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The Impact of Biochar and $FeCl_3$ Administration on Fe Toxicity Symptoms and Productivity of Bok Choy Mustard Plants (*Brassica rapa* L.) Nauli Variety

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ABSTRACT

Fe^{3+} pollution is a factor that reduces bok choy production. Its productivity can be increased by adding biochar, which can adsorb inorganic ions, including iron. This study examines the impact of rice husk biochar and $FeCl_3$ on bok choy productivity, using a completely randomized design with a 3×3 factorial pattern. The first factor was the concentration of rice husk biochar (0, 2.5, and 7.5 g/kg), and the second was the concentration of $FeCl_3$ (0, 25, and 50 mg/L). The criteria measured were the number of leaves, leaf area, chlorophyll content, and vitamin C levels. The combination of 0 g/kg biochar and 0 mg/L $FeCl_3$ proved to be the most effective treatment for boosting bok choy growth. The treatment yielded 12.6 leaf count, 73.3 cm^2 leaf area, 2.0 mg/g chlorophyll, and 30.8 ppm vitamin C. Fe^{3+} treatments at 25–50 mg/L drastically lowered all growth metrics. The applied biochar doses were insufficient to efficiently absorb Fe^{3+} and boost the yield of bok choy.

Keywords: biochar, bok choy, chinese mustard, $FeCl_3$, toxicity

INTRODUCTION

Bok choy mustard (*Brassica rapa* subsp. chinensis) is a popular horticultural plant in Indonesia, with high potential for production (Subakti *et al.* 2022). This is due to its nutritional value, which includes vitamins and minerals that are good for your health and prevent diseases including cancer, hypertension, heart disease, cataracts, congenital impairments, and stroke (Subakti *et al.* 2022). However, the production of bok choy mustard fell in 2022. This drop was caused by two major factors: decreased soil fertility and overuse of inorganic fertilizers (Rizal 2024). Long-term usage of inorganic fertilizers can harm soil structure and contribute to environmental contamination. One method for addressing soil fertility issues is to use organic materials such as compost, manure, or other organic substances. The incorporation of organic materials into soil raises the organic carbon (C-organic) content and improves soil microporosity. These micropores serve as water retainers (Angst *et al.* 2021). Biochar is an organic material formed during the pyrolysis process, which involves incomplete combustion with little or no oxygen (Yaashikaa *et al.* 2020).

The use of biochar obtained from agricultural waste is one way for improving soil structure, increasing organic carbon content, increasing water retention and absorption capacity, and improving soil fertility and plant growth (Nepal *et al.* 2023). Biochar is not only used as a soil amendment, but it may also absorb excess heavy metals in the soil (Zhou *et al.* 2022). Another advantage of biochar is its environmental friendliness, which indirectly contributes to waste reduction while aiding sustainable agriculture (Janah *et al.* 2023). Biochar is widely used as a highly promising resource to assist environmental sustainability and current agriculture techniques (Mensah and Frimpong, 2018). Biochar treatment has the effect of lowering soil and water pollution by binding heavy metals and toxic inorganic compounds, hence minimizing contamination risk. Previous research has demonstrated that the use of rice husk biochar can improve the growth of mustard greens (*Brassica juncea* L.).

This study examines the effect of rice husk biochar treatment combinations in lowering $FeCl_3$ toxicity on the growth and production of bok choy plants. Fe^{3+} is extensively used in insecticides sprayed on agricultural areas. Fe^{3+} contamination can occur from industrial waste that enters water bodies without sufficient treatment (Fermindo 2015).

METHODS

This research was carried out from March to October 2023 at the Experimental Garden of the Department of Biology, Faculty of Science and

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Mathematics, Diponegoro University, Semarang, Central Java, Indonesia. The Plant Structure and Function Biology Laboratory, Department of Biology, Faculty of Science and Mathematics, Diponegoro University, conducted chlorophyll content tests and vitamin C analyses on bok choy. This study included bok choy seeds from the Nauli variety, planting soil, rice husk charcoal, $FeCl_3$, 20 cm pots, acetone, distilled water, and ascorbic acid.

