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Detrimental Effects of Eucalyptus and Rice Husk Biochars on Three Consecutive Corn Cropping Cycles in a Tropical Acidic Sandy Soil

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ABSTRACT

Supplementing chemically fertilized soil with biochar might enhance plant growth and decrease fertilizer needs. Biochar could impact not only the initial cropping cycle but also subsequent cycles, even without additional applications. Seven soil amendments were investigated: unamended, 100% of the recommended chemical fertilizer (100%CF), 50%CF+eucalyptus biochar (EUB), 100%CF+EUB, 50%CF+rice husk biochar (RHB), and 100%CF+RHB. Corn was used as the plant bioassay over three cropping cycles, with biochars applied only before the first cycle. Biochar supplementation generally had a negative effect on plant growth, so more EUB than RHB. The effects of biochars were classified into two phases. The first phase showed an immediate negative effect during the first two cropping cycles. Compared to 100%CF, the 50%CF+EUB decreased corn shoot biomass by –35.8% and –26.9% (1st and 2nd cycles); 100%CF+EUB by –13.7% and –20.2%; 50%CF+RHB by –34.8% and increased by +5.2%; and 100%CF+RHB decreased by –3.4% and increased by +72.1%. The second phase showed a delayed negative effect in the third cycle, with depression in corn shoot biomass of –51.8% and –47.4% for 50%CF and 100%CF+EUB, and –15.2% and –23.7% for 50%CF and 100%CF+RHB.

Keywords: Biochar quality, coarse textured soil, delayed effect, immediate effect, negative effect

INTRODUCTION

A significant global challenge in crop cultivation involves the management of acidic sandy soils, which cover approximately 900 million ha, representing nearly 50% of the Earth's total soil (Yost and Hartemink, 2019). Moreover, acidic soils constitute 30–40% of the world's arable lands (von Uexküll and Mutert, 1995; Samac and Tesfaye, 2003). These soils are characterized by their inherent low fertility and difficulty in amendment, issues compounded by intensive agricultural practices and the impact of global warming (Mehmood *et al.*, 2020). This problem is particularly pronounced in Northeast Thailand, a vital agricultural region within the country (Vityakon, 2007).

Although chemical fertilizers have traditionally been employed to enhance soil fertility, their application raises concerns regarding environmental pollution (Pahalvi *et al.*, 2021). The recent fertilizer price crisis underscores the urgent need to reduce chemical fertilizer use or improve fertilizer efficiency (Abebe *et al.*, 2022; Eisa *et al.*, 2022). Consequently, integrating organic materials to supplement chemical fertilizers is increasingly regarded as a viable and environmentally sustainable strategy (Sharma *et al.*, 2019; Durán-Lara *et al.*, 2020). Among these organic amendments, biochar has gained prominence due to its potential to enhance soil quality and contribute to climate change mitigation, as recognized by the Intergovernmental Panel on Climate Change (IPCC, 2019). Biochar, derived from biomass through pyrolysis in an oxygen-limited condition, serves primarily as a valuable soil amendment (Saletnik *et al.*, 2019; Wang *et al.*, 2020; Kumar and Bhattacharya, 2021).

Rice husk, a by-product of rice production, is abundant in countries with high rice consumption and exportation, such as Thailand (Thambhitaks and Kitchaicharoen, 2021). In addition, while rice is not a staple food in countries like the United States and Australia, these nations are leading producers and exporters of rice (Muthayya *et al.*, 2014). Similarly, eucalyptus, although not indigenous to Thailand, is extensively cultivated there for use as raw material in paper production, both in monoculture and agroforestry systems (Boulay and Tacconi, 2012). Eucalyptus also yields considerable biomass, particularly in the form of branches that are unsuitable for processing in paper mills (Butnan *et al.*, 2015). Therefore, both rice husk and eucalyptus branches are recognized as promising feedstocks for biochar production.

