SUPPLEMENTARY INFORMATION

Reprogramming of black bean leaf metabolism by zinc nanofertilizers and biofertilizers revealed through NMR metabolomics

Table S1. Physical and chemical characteristics of the soil (0-30 cm depth)

Parameters	Values
pH (1:2)	7.2 (neutral)
CE	1.281 dS m^{-1}
CIC	38.02
$N\text{-}NO_3^-$	45.20 mg Kg^{-1}
K^+	840 mg Kg^{-1}
P available	16.01 mg Kg^{-1}
Ca^{++}	5900 mg Kg^{-1}
Mg^+	730 mg Kg^{-1}
B	1.30 mg Kg^{-1}
Na^+	97.00 mg Kg^{-1}
MO	3.29%
Clayey texture (sand 20 %, clay 53 % and silt 27 %)	
Low content of microelements such as Fe^{++}, Zn^{++}, Mn^{++} y Cu^{++}	

pH: hydrogen potential; EC: electrical conductivity; CEC: cation exchange capacity; N: nitrogen; NO_3: nitrates; K: potassium; P: phosphorus; Ca: calcium; Mg: magnesium; B: boron; Na: sodium; OM: organic matter; Fe: iron; Zn: zinc; Mn: manganese; Cu: copper.

Table S2. Characteristics of the biofertilizers evaluated in black bean seedlings.

Nopal extract	Worm-humus Biojal®
N (600-670 mg L^{-1})	NO_3 (531.42 mg L^{-1})
P (154-170 mg L^{-1})	NH_4 (212 mg L^{-1})
Ca (1830-1950 mg L^{-1})	Phosphates (458.20 mg L^{-1})
Mg (700-800 mg L^{-1})	Sulfates (1200 mg L^{-1})
Fe (5-10 mg L^{-1})	Ca (560 mg L^{-1})
Zn (> 1 mg L^{-1})	S (400 mg L^{-1})
Cu (> 1 mg L^{-1})	Cl^- (1701.60 mg L^{-1})
Na (600 mg L^{-1})	Fe (53 mg L^{-1})
Humic acids (600 mg L^{-1})	Zn (1 mg L^{-1})
	Mg (100 mg L^{-1})
Total chlorophyll (4.7 mg L^{-1})	Mn (1 mg L^{-1})
	B (12 mg L^{-1})
Organic matter (250-302 mg L^{-1})	Organic matter (0.54 %)

N: nitrogen; P: phosphorus; Ca: calcium; Mg: magnesium; Fe: iron; Zn: zinc; Cu: copper; Na: sodium; NO_3: nitrates; NH4: ammonium; S: sulfur; Cl: chlorine; Mn: manganese; B: boron.

Table S3. R^2 and Q^2 values with their corresponding CV-ANOVA, PCA, and OPLS-DA permutations obtained from bean leaves accessions models.

	PCA		OPLS-DA			CV-ANOVA	Permutation
	R^2X	**Q^2**	**R^2X**	**R^2Y**	**Q^2**		
C vs ZnC vs NE+ZnC vs WH+ZnC	0.788	0.641	0.837	0.897	0.813	6.95438e-014	
C vs ZnCh vs NE+ZnCh vs WH+ZnCh	0.809	0.543	0.820	0.826	0.604	0.000195951	
C vs ZnC vs ZnCh vs NE vs WH	0.792	0.510	0.825	0.873	0.723	6.48786e-014	
C vs NE+Zn C vs NE+ZnCh vs WH+ZnC vs WH+ZnCh	0.788	0.584	0.818	0.727	0.535	1.50868e-009	

Table S4. ^{1}H NMR signals used for the identification of metabolites in aqueous extracts of black bean leaves treated with NFs and BFs.

