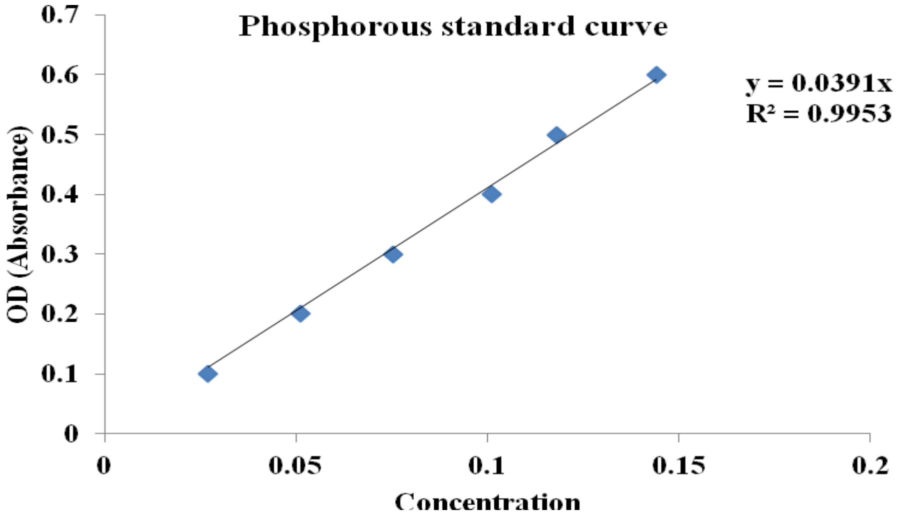
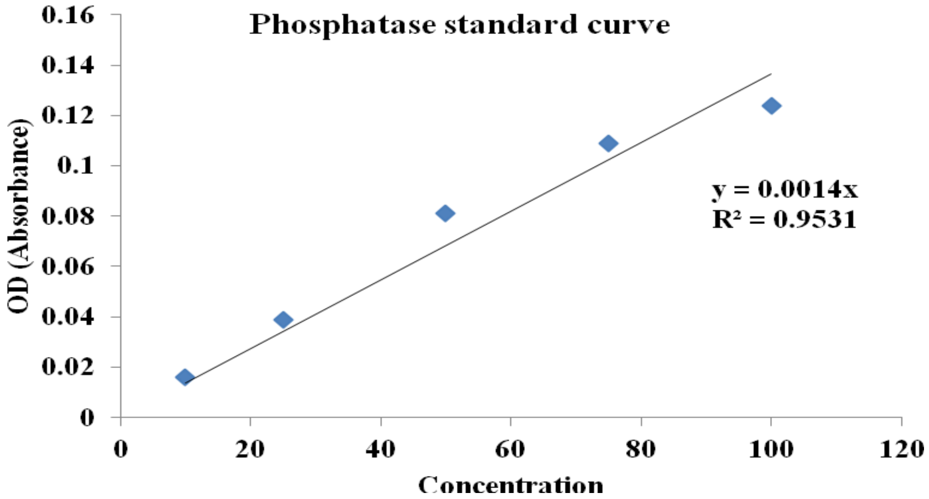
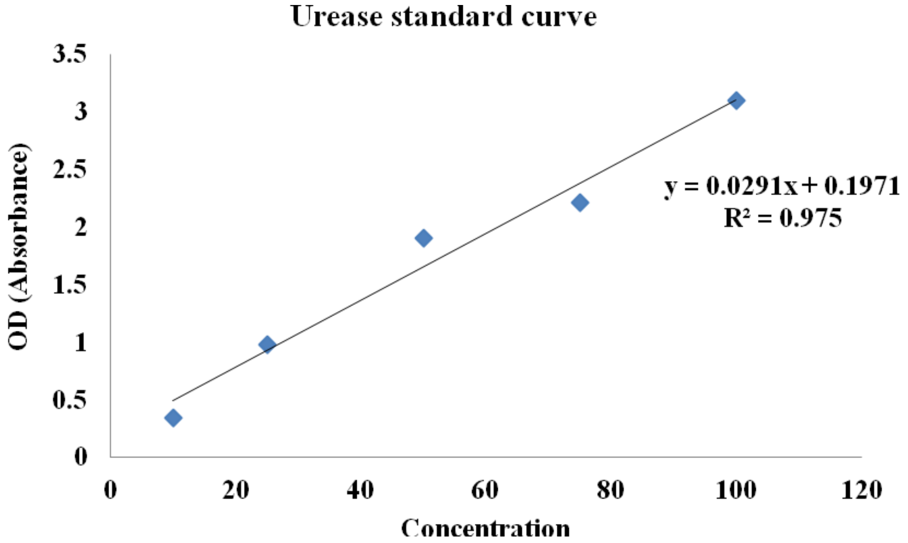
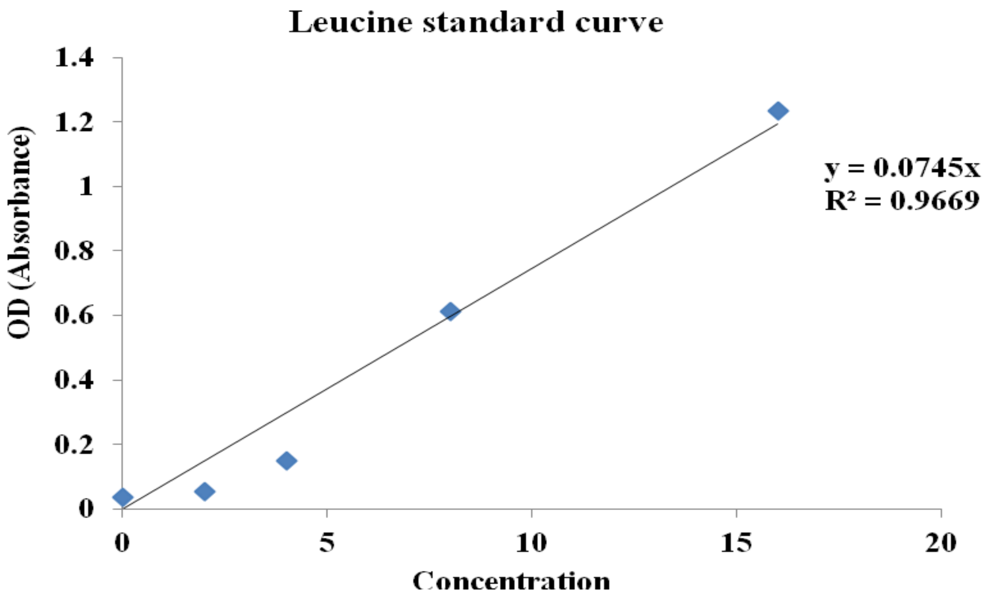
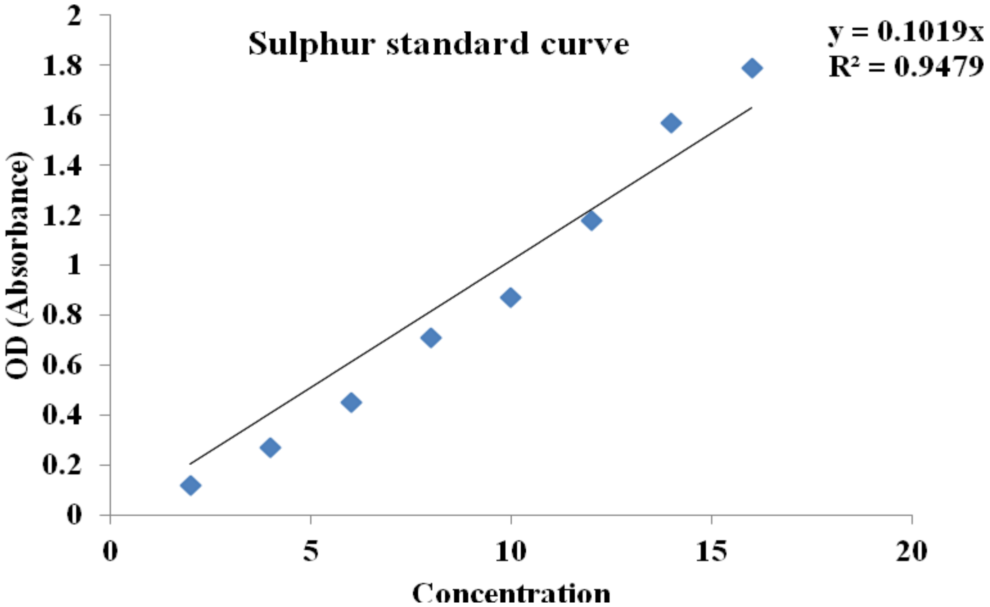
ANNEXURE