Each type of biochar varies in quality, impacting plant growth differently. Factors such as feedstock types and pyrolysis techniques play a critical role in determining biochar quality, affecting parameters such as fixed carbon, volatile matter, and ash content (Deenik *et al.*, 2010; Wang *et al.*, 2020). Fixed carbon is essential for soil structure and nutrient retention, primarily due to its graphitic structure, which influences porosity and adsorption capacity (Wang *et al.*, 2020). Volatile matter comprises stable pools, including pyranones, ethers, and quinones; as well as labile pools with functional groups such as hydroxyl, carbonyl, carboxyl, and hemiacetal, serving as a carbon source for soil microorganisms (Tomczyk *et al.*, 2020). Studies have demonstrated that polycyclic aromatic hydrocarbons present in volatile matter can be detrimental to soil microorganisms and plants (Tarigholizadeh *et al.*, 2024). Ash content consists of vital plant nutrients like P, K, Ca, Mg, and Si, which are preserved during pyrolysis as oxides, hydroxides, and carbonates (Butnan and Vityakon, 2023).

Biochar production encompasses complex processes, including biomass burning, grinding, and sometimes sieving prior to application, rendering it a multifaceted endeavor. Field–level applications often require substantial quantities of biochar; for instance, to attain a 1% w/w application rate in sandy soils, up to 22 tons/ha of biochar may be required (Butnan *et al.*, 2018). Additionally, the application of biochar may generate fine dust particles, posing potential environmental and health risks to users and individuals in the vicinity. Effectively applying biochar once to influence subsequent cropping cycles could help mitigate these concerns.

While a considerable body of studies has investigated the use of biochar alongside chemical fertilizers, most studies focus on single cropping cycles without addressing the continuous effects of biochar applications across multiple cycles. For example, studies conducted by Mete *et al.* (2015), Bi *et al.* (2017), Kim *et al.* (2017), Yu *et al.* (2017), Cheng *et al.* (2018), Graef *et al.* (2018), Yu *et al.* (2018), Ijaz *et al.* (2019), Ali *et al.* (2021), Li *et al.* (2022), Liu *et al.* (2022), Usevičiūtė *et al.* (2022), and others analyze outcomes over multiple cycles but apply biochar afresh with each cycle, overlooking the residual effects. This is similarly noted in the work of Šimanský *et al.* (2018), Horák *et al.* (2019), Song *et al.* (2020), Tian *et al.* (2020), Cao *et al.* (2021), Peng *et al.* (2021), and Dong *et al.* (2022). There is a conspicuous gap in the literature regarding plant responses over successive cropping cycles following a single application of biochar, especially in acidic sandy soil supplemented with various biochar and chemical fertilizers. Previous studies by Kamau *et al.* (2019) and Wu *et al.* (2023) focused on non–acidic fine-textured soil, whereas Ning *et al.* (2022) conducted similar investigations but did not account for specific soil characteristics. Therefore, the residual effects of biochar in acidic sandy soils remain underexplored, highlighting an urgent need for further investigation into these unique soil reactions and mechanisms in relation to biochar usage.

The current study hypothesized that supplementing chemically fertilized soil with different types of biochar would enhance plant growth, reduce chemical fertilizer use, and sustain beneficial effects over successive cropping cycles. This study therefore aimed to evaluate the effect of different application rates of chemical fertilizers supplemented with eucalyptus and rice husk biochars applied once, on plant growth over multiple consecutive cropping cycles.

MATERIALS AND METHODS

Planting media

The soil used in this study was the Korat soil series (isohyperthermic Typic Oxyaquic Kandiustults), which is the predominant acidic sandy soil in Thailand (Keerati-Kasikorn, 1984). Samples were collected from agricultural areas in the Waritchaphum district of Sakon Nakhon province (17°20'28.1"N, 103°41'53.3"E) at 0–15 cm depth. Prior to the experiment, the soil was air-dried and sieved through a 2–mm mesh. The soil properties before the experiment are presented in **Table 1**.

Table 1. Initial soil properties of the current study.

Characteristic	Soil
Soil particle size distribution	
Sand (g/kg)	865.6
Silt (g/kg)	90.8
Clay (g/kg)	43.6
Texture	Loamy Sand
Bulk density (g/cm^3)	1.45
Water holding capacity (g/kg)	205
pH (material: water =1:1)	5.41
Organic matter (g/kg)	4.7
Electrical conductivity (mS/cm)	0.021
NH_4^+–N (mg/kg)	6.3
NO_3^-–N (mg/kg)	3.5
P (mg/kg)	9.5
K (mg/kg)	69.7
Ca (mg/kg)	117.2
Mg (mg/kg)	21.0
Fe (mg/kg)	112

Biochars were produced from eucalyptus branches and rice husks using identical pyrolysis techniques. Both types of biomass were processed in a 200–L metal drum furnace commonly used in Sakon Nakhon and nearby areas. Pyrolysis was conducted at approximately 450°C for two hours, followed by a cooling period of six hours. The eucalyptus biochar was further ground and sieved through a 2–mm mesh before being used in the experiments. The properties of the biochars are detailed in **Table 2**.