Experimental Design

This study used a 3×3 randomized factorial design (CRD). The first factor was rice husk biochar at concentrations of 0, 2.5, and 7.5 g/kg. The second component was $FeCl_3$ at concentrations of 0, 25, and 50 mg/L. Each treatment combination was replicated three times, for a total of 27 experimental pots.

Nursery, Planting, and Maintenance of Bok Choy

The bok choy seeds utilized in this study were of the Nauli variety, purchased from PT East West Indonesia under the Cap Panah Merah label. The seeds were initially picked by soaking them in water for around 3 hours. The selected seeds were then sown in seedling trays, scattered on the soil surface, lightly covered with soil, and covered with plastic to retain moisture.

Bok choy seedlings were seven days old and ready to be put into the treatment pots. Before transplanting, the planting media, which included soil and rice husk biochar, was created at predefined concentrations of 0, 2.5, and 7.5 g/kg. A total of 4 kg of the soil-biochar combination was placed in 20 cm diameter pots. Seven days before planting, $FeCl_3$ was added to each treatment pot in powder form at the appropriate concentration. The seedlings were planted 5 cm deep, one plant per pot. Watering, replanting, weeding, and pest and disease control were all required to keep the plants healthy. Watering was done by measuring 500 mL of water into each treatment pot (Anjarwati 2022). Maintenance continued until the plants reached 42 days after planting (DAP).

Measurement of Plant Growth, Chlorophyll and Vitamin C Analyses

The characteristics studied were the number of leaves, leaf area, chlorophyll concentration, and vitamin C content. To analyze the growth of bok choy plants, the number of leaves and leaf area were measured initially. Chlorophyll and vitamin C content were measured on the 21st DAP. A UV-Vis spectrophotometer was used to measure chlorophyll content at wavelengths of 645, 663, and 480 nm (Vaghela *et al.* 2022). The vitamin C test was carried out destructively using the spectrophotometric method (Pathy 2018).

Data Analysis

The data were analyzed using Two-Way ANOVA to see if there was a significant difference between treatment combinations. If a significant effect was discovered, Duncan's Multiple Range Test (DMRT) was used at a 95% confidence level to determine differences between treatments.

RESULTS AND DISCUSSION

Number of Leaves

The ANOVA results show an interaction impact between rice husk biochar and $FeCl_3$ on the number of leaves (Table 1. Number of bok choy leaves after treatment with rice husk biochar and $FeCl_3$). Increased $FeCl_3$ concentration led to fewer bok choy leaves than treatments without rice husk charcoal or $FeCl_3$. The B0F0 treatment produced the most leaves (12.6) (Figure 1. Bok choy plants after treatment with the combination of rice husk biochar and $FeCl_3$.). Combining 0 g/kg rice husk biochar with F1 (25 mg/L) or F2 (50 mg/L) resulted in a substantial reduction in leaf number ($p < 0.05$). The B0F0 treatment (control) produced the most leaves. Increased $FeCl_3$ concentration led to fewer leaves than treatments without rice husk charcoal or $FeCl_3$. This is because planting soil without rice husk biochar has a high cation exchange capacity, allowing nutrients to be retained effectively and resulting in optimal leaf formation (Singh et al. 2022).

A decrease in the number of leaves might result in inefficient photosynthesis, as leaves play an important role in the process. The number of leaves increases photosynthetic output, resulting in optimal plant growth (Sales *et al.* 2021). This study found that the number of leaves observed may be influenced by various factors beyond the $FeCl_3$ content in the growing media and bok choy plants, including the presence of other critical nutrients in the soil. Carreño *et al.* (2021) found that

Table 1 Number of bok choy leaves after treatment with rice husk biochar and $FeCl_3$

Biochar (g/kg)	$FeCl_3$ (mg/L)			
	F0	F1	F2	Average
B0	12.6± 0.5^{a}	11.2± 1.3^{bc}	10.4± 0.5^{cd}	11.4 ± 1.2^{q}
B1	12.4± 0.5^{ab}	11.8 ± 0.8^{ab}	7.8 ± 0.8^{e}	10.7 ± 2.2^{q}
B2	11.8± 1.5^{ab}	9.4 ± 0.9^{d}	9.4 ± 0.9^{d}	10.2 ± 1.6^{q}
Average	12.3 ± 1^{p}	10.8 ± 1.4^{p}	9.2 ± 1.3^{p}	

Description: Numbers followed by the same letter indicate no significant difference based on the DMRT test at a 95% significance level (α = 5%).

having more leaves correlates with increased leaf area. Plants with more non-overlapping leaves can capture more sunlight for photosynthesis, resulting in increased assimilate production.

