Biochar Extract Compounds Alter Germination and Growth of Crop Seed

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Positive or negative plant growth effects due to soluble organic biochar compounds are not well understood. Increasing quantities (0 g, 0.5 g, 2.5 g, and 5 g) of four different biochars were extracted with 0.1 M HCl, which were separated and marked as W, X, Y, and Z. Corn and rice seeds were treated with biochar extract solution. The rice straw Y and Z solutions positively affected corn germination, while the cotton straw Z solution negatively affected it. When differences were present, the biochar extract solutions always caused reduction in rice seed germination. The rice straw and cotton straw solutions tended to increase, the wheat straw Y and Z solutions tended to decrease, while little differences were evident in terms of the effects of the *Spartina alterniflora* solution on corn bud and root length when compared to the control. Increasing the biochar solution extract treatments reduced the rice bud and root length when compared to the control, especially with solution Z. Organic compounds, ***e.g.***, triethyl phosphate, 2,4-bisphenol, were present in the solutions, which likely promoted seed growth at lower concentrations. Determining the presence of biochar organic moieties helped with designing biochars for enhanced seed germination, growth, and crop productivity.

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INTRODUCTION

Biochar is a solid porous product, with an alkaline pH, that contains necessary plant nutrients and has the ability to sorb and retain nutrients due to its cation exchange properties (Lehmann 2007). These characteristics make biochar potentially useful for overall crop growth and production purposes (Ochiai *et al*. 2021). However, these properties likely do not affect seed germination and initial plant growth to the extent that the organic compounds present within biochar might. This area of research needs further exploration, with focus on the mechanisms and effects of biochar extract solutions on seed germination and growth (Sun *et al*. 2017).

The initial research in this area, performed by a variety of scientists, focused its attention on the presence of organic compounds in the biochar extract. For example, Lin *et al*. (2012) showed that relatively low pyrolysis temperatures (less than 450 °C) favored the creation of *Acacia saligna* wood chip biochar extracts that contained low molecular weight neutral and humic-acid-like components, while greater pyrolysis temperatures produced

lower quantities of soluble organic compounds. Graber *et al*. (2010) studied compounds present in low temperature biochars (less than 500 °C) and observed the presence of low molecular weight organic acids, phenols, aromatic hydrocarbons, and alkanes. A subsequent study by Graber *et al*. (2014) found that biochar water-soluble organic carbon components contained a variety of small molecular organic acids and humic acid-like macromolecular organic compounds. Similarly, Quan *et al*. (2020) showed that fulvic and humic acid-like substances were released as the primary water-soluble products from biochar over time.

More specific research focused on biochar use and its soluble components, in terms of improving seed germination and root elongation, has been performed. Ahmad *et al*. (2012) showed that oak wood biochar, applied at 5% (w/w) in a Pb-contaminated soil, helped to improve lettuce seed germination and root length by 360% and 189%, respectively, as compared to the control; concomitantly, the biochar addition contributed to biologically active soil carbon. Lou *et al*. (2016) suggested that aqueous biochar extracts can contain organic moieties that increase crop yield and quality. The authors studied extracts from wheat straw and maize stalk biochars and observed the presence of aliphatic compounds, ketones, quinones, and aromatic humic substances; humic substances have been linked to promoting root growth (Eyheraguibel *et al*. 2008). Yang *et al*. (2020) quantified organic moieties released from biochar dissolved organic carbon phases and observed aliphatic, fulvic, and humic acid-like compounds; these compounds have been shown to stimulate seed germination (Zhang *et al*. 2020). Phoungthong *et al*. (2018) suggested that biochar leachate composition, and its effect on wheat seed germination, was dependent on the pyrolysis temperature; root growth appeared stimulated with extracts from biochars produced between a temperature in the range of 400 to 500 °C, yet root growth was reduced when the pyrolysis temperatures were increased to a range of 500 to 900 °C. However, the exact opposite effect on seedling growth and the pyrolysis temperature was observed by Kong *et al*. (2021). Fregolente *et al*. (2021) created hydrochar extracts *via* a phosphoric acid treatment and observed elevated bioavailable lignin-derived compounds. These compounds appeared to enhance corn seedling growth. However, others studying corn growth have found no significant seed germination and root elongation responses due to the presence of several different biochars or their organic compounds (Free *et al*. 2010).

Even given the above literature, biochar characteristics greatly vary as related to feedstock and pyrolysis temperature, and thus the mechanisms of biochar extract solutions on plant growth continue to remain unclear (Ippolito *et al*. 2020). In order to close the knowledge gap on this subject, biochars were prepared at the same temperature using four different feedstocks. Biochar solutions, using increasing biochar quantities extracted with 0.1 M HCl, were utilized to determine corn and rice germination, bud and root elongation, and seedling physiological indices. The novelty of this research lies in the comparison between seeds that have a true seed coat, *i.e.*, corn, or a fused seed coat, *i.e.*, rice, in the presence of the compounds found in the biochar extracts.