Metabolite		Chemical shifts (ppm), *J* (Hz), multiplicity
Sugars		
1	Arabinose	5.25 (d, *J* = 3.5)
2	Fructose	3.98 (m), 4.01 (dd, *J* = 12.7, 1.3 Hz)
3	Galactose	4.57 (d, *J* = 7.9 Hz)
4	Glucose	4.63 (d, *J* = 7.9 Hz), 5.22 (d, *J* = 3.7 Hz).
5	Mannose	5.17 (d, *J* = 1.23 Hz)
6	Myo-inositol	3.26 (t, *J* = 9.4 Hz)
7	Sucrose	4.20 (d, *J* = 8.8 Hz), 5.40 (d, *J* = 3.8 Hz) CH-7
Amino acids		
8	Alanine	1.47 (d, *J* = 7.2 Hz)
9	Arginine	1.72 (m), 1.91 (m), 3.20 (t, *J* = 6.9)
10	Asparagine	2.85 (dd, *J* = 16.9, 7.8 Hz), 2.94 (dd, *J* = 16.9, 4.2 Hz)
11	Aspartic acid	2.68 (dd, *J* = 17.5, 7.4 Hz), 2.80 (dd, *J* = 17.5, 3.7 Hz)
12	GABA	1.89 (m), 2.29 (t, *J* = 7.4 Hz)
13	Glutamic acid	2.15 (m), 2.36 (m)
14	Glutamine	2.14 (m), 2.46 (m)
15	Histidine	8.30 (m), 7.25 (m)
16	Isoleucine	0.92 (t, *J* = 7.4 Hz), 1.00 (d, *J* = 7.0 Hz)
17	Leucine	0.94 (d, *J* = 6.2 Hz) , 0.95 (d, *J* = 6.2 Hz)
18	Methionine	2.12 (s), 2.65 (t, *J* = 7.6 Hz)
19	Phenylalanine	7.31 (d, J = 7.5), 7.36 (m), 7.41 (t, *J* = 7.5 Hz)
20	Proline	2.01 (m), 2.3 (m)
21	Threonine	1.32 (d, *J* = 6.6 Hz)
22	Tryptophan	7.52 (d, *J* = 8.0 Hz), 7.71 (d, *J* = 8.0 Hz)
23	Tyrosine	7.18 (d, *J* = 7.18 Hz), 6.87 (d, *J* = 7.18 Hz)
24	Valine	0.98 (d, *J* = 7.0 Hz), 1.03 (d, *J* = 7.0 Hz)
Organic acids		
25	Acetic acid	1.92 (s)

26	Citric acid	2.52 (d, *J* = 15.6 Hz), 2.71 (d, *J* = 15.6 Hz)
27	Formic acid	8.44 (s)
28	Fumaric acid	6.51 (s)
29	Malic acid	2.53 (dd, *J* = 15.5, 9.9 Hz), 2.69 (dd, *J* = 15.5, 3.2 Hz)
30	Pyruvic acid	2.34 (s)
31	Succinic acid	2.43 (s)
32	Tartaric acid	4.38 (s)
33	2-Hydroxyisobutyric acid	1.36 (s)
34	3-O-Caffeoylquinic acid	6.42 (d, *J* = 15.9 Hz)
35	4-O-Caffeoylquinic acid	6.46 (d, *J* = 15.9 Hz)
36	5-O-Caffeoylquinic acid	6.45 (d, *J* = 15.9 Hz)
Alcohols		
37	Methanol	3.34 (s)
Nucleosides		
38	Adenosine	6.06 (d, *J* = 6.1 Hz), 8.17 (s), 8.22 (s)
39	Cytidine	6.05 (d, *J* = 7.6 Hz), 7.84 (d, *J* = 7.6 Hz)
40	Guanosine	5.90 (d, *J* = 6.0 Hz), 7.78 (s)
41	Uridine	5.88 (m), 5.90 (m), 7.86 (d, *J* = 8.1 Hz)
Other compounds		
42	Choline	3.18 (s)
43	NAD^+	9.45 (s)
44	Trigonelline	8.83 (m), 9.11 (s)

