Supplementary Material

Role of nano-Biochar in attenuating allelopathic effects from *Imperata cylindrica* on rice seedlings

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Content

Table S1 Kimura B nutrient solution (μmol L^{-1})

Composition	Concentration
KH_2PO_4	90
MgSO4	270
$(NH_4)_2SO_4$	180
KNO_3	90
$Ca(NO_3)_2$	180
H_3BO_3	3
$MnCl_2$	0.5
$(NH_4)_6Mo_7O_{24}$	1
$ZnSO_4$	0.4
Fe(III)-EDTA	20

Table S2 DNA primer information

Gene name	Consequence
*OsNCED1*_F	AGCCTCGGTCTTCCAATTTT
*OsNCED1*_R	CACCCAACACAAAAGCTACG
*OsABA8ox1*_F	AAGCTGGCAAAACCAACATC
*OsABA8ox1*_R	CCGTGCTAATACGGAATCCA
*OsPR10a*_F	GCTACAGGCATCAGTGGTCA
*OsPR10a*_R	GACTCAAACGCCACGAGAAT

Nano-biochar preparation and physical characteristics

Biochar preparation

Fresh wheat, rice and corn straws collected from Jiangning District, Nanjing, China, were air dried and cut to small pieces (30-50 mm) and separated into six groups. Known quantity of air-dried material was taken in closed perforated stain less steel boxes and heated in muffle furnace at two different temperatures (350 and 650 ^{o}C) for 120 min, and 35% of biomass was converted to biochar. When the boxes were cooled down, six biochars were obtained. The biochar preparation method was followed by Zhang et al. (2012)[1].

Nano particle preparation and physical characteristics

Planetary Ball Mill (TJ-800D, Tianjing, China) was applied to prepare nano biochar particles. The biochar powders were inserted into a planetary ball mill made from stainless steel with diameter of 3-15 mm Tungsten carbide (WC)-Co ball, and ethanol worked as grinding aid in this system. The weight ratio between ball and powder was 15:1. The vials were rotated at 350 rpm for 12 h. The method was followed by Hong et al. (2015). After milling, the powders were reduced in H_2 atmosphere at 150 °C, and then we tested these twelve particles physical characteristics.

Particle size, pH and electrical conductivity

All powders' samples (six biochar particles and their corresponding nano-particles) were characterized with particle sizes by Particle Size Analyser (90 Plus, Brookhaven, USA) and electrical conductivity and pH by Electronic Conductor

(Accumet XL200, Fisher Scientific,USA). The detailed information is below in Table S3.

Table S3 Biochar physical characteristics

Biochar Label	Description	Particle size (nm)	Zeta Potential (mV)	pH	Electric Conductivity (μS/cm)
R3	Rice straw - 350℃ treated	1776.6	-35.39	9.15	58.54±0.45
R6	Rice straw - 650℃ treated	1275.6	-34.05	9.18	55.49±0.14
W3	Wheat straw - 350℃ treated	2560.3	-34.30	9.36	65.19±0.34
W6	Wheat straw - 650℃ treated	2119.1	-33.62	9.37	60.55±6.64
C3	Corn straw - 350℃ treated	1029.1	-31.99	9.24	61.01±0.31
C6	Corn straw - 650℃ treated	1479.7	-35.92	9.23	57.75±0.91
N1	Nano rice biochar - 650℃ treated	201.7	-38.58	9.44	78.43±0.64
N2	Nano wheat biochar - 650℃ treated	224.2	-41.17	9.43	96.70±0.82
N3	Nano rice biochar - 350℃ treated	253.2	-43.80	9.40	74.75±0.82
N4	Nano wheat biochar - 350℃ treated	284.6	-38.80	9.41	87.83±3.56
N5	Nano corn biochar - 350℃ treated	259.7	-38.47	9.42	72.38±2.49
N6	Nano corn biochar - 650℃ treated	252.1	-39.96	9.41	94.36±3.57

Note: Label, biochar sample shorthand.