EXPERIMENTAL

Biochar Production and Extract Solution Preparation

Biochars were produced from rice straw (RSB), wheat straw (WSB), cotton straw (CSB), and *Spartina alterniflora* (SSB). All the feedstocks were air-dried and crushed with

a pulverizer (FW80, Taisite Instrument Company, Tianjin, China). Then the powder was passed through a 2 mm sieve and pyrolyzed at a temperature of 450 °C with 10 °C min^{-1} under a continuous flow of N_2 gas for 4 h. The ash content of the RSB, WSB, CSB, and SSB were determined as 35, 22, 11, and 18%, respectively.

After biochar creation, the biochar extract solutions were prepared by placing either 0 (W), 0.5 (X), 2.5 (Y), or 5 g (Z) of each biochar and 75 mL of a 0.1 M HCl solution in 125 mL plastic bottles, then shaking at 180 rpm at room temperature (25 °C) for 24 h. The supernatant was separated from the biochars *via* centrifugation at 10,000 rpm, then filtered twice through a glass fiber filter (Hill *et al*. 2019). The extract was used immediately for the seed germination and vigor test (as outlined below), with the remaining extract stored at a temperature of 4 °C for additional characterization and organic moiety analyses within one week. The extracted biochar was stored for subsequent organic functional group determination.

Seed Germination Rate, Bud, and Root Length Determination

The seed germination, bud, and root growth were determined using 50 seeds as a single replicate, with three replicates used for each biochar extract solution. The rice and corn seeds were randomly placed in petri dishes, and then 10 mL of the biochar extract solutions, adjusted to a pH of 6.5 using 0.1 M NaOH, was added. The seeds were incubated in a constant temperature incubator for 96 h at a temperature of 25 °C. After incubation, the number of germinated seeds was recorded, and all seedlings were measured with a vernier caliper to determine both the bud and root length.

Characterization of the Biochar Extract Solutions

The properties of the biochar extract solution were determined by the following methods recommended by the International Biochar Initiative, using three replicates of each biochar (Table 1) (IBI 2012). The solution was filtered through a glass fiber filter, and then the pH was measured using a glass electrode (pHS-3C, LEICI, Shanghai, China). Magnesium and calcium concentrations were determined by flame atomic adsorption spectrophotometry (FAAS) (TAS-990F, Beijing Purkinje General Instrument Co., Ltd., Beijing, China) (Ndour *et al*. 2008). The total nitrogen was determined *via* the alkaline potassium persulfate digestion UV spectrophotometric method. The total phosphorus was determined *via* the ammonium molybdate method followed by UV spectrophotometry analysis (TU-1901, Beijing Purkinje General Instrument Co., Ltd. China) (Liu *et al*. 2019).

The extract solutions were pretreated, and the organic phases present were determined as follows: 2 g of NaCl was added to 50 mL of the biochar extract solution and completely dissolved in a separatory funnel. Then, 4 mL of n-hexane was added, and the funnel was shaken *via* hand for 5 min followed by standing for 10 min. Then, the organic compounds in the aqueous phase was released from the bottom of the separatory funnel into a beaker. The organic compounds in the aqueous phase was extracted again following the same procedure. Afterwards, the organic phase was passed through a funnel containing anhydrous sodium sulfate to remove the water. The organic phase was subsequently transferred to a rotary evaporator (RE-52A, Shanghai Yarong Biochemical Instrument Factory, Shanghai, China) with N_2 gas blown across the liquid phase until 1 mL remained, and then the organic phases present in the solution were quantified *via* a Thermo Trace DSQ II gas chromatography-mass spectrometer (GC-MS) (TRACE 1310- ISQ, Thermo Fisher Scientific, Waltham, MA) equipped with a TG-5MS capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness). The column temperature was initially 80 °C for 1 min,

and then it increased to 350 °C at a rate of 10 °C/min. The carrier gas was helium at a flow rate of 1 mL/min, and the injection volume was 1 µL; the injector was operated in the split mode after sample injection (Cui *et al*. 2017a).

A portion of the biochar extract solution was placed in a 10 mm quartz cuvette. Then, the biochar extract solution organic moiety chemical characteristics were detected *via* three-dimensional excitation-emission matrix spectrophotometry (3DEEM) (Aqualog, HORIBA Scientific, Kyoto, Japan) with an excitation spectral range (λ_{Ex}) of 240 to 340 nm and emission spectral range of 213 to 450 nm; the width of the excitation and emission slits were both 5 nm, and the exposure time was 0.5 s.

Seedling Malondialdehyde (MDA) Content

Malondialdehyde (MDA) is the final decomposition product of the membrane lipid peroxidation (Morales and Munné-Bosch 2019). Malondialdehyde can loosen the bridge bonds between cellulose molecules, can inhibit protein synthesis, and thus can be used as a marker for oxidation stress. The seedling MDA content was determined according to the thiobarbituric acid (TBA) developing method (Shang *et al*. 2019). After the germination and bud length characteristics were determined, 1.0 g of fresh bud was placed in a mortar, 2 mL of 10% trichloroacetic acid and approximately 0.1 g of quartz sand was added, and the bud was ground with a pestle. Next, 8 mL of 10% trichloroacetic acid was added, followed again by grinding. The homogenate was placed into a 50 mL centrifuge tube and then centrifuged at 6000 rpm for 10 min. Next, 2 mL of the supernatant and 2 mL of a 0.6% thiobarbituric acid solution were added to another centrifuge tube, boiled for 15 min in a water bath, cooled, and centrifuged again. The MDA in the supernatant was quantified with a UV spectrophotometer (TU-1901, Beijing Purkinje General Instrument Co., Ltd. China) at 450, 532, and 600 nm.

