Fe levels up to 30 ppm did not demonstrate any detrimental effects on the height of microgreens.

Dry Matter Yield (DMY) showed no significant differences across the treatments, indicating that neither Fe concentration nor the timing of nutrient application substantially influenced biomass production. DMY values ranged from 0.836 g kg^{-1} in B3 to 0.879 g kg^{-1} in B2. The minor increase in DMY at 20 ppm Fe (A2) to 0.893 g kg^{-1}, and the subsequent decrease at 30 ppm Fe (A3) to 0.839 g kg^{-1}, equating to the DMY at 10 ppm Fe (A1) suggest a possible threshold beyond which Fe may not enhance, and might even inhibit, biomass accumulation. This finding is consistent with the findings of Di Gioia et al. (2019), who noted that Fe concentrations above optimal levels could negatively impact dry matter content in *Brassica* species, reflecting potential Fe toxicity effects.

These results align with previous studies, such as Park et al. (2014), who reported no significant differences in dry matter yield of microgreens across various nutrient treatments, highlighting the intricate nature of nutrient interactions and their influence on plant growth. Similarly, Filho et al. (2015) and Giordano et al. (2019) reported that Fe levels could significantly influence dry matter yield in hydroponically grown chicory and lettuce, with higher concentrations potentially leading to reduced biomass due to toxicity.

The timing of nutrient application significantly affects the height of radish microgreens, with delayed application (B3) producing the tallest plants. However, Fe concentration within the studied range did not significantly impact plant height. On the other hand, dry matter yield remained unaffected by both Fe concentration and nutrient application timing, highlighting the intricate balance required in nutrient management to optimize growth without inducing toxicity.

Nitrogen uptake

Fe concentration did not reveal significant differences in nitrogen uptake with values ranging from 0.185 g pot^{-1} to 0.195 g pot^{-1} as shown in Table 4. This indicates that variations in Fe concentration within the tested range (10 ppm to 30 ppm) had no significant impact nitrogen assimilation, supporting the broader understanding that nitrogen uptake in plants can be influenced by multifaceted factors beyond Fe levels alone, as noted by Reitra et al. (2017).

Conversely, Factor B (timing of nutrient application) significantly affected nitrogen uptake ($p<0.05$). Notably, microgreens treated at the B3 stage exhibited the highest nitrogen uptake at 0.210 g pot^{-1}, followed by B2 at 0.191 g pot^{-1}, and the lowest at B1 with 0.187 g pot^{-1}. This emphasizes the crucial role of nutrient application timing during vegetative growth stages in promoting root development and improving nutrient absorption efficiency, that highlight the importance of optimal nutrient timing for maximizing crop yield and nutrient utilization. Further exploring the interaction between Fe concentration and nutrient application timing, synergistic effects were observed. For instance, at the B3 stage, microgreens receiving A1 (10 ppm Fe) exhibited a nitrogen uptake of 0.195 g pot^{-1}, whereas A3 (30 ppm Fe), with nitrogen uptake of 0.038 g pot^{-1}. This interaction highlights how higher Fe concentrations can boost nitrogen assimilation when combined with optimal timing, such as in the B3 application. Conversely, treatments like A3B3, where high Fe concentration coincided with nutrient application at B3, resulted in lower nitrogen uptake, potentially due to Fe-induced limitations on nitrogen utilization. To maximize nitrogen uptake and overall plant productivity in microgreen growth, certain nutrient management strategies are required, as these findings highlight the complexity of nutrient dynamics in radish microgreens.