Ash content and element analysis of particles

We used high temperature calcination to test the ash content of each biochar sample and applied Elemental Analyzer (EA2400II, PerkinElmer, USA) to analyze the C, H, O, N, and S contents in biochar particles. The detailed information is below.

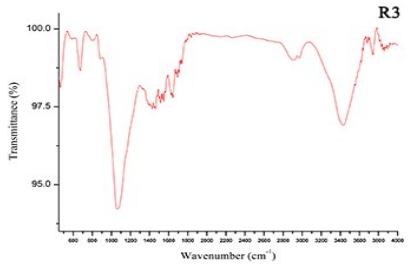
Table S4 Biochar and nano-biochar particle ash content and element (C, H, O, N and S) contents

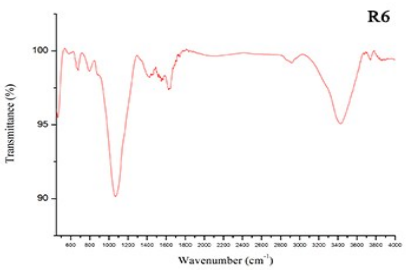
Label	Ash contents (%)	C (%)	H (%)	O (%)	N (%)	S (%)
R3	20.23	48.82	1.92	7.59	0.87	0.72
R6	16.10	49.58	1.81	7.69	0.88	0.30
W3	32.26	62.99	2.01	8.02	1.46	0.30
W6	34.40	64.67	2.05	7.56	1.48	0.27
C3	27.16	62.13	1.68	7.22	1.38	0.45
C6	30.08	62.82	1.48	7.46	1.32	0.41
N1	37.90	54.56	1.56	7.52	1.10	0.40
N2	33.62	56.24	1.68	7.68	1.19	0.33
N3	27.33	60.75	1.79	8.27	1.27	0.62
N4	31.95	56.99	1.82	7.90	1.25	0.14
N5	42.18	51.21	1.68	6.91	1.06	0.24
N6	34.09	55.36	1.78	7.61	1.15	0.28

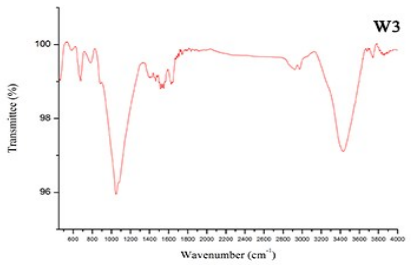
Note: Label, biochar sample shorthand.

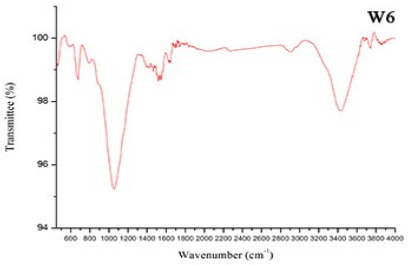
Infrared Spectra

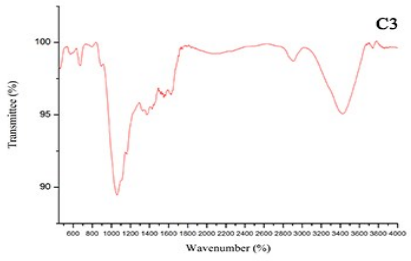
In addition, we also recorded the infrared spectra (FT-IR) of all biochar samples for our study. We applied FT-IR Spectrometer (Spectrum One, PerkinElmer, USA) to test the biochar samples' FT-IR. The information is below.

