

Upcycling plant waste: Iron nanoparticles synthesized from *Cannabis sativa* enhance biomass and antioxidative properties in soybean (*Glycine max*)

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Supplementary Material

Table S1: Wavenumber range assigned to different bonds and compounds

Wavenumber range	Bonds	Compounds
3591 – 3221	O-H stretching	Alcohols, phenols
3169 - 3013	C-H stretching	Aromatics
2989 - 2805	C-H stretching	Alkanes
1751 - 1703	C=O stretching	Esters
1740 - 1711	H-C=O: C-H stretching	Aldehydes
1628 - 1579	C-C stretching (in ring)	Aromatics
1476 - 1316	C-H bending and C-H rocking	Alkanes
1346 - 1305	C-O-C stretching	Ethers
1268 - 1132	C-N stretching	Aliphatic amines
911 - 822	C-O bend	Carboxylic acids

Table S2: Composition of extract from *Cannabis sativa* ssp. *sativa* and *Cannabis sativa* ssp. *indica*

Compounds present in both extracts	Compounds present in extract from <i>Cannabis sativa</i> ssp. <i>sativa</i>	Compounds present in extract from <i>Cannabis sativa</i> ssp. <i>indica</i>
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<i>Compound</i>	<i>Target response</i>	<i>Compound</i>	<i>Target response</i>	<i>Compound</i>	<i>Target response</i>
Bicyclo[3.1.1]hept-2-ene	6332.3s; 12529.1i	alpha-Gurjunene	42224	Alloaromadendrene	52840
Caryophyllene	114662s; 61714i	Guaiol	157841	Eudesma-3,7(11)-diene	15566.5
(E)-beta-Famesene	132968s; 241704i	10-epi-gamma-eudesmol	137041	gamma-Selinene	106021
beta-Bisabolene	47549s; 2156i	1H-4-Oxabenzo(f)cyclobut(cd)inden-8-ol	589	1,5-Cyclodecadiene	117654
alpha-Humulene	105920s; 45452i	Ethyl (E)-3-fluoro-2-[(trimethylsilyl)methyl]-2-butenolate	3145	Ethyl 9-hexadecenoate	5083.1
alpha-Bisabolol	661806s; 162137i	Octacosane	3284899	Methyl stearate	16474.1
2-(4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydro-2-naphthalenyl)-2-propanol	3321s;1945i	Squalene	15036	8,11-Octadecadienoic acid	24821
2-Naphthalenemethanol	205615s; 53309i	Docosane	2341	1-Amino-3,4-dihydro-3-methyl-4-phenyl-2-naphthalenecarbonitrile	1078
Benzoic acid	51629s; 29675i	Valencene	30486	4-(2-furyl)-6-(1-piperidinyl)-1,3,5-triazin-2-ylamine	692
Hexadecanoic acid	33077s; 34849i	(+)-Aromadendrene	895	3-Chloroformanilide	112173
9,12,15-Octadecatrienoic acid	3820s; 8294i	8-hydroxy-1-methoxycarbonyl-5,6,7,8-tetrahydronaphtalene	895	Geranyl linalool isomer	172735
Phytol	183491s; 17398i	(3E)-4-(1,2-Epoxy-2,6,6-trimethylcyclohexyl)-3-buten-2-on-ethylenacetal	9885	benzo[a]phenanthridin-8(7H)-one	172735
Octadecanoic acid	17949.5s; 6065.5i	1H-Cycloprop[e]azulene	689	1H-Indole-3-butanoic acid	400459
Dronabiol	61812s; 43933i	Linoleic acid ethyl ester	20218	4,4-Dimethyl-13-alpha-androst-5-ene	407389
Cannabichromene	2465125s; 33027195i	6-acetyl-7-hydroxy-2,2-dimethylbenzopyran	7913	5H,10H-Diimidazo[1,5-a:1',5'-d]pyrazine-5,10-dione	210197
THC	8806414s; 18968384i	7-(bis(4-methoxyphenyl)methylene)-7H-bicyclo[4.1.0]hepta-1,3,5-triene	8765	ethyl 2-(2-methyl-1-hexenyl)indole-3-carboxylate	158295
CBD	5369s; 1876339i	p-Octylacetophenone	58613	4-Cyclohexyl-2-methyl-1-(4-methylphenyl)-6-phenyl-5-(3-propynyl)-1,2-dihydropyrimidine	183809
Naphtalene	1610836s; 424977i	Muscovopyridine	427237	1,3,5,7,9,11,13,15,17,19,21,23-Cyclotetracosadodecaene	97456
6H-Dibenzo[b,d]pyran-1-ol	64455s; 622i	6-Imino-8-(4'-nitrophenyl)-2,3,4,6-tetrahydropyrido[2,1-b][1,3]thiazine-7-carbonitrile	173639	4(3H)-Quinazolinone	186800
2H-1-benzopyran-5-ol	34s; 46i	2-benzoyl-6,7-dimethoxy-4-methylidene-2H-1,3-benzothiazine	3125	cis-5,6,6a,11,12,12a-Hexahydro-5,11-dimethylpentaleno[2.1-b:5,4-b]diindole	2638072
Nonanoic acid	95417s; 208253i	5,10-seco-3'H-cyclopropa[2,3]cholestan-1(10)-		beta-Amyrin	102363
2,6,10,14,18,22-tetracosahexane	82307s; 57453i			alpha-Amyrin	191026
Anthracene	20584s; 7386i			12-Oleanen-3-yl acetate	105367
Neophytadiene	49180s; 43544i			Morphinan	1826
Vitamin E	8914s;3524i				
Campesterol					