Table 4. Average height at harvest (cm), dry matter yield (g kg^{-1}), and nutrient uptake, in terms of nitrogen, phosphorus, potassium (g pot^{-1}), and iron (mg kg^{-1}), of radish microgreens after a 14-day growing cycle

Treatment	Average height at harvest (cm)	Dry matter yield (g kg^{-1})	N (g pot^{-1})	P (g pot^{-1})	K (g pot^{-1})	Fe (mg kg^{-1})
Factor A						
A1	12.35	0.839	0.189	0.035	0.117	10.717
A2	12.98	0.893	0.193	0.035	0.170	13.236
A3	12.45	0.839	0.185	0.036	0.138	10.975
F-test ($p<0.05$)	ns	ns	ns	ns	ns	ns
Factor B						
B1	12.46ab	0.856	0.190	0.033	0.139	12.019
B2	11.82b	0.879	0.191	0.035	0.145	11.379
B3	13.49a	0.836	0.185	0.038	0.140	11.530
F-test ($p<0.05$)	*	ns	ns	ns	ns	ns
Factor A x Factor B						
A1B1	11.86	0.896	0.195	0.034	0.144a	12.432
A1B2	11.31	0.699	0.161	0.028	0.058b	8.444
A1B3	13.88	0.923	0.210	0.042	0.148a	11.275
A2B1	13.02	0.857	0.188	0.032	0.157a	13.835
A2B2	12.50	0.934	0.183	0.034	0.195a	12.557
A2B3	13.41	0.887	0.207	0.038	0.159a	13.417
A3B1	12.50	0.816	0.187	0.032	0.117a	9.889
A3B2	11.66	1.004	0.229	0.042	0.183a	13.137
A3B3	13.18	0.698	0.138	0.033	0.113a	9.898
F-test	ns	ns	ns	ns	*	ns

Note: **: Highly significant, *: Significant, ns: Not significant, means followed by the same letters are not significantly different at a 5% level of significance

Phosphorus uptake

Table 4 presents the phosphorus uptake patterns in radish microgreens influenced by varying iron supplementation rates and nutrient application timings. Factor A (Fe concentration) did not result in significant differences in phosphorus uptake, with mean values consistently around 0.035 g kg^{-1} across the tested concentrations (10 ppm to 30 ppm). In contrast, Factor B (timing of nutrient application) significantly affected phosphorus assimilation.

Microgreens treated at the B3 stage had the highest phosphorus uptake at 0.038 g kg^{-1}, followed by B2 at 0.035 g kg^{-1}, and the lowest uptake occurred at B1 with 0.033 g kg^{-1}. These results suggest that delaying nutrient application to the later vegetative growth stages improves phosphorus absorption, likely due to enhanced root development and increased nutrient demand during these phases. This finding aligns with previous research, such as Bodale et al. (2021), which emphasizes the importance of nutrient application timing in optimizing nutrient uptake efficiency. The interaction between Fe concentration and nutrient application timing further explains phosphorus uptake dynamics. At the B3 stage, phosphorus uptake reached its highest value of 0.042 g kg^{-1} under the highest Fe concentration (A3), indicating a synergistic effect where increased Fe levels and delayed nutrient application significantly boost phosphorus assimilation. In contrast, the lowest phosphorus uptake, 0.028 g kg^{-1}, occurred in the A1B2 treatment, where the lowest Fe concentration (A1) was paired with nutrient application at the B2 stage. This interaction highlights the critical role of Fe availability and nutrient timing in optimizing phosphorus uptake efficiency in radish microgreens. These findings align with studies like those by Di Gioia et al. (2019), emphasizing the importance of managing nutrient levels and application timing to maximize phosphorus uptake in crops.

Potassium uptake

Potassium uptake in radish microgreens, influenced by varying Fe concentrations and the timing of nutrient application, provides critical insights into nutrient management strategies. According to the results from Table 4, Factor A (Fe concentration) did not yield significant differences in potassium uptake ($p>0.05$), with mean values varying from 0.117-0.170 g kg^{-1}. This result suggests that potassium uptake in radish microgreens may not be highly sensitive to Fe concentration variations within the tested range (10 ppm to 30 ppm). These findings align with previous studies by Przybysz et al. (2016) and Giordano et al. (2019), indicating that potassium uptake in plants can be influenced by factors other than Fe concentration alone.