Table 2. Properties of the eucalyptus and rice husk biochars used in the current study

Characteristic	Eucalyptus biochar	Rice husk biochar
Proximate analysis		
Volatile matter (g/kg)	219	181
Ash (g/kg)	91	303
Fixed carbon (g/kg)	690	516
P (mg/kg)	610	3680
K (mg/kg)	3590	5050
Ca (mg/kg)	24290	1750
Mg (mg/kg)	1220	1150
Si (mg/kg)	156300	125900

Experimental setup

The experiment was conducted in greenhouse pots from May to August 2018, following a randomized complete block design with three replications and seven treatments: (i) no soil amendment (none), (ii) recommended chemical fertilizer (100%CF), (iii) half of 100%CF supplemented with eucalyptus biochar (50%CF+EUB), (iv) 100%CF+EUB, (v) 50%CF supplemented with rice husk biochar (50%CF+RHB), and (vi) 100%CF+RHB. Biochar was applied at a rate of 2% w/w; this rate was chosen based on its suitability for sandy textured soil (Butnan *et al.,* 2015). The recommended chemical fertilizer rates for Ultisols sandy soils in Thailand were 125 kg N/ha, 25 kg P_2O_5/ha, and 84.4 kg K_2O/ha (Department of Agriculture, 2005). A commercial corn variety was used as the bioassay plant for soil fertility assessment, grown continuously over three cropping cycles with biochar applied once before the first planting cycle.

For the first cropping cycle, each pot (top *d* = 18 cm, bottom *d* = 13.5 cm, *h* = 14.3 cm, *V* = 2805 cm^3) was filled with 2 kg of soil mixed with 40 g of biochar and incubated for 14 days. Nine–day–old corn seedlings, at the two-true leaf stage and selected for their vigor and uniformity from plug trays, were transplanted at a rate of one seedling per pot. To provide nutrients at the recommended rate, three fertilizer grades were used: 15–0–0 (15% N from calcium nitrate), 0–52–34 (52% P_2O_5 and 34% K_2O from monopotassium phosphate), and 0–0–50 (50% K_2O from potassium sulfate). Fertilizers were applied twice, at equal rates, when the corn was 1 and 20 days after transplanting in each cycle. During each application, 0.367 g of calcium nitrate, 0.086 g of monopotassium phosphate, and 0.017 g of potassium sulfate were applied to each pot, with the half rates adjusted accordingly. Soil moisture was maintained at 70% of the water holding capacity (287 ml H_2O/pot) and monitored daily through pot weighing.

Corn shoot biomass was measured when the corn plants were 42 days old, during the vegetative growth stage, which has the highest nutrient demand (Reuter and Robinson, 1997). The biomass was dried in an oven at 65°C until a constant weight was achieved, then ground to pass through a 1–mm sieve for nutrient analysis. Concurrently, soil bulk density was measured using the core method with a modified soil core (25 mm *d* and 50 mm *h*), and fresh soil samples were collected for NH_4^+ and NO_3^- analysis. The remaining soil was air-dry and passed through a 2–mm sieve with roots removed. Samples of air–dry soil were taken for analysis of other soil properties, and the biochar was returned to the pots for subsequent cycles.

In the second and third cropping cycles, similar procedures for treatment management, experimental maintenance, and sample collection were followed shortly after the first cycle. However, biochar was not re-applied.