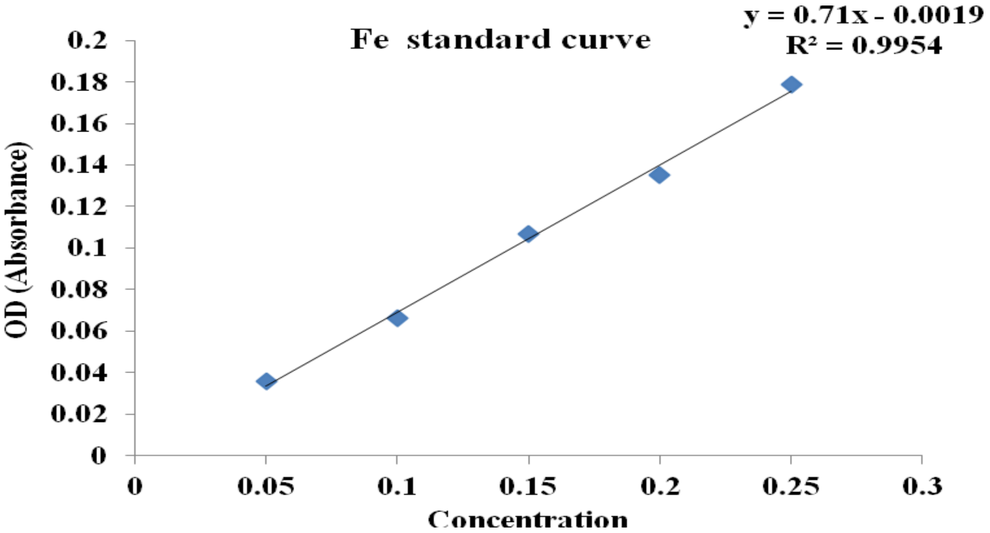

Phosphorous standard curve
y = 0.0391x
R² = 0.9953
OD (Absorbance)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
0.05
0.1
0.15
0.2
Concentration

