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Research Article



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Iron uptake in spinach (*Spinacia oleracea*) using biochar-iron complex derived from sugarcane bagasse under greenhouse conditions

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ABSTRACT

Iron deficiency limits spinach (*Spinacia oleracea*) growth and nutritional quality, particularly in alkaline soils. By investigating the complexation of iron with sugarcane bagasse-derived biochar, this study aimed to bridge that gap and offer insights into an innovative approach for improving iron nutrition in spinach crops. This study also evaluated the efficacy of sugarcane bagasse-derived biochar-iron complexes in enhancing iron uptake under controlled greenhouse conditions. Biochar-iron complexes were synthesized via pyrolysis and treated with iron ($FeCl_3$) at 0.25 M, 0.50 M, and 0.75 M. In the greenhouse, using a Randomized Complete Design (RCD), the experiment compared treatments including $FeCl_3$ only, biochar only and biochar-iron complexes at 0.25 M, 0.50 M, and 0.75 M concentrations against an untreated control. Iron uptake in spinach leaves was measured over three months using Atomic Absorption Spectroscopy (AAS). Infrared Fourier Transform Spectroscopy (FT-IR) analysis confirmed key functional groups involved in biochar-iron chelation, while X-Ray Fluorescence (XRF) revealed increased iron loading, peaking at 0.75 M. Analysis of variance from the greenhouse study revealed significant effects of treatment, time, and their interaction ($p<0.001$). Although the 0.75 M treatment produced the highest iron content (220 to 300 ppm), the 0.50 M biochar-iron complex is considered optimal, as it achieved iron levels within the FAO guideline range (80-250 ppm). In contrast, conventional $FeCl_3$ treatment-maintained leaf iron at only 1.7-1.9 ppm throughout the three months, reflecting poor soil retention and rapid leaching of $FeCl_3$. These findings highlight the composite's superior magnitude and temporal stability of iron delivery. Sugarcane bagasse biochar–iron composite thus emerges as a promising, eco-friendly alternative to synthetic iron fertilizers for improving soil fertility and crop nutrition, addressing iron deficiency in sustainable food production.

KEYWORDS: Biochar, Green house, Iron Complexation, Iron uptake, Spinach

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INTRODUCTION

Iron is an essential micronutrient for plant metabolism, playing a critical role in chlorophyll synthesis, respiration, DNA synthesis, and photosynthetic electron transport. Its deficiency is widespread in alkaline soils, where high pH leads to iron precipitation, reducing its bioavailability despite adequate total iron levels (Ahmad *et al.*, 2014). As a result, crops grown under such conditions often exhibit interveinal chlorosis, stunted growth, and reduced productivity (Huang *et al.*, 2022; Bhat *et al.*, 2024). Leafy vegetables like Spinach (*S. Oleracea*) are especially sensitive to iron deficiency, which adversely affects both plant vigour and nutritional quality. Spinach has high iron demands due to its rapid growth rate and physiological iron functions (Turan *et al.*, 2022). However, the use of conventional iron fertilizers such as $FeCl_3$ or Iron Sulphate ($FeSO_4$) is often ineffective, as these compounds precipitate quickly in alkaline soils, leading to limited uptake by plants and environmental loss (Saleem *et al.*, 2022). Biochar, produced via the pyrolysis of organic waste materials, has emerged as a promising soil amendment due to its high porosity and abundance of functional groups that facilitate nutrient retention and slow release (Wang *et al.*, 2020; Tang *et al.*, 2022). Sugarcane bagasse, an abundant agricultural by-product, is an ideal material for biochar production. Converting bagasse into biochar not only reduces waste but also creates a value-added product that can enhance soil fertility. Additionally, the role of biochar in enhancing soil microbial activity and health, which indirectly aids in nutrient uptake and nutrient retention, has been highlighted by other researchers (Ahmad *et al.*, 2014; Singh *et al.*, 2018).