Stigmasterol	997647s; 389939i	en-5-one	12895	Urs-12-en-24-oic acid	60408
Propanol	12028s; 14348i	1-Triacontanol	906	Methyl 8-hydroxy-3,6-dimethoxy-1-methylanthraquinone-2-carboxylate	185784
Limonene	59030s; 73632i	Cholest-5-en-3-ol			47142
1-Hydroxy-2-propanone	5464s; 6875i	alpha-Eudesmol	463729	Myrcene	
1-Dodecene	60114s; 12704i	Cannabicitran	25281	4-dodecyne	3255
Elemene	6070s; 6632i	2,2,3,3-tetrafluoro-5-(1,1,2,2-tetrafluoroethoxy)-2,3-dihydrobenzofuran	488468		172735
(23S)-ethylcholest-5-en-3-beta-ol	463729s; 303939i		57672066		
Ionone	2879s; 7312i				
Glycerin	3291s; 14058i				
4-Vinylphenol	184321s; 282395i				
Dibenz[b,d]cycloheptane	32346s; 3205i				
Resorcinol	6953s; 53343i				
	1811705s; 34168				

s-ssp. *sativa*; i- ssp. *indica*; THC-Tetrahydrocannabinol; CBD-Cannabidiol.

Table S3: Zeta potential, hydrodynamic diameter and dry size of nanoparticles synthesized using *Cannabis sativa* ssp. *sativa* (Fe NP-S) and *Cannabis sativa* ssp. *indica* (Fe NP-I)

Sample	Hydrodynamic diameter (nm)	Zeta potential (mV)	Dry size (nm)

Fe NP-S	1739 ± 146	+4.3 ± 0.1	25.4 ± 3.5
Fe NP-I	164 ± 47	- 27.2 ± 0.2	9.7 ± 2.1

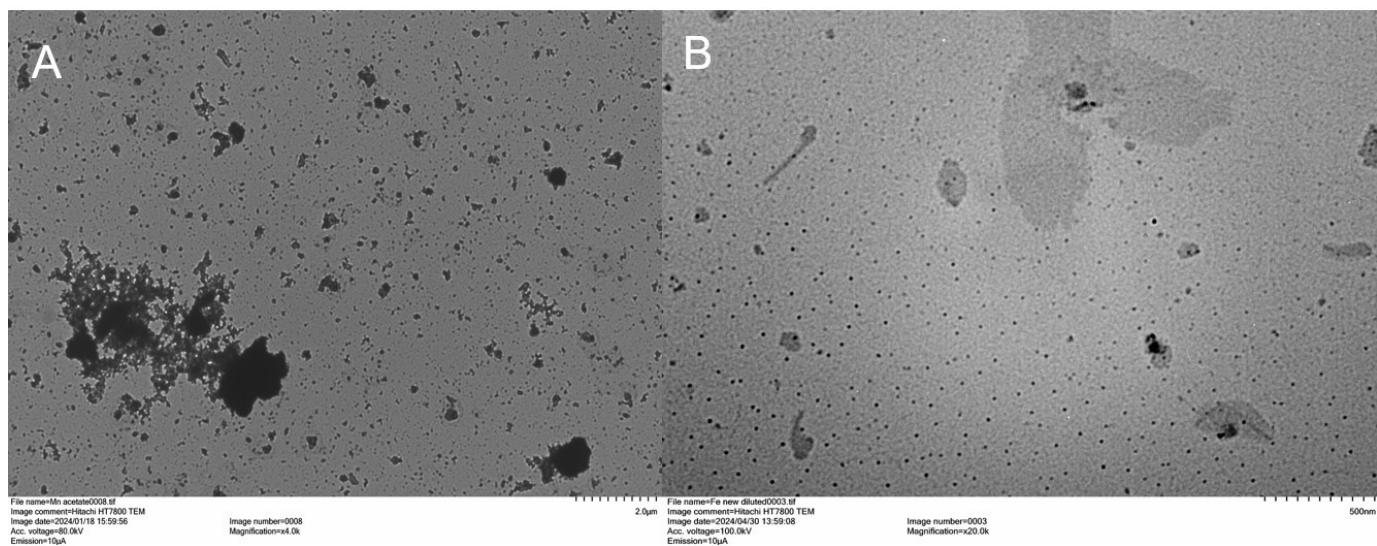


Figure S1. Transmission electron micrographs of iron nanoparticles. A-Iron nanoparticles synthesized from *Cannabis sativa* ssp. *sativa*; B- Iron nanoparticles synthesized from *Cannabis sativa* ssp. *indica*