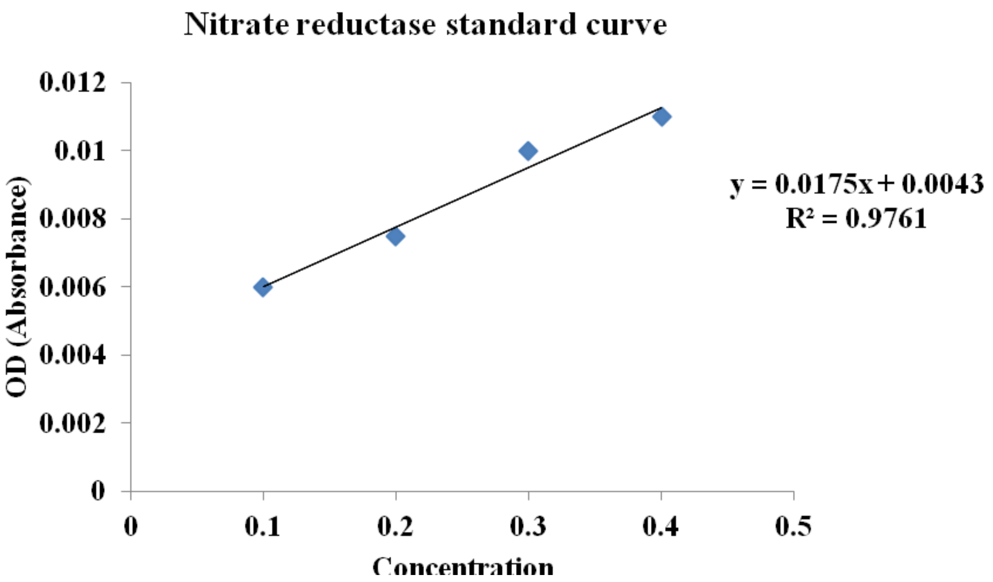

Phosphatase standard curve
y = 0.0014x
R² = 0.9531
OD (Absorbance)
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0
20
40
60
80
100
120
Concentration

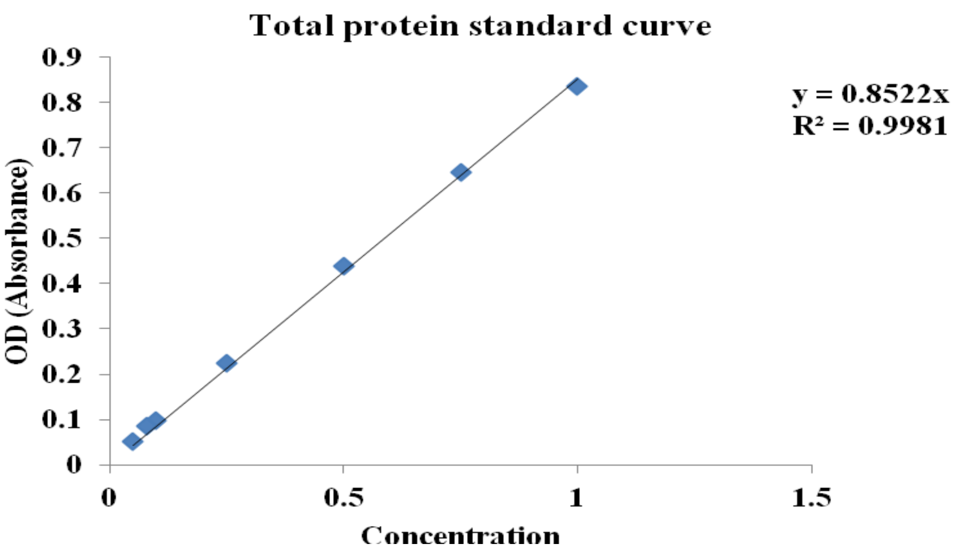

Urease standard curve
y = 0.0291x + 0.1971
R² = 0.975
OD (Absorbance)
0
0.5
1
1.5
2
2.5
3
3.5
0
20
40
60
80
100
120
Concentration

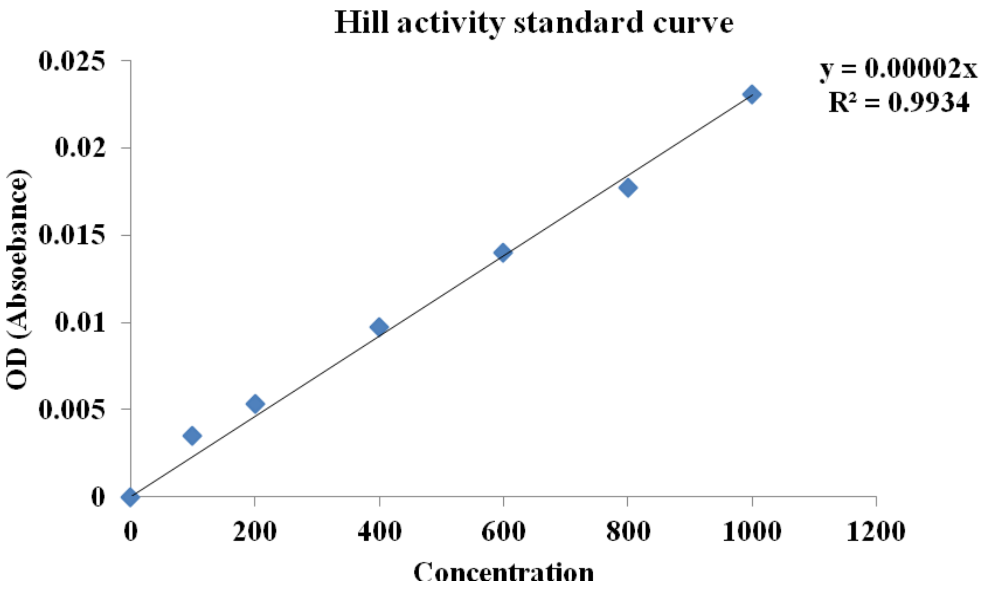

Leucine standard curve
y = 0.0745x
R² = 0.9669
OD (Absorbance)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
Concentration

