

Supplemental Information

Biochar nanoparticle-induced plant immunity and its application with the elicitor methoxyindole in *Nicotiana benthamiana*

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Table S1 Properties of the biochar particles

Biochar label	Description Particle size in water (nm)	Zeta Potential (mV)	pH	Electric Conductivity ($\mu\text{S}/\text{cm}$)
Corn stalks- 350°C treated	1029.1	-31.99 $\pm$ 3.22	9.24	61.01 $\pm$ 0.31
Corn stalks- 650°C treated	1479.7	-35.92 $\pm$ 1.54	9.23	57.75 $\pm$ 0.91
Nano corn biochar - 350°C treated	259.7	-38.47 $\pm$ 2.11	9.42	72.38 $\pm$ 2.49
Nano corn biochar - 650°C treated	252.1	-39.96 $\pm$ 2.92	9.41	94.36 $\pm$ 3.57

Table S2 Biochar and nano-biochar particle ash content (%) and C (%), H (%), O (%), N (%) and S (%)

	Ash content (%)	C (%)	H (%)	O (%)	N (%)	S (%)
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
N3	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

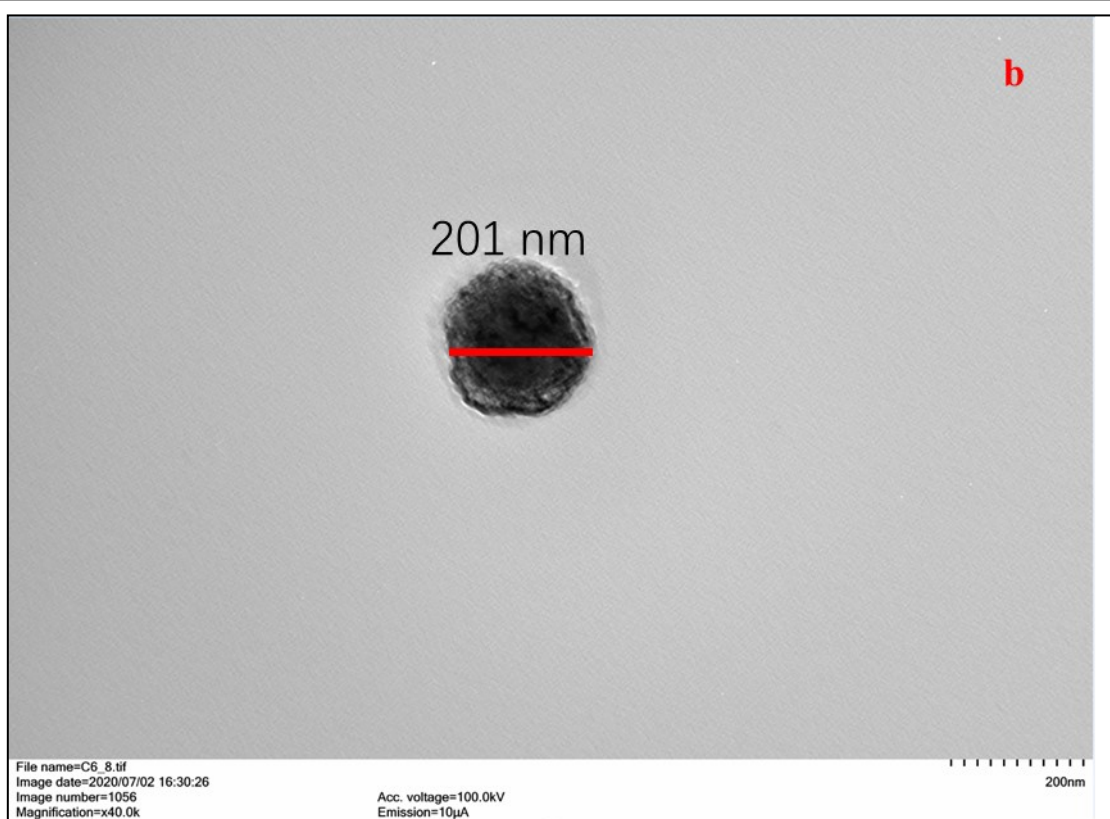
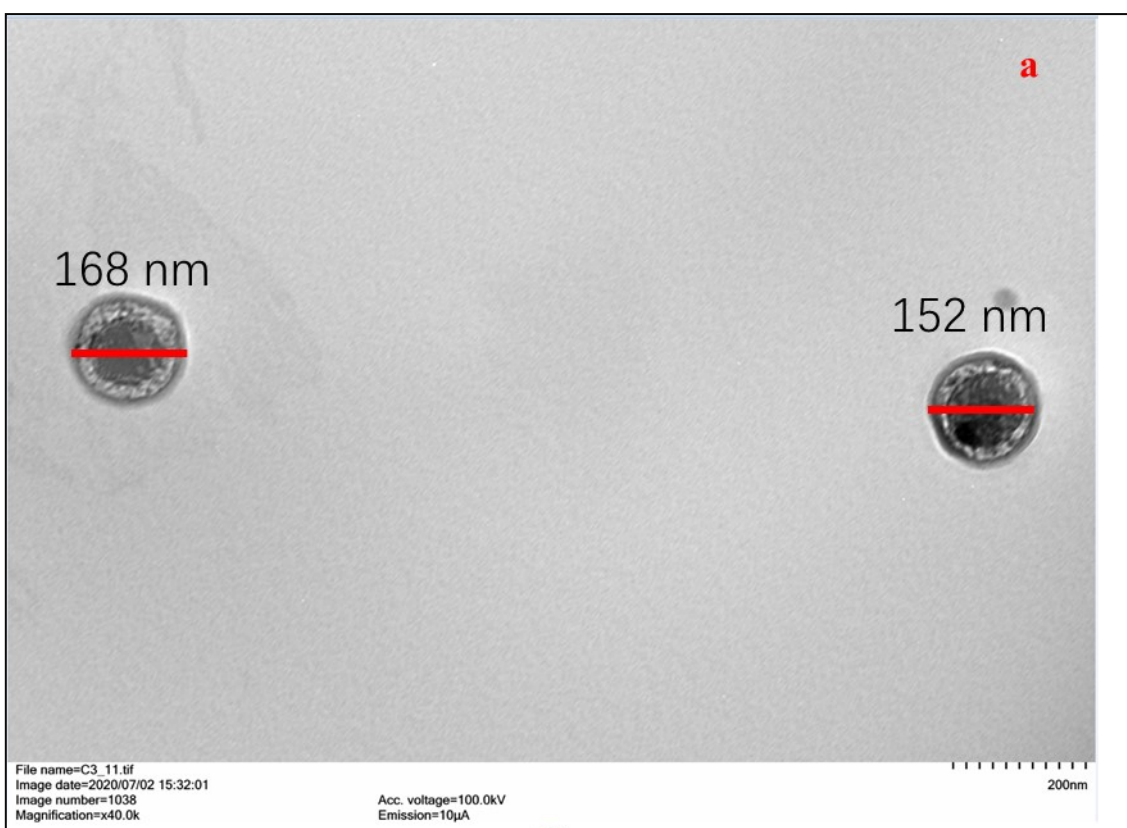


