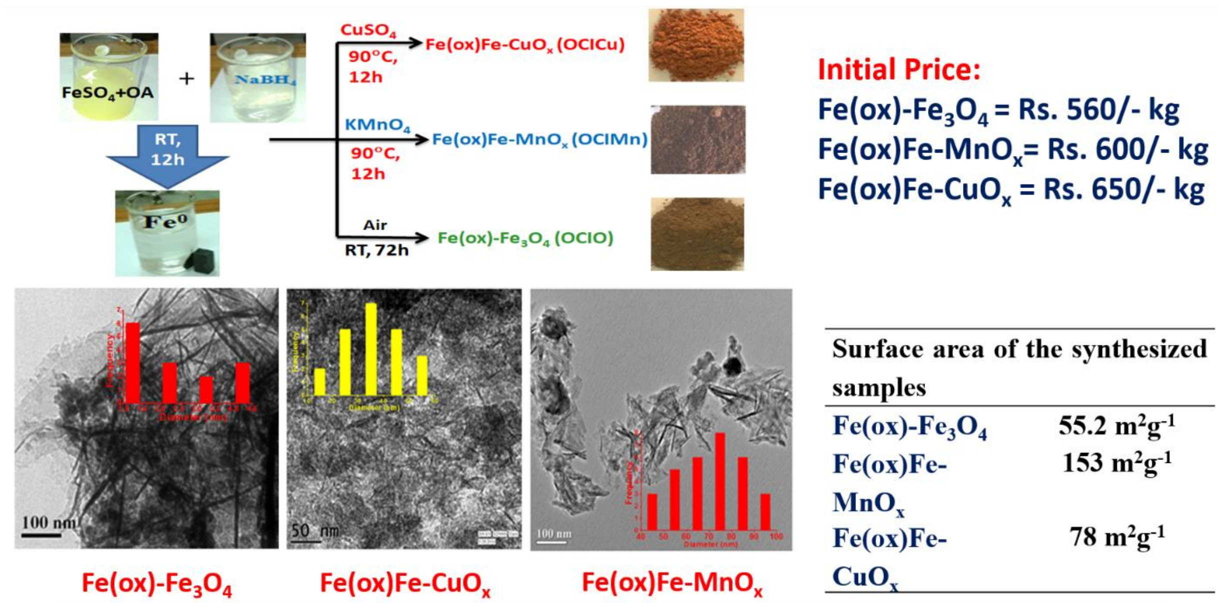
<u>**Final Technical Report**</u>

(9th May 2018 – 30th June 2022)

Nano-Based Soil Conditioner for Agricultural Applications



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Part I

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Abstract

The growing field of nano-enabled agriculture is gaining immense importance under the current perspectives of food security and environmental safety challenges. Agriculture is a field where new techniques are frequently used to boost agricultural yields. Nanoparticles are being used in agriculture systems to provide favorable impacts on crop productivity in the form of nano fertilizers. Nano-enabled agriculture is a vibrant research area; nonetheless, reports on effective nano fertilizers are rather scant. Hence, we evolved novel, easy, and scalable synthetic routes for environmentally benign Fe-oxalate capped metal oxide nanomaterials (OCIO, OCIMnox, and OCICuox) for agricultural use (Patent no. 343590). The materials were systematically characterized through PXRD, FT-IR, HR-SEM, HR-TEM, and XPS analyses, which induced the least toxicity to plants, soil microbes, and earthworms. These materials corrected soil acidity by maintaining the soil pH above 6. Their application increased the surface area by ∼1.5–1.7-fold, water holding capacity by ∼1.4–1.5 fold, and organic C by ∼1.3–1.4 fold in the soil as compared to non-nanoscale fertilizers (sulfates and EDTAs of Fe/Mn/Cu). Their application in nutrient-fatigued soil could efficiently alleviate Fe, Mn, and Cu deficiency, enhance soil bacterial growth by ∼4–9 fold, and augment siderophore-producing bacteria. Furthermore, oxidative stress factors were minimized in earthworms and plants cultivated in nano-exposed soil. Focused experimentations showed that OCIO, OCICuox, and OCIMnox stabilized soil pH, probably by adjusting the H+ abundance, and optimized nutrient availability by increasing the net-negative charge in soil. Eventually, plant productivity was stimulated through greater N uptake (>6%), 3–10 fold greater up-regulation of expression of vital genes [glutamine synthetase-2 (GS2), glutamate synthase (GOGAT), and nitrate reductase (NR)], and a higher rate of photosynthesis than FeSO4. In conclusion, the innovatively synthesized OCIO, $OCIMn_{ox}$, and $OCICu_{ox}$ showed great promise as micronutrient nanofertilizers for future applications.