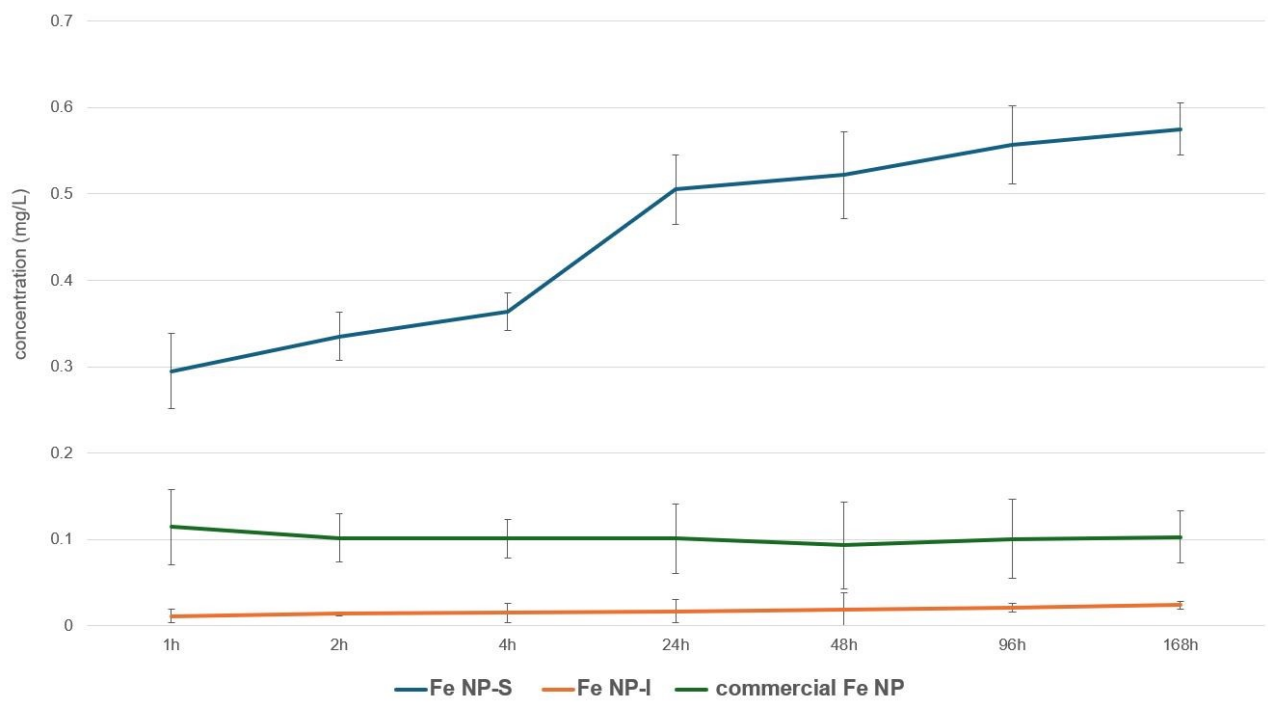


Figure S2. Dissolution analysis of iron nanoparticles. Fe NP-S - Iron nanoparticles synthesized from *Cannabis sativa* ssp. *sativa*; Fe NP-I- Iron nanoparticles synthesized from *Cannabis sativa* ssp. *Indica*; Fe commercial NP - commercially available iron oxide nanoparticles