Leaf Area

ANOVA results show that the B0F0 treatment had the highest average leaf area (73.3 cm², Table 2. Leaf area of bok choy after treatment with rice husk biochar and $FeCl_3$), while the B2F2 treatment had the lowest (25.2 cm²). Combining rice husk biochar and $FeCl_3$ treatments resulted in an interaction effect on leaf area. Leaf area decreased as $FeCl_3$ concentration increased. This is most likely due to the failure of the biochar concentrations used in treatments B1 (2.5 g/kg) and B2 (7.5 g/kg) to effectively absorb heavy metals, resulting in slowed leaf development.

The findings of this study contradict recent research conducted by Adebajo *et al.* (2022), which found that applying rice husk biochar at doses ranging from 0 to 16 g/kg increased the number and leaf area of tomato plants. According to Parjono *et al.* (2019), high levels of metal contamination, including $FeCl_3$, can harm plant growth. Hamblin *et al.* (2014) stated that leaf area determines the amount of chlorophyll manufactured by plants since chlorophyll is related to photosynthesis and a wider leaf surface area allows for more chlorophyll production.

According to Badria and Aboelmaaty (2019), $FeCl_3$ inhibits leaf area expansion in plants by reacting with tannins, which are key components in plant cells. Tomasi *et al.* (2022) defined tannins as polyphenolic chemicals found in plant cells, notably leaves. When $FeCl_3$ combines with tannins, the tannin component turns dark black, indicating a favorable result in tannin identification tests. Tannins contain oxygen atoms with lone electron pairs that can coordinate as ligands, while the core atom in $FeCl_3$ is the Fe^{3+} ion. Espina *et al.* (2022) found that Fe^{3+} ions can bind three tannin molecules, each with two oxygen atoms functioning as donor atoms at the 4' and 5' dihydroxy sites, allowing the central atom to coordinate with six lone pairs. The oxygen atoms at the 4' and 5' dihydroxy sites can act as ligands since they require the least amount of energy to build complex molecules.

In line with Gonçalves *et al.* (2010), tannin chemicals accumulate in plant cells and impede leaf area expansion by disturbing the water and mineral balance inside the cells, resulting in cellular drought conditions. Tannins can also interfere with photosynthesis and plant development, all of which can contribute to a loss in leaf area.

Chlorophyll Content

In the B0F0 treatment (control), bok choy had the highest chlorophyll content (2.97 mg/g), while the B0F2

Figure 1 Bok choy plants after treatment with the combination of rice husk biochar and $FeCl_3$. (A) B0F0 (control), exhibiting the best plant growth; (B) B0F2 (0 g/kg rice husk biochar + $FeCl_3$), plants with the lowest chlorophyll content; (C) B2F2 (7.5 g/kg rice husk biochar + 50 mg/L $FeCl_3$), plants with the lowest vitamin C content.

Table 2 Leaf area of bok choy after treatment with rice husk biochar and $FeCl_3$

Biochar (g/kg)	$FeCl_3$ (mg/L)			
	F0	F1	F2	Average
B0	73.3 ± 3.3[a]	52.3 ± 2.8[b]	46.2 ± 7.7[d]	52.1 ± 6.9
B1	57.8 ± 4.8[b]	54 ± 2.5[b]	34.3 ± 4.4[d]	53.9 ± 17.1
B2	42.8 ± 1.8[e]	59.8 ± 0.8[b]	25.2 ± 4[e]	42.6 ± 15.1
Average	57.9 ± 13.5	55.3 ± 3.9	35.2 ± 10.3	

Description: Numbers followed by the same letter indicate no significant difference based on the DMRT test at a 95% significance level (α = 5%).