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In synthesis protocols, Rajapaksha *et al.* (2016) optimized conditions for mixing and reacting biochar with $FeCl_3$, laying a strong foundation for subsequent studies aiming at enhanced iron loading. These complexes may serve as controlled-release systems, gradually supplying bioavailable iron to plants and reducing leaching (Wu *et al.*, 2019). Similarly, Niu *et al.* (2024) demonstrated that stable biochar-iron complexes significantly improve the availability of iron in nutrient-deficient soils, especially in systems with high pH. Further Tang *et al.* (2022) reported that the efficiency of such complexation is significantly influenced by the concentration of the iron precursor. These studies underscore the potential to improve iron availability in soil through strategic biochar modification, a concept that this work further investigates through detailed characterization. Despite these advantages, limited studies have explored the synthesis of biochar–iron complexes using sugarcane bagasse and their potential for improved nutrient retention. Additionally, most of the studies on biochar characterization have been based on its potential to reclaim contaminated soils and supply macronutrients such as phosphorus. Limited studies have evaluated the biochar-iron complexes in light of their capability to retain and slowly release the micronutrients. Furthermore, while biochar's properties and its potential to enhance iron uptake are well established in laboratory studies, few have explored its efficiency on crops grown in greenhouse or field conditions. Therefore, this study sought to characterize the biochar-iron complexes in terms of nutrient retention and evaluate the impact of sugarcane bagasse-derived biochar-iron complexes on the growth and iron uptake in spinach under greenhouse conditions.

MATERIALS AND METHODS

Biochar Synthesis

Sugarcane bagasse was collected from Mtito Andei Market, then completely cleaned with distilled water and oven-dried for 24 hours at 105 °C (Melesse *et al.*, 2022). In a Thermo Scientific muffle furnace, the dried bagasse was pyrolyzed under conditions that were optimized based on the procedure described by Huang *et al.* (2022) to create biochar with a high carbon content and functional groups that were well-preserved.

Biochar-Iron Complex Preparation

To guarantee a consistent particle size, the generated biochar was sieved and separated into three sections. An aqueous solution of $FeCl_3$ was added to each fraction in weight-to-volume ratios of 1:5, 1:10, and 1:15, respectively, at concentrations of 0.25 M, 0.50 M, and 0.75 M. The mixtures were agitated for 24 hours at room temperature, filtered, and then cleaned with deionized water before being dried for 12 hours at 105 °C.

Techniques for Characterization

FT-IR analysis

FT-IR was used to detect functional groups in both the raw biochar and each biochar-iron combination. KBr pellets were used to produce the samples, and spectra were obtained between 4000 and 400 cm^{-1}. To verify iron chelation, shifts in distinctive peaks (such as -OH and -COOH) were employed (Bonvin *et al.*, 2017).

XRF analysis

The iron concentration that was preserved in the biochar–iron complexes was measured. An XRF spectrometer was used to analyse the powdered samples, and the iron content was expressed as a percentage by weight along with the standard deviations that were determined.

Greenhouse Experimental Site and Soil Collection

The experiment was conducted in a greenhouse at Lukenya University. Soil was collected from an agricultural field characterized as alkaline loamy soil. Before treatment application, baseline iron levels in the soil were determined through wet digestion and analysis using AAS.

Planting material

The spinach variety used was *Spinacia oleracea* Fordhook Giant, known for its sensitivity to micronutrient availability and rapid vegetative growth. The spinach seeds were planted straight into 5-liter buckets that held 2 kg of uniform soil-treatment solutions. Following germination, thinning was done to ensure uniform growth conditions and eliminate competition for resources by keeping one healthy seedling per bucket.

Experimental design

The study adopted an RCD in a greenhouse to minimize variability, and ten treatments were established. The treatments included biochar-iron complexes at three $FeCl_3$ concentrations (0.25 M, 0.50 M, and 0.75 M), synthetic $FeCl_3$ solutions at matching concentrations, biochar-only treatments of soil amended with sugarcane bagasse-derived biochar only (applied at 20%, 10%, and 6.65% by weight), and a control with no iron supplementation. Treatments were replicated three times and applied to spinach grown in 2 kg of soil in a 5 L bucket. Baseline soil analysis showed an iron concentration of approximately 2.0 ppm. Three times a week (Monday, Wednesday, and Friday), a graduated cylinder was used to apply 1 L of water per bucket to standardize soil moisture. By avoiding waterlogging, this frequency preserved the ideal level of soil moisture.

Spinach growth

The spinach was grown over three months. Leaf samples were collected monthly and analysed for iron content using AAS.