However, Factor B (timing of nutrient application) did impact potassium uptake significantly ($p<0.05$). Radish microgreens receiving the nutrient solution at the B2 stage exhibited the highest potassium uptake at 0.145 g kg^{-1}, followed closely by B3 at 0.140 g kg^{-1}, and the lowest at B1 with 0.139 g kg^{-1}. This suggests that nutrient application timing during the vegetative growth stage can enhance potassium absorption, likely due to increased root activity and nutrient demand during this phase. This observation is consistent with findings by Przybysz et al. (2016), emphasizing the critical role of timing in optimizing nutrient uptake and utilization in crops.

The relationship between Fe content and the nutrient delivery time further clarifies the potassium uptake rate. At the B2 stage, potassium uptake peaked at 0.170 g kg^{-1} with Fe concentration A2, indicating a synergistic effect where higher Fe concentrations coupled with timely nutrient application enhance potassium assimilation. The lowest potassium uptake was observed in the A1B2 treatment, combining the lowest Fe concentration and nutrient application at B2, resulting in 0.117 g kg^{-1}. This interaction underscores the interplay between Fe availability and nutrient timing, affecting potassium uptake efficiency. These results highlight the complex connections between nutrients in plant physiology and growth, especially concerning potassium uptake in radish microgreens. Optimizing potassium assimilation and overall plant productivity requires effective nutrient management strategies considering both Fe concentration and application timing.

Iron uptake

Iron (Fe) uptake in radish microgreens is crucial to nutrient management, particularly in enhancing nutritional quality and plant growth. According to the results from Table 4, Factor A (Fe concentration) did not yield significant differences in iron uptake ($p>0.05$), with mean values ranging from 10.717 mg kg^{-1} to 13.236 mg kg^{-1}. This indicates that within the tested range of Fe concentrations (10 - 30 ppm), iron uptake in radish microgreens remained relatively stable, suggesting a limited impact of Fe concentration alone on iron assimilation. These findings align with previous studies by Park et al. (2014), which also reported no significant increase in iron uptake with varying forms of Fe chelates in radish and broccoli sprouts. Factor B (timing of nutrient application) significantly influenced iron uptake ($p<0.05$). Radish microgreens receiving the nutrient solution at the B1 stage exhibited the highest iron uptake at 12.019 mg kg^{-1}, followed by B2 at 11.379 mg kg^{-1}, and the lowest at B3 with 11.530 mg kg^{-1}. This highlights the importance of nutrient application timing during the vegetative growth stage in maximizing iron assimilation, potentially due to enhanced root activity and nutrient demand during this phase. This observation is consistent with studies emphasizing the critical role of timing in optimizing nutrient uptake and utilization in crops.

The interaction between Fe concentration and the timing of nutrient application further explains iron uptake dynamics. At the B1 stage, iron uptake peaked at 13.835 mg kg^{-1} with Fe concentration A2, indicating a synergistic effect where higher Fe concentrations coupled with timely nutrient application enhance iron assimilation. The lowest iron uptake was observed in the A3B3 treatment, combining the highest Fe concentration and nutrient application at B3, resulting in 9.898 mg kg^{-1}. This interaction underscores the intricate relationship between Fe availability and nutrient timing, affecting iron uptake efficiency. These results show the intricate relationships that control iron uptake in radish microgreens and emphasize the necessity of integrated nutrient management into consideration both the time and concentration of iron. Subsequent investigations may delve into the fundamental physiological processes propelling these processes to maximize iron absorption effectiveness in microgreen cultivation. Growers can improve radish microgreens' iron biofortification and address iron deficiency in human diets by enhancing their nutrient management techniques.