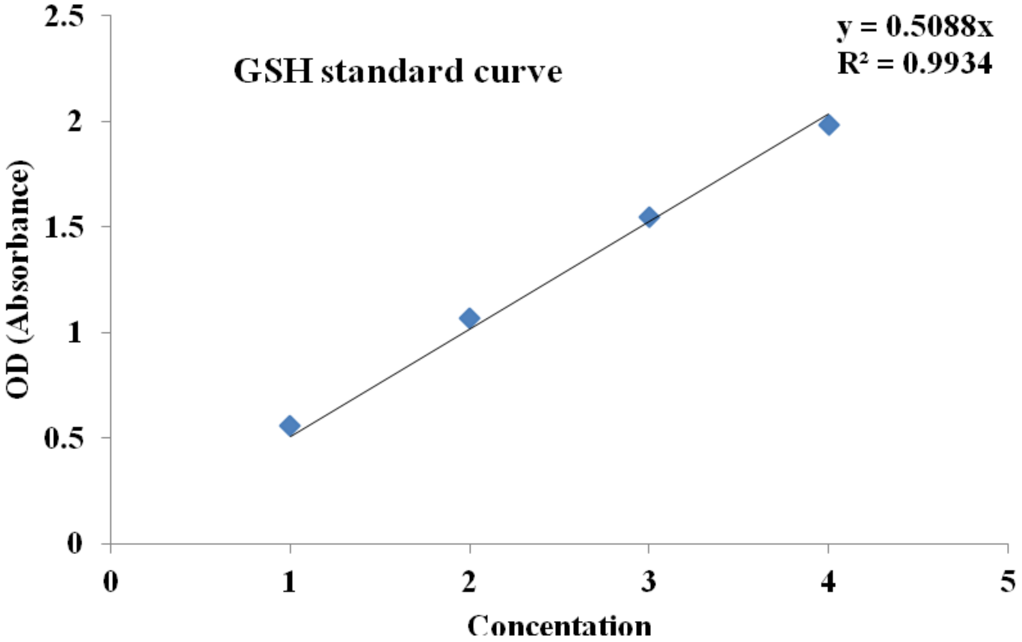

Sulphur standard curve
y = 0.1019x
R² = 0.9479
OD (Absorbance)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
Concentration


Fe standard curve
y = 0.71x - 0.0019
R² = 0.9954
OD (Absorbance)
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
0.05
0.1
0.15
0.2
0.25
0.3
Concentration


Nitrate reductase standard curve
OD (Absorbance)
0.012
0.01
0.008
0.006
0.004
0.002
0
0
0.1
0.2
0.3
0.4
0.5
Concentration
y = 0.0175x + 0.0043
R² = 0.9761


Total protein standard curve
OD (Absorbance)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.5
1
1.5
Concentration
y = 0.8522x
R² = 0.9981


Hill activity standard curve
OD (Absoebance)
0.025
0.02
0.015
0.01
0.005
0
0
200
400
600
800
1000
1200
Concentration
y = 0.00002x
R² = 0.9934


GSH standard curve
y = 0.5088x
R² = 0.9934
OD (Absorbance)
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
Concentation

ANNEXURE 1

The detailed agronomic practice followed for AgNP NMs

Sl. No.	Agronomic practice	Time 2015	 2016
1.	Preparation of the nursery bed	16.08.15	21.08.16
2.	Seed sowing	25.08.15	02.09.16
3.	Land preparation	09.09.15	10.09.16
4.	Plot size demarcation (2m×3m) by preparation of bunding	11.09.15	13.09.16
5.	Application of AgNP in different levels	21.09.15	23.09.16
6.	Transplantation and basal application of urea (1/3 of N), full SSP, and full potash.	29.09.15	30.09.16
7.	Application of 1/3 of N, weeding and cleaning	14.10.15	15.10.16
8.	Application of 1/3 of N, cleaning and irrigation	28.10.15	30.10.16
9.	Harvesting	20.11.15	23.11.16

Agronomic practices followed for OCIO NMs

Sl. No.	Agronomic practice	Time 2015	 2016
1.	Preparation of the nursery land	15.08.15	20.08.16
2.	Seed sowing	25.08.15	02.09.16
3.	Land preparation	08.09.15	09.09.16
4.	Plot size demarcation (2m×3m) by preparation of bunding	10.09.15	12.09.16
5.	Application of nano treatments	20.09.15	22.09.16
6.	Transplantation and basal application of urea (1/3 of N), Full SSP, full potash.	28.09.15	30.09.16
7.	Application of 1/3 of N, weeding and cleaning	13.10.15	15.10.16
8.	Application of 1/3 of N, cleaning and irrigation	28.10.15	30.10.16
9.	Harvesting	20.11.15	22.11.16

Impact of OCIO on pericarp thickness, total soluble solid, and total acidity of field grown tomato crops

Treatments	Pericarp thickness (cm)		Total soluble solid (°Brix)		Total acidity (%)	
	First yr	Second yr	First yr	Second yr	First yr	Second yr
Control	0.45±0.03	0.43±0.04	2.9±0.1	2.92±0.07	1.09±0.06	0.55±0.01
OCIO 2	0.67±0.06	0.83±0.01	4±0.13	5.2±0.1	1.66±0.03	1.85±0.01
OCIO 5	0.6±0.01	0.81±0.02	3.8±0.28	4.9±0.17	1.38±0.06	1.82±0.02
OCIO 10	0.53±0.06	0.78±0.02	4±0.14	4.7±0.17	1.44±0.03	1.77±0.01
OCIO 20	0.6±0.01	0.71±0.03	4.4±0.04	4.1±0.1	0.8±0.01	1.53±0.01
Fe-EDTA 2	0.5±0.01	0.47±0.02	3.8±0.17	3.4±0.1	0.71±0.01	0.67±0.01
Fe-EDTA 5	0.48±0.02	0.42±0.03	3.2±0.03	3.1±0.1	0.8±0.01	0.7±0.01
Fe-EDTA 10	0.45±0.01	0.4±0.02	3.2±0.06	3±0.1	0.74±0.01	0.67±0.01
Fe-EDTA 20	0.41±0.02	0.38±0.01	2.9±0.07	2.7±0.1	0.79±0.02	0.69±0.02
$FeSO_4$ 20	0.5±0.01	0.45±0.02	3.4±0.22	3.1±0.1	0.8±0.01	0.77±0.03
P (yr)	0.000		0.000		0.000	
P (trt)	0.000		0.000		0.000	
P (yr×trt)	0.000		0.000		0.000	
LSD (trt)	0.01		0.07		0.03	