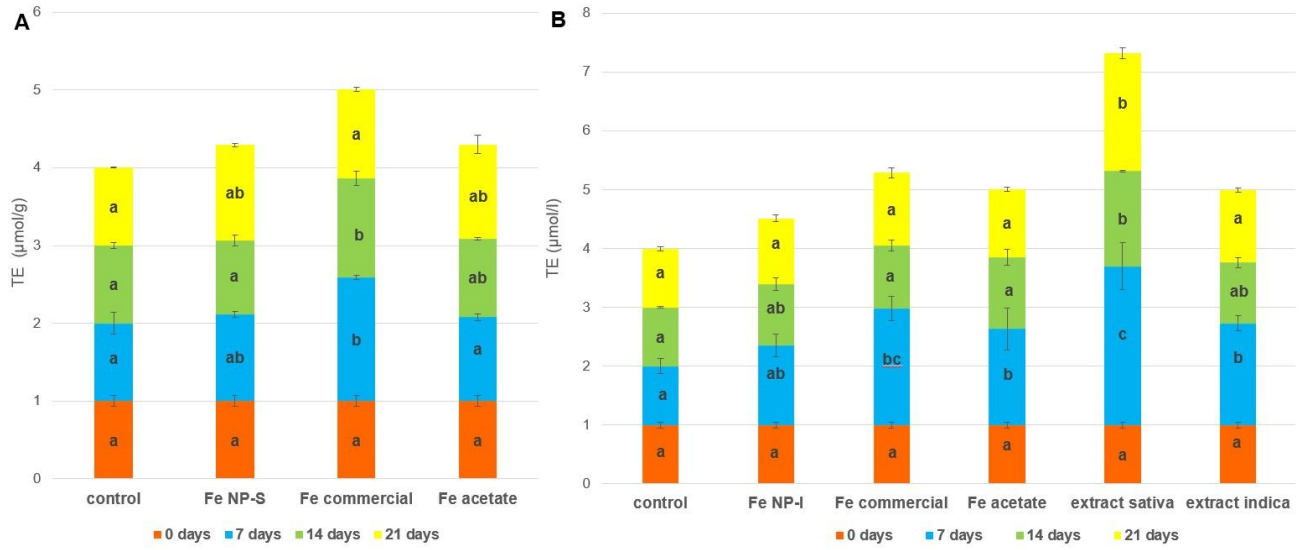


Figure S3. Changes in normalized antioxidant content in soybean exposed to Fe NPs (as detected by ABTS assay). A- Experiment with iron nanoparticles synthesized from *Cannabis sativa* ssp. *sativa* extract; B- Experiment with iron nanoparticle synthesized from *Cannabis sativa* ssp. *indica* extract. Fe NP-S - Fe nanoparticles synthesized from *Cannabis sativa* ssp. *sativa* extract; Fe commercial- commercial Fe₂O₃ nanoparticles; extract sativa- *Cannabis sativa* ssp. *sativa* extract; extract indica- *Cannabis sativa* ssp. *indica* extract. Different letters indicate that samples were statistically different (as determined by Tukey test at p < 0.05).

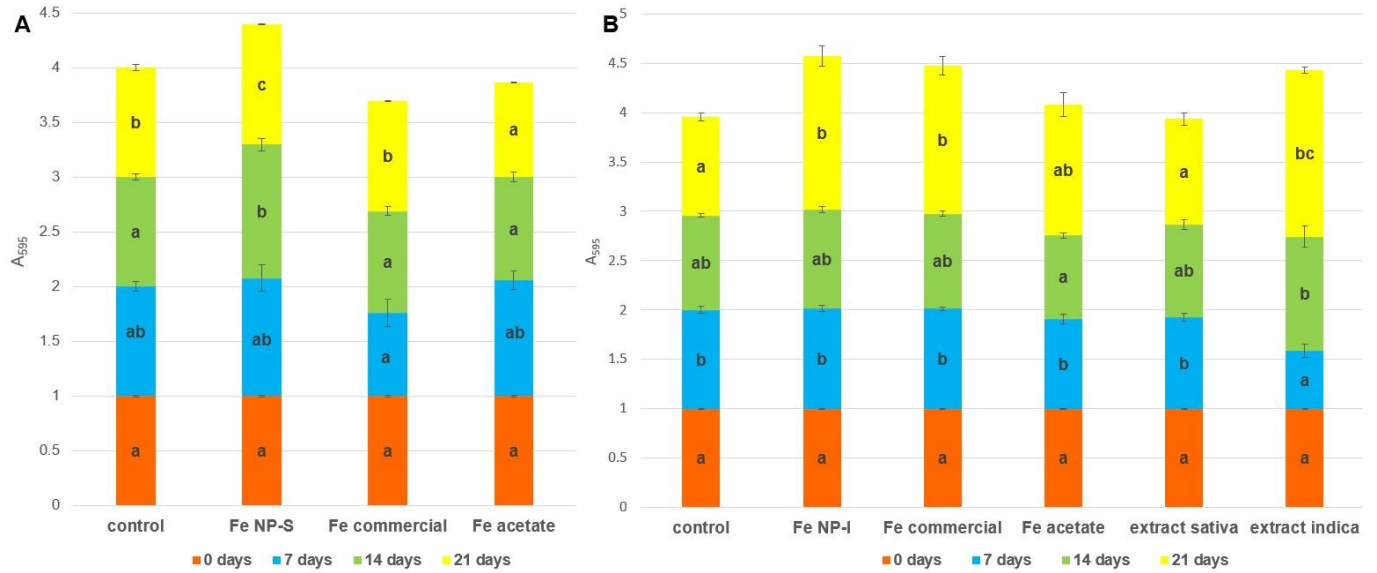


Figure S4. Changes in normalized plant cell viability in soybean exposed to Fe NPs. A- Experiment with iron nanoparticles synthesized from *Cannabis sativa* ssp. *sativa* extract; B- Experiment with iron nanoparticle synthesized from *Cannabis sativa* ssp. *indica* extract. Fe NP-S - Fe nanoparticles synthesized from *Cannabis sativa* ssp. *sativa* extract; Fe commercial- commercial Fe₂O₃ nanoparticles; extract sativa- *Cannabis sativa* ssp. *sativa* extract; extract indica- *Cannabis sativa* ssp. *indica* extract. Different letters indicate that samples were statistically different (as determined by Tukey test at $p < 0.05$).