Extracted Biochar Functional Groups Determination

The biochar functional groups, before and after extraction, were determined utilizing pulverized biochar and KBr powder to create pellets. The pellets were then analyzed for the presence of functional groups *via* ATR-FTIR (NEXUS-670, NICOLET, Thermo Nicolet Corporation, Waltham, MA) from 4000 cm^{-1} to 400 cm^{-1} with a resolution of 1.0 cm^{-1}.

Statistical Analysis

The data were expressed as the means ± standard error of the mean. Significant differences between the treatments were determined using analyses of variance (ANOVA) at a p-value less than 0.05, and when present, the differences between the means were determined using a Tukey post-hoc test. All statistical analyses were carried out using SPSS, version 22.0 (SPSS Institute, USA) and Origin 9.0 (Origin Lab, USA).

RESULTS AND DISCUSSION

Characterization of the Biochar Extract Solutions

The basic properties of the biochar extract solution, averaged across the three extracts replicates for a given biochar type, are shown in Table 1. After extraction with 0.1 M HCl (pH = 1.08), the biochar solution pH values ranged from 1.55 to 3.74. All the biochar extract pH values were greater than the initial extract solution pH, likely due to the alkaline materials created during pyrolysis. In a biochar meta-analyses review, Ippolito *et*

al. (2020) showed that as the pyrolysis temperature increased, the biochar pH increased. The authors also showed that biochar pH was greater than or equal to 8.5 when the pyrolysis temperature was greater than 400 °C; in the current study, the pyrolysis temperature was 450 °C. Increasing the pyrolysis temperature increases the ash content and concomitantly the biochar pH, as Ca and Mg carbonate and hydroxide phases are present within the ash (Yuan *et al.* 2011; Ippolito *et al.* 2020). This information also helps explain the relatively elevated Ca and Mg concentrations within the biochar extracts. The total organic C, N, and P contents in all the extracts were in-line with the values published by Ippolito *et al.* (2020).

Table 1. Basic Properties of Biochar Extract Solutions ($mg \cdot L^{-1}$)

	pH	TOC	TN	TP	Ca	Mg
RSB*	3.18 ± 0.05	35.82 ± 0.08	1.95 ± 0.04	5.65 ± 0.02	81.54 ± 0.00	3.18 ± 0.00
WSB	3.74 ± 0.09	47.76 ± 0.00	1.76 ± 0.00	6.5 ± 0.01	79.41 ± 0.00	3.33 ± 0.00
CSB	3.45 ± 0.01	59.7 ± 0.08	1.84 ± 0.03	8.86 ± 0.03	86.87 ± 0.00	3.20 ± 0.00
SSB	1.55 ± 0.01	23.88 ± 0.08	2.12 ± 0.03	9.28 ± 0.00	75.08 ± 0.00	3.30 ± 0.01
	K	S				
RSB*	18.24±0.45	7.90±0.01				
WSB	23.21±0.56	28.49±0.12				
CSB	19.21 ±0.67	24.70 ±0.23				
SSB	20.76±0.34	118.90±2.15				

* RSB = rice straw biochar; WSB = wheat straw biochar; CSB = cotton straw biochar; SSB = Spartina alterniflora biochar; TOC = total organic carbon; TN = total nitrogen; and TP = total phosphorus

The Effect of the Different Biochar Extract Solutions on the Corn and Rice Seed Germination, Bud, and Root Length

Various biochars and increasing biochar quantities in the extract solutions, and the subsequent effects of the extract solution on corn and rice germination rate (Table 2), bud, and root length, are shown in Fig. 1. The RSB biochar extract solutions Y and Z increased corn germination compared to the control, while CSB extract Z decreased the corn germination relative to the other solutions; all the WSB and SSB extract solutions had no effect on corn germination (Table 2). Results similar to those by RSB solutions Y and Z were found by Hille and Ouden (2005), who applied a phytotoxin with either granulated or powdered pine biochars to seedlings. The authors noted a positive germination response when the biochar was introduced to the solutions containing a relatively greater toxin concentrations compared to lower concentrations. Results similar to the WSB and SSB treatments were also found by Free *et al.* (2010), who used sludge-, corn stover-, and wood-biochar (at 550 °C) as well as by Kamara *et al.* (2014) who used corn stalk biochar, with all biochars applied to corn seeds; both sets of researchers observed no effect on corn germination. The RSB and CSB extract solution Y increased the corn bud length compared to the control, while WSB extract solution Z decreased the bud length over compared to the control; all other extract solutions, when compared to the control, affected the bud length similarly (Fig. 1a). The RSB extract solution X and Y, the CSB extract solutions Y and Z, and the SSB extract solution X increased the root length compared to the control (Fig. 1b). However, the WSB extract solutions Y and Z both decreased the root length compared to the control.

When the biochar extract effects were evident for rice germination, they were always expressed as a negative response as compared to the control (Table 2). A similar

response was observed for the rice bud and root length (as shown in Fig. 1c and Fig. 1d). Often, extract Y from all biochars, and always extract Z from all biochars, reduced the bud and root length when compared to the control. Obviously, rice seeds did not prefer solutions extracted with greater biochar quantities compared to corn seeds.