Impact of OCIO on height of tomato plant grown under field condition

Treatments	30 days		45 days	
	1st year	2nd year	1st year	2nd year
Control	18.5±0.89	21.65±0.54	27.85±0.31	49.38±0.24
OCIO 2	32.13±0.78	52±0.92	54.1±0.17	101.93±0.39
OCIO 5	28.83±1.2	56.73±0.28	48.88±0.36	97.83±0.30
OCIO 10	27.95±0.48	55.23±0.62	49.85±0.33	96.6±0.26
OCIO 20	26.23±0.62	51.25±0.48	44.25±0.22	86.38±0.24
Fe-EDTA 2	15.98±0.52	28.33±0.25	21.5±0.26	35.5±0.3
Fe-EDTA 5	15.7±0.44	26.15±0.30	20.15±0.15	33.08±0.29
Fe-EDTA 10	15.28±0.28	24.18±0.23	20.43±0.20	34.33±0.20
Fe-EDTA 20	14.78±0.42	23.7±0.26	21.13±0.42	31.33±1.7
$FeSO_4$ 20	14.83±0.37	23.3±0.26	18.38±0.25	28.18±0.17
P value	0.000	0.000	0.000	0.000
LSD	0.537	0.365	0.231	0.45

Impact of OCIO on leaf number of tomato plant grown under field condition

Treatments	30 days		45 days	
	1st year	2nd year	1st year	2nd year
Control	20±0.9	41±1.5	38±1.1	65±3.6
OCIO 2	48±1.1	85±3.5	97±4.5	235±9.8
OCIO 5	43±1.3	80±3.2	94±4	318±11.6
OCIO 10	42±1.3	78±2.9	92±4.1	204±10.4
OCIO 20	38±1	74±2.5	88±3.8	180±9
Fe-EDTA 2	18±0.6	42±0.8	46±2.2	72±3.6
Fe-EDTA 5	18±0.8	41±0.8	43±2.1	63±3.4
Fe-EDTA 10	16±0.9	39±0.7	42±2	62±3.2
Fe-EDTA 20	17±0.8	35±0.7	38±1.9	52±2.9
$FeSO_4$ 20	16±0.9	32±0.7	35±1.9	52±2.9
P value	0.000	0.000	0.000	0.000
LSD	0.476	0.581	0.639	0.45

The ANOVA output of mean square root, degrees of freedom and their interaction in regard to various parameters of AgNP treated sample

		Mean Square												
	Degrees of freedom	Lipid peroxidation	Proline	GS	GOGAT	Hill activity	PS rate	Uptake K	Uptake P	Uptake Ag	Uptake N	Chlorophyll	NR	Yield
Between Groups	1	0.0000011	85181669	0.001706	8.74006	528067	9.6164	188.16	5.02	0.057918	47.04	32.202	11.093	12.36
Within Groups	4	0.0000001	1177	0.000003	0.00298	376.833	0.0394	0.02	0.04	0.000004	6.86	0.002	0.027	0.07
Total	5													

The ANOVA output of degrees of freedom, F ration and their interaction in regard to various parameters of AgNP treated sample

		F ration												
	Degrees of freedom	Lipid peroxidation	Proline	GS	GOGAT	Hill activity	PS rate	Uptake K	Uptake P	Uptake Ag	Uptake N	Chlorophyll	NR	Yield
Between Groups	1	9.03	72402.51	539.84	2933.97	1401.33	244.06	10263.27	112.93	13333.73	6.86	19321	410.47	188.20
Within Groups	4													
Total	5													

The ANOVA output of F ration, degrees of freedom, and their interaction in regard to various parameters of OCIO treated samp

	Degrees of freedom	F ration												
		Fd	*NR*	*GOGAT*	*GS 2*	GS	GOGAT	NR	Hill activity	Uptake P	Uptake Fe	Lipid peroxidation	Catalase	SOD
Between Groups	3	1693.9	187.35	218.6	1462.1	48899.5	3.6	20564.4	617.4	2680386	2945.19	93.35	1.33	13807.03
Within Groups	8													
Total	11													

The ANOVA output of degrees of freedom, mean square root and their interaction in regard to various parameters of OCIO treated sample

	Degrees of freedom	Mean Square												
		Fd	*NR*	*GOGAT*	*GS 2*	GS	GOGAT	NR	Hill activity	Uptake P	Uptake Fe	Lipid peroxidation	Catalase	SOD
Between Groups	3	140.8	1.54	1.80	11.37	39.413	0.0007	328.53	4161483.33	3685.531	2132.98	0.00007	10.67	363.06
Within Groups	8	0.1	0.01	0.01	0.01	0.001	0.0002	0.02	6740.8	0.001	0.72	0.00000	8	0.03
Total	11													