Figure S1 Transmission electron microscopy (TEM) images of biochar nanoparticles.
(Note, a, Nano corn biochar - 350°C treated; b, Nano corn biochar - 650°C treated.)



Figure S2. Images of *Nicotiana tabacum* growing in the presence of *Phytophthora nicotianae* after treatment with biochar particles.

(Note, from left to right, control; 350 °C synthesized biochar treatment; 650 °C synthesized biochar treatment; 350 °C synthesized biochar nanoparticle treatment; 650 °C synthesized biochar nanoparticle treatment.)

We applied FT-IR Spectrometer (Spectrum One, PerkinElmer, USA) to test the biochar samples' FT-IR. The information is below,

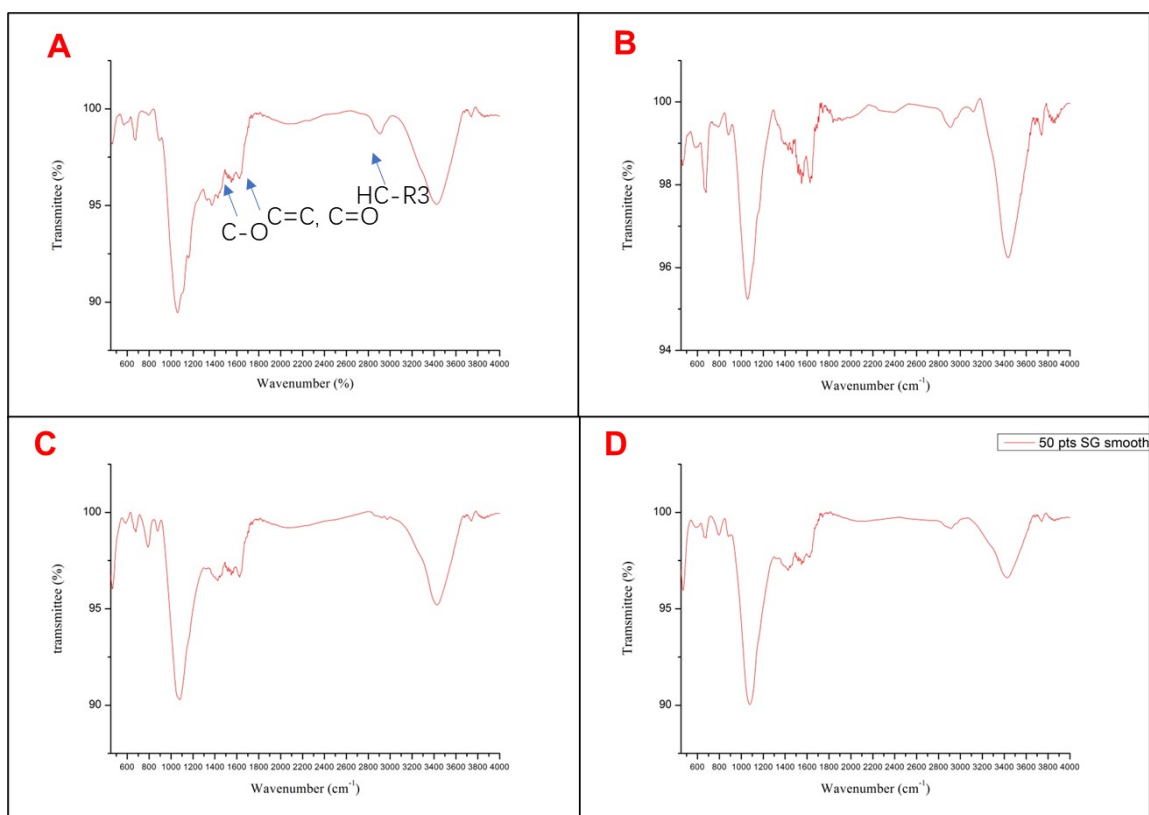


Figure S3 The infrared spectra (FT-IR) of all biochar samples

Note, A, C3, 350 °C prepared biochar; B, C6, 650 °C prepared biochar; C, N3, 350 °C prepared biochar nanoparticle; and, D, N6, 650 °C prepared biochar nanoparticle.

Table S3 Oligo DNA primers used in RT-PCR and qRT-PCR analysis in this study

Name		Sequence (5'-3')	
<i>EF1α</i> -F	ATGATTACTGGTACCTCCCG	<i>EF1α</i> -R	ACCTAGCCTTGGAATACTTG
<i>rbohA</i> -F	GAAGGCGGAGTTAAGGAGAT	<i>rbohA</i> -R	GAGCTCTATGAGCGCTGGA A
<i>rbohB</i> -F	GTGATGCTCGTTCTGCTCTT	<i>rbohB</i> -R	CTTTAGCCTCAGGGTGGTTG
<i>NIA1</i> -F	CATTCCTCGACGTGAAAGGT	<i>NIA1</i> -R	AGAATTGCCTGCATGACTTG
<i>NIA2</i> -F	GTGTGGCCCTAATTCCAAGA	<i>NIA2</i> -R	CGTCAATAACGGCACAGAG A
<i>PR1a</i> -F	CGTTGAGATGTGGGTCAATG	<i>PR1a</i> -R	CCTAGCACATCCAACACGA A
<i>ERF1</i> -F	GCTCTTAACGTCGGATGGTC	<i>ERF1</i> -R	AGCCAAACCCTAGCTCCATT
<i>LOX</i> -F	CCTTAAGAGGAGATGGAACT	<i>LOX</i> -R	TCTAAGCTCATAAGCAATGG

Table S4 Lesion area in *N. benthamiana* leaves

	Control	C3	C6	N3	N6
Area (%)	1.02 ± 0.0016	0.70 ± 0.0008	0.67 ± 0.0039	0.56 ± 0.0098	0.56 ± 0.0087

Note, C3, 350 °C prepared biochar; C6, 650 °C prepared biochar; N3, 350 °C prepared biochar nanoparticle; N6, 650 °C prepared biochar nanoparticles.