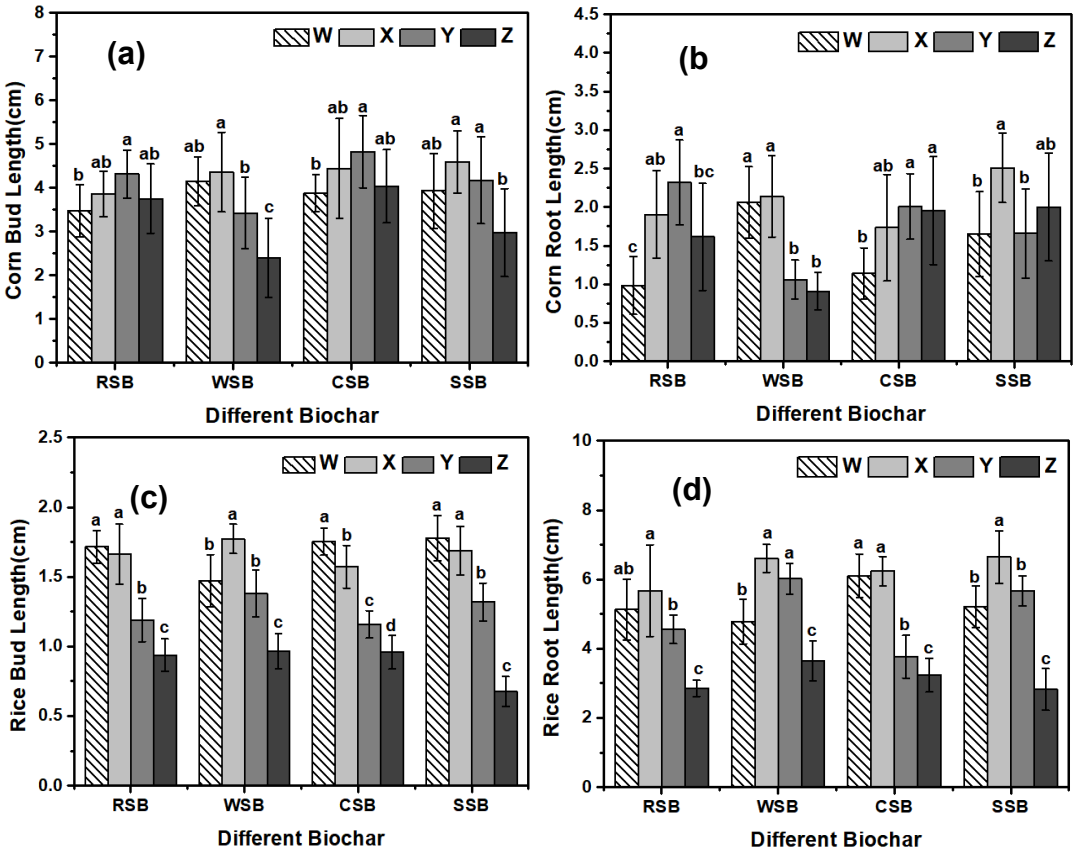

Fig. 1. The effect of the biochar extract solution [from rice straw (RSB), wheat straw (WSB), cotton straw (CSB), and *Spartina alterniflora* (SSB) biochars] on corn and rice growth [corn seed bud length (a), root length (b), and rice seed bud length (c), root length (d)] Note: different lower-case letters above error bars (standard deviation of the mean; n = 3; p-value less than 0.05, determined *via* a Tukey post-hoc test) indicate significant differences between the same biochar yet extracted with different biochar to solution ratios [Figure legends: 0 (W), 0.5 (X), 2.5 (Y), or 5 g (Z) of biochar to 75 mL of 0.1 M HCl]

Rice seeds are a fusion of the seed coat and embryo, while corn seeds contain a seed coat that protects the embryo. The lack of a true seed coat in rice likely did not protect the seed from the adverse effects of the compounds present within the biochar extracts. A similar response was reported by Oh *et al.* (2012), who suggested that pyrolysis may or may not remove substances toxic to seed germination. Thus, the results from the current study suggested that biochar and its extracts, and the potential detrimental effects, should be considered prior to its addition to certain seeds within cropping systems.

Table 2. Effect of Biochar Extract Solution on Corn and Rice Seed Germination Percentage

Seed	Biochars	Biochar Extract Solution Treatments			
		W	X	Y	Z
		Seed Germination (%)			
Rice	RSB	91.33±2.40a	82.67±0.67b	90.67±2.67a	89.33±4.06a
	WSB	91.33±5.21a	89.00±7.35a	90.67±2.40a	96.00±4.00a
	CSB	96.67±3.33a	84.67±2.91b	87.67±3.53b	94.00±3.46ab
	SSB	92.00±1.15a	96.00±1.76a	94.00±1.76b	88.00±2.00a
Corn	RSB	78.00±4.00b	88.00±4.16ab	83.33±2.67a	88.67±1.33a
	WSB	90.67±1.33a	92.67±2.00a	88±4.16a	85.33±3.71a
	CSB	91.33±1.76a	92.67±2.40a	90.67±3.33a	68.67±7.42b
	SSB	85.33±3.71a	89.33±1.76a	81.33±3.53a	76.67±7.69a

Different lower-case letters above error bars (standard deviation of the mean; n=3; $p<0.05$, determined via a Tukey post-hoc test) indicate significant differences between the same biochar yet extracted with different biochar to solution ratios.

