Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting Information

Assessment of photo-modulation, nutrient-use efficiency and toxicity of iron nanoparticles in *Vigna radiata*

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EXPERIMENTAL SECTION

Isolation of chloroplast

Fresh leaf tissues of mung bean plants were homogenized in chloroplast isolation buffer; 330 mM mannitol, 30 mM HEPES, 2 mM EDTA, 3mM MgCl, and 0.1% w/v BSA, pH 7.8 and filtered. The filtrate was then centrifuged at cold for 5 min at 250 ×g. Supernatant were further centrifuged at 1000 ×g for 5 min at 4ºC. A pellet of chloroplast was then suspended in isolation buffer and stored at - 80ºC, till further use.

Photo reduction Activities

Whole chain electron transport

Polarographic estimation of the electron transport through the whole chain of photosynthesis was performed with an Oxygraph oxygen electrode (Hansatech Instruments, UK). Chloroplast was added to the reaction mixture (50 mM HEPES (pH 7.5), 10 mM NaCl, 1 mM NH_4Cl, 3 mM $MgCl_2$, 1. mM NaN_3, and 0.5 mM MV) to a total concentration of 378 µg/ mL. Entire reaction was monitored continuously for 5 min to determine the activity of ETC.

Oxygen evolution measurement

Oxygen evolution was analyzed polarographically in reaction buffer (0.33 M sorbitol, 2 mM NaEDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, and 50 mM HEPES, pH 7.6) containing chloroplasts equivalent to 378 µg/mL of chlorophyll. 0.88 mM Sodium 2, 6-dichlorophenolindophenol was added to the reaction just before the addition of the chloroplast. The isolated Chlorophyll solution was illuminated for 1 min with 500 µmol m^{-2} s^{-1} visible light (400–700 nm).

Hill reaction in chloroplast

Hill activity of the chloroplast solution, dissolved in sucrose phosphate buffer, was determined by measuring absorbance of 2, 6-dichlorophenol indophenol (0.03%) at 610 nm. Hill activity was expressed as μmole DCPIP reduced per hour per mg chlorophyll.

Photophosphorylation

Ferricyanide reduction was measured using the reaction mixture containing chloroplasts (378 μg/ mL); 86 mM sorbitol, 50 mM Tricine (pH 8.1), 50 mM NaCl, 5 mM $MgCl_2$, 2 mM K_2HPO_4, 2 mM ADP, and 1 mM ferricyanide. Immediately following 1 min of saturating irradiance, trichloroacetic acid was added. The mixture was centrifuged and the absorbance of the supernatant was determined at 420 nm. Dark control was used for negative control. The experimental conditions for measuring NADP reduction were identical except for the deletion of ferricyanide from the reaction medium and the addition of 3 μM purified spinach ferredoxin (Sigma) and 0.66 mM NADP. After 1 min of irradiation the reaction mixture was centrifuged and absorbance was determined at 340 nm.

Measurement of ATP content

Chloroplast solution (30 μg chlorophyll/ mL) suspended in the reaction mixture containing 0.4 mM sucrose, 50 mM Tris-HCl (pH 7.6), 10 mM NaCl, 5mM $MgCl_2$, 2 mM ADP and 10 mM Na_2HPO_4 was illuminated for 1 minand 10% TCA was then added. 3M Na_2CO_3 was used to neutralize the reaction. Solution was then centrifuged at 2000 rpm for 5 min to obtain the soluble fraction. This soluble fraction was subjected to ATP assay using Lambda 25 Perkin Elmer UV-Vis spectrophotometer and ATP colorimetric/Fluorimetric assay kit (Biovision, Catalog No. K354).

Synthesis of amine functionalized FeNPs

Amine functionalization of FeNP was carried out by using 3-aminopropyltriethoxysilane (APTES) via co-condensation reaction. In brief, FeNP, dispersed in DMSO solution, was subjected to ultra-bath sonication for 30 min. Measured amount of APTES was then added to the solution and the solution was refluxed at 120ºC for about 3 h. Then the mixture was centrifuged washed several times to remove the unreacted APTES. Finally the product was dried at 60ºC for overnight to produce amine functionalized FeNPs.

Conjugation of FITC to amine functionalized FeNPs

Aminated FeNPs of appropriate concentration were dispersed in 0.1 M Na_2CO_3 solution. To it, FITC dissolved in DMSO was added and the resulting solution was stirred at room temperature for 24 h in a dark condition. FeNP-FITC was separated by centrifugation at 4ºC and washed repeatedly to remove excess of FITC. This FITC labeled FeNPs were used for uptake study.

Isolation of FeS protein

FeS proteins from control and treated chloroplast samples were isolated following the procedure as mentioned by Malkin *et al.* 1974. In brief, the lyophilized chloroplast fragments (approximately 1.5 g of chlorophyll) were suspended in 1 L of absolute methanol (precooled to -20 °C) containing 1 mL of 2-mercaptoethanol. This suspension was quickly mixed with 15 L of acetone (precooled to -20 °C) containing 10 mL of 2-mercaptoethanol. The resulting suspension was stirred for approximately 1 min and rapidly filtered through a glass cintered funnel. The residue remaining on the funnel was dried under N_2 atmosphere; resuspended stirred for 2 h in a solution of 500 mL 50 mM Tris HCl buffer (pH 7.6) containing 2 mM cysteine-hydrochloride (hereafter referred to as buffer). The suspension was centrifuged at 35,000 g for 10 min and the pellet was discarded. The pale yellow brown supernatent constituted the soluble fraction used for

further purification of the iron-sulfur protein. The soluble fraction contained approximately half of the total inorganic sulfide, assayed as described below, in the chloroplast fragments prior to extraction. The supernatent solution was applied to a 3 X 25-cm DEAE-cellulose column (equilibrated with buffer) and the column was developed with a linear salt gradient (0.1-0.6 M NaCl). The sulfide-containing fractions were combined and diluted 5-fold with buffer free of cysteine. The dilute solution was applied to a 2 X 8-cm DEAE-cellulose column (equilibrated with buffer) and the adsorbed brown material was eluted with buffer containing 0.5 M NaCl. This concentrated fraction was applied to a 2.5 X 90-cm Sephadex G-75 column (equilibrated with a buffer containing 50 mM NaCl) and the column was eluted with the same buffer. The inorganic sulfide content of the fractions were concentrated and stored at -80 ºC for further study.