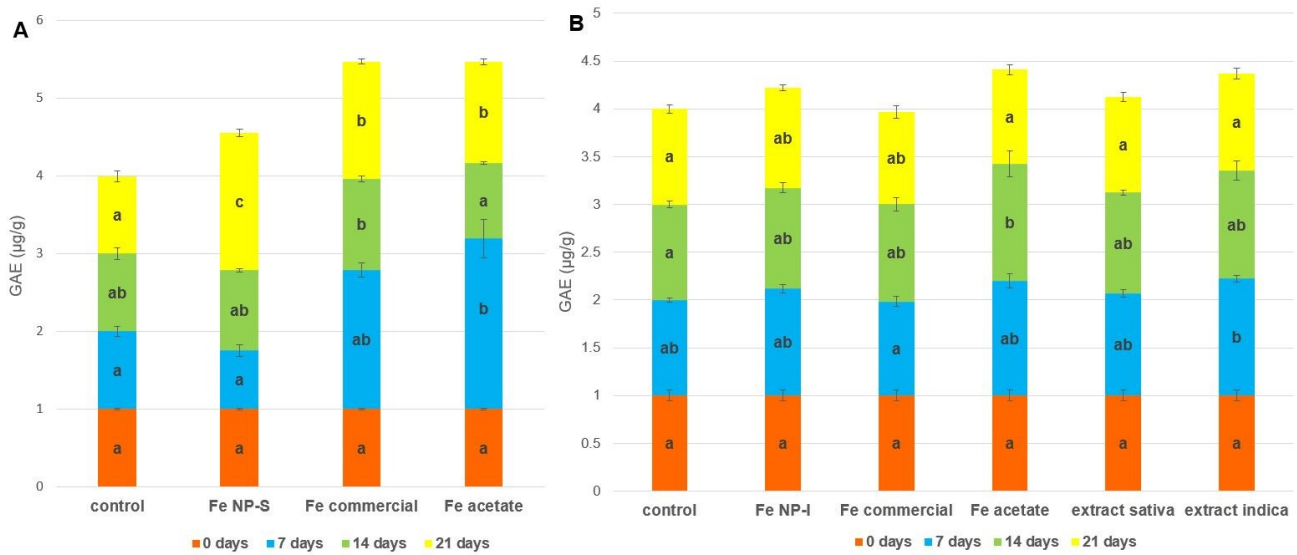


Figure S5. Changes in normalized total polyphenolic content in soybean exposed to Fe NPs. A- Experiment with iron nanoparticles synthesized from *Cannabis sativa* ssp. *sativa* extract; B- Experiment with iron nanoparticle synthesized from *Cannabis sativa* ssp. *indica* extract. Fe NP-S - Fe nanoparticles synthesized from *Cannabis sativa* ssp. *sativa* extract; Fe commercial- commercial Fe₂O₃ nanoparticles; Fe NP-I - Fe nanoparticles synthesized from *Cannabis sativa* ssp. *indica* extract; extract sativa- *Cannabis sativa* ssp. *sativa* extract; extract indica- *Cannabis sativa* ssp. *indica* extract. Different letters indicate that samples were statistically different (as determined by Tukey test at p < 0.05).