Organic Moieties Present in the Biochar Extract Solutions

The biochar extract solutions contained a variety of water-soluble organic phases, including triethyl phosphate ($C_6H_{15}PO_4$), 2,4-bis (1,1-dimethylethyl)-phenol ($C_{15}H_{24}O$), and alkanes (C_nH_{2n+2} species), as determined by GC-MS (as shown in Table 3). Other studies have shown that the hydrophilic-fraction of biochar extract solutions primarily contains simple organic acids, carbohydrates, phenols, amino acids, and amino sugars, while the hydrophobic-fraction consists of hydrocarbons, fatty acids, nucleic acids, and quinones (Sun *et al.* 2021). These compounds may play positive or negative roles in terms of seed germination and plant growth. In the current study, the biochars contained various organic moieties, with those higher molecular weight compounds potentially promoting plant growth, as shown by (Henner *et al.* 1999). Li *et al.* (2015) found that corn stover biochar water extracts contained polycyclic aromatic hydrocarbon (PAH)-like compounds, *e.g.*, 2-ring (naphthalene) and 4-ring (chrysene) PAHs; these compounds improved tomato germination rates at relatively low doses but inhibited germination at higher doses.

Table 3. List of Organic Compounds in Biochar Extract Solutions as Determined *via* Gas Chromatography-Mass Spectrometer (GC-MS)

Number	Organic Compound			
	RSB*	WSB	CSB	SSB
1	$C_6H_{15}O_4P$	$C_6H_{15}O_4P$	$C_6H_{15}O_4P$	$C_6H_{15}O_4P$
2	$C_{15}H_{24}O$	$C_{15}H_{24}O$	$C_{15}H_{24}O$	$C_{15}H_{24}O$
3	$C_{14}H_{30}$	$C_{24}H_{50}$	$C_{20}H_{42}$	$C_{17}H_{36}$
4	$C_{15}H_{32}$	$C_4H_{12}Si$	$C_{16}H_{34}$	$C_{16}H_{34}$
5	$C_{12}H_{26}$	$C_{14}H_{30}$	$C_{15}H_{32}$	$C_{15}H_{32}$
6	$C_{22}H_{25}NO_6$	$C_{22}H_{25}NO_6$	$C_{17}H_{36}$	$C_{14}H_{30}$
7	$C_{16}H_{34}$	$C_{28}H_{58}$	$C_{24}H_{50}$	$C_{12}H_{26}$

* RSB = rice straw biochar, WSB = wheat straw biochar, CSB = cotton straw biochar, SSB = *Spartina alterniflora* biochar

Three-Dimensional Excitation-Emission Matrix Spectrophotometry Analysis

The biochar extract solution soluble organic compound spectral characteristics, as detected using the three-dimensional excitation-emission matrix (3DEEM), are presented in Fig. 2. The fluorescence intensity suggests that these biochar extract solutions primarily contained humic- and fulvic-like acids, and a tryptophan-like compound in the region of $\lambda_{Em}/\lambda_{Ex}$ = (220 to 370 nm) /(330 to 470 nm) (as shown in Table 4). However, these organic moieties were present to lesser or greater extents (based on the fluorescence intensity) depending on the biochar type. The organic moiety disparities present are most likely due to the feedstock choice, as shown by Rajapaksha *et al.* (2019), who used the same excitation technique.

The 3DEEM not only shows the change of the fluorescence intensity, but it also shows the change in the peak position varying across all four biochars (Table 4). The change in the peak position, with respect to changing the biochar type, suggests alterations in the organic moieties present. However, the biochar soluble organic compounds were primarily derived from humic acid-like compounds. Although this technique cannot distinguish the specific compound present, humic acid-like substances are aromatic, and these aromatic compounds could have played a role in the corn and rice seed germination, bud, and root length observations.