Biosafety study using mice model

The Biosafety study was carried out using young female non-pregnant mice weighing 20-22 g each. The animals were randomly distributed in control and 3 treated groups, containing 5 animals/group. They were allowed to acclimatize for 7 d before experiment in animal house with controlled temperature (23°C ± 2°C), humidity (60 % ± 10 %) and 12 h light/dark cycle. The control group received 1 mL of distilled water while treated groups received 1 mL of FeNPs mixed in distilled water at the dose of 1 mg/mL, 10 mg/mL and 25 mg/mL within 24 h. After administration, mice were observed daily for total 14 d. The symptoms of observation included changes in skin and fur, eyes membrane, behavior pattern. Special attention was paid to the clinical signs of toxicity including tremors, convulsions, salivation, nausea, vomiting, diarrhea, lethargy etc. After 14 days of treatment they were sacrificed. Blood and serum from control and treated mice were analyzed for TC (total count), DC (differential count), Plt (platelet count), LDH (lactate dehydrogenase), creatinine, ALP (alkaline phosphatase), TP (total protein),

cholesterol, TG (triglyceride), uric acid, BUN (blood urea nitrogen), SGOT (serum glutamic oxaloacetic transaminase), SGPT (serum glutamic pyruvic transaminase) and phosphorous level. Brain, heart, lungs, liver, kidney, spleen and testis were carefully removed and fixed in 10 % formalin solution containing neutral phosphate buffer saline. Thereafter the organs were embedded in paraffin, sectioned at 5 μM, mounted on glass slide and stained with eosin–hematoxylin using standard histopathological techniques. Sections were examined under light microscope. All the experiments were performed following OECD guideline.

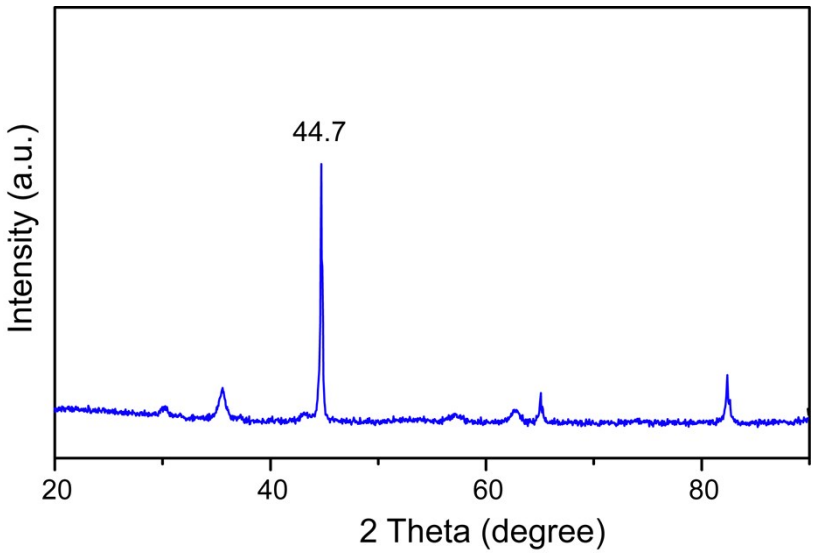

Figure S1. PXRD pattern of FeNPs, which shows characteristic diffraction pattern of zero valent iron nanoparticles at 2θ = 35.6°, 65.2°, 82.3°.

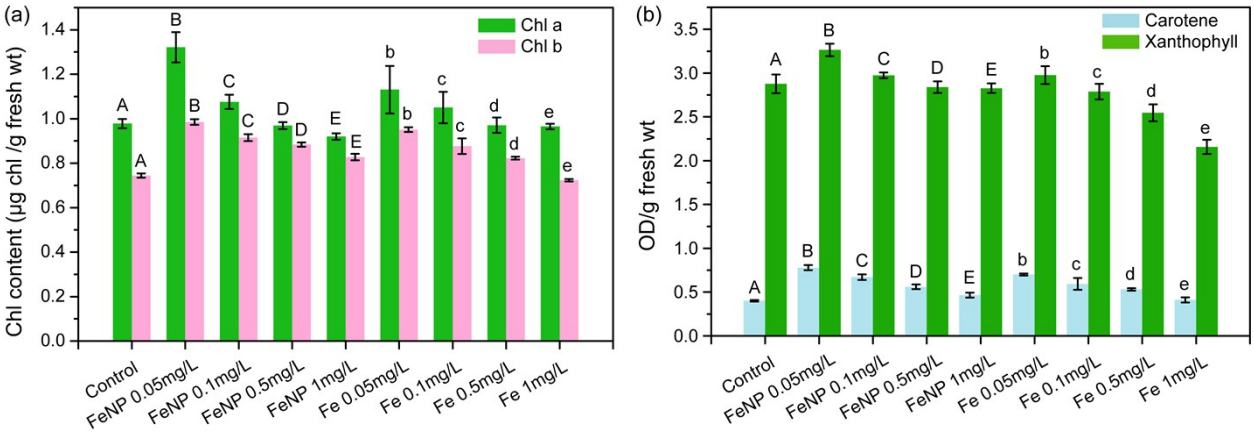

Figure S2. (a) Effect of FeNP and Fe on chlorophyll contents of 15days treated mung bean plants. (b) Effect of FeNP and Fe on carotenoid content of 15days treated mung bean plants. Data represents mean ± SE (n, no. of samples = 3). Within each type of treatment mean data (± SE, n=3) the upper case letter is used to denote FeNPs treatment for a particular dose, the lower case letter is used to denote Fe treatment for a particular dose; Tukey-Kramer HSD test.