PHOTOGRAPHS

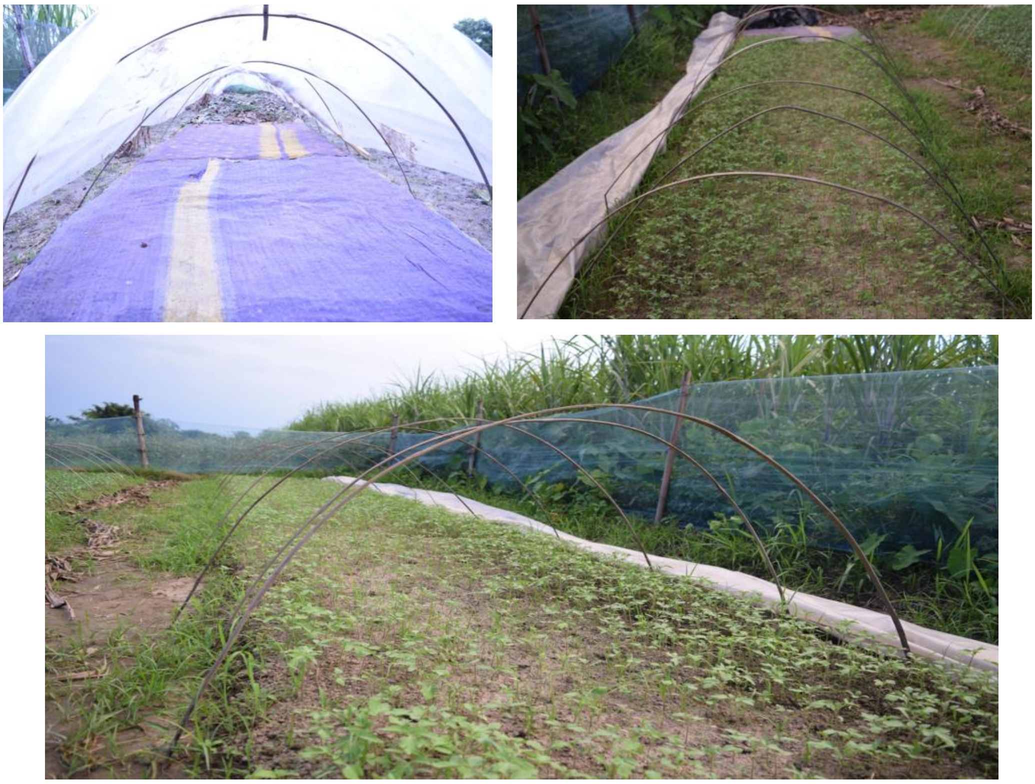


Photo 1: Nursery preparation

Photo 2: Field trial with OCIO treatments

Photo 3: Field trial with AgNP treatments

Photo 4: Pot culture experiments with both the nanoparticles

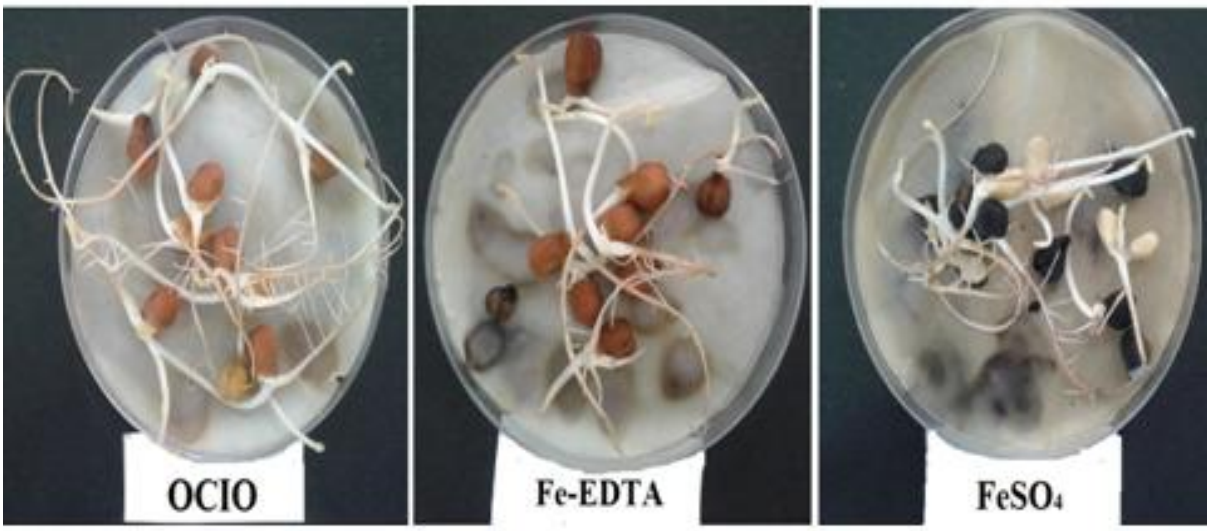


Photo 5: Seed germination assay

Photo 6: Synthesis of OCIO and AgNP

RSC Advances

PAPER

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Novel synthesis of an iron oxalate capped iron oxide nanomaterial: a unique soil conditioner and slow release eco-friendly source of iron sustenance in plants†

Pallabi Das,[a] Kasturi Sarmah,[b] Nazneen Hussain,[a] Sanjay Pratihar,*[b] Subhasish Das,[a] Pradip Bhattacharyya,[c] Supriya A. Patil,[de] Hak-Sung Kim,[d] Mohammed Iqbal A. Khazi[e] and Satya Sundar Bhattacharya*[a]

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Iron (Fe) is a vital plant-derived micronutrient in the human diet. Fe availability in soil largely depends on the pH and leaching behaviour of the soil. Although common salts ($FeSO_4$) and chelates (EDTA) of Fe ensure high availability of the nutrient, they often interfere with P availability in the soil. Considering such disadvantages of the well-known Fe sources, we attempted to evolve efficient Fe_3O_4 nanomaterials that are independent of soil reaction (*i.e.* pH) and do not prevent P solubility in soil. The present investigation resulted in a novel, green and an easy pathway of large-scale synthesis of orthorhombic Fe–oxalate capped-Fe-oxide (Fe_3O_4) (OCIO) nanomaterial with a prolific agricultural applicability. This nanomaterial did not affect the growth of beneficial soil bacteria and had no phytotoxic effects on seed germination. The Fe release profile from the OCIO was uniform at different pH (4 to 9) conditions due to its exceptional H^+ ion scavenging quality. Significantly higher P availability was recorded in aqueous and soil media treated with OCIO as compared to $FeSO_4$ and Fe–EDTA. Additionally, application of OCIO@10–20 mg kg^{-1} considerably increased organic C, N, P, and enzyme activity in soil. Furthermore, the OCIO dramatically recovered Fe deficiency, maintained steady P availability, and stabilized pH in poorly fertile soil which promoted healthy growth and productivity of tomato.