RESULTS AND DISCUSSIONS

FT-IR Analysis of Biochar Derived from Sugarcane Bagasse

The FT-IR spectra revealed the chemical interactions between biochar and iron at different concentrations, showing the -OH

and -COOH functional groups indicated by the absorption peaks (Figure 1). These findings are consistent with earlier research showing that the high surface functionality of biochar promotes metal adsorption, increasing its suitability as a soil amendment (Tan *et al.*, 2020). Strong absorption bands corresponding to -OH, -COOH, and -PhOH groups were visible in the FT-IR spectra of the biochar made from sugarcane bagasse. These functional groups are essential for iron binding during complexation and play a role in nutrient retention, water adsorption, and buffering soil pH. Their presence demonstrates that pyrolysis preserved important surface functionalities critical for biochar's performance as a soil amendment (Wu *et al.*, 2019). The sugarcane bagasse-derived biochar's FT-IR spectrum reveals its functional groups, which are essential for metal chelation and nutrient retention. The -OH stretching vibrations, mostly from leftover cellulose, hemicellulose, and lignin, are responsible for the broad absorption at 3440.10 cm^{-1}. These hydroxyl groups improve water retention and facilitate metal adsorption by increasing the hydrophilicity and hydrogen bonding capacity of biochar. The -COOH groups, which offer negatively charged sites for attaching metal ions like Fe^{3+} by electrostatic attraction or chelation, are responsible for the signal at 1383.95 cm^{-1}. The band at 1117.77 cm^{-1} also contributes to cation exchange capacity and is associated with phenolic –OH or ether functionalities. These oxygen-containing groups presence indicate the preservation of critical surface functions during pyrolysis, which are necessary for the iron complexation that follows. These findings are consistent with other research that has shown that lignocellulosic biochars retain functional groups similarly (Wu *et al.*, 2019; Fan *et al.*, 2021), thus confirming that the produced biochar used in this study is chemically suitable for applications involving soil fertility and iron loading.

Biochar-Iron Complex Characterization using FT-IR

The analysis verified that iron was successfully incorporated into the biochar matrix at all $FeCl_3$ concentrations (Figure 2). Strong chelation between iron ions and the surface of the biochar was demonstrated by distinct peak shifts in the -OH and -COOH functional groups (Figure 2). The spectra also showed the development of Fe-O links, indicating enhanced stability and direct chemical bonding. According to earlier publications, these alterations show that the surface chemistry of biochar has changed without sacrificing its structural integrity (Rajapaksha *et al.*, 2016; Tang *et al.*, 2022). Coordination between Fe^{3+} ions and -OH groups is suggested by the shift of the hydroxyl band from 3440.10 cm^{-1} (in raw biochar) to 3429.43 cm^{-1} in the FT-IR spectrum of the biochar-iron complex treated with 0.25 M $FeCl_3$. The successful integration of iron into the structure of the biochar is confirmed by the emergence of additional bands at 671.23 cm^{-1} and 507.28 cm^{-1}, which are indicative of Fe-O vibrations. Furthermore, aromatic C=C stretching is indicated by peaks at 1633.71 cm^{-1} and 1539.20 cm^{-1}, which show that the carbonized structure of the biochar has been retained, which is essential for long-term stability. A faint band at 2358.94 cm^{-1} might be a sign of slight CO_2 adsorption, which happens frequently when metal is modified. Overall, effective iron binding without over-saturation was achieved at a concentration of 0.25 M, indicating that it might be the best choice for balanced iron enrichment and structural stability (Figure 2).

A large absorption band at 3406.29 cm^{-1} in the FT-IR spectra of the 0.5 M Biochar-Iron Complex indicates -OH stretching, which is suggestive of water molecules or surface hydroxyl groups (Figure 3). The existence of CO_2 or C≡C stretching vibrations is suggested by the peak at 2360.87 cm^{-1}, which may be related to surface oxidation processes or adsorbed ambient CO_2. C-O stretching is represented by the peak at 1114.86 cm^{-1}, which is probably caused by oxygen-containing functional groups like carboxylates or phenols (Figure 3). These groups boost the complex's sorption capability by providing active sites for iron coordination. The hydroxyl signal, which emerges at 3406.29 cm^{-1} in the FT-IR spectra of the biochar-iron complex treated with 0.50 M $FeCl_3$, indicates that the