Laboratory analysis

The proximate values of biochar, including fixed carbon, volatile matter, and ash, were analyzed according to the ASTM D 7582 method (American Standard of Testing Material, 2015). Soil pH and electrical conductivity were measured at soil–to–water ratios of 1:1 and 1:5, respectively. Soil organic matter was quantified using wet oxidation following the Walkley and Black method (Nelson and Sommers, 1982). Phosphate extraction was performed using Bray2 solution (0.1 *M* HCl + 0.03 *M* NH_4F at pH 1.5), and the measurement was conducted with a UV–Vis spectrophotometer (Specord250 plus, Analytik Jena, Germany) at a wavelength of 820 nm (Fixen and Grove, 1990). Sodium, Ca, Mg, and Fe were extracted using 1 *M* NH_4OAc at pH 7.0 and quantified using spectrophotometric techniques (Pansu and Gautheyrou, 2006) with an atomic absorption spectrometer (AAS) (novAA® 350, Analytik Jena, Germany). Total N was analyzed using the steam distillation method (Bremner and Mulvaney, 1982) on a micro-Kjeldahl machine (Pro–Nitro S 4002851, JP Selecta, Barcelona, Spain), while NH_4^+ and NO_3^- were extracted with 2 *M* KCl and measured using the same technique.

After performing nitric-perchloric wet digestion to extract nutrients from corn shoot tissue, N was measured using the steam distillation method (Miller, 1998). Phosphorus was quantified using UV–Vis spectrophotometry; while cations, including K, Ca, Mg, and Fe, were assessed using AAS.

Statistical analysis

An analysis of variance was conducted to evaluate the effects of chemical fertilizer rates and biochar types on soil properties, plant growth, and tissue nutrient content. Tukey's honestly significant difference (HSD) test was applied for multiple comparisons, with statistical significance set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Immediate adverse effects of biochars on corn directly driven by their volatile matter

Biochar supplementation of strongly acidic sandy soil (pH 5.41) using eucalyptus and rice husk biochars showed variable effects on corn growth across cropping cycles, suggesting potential aging effects of biochar (**Figure 1**). Compared to 100%CF (**Table 3**), both biochars immediately had a negative effect during the first two cycles, with the most pronounced negative effect observed in the third cycle. This observation aligns with findings by Shi *et al.* (2023), which indicated that biochar toxicity increases with age; however, the mechanisms identified in this study differ from those in Shi *et al.* (2023).

Eucalyptus biochar, which has a higher volatile matter content (219 g/kg) than rice husk biochar (181 g/kg) (**Table 2**), led to a 13.7% decrease in corn dry weight during the first cycle, whereas rice husk biochar had no effect. In the second cycle, eucalyptus biochar continued to exert a slight negative influence on growth, while rice husk biochar resulted in a 72.1% improvement. This immediate positive effect from rice husk biochar could not be explained by soil (**Table 4**) or tissue nutrient analyses (**Table 5**). However, this phenomenon could be attributed to the volatile matter in the biochar, particularly polycyclic aromatic hydrocarbons and labile carbons, which are toxic to plants and can impair essential physiological processes such as photosynthesis and respiration (Tarigholizadeh *et al.*, 2024).

The presence of labile carbons may have induced N deficiency through immobilization; however, this effect diminished over time, as noted by Oleszczuk and Kołtowski (2018). By the third cycle, soil N (**Table 4**) and shoot N content (**Table 5**) in the eucalyptus biochar treatment had increased, indicating reduced N immobilization and enhanced N uptake by the corn plants. In contrast, the lower volatile matter content of rice husk biochar resulted in no immediate negative effects and ultimately facilitated growth enhancement (**Table 3**).

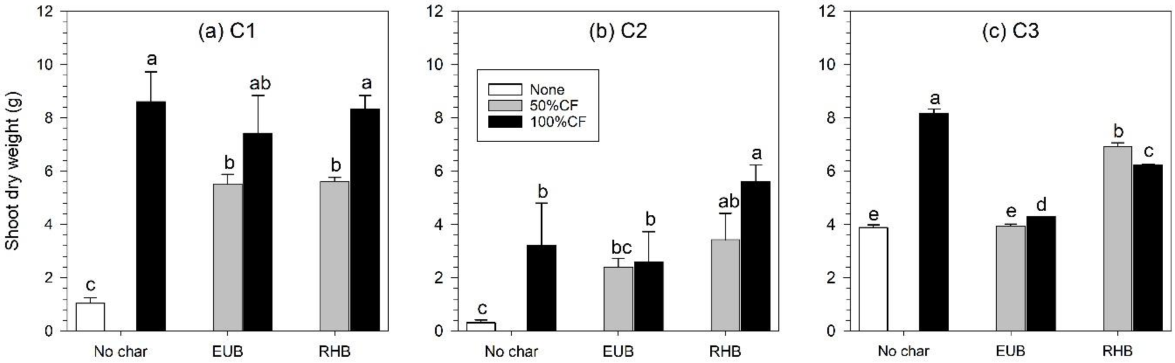

Figure 1. Corn shoot biomass as affected by varied chemical fertilizer rates supplemented with eucalyptus (EUB) and rice husk biochars (RHB) in three consecutive cropping cycles. Different letters on the bars for each cropping cycle indicate statistically significant differences at a significance level of $p \leq 0.05$ based on Tukey's HSD test. Error bars indicate the standard error of the means. C1, C2, and C3 refer to Cropping cycles 1, 2, and 3, respectively.