Introduction

Global food production has to be augmented by 60% as compared to the 2005 levels to feed about 9.8 billion people by 2050 (Kah et al., 2019, 2018). According to the FAO, over 820 million people in the world are hungry; yet the incidences of obesity as well as undernourishment are sharply increasing (Kah and Kookana, 2020). It is indeed important to safeguard the nutrient balance in arable lands in order to meet the food security demand and ensure balanced nutrition for all. Ironically, deterioration in soil quality and widespread deficiency of micronutrients due to the indiscriminate use of macronutrient fertilizers is a stark reality (Gogos et al., 2012; Raliya et al., 2018). Hence, it is necessary to develop innovative and sustainable alternatives for improved production of nutritious crops through efficient use of resources and reduced application of synthetic fertilizers. At this juncture, nano-enabled soil health management can be a reliable platform to achieve the goal of sustainable crop production (Kopittke et al., 2019; Lowry et al., 2019). Nano-form fertilizers contain micro or macronutrients like P (nano-apatite), Ca (nano-calcite), Fe (nano-iron oxide), nanoscale Mn, etc. either in particle or emulsion forms (Xin et al., 2020). For instance, FeO and Fe2O3 nanomaterials significantly improved total leaf chlorophylls, branching, and root biomass of several crops (soybean, black-eyed pea, and groundnuts) (Adisa et al., 2019). Pradhan et al., 2014 demonstrated that a group of nanomaterials (ZnO, CuO, and B2O3) enhanced soybean productivity by 36% under water stress conditions. Low-dose (0.05 mg L^{-1}) application of engineered Mn nanomaterials has improved biomass, root growth, and shoot length by 38–52% as compared to non-nanoscale $MnSO_4$. In the past few years, engineered carbon-based materials, graphene, fullerenes, and various forms of carbon nanotubes (helical multiwalled, long multi-walled, short multi-walled, single walled, etc.) have also been successfully utilized by various research groups to increase root length, stimulate seed germination, and increase plant biomass (Lahiani et al., 2016, 2015; Patel et al., 2018; Tripathi et al., 2011). Sophisticated hybrid nano-carriers have also been proposed as sustainable micronutrient nanofertilizers (Shakiba et al., 2020). For example, chitosan (Adisa et al., 2019), plant derived saccharides like zein (Derosa et al., 2010), clay (Mitter et al., 2017), tannic acid (Yao et al., 2019), etc. have been found to be effective in improving nutrient delivery systems. Several nanofertilizers are composed either of metal/metal oxide or of various forms of carbon-based nanomaterials, in whole or in part, of nanostructured formulation. However, the lack of focused scientific research in this domain has hindered the practical application of nanotechnology in agriculture. There is a considerable research gap related to the interactions of metal oxide nanomaterials with the inherent physico-chemical properties of soil. Moreover, very little is known about the uptake mechanism of nanostructured elements in plants and how the molecular metabolism of plants responds to these materials. Nutrient deficiency problems in soils have been greatly aggravated because of low fertilizer use efficiency (Chugh et al., 2021; Kah et al., 2019). Studies have shown that about 3.7 billion people suffer from acute Fe deficiency, while plants are the major source of Fe in the human diet (Majumder, 2013; Robinson et al., 1999). As such, the bioavailability of Fe largely depends on soil pH and the abundance of anionic species like phosphates (Das et al., 2016). Copper (Cu) and manganese (Mn) deficiency are even greater (10–15%) than Fe in tropical countries (Das and Bhattacharya, 2019). Soluble salts (ferrous sulphate, manganese sulphate, and copper sulphate) are often used in agriculture, which greatly acidifies soils and creates acute P deficiency; but the efficacy of micronutrient chelates (EDTA) is strongly pH dependent and is often unstable in many soils (Flaten et al., 2004). Moreover, their prolonged use deteriorates soil quality through acidification and aggravation of P deficiency. On the other hand, the use of metal oxide nanomaterials is expanding and their exposure to the soil and water environment is also on the rise. These nanomaterials can greatly reduce the micronutrient deficiency in agriculture (Conway et al., 2015; Kim et al., 2014). However, about 20% of the micronutrient nanofertilizers have demonstrated poorer efficiency as compared to their non-nano counterparts (Kah et al., 2018). Kah et al., 2018 also apprehended that such poor efficiency was mainly attributed to the toxic effects of nanomaterials due to excessive accumulation of the target elements in plants. Nevertheless, eco-toxicity issues are one of the greatest obstacles to the use of nano-materials in agriculture. Several nanomaterials (TiO_2, CeO_2, ZnO, and CuO) have been reported to inhibit photosynthesis, CO_2 assimilation efficiency, and promote oxidative stress in plants and microbes (Das et al., 2021; P. Das et al., 2018). In addition, literature-based research gap analysis reveals that the majority of studies have failed to derive the actual benefit from nano-sources (Kah and Kookana, 2020). Most of the experiments have been performed on a miniature scale (i.e., with seeds or seedlings) in Petri-dishes and under controlled conditions. These studies often fail to show statistically significant results (Kah et al., 2018). Yin et al., 2018 also stated that the absence of non-nano controls in experimental designs is one of the vital flaws in the majority of studies. As such, the efficiency of the nano-form micronutrients (particularly, Fe) has been rarely assessed in comparison with non-nano scale controls. It is also not clear whether the apparent beneficial effects were because of the nano-forms of the target elements or not; how the micronutrient (Fe, Cu, and Mn) nanomaterials influenced the internal metabolism of plants is still unclear. From these perspectives, we have synthesized efficient micronutrient soil conditioners such as: Iron oxalate capped iron oxide (OCIO), orthorhombic iron oxalate capped iron-copper oxide (hereafter OCICuox) and iron manganese oxide (hereafter OCIMnox) through an easy, scalable, and environmentally benign chemical method (Patent Grant No: 343590 with effect from 29.03.2016). We have confirmed the non-toxic nature of the synthesized nanomaterials through standard toxicity assays with seeds, earthworms, and soil microorganisms. Their potential to recharge nutrient deficiency in soil was verified through pot-scale soil–plant experiments in comparison with non-nano scale conventional fertilizers ($FeSO_4$, $CuSO_4$, and