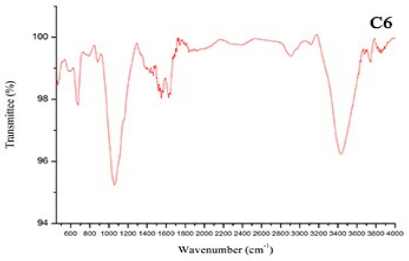

R3
Transmittance (%)
Wavenumber (cm⁻¹)

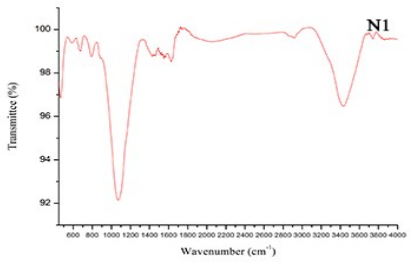

R6
Transmittance (%)
Wavenumber (cm⁻¹)

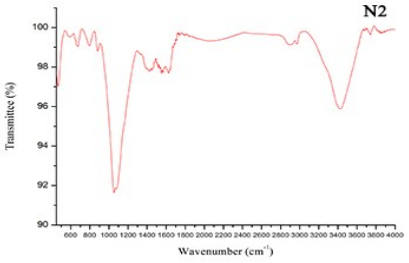

W3
Transmittee (%)
Wavenumber (cm⁻¹)

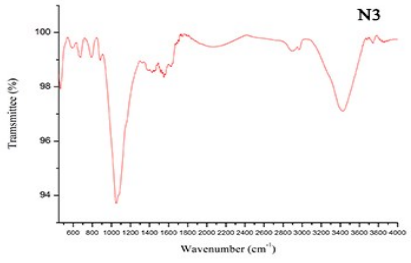

W6
Transmittee (%)
Wavenumber (cm⁻¹)

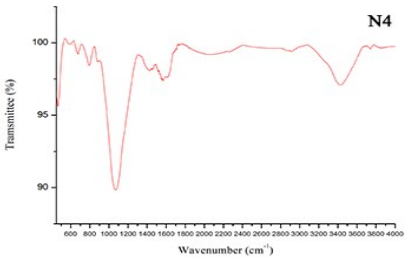

C3
Transmittee (%)
Wavenumber (%)

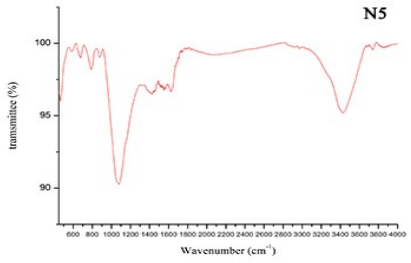

C6
Transmittee (%)
Wavenumber (cm⁻¹)


N1
Transmittee (%)
Wavenumber (cm⁻¹)


N2
Transmittee (%)
Wavenumber (cm⁻¹)


N3
Transmittee (%)
Wavenumber (cm⁻¹)


N4
Transmittee (%)
Wavenumber (cm⁻¹)


N5
transmittee (%)
Wavenumber (cm⁻¹)

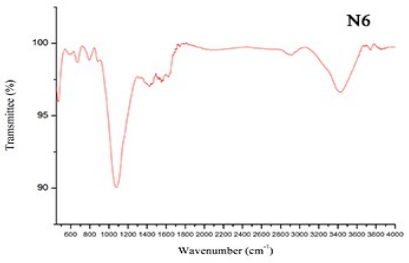

N6
Transmittee (%)
Wavenumber (cm⁻¹)

Figure S1 the infrared spectra (FT-IR) of all biochar samples

Note: R3, Rice straw – 350 ℃ treated; R6, Rice straw – 650 ℃ treated; W3, Wheat straw – 350 ℃ treated; W6, Wheat straw – 650 ℃ treated; C3, Corn straw – 350 ℃ treated; C6, Corn straw – 650 ℃ treated; N1, Nano rice biochar – 650 ℃ treated; N2, Nano wheat biochar – 650 ℃ treated; N3, Nano rice biochar – 350 ℃ treated; N4, Nano wheat biochar – 350 ℃ treated; N5, Nano corn biochar – 350 ℃ treated; N6, Nano corn biochar – 650 ℃ treated)

Biochar screening experiment for ferulic acid adsorption

In this study, we needed to find a best biochar particle for the further experiment. We applied the adsorption experiment to find the best particles for ferulic acid adsorption.