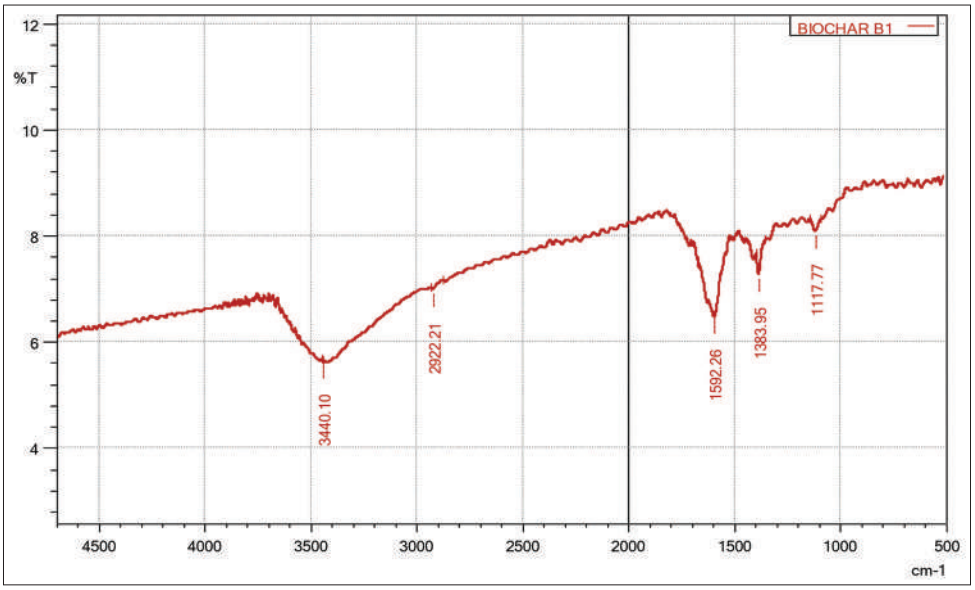


Figure 1: FT-IR characterization of biochar made from sugarcane bagasse

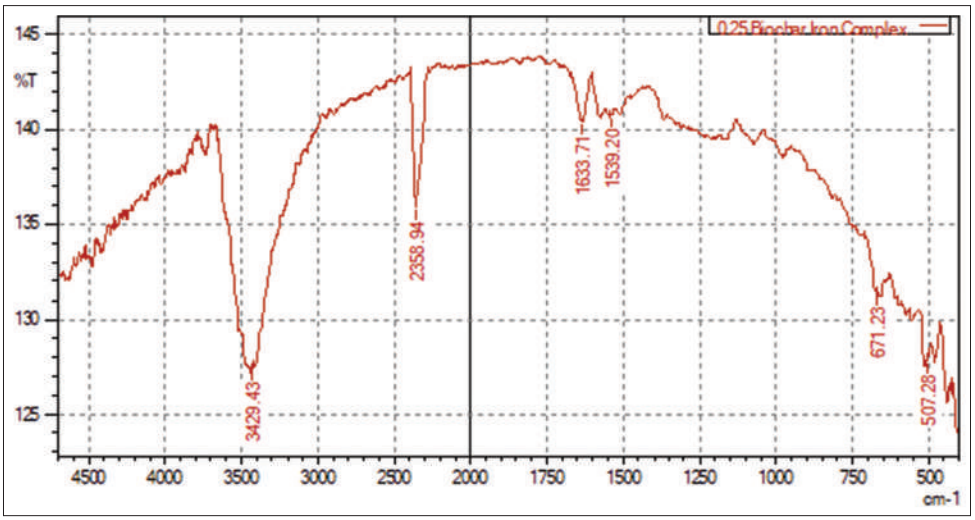


Figure 2: Biochar-iron complex characterization by FT-IR (0.25 M $FeCl_3$ (1:5w/v))

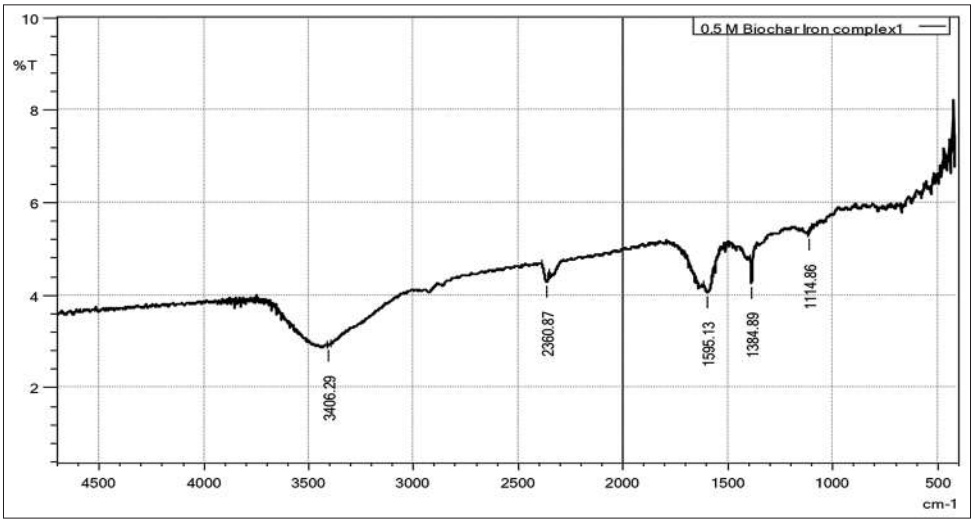


Figure 3: FT-IR characterization of the biochar-iron complex (0.5 M $FeCl_3$ (1:10w/v))