In the second cycle, 100%CF+EUB maintained a slight negative effect (**Table 3**), while 100%CF+RHB significantly improved growth. Godlewska *et al.* (2021) noted that the composition of volatile matter varies based on feedstock and pyrolysis conditions, suggesting that eucalyptus biochar might contain higher levels of persistent toxic compounds, such as polycyclic aromatic hydrocarbons, particularly naphthalene. In contrast, rice husk biochar, with its lower volatile matter and reduced presence of toxic substances, demonstrated positive effects, supporting the observed growth patterns across the cropping cycles.

Table 3. The responses of corn in soil amended with recommended rates of chemical fertilizer supplemented with eucalyptus or rice husk biochar, shown by changes in shoot dry weight relative to soil amended with chemical fertilizer alone (100%CF).

Cropping cycle	Relative change of corn dry weight (%)			
	Eucalyptus biochar		Rice husk biochar	
	50%CF+EUB	100%CF+EUB	50%CF+RHB	100%CF+RHB
C1	–35.8 (– –)	–13.7 (–)	–34.8 (– –)	–3.4 (0)
C2	–26.9 (– –)	–20.2 (–)	+5.2 (+)	+72.1 (+ + +)
C3	–51.8 (– – –)	–47.4 (– –)	–15.2 (–)	–23.7 (–)

Note: The plus (+) and minus (–) signs within parentheses following the relative change of corn dry weight indicate the following: <5% = 0 denotes no effect; 5-25% = – or + denotes slight negative or positive effects; 26–50% = – – or ++ denotes significant negative or positive effects; 51–75% = – – – or + + + denotes very significant negative or positive effects; and 76–100% = – – – – or ++++ denotes extremely significant negative or positive effects.
C1, C2, and C3 refer to cropping cycles 1, 2, and 3, respectively.

Delayed adverse effects of biochars on corn indirectly driven by their volatile and directly driven by ash

The detrimental effects of biochars on corn growth became more pronounced during the third consecutive cropping cycle. Compared to 100%CF, the application of 100%CF+EUB resulted in a highly negative effect, while 100%CF+RHB had a slightly negative effect on corn growth of corn (**Table 3**). Soil properties affected by both treatments revealed significantly increased concentrations of soil P and K in 100%CF+EUB, and soil Ca concentrations in both 100%CF+EUB and 100%CF+RHB during the third cropping cycle, as opposed to the other treatments (**Table 4b**).

The shoot tissue contents of P, K, and Ca during this cycle indicated levels exceeding toxic thresholds: >5 mg P/kg, >40 mg K/kg, and >7 mg Ca/kg (Reuter *et al.*, 1997), in all instances of 100%CF+EUB and 100%CF+RHB, applied once before the first cropping cycle (**Table 5**). Specifically, the tissue P, K, and Ca contents under 100%CF supplemented with eucalyptus and rice husk biochars were 6.65 and 4.90 mg P/kg, 63.9 and 53.6 mg K/kg, and 21 and 13 mg Ca/kg for 100%CF+EUB and 100%CF+RHB, respectively (**Table 5**). This increase could be attributed to the high nutrient contents in eucalyptus biochar (610 mg P/kg, 3590 mg K/kg, 24290 mg Ca/kg) and rice husk biochar (3680 mg P/kg, 5050 mg K/kg, 1750 mg Ca/kg) (**Table 1**). These nutrients were released in large quantities during the third cropping cycle, consistent with Ulery *et al.* (1993), who identified that nutrient release from biochar ash occurred in two phases: immediate dissolution from soluble quicklime and slow dissolution from oxides and carbonates. Therefore, the increased soil concentrations of P, K, and Ca observed during the third cropping cycle, potentially toxic to plants, might result from the dissolution of slow–soluble oxides and carbonates.