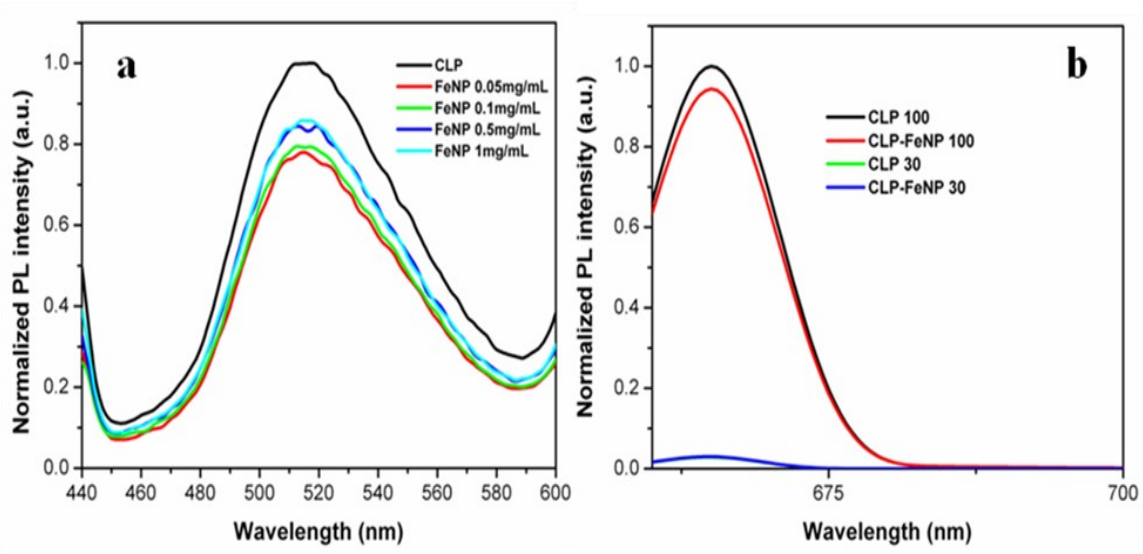

Figure S3. (a) PL measurements (b) PI index of control and FeNP treated chloroplast.

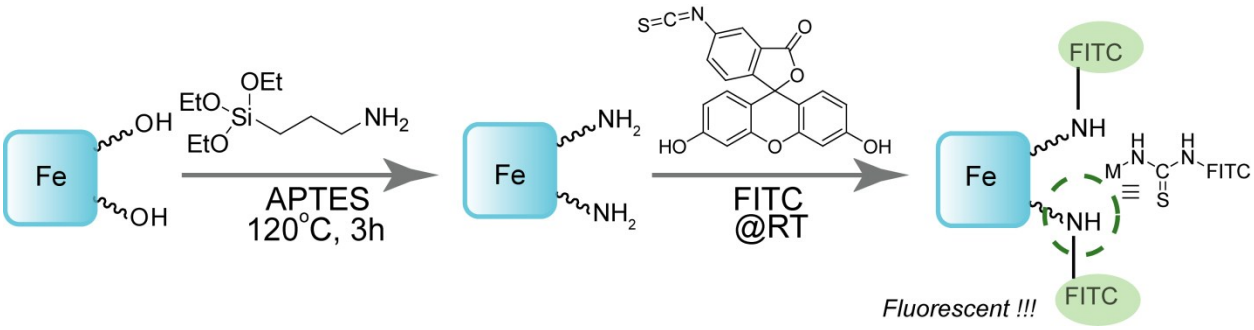

Figure S4. Schematic representation of fluorescent functionalization for qualitative uptake study of FeNPs. The uptake study is qualitative in nature. We functionalized the FeNPs using 3-aminopropyltriethoxy silane to yield amine functionalized FeNPs and then fluorescent counterpart fluoroscein isothiocyanate (FITC) was conjugated with it. That resulted in FITC conjugated FeNPs which was used for uptake studies of FeNPs.

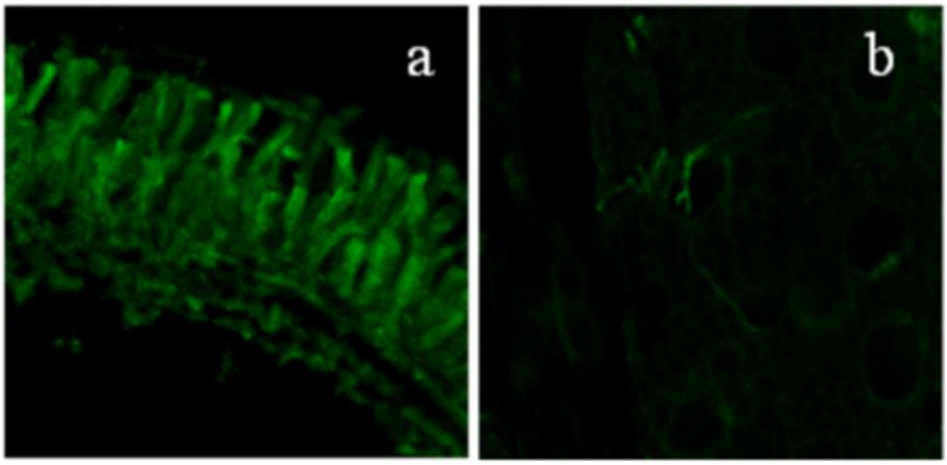

Figure S5. Uptake of FeNPs in (a) leaf and (b) root.