Table 3 Chlorophyll content of bok choy plants after treatment with rice husk biochar and $FeCl_3$

Biochar (g/kg)	$FeCl_3$ (mg/L)			
	F0	F1	F2	Average
B0	2.0± 0.1[a]	1.47± 0.2[de]	1.37± 0.2[e]	1.61 ± 0.3
B1	1.83± 0.1[ab]	1.53± 0.1[de]	1.73 ± 0.1[bc]	1.7 ± 0.1
B2	1.8± 0.0[ab]	1.6± 0.1[cd]	1.6 ± 0.1[cd]	1.67 ± 0.6
Average	1.88 ± 0.1	1.53 ± 0.1	1.57 ± 0.2	

Description: Numbers followed by the same letter indicate no significant difference based on the DMRT test at a 95% significance level (α = 5%).

treatment had the lowest chlorophyll content (1.3 mg/g) (p<0.05) (Table 3). These findings are consistent with prior studies, which found that mustard greens had chlorophyll concentration ranging from 0.383 to 2.356 mg/g.

Research indicates that increasing $FeCl_3$ concentration can cause yellowing and decreased chlorophyll content in bok choy plants (Figure 1). Bernado *et al.* (2021) found that increasing Fe^{3+} concentration on the 21st day of cultivation increased chlorophyll a and b content, however this rise stabilized on the same day in *Chlorella* sp. Manzoor *et al.* (2023) found that the impact of Fe^{3+} concentration on chlorophyll content varies based on environmental circumstances and plant species. According to Barrow and Hartemink (2023), other factors influencing Fe uptake and utilization by plants include soil pH and interactions with other chemicals in the soil. The decrease in chlorophyll content may be owing to excessive $FeCl_3$ concentration, which is hypothesized to come from rice husk biochar's inadequate performance in absorbing excess $FeCl_3$.

Biochar's high porosity and surface area assist the physical adsorption of Fe ions, allowing them to be absorbed. The pore structure of biochar provides opportunities for capturing Fe ions from the surrounding environment. The next step is ion exchange, which can activate functional groups on the biochar surface, such as carboxyl (–COOH) and hydroxyl (–OH) groups, which aid in ion exchange. Fe^{3+} ions can replace other ions on the biochar surface through chemical interactions, followed by electrostatic interactions that attract positively charged Fe ions to negatively charged biochar surfaces. Biochar's relatively high cation exchange capacity helps to assist this process. Complexation then happens, in which organic functional groups in biochar, such as phenolics, form complexes with Fe ions, increasing the biochar's attraction for these metal ions and resulting in precipitation. Under some conditions, Fe ions can precipitate on the charcoal surface, especially if the biochar has an alkaline pH that facilitates this process (Hidayat 2015).

$FeCl_3$ decreases chlorophyll levels in bok choy by interacting with the plant's chlorophyll and modifying its structure, rendering it inactive or non-functional. Dey *et al.* (2019) reported that this reaction involves electron transfer from chlorophyll to $FeCl_3$, resulting in color changes or reduced photosynthetic activity. According to Putri *et al.* (2009), $FeCl_3$ can reduce photosynthetic activity in mustard plants, leading to lower productivity, including fewer leaves and less chlorophyll content.

Rice husk biochar can improve soil's physical, chemical, and biological quality while lowering hazardous metal concentrations (Li *et al.* 2023). Additionally, rice husk biochar can improve soil quality and plant productivity. However, its impact on the absorption of excess metal elements varies depending on climatic conditions and plant species. As a result, using rice husk biochar in plants can help reduce the detrimental effects of high metal levels in the soil, allowing plants to grow and develop healthily (Campos and de la Rosa 2020). However, in this investigation, rice husk biochar did not exhibit a significant function in heavy metal absorption. As the $FeCl_3$ concentration increased in the biochar treatments, the chlorophyll content decreased continuously.