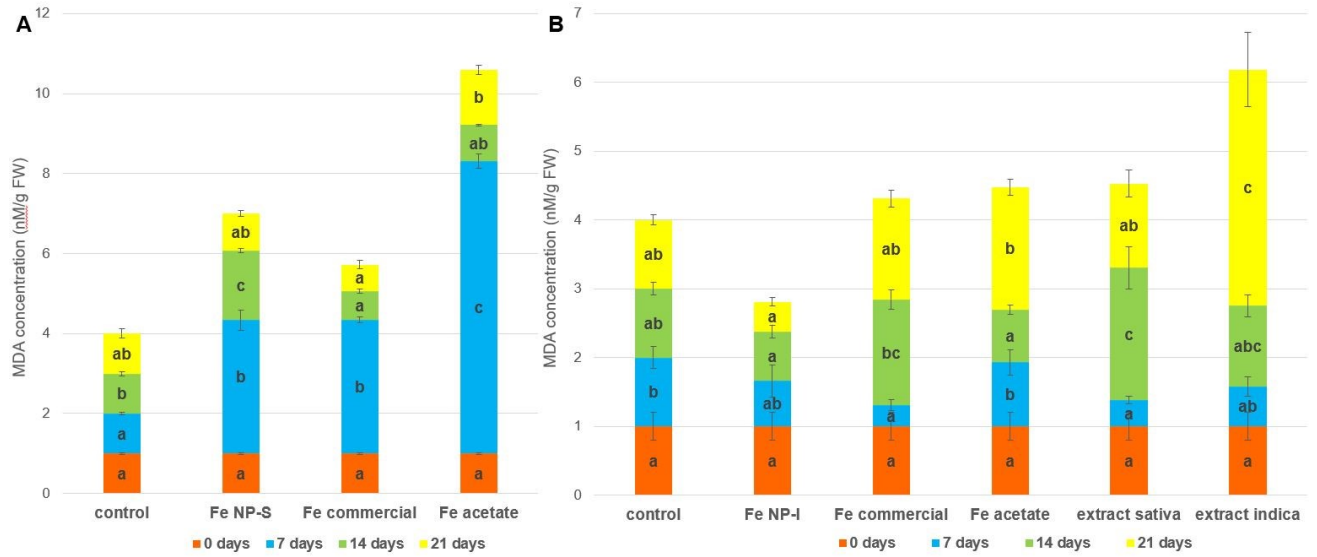


Figure S6. Changes in normalized malondialdehyde content in soybean exposed to Fe NPs. A- Experiment with iron nanoparticles synthesized from *Cannabis sativa* ssp. *sativa* extract; B- Experiment with iron nanoparticle synthesized from *Cannabis sativa* ssp. *indica* extract. Fe NP-S - Fe nanoparticles synthesized from *Cannabis sativa* ssp. *sativa* extract; Fe commercial- commercial Fe₂O₃ nanoparticles; extract sativa- *Cannabis sativa* ssp. *sativa* extract; extract indica- *Cannabis sativa* ssp. *indica* extract. Different letters indicate that samples were statistically different (as determined by Tukey test at $p < 0.05$).

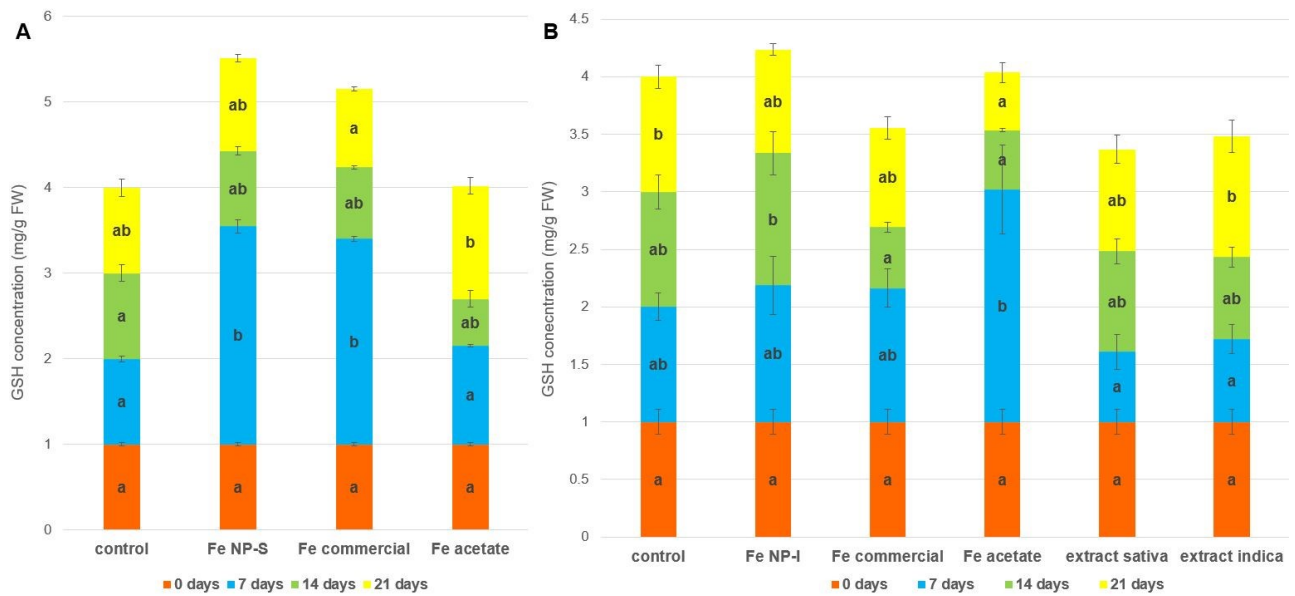


Figure S7. Changes in normalized glutathione content in soybean exposed to Fe NPs. A- Experiment with iron nanoparticles synthesized from *Cannabis sativa* ssp. *sativa* extract; B- Experiment with iron nanoparticle synthesized from *Cannabis sativa* ssp. *indica* extract. Fe NP-S - Fe nanoparticles synthesized from *Cannabis sativa* ssp. *sativa* extract; Fe commercial- commercial Fe₂O₃ nanoparticles; extract sativa- *Cannabis sativa* ssp. *sativa* extract; extract indica- *Cannabis sativa* ssp. *indica* extract. Different letters indicate that samples were statistically different (as determined by Tukey test at $p < 0.05$).