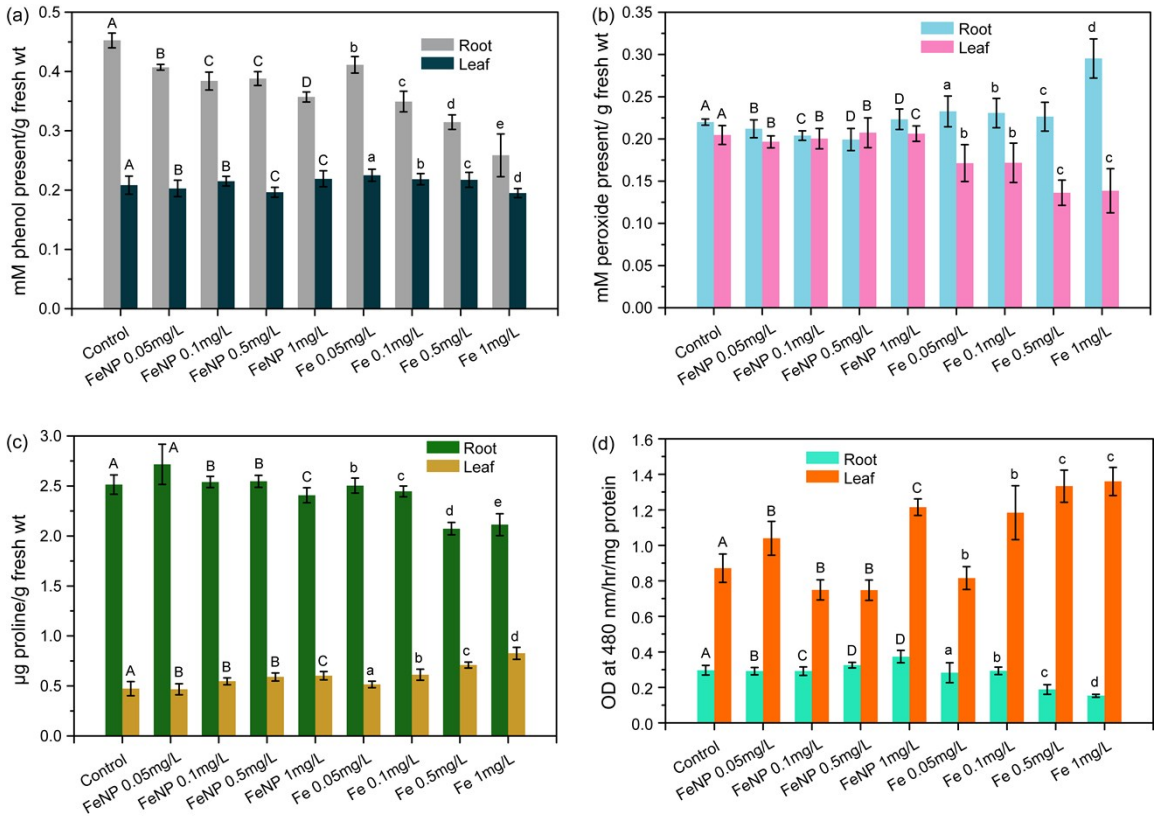

Figure S6. (a) Phenol content, (b) Total peroxide, (c) Proline, (d) PPO content of 15days treated plants. Within each type of treatment mean data (± SE, n=3) the upper case letter is used to denote FeNPs treatment for a particular dose, the lower case letter is used to denote Fe treatment for a particular dose; Tukey-Kramer HSD test.

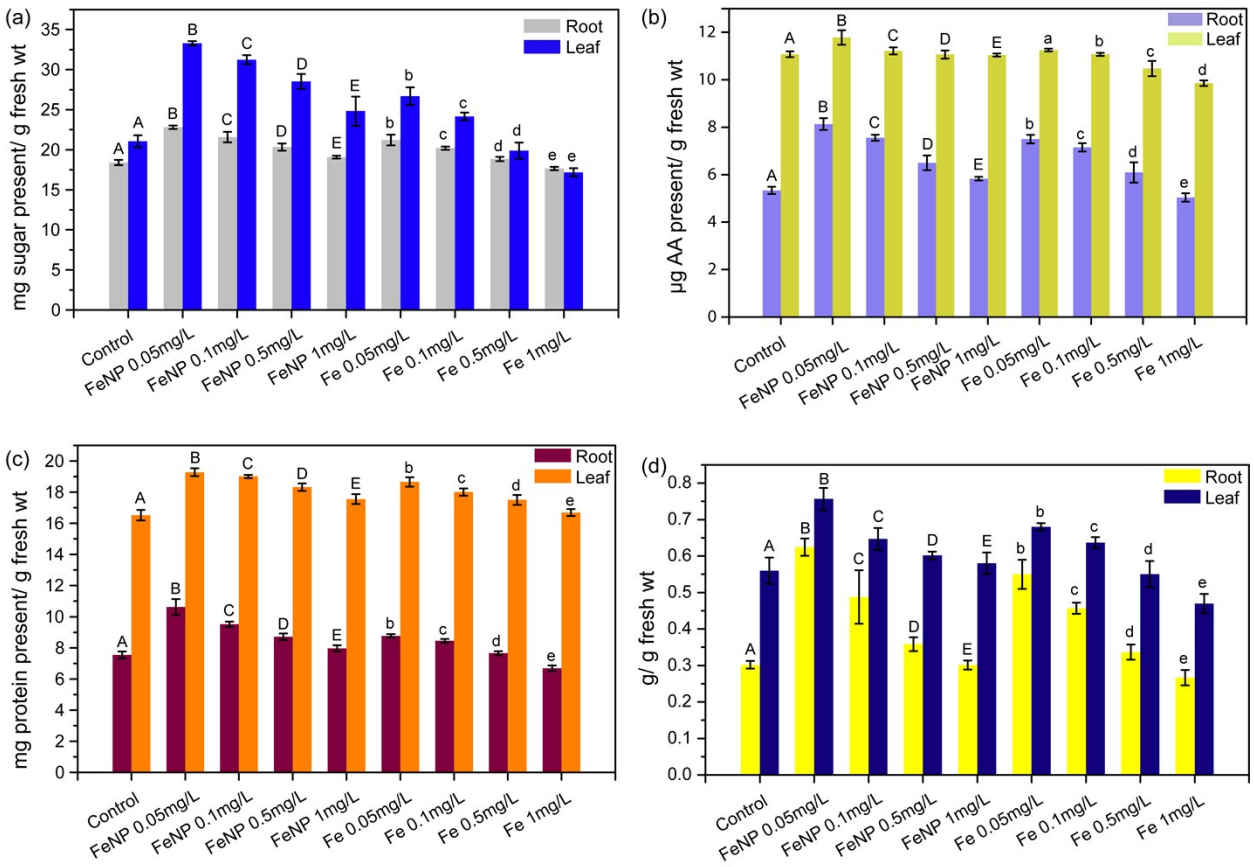

Figure S7. (a) Sugar content, (b) AA content, (c) Protein content, (d) Lipid content of 15days treated plants. Within each type of treatment mean data (± SE, n=3) the upper case letter is used to denote FeNPs treatment for a particular dose, the lower case letter is used to denote Fe treatment for a particular dose; Tukey-Kramer HSD test.

Table T1. Iron content estimation by ICP-OES

Type of Sample	Iron content in Control (mg/Kg)	Iron content in treatment (mg/Kg)
Root	22.82 ±0.025	23.5 ±0.4
Leaf	26.51 ±0.05	28.48 ±1

Table T2. Body weight of FeNP treated and untreated (control) mice in acute oral toxicity assay.

Animal	Control	FeNP-1mg/L	FeNP-10mg/L	FeNP-25mg/L
Mice body weight (g)	21.6 ±0.153	22.42 ±0.0208	211.87 ±0.58	22.28 ±1

Table T3. Comparison of blood biochemical parameters between control and FeNP treated mice.