We weighted 200 mg of each biochar particle, prepared 800 mg L^{-1} ferulic acid (FA) solution [2], and adjusted the solution pH to 5.5 which is the best pH for rice culture in agriculture [3]. Then the mixture was agitated on a reciprocating shaker at room temperature (25 ± 2 ^{o}C) with the rotating speed of 120 rpm. The mixture solution was collected at 4, 8, 16, 24, 48, 72 and 96 h, and 1 mL supernatant was taken from the solution. FA concentration was determined by HPLC. We calculated and fitted the particle adsorption curves of 12 samples on FA in Fig. S1.

In our results (Fig. S1), we found that N5 (Nano corn biochar – 350 ^{o}C treated) is the best biochar particle to adsorb FA in solution. This particle could adsorb about 3.4 g FA per 1g particles, and the adsorption rate is about 85% at 96 h. Therefore, we selected nano corn biochar treated with 350 ^{o}C for the further experiment in this study.

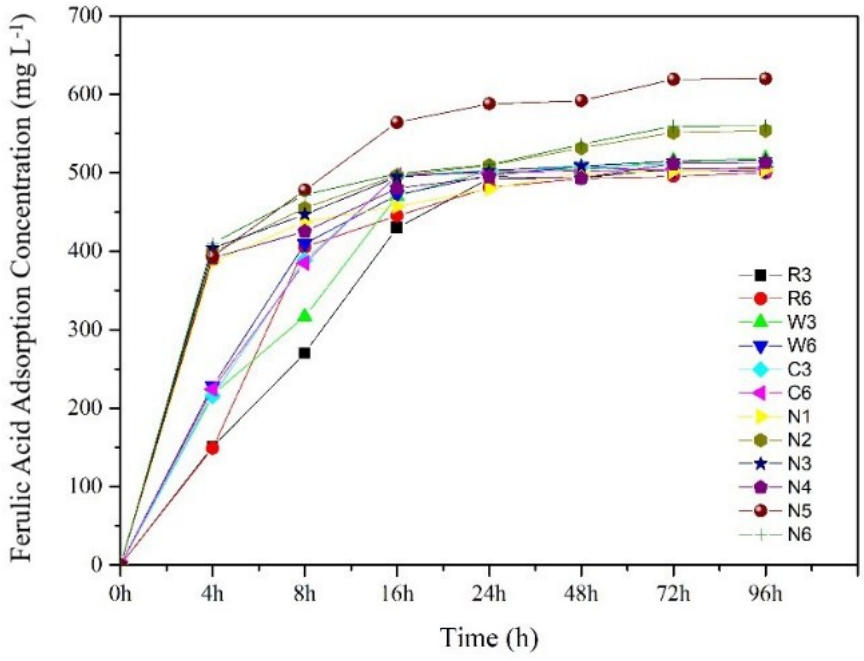


Figure S2 Ferulic acid adsorption curve by biochar particles in 96 h.

Note: R3, Rice straw – 350 ℃ treated; R6, Rice straw – 650 ℃ treated; W3, Wheat straw – 350 ℃ treated; W6, Wheat straw – 650 ℃ treated; C3, Corn straw – 350 ℃ treated; C6, Corn straw – 650 ℃ treated; N1, Nano rice biochar – 650 ℃ treated; N2, Nano wheat biochar – 650 ℃ treated; N3, Nano rice biochar – 350 ℃ treated; N4, Nano wheat biochar – 350 ℃ treated; N5, Nano corn biochar – 350 ℃ treated; N6, Nano corn biochar – 650 ℃ treated)

At the end of 30 days of treatment, we applied external standard method with standard ferulic acid via HPLC to test the final ferulic acid concentration in the solution. The method was described by Sellappan et al. (2002), and with some modification. The detailed information is below in Table S5.