MnSO4, Fe-EDTA, Mn-EDTA, and Cu-EDTA). Nutrient release mechanisms were assessed through lab-scale batch experiments. Moreover, the impacts of the synthesized materials on some of the vital molecular responses in plants were assessed in a parallel manner with non-scale controls.

Objective:

1. Standardization of large-scale synthetic route of oxalate capped Fe(ox)-Fe3O4, Fe(ox)MnOx, and Fe(ox)Fe-CuOx nanomaterial
2. Impact assessment of the synthesized products with special emphasis on solubility dynamics
3. Evaluation of eco-toxicity potential and environmental compatibility of the synthesized nanomaterial
4. Impact assessment of the synthesized products in different agroclimatic and cropping system

Objective 1. Standardization of large-scale synthetic route of oxalate capped Fe(ox)-Fe3O4, Fe(ox)MnOx, and Fe(ox)Fe-CuOx nanomaterial

1.1.Industrially scalable large-scale synthesis of the nanomaterials

The Fe(Ox)-Fe_3O_4 (hereafter, OCIO) was synthesized with ferrous sulphate as the starting chemical following details methods described by Das et al. (2016). Ferrous sulphate was also used as one of the starting chemicals for synthesizing the Fe(ox)-$FeMn_{ox}$ (hereafter, OCIMnox) and Fe(ox)-$FeCu_{ox}$ (hereafter, OCICuox) materials along with $MnSO_4$ and $CuSO_4$, respectively as detailed by Das et al. (2021).

1.2.Characterization of Synthesised nanomaterial

The materials were characterised with PXRD, SEM and TEM and it was observed that materials of all the batches are showing similar morphology and structure without any change in its plane. Another interesting observation was made that with increase in quantity of starting materials use of $NaBH_4$ decreases which may be due to the redox potentials of the metal salts.