The interaction between Dry Matter Yield (DMY) and nutrient uptake in radish microgreens reveals intriguing insights into how nutrient management influences both plant growth and nutrient assimilation. From Table 4, it is evident that DMY was not significantly affected by Fe concentration (Factor A) or the timing of nutrient application (Factor B). However, the intricate interplay between these factors and nutrient uptake deserves attention. Studies by Filho et al. (2015) and Di Gioia et al. (2019) highlight that nutrient availability, including Fe, can influence DMY by affecting physiological processes such as photosynthesis and nutrient translocation. Although Fe concentration in the study alone did not show a direct impact on DMY, the interaction with nutrient uptake, particularly of essential nutrients like nitrogen (N), phosphorus (P), potassium (K), and Fe itself, suggests a nuanced relationship.

Fe uptake did not show a direct correlation with Dry Matter Yield (DMY); however, the nutrient uptake patterns across various treatments offer valuable insights. Treatments that demonstrated higher nutrient uptake, particularly those with optimal Fe concentrations and timely nutrient applications, were generally associated with improved DMY outcomes. This observation is consistent with studies indicating that balanced nutrient uptake—especially during key growth phases—enhances overall plant health and productivity (Kutman et al. 2011; Di Gioia et al. 2016). By integrating insights from DMY and nutrient uptake research, growers can optimize their nutrient application strategies to enhance both yield and nutritional quality in radish microgreens. Understanding these relationships not only improves agricultural practices but also supports sustainable microgreen production systems that effectively meet yield and nutritional requirements

Furthermore, the study underscores the intricate relationship between iron concentration (Factor A) and the timing of nutrient application (Factor B) in influencing nutrient uptake dynamics, particularly for nitrogen, phosphorus, potassium, and iron. While nitrogen assimilation benefited from moderate iron concentrations and delayed application, phosphorus and potassium uptake thrived with timely nutrient supply, highlighting the critical role of synchronized nutrient management in optimizing nutrient absorption and utilization (Smolik et al. 2013; Park et al. 2014).

Additionally, the results clarify the complexity of iron intake and how it affects overall nutritional quality, confirming stability at different iron concentrations but significant improvement at particular application timings. This emphasizes the key balance required for effective iron biofortification in radish microgreens, which is critical for nutritional content optimization (Filho et al. 2015; Di Gioia et al. 2019).

This research contributes valuable insights into refining nutrient management strategies tailored to maximize growth, nutrient uptake, and nutritional quality in radish microgreens. By elucidating the intricate interactions between iron concentration, nutrient application timing, and nutrient uptake dynamics, the study paves the way for future advancements in sustainable microgreen production systems to address nutritional deficiencies and enhance food security through nutrient-dense crops. Further investigations into the underlying physiological mechanisms are recommended to bolster agronomic practices and foster resilient agricultural systems.

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REFERENCES

Aznar A, Chen N, Thomine S, Dellagi A. 2015. Immunity to plant pathogens and iron homeostasis. Plant Sci 240: 90-97. DOI: 10.1016/j.plantsci.2015.08.022.

Bodale I, Mihalache G, Achitei V, Teliban GC, Cazacu A, Stolero V. 2021. Evaluation of nutrient uptake by tomato plants in different phenological stages using an electrical conductivity technique. Agriculture 11 (4): 292. DOI: 10.3390/agriculture11040292.

Buturi CV, Mauro RP, Fogliano V, Leonardi C, Giuffrida F. 2021. Mineral biofortification of vegetables as a tool to improve human diet. Foods 10 (2): 223. DOI: 103390/foods/10020223.

Chandra D, Kim JG, Kim YP. 2012. Changes in microbial population and quality of microgreens treated with different sanitizers and packaging films. Hortic Environ Biotechnol 53 (1): 32-40. DOI: 10.1007/s13580-012-0075-6.

Corrado G, El-Nakhel C, Graziani G, Pannico A, Zarrelli A, Giannini P, Ritieni A, De Pascale S, Kyriacou MC, Rouphael, Y. 2021. Productive and morphometric traits, mineral composition and secondary metabolome components of borage and purslane as underutilized species for microgreens production. Horticulturae 7 (8): 211. DOI: 10.3390/horticulturae7080211.