Table S5 Final ferulic acid concentration in hydroponic experiment (30th day)

	CK	SA	FA	Nano-BC	FA+0.5 nano-BC	FA+ nano-BC
concentration (mg L^{-1})	N.D.	N.D.	350.5 ± 7.10	N.D.	284.1 ± 10.07	112.8 ±8.32

Note: CK, control; FA, 400 mg L^{-1} ferulic acid; FA+0.5 nano-BC, 400 mg L^{-1} FA + 200 mg L^{-1} nano-BC; FA+ nano-BC, 400 mg L^{-1} FA + 400 mg L^{-1} nano-BC. Data are shown as mean ± standard deviation. N.D., not detected.

References

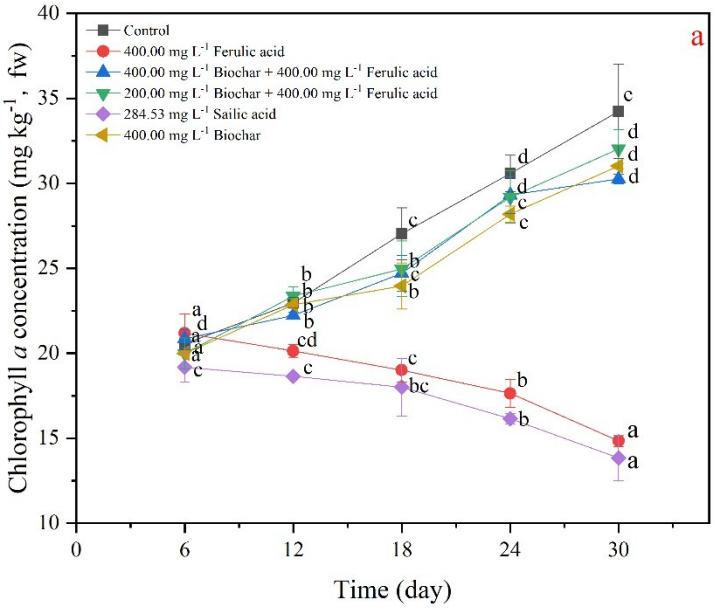
1. A. Zhang, R. Bian, G. Pan, L. Cui, Q. Hussain, L. Li, J. Zheng, J. Zheng, X. Zhang, X. Han and X. Yu, Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: A field study of 2 consecutive rice growing cycles, Field Crop. Res., 2012, 127, 153-160.
2. F. L. Mota, A. J. Queimada, S. P. Pinho and E. A. Macedo, Aqueous solubility of some natural phenolic compounds, Ind. Eng. Chem. Res., 2008, 47, 5182-5189.
3. J. Yan, P. Wang, P. Wang, M. Yang, X. Lian, Z. Tang, C. F. Huang, D. E. Salt and F. J. Zhao, A loss-of-function allele of OsHMA3 associated with high cadmium accumulation in shoots and grain of Japonica rice cultivars, Plant Cell Environ., 2016, 39, 1941-1954.
4. S. Sellappan, C. C. Akoh, G. Krewer, Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries, *J. Agr. Food Chem.*, 2002, 50, 2432-2438.

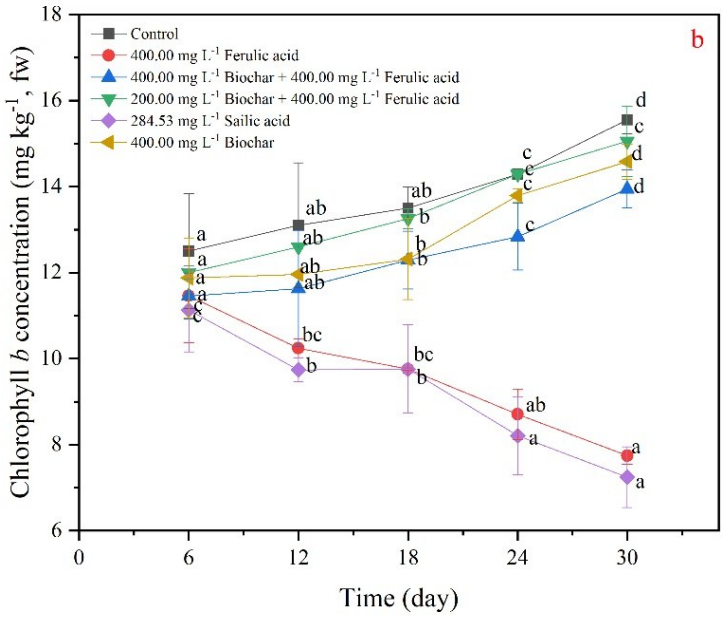
Chlorophyll *a* and chlorophyll *b* concentration and root length measurement