Parameters		Control	FeNP		
			1mg/L	10mg/L	25mg/L
Hemoglobin (g/dl)		15.63±0.551	12±0.87	15.67±.45	11.66±1.53
Total Count	**RBC (million/ mm^3)**	5.03±0.208	4.73± 0.25	4.96±.15	4.76±.058
	WBC(million/ mm^3	6400±50	7550±327.88	6766.08±305.44	5433.33±305.56
Differential Count (%)	**Neutrophils**	42±6.03	43.33±1.528	45±4	43.33±2.52
	Lymphocytes	25±3	27.67±2.081	32±3.6	32±2.65
	Monocytes	2±0	1±0	2±0	2±0
	Eosinophils	2±0	1.33±0.58	2±0	2±0
	Basophils	0±0	0±0	0±0	0±0
Plt (lakh/ mm^3)		1.65±0.05	1.69±0.04	1.74±0.04	1.64±.04
LDH (IU/L)		224±6	210±11.14	234±14.53	252.67±11.59
Creatinine (mg/dl)		0.83±0.025	0.77 ±.05	0.753±.02	0.787±.03
ALP (U/L)		73.67±2.52	75±3	68.33±8.74	80.67±5.13
Total Protein (gm/dl)		6.77±0.153	6.87±.45	6.4±.3	7.03±.15
Cholesterol (mg/dl)		140±2.65	160±35.08	138.33±8.74	140±5.29

Triglyceride (mg/dl)	91.33±2.52	116.33±15.57	132.77±13.2	139±9.2
Uric Acid (mg/dl)	6.33±0.252	6.333±.61	7.7±.56	7.23±0.25
BUN (mg/dl)	11.67±1.15	11±2.65	11.33±2.52	14.67±2.87
SGOT (Unit/L)	32.33±1.53	30.11±3.05	22.67±4.73	35.67±6.11
SGPT (Unit/L)	22.33±1.15	28.33±3.51	31±4	55.33±3.51
Phosphorous (mg/dl)	3.67±0.058	3.467±.513	3.6±.89	3.33±0.31

Statistical analyses

1. Morphology

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment type	Pillai's Trace	.991	924.912[a]	4.000	33.000	.000
	Wilks' Lambda	.009	924.912[a]	4.000	33.000	.000
	Hotelling's Trace	112.111	924.912[a]	4.000	33.000	.000
	Roy's Largest Root	112.111	924.912[a]	4.000	33.000	.000
Concentration	Pillai's Trace	1.788	12.913	12.000	105.000	.000
	Wilks' Lambda	.002	70.620	12.000	87.601	.000
	Hotelling's Trace	150.399	396.886	12.000	95.000	.000
	Roy's Largest Root	148.152	1296.334[b]	4.000	35.000	.000
Treatment type * Concentration	Pillai's Trace	1.697	11.396	12.000	105.000	.000
	Wilks' Lambda	.056	14.457	12.000	87.601	.000
	Hotelling's Trace	5.886	15.531	12.000	95.000	.000
	Roy's Largest Root	3.750	32.817[b]	4.000	35.000	.000

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment type	Root Length	41.534	1	41.534	913.536	.000
	Shoot Length	31.952	1	31.952	1777.830	.000
	Fresh weight	.072	1	.072	894.714	.000
	Dry weight	.001	1	.001	686.940	.000
Concentration	Root Length	59.780	3	19.927	438.280	.000
	Shoot Length	32.632	3	10.877	605.234	.000
	Fresh weight	.113	3	.038	468.381	.000
	Dry weight	.002	3	.001	332.450	.000
Treatment type * Concentration	Root Length	1.865	3	.622	13.675	.000
	Shoot Length	1.667	3	.556	30.912	.000
	Fresh weight	.001	3	.000	4.762	.007
	Dry weight	.000	3	3.792E-5	22.750	.000

2. Chlorophyll content

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment type	Pillai's Trace	.828	40.933[a]	2.000	17.000	.000
	Wilks' Lambda	.172	40.933[a]	2.000	17.000	.000
	Hotelling's Trace	4.816	40.933[a]	2.000	17.000	.000
	Roy's Largest Root	4.816	40.933[a]	2.000	17.000	.000
Concentration	Pillai's Trace	1.346	12.341	6.000	36.000	.000
	Wilks' Lambda	.020	34.157[a]	6.000	34.000	.000
	Hotelling's Trace	30.314	80.836	6.000	32.000	.000
	Roy's Largest Root	29.705	178.231[b]	3.000	18.000	.000
Treatment type * Concentration	Pillai's Trace	.848	4.418	6.000	36.000	.002
	Wilks' Lambda	.277	5.094[a]	6.000	34.000	.001
	Hotelling's Trace	2.154	5.744	6.000	32.000	.000
	Roy's Largest Root	1.918	11.509[b]	3.000	18.000	.000

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment type	chlorophyll a content	.011	1	.011	3.959	.062
	chlorophyll b content	.021	1	.021	85.157	.000
Concentration	chlorophyll a content	.294	3	.098	36.255	.000
	chlorophyll b content	.116	3	.039	156.040	.000
Treatment type * Concentration	chlorophyll a content	.048	3	.016	5.899	.005
	chlorophyll b content	.005	3	.002	6.195	.004

3. Carotenoid content

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment type	Pillai's Trace	.828	40.933[a]	2.000	17.000	.000
	Wilks' Lambda	.172	40.933[a]	2.000	17.000	.000
	Hotelling's Trace	4.816	40.933[a]	2.000	17.000	.000
	Roy's Largest Root	4.816	40.933[a]	2.000	17.000	.000
Concentration	Pillai's Trace	1.346	12.341	6.000	36.000	.000
	Wilks' Lambda	.020	34.157[a]	6.000	34.000	.000
	Hotelling's Trace	30.314	80.836	6.000	32.000	.000
	Roy's Largest Root	29.705	178.231[b]	3.000	18.000	.000
Treatment type * Concentration	Pillai's Trace	.848	4.418	6.000	36.000	.002
	Wilks' Lambda	.277	5.094[a]	6.000	34.000	.001
	Hotelling's Trace	2.154	5.744	6.000	32.000	.000
	Roy's Largest Root	1.918	11.509[b]	3.000	18.000	.000

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment type	carotene content	.011	1	.011	3.959	.062
	xanthophyll content	.021	1	.021	85.157	.000
Concentration	carotene content	.294	3	.098	36.255	.000
	xanthophyll content	.116	3	.039	156.040	.000
Treatment type * Concentration	carotene content	.048	3	.016	5.899	.005
	xanthophyll content	.005	3	.002	6.195	.004