Fe^{3+} ions and surface –OH groups are still strongly interacting. The band at 2360.87 cm^{-1} indicates greater surface carbonate production or CO_2 adsorption, which frequently happens at higher metal loading levels. Reactive oxygen sites stay active even after alteration thanks to a noticeable band at 1114.86 cm^{-1} (C-O stretching) that verifies the persistence of phenolic or ether groups. This complex exhibits stronger iron binding than 0.25 M; however, surface accessibility may be impacted by possible pore obstruction at higher loading. These observations suggest 0.50 M is highly effective for iron enrichment while maintaining biochar functionality.

Key functional group interactions in the 0.75 M biochar-iron complex's FT-IR spectra are comparable to those in the 0.5 M biochar-iron complex, although there are minor peak position shifts that could suggest stronger or different bonding at higher iron concentrations (Figure 4). The wide hydroxyl bond in the 0.75 M biochar-iron complex was located at 3419.79 cm^{-1}. The small shift in this peak from 3406.29 cm^{-1} in the 0.5 M complex raises the possibility that increased iron loading has altered hydrogen bonding. In contrast to the 0.5 M complex's peak at 1595.13 cm^{-1}, the carboxyl group peak was located at 1593.20 cm^{-1} (Figure 4). Similar to the 0.5 M complex's 1114.86 cm^{-1}, the 1118.71 cm^{-1} stretching from phenolic or ether groups shows that functional groups that contain oxygen are still important in both complexes. Although there are minor changes that point to improved interaction with iron, overall, the 0.75 M biochar-iron complex maintains the main functional groups of biochar. For uses like heavy metal adsorption or soil remediation, this could enhance its metal-binding qualities and increase its potential effectiveness.

The greatest precursor concentration of 0.75 M $FeCl_3$ was used to produce the biochar-iron complex, and its FT-IR spectra were examined. Both the carboxyl peak and the hydroxyl band are somewhat displaced from lower concentrations, appearing at 1593.20 cm^{-1} and 3419.79 cm^{-1}, respectively. Stronger or more extensive iron coordination is indicated by these changes. Iron incorporation is confirmed by the presence of Fe-O peaks, although the small variations between 0.50 M and 0.75 M imply

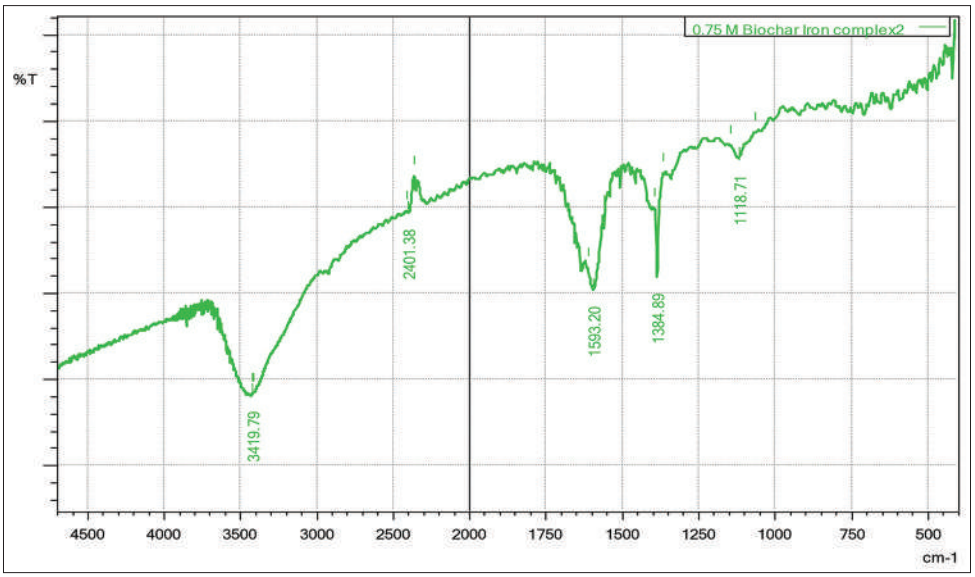


Figure 4: Biochar-iron complex characterization by FT-IR (0.75 M $FeCl_3$ (1:15w/v)

that the majority of reactive sites were already occupied at 0.50 M. The extra iron is probably present as surface precipitates rather than as new coordination bonds, which could limit functional performance and decrease porosity. Consequently, even if 0.75 M optimizes iron loading, the practical advantages seem to be negligible above 0.50 M, underscoring the need to maximize precursor concentration for both cost-effectiveness and soil application success.