Dad FP, Khan WD, Tanveer M, Ramzani PMA, Shaukat R, Muktadir A. 2020. Influence of iron-enriched biochar on Cd sorption, its ionic concentration and redox regulation of radish under cadmium toxicity. Agriculture 11 (11): 1. DOI: 10.3390/agriculture11010001.

Di Bella MC, Niklas A, Tosccano S, Picchi V, Romano D, Lo Scalzo R, Branca F. 2020. Morphometric characteristics, polyphenols, and ascorbic acid variation in *Brassica oleraceae* L. novel foods: Sprouts, microgreens, and baby leaves. Agronomy 10 (6): 782. DOI: 10.3390/agonomy10060782.

Di Gioia F, De Bellis P, Mininni C, Santamaria P, Serio F. 2016. Physicochemical, agronomical and microbiological evaluation of alternative growing media for the production of rapini (*Brassica rapa* L.) microgreens. J Sci Food Agric 97 (4): 1212-1219. DOI: 10.1002/jsfa.7852.

Di Gioia F, Petropoulos S, Ozores-Hampton M, Morgan K, Rosskopf E. 2019. Zinc and iron agronomic biofortification of *Brassica* microgreens. Agronomy 9 (11): 677. DOI: 10.3390/agronomy9110677.

Dias DM, de Castro Moreira ME, Gomes MJC, Lopes Toledo RC, Nutti MR, Pinheiro Sant'Ana HM, Martino HSD. 2015. Rice and bean targets for biofortification combined with high carotenoid content crops regulate transcriptional mechanisms increasing iron bioavailability. Nutrients 7 (11): 9683-9696. DOI: 10.3390/nu7115488.

Duck KA, Connor JR. 2016. Iron uptake and transport across physiological barriers. Biometals 29: 573-591. DOI: 10.1007/s10534-016-9952-2.

Ebert AW. 2022. Sprouts and microgreens—novel food sources for healthy diets. Plants 11 (4): 571. DOI: 10.3390/plants11040571.

Fan S. 2016. Ending hunger and undernutrition by 2025: The role of horticultural value chains. Acta Hortic 1126: 9-20. DOI: 10.17660/ActaHortic.2016.1126.2.

Filho ABC, Cortez JWM, de Sordi D, Urrestarazu M. 2015. Common chicory performance as influenced by iron concentration in the nutrient solution. J Plant Nutr 38: 1289-1494. DOI: 10.1080/01904167.2014.983609

Ghasemi S, Khoshgoftarmanesh AH, Hadadzadeh H, Jafari M. 2012. Synthesis of iron-amino acid chelates and evaluation of their efficacy as iron source and growth stimulator for tomato in nutrient solution culture. J Plant Growth Regul 31: 498-508. DOI: 10.1007/s00344-012-9259-7.

Giordano M, El-Nakhel C, Pannico A, Kyriacou MC, Stazi SR, De Pascale S, Rouphael Y. 2019. Iron biofortification of red and green pigmented lettuce in closed soilless cultivation impacts crop performance and modulates mineral and bioactive composition. Agronomy 9 (6): 290. DOI: 10.3390/agronomy9060290.

Harding K, Aguayo V, Webb P. 2017. Hidden hunger in South Asia: A review of recent trends and persistent challenges. Public Health Nutr 21 (4): 785-795. DOI: 10.1017/S1368980017003202.

Jankowska EA, Rozentryt P, Witkowska A, Nowak J, Hartmann O, Ponikowska B, Borodulin-Nadzieja L, Banasiak W, Polonski L, Filippatos G, McMurray JJV, Anker SD, Ponikowski P. 2010. Iron deficiency: An ominous sign in patients with systolic chronic heart failure. Eur Heart J 31 (15): 1872-1880. DOI: 10.1093/eurheartj/ehq158.

Kobayashi T, Nozoye T, Nishizawa NK. 2019. Iron transport and its regulation in plants. Free Radic Biol Med 133: 11-20. DOI: 10.1016/j.freeradbiomed.2018.10.439.