4. Photosynthetic pathway analysis

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment type	Pillai's Trace	.971	71.877[a]	6.000	13.000	.000
	Wilks' Lambda	.029	71.877[a]	6.000	13.000	.000
	Hotelling's Trace	33.174	71.877[a]	6.000	13.000	.000
	Roy's Largest Root	33.174	71.877[a]	6.000	13.000	.000
Concentration	Pillai's Trace	1.944	4.599	18.000	45.000	.000
	Wilks' Lambda	.004	12.859	18.000	37.255	.000
	Hotelling's Trace	70.884	45.943	18.000	35.000	.000
	Roy's Largest Root	68.897	172.243[b]	6.000	15.000	.000
Treatment type * Concentration	Pillai's Trace	1.928	4.496	18.000	45.000	.000
	Wilks' Lambda	.021	6.002	18.000	37.255	.000

	Hotelling's Trace	11.275	7.308	18.000	35.000	.000
	Roy's Largest Root	8.492	21.231[b]	6.000	15.000	.000

a. Exact statistic
b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment type	oxygen uptake	9.493	1	9.493	303.104	.000
	oxygen evolution	5.558	1	5.558	181.363	.000
	Hill activity	.004	1	.004	142.954	.000
	PS II activity	.093	1	.093	98.725	.000
	PS I activity	.373	1	.373	70.463	.000
	ATP content	2.072E-5	1	2.072E-5	17.407	.001
Concentration	oxygen uptake	13.443	3	4.481	143.078	.000
	oxygen evolution	16.146	3	5.382	175.608	.000
	Hill activity	.008	3	.003	96.637	.000
	PS II activity	.330	3	.110	116.144	.000
	PS I activity	.533	3	.178	33.567	.000
	ATP content	7.465E-5	3	2.488E-5	20.905	.000
Treatment type * Concentration	oxygen uptake	.215	3	.072	2.288	.113
	oxygen evolution	.175	3	.058	1.907	.165
	Hill activity	.001	3	.000	12.742	.000
	PS II activity	.069	3	.023	24.254	.000
	PS I activity	.036	3	.012	2.241	.118
	ATP content	9.346E-7	3	3.115E-7	.262	.852

5. Lipid content

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment type	Pillai's Trace	.727	22.586[a]	2.000	17.000	.000
	Wilks' Lambda	.273	22.586[a]	2.000	17.000	.000
	Hotelling's Trace	2.657	22.586[a]	2.000	17.000	.000
	Roy's Largest Root	2.657	22.586[a]	2.000	17.000	.000
concentration	Pillai's Trace	.992	5.908	6.000	36.000	.000
	Wilks' Lambda	.030	27.211[a]	6.000	34.000	.000
	Hotelling's Trace	31.921	85.123	6.000	32.000	.000
	Roy's Largest Root	31.898	191.387[b]	3.000	18.000	.000
Treatment type * concentration	Pillai's Trace	.504	2.019	6.000	36.000	.088
	Wilks' Lambda	.536	2.071[a]	6.000	34.000	.083
	Hotelling's Trace	.790	2.106	6.000	32.000	.080
	Roy's Largest Root	.680	4.079[b]	3.000	18.000	.023

a. Exact statistic
b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment type	lipid content in root	.010	1	.010	9.502	.006
	lipid content in leaves	.023	1	.023	31.980	.000
concentration	lipid content in root	.326	3	.109	105.454	.000
	lipid content in leaves	.126	3	.042	58.142	.000
Treatment type * concentration	lipid content in root	.002	3	.001	.787	.517
	lipid content in leaves	.008	3	.003	3.685	.031

6. Protein content

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment type	Pillai's Trace	.933	118.361[a]	2.000	17.000	.000
	Wilks' Lambda	.067	118.361[a]	2.000	17.000	.000
	Hotelling's Trace	13.925	118.361[a]	2.000	17.000	.000
	Roy's Largest Root	13.925	118.361[a]	2.000	17.000	.000
Concentration	Pillai's Trace	1.003	6.032	6.000	36.000	.000
	Wilks' Lambda	.033	25.725[a]	6.000	34.000	.000
	Hotelling's Trace	28.606	76.283	6.000	32.000	.000
	Roy's Largest Root	28.568	171.411[b]	3.000	18.000	.000
Treatment type * concentration	Pillai's Trace	.430	1.644	6.000	36.000	.164
	Wilks' Lambda	.577	1.795[a]	6.000	34.000	.130
	Hotelling's Trace	.722	1.925	6.000	32.000	.107
	Roy's Largest Root	.705	4.229[b]	3.000	18.000	.020

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment type	protein in root	10.350	1	10.350	188.027	.000
	protein in leaves	4.100	1	4.100	57.110	.000
concentration	protein in root	18.897	3	6.299	114.431	.000
	protein in leaves	11.434	3	3.811	53.086	.000
Treatment type * concentration	protein in root	.642	3	.214	3.886	.026
	protein in leaves	.109	3	.036	.505	.684

7. Sugar content

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment type	Pillai's Trace	.972	298.432[a]	2.000	17.000	.000
	Wilks' Lambda	.028	298.432[a]	2.000	17.000	.000
	Hotelling's Trace	35.110	298.432[a]	2.000	17.000	.000
	Roy's Largest Root	35.110	298.432[a]	2.000	17.000	.000
Concentration	Pillai's Trace	.999	5.987	6.000	36.000	.000
	Wilks' Lambda	.020	34.278[a]	6.000	34.000	.000
	Hotelling's Trace	47.743	127.313	6.000	32.000	.000
	Roy's Largest Root	47.723	286.336[b]	3.000	18.000	.000
Treatment type * concentration	Pillai's Trace	.206	.689	6.000	36.000	.660
	Wilks' Lambda	.797	.682[a]	6.000	34.000	.666
	Hotelling's Trace	.252	.671	6.000	32.000	.674
	Roy's Largest Root	.238	1.425[b]	3.000	18.000	.268

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment type	sugar in root	12.995	1	12.995	82.460	.000
	sugar in leaves	336.676	1	336.676	390.990	.000
concentration	sugar in root	44.810	3	14.937	94.782	.000
	sugar in leaves	279.732	3	93.244	108.287	.000
Treatment type * concentration	sugar in root	.041	3	.014	.087	.966
	sugar in leaves	3.464	3	1.155	1.341	.292