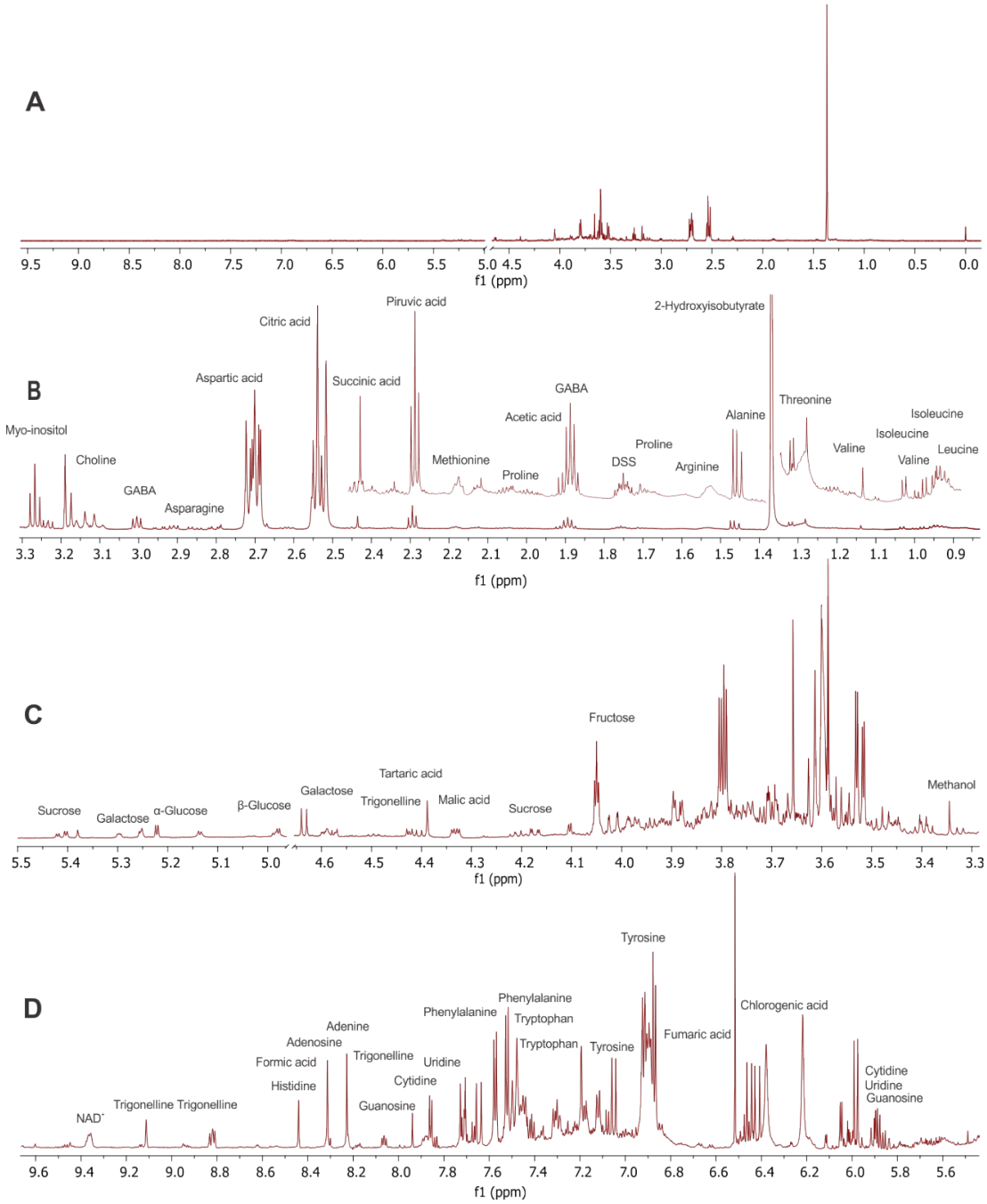

Fig. S1. ^{1}H NMR spectrum from leaves of black bean. The spectrum obtained at 750 MHz was extended from 0.00 to 9.50 ppm (A); from 0.90 to 3.30 ppm (B); from 3.3 to 5.5 ppm (C) and from 5.60 to 9.60 ppm (D). Signal assignments were based on 2D NMR experiments and the literature (Hernández-Guerrero et al., 2021).