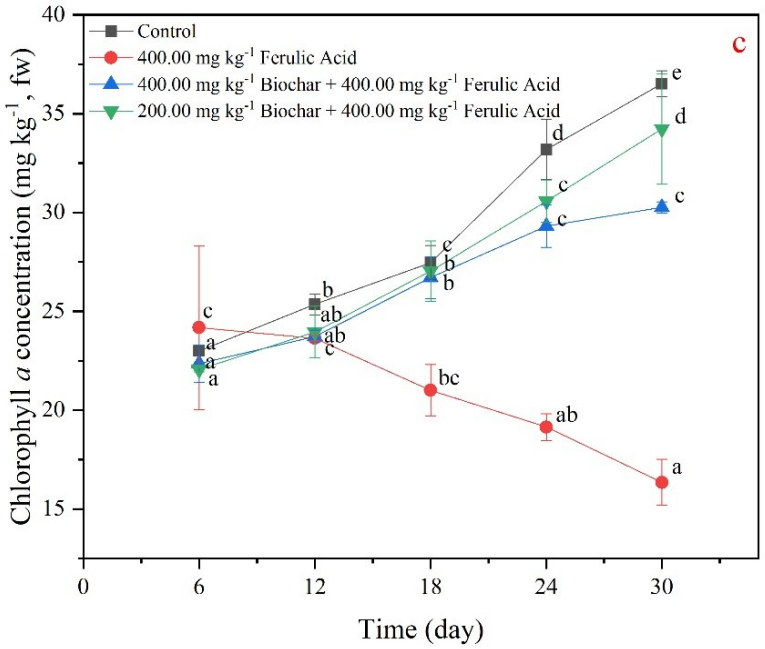
In this study, we tested the chlorophyll concentration changes in rice seedlings in pot and hydroponic experiments. We recorded the chlorophyll concentrations at day 6, 12, 18, 24 and 30. For chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) measurements, 0.5 g fresh rice leaf samples were extracted with 10 mL ethanol/95% acetone (1:1, v/v) mixture in darkness for 48 h. Colorimetry was used to measure chlorophyll concentrations (Moran, 1982), and the formula are below:

Chl $a = 12.21 \times OD_{664} - 2.81 \times OD_{647}$;

Chl $b = 20.13 \times OD_{647} - 5.03 \times OD_{664}$.




b
Control
400.00 mg L-1 Ferulic acid
400.00 mg L-1 Biochar + 400.00 mg L-1 Ferulic acid
200.00 mg L-1 Biochar + 400.00 mg L-1 Ferulic acid
284.53 mg L-1 Sailic acid
400.00 mg L-1 Biochar
Chlorophyll b concentration (mg kg-1, fw)
Time (day)


c
Control
400.00 mg kg-1 Ferulic Acid
400.00 mg kg-1 Biochar + 400.00 mg kg-1 Ferulic Acid
200.00 mg kg-1 Biochar + 400.00 mg kg-1 Ferulic Acid
Chlorophyll a concentration (mg kg-1, fw)
Time (day)