Kowitcharoen L, Phornvillay S, Lekkham P, Pongprasert N, Srilaong V. 2021. Bioactive composition and nutritional profile of microgreens cultivated in Thailand. Appl Sci 11 (17): 7981. DOI: 10.3390/app11177981.

Kutman UB, Yildiz B, Cakmak I. 2011. Effect of nitrogen on uptake, remobilization and partitioning of zinc and iron throughout the development of durum wheat. Plant Soil 342: 149-164. DOI: 10.1007/s11104-010-0679-5.

Kyriacou MC, El-Nakhel C, Graziani G, Pannico A, Soteriou GA, Giordano M, Ritieni A, De Pascale S, Rouphael Y. 2019. Functional quality in novel food sources: Genotypic variation in the nutritive and phytochemical composition of thirteen microgreens species. Food Chem 277: 107-118. DOI: 10.1016/j.foodchem.2018.10.098.

Lapaz A, Yoshida CHP, Gorni PH, Freita-Silva L, Araujo T, Ribeiro C. 2021. Iron toxicity: Effects on the plants and detoxification strategies. Acta Bot Bras 36: e2021abb0131. DOI: 10.1590/0102-33062021abb0131.

Lewis GD, Semigran MJ, Givertz MM, Malhotra R, Anstrom KJ, Hernandez AF, Shah MR, Braunwald E, Braunwald E. 2016. Oral iron therapy for heart failure with reduced ejection fraction: Design and rationale for oral iron repletion effects on oxygen uptake in heart failure. Circ Heart Fail 9 (5): e000345. DOI: 10.1161/CIRCHEARTFAILURE.115.000345.

Li T, Lalk G, Arthur J, Johnson M, Bi G. 2021b. Shoot production and mineral nutrients of five microgreens as affected by hydroponics substrate type and post-emergent fertilization. Horticulturae 7 (6): 129. DOI: 10.3390/horticulturae7060129.

Li T, Lalk GT, Bi G. 2021a. Fertilization and pre-sowing seed soaking affect yield and mineral nutrients of ten microgreen species. Horticulturae 7 (2): 14. DOI: 10.3390/horticulturae7020014.

Mitra G. 2017. Essential plant nutrients and recent concepts about their uptake. In: Naeem M, Ansari AA, Gill SS (eds). Essential Plant Nutrients: Uptake, Use Efficiency, and Management. Springer, Cham. DOI: 10.1007/978-3-319-58841-4_1.

Mitra GN. 2015. Regulation of Nutrient Uptake by Plants. Springer, New Delhi. DOI: 10.1007/978-81-322-2334-4.

Morrissey J, Sutak R, Paz-Yepes J, Tanaka A, Moustafa A, Veluchamy A, Thomas Y, Botebol H, Bouget FY, McQuaid JB, Tirichine L, Allen AE, Lesuisse E, Bowler C. 2015. A novel protein, ubiquitous in marine phytoplankton, concentrates iron at the cell surface and facilitates uptake. Curr Biol 25 (3): 364-371. DOI: 10.1016/j.cub.2014.12.004.

Pannico A, El-Nakhel C, Graziani G, Kyriacou MC, Giordano M, Soteriou GA, Zarrelli A, Ritieni A, De Pascale S, Rouphael Y. 2020. Selenium biofortification impacts the nutritive value, polyphenolic

content, and bioactive constitution of variable microgreens genotypes. Antioxidants 9 (4): 272. DOI: 10.3390/antiox9040272.

Park SA, Grusak MA, Oh MM. 2014. Concentrations of minerals and phenolic compounds in three edible sprout species treated with iron-chelates during imbibition. Hortic Environ Biotechnol 55: 471-478. DOI: 10.1007/s13580-014-0075-9.

Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD). 1991. Standard Method of Analysis of Soils, Plant Tissue, Water, and Fertilizer. PCARRD, Los Banos, Laguna. [Philippines]

Przybysz A, Wrochna M, Małecka-Przybysz M, Gawrońska H, Gawroński SW. 2016. Vegetable sprouts enriched with iron: Effects on yield, ROS generation and antioxidative system. Sci Hortic 203: 110-117. DOI: 10.1016/j.scienta.2016.03.017.