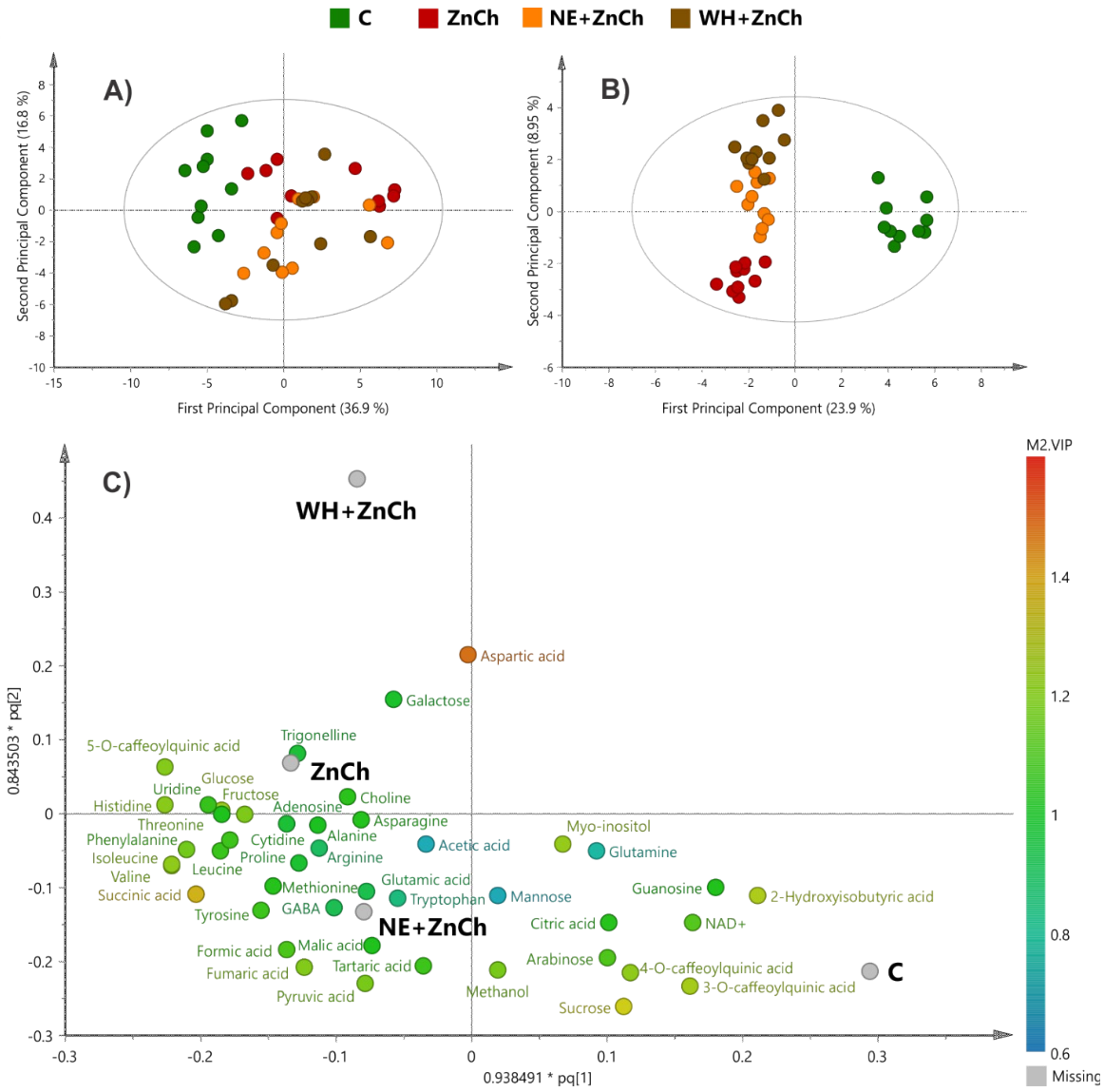

Fig. S2. The score scatter plot of PCA model (A), score scatter plot of OPLS-DA model (B) and the loadings scatter plot of OPLS-DA model (C) generated from ^{1}H NMR spectra (750 MHz) of black bean leaves treated with zinc nanoparticles synthesized using chitosan and their combination with biofertilizers. C: absolute control; ZnCh: zinc-chitosan nanoparticles; NE+ZnCh: nopal extract + zinc-chitosan nanoparticles; WH+ ZnCh: worm-humus Biojal® + zinc-chitosan nanoparticles.