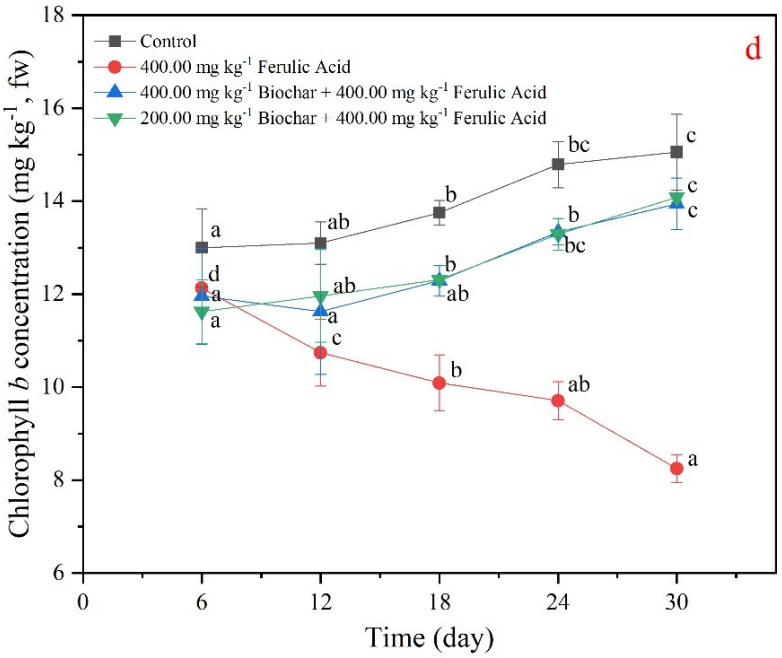


Figure S3 Chlorophyll *a* and chlorophyll *b* concentrations (mg kg^{-1}) in rice seedlings under salicylic acid, ferulic acid and nano-biochar added treatments in hydroponic experiment (a); and under ferulic acid and nano-biochar added treatments in pot experiment (b) after 30 days. (fw, fresh weight). Bars with different letters are significantly different (One-way ANOVA with a *Ducan* test).

We also recorded the root length at day 6, 12, 18, 24 and 30 in hydroponic experiment.

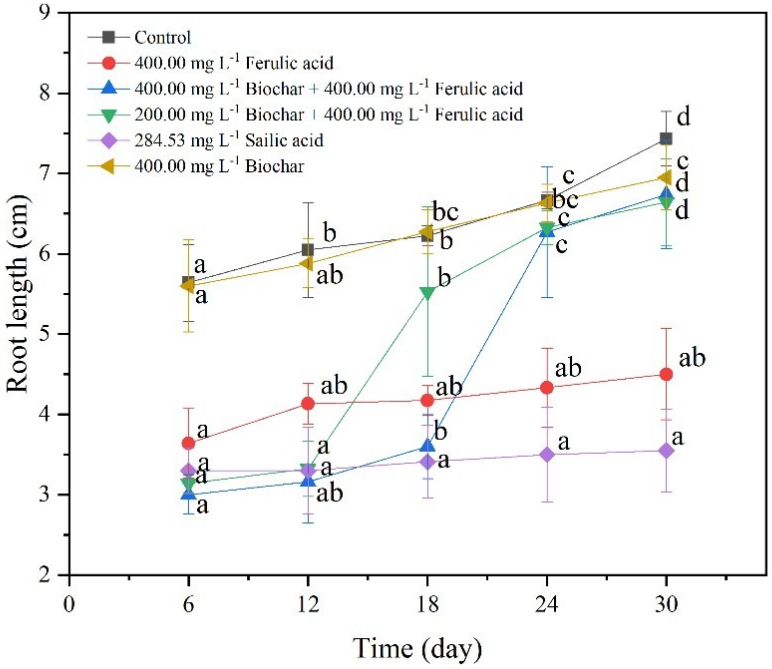


Figure S4 Root length of rice seedlings under salicylic acid, ferulic acid and nano-biochar added treatments in hydroponic experiment (a); and under ferulic acid and nano-biochar added treatments in pot experiment (b) after 30 days. Bars with different letters are significantly different (One-way ANOVA with a *Ducan* test).

Reference

Moran, R., 1982. Formulae for determination of chlorophyllous pigments extracted with N, N-dimethylformamide. Plant Physiol. 69, 1376-1381.