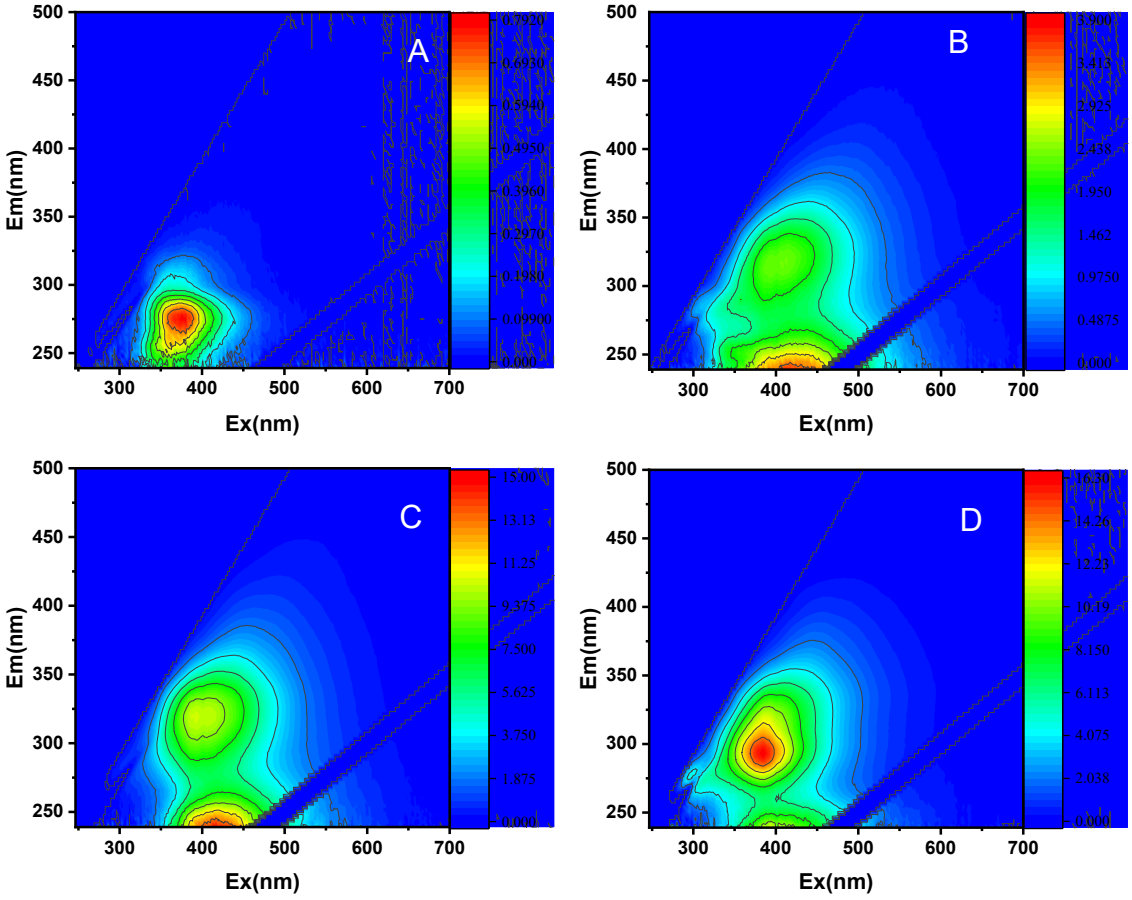

Fig. 2. Three-dimensional fluorescence spectrum of the water-soluble organic matter in the biochar extract solution (A: RSB, rice straw biochar; B: WSB, wheat straw biochar; C: CSB, cotton straw biochar; D: SSB, *Spartina alterniflora* biochar)

Table 4. Characteristics of Organic Matter Components in Different Biochars Extract Solutions

Biochar Species	Component	λ_{Em} / λ_{Ex}	Signal Strength
RSB	UVA humic-like	419/254	0.199
	UVC humic-like	424/305	0.077
	Tryptophan-like	340/275	0.381
	Fulvic acid-like	368/245	0.442
WSB	UVA humic-like	428/248	3.049
	UVC humic-like	484/320	1.147
	Fulvic acid-like	378/240	2.966
CSB	UVA humic-like	424/245	12.787
	UVC humic-like	437/326	8.679
	tryptophan-like	340/275	2.252
	Fulvic acid-like	516/269	2.904
SSB	UVA humic-like	414/239	10.507
	UVC humic-like	479/353	2.458
	Tryptophan-like	340/275	6.726
	Fulvic acid-like	368/245	7.069
RSB = rice straw biochar, WSB = wheat straw biochar, CSB = cotton straw biochar, SSB = Spartina alterniflora biochar			

Others have shown that relatively high concentrations of biochar extracts inhibited seed vitality, primarily due to relatively high organic compound concentrations present (Xia *et al.* 2020). These authors contended that the antagonistic effect on seed vitality was likely due to the presence of bio-oil, *i.e.*, aromatic compounds, in the biochar, creating biological toxicity. Oppositely, Fregolente *et al.* (2021) found that the benzene derivatives and phenolic compounds, *i.e.*, both aromatic compounds, present in the biochars were responsible for stimulating the shoot and root elongation.

Malondialdehyde (MDA) Content of the Seed Generation

Malondialdehyde is one of the major products of lipid peroxidation, and its accumulation reflects the degree of damage from oxidative stress (Jia *et al.* 2019). Malondialdehyde is typically employed as a general indicator to evaluate the extent of lipid peroxidation resulting from oxidative stress, which can be related to antioxidant enzyme activity. The MDA content in the corn or rice buds, as a function of biochar extract type, is presented in Fig. 3. In general, the MDA content of the corn and rice buds usually increased as the biochar extract ratio increased. In the RSB and CSB X, Y, and Z extract solutions, the MDA content in corn bud was increased by 24.6% to 62.9% compared to the control (Fig. 3a). However, the corn bud MDA content decreased with WSB Z solution and with the increasing SSB solutions, compared to the control. In the rice buds, the MDA content increased by 2.8% to 59.9% in all biochar extract solutions Y and Z, compared to the control (Fig. 3b). This result suggests that greater oxidative stress was present with greater biochar extract solution treatments.