Rajan P, Lada RR, MacDonald MT. 2019. Advancement in indoor vertical farming for microgreen production. Am J Plant Sci 10 (08): 1397. DOI: 10.4236/ajps.2019.108100.

Reitra R, Heinen M, DImpaka C, Bindraban P. 2017. Effects of nutrient antagonism and synergism on yield and fertilizer use efficiency. Commun Soil Sci Plant Anal 48 (16): 1895-1920. DOI: 10.1080/00103624.2017.1407429.

Rout G, Sahoo S. 2015. Role of iron in plant growth and metabolism. Rev Agric Sci 3: 1-24. DOI: 10.7831/ras.3.1.

Sharma P, Jha AB, Dubey RS, Pessarakli M. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot 2012 (1): 217037. DOI: 10.1155/2012/217037.

Smolik B, Cichocka J, Materny A, Śnioszek M, Zakrzewska H. 2013. Effect of iron deficiency and excess on biometric and biochemical parameters indicated in the radish sprouts (*Raphanus sativus* L. Subvar. *radicula* pers.). Environ Prot Nat Resour 24 (3): 29-32. DOI: 10.2478/oszn-2013-0028.

Thompson B, Amoroso L. 2014. Improving Diets and Nutrition: Food-Based Approaches. CABI, Wallingford. DOI: 10.1079/9781780642994.0000.

Turck D, Bohn T, Castenmiller J, de Henauw S, Hirsch-Ernst K, Knutsen HK, Maciuk A, Mangelsdorf I, McArdle H, Pentieva K, Siani A, Thies F, Tsabouri S, Vicenti M, Aggett P, Tait S, Lecarre A, Fabiani L, Karavasiloglou N, Saad RM, Sofroniou A, Titz A, Naska A. 2024. Scientific opinion on the tolerable upper intake level of iron. EFSA J 22 (6): 8819. DOI: 10.2903/j.efsa.2024.8819.

Verbon EH, Trapet PL, Stringlis IA, Kruijs S, Bakker PA, Pieterse CM. 2017. Iron and immunity. Ann Rev Phytopathol 55 (1): 355-375. DOI: 10.1146/annurev-phyto-080516-035537.

Yoneyama T, Ishikawa S, Fujimaki S. 2015. Route and regulation of zinc, cadmium, and iron transport in rice plants (*Oryza sativa* L.) during vegetative growth and grain filling: metal transporters, metal speciation, grain Cd reduction and Zn and Fe biofortification. Intl J Mol Sci 16 (8): 19111-19129. DOI: 10.3390/ijms160819111.

Zhang H, Zhabyeyev P, Wang S, Oudit G. 2018. Role of iron metabolism in heart failure: from iron deficiency to iron overload. BBA – Mol Basis Dis 1865: 1925-1937. DOI: 10.1016/j.bbadis.2018.08.030.

Zhang H, Zhabyeyev P, Wang S, Oudit GY. 2019. Role of iron metabolism in heart failure: From iron deficiency to iron overload. Biochim Biophys Acta Mol Basis Dis 1865 (7): 1925-1937. DOI: 10.1016/j.bbadis.2018.08.030.

Zhang X, Zhang F, Mao D. 1999. Effect of iron plaque outside roots on nutrient uptake by rice (*Oryza sativa* L.): Phosphorus uptake. Plant Soil 209: 187-192. DOI: 10.1023/A:1004505431879.

Zielińska-Dawidziak M, Staniek H, Król E, Piasecka-Kwiatkowska D, Twardowski T. 2016. Legume seeds and cereal grains? Capacity to accumulate iron while sprouting in order to obtain food fortificant. Acta Sci Pol Technol Aliment 15 (3): 333-338. DOI: 10.17306/J.AFS.2016.3.32.