The Content of Vitamin C

The vitamin C content was determined using the spectrophotometric technique with ascorbic acid as a standard solution. The maximum wavelength must first be identified. The absorbance of the ascorbic acid solution was measured at wavelengths between 260 and 270 nm (Khafid *et al.* 2023). Based on the observations, the highest absorption value was 0.776 at 266 nm (Figure 2). The combined treatment of rice husk biochar and $FeCl_3$ on the vitamin C content was found to interact, according to ANOVA results. The B1F2 treatment had the highest vitamin C concentration (30.8 ppm). The B0F2 and B1F2 treatments differed significantly as the biochar concentration increased in B1 (2.5 g/kg rice husk biochar) (p < 0.05). The B2F2 treatment had the lowest vitamin C level (8.9 ppm) (Table 4). Vitamin C content of bok choy after treatment with rice husk biochar and $FeCl_3$). This suggests that increasing $FeCl_3$ concentration can decrease vitamin C levels. This decline is thought to be related to tissue damage in the leaves, which prevents optimal vitamin C production. Faster tissue damage might impede plant growth and development.

Using rice husk charcoal and $FeCl_3$ in the growing media reduces vitamin C concentration in bok choy leaves, leading to decreased plant development. This occurs because the precursor for vitamin C production is a main metabolite, specifically glucose. As said by Bernado *et al.* (2021), glucose produced by plants is

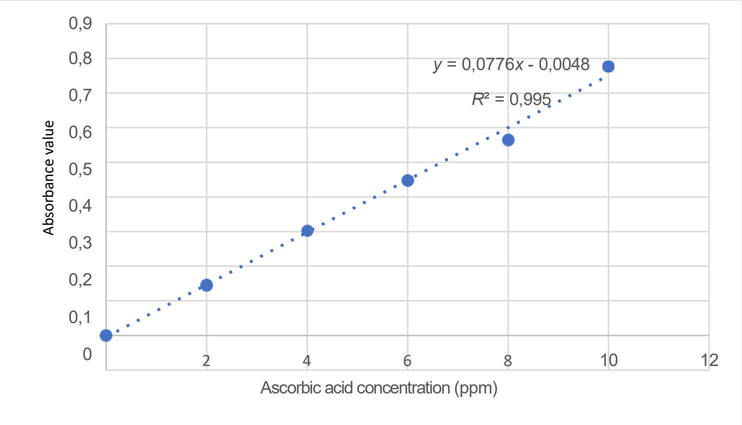

Figure 2 Standard curve of ascorbic acid.

Table 4 Vitamin C content of bok choy after treatment with rice husk biochar and $FeCl_3$

Biochar (g/kg)	FeCl₃ (mg/L)			
	F0	F1	F2	Average
B0	25.1± 2.3[b]	14.3± 0.5[d]	29.9± 2.3[a]	23.1 ± 7.1
B1	19.4 ± 0.8[c]	12.4 ± 0.8[d]	30.8 ± 0.1[a]	20.9 ± 8.1
B2	26.2 ± 2.4[b]	24.1 ± 0.7[b]	8.9 ± 0.1[e]	19.7 ± 8.3
Average	23.6 ± 3.6	16.9 ± 5.5	23.2 ± 10.8	

Description: Numbers followed by the same letter indicate no significant difference based on the DMRT test at a 95% significance level (α = 5%).

used not only for growth but also for secondary metabolism, such as vitamin C production. Similarly, Castro *et al.* (2023) stated that the acquired glucose enters the *D*-glucuronic acid and *L*-gulonic acid routes, which then generate *L*-ascorbate, resulting in vitamin C production.

Vegetables abundant in vitamin C are regarded high-quality vegetables for health (Hermsdorff *et al.* 2012). Vitamin C is an essential component that serves as an antioxidant and contributes to a healthy immune system. Vitamin C-rich vegetables include broccoli, mustard greens, red bell peppers, strawberries, and papaya. Carr and Rowe (2020) noted that eating vegetables high in vitamin C can assist meet daily vitamin C requirements, which are critical for overall health. As a result, the higher the vitamin C level in veggies, the better they are for your health and daily nutritional demands.

CONCLUSION

The combination of rice husk biochar and $FeCl_3$ reduces the development of bok choy plants. The B0F0 treatment (control) produced the best productivity of this particular plant. Increasing the concentration of rice husk charcoal and $FeCl_3$ reduced the number of leaves, leaf area, and chlorophyll content, but had no effect on vitamin C levels.

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