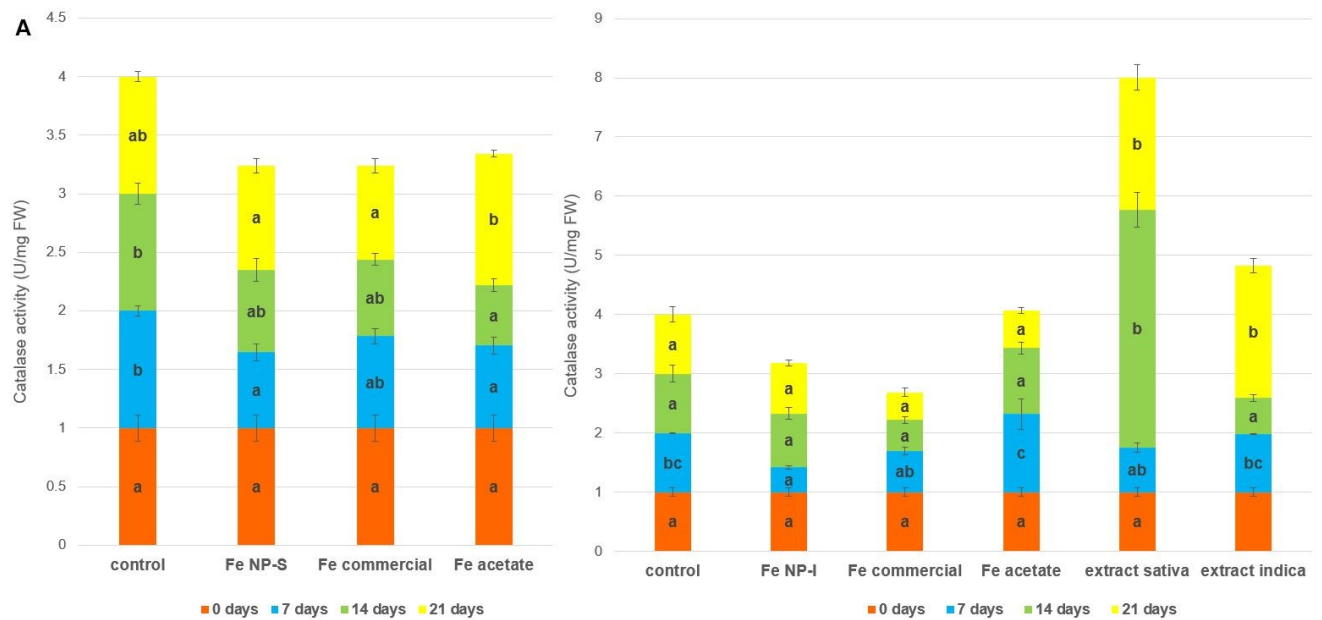


Figure S8. Changes in normalized catalase activity in soybean exposed to Fe NPs. A- Experiment with iron nanoparticles synthesized from *Cannabis sativa* ssp. *sativa* extract; B- Experiment with iron nanoparticle synthesized from *Cannabis sativa* ssp. *indica* extract. Fe NP-S - Fe nanoparticles synthesized from *Cannabis sativa* ssp. *sativa* extract; Fe commercial- commercial Fe₂O₃ nanoparticles; extract sativa- *Cannabis sativa* ssp. *sativa* extract; extract indica- *Cannabis sativa* ssp. *indica* extract. Different letters indicate that samples were statistically different (as determined by Tukey test at p < 0.05).