In general, in both corn and rice, the MDA content remained relatively low in the presence of low biochar extract solution concentrations. As suggested by others, this may indicate that the reactive oxygen species, *i.e.*, indicators of oxidative stress, were effectively eliminated by antioxidant enzymes (Li *et al.* 2013) or helped induce systemic resistance to oxidative stress (Graber *et al.* 2010). In addition, in general, in both the corn and rice, the MDA content was relatively elevated in the presence of high biochar extract solution concentrations. This may have been due to the increased presence of aromatic

organic moieties that lead to oxidative stress. Similarly, Li *et al.* (2015) found that MDA content and antioxidant enzymatic activity in tomato seedling leaves and roots increased as the biochar extract solution concentrations increased, potentially due to the presence of PAHs. It is interesting to note that the work from Li *et al.* (2015) suggested that the toxicity to seedlings may be alleviated as the plants mature.

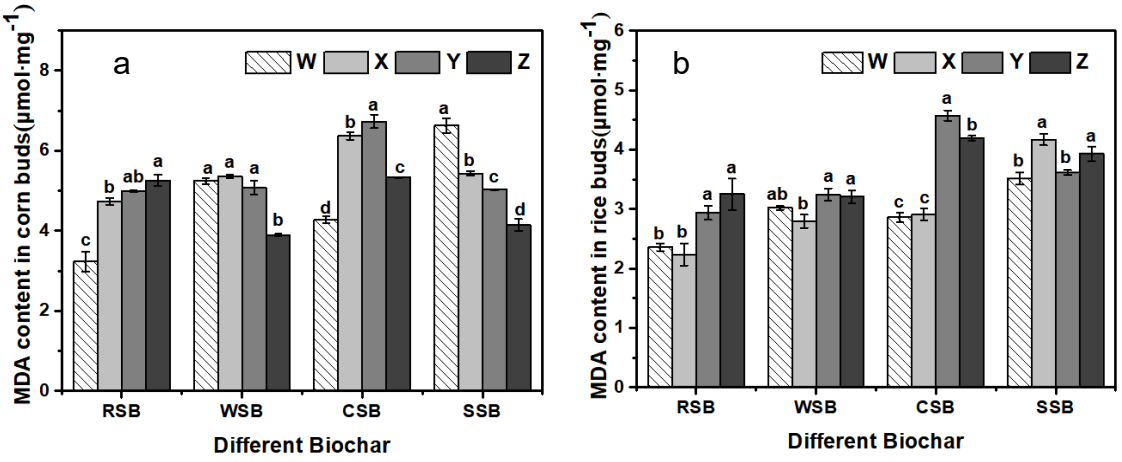

Fig. 3. The effect of the biochar extract solution [from rice straw (RSB), wheat straw (WSB), cotton straw (CSB), and *Spartina alterniflora* (SSB) biochars] on the content of malondialdehyde (MDA) in buds of corn (a) and rice (b). Note: different lower-case letters above error bars (standard deviation of the mean; n = 3; *p*-value less than 0.05, determined *via* a Tukey post-hoc test) indicate significant differences between the same biochar yet extracted with different biochar to solution ratios [Figure legends: 0 (W), 0.5 (X), 2.5 (Y), or 5 g (Z) of biochar to 75 mL of 0.1 M HCl]

Biochar Functional Groups

The presence of biochar surface functional groups in fresh or extracted biochars, as determined *via* Fourier-transform infrared spectroscopy (FTIR), are presented in Fig. 4a through 4d. In general, peak comparisons between the fresh and extracted biochars varied greatly, which likely indicated a stripping of the biochar-borne surface organic functional groups with subsequent accumulation of the biochar extracts. This observation further supports the findings presented above with respect to the presence of organic moieties in biochar extracts.

Table 5 outlines the FTIR peak assignments, which are presented in Fig 4a through 4d. Specifically, the rice, wheat, cotton, and *S. alterniflora* biochars and extracted biochars had peaks at 3430, 2858, 1586, and 1150 cm^{-1}, yet the peak intensities were different and additional peaks were present in the fresh biochars. Fresh biochars contained more functional groups, especially from 673 to 1673 cm^{-1}, which were lost in the extracted biochars. Various peaks, *e.g.*, 1558 and 1409 cm^{-1} in RSB cm^{-1}, 1409, 871, and 755 cm^{-1} in WSB, 1411, 1012, 869, and 750 cm^{-1} in CSB, and 1074, 863, 809, and 754 cm^{-1} in SSB, were present in fresh biochars but lost in extracted biochars. The peak located at 1037 to 1078 cm^{-1} was typically greater in the extracted biochars, likely due to the C-O-C or alcohol -OH group enhancement on the biochar surface. The peak at 3430 cm^{-1} was the result of the -OH stretching vibration of phenolic hydroxyl or aliphatic groups, with this peak becoming more pronounced in extracted biochars; a similar phenomenon was found with the peak at 1579 to 1612 cm^{-1}, which is likely due to increased aromatic C=C stretching on the extracted biochar (Parshetti *et al.* 2012). Choudhary *et al.* (2017) found similar shifts

between the fresh and washed *Eucalyptus* bark biochar; a rightward shift (10 cm^{-1}) and enhancement of the 1621 cm^{-1} peak, the disappearance of peaks 1412 and 1370 cm^{-1}, and suppression of the 874 cm^{-1} peak. The authors also noted a new peak located at 1384 cm^{-1}, which corresponded to carboxylate C=O stretching in the fresh biochar, potentially indicating a carboxylic acid compound in the extracted biochar.