XRF Analysis of Iron Content in the Biochar-Iron Complex

The amount of iron incorporated into the biochar matrix was measured, and a distinct pattern of greater iron retention with higher $FeCl_3$ concentrations was observed. Table 1 shows that the iron concentration increased from 51.694% at 0.25 M to 65.754% at 0.5 M and 72.049% at 0.75 M. As concentration rose, standard deviation values marginally decreased (from 1.318 to 1.159), suggesting more consistent iron deposition at higher molarities. This implies that increased ion-exchange interactions and surface complexation caused by an increase in $FeCl_3$ concentration improve iron loading. A possible saturation point or restricted availability of reactive sites may be indicated by the fact that the incremental gain between 0.5 M and 0.75 M (from 65.754% to 72.049%) was less noticeable than between 0.25 M and 0.5 M. These results are consistent with those of Singh and Maiti (2024), who found that high precursor concentrations result in decreasing returns because of steric hindrance or surface precipitation, and those of Kuang *et al.* (2023), who found that biochar composites treated with higher $FeCl_3$ levels had greater iron sorption capacities. As a result, although 0.75 M reaches the maximum loading, 0.5 M might be the best balance between material efficiency and iron enrichment. These results highlight the significance of dosage optimization in balancing cost, performance, and environmental factors for the practical use of soil amendments. Higher precursor concentrations result in improved iron loading on the biochar, as the data show that the iron content raised as the concentration of $FeCl_3$ used increased. Additionally, a more equal distribution of iron is shown by the standard deviation decreasing as concentration rises.

Table 1: Determination of iron percentage in different biochar-iron complex concentration using XRF

Biochar-iron complex concentration	Iron concentration (%)	Std. deviation
0.25M	51.694	1.318
0.5M	65.754	1.275
0.75M	72.049	1.159

Iron Concentration of the Experimental Soil

The untreated soil had an average iron concentration of 2.0 ppm (Table 2). This baseline served as a reference for assessing the efficacy of the various treatments in enhancing iron availability. The AAS analysis quantified iron availability in soil, indicating an average iron concentration of 2.0 ppm (Table 2).

Effect of Treatment on iron Concentration

The iron concentration in spinach was significantly influenced by treatment, time, and their interaction, as shown in Table 3. The treatment effect was highly significant ($p<0.001$), suggesting that different treatments led to substantial variations in iron content. This implies that certain treatments were more effective in enhancing iron concentration than others. Similarly, the time effect was also highly significant ($p<0.001$), indicating that iron concentration varied significantly across different time points. This variation may be attributed to factors such as the growth stage of the spinach, nutrient uptake efficiency, or environmental conditions over time (Table 3). Moreover, the interaction between treatment and time was found to be statistically significant ($p<0.001$), indicating that the impact of specific treatments varied depending on the time of

Table 2: The average levels of iron in the soil in ppm

Element	Absorbance	Concentration (ppm)
Fe	0.100	1.9
	0.110	2.0
	0.105	2.1
Average		2.0

Table 3: Effect of treatment on iron concentration

Source of variation	df	Mean Square
Treatment	9	66941.80***
Time	2	1503.08***
Treatment*Time	18	533.02***
Residuals	60	0.90

***- Significant at $P<0.001$

measurement, highlighting the importance of considering both factors when evaluating treatment effectiveness. The observed variation over time suggests that iron availability is influenced by soil dynamics and plant physiological needs. Studies have shown that iron bioavailability in soil is dependent on microbial activity and redox conditions, which can fluctuate over time (Kappler *et al.*, 2021). The significant interaction between treatment and time indicates that different biochar-iron formulations release iron at varying rates, an observation consistent with reports by Kuang *et al.* (2023) that biochar modifications alter nutrient release kinetics. This slow-release mechanism helps ensure consistent nutrient availability throughout the crop's growth cycle, reducing the need for frequent fertilizer applications.