Introduction

A large portion of the world's population suffers from iron deficiency and plants are the prime contributor of iron in the human diet.[1] Fe deficiency is one of the most prominent nutritional disorders in many economically important crops.[2,3] The most easily detectable symptom of Fe deficiency in plants is the yellowing of leaves, technically known as leaf chlorosis. Although iron is the fourth most abundant element on earth, its bioavailability is noticeably low in the soil. Various factors like high soil pH, 'iron-insufficient' plant species, predominance of bicarbonates, and abiotic stress reduce the bioavailability of Fe in the soil. Primarily, Fe is held on the organic inorganic interfaces in soils.[4] The solubility of this element in soils is determined by $Fe(OH)_3$, $Fe_3(OH)_8$ (ferric hydroxide) or by $FeCO_3$ (siderite) depending on the prevailing oxidation state in the soil.[5] The dissolution–precipitation dynamics of ferric oxides in aerated soils largely governs Fe solubility, which is highly pH dependent. For example, the Fe^{3+} precipitation in soil increases by 1000 folds for each unit increase in soil pH.[5] Plants can absorb iron as ferrous iron (Fe^{2+}). However, the Fe^{2+} iron is readily oxidized in soil and transforms into plant-unavailable ferric (Fe^{3+}) form when soil pH is greater than 5.3.[6] Under such situations, if soluble Fe salts (*e.g.*, $FeSO_4$) are applied to correct Fe deficiency, they rapidly precipitate as amorphous $Fe(OH)_3$ and decrease Fe availability over time.[4]

Various iron complexes conjugated with ligands (EDTA, DTPA and EDDHA) are used as slow releasing iron fertilizer to improve iron availability in the soil. Although these complexes are greener and rather more efficient compared to $FeSO_4$ fertilization, the impending global demand for iron in soil has prompted intense research on the development of various types of sustainable and cost effective iron source.[7] Incidentally, the rapidly emerging nanotechnology may render smart and

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[a] *Department of Environmental Science, Tezpur University, Tezpur 784028, Assam, India. E-mail: satyasundarb@yahoo.co.in; satya72@tezu.ernet.in; Tel: +91-3712-267007 ext. 5610; +91-3712-267008 ext. 5610; +91-3712-2670079 ext. 5610*

[b] *Department of Chemical Sciences, Tezpur University, Tezpur 784028, Assam, India. E-mail: spratihar29@gmail.com*

[c] *Agricultural and Ecological Research Unit, Indian Statistical Institute, Giridih 815301, Jharkhand, India*

[d] *Department of Mechanical Engineering, Hanyang University, Haengdang-dong, Seongdong-gu, Seoul 133-791, South Korea*

[e] *Institute of Nano Science and Technology, Hanyang University, Seoul, 133-791, South Korea*

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Mechanism of toxicity and transformation of silver nanoparticles: Inclusive assessment in earthworm-microbe-soil-plant system

Pallabi Das[a], Shaswat Barua[b], Shuvasree Sarkar[c], Subhendu Kumar Chatterjee[c], Sandip Mukherjee[c], Linee Goswami[d], Subhasish Das[a], Shelley Bhattacharya[c], Niranjan Karak[b], Satya Sundar Bhattacharya[a,*]

[a] Soil and Agro Bio-engineering Lab, Department of Environmental Science, Tezpur University, Tezpur 784 028, India
[b] Advanced Polymer and Nanomaterial Laboratory, Department of Chemical Sciences, Tezpur University, Tezpur 784028, India
[c] Department of Zoology, Siksha Bhavana, Visva Bharati, Santiniketan 731 235, India
[d] Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur 784028, India

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Soil and plant health
N-metabolism
Gene expression

ABSTRACT

Long term and inclusive toxicity studies encompassing soil, plants, and organisms are rare in literature for AgNPs. This study examines AgNP behavior in soil-plant system through 72 weeks long soil experiment, earthworm response, and plant metabolic analysis. AgNP exposed earthworms did not show reproductive failure; yet high oxidative stress and reduced protein synthesis led to significant weight loss. Such stress was highest with $AgNP_{50}$ exposure. Correspondingly, the 50 ppm exposure of AgNP was capable to reduce nutrient availability and microbial growth in soil. Contrary to previous reports, we demonstrated that dissolution rate of AgNP increased with time in soil. Dynamic Light Scattering and UV-VIS assessments exhibited concentration and time dependent agglomeration of AgNP in soil and aqueous media. Moreover, lab based experiments in aqueous medium revealed that significant reduction in silver availability was due to formation of Ag_2S or Ag_3PO_4; which also greatly affected the P and S availability. Although the vegetative growth of tomato was normal, AgNP (10 mg kg^{-1}) treatment markedly upset the fruit yield. The 10 mg kg^{-1} AgNP exposure significantly enhanced oxidative stress and Ag uptake in plants; consequently, retarded N-assimilating enzyme (glutamate synthase, glutamine synthetase, and nitrate reductase) activity by suppressing their genes in plants. Eventually, photosynthesis and CO_2 assimilating efficiency were severely disrupted. These assays were vital to appreciate the true toxicity and are not well attended in most of the studies with AgNPs.