8. AA content

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment type	Pillai's Trace	.854	49.913[a]	2.000	17.000	.000
	Wilks' Lambda	.146	49.913[a]	2.000	17.000	.000
	Hotelling's Trace	5.872	49.913[a]	2.000	17.000	.000
	Roy's Largest Root	5.872	49.913[a]	2.000	17.000	.000
Concentration	Pillai's Trace	1.082	7.065	6.000	36.000	.000
	Wilks' Lambda	.032	26.026[a]	6.000	34.000	.000
	Hotelling's Trace	26.729	71.278	6.000	32.000	.000
	Roy's Largest Root	26.596	159.575[b]	3.000	18.000	.000
Treatment type * concentration	Pillai's Trace	.648	2.878	6.000	36.000	.021
	Wilks' Lambda	.375	3.588[a]	6.000	34.000	.007
	Hotelling's Trace	1.605	4.280	6.000	32.000	.003
	Roy's Largest Root	1.565	9.391[b]	3.000	18.000	.001

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment type	Amino acid in root	1.893	1	1.893	35.229	.000
	Amino acid in leaves	2.251	1	2.251	80.850	.000
Concentration	Amino acid in root	20.566	3	6.855	127.587	.000
	Amino acid in leaves	3.986	3	1.329	47.718	.000
Treatment type * concentration	Amino acid in root	.159	3	.053	.989	.420
	Amino acid in leaves	.760	3	.253	9.094	.001

9. CAT activity

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment type	Pillai's Trace	.600	12.750[a]	2.000	17.000	.000
	Wilks' Lambda	.400	12.750[a]	2.000	17.000	.000
	Hotelling's Trace	1.500	12.750[a]	2.000	17.000	.000
	Roy's Largest Root	1.500	12.750[a]	2.000	17.000	.000
Concentration	Pillai's Trace	.832	4.276	6.000	36.000	.002
	Wilks' Lambda	.237	5.962[a]	6.000	34.000	.000
	Hotelling's Trace	2.918	7.781	6.000	32.000	.000
	Roy's Largest Root	2.813	16.880[b]	3.000	18.000	.000
Treatment type * concentration	Pillai's Trace	.648	2.874	6.000	36.000	.022
	Wilks' Lambda	.413	3.152[a]	6.000	34.000	.014
	Hotelling's Trace	1.275	3.401	6.000	32.000	.010
	Roy's Largest Root	1.148	6.886[b]	3.000	18.000	.003

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment type	CAT in root	.627	1	.627	19.418	.000
	CAT in leaves	.011	1	.011	1.358	.259
Concentration	CAT in root	.236	3	.079	2.436	.098
	CAT in leaves	.252	3	.084	10.573	.000
Treatment type *	CAT in root	.269	3	.090	2.778	.071

concentration	CAT in leaves	.069	3	.023	2.879	.065

10. GR activity

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment type	Pillai's Trace	.851	48.489[a]	2.000	17.000	.000
	Wilks' Lambda	.149	48.489[a]	2.000	17.000	.000
	Hotelling's Trace	5.705	48.489[a]	2.000	17.000	.000
	Roy's Largest Root	5.705	48.489[a]	2.000	17.000	.000
Concentration	Pillai's Trace	.933	5.244	6.000	36.000	.001
	Wilks' Lambda	.158	8.586[a]	6.000	34.000	.000
	Hotelling's Trace	4.751	12.669	6.000	32.000	.000
	Roy's Largest Root	4.627	27.760[b]	3.000	18.000	.000
Treatment type * concentration	Pillai's Trace	.763	3.702	6.000	36.000	.006
	Wilks' Lambda	.288	4.900[a]	6.000	34.000	.001
	Hotelling's Trace	2.301	6.136	6.000	32.000	.000
	Roy's Largest Root	2.221	13.329[b]	3.000	18.000	.000

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment type	GR in root	.007	1	.007	54.245	.000
	GR in leaves	.015	1	.015	55.414	.000
Concentration	GR in root	.008	3	.003	21.984	.000
	GR in leaves	.007	3	.002	8.095	.001
Treatment type * concentration	GR in root	.004	3	.001	10.799	.000
	GR in leaves	.003	3	.001	3.735	.030

11. POD activity

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment type	Pillai's Trace	.440	6.678[a]	2.000	17.000	.007
	Wilks' Lambda	.560	6.678[a]	2.000	17.000	.007
	Hotelling's Trace	.786	6.678[a]	2.000	17.000	.007
	Roy's Largest Root	.786	6.678[a]	2.000	17.000	.007
Concentration	Pillai's Trace	.769	3.745	6.000	36.000	.005
	Wilks' Lambda	.290	4.854[a]	6.000	34.000	.001
	Hotelling's Trace	2.245	5.987	6.000	32.000	.000
	Roy's Largest Root	2.151	12.907[b]	3.000	18.000	.000
Treatment type * concentration	Pillai's Trace	.451	1.747	6.000	36.000	.138
	Wilks' Lambda	.550	1.976[a]	6.000	34.000	.097
	Hotelling's Trace	.818	2.180	6.000	32.000	.071
	Roy's Largest Root	.816	4.896[b]	3.000	18.000	.012

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment type	POD in root	.001	1	.001	5.423	.032
	POD in leaves	.001	1	.001	8.202	.010
Concentration	POD in root	.003	3	.001	6.177	.004

	POD in leaves	.002	3	.001	6.835	.003
Treatment type * concentration	POD in root	.001	3	.000	2.570	.086
	POD in leaves	.001	3	.000	2.155	.129

12. Phenol content

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment type	Pillai's Trace	.777	29.624[a]	2.000	17.000	.000
	Wilks' Lambda	.223	29.624[a]	2.000	17.000	.000
	Hotelling's Trace	3.485	29.624[a]	2.000	17.000	.000
	Roy's Largest Root	3.485	29.624[a]	2.000	17.000	.000
Concentration	Pillai's Trace	.959	5.528	6.000	36.000	.000
	Wilks' Lambda	.125	10.375[a]	6.000	34.000	.000
	Hotelling's Trace	6.342	16.912	6.000	32.000	.000
	Roy's Largest Root	6.234	37.405[b]	3.000	18.000	.000
Treatment type * concentration	Pillai's Trace	.988	5.856	6.000	36.000	.000
	Wilks' Lambda	.229	6.180[a]	6.000	34.000	.000
	Hotelling's Trace	2.424	6.464	6.000	32.000	.000
	Roy's Largest Root	1.934	11.607[b]	3.000	18.000	.000