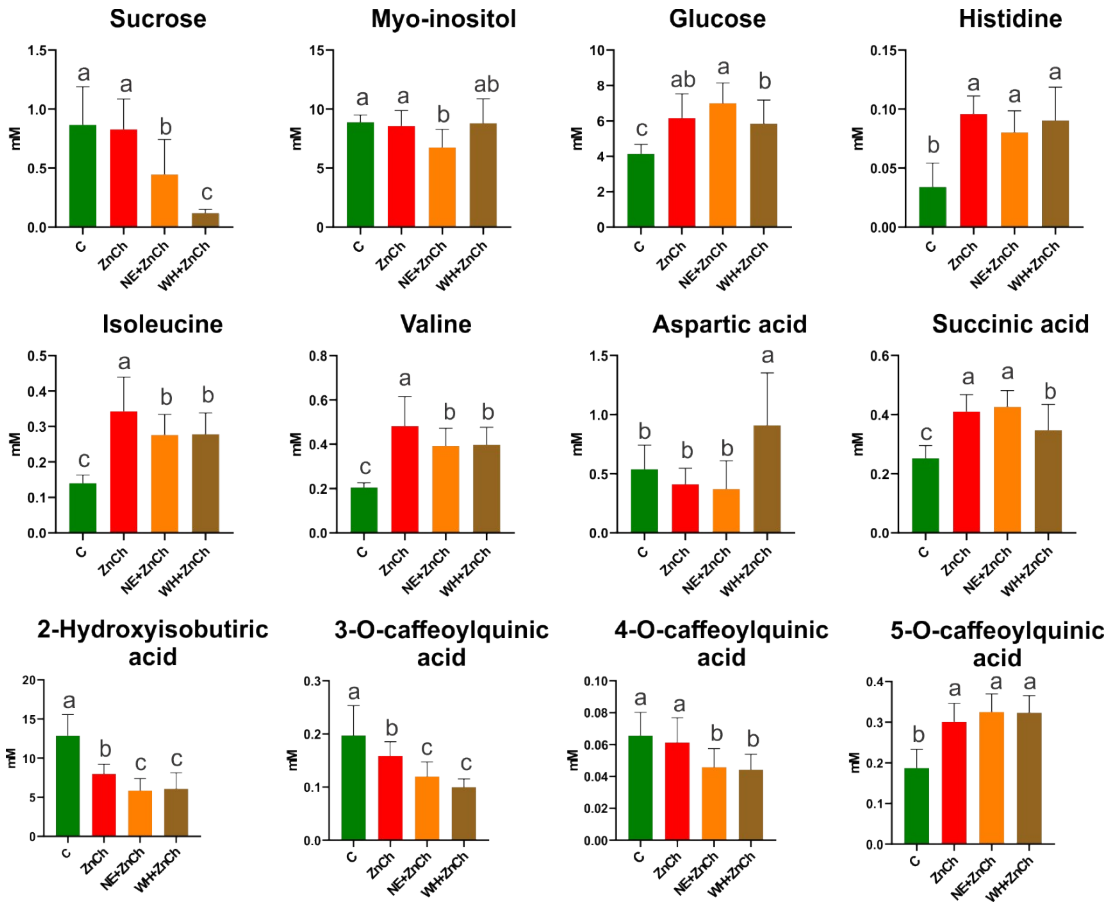

Fig. S3. The relative abundance of differential metabolites found in black bean leaves treated with zinc nanoparticles synthesized using chitosan and biofertilizers, as well as their respective combinations, is illustrated. Each data point is the average of ten replicates ± standard error (n=40). Different letters indicate significant differences between treatments according to Duncan's test ($p \leq 0.05$). C: absolute control; ZnCh: zinc-chitosan nanoparticles; NE+ZnCh: nopal extract + zinc-chitosan nanoparticles; WH+ ZnC: worm-humus Biojal® + zinc-Chitosan nanoparticles.