1. Introduction

World's land and water resources are considerably exposed to silver nanomaterials, since silver (Ag) is the most widely used nanomaterial (Rejeski, 2009; Lee et al., 2012). An estimate has shown that the exposure levels of silver nanoparticles (AgNPs) were likely to be 1581 ng kg^{-1} h^{-1} for the contaminated lands of Europe (Gottschalk et al., 2009). Therefore, it is important to derive mechanistic interpretations through focused as well as holistic experimentations to ascertain the true impacts of AgNPs on soil environment.

The behavior and effects of nanoparticles (NPs) in soil-plant systems are rather unpredictable because of influence of numerous factors (inherent soil chemistry, soil porosity, water retention capacity, size of NPs, coating materials, time, and level of exposure) (Dinesh et al., 2012; Goswami et al., 2017). Studies have revealed concentration driven agglomeration property of engineered nanomaterials in soil greatly influences microbial diversity, nitrogen metabolism, photosynthesis, and plant growth (Li et al., 2017; Yang et al., 2017). However, AgNP toxicity to plant have been more severe in soil-less media than in soil (Lee et al., 2012; Musante and White, 2012; Dimkpa et al., 2013). Contrarily, AgNP exposure has been reported to promote root nodulation and shoot growth in plants (Lee et al., 2012; Pallavi et al., 2016). However, to which extent the AgNP exposure disturbs molecular functions in plants and how AgNP affects soil quality is a least attended question.

The unique features of AgNPs (aggregation/agglomeration, dissolution, dispersibility, charge, surface area, and surface chemistry) greatly modify the stability and migration of the nanomaterials within soil systems; which may also alter the physico-chemical character of the contaminated soils (Anjum et al., 2013). As such, the distinctive

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[*] Corresponding author at: Department of Environmental Science, Tezpur University, Assam 784028, India.
E-mail addresses: satya72@tezu.ernet.in, satyasundarb@yahoo.co.in (S.S. Bhattacharya).

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Plant extract–mediated green silver nanoparticles: Efficacy as soil conditioner and plant growth promoter

Pallabi Das[a], Shaswat Barua[b], Shuvasree Sarkar[c], Niranjan Karak[b], Pradip Bhattacharyya[d], Nadeem Raza[e], Ki-Hyun Kim[f,*], Satya Sundar Bhattacharya[a,*]

[a] Soil and Agro-Bioengineering Laboratory, Department of Environmental Science, Tezpur University, Assam, 784028, India
[b] Advanced Polymer and Nanomaterial Laboratory, Department of Chemical Sciences (Centre for Polymer Science and Technology), Tezpur University, Assam, 784028, India
[c] Department of Zoology, Visva-Bharati, Santiniketan, West Bengal, India
[d] Agricultural and Ecological Research Unit, Indian Statistical Institute, Giridih, Jharkhand 815301, India
[e] Govt. Emerson College affiliated with Bahauddin Zakaryia University, Multan, Pakistan
[f] Dept. of Civil & Environmental Engineering, Hanyang University, 222 Wangsimni-Ro, Seoul 04763, South Korea

HIGHLIGHTS

- The remarkable antimicrobial activity of silver nanoparticles (SNPs) is well known.
- Extensive industrial use of SNPs has led to their large-scale disposal as waste materials.
- The effects of SNPs on plant metabolism are assessed in terms of NR and Fd expression.
- We provide evidence of an overall beneficial impact of SNPs on soil properties.

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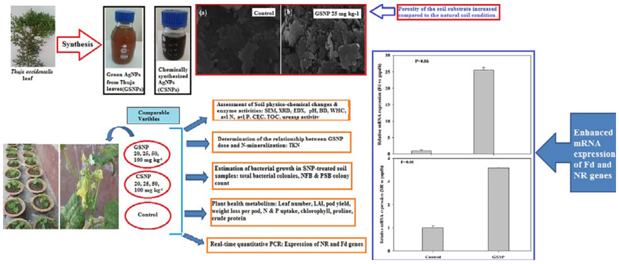
Fd gene expression

GRAPHICAL ABSTRACT



ABSTRACT

Recently, concerns have been raised regarding the ultimate fate of silver nanoparticles (SNPs) after their release into the environment. In this study, the environmental feasibility of plant leaf (*Thuja occidentalis*) extract–mediated green SNPs (GSNPs) was assessed in terms of their effects on soil physicochemical properties and crop growth in comparison to conventionally synthesized silver nanoparticles (CSNPs). Upon application of GSNPs, soil pH shifted toward neutrality, and substantial increments were observed in water holding capacity (WHC), cation exchange capacity (CEC), and N/P availability. The mechanism behind the enhanced availability of N was verified through lab-scale experiments in which GSNP-treated soils efficiently resisted nitrate leaching, thereby sustaining N availability in root zone soil layers. However, retardation in nutrient availability and enzyme activity was apparent in soils treated with 100 mg kg^{-1} of either CSNPs or GSNPs. Remarkable improvements in leaf area index (LAI), leaf number, chlorophyll content, nitrate reductase (NR) activity, and *Phaseolus vulgaris* pod yield were observed after the application of low doses of GSNPs (25–50 mg kg^{-1}). The true benefit of GSNP application to soil was substantiated through experiments on plant uptake of nutrients, NR expression, and ferredoxin gene expression in *P. vulgaris* leaves.

1. Introduction

Nanotechnology is a leading area in modern science. The community is expecting great improvements in living quality with the

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* Corresponding authors.
E-mail addresses: kkim61@hanyang.ac.kr (K.-H. Kim), satyasundarb@yahoo.co.in, satya72@tezu.ernet.in (S.S. Bhattacharya).

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