Additionally, both eucalyptus and rice husk biochars demonstrated exceptionally high Si concentrations: 156300 and 125900 mg Si/kg, respectively (**Table 1**). While this study did not measure Si contents in soil or plant tissue, comparing Si levels with other elements in both biochar types suggested potential Si toxicity emerging in the third cropping cycle. This observation supported findings by Butnan and Vityakon (2023), who reported that prolonged or aged biochar presence in soil increased Si content, leading to decreased rice growth and yield due to excessive Si dissolution and antagonistic effects.

Moreover, as for the 100%CF+EUB, corn plants may have faced NO_3^- toxicity, as indicated by high soil NO_3^- concentrations reaching up to 31.38 mg NO_3^-–N/kg (**Table 4a**). High NO_3^- accumulation in plants resulted in NO_2^- formation, which could further convert to NO and O_2^-, ultimately producing peroxynitrite ($ONOO^-$), a highly toxic compound for plants (Reddy and Menary, 1990; Durner and Klessig, 1999; Lamattina *et al.*, 2003). Optimal soil NO_3^- levels for corn had not been established; however, Ferrario-Méry *et al.* (2005) reported symptoms of NO_3^- toxicity in Arabidopsis at 10 m*M* NO_3^-. In the current study, the concentration of 31.38 mg NO_3^-–N/kg (**Table 4a**) translated to 9.83 m*M* NO_3^-–N or 43.5 m*M* NO_3^- for an individual corn plant, using the Arabidopsis toxicity scenario for comparison. This indicated that the soil NO_3^- concentration in 100%CF+EUB during the third cropping cycle is potentially toxic to corn. Therefore, the observed NO_3^- toxicity might arise from the indirect and lasting effects of volatile matter on corn growth, which serves as a carbon source for nitrifying microorganisms, thereby enhancing their activity.

Table 4. Selected soil physicochemical properties and elemental concentrations at the harvest as affected by the effects of eucalyptus (EUB) and rice husk biochars (RHB) supplemented to the different rates of the recommended chemical fertilizer (CF).

(a) Selected soil physicochemical properties

Amendment	BD (g/cm3)			SOM (g/kg)			C/N ratio			pH (1:1)			EC (mS/cm)			NH4+-N (mg/kg)			NO3–N (mg/kg)		
	C1†	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3
None	1.46 a‡	1.37 a	1.33 a	3.77 b	4.60 c	4.65 d	0.24 b	0.18 b	0.18 b	5.98 c	5.91 c	5.99 d	0.0130 b	0.0220 c	0.024 d	2.63 bc	2.92	3.15 a-c	3.24 b	3.85 b	3.03 b
100%CF	1.44 a	1.33 a	1.29 a	3.70 b	4.67 c	5.65 c	0.27 ab	0.18 b	0.22 b	6.13 bc	6.60 b	6.90 c	0.0127 b	0.0300 b	0.037 c	3.06 ab	2.92	4.08 a	3.85 b	4.90 ab	2.68 b
50%CF+EUB	1.34 ab	1.24 b	1.22 b	4.87 ab	5.65 bc	5.83 bc	0.32 ab	0.29 b	0.15 b	7.40 a	7.66 a	7.61 a	0.0263 a	0.0423 a	0.074 b	2.19 c	2.92	2.10 c	4.29 b	3.62 b	9.10 b
100%CF+EUB	1.28 bc	1.25 b	1.23 b	4.50 ab	6.70 ab	6.70 a	0.23 b	0.19 b	0.12 b	7.25 a	7.56 a	7.39 b	0.0237 a	0.0463 a	0.109 a	2.63 bc	2.92	2.45 bc	7.27 a	7.35 a	31.38 a
50%CF+RHB	1.20 bc	1.23 b	1.21 b	5.90 a	7.65 a	5.70 c	0.43 a	0.53 a	0.37 a	6.40 b	6.65 b	6.82 c	0.0150 b	0.0280 bc	0.036 c	3.33 a	3.03	3.97 a	3.33 b	3.62 b	3.50 b
100%CF+RHB	1.19 c	1.26 b	1.24 b	5.83 a	7.65 a	6.35 ab	0.39 ab	0.47 a	0.19 b	6.27 bc	6.71 b	6.96 c	0.0127 b	0.0297 bc	0.044 c	3.24 a	2.92	3.85 ab	3.94 b	4.20 b	4.32 b
p-value	< 0.001	< 0.001	< 0.001	0.01	< 0.001	< 0.001	0.0158	<0.001	0.0012	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001	0.999	0.0025	<0.001	0.0131	< 0.001
F-test	***	***	***	**	***	***	*	***	**	***	***	***	***	***	***	***	ns	**	***	*	***
CV (%)	3.88	1.36	1.36	14.74	8.12	3.15	21.36	21.54	25.99	1.94	1.07	0.98	13.56	8.3	5.24	7.52	21.73	15.53	11.93	23.78	37.88