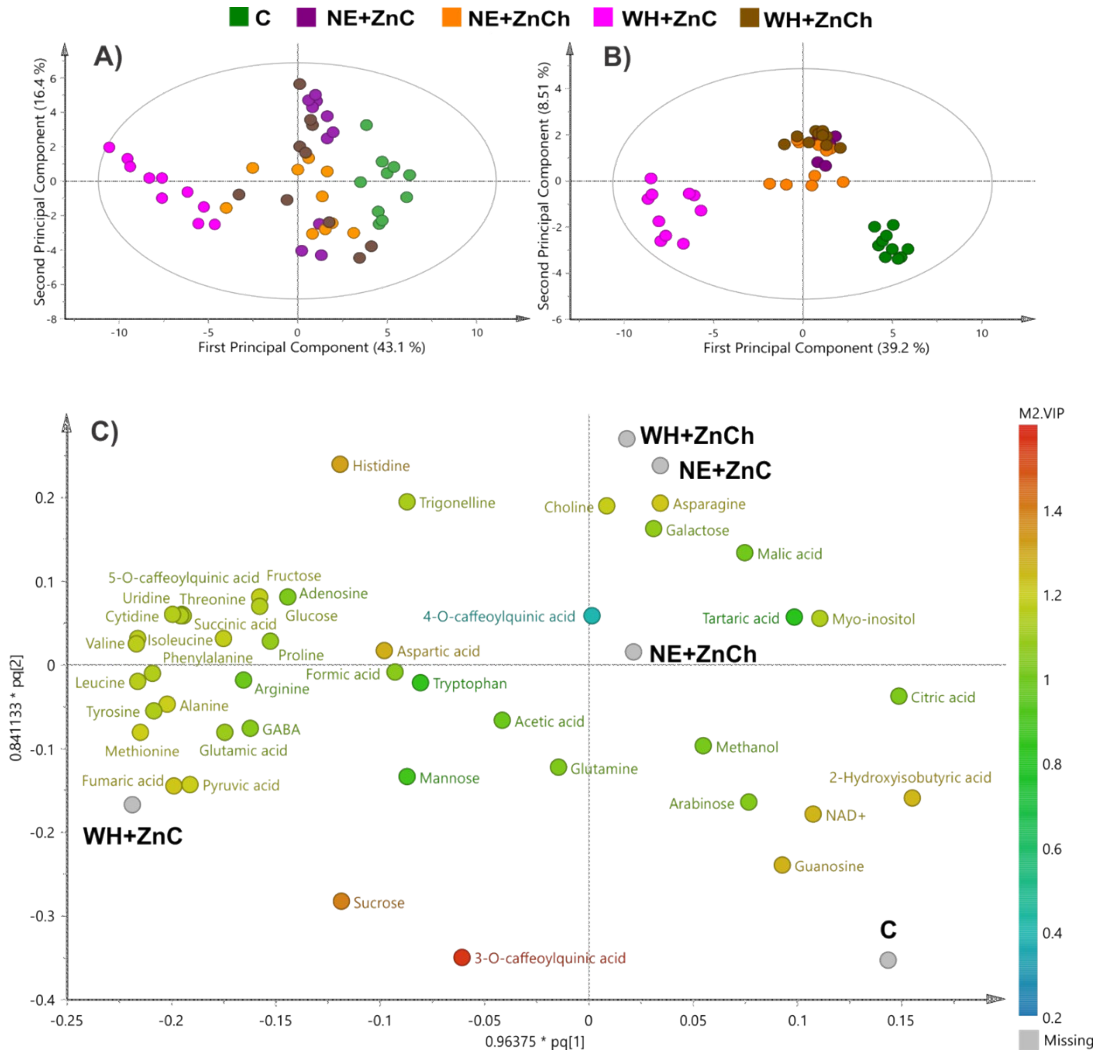

Fig. S4. The score scatter plot of PCA model (A), score scatter plots of OPLS-DA model (B) and the loadings scatter plot of OPLS-DA model (C) from leaves of black bean treated with the combination of nanofertilizers and biofertilizers. NE+ZnC: nopal extract + zinc-cotton nanoparticles; WH+ ZnC: worm-humus Biojal® + zinc-cotton nanoparticles; NE+ZnCh: nopal extract + zinc-chitosan nanoparticles; WH+ ZnC: worm-humus Biojal® + zinc-Chitosan nanoparticles; C: absolute control.