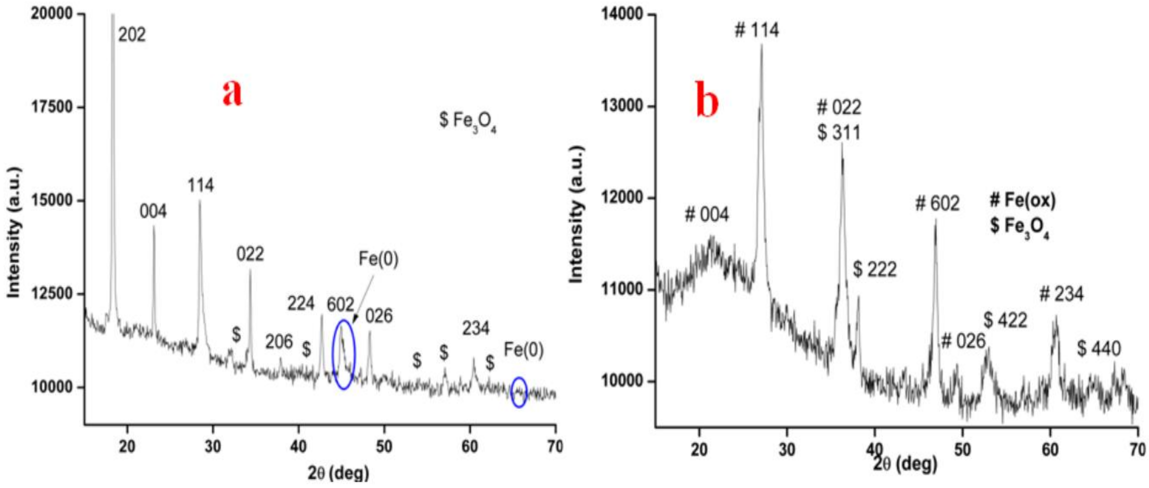


Figure 1: (a) XRD spectrum of Fe(ox)-Fe (0) (b) Fe(ox)-Fe3O4

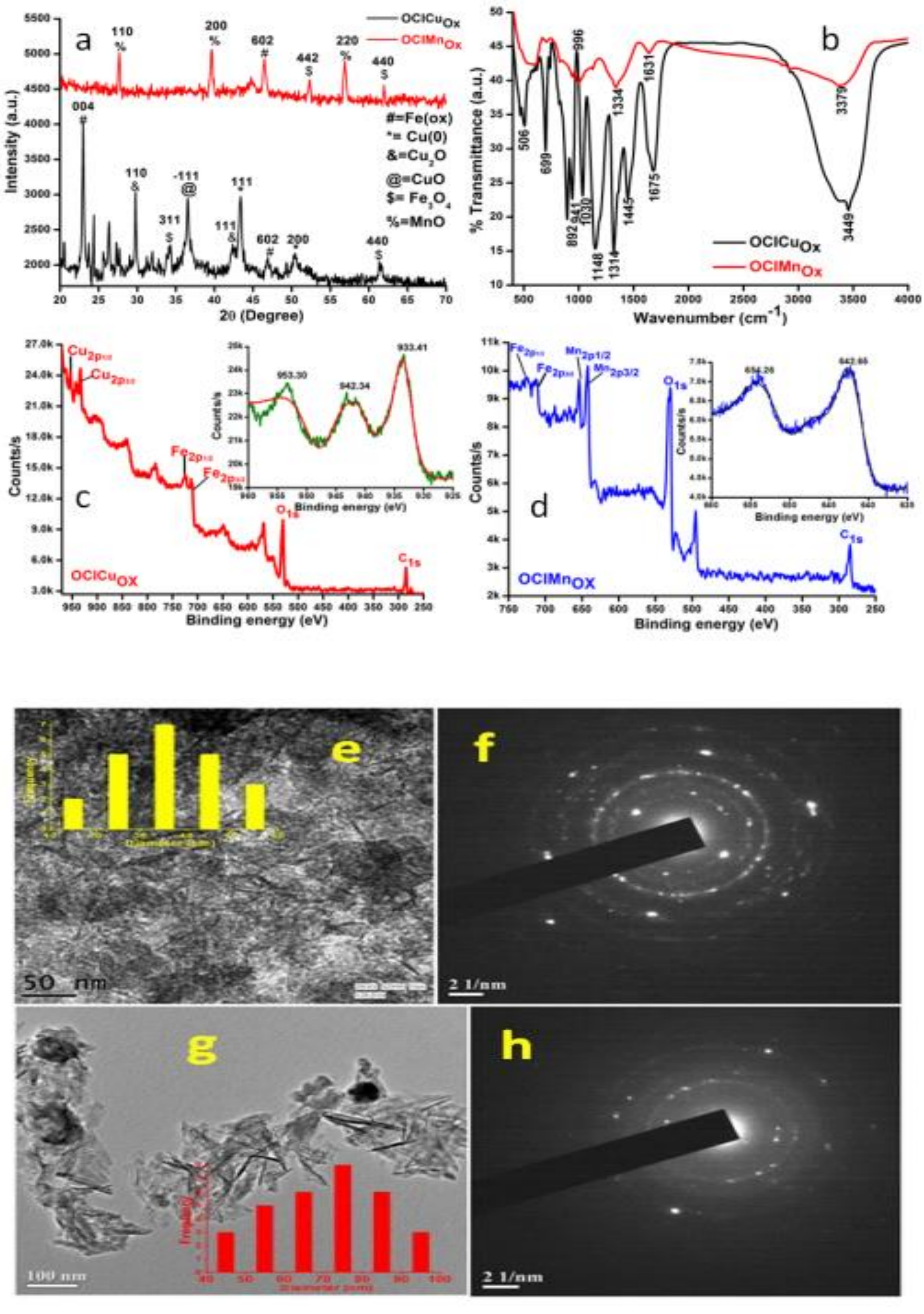


Figure 2: (a) XRD spectrum, (b) FTIR, (c-d) XPS spectrum, (e-f) TEM of $OCICu_{ox}$, and (g-h) TEM of $OCIMn_{ox}$.

Objective 2: Impact assessment of the synthesized products with special emphasis on solubility dynamics