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment type	Phenol content in root	.015	1	.015	53.808	.000
	Phenol content in leaves	.000	1	.000	1.513	.235
Concentration	Phenol content in root	.032	3	.011	36.936	.000
	Phenol content in leaves	.000	3	.000	1.154	.355
Treatment type * concentration	Phenol content in root	.009	3	.003	10.627	.000
	Phenol content in leaves	.002	3	.001	5.491	.007

13. PPO activity

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment type	Pillai's Trace	.767	28.029[a]	2.000	17.000	.000
	Wilks' Lambda	.233	28.029[a]	2.000	17.000	.000
	Hotelling's Trace	3.298	28.029[a]	2.000	17.000	.000
	Roy's Largest Root	3.298	28.029[a]	2.000	17.000	.000
Concentration	Pillai's Trace	.390	1.454	6.000	36.000	.222
	Wilks' Lambda	.639	1.420[a]	6.000	34.000	.236
	Hotelling's Trace	.518	1.382	6.000	32.000	.252
	Roy's Largest Root	.404	2.425[b]	3.000	18.000	.099
Treatment type * concentration	Pillai's Trace	.979	5.754	6.000	36.000	.000
	Wilks' Lambda	.177	7.806[a]	6.000	34.000	.000
	Hotelling's Trace	3.771	10.056	6.000	32.000	.000
	Roy's Largest Root	3.520	21.123[b]	3.000	18.000	.000

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment type	Polyphenol oxidase in root	.050	1	.050	59.320	.000

	Polyphenol oxidase in leaves	.332	1	.332	2.678	.119
Concentration	Polyphenol oxidase in root	.006	3	.002	2.192	.124
	Polyphenol oxidase in leaves	.469	3	.156	1.260	.318
Treatment type * concentration	Polyphenol oxidase in root	.051	3	.017	20.373	.000
	Polyphenol oxidase in leaves	.572	3	.191	1.537	.239

14. Proline content

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment type	Pillai's Trace	.774	29.054[a]	2.000	17.000	.000
	Wilks' Lambda	.226	29.054[a]	2.000	17.000	.000
	Hotelling's Trace	3.418	29.054[a]	2.000	17.000	.000
	Roy's Largest Root	3.418	29.054[a]	2.000	17.000	.000
Concentration	Pillai's Trace	.910	5.013	6.000	36.000	.001
	Wilks' Lambda	.150	8.962[a]	6.000	34.000	.000
	Hotelling's Trace	5.261	14.030	6.000	32.000	.000
	Roy's Largest Root	5.184	31.102[b]	3.000	18.000	.000
Treatment type * concentration	Pillai's Trace	.769	3.750	6.000	36.000	.005
	Wilks' Lambda	.377	3.558[a]	6.000	34.000	.008
	Hotelling's Trace	1.262	3.364	6.000	32.000	.011
	Roy's Largest Root	.727	4.364[b]	3.000	18.000	.018

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment type	Proline in root	.433	1	.433	45.228	.000
	Proline in leaves	.079	1	.079	33.270	.000
Concentration	Proline in root	.475	3	.158	16.543	.000
	Proline in leaves	.166	3	.055	23.416	.000
Treatment type * concentration	Proline in root	.114	3	.038	3.980	.024
	Proline in leaves	.028	3	.009	3.915	.026

15. SOD activity

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment type	Pillai's Trace	.732	23.174[a]	2.000	17.000	.000
	Wilks' Lambda	.268	23.174[a]	2.000	17.000	.000
	Hotelling's Trace	2.726	23.174[a]	2.000	17.000	.000
	Roy's Largest Root	2.726	23.174[a]	2.000	17.000	.000
Concentration	Pillai's Trace	1.130	7.787	6.000	36.000	.000
	Wilks' Lambda	.107	11.670[a]	6.000	34.000	.000
	Hotelling's Trace	6.147	16.393	6.000	32.000	.000
	Roy's Largest Root	5.763	34.580[b]	3.000	18.000	.000
Treatment type * concentration	Pillai's Trace	.959	5.530	6.000	36.000	.000
	Wilks' Lambda	.118	10.801[a]	6.000	34.000	.000
	Hotelling's Trace	6.789	18.105	6.000	32.000	.000
	Roy's Largest Root	6.691	40.148[b]	3.000	18.000	.000

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment type	SOD in root	12.070	1	12.070	12.573	.002
	SOD in leaves	27.243	1	27.243	37.221	.000
Concentration	SOD in root	20.736	3	6.912	7.200	.002
	SOD in leaves	66.026	3	22.009	30.070	.000
Treatment type * concentration	SOD in root	35.517	3	11.839	12.332	.000
	SOD in leaves	63.704	3	21.235	29.012	.000

16. Total peroxide content

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment type	Pillai's Trace	.794	32.715[a]	2.000	17.000	.000
	Wilks' Lambda	.206	32.715[a]	2.000	17.000	.000
	Hotelling's Trace	3.849	32.715[a]	2.000	17.000	.000
	Roy's Largest Root	3.849	32.715[a]	2.000	17.000	.000
Concentration	Pillai's Trace	.858	4.507	6.000	36.000	.002
	Wilks' Lambda	.242	5.843[a]	6.000	34.000	.000
	Hotelling's Trace	2.712	7.232	6.000	32.000	.000
	Roy's Largest Root	2.550	15.298[b]	3.000	18.000	.000
Treatment type * concentration	Pillai's Trace	.751	3.606	6.000	36.000	.007
	Wilks' Lambda	.377	3.564[a]	6.000	34.000	.008
	Hotelling's Trace	1.315	3.507	6.000	32.000	.009
	Roy's Largest Root	.964	5.783[b]	3.000	18.000	.006

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment type	total peroxide in root	.009	1	.009	40.234	.000
	Total peroxide in leaves	.014	1	.014	47.473	.000
Concentration	total peroxide in root	.010	3	.003	14.918	.000
	Total peroxide in leaves	.001	3	.000	1.138	.360
Treatment type * concentration	total peroxide in root	.004	3	.001	5.525	.007
	Total peroxide in leaves	.003	3	.001	3.075	.054