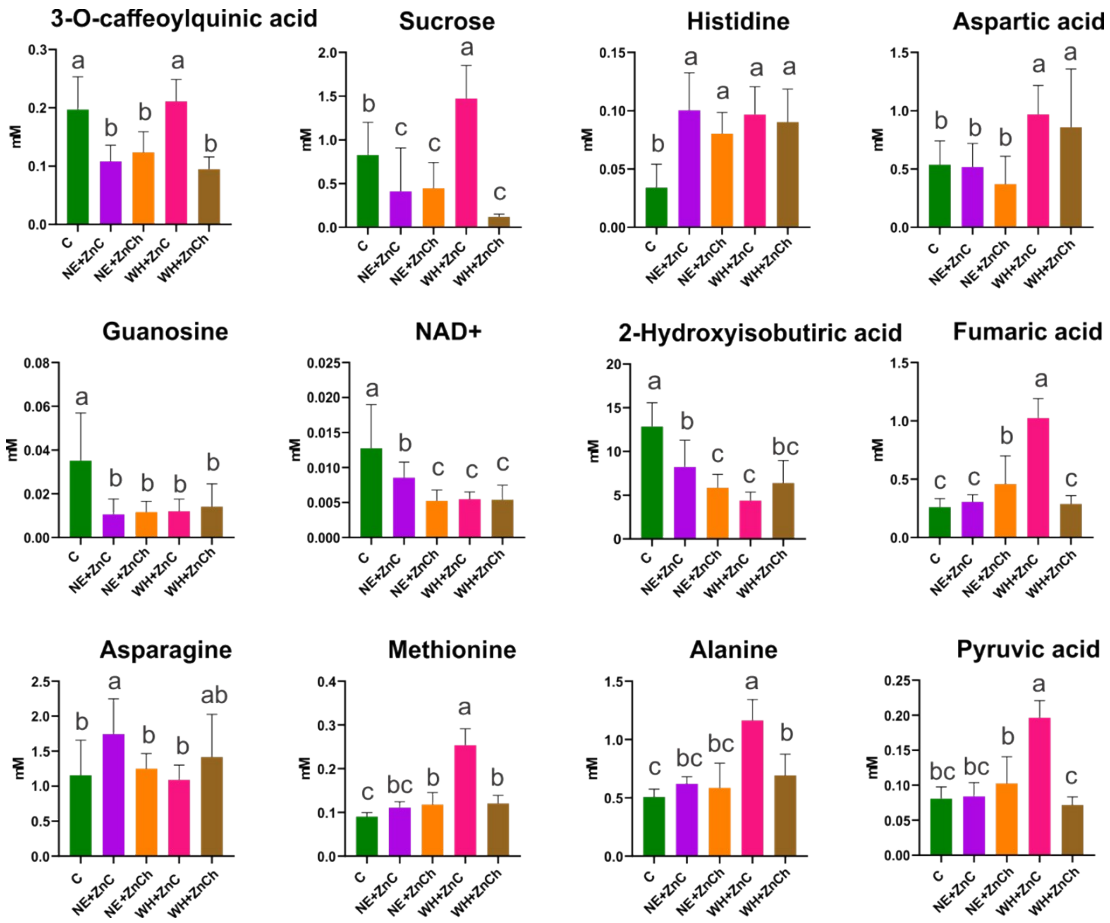

Fig. S5. The relative abundance of differential metabolites found in black bean leaves treated with the combination of nanofertilizers and biofertilizers is illustrated. Each data point is the average of ten replicates ± standard error (n=40). Different letters indicate significant differences between treatments according to Duncan's test ($p \leq 0.05$). NE+ZnC: nopal extract + zinc-cotton nanoparticles; WH+ ZnC: worm-humus Biojal® + zinc-cotton nanoparticles; NE+ZnCh: nopal extract + zinc-chitosan nanoparticles; WH+ ZnC: worm-humus Biojal® + zinc-Chitosan nanoparticles; C: absolute control.