Effect of Treatment on the Iron Concentration of Spinach

The iron concentration (ppm) in spinach leaves varied significantly across treatments and months, as shown in Table 4. The highest iron concentrations were consistently recorded in the biochar-iron complex 0.75M treatment, with values of 220 ppm in November, 260 ppm in December, and 300 ppm in January. These values were significantly higher than those observed in all other treatments ($p\leq0.001$). This consistent upward trend suggests that the biochar-iron complex (0.75 M) treatment provides sustained benefits, potentially through a gradual nutrient release or enhanced soil conditions over time. The biochar-iron complex 0.50 M treatment resulted in moderately high iron concentrations, measuring 110 ppm, 130 ppm, and 150 ppm in November, December, and January, respectively. These values were significantly lower than those of the 0.75 M treatment but higher than those of the other treatments. A further reduction in iron concentration was observed in the biochar-iron complex 0.25 M treatment, which yielded 55 ppm in November, 65 ppm in December, and 75 ppm in January. While this treatment improved iron content compared to the control and other non-complexed treatments, it was significantly less effective than the higher concentrations of the iron complex. Although this increase was smaller in magnitude, it indicates that even lower concentrations of this treatment offer some beneficial effects. Treatments involving $FeCl_3$ alone (0.25 M, 0.50 M, and 0.75 M), plain biochar (10%, 20%, and 6.65%), and the control resulted in considerably lower iron concentrations, ranging from 1.3 to 2.1 ppm across

Table 4: Effect of treatment on iron concentration of spinach

Treatment	Iron concentration (ppm)		
	November	December	January
Biochar-iron complex 0.75 M	220[a]	260[a]	300[a]
Biochar-iron complex 0.50 M	110[b]	130[b]	150[b]
Biochar-iron complex 0.25 M	55[c]	65[c]	75[c]
$FeCl_3$ 0.25 M	1.8[d]	2.0[d]	2.1[d]
$FeCl_3$ 0.50 M	1.7[d]	1.8[d]	1.9[d]
Biochar 10%	1.6[d]	1.7[d]	1.8[d]
Biochar 20%	1.6[d]	1.7[d]	1.8[d]
Biochar 6.65%	1.6[d]	1.7[d]	1.8[d]
Control	1.6[d]	1.6[d]	1.8[d]
$FeCl_3$ 0.75 M	1.3[d]	1.5[d]	1.6[d]
Standard error (±)	12.8	15.2	17.5
p-value	<0.001	<0.001	<0.001

Means followed by different letters within a column are significantly different from each other at $\alpha\leq0.05$

the months. These treatments were statistically similar to each other, as indicated by the common letter, and did not differ significantly from the control.

The biochar-iron complex 0.75 M treatment consistently resulted in iron concentrations exceeding the upper threshold of the recommended range for spinach (80-250 ppm), reaching 300 ppm in January. While this treatment significantly improved iron uptake, the recorded levels surpassed the upper limit of the optimal range, potentially raising concerns regarding iron accumulation beyond nutritional needs. The biochar-iron complex 0.50 M treatment produced iron concentrations that remained well within the recommended range (110-150 ppm), suggesting its potential as a balanced and effective strategy for biofortifying spinach without exceeding safe limits. In contrast, treatments such as the Biochar 10% and $FeCl_3$ treatments showed consistently low iron concentrations across all time points. These results suggest that these treatments either lacked effective nutrient delivery mechanisms or experienced rapid iron leaching. The findings from the greenhouse study suggest that chelation of iron with biochar improves its availability and uptake, which could be a promising strategy for improving iron nutrition in leafy vegetables. A distinct trend was observed over the three sampling times. The Biochar-Iron complex treatments showed a steady increase in concentration, indicating a cumulative effect or progressive nutrient release. This trend suggests that these treatments may improve outcomes gradually rather than delivering an immediate impact. Conversely, the treatments that maintained low concentration values, such as the control and $FeCl_3$ treatments showed no change over time, implying that any potential effects were either weak or were for a short time duration.

Results from the comparative performance of Biochar-iron complexes to conventional $FeCl_3$ showed a significant effect (Figure 5). In November, the biochar-iron complex delivered 110 ppm of iron in spinach leaves, compared to 1.7 ppm from $FeCl_3$- a>60 × increase. By January, this difference peaked at 150 ppm, clearly reflecting the sustained release of iron from the biochar complex as opposed to the rapid precipitation and leaching associated with $FeCl_3$. The levels of iron under $FeCl_3$ treatment remained relatively static, ranging between

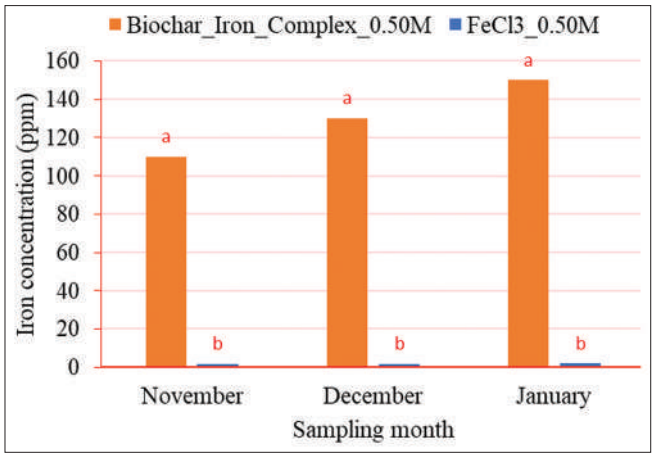


Figure 5: Tukey's mean separation of iron concentration (ppm) in spinach leaves treated with biochar-iron complex and $FeCl_3$ (0.50 M) across three sampling periods. Means followed by different letters within a column differ significantly at $\alpha \leq 0.05$.

