Supporting Information for

Mn_3O_4 Nanoenzyme Boost Endogenous Antioxidant Metabolites in Cucumber (*Cucumis sativus*) Plant and Enhance Resistance to Salinity Stress

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GC-MS Method. Helium (>99.999%) was used as the carrier gas at a constant flow rate of 1.0 mL min−1 through the column. The initial oven temperature was 60 °C, ramped to 125 °C at a rate of 8 °C min−1, to 210 °C at a rate of 4 °C min−1, to 270 °C at a rate of 5 °C min−1, to 305 °C at a rate of 10 °C min−1, and finally, held at 305 °C for 3 min. The injection volume was 1 μL with an injector temperature of 260 °C in splitless mode. The temperature of the MS quadrupole and ion source (electron ionisation) was set to 150 and 230 °C, respectively. The ionisation energy was 70 eV. Mass data were acquired in a full-scan mode (m/z 50–500), and the solvent delay time was set to 5 min. Quality control samples, which were prepared by applying small aliquots from each sample with L-2-chlorophenylalanine as an internal standard, were injected at regular intervals (every 10 samples) throughout the analytical run (Zhao et al., 2019).

Reference:

Zhao et al. Metabolomics reveals that engineered nanomaterial exposure in soil alters both soil rhizosphere metabolite profiles and maize metabolic pathways. Environ. Sci.: Nano, 2019, 6, 1716.

Dissolution test of Mn_3O_4 NPs. Prepare a suspension of 20 mg/L Mn_3O_4 NPs and sonicated for 30 min to get a well dispersed suspension. Solution samples were taken at 24 and 96 h. To separate released Mn ions from Mn_3O_4 NPs, each sample was centrifuged in ultrafiltration concentration centrifugal tube (2-5 nm pore size, molecular weight cutoff 10K, PALL, America) at 10000 rpm for 15 minutes. The filtrate containing only Mn ions was collected and analyzed with ICP-MS (1% HNO_3). The experiment was run for triplicate.

Table S1. ROS scavenging capacities of Mn_3O_4 NPs

Material	Concentration	Clearance rate		
		$\bullet O_2^-$	H_2O_2	•OH
Mn_3O_4	**20 ppm**	**7.62%**	**5.31%**	**19.14%**
Mn_3O_4	**100 ppm**	**46.29%**	**40.36%**	**30.21%**

Table S2.

Macro- and micro elements in cucumber tissues (mg/kg dry weight)

	Al	Cu	Zn	Fe	Na	K	Ca	Mg	Si	P
					Leaf					
Control	95.3 ± 3.4 a	40.9 ± 1.2 ab	57.1 ± 5.9 a	182 ± 31 a	751 ± 134 a	36029 ± 4350 a	16244 ± 711 b	6570 ± 445 b	2736 ± 174 a	4271 ± 601 a
20 ppm Mn_3O_4	84.6 ± 7.6 a	42.6 ± 0.6 a	62.4 ± 6.3 a	185 ± 8 a	743 ± 107 a	37954 ± 4648 a	**19680 ± 2028 a**	**7424 ± 515 a**	2786 ± 129 a	5071 ± 1241 a
100 ppm Mn_3O_4	87.8 ± 5.3 a	39.6 ± 2.2 b	54.1 ± 4.5 a	145 ± 12 b	560 ± 99 a	33120 ± 2043 a	15831 ± 652 b	6047 ± 236 b	2580 ± 210 a	3795 ± 234 a
					Stem					
Control	35.2 ± 3.4 a	36.6 ± 1.4 a	27.7 ± 1.8 a	97 ± 8 a	5762 ± 1530 a	92902 ± 2149 ab	5963 ± 171 b	3161 ± 269 a	570 ± 31 a	11345 ± 2865 a
20 ppm Mn_3O_4	32.8 ± 7.6 a	35.9 ± 0.6 a	26.9 ± 2.8 a	99 ± 9 a	4363 ± 1064 a	96904 ± 7161 a	6687 ± 184 a	3181 ± 211 a	543 ± 33 a	10978 ± 2310 a
100 ppm Mn_3O_4	32.8 ± 5.3 a	35.4 ± 0.9 a	23.3 ± 5.4 a	85 ± 7 a	5247 ± 195 a	82389 ± 7264 b	5990 ± 461 b	3333 ± 199 a	431 ± 12 b	10165 ± 1003 a
					Root					
Control	117.9 ± 17.0 a	37.6 ± 1.3 a	80.3 ± 5.2 a	131 ± 18 a	3732 ± 357 a	59024 ± 5556 a	10436 ± 533 a	2187 ± 81 a	454 ± 74 a	4684 ± 433 a
20 ppm Mn_3O_4	89.3 ± 7.9 b	38.3 ± 1.3 a	78.2 ± 18.7 a	138 ± 47 a	4341 ± 865 a	61581 ± 4401 a	9517 ± 481 a	1980 ± 204 a	432 ± 36 a	4859 ± 891 a
100 ppm Mn_3O_4	98.9 ± 15.3 ab	39.0 ± 1.9 a	82.2 ± 18.1 a	112 ± 6 a	3621 ± 253 a	57909 ± 3242 a	9758 ± 652 a	2054 ± 93 a	402 ± 40 a	4381 ± 296 a