Table 5. Band Assignments in the Fourier-transform Infrared (FTIR) Spectra of the Fresh and Extracted Biochars

Bands (cm^{-1})	Assignments	References
3400 to 3320	-OH stretching	Chen *et al*. (2015)
3000 to 2800	Aliphatic CH stretching	Cui *et al*. (2017b)
1630 to 1700	Aromatic carbonyl/carboxyl C=O/C=C stretching	Cimò *et al*. (2014)
1430 to 1420	Aromatic C=C stretching	Cui *et al*. (2019)
1000 to 1157	C-O-C	Fan *et al*. (2018)
840 to 880	Glucoside CH_2 deformation	Mia *et al*. (2017)
750 to 820	Aromatic rings	Li *et al*. (2017)

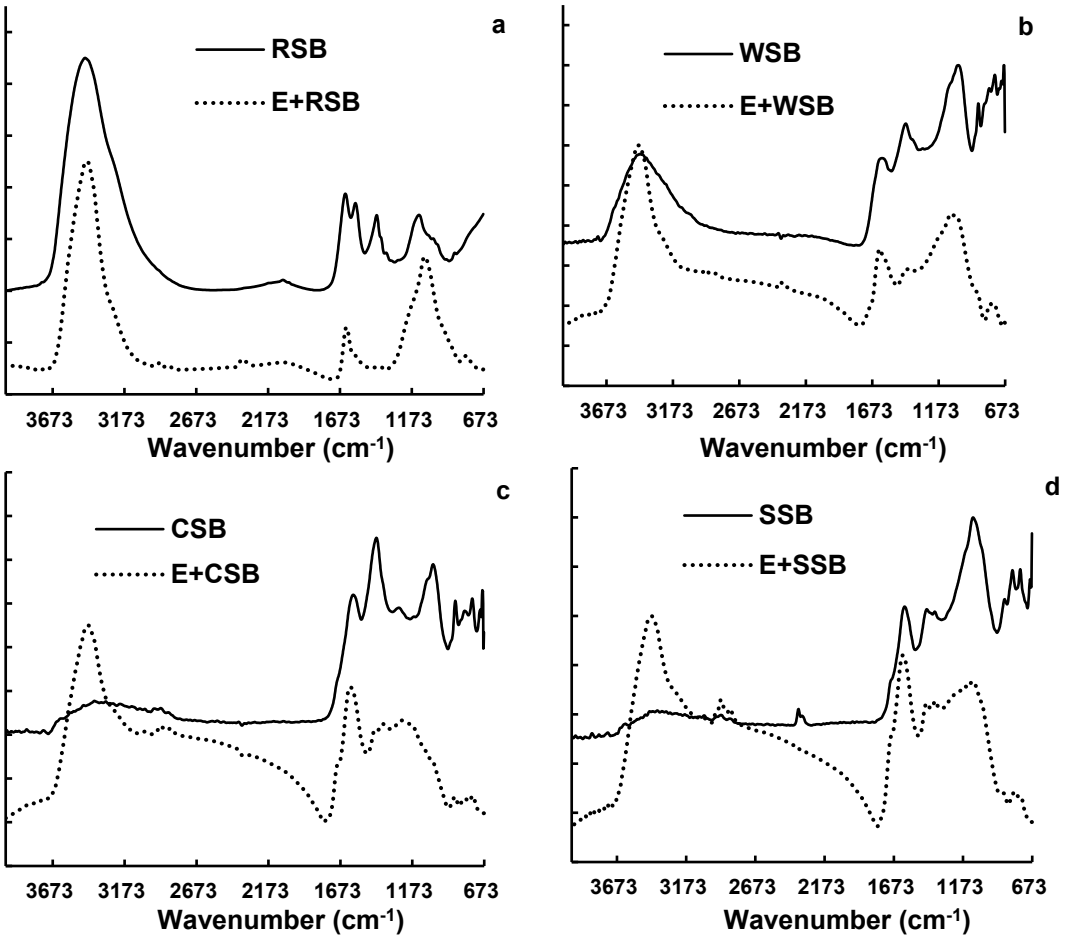

Fig. 4. The changes in the fresh and extracted (E+) biochar functional groups (Note: RSB = rice straw biochar (a), WSB = wheat straw biochar (b), CSB = cotton straw biochar (c), SSB = Spartina alterniflora biochar (d))

CONCLUSIONS

1. Increasing the amounts of biochar in the extracts tended to improve the corn seed bud and root length; however, the opposite was observed in rice seeds. This was likely a function of the corn seeds containing a seed coat, and thus the seed embryo was protected from growth-inhibiting organic compounds in the biochar extracts.
2. A variety of the organic moieties present in the biochar extract solutions were quantified *via* FTIR and 3DEEM analysis, *e.g.*, triethyl phosphate, 2,4-bisphenol, alkanes, and other humic-like aromatic compounds, which likely influenced the corn and rice bud and root growth.
3. It was possible that the presence of PAHs may have enhanced or inhibited seed growth at either low or high concentrations, respectively, with MDA analysis supporting this contention.
4. Determining the presence of the biochar organic moieties may help with designing biochars for enhanced seed germination, growth, and crop productivity.

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