2.1. Impact of nanomaterials on changes of cations and anions in aqueous and soil mixed aqueous media.

2.1.1. Release profile of Fe, Mn, and Cu in aqueous solutions of different pH

Solutions of three different pH (4, 7 and 9) were prepared adding HCL and NH4OH respectively. Subsequently, a uniform amount (0.1 g) of $OCIMn_{ox}$, $OCICu_{ox}$, EDTA (Fe, Mn, and Cu) and salts (FeSO4, MnSO4 and CuSO4) were added separately in such solutions and were kept under continuous shaking @ 120 rpm for 72 hours in a mechanical shaker. Shift in pH as well as Fe, Mn, and Cu release were recorded at 24, 48 and 72 hours in ICP-OES (APHA, 1999). The pH of the strongly acidic solution (pH 4) significantly shifted towards near neutral value in $OCIMn_{ox}$ (pH 4 to 6.41 in 72 hours) and $OCICu_{ox}$ (pH 4 to 6.34 in 72 hours) treated solutions. The pH of the neutral (pH 7) solutions fractionally increased from 7 to 7.19 under OCIMnox and 7 to 7.21 under OCICuox treatments. However, the pH in alkaline solutions considerably decreased from 9 to 8.65 and 8.67 under $OCIMn_{ox}$ and $OCICu_{ox}$ treatments respectively. Whereas, sharp acidification was noted in salt ($FeSO_4$, $MnSO_4$, and $CuSO_4$) and EDTA treated 9 solutions of all pH. Although in Fe release was highest in $FeSO_4$ treated solutions (20.47±0.1 mg kg^{-1}) followed by $OCIMn_{ox}$ (18.85±1.0 mg kg^{-1}) and $OCICu_{ox}$ (17.58±0.6 mg kg^{-1}) treated solutions in strongly acidic pH solution. Release of Fe was significantly higher in OCIMnox (pH 7: 23.58±0.5 mg kg^{-1}; pH 9: 15.11±0.5 mg kg^{-1}) and $OCICu_{ox}$ (pH 7: 20.57±0.8 mg kg^{-1}; pH 9: 14.78±0.7 mg kg^{-1}) treated solutions than $FeSO_4$ (pH 7: 15.28±1.1 mg kg^{-1}; pH 9: 6.87±1.0 mg kg^{-1}) treatment when the pH was neutral and alkaline. On the other hand, the Mn release in OCIMnox treated solutions (pH 4: 10.38±1.1 mg kg^{-1}; pH 7: 22.45±1.2 mg kg^{-1}; pH 9: 19.35±1.5 mg kg^{-1}) was significantly higher than Mn-EDTA (pH 4: 1.34±0.05 mg kg^{-1}; pH 7: 5.74±0.2 mg kg^{-1}; pH 9: 3.74±0.05 mg kg^{-1}) treatments under all pH conditions. Interestingly, the Cu release profile in $OCICu_{ox}$ treated solutions (pH 4: 5.87±0.05 mg kg^{-1}; pH 7: 9.98±0.8 mg kg^{-1}; pH 9: 8.74±0.8 mg kg^{-1}) was significantly higher than Cu-EDTA (pH 4: 4.45±0.04 mg kg^{-1}; pH 7: 10.47±0.8 mg kg^{-1}; pH 9: 7.89±0.8 mg kg^{-1}) in acidic and alkaline pH solutions. However, there was no significant difference between $CuSO_4$ and $OCICu_{ox}$ in regard to Cu release in all the three solutions of 4, 7, and 9 pH.

2.1.2. pH- Buffering capacity and release profiles of Fe, Mn, and Cu in soil and aqueous media

A typical alluvial soil was incubated with the $OCIMn_{ox}$, $OCICu_{ox}$, Fe/Mn/Cu-EDTAs and sulphates. The soil pH increased approximately from 5.5 to 5.6 under 10 mg kg-1 application of $OCIMn_{ox}$ and $OCICu_{ox}$ in 90 days. Conversely, the soil pH was reduced by 1.27 times due to application of FeSO4. Sharp reduction in pH was observed in presence of Fe-EDTA in both aqueous and soil mediums. Aqueous solutions of three different pH values (pH: 4, 7, and 9) were treated with OCIMnox and OCICuox to appreciate the relationship between pH and release profile of micronutrients. In acidic solution (pH 4), the pH significantly shifted towards neutral value (pH 6.3-6.4) due to OCICuox and OCIMnox addition. The pH of the neutral (pH 7) solutions fractionally increased from 7 to 7.21 under $OCIMn_{ox}$ and 7 to 7.19 under $OCICu_{ox}$ treatments. The alkaline pH considerably decreased from 9.0 to 8.65 in OCIMnox and OCICuox solutions respectively. However, sharp acidification was noted in Fe/Mn/Cu-EDTAs and sulphates treated solutions of all pH. Although Fe release was highest in $FeSO_4$ treated solutions followed by $OCIMn_{ox}$ and $OCICu_{ox}$ solutions in acid (pH 4) solution; the release was significantly higher in neutral and alkaline solutions due to $OCIMn_{ox}$ (pH 7: 23.58±0.5 ppm; pH 9: 15.11±0.5 ppm) and OCICuox (pH 7: 20.57±0.8 ppm; pH 9: 14.78±0.7 ppm) incorporation than $FeSO_4$ (pH 7: 15.28±1.1 ppm; pH 9: 6.87±1.0 ppm).