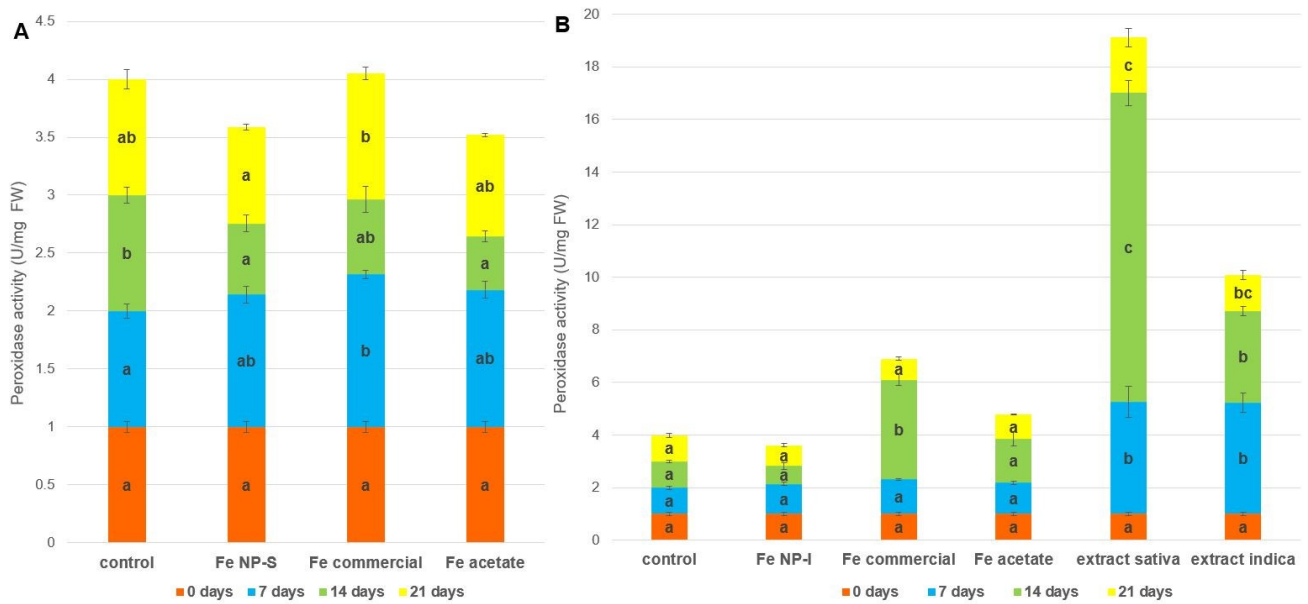


Figure S9. Changes in normalized peroxidase activity in soybean exposed to Fe NPs. A- Experiment with iron nanoparticles synthesized from *Cannabis sativa* ssp. *sativa* extract; B- Experiment with iron nanoparticle synthesized from *Cannabis sativa* ssp. *indica* extract. Fe NP-S - Fe nanoparticles synthesized from *Cannabis sativa* ssp. *sativa* extract; Fe commercial- commercial Fe₂O₃ nanoparticles; extract sativa- *Cannabis sativa* ssp. *sativa* extract; extract indica- *Cannabis sativa* ssp. *indica* extract. Different letters indicate that samples were statistically different (as determined by Tukey test at p < 0.05).

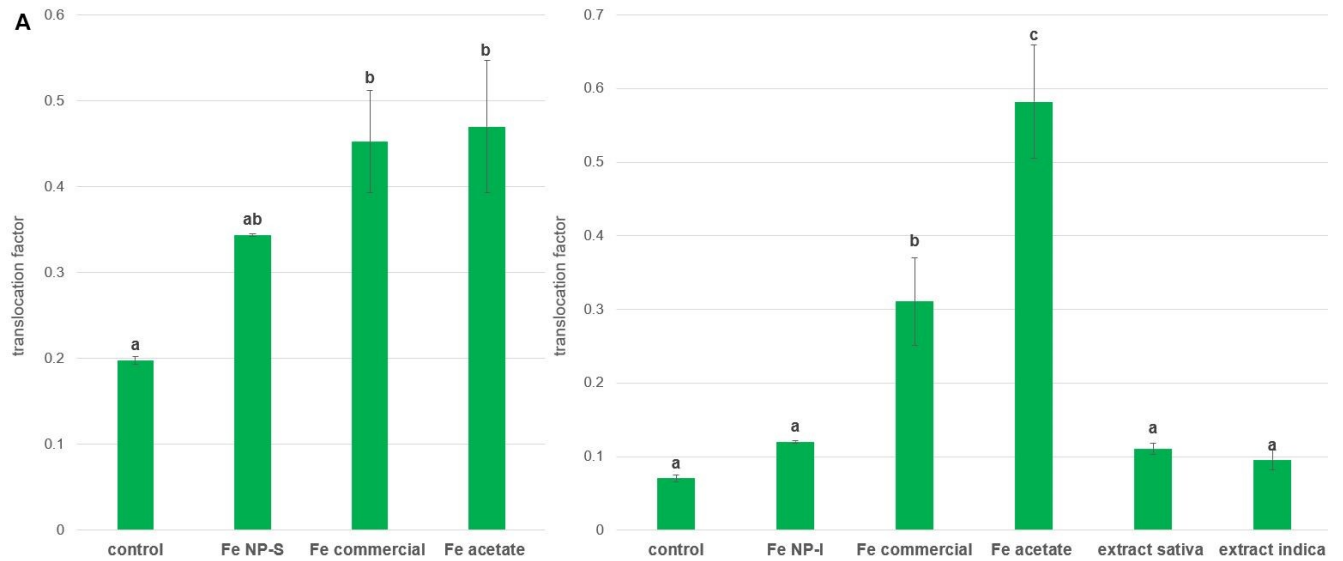


Figure S10. Translocation factor of Fe NPs in soybean. A- Experiment with iron nanoparticles synthesized from *Cannabis sativa* ssp. *sativa* extract; B- Experiment with iron nanoparticle synthesized from *Cannabis sativa* ssp. *indica* extract. Fe NP-S - Fe nanoparticles synthesized from *Cannabis sativa* ssp. *sativa* extract; Fe commercial- commercial Fe₂O₃ nanoparticles; extract sativa- *Cannabis sativa* ssp. *sativa* extract; extract indica- *Cannabis sativa* ssp. *indica* extract. Different letters indicate that samples were statistically different (as determined by Tukey test at p < 0.05).