Table S3. Details regarding the responsible metabolites leading to the grouping between control and Mn_3O_4 NPs groups

Metabolite name	Average Rt(min)	Quant mass	Total score	Database	HMDB ID	p-value
Dihydroxycylohexane	7.74	258	76.7	LUG	/	0.00534
Deoxyguanosine	24.454	281	67.9	LUG	HMDB0000085	0.11891
Ketoglucose dimethylacetal	10.91	102	80.7	LUG	HMDB0029932	0.33458
Dimethylaminoazobenzene	20.779	282	68.5	LUG	HMDB0032141	0.00536
Adenine	25.095	264	84.1	LUG	HMDB0000034	0.02996
Alanine	6.634	176	84.5	LUG	HMDB0000056	0.02140
Citrulline	24.241	157	77.8	LUG	HMDB0000904	0.07148
Cyanoalanine	12.688	141	82.6	LUG	/	0.23581
Xylose	9.559	160	85.5	LUG	HMDB0000098	0.15794
Ferulic acid	30.557	338	80.6	LUG	HMDB0000954	0.03663
Aminobutyric acid	16.639	174	93.2	LUG	HMDB0000112	0.18669
Glycerol	10.576	147	97.7	LUG	HMDB0000131	0.01764
Indoxyl sulfate	7.276	163	67.9	LUG	HMDB0000682	0.11088
Alanine	7.481	116	95.3	LUG	HMDB0000161	0.12491
Isoleucine	10.981	158	96.2	LUG	HMDB0000172	0.00713
Lysine	26.634	174	92.2	LUG	HMDB0000182	0.00921
Phenylalanine	19.04	218	94.5	LUG	HMDB0000159	0.01764
Sorbose	25.406	103	95.9	LUG	HMDB0001266	0.02698
Threonic acid	13.669	148	84.2	LUG	HMDB0062620	0.06146
Tyrosine	26.968	218	86.2	LUG	HMDB0000158	0.04552
Valine	9.452	144	97.3	LUG	HMDB0000883	0.06465
Maltitol	19.926	288	71.9	LUG	HMDB0002928	0.40746
Methionine	16.334	176	84.9	LUG	HMDB0000696	0.11891
Methyl glucopyranoside	24.897	204	97.7	LUG	HMDB0029965	0.11036
Methylalanine	5.159	130	83.3	LUG	HMDB0094692	0.35077
Palatinitol	29.21	204	81	LUG	/	0.03012
Phosphenodiimidic amide	14.662	350	76.3	LUG	/	0.05135
Piceatannol	40.783	194	43.5	LUG	HMDB0004215	0.06879
Pyruvic acid	6.583	174	97.5	LUG	HMDB0000243	0.10869
Spermine	33.841	174	77.5	LUG	HMDB0001256	0.11036

LUG: Untarget database of GC-MS from Lumingbio.
HMDB ID: The Human Metabolome Database.