1.7 and 1.9 ppm throughout the three months, indicating poor iron retention in the soil. In contrast, the biochar-Fe complex exhibited a steady and continuous increase in iron concentration, rising from 110 ppm in November to 150 ppm in January. This progressive increase is critical for supporting the multi-stage nutrient uptake essential for healthy plant growth (Figure 5). These findings align with previous research indicating that biochar amendments can improve micronutrient availability by modifying soil physicochemical properties (Joseph *et al.*, 2021).

The significant effect of treatment highlights the role of biochar in facilitating iron retention and release, likely through increased cation exchange capacity and reduced iron leaching (Dey *et al.*, 2023). Biochar's carbonaceous structure, which resists microbial degradation, plays a key role in this behaviour (Kumar *et al.*, 2023). Studies have shown that biochar forms strong complexes with metal ions, gradually releasing nutrients as environmental conditions fluctuate. The sustained increase in iron levels observed with the 0.75 M and 0.50 M treatments aligns with findings by Roy *et al.* (2024), who observed prolonged nutrient release from biochar-treated soils. This slow-release mechanism helps ensure consistent nutrient availability throughout the crop's growth cycle, reducing the need for frequent fertilizer applications. The superior performance of the biochar-iron complexes can also be attributed to biochar's ability to improve cation exchange capacity (CEC) and stabilize soil nutrients. The presence of oxygen-containing functional groups such as carboxyl, hydroxyl, and phenolic groups enhances biochar's capacity to retain positively charged ions like Fe^{3+} (Fan *et al.*, 2021). These functional groups provide active binding sites, reducing the likelihood of iron leaching and improving nutrient retention in the root zone.

In comparison with other studies, the enhanced iron concentration in spinach aligns with findings by Nigussie *et al.* (2012), Carter *et al.* (2013) and Liu *et al.* (2019) who reported improved micronutrient uptake in leafy vegetables with biochar application. However, discrepancies arise when considering the extent of improvement, as some studies report more gradual increases in iron concentration, possibly due to differences in soil type, biochar feedstock, or plant species. For instance, contrasting results by Baigorri *et al.* (2020) suggest that in alkaline soils, iron availability may still be limited despite biochar application due to precipitation of iron oxides. This difference underscores the importance of soil conditions in mediating biochar effectiveness.

CONCLUSIONS

This study successfully synthesized sugarcane bagasse-derived biochar and complexed it with $FeCl_3$ to form a biochar-iron complex. FT-IR analysis confirmed the binding of iron ions to key functional groups on the biochar, and XRF analysis quantitatively demonstrated enhanced iron retention, particularly at the 0.75 M concentration. The biochar-iron complex shows significant potential as a sustainable soil amendment for improving nutrient retention and could contribute to enhanced soil fertility and crop productivity. The 0.50 M biochar-Fe complex maintains leaf iron concentrations within the FAO's recommended range of 80-250 ppm for spinach, thus ensuring optimal fortification without risking iron toxicity. The 0.50 M biochar-iron complex experimentally outperforms conventional $FeCl_3$ in both magnitude and temporal stability of iron uptake, making it a viable, sustainable alternative for correcting iron deficiency in spinach. Therefore, the spinach grown in biochar-iron-amended soil exhibited higher iron concentrations compared to those in untreated soil, biochar-only, and $FeCl_3$-treated soils. This suggests that biochar-iron complexes enhance iron uptake in plants more effectively than $FeCl_3$ alone, potentially due to better iron stabilization and controlled release. Based on the study's findings, Biochar-iron complex (0.50 M) is recommended as the most effective treatment for boosting iron concentration in spinach that falls within the range stipulated by FAO, particularly in iron-deficient soils. The observed cumulative effect suggests that these treatments should be applied early in the planting season to provide sustained iron availability throughout crop development. Future trials are recommended to explore how biochar-iron complexes perform under varying environmental conditions, such as soil pH, moisture levels, and different crop species.

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