Further, Mn release in $OCIMn_{ox}$ treated solutions (pH 4: 10.38±1.1 ppm; pH 7: 22.45±1.2 ppm; pH 9: 19.35±1.5 ppm) was significantly higher than Mn-EDTA (pH 4: 8.34±0.05 ppm; pH 7: 17.74±0.2 ppm; pH 9: 15.74±0.05 ppm) at all pH. Similarly, the Cu release profile was greater in $OCICu_{ox}$ treated solutions (pH 4: 5.87±0.5 ppm; pH 7: 9.98±0.8 ppm; pH 9: 8.74±0.8 ppm) than Cu-EDTA (pH 4: 4.51±0.04 ppm; pH 7: 10.47±0.8 ppm; pH 9: 7.89±0.8 ppm) at acidic and alkaline pH. However, (NH4)2SO4 incorporation sharply raised the solution pH within 24 hours; probably due to NH3 production, which sufficiently neutralized later by OCIMnox and OCICuox. In (NH4)2SO4 solutions, the Fe release pattern was in the order: $OCIMn_{ox}$ = $FeSO_4$> $OCICu_{ox}$ > Fe-EDTA ($P<0.01$; $LSD_{treatment}$=0.33); while, the Mn and Cu release profiles were in the orders: $OCIMn_{ox}$ > Mn-EDTA> $MnSO_4$ ($P<0.01$; $LSD_{treatment}$=0.43) and $OCICu_{ox}$ > $CuSO_4$> Cu-EDTA respectively ($P<0.01$; $LSD_{treatment}$=0.44). The remarkable buffering capacity of OCIMnox and OCICuox materials were further evidenced upon introduction to KH2PO4 solutions. The pH sharply reduced by 1.63–1.74 and 2.26–3.41 times respectively in EDTAs and sulphates treated solutions. Whereas, the pH increased over time to near neutral state (6.87) in OCIMnox-KH2PO4 solution. In OCICuox-KH2PO4 solution, the pH was stable (6.5-6.6) throughout the study-period (21 days). On the other hand, the pH of the KCl mixed solutions sharply shifted to acidic range (3.2-4.7) within

24 hours irrespective of treatments. Interestingly, the pH increased from 4.7±0.1 to 5.8 ±0.5 and 4.5±0.1 to 5.2 ±0.1 respectively in OCIMnox-KCl and OCICuox-KCl solutions. Although, the pH reduction resulted in profuse iron release in FeSO4-KCl solution, iron release was uniform in OCIMnox-KCl solution and sharply enhanced in OCICuox-KCl solution. Moreover, Mn release was significantly greater with OCIMnox as compared to Mn-EDTA, MnSO4 ($P<0.01$; $LSD_{treatment}=0.18$). Whereas, availability of Cu was greatest in OCICuox-KCl solution ($P<0.01$; $LSD_{treatment}=0.35$).

2.1.3. Release profile of, Fe, Mn, and Cu in soil and salt mixed aqueous medium

The temporal change in the release profile of Fe, Mn, and Cu from OCIMnox and OCICuox in salt mixed [(NH4)2SO4, KH2PO4, and KCl] aqueous solutions are presented. The release of Fe in OCIMnox-KH2PO4 and OCICuox-KH2PO4 solutions was significantly higher than Fe-EDTA-KH2PO4 solutions. Similarly, the Mn release profile under OCIMnox-KH2PO4 was significantly higher than Mn-EDTA-KH2PO4 and MnSO4-KH2PO4 solutions ($P<0.01$; $LSD_{treatment}=0.49$). Moreover, OCICuox showed significantly greater release of Cu than Cu-EDTA in such solutions. Interestingly, similarity between aqueous and soil mediums was distinct in regard to release profile of micronutrients. Fe availability in soil increased by 1.23-1.29 times and 1.24-1.27 times in 5 to 90 days under OCIMnox and OCICuox respectively ($P<0.01$; $LSD_{treatment}=0.83$). Significantly higher Mn content was recorded under OCIMnox (20 and 10 mg kg^{-1}) application as compared to Mn-EDTA and MnSO4 ($P<0.01$; $LSD_{treatment}=0.74$). Likewise, Cu content was greater in OCICuox treated soils [@ 10-50 mg kg^{-1}] as compared to Cu-EDTA and CuSO4 treatments ($P<0.01$; $LSD_{treatment}=0.56$). The characteristic relationships between soil pH and nutrient (Fe, Mn, and Cu) release from OCIMnox and OCICuox in tomato field exhibited a close resemblance with the lab-based experiments. The pH of the tomato soil significantly increased from 5.49 to 5.70 under OCIMnox and OCICuox treatments. The acidifying effect of Fe/Mn/Cu-EDTAs and sulphates was evidenced in cropped soil. Fe availability was highest under OCIMnox followed by OCICuox, FeSO4, Fe-EDTA ($P<0.01$; $LSD_{treatment}=0.76$). Moreover, Mn and Cu availability was significantly greater under OCIMnox and OCICuox compared to all other.