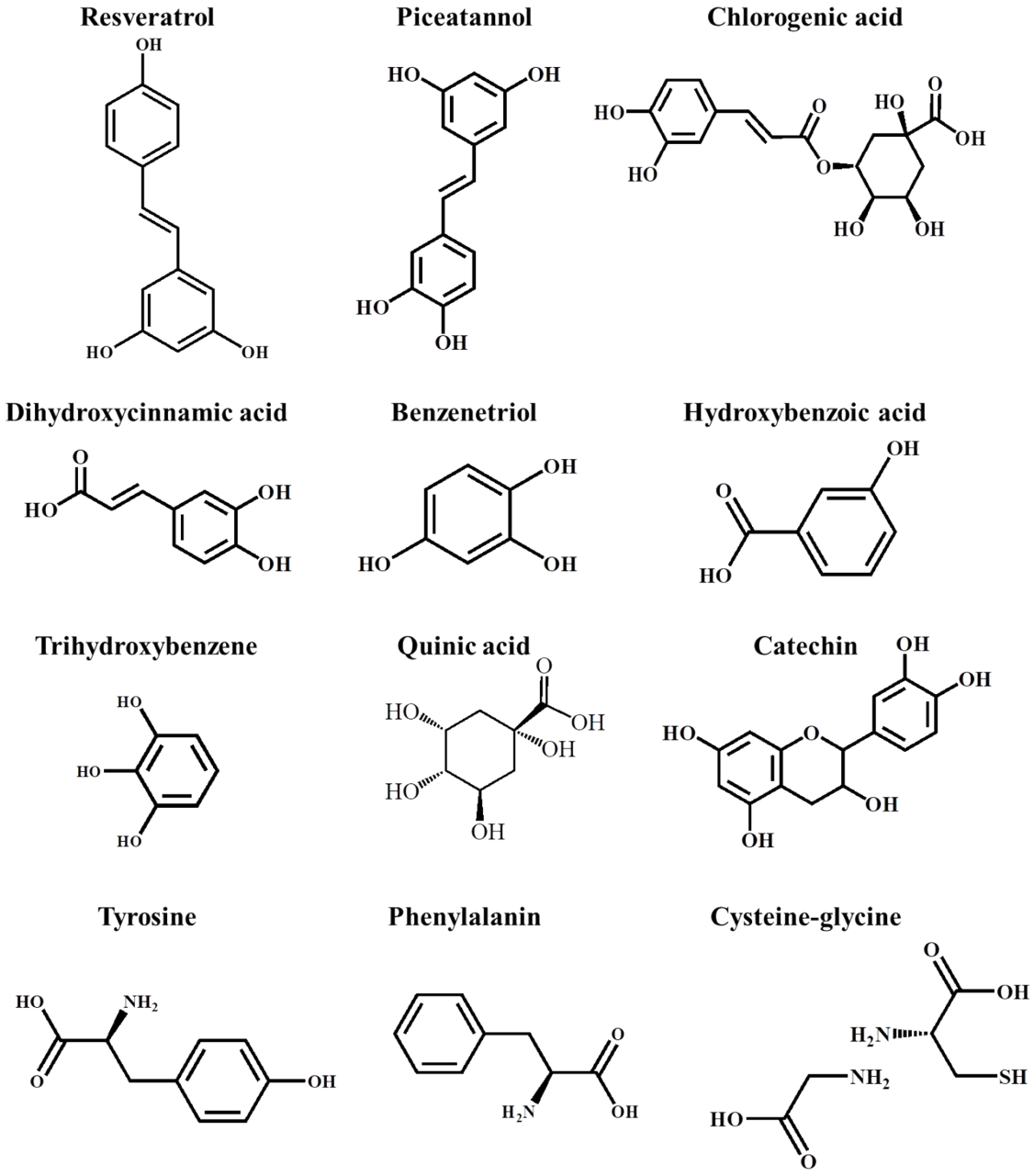


Figure S1. Structure of antioxidant compounds

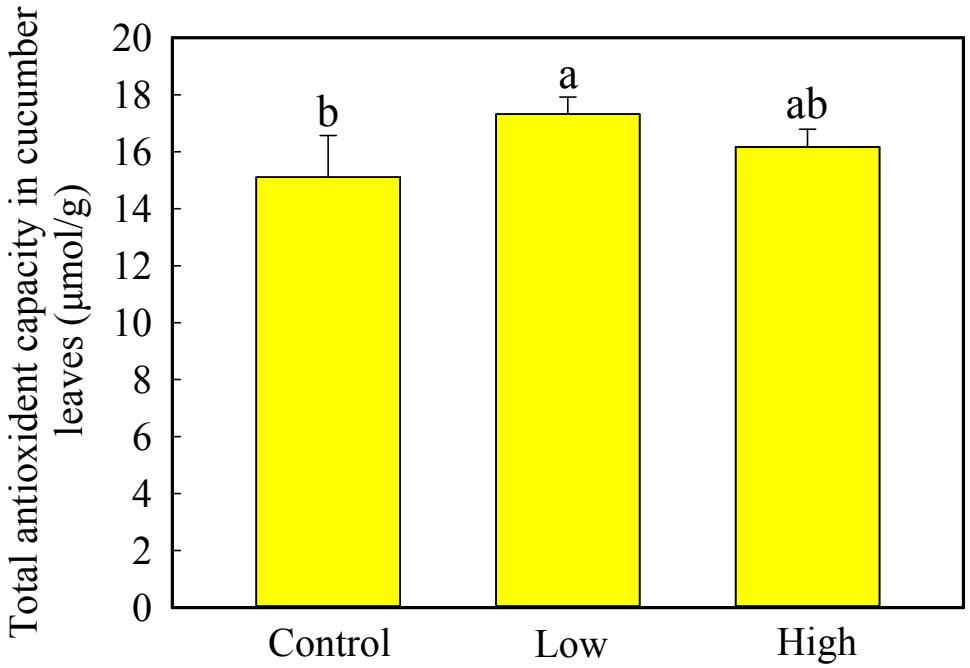
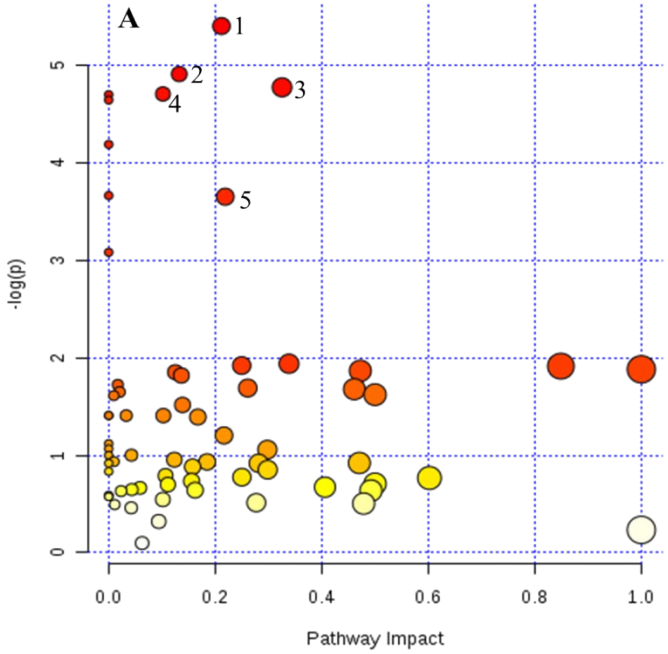
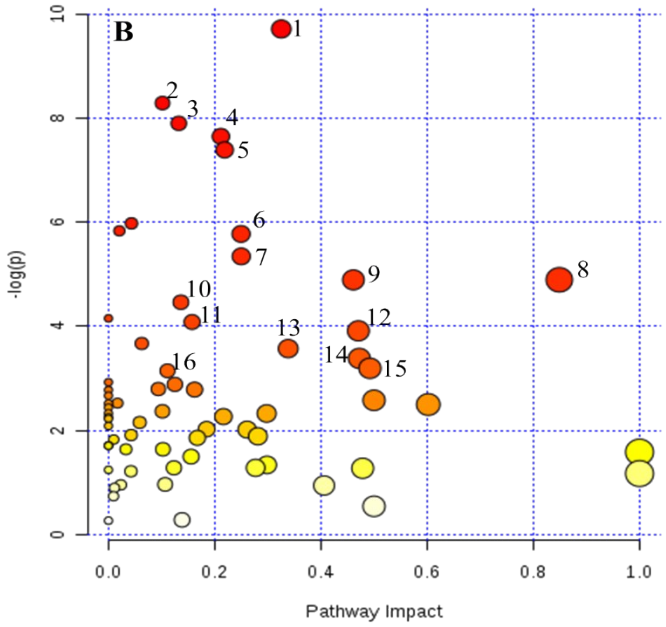


Figure S2. Total antioxidant capacities of cucumber leaves treated with Mn_3O_4 NPs



(1) Pantothenate and CoA biosynthesis;(2) Stilbenoid, diarylheptanoid and gingerol biosynthesis;(3) beta-Alanine metabolism;(4) Phenylpropanoid biosynthesis;(5) Pentose and glucuronate interconversions.



(1) beta-Alanine metabolism; (2) Phenylpropanoid biosynthesis;(3) Stilbenoid, diarylheptanoid and gingerol biosynthesis; (4) Pantothenate and CoA biosynthesis; (5) Pentose and glucuronate interconversions; (6) Pyrimidine metabolism; (7) Lysine degradation; (8) Alanine, aspartate and glutamate metabolism; (9) Arginine and proline metabolism; (10) Butanoate metabolism; (11) Tryptophan metabolism; (12) Phenylalanine metabolism; (13) Citrate cycle (TCA cycle); (14) Glyoxylate and dicarboxylate metabolism; (15) Glutathione metabolism; (16) Aminoacyl-tRNA biosynthesis

Figure S3. Perturbed metabolic pathways in cucumber leaves in response to Mn_3O_4 NPs