(b) Soil elemental concentrations

Amendment	Total N (g/kg)			P (mg/kg)			K (mg/kg)			Ca (mg/kg)			Mg (mg/kg)			Fe (mg/kg)		
	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3
None	9.3	15.0 ab	14.2 c	6.9 d	7.1 d	9.0 d	20.7 b	71.5 c	67.0 c	109 b	129 e	125 f	15.3 b	17.4 ab	14.8 a	80.9 a	90.7	102.7 a
100%CF	7.9	16.6 ab	13.3 cd	14.4 c	29.0 b	48.0 b	23.3 b	76.7 bc	65.0 c	132 b	232 d	331 c	9.9 c	10.4 c	7.1 b	59.7 ab	67.1	56.8 b
50%CF+EUB	9.1	13.1 b	22.8 b	15.4 c	22.1 c	35.0 c	52.4 a	95.7 a	102.3 a	275 a	447 b	449 b	16.3 b	19.8 a	15.0 a	40.2 ab	21.6	20.7 d
100%CF+EUB	11.5	21.2 a	31.5 a	19.5 a	32.4 ab	55.6 a	35.4 ab	84.8 a-c	102.5 a	295 a	532 a	669 a	15.6 b	15.6 b	13.5 a	27.5 b	23.1	20.9 d
50%CF+RHB	8.1	8.9 b	10.6 d	16.0 bc	23.5 c	31.0 c	42.1 ab	90.0 ab	72.9 bc	161 b	219 d	248 e	18.5 a	20.2 a	13.7 a	69.6 ab	54.6	54.8 bc
100%CF+RHB	8.9	9.3 b	22.0 b	18.3 ab	32.9 a	49.0 b	33.7 ab	99.3 a	88.3 ab	147 b	253 c	294 d	15.1 b	14.4 b	8.6 b	66.0 ab	51.6	39.6 c
p-value	0.102	0.0474	<0.001	< 0.001	< 0.001	< 0.001	0.008	0.0017	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.022	< 0.001	< 0.001
F-test	ns	*	***	***	***	***	**	**	***	***	***	***	***	***	***	*	***	***
CV (%)	15.43	31.38	8.71	6.15	5.16	4.14	24.01	7.17	6.98	11.27	1.71	3.26	3.99	6.54	4.68	28.61	5.73	11.22

* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, ns = not significantly different.

† C1, C2, and C3 are cropping cycles 1, 2, and 3.

‡ Different letters following the mean values in the same column for each parameter indicate statistically significant differences at a significance level of $p \leq 0.05$ based on Tukey's HSD test.

Table 5. Tissue nutrient contents in corn shoot of each cropping cycle as affected by the effects of eucalyptus (EUB) and rice husk biochars (RHB) supplemented to the different rates of the recommended chemical fertilizer (CF).