2.1.4. Interactions of OCIMnox with N, P, and K availability in soil and aqueous media

The spectacular influence of the synthesized nanomaterials on pH encouraged us to understand their interactions with fertilizer-N as well as inherent soil-N. Nitrogen release from ammonium sulphate sharply elevated in presence of OCIMnox and OCICuox treatment from the 7th day onwards ($P<0.001$), while meagre retardation in N release was noted after 5 days with Fe/Mn/Cu-EDTAs and sulphates. Significant increment (0.4-1.3 folds) in mineralizable N in soil was recorded under [OCIMnox] 10 mg kg^{-1} followed by [OCICuox]10 mg kg^{-1} and [OCIMnox] 20 mg kg^{-1} as compared to their initial values ($P<0.01$; $LSD_{treatment}=4.08$). However, soil-N availability reduced due to 20 and 50 mg kg^{-1} application of Fe/Mn/Cu-EDTAs and sulphates. Hence, it appeared that efficacy of the synthesized materials was best when applied @ 10 mg kg^{-1}. The 10 mg kg-1 dose was used to grow tomato in nutrient deficient alluvial soil. Overall, N availability was significantly greater with OCIMnox followed by OCICuox as compared to other treatments ($P<0.01$; LSD=24.14). About 2.4- and 2.3-folds increment in N-availability was noted with OCIMnox and OCICuox application in leached. Interestingly, strong positive correlation was achieved between N release from (NH4)2SO4 in aqueous medium and N availability in soil without crop (r: 0.97; $P<0.01$). Correspondingly, the correlation for N- availability was significant between cultivated soil and uncultivated soil (r value: 0.88, $P<0.01$).

Phosphate ($H2PO^-$) release from KH2PO4 significantly enhanced in presence of OCIMnox and OCICuox in aqueous media ($P<0.01$, $LSD_{treatment}=0.13$) but sharply reduced (1.5-6.6 folds) in presence of Fe/Mn/Cu-sulphates and EDTAs. The test soil in our experiment was acidic and the problem of P precipitation in the form of Fe/Al-PO42- is well known in such soils. Fascinatingly, P availability significantly improved due to [OCIMnox]10 mg kg^{-1} application followed by [$OCICu_{ox}$]10 mg kg^{-1} and [$OCIMn_{ox}$]20 mg kg^{-1} ($P<0.01$; $LSD_{treatment}=0.16$), we also found strong positive correlation between P increment patterns in aqueous medium, in soil under laboratory condition, and under tomato cultivation. K release from KCl was monitored in aqueous medium in presence of OCIMnox and OCICuox. Manifold increment in K release from KCl was evidenced due to presence of OCIMnox (1.67 folds) and OCICuox (1.62 folds). K availability was greatest under OCIMnox followed by $OCICu_{ox}$, Fe-EDTA, Cu-EDTA, $FeSO_4$, and Mn-EDTA treatments after 21 days ($P<0.01$; $LSD_{treatment}=0.65$). Similar performance of OCIMnox and OCICuox was recorded when these materials were applied to soil under laboratory condition. Consequently, the K availability in cultivated nutrient deficient soil significantly improved due to OCIMnox application followed by $OCICu_{ox}$ and Fe/Mn/Cu-sulphates and EDTAs (natural soil: $P<0.01$; $LSD_{treatment}=0.5$; leached soil: $P<0.01$; $LSD_{treatment}=0.39$). Moreover, we recorded strong positive correlation between the K release profile in aqueous solution and in soil under laboratory condition (r: 0.62; $P<0.01$).

Objective 3: Evaluation of eco-toxicity potential and environmental compatibility of the synthesized nanomaterial

3.1. Eco-safety analyses in plant (germination assay), earthworm, and microbial models

3.1.1. Methodology

The viability of $OCIMn_{ox}$ and $OCICu_{ox}$ exposed tomato (*Lycopersicon esculentum*, cv. Badshah *F1 hybri*d) seeds was evaluated. 2 mg of $OCIMn_{ox}$ and $OCICu_{ox}$, $FeSO_4$, and Fe–EDTA were mixed in 10 ml deionized water and sonicated for 15 minutes. Meanwhile, 20 seeds of the selected species were placed on tissue papers in sterilized glass petriplates, inoculated with previously prepared solution mixtures and kept in dark at 25^0C for 48 hours. Germination index (GI), relative root growth (RRG), and relative seed germination (RSG) were measured following (Das et al., 2016) as below:

$$\text{RSG (\%)} = \frac{\text{Number of seeds germinated with treatments}}{\text{Number of seeds germinated in distilled water}} \times 100$$

$$\text{RRG (\%)} = \frac{\text{Mean root length of seeds receiving treated solutions}}{\text{Mean root length of seeds receiving distilled water}} \times 100$$

$$\text{GI(\%)} = \frac{\text{RSG} \times \text{RRG}}{100}$$

Eisenia fetida is a model earthworm for standard soil toxicity tests according to OECD and US-EPA guidelines. Well grown clitelleted (i.e., adult) specimens of *E. fetida*, weighing about 400-500 mg were collected from the departmental vermiculture unit and kept overnight in moist filter papers to ensure evacuation of previously ingested feed materials from earthworm guts. Concurrently, urine free cow dung-based substrates (1 kg each) were prepared in earthen pots and synthesized nanomaterials and $FeSO_4$ (as non-nanoscale control) were homogenized with the cow dung-based substrates in the pots at 10 and 50 ppm concentrations. The final combinations of treatments in the substrates were as below:

Control	Nano fertilizer spiked substrate	Non-nanoscale fertilizer spiked substrate
Only substrate (cow dung)	$(OCIMn_{ox})_{10}$ – 10 mg kg^{-1}	$FeSO_4$ – 20 mg kg^{-1}
	$(OCIMn_{ox})_{50}$ – 50 mg kg^{-1}	$FeSO_4$ – 50 mg kg^{-1}
	$(OCICu_{ox})_{10}$ – 10 mg kg^{-1}	
	$(OCICu_{ox})_{50}$ – 50 mg kg^{-1}	

After 7 days of treatment the evacuated earthworm specimens were uniformly (10 per pot) introduced to different pots. The incubation was continued for 30 days and the whole experiment was replicated thrice.

A group of earthworm specimens were collected from each pot after 30 days. Subsequently, both treated and un-treated earthworms specimens were gut cleaned, freeze-killed, and sonicated. Then the whole body homogenate of the earthworms was used for analyzing the activity of catalase (AEBI et al., 1974), reduced glutathione (GSH) (Ellman, 1958), glutathione-S-transferase (GST) (Nimmo et al., 1979), and superoxide dismutase (SOD) (Liochev et al., 1996) following standardized methods. Concurrently, another group of earthworm specimens were gut evacuated as detailed above and digested in acid mixture [HNO_3:$HClO_4$ (6:1)]. The digested samples were further used for estimation of Fe, Mn, and Cu in ICP-OES as detailed for other experiments.

It was also necessary to assess whether the $OCIMn_{ox}$ and $OCICu_{ox}$ impart any inhibitory effect on beneficial soil microorganisms or not. Therefore, well-characterized strains of one symbiotic N-fixing (*Rhizobium sp.*) and one P-solubilizing (*Serratia marcescens*) were selected for the investigation. We performed this qualitative assay following the agar well diffusion method (Fernandez-Garayzabal et al., 1992). Eventually, suspensions of the selected bacterial strains were prepared in nutrient agar (NA) broth and then 100 μl of the bacterial suspensions were uniformly spreaded on NA plates using glass rod spreader. Then, wells were formed in the petriplates with the help of cork-borer and these wells were inoculated with 50 μl of 50 μg ml^{-1} concentrations of $OCIMn_{ox}$, $OCICu_{ox}$, $FeSO_4$, and Fe-EDTA solutions, respectively. These tasks were performed in a laminar air flow chamber. Subsequently, the inoculated petriplates were incubated for 72 hours at 28 ºC and zones of inhibition were observed after visualizing the bacterial growth (Fernandez-Garayzabal et al., 1992).

3.1.2. Results and Discussion

A few enzymes [catalase (CAT) and superoxide dismutase (SOD)] are induced in plant cells to counter oxidative stress. In our research, CAT activity was significantly lower in $OCIMn_{ox}$ and $OCICu_{ox}$ treated plants than the Fe/Mn/Cu-sulphates ($p<0.01$; LSD=0.58), while SOD activity was highest under Mn-EDTA and lowest under $OCIMn_{ox}$ and $OCICu_{ox}$ ($p <0.01$; LSD=0.76) (Table 1). Interestingly, lipid peroxidation indicating cell damage was significantly lower in $OCIMn_{ox}$ and $OCICu_{ox}$ treated plants as compared to sulphates and EDTAs ($p<0.01$; LSD=0.007) (Table 1). Such low levels of these stress indicators in treated plants showed the eco-friendly nature of the synthesized nano-materials. Correspondingly, we recorded 100% germination with $OCIMn_{ox}$ and $OCICu_{ox}$ treatments while germination index was extremely poor with Fe/Mn/Cu-sulphates leading to significantly high relative seed germination (RSG) and root growths (RRG) in $OCIMn_{ox}$ and $OCICu_{ox}$ treated seeds (Table 2). However, some engineered Fe, Mn, Cu, and Zn-nanoparticles were reported to be non-toxic in low concentration to lettuce, and significantly augmented plant growth (Liu et al., 2016).

Table 1: Effect on Catalase activity, Lipid peroxidation, Super oxide dismutase, Photosynthetic rate, Hill activity, Chlorophyll content, total protein content and shelf life in tomato leaves treated with $OCIMn_{ox}$ and $OCICu_{ox}$

	Attributes							
Treatment	Catalase (ml $min^{-1}g^{-1}$)	Lipid peroxidation (μM g^{-1})	Super oxide dismutase (min^{-1} mg^{-1})	Photosynthetic rate (μM CO_2 