Amendment	N (g/kg)			P (g/kg)			K (g/kg)			Ca (g/kg)			Mg (g/kg)			Fe (g/kg)		
	C1†	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3
None	9.27	13.7 b‡	13.4 c	0.70	0.85 c	0.70 f	12.9 cd	44.6 b	31.9 c	6.73 a	12.3 a	13.2 b	4.23 a	5.87 a	4.85 a	0.37	1.51 a	1.89 a
100%CF	7.87	12.7 bc	13.3 c	0.92	1.87 b	2.10 d	7.4 d	64.6 ab	38.1 c	4.40 bc	9.8 a-c	11.6 b	2.17 b	3.33 ab	1.87 d	0.29	0.20 cd	0.37 bc
50%CF+EUB	9.13	10.8 bc	21.2 b	0.84	1.35 bc	2.70 c	36.7 a	78.7 a	62.5 a	4.53 bc	10.5 ab	20.4 a	1.40 c	4.00 ab	3.95 b	0.22	0.35 b	0.44 b
100%CF+EUB	11.47	17.7 a	32.5 a	1.08	3.20 a	6.65 a	37.6 a	74.7 a	63.9 a	5.40 ab	11.0 ab	21.0 a	1.63 bc	3.43 ab	3.01 c	0.28	0.32 bc	0.36 bc
50%CF+RHB	8.07	8.9 c	11.5 c	1.08	1.30 bc	1.40 e	23.6 b	50.5 b	35.7 c	3.53 c	6.9 c	8.9 c	1.50 bc	2.25 b	2.87 c	0.4	0.15 d	0.25 c
100%CF+RHB	8.9	9.3 c	23.5 b	0.9	1.73 b	4.90 b	18.9 bc	51.2 b	53.6 b	4.33 bc	8.1 bc	13.0 b	1.80 bc	2.10 b	2.85 c	0.76	0.22 b-d	0.26 c
p-value	0.102	<0.001	<0.001	0.07	<0.001	<0.001	<0.001	0.03	<0.001	0.001	0.002	<0.001	<0.001	0.031	<0.001	0.087	<0.001	<0.001
F-test	ns	***	***	ns	***	***	***	*	***	***	**	***	***	*	***	ns	***	***
CV (%)	15.43	11.14	4.33	16.13	14.06	3.7	14.46	20.07	6.36	12.56	11.31	4.29	12.58	34.27	3.48	52.9	11.59	8.89

* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, ns = not significantly different.
† C1, C2, and C3 are cropping cycles 1, 2, and 3.
‡ Different letters following the mean values in the same column for each parameter indicate statistically significant differences at a significance level of $p \leq 0.05$ based on Tukey's HSD test.

Could biochars substitute chemical fertilizer use?

The question remained whether biochars would reduce the need for chemical fertilizer. Both eucalyptus and rice husk biochars failed to achieve this purpose. This was evident from the diminished corn growth observed with 50%CF+EUB and 50%CF+RHB (**Table 3**). Corn shoot dry weights decreased by –35.8%, –26.9%, and –51.8% in 50%CF+EUB, and –34.8%, +5.2%, and –15.2% in 50%CF+RHB during the first, second, and third cropping cycles respectively (**Table 3**). Although 50%CF+RHB initially had a slightly positive effect on corn growth during the second cropping cycle, it turned negative in the third cycle. The decrease in corn growth could be attributed similarly to the adverse effects observed with 100%CF+EUB and to the effects of 100%CF+RHB. This reduction in corn nutrients in 50%CF+EUB was consistent across all three cropping cycles, resulting in lower soil nutrients (**Table 4**) and shoot tissue nutrients in both 50%CF+EUB and 50%CF+RHB (**Table 5**) compared to 100%CF+EUB and 100%CF+RHB respectively.

Using both biochars at recommended chemical fertilizer rates, either half (50%CF) or full (100%CF), produced immediate and delayed effects on corn. These effects stemmed from biochars applied once before the first cropping cycle, which predominantly harmed the plants, except for a residual beneficial effect of rice husk biochar in the second cycle, which was later followed by negative effects. These adverse outcomes likely resulted from the excessive rates of biochar applied. Deenik *et al.* (2010) indicated that biochar levels exceeding 1% w/w could negatively affect plant growth due to N immobilization from high volatile matter content and nutrient antagonistic effects associated with a high ash biochar. Therefore, studying appropriate biochar rates when using biochar with chemical fertilizers was advisable.

From the perspective of crop production in acidic sandy soil and evaluating corn responses over three cropping cycles, the current study showed that biochars could not substitute for chemical fertilizers. Using these biochars with chemical fertilizers was not recommended due to increased production costs and adverse effects on plant growth.

CONCLUSION

This study clearly showed that supplementing the chemically fertilized soil with eucalyptus and rice husk biochars did not improve corn growth or reduce amount of chemical fertilizer needed. Instead, both biochars caused immediate negative effects on growth in the first two cropping cycles, possibly due to volatile matter, while both volatile matter and ash of biochars contributed to delayed negative effects occurring in the third cycle. Therefore, from a crop production perspective, high application rates of these biochars with chemical fertilizers in the loamy sandy soil are not recommended. Future research should focus on determining optimal biochar application rates when used together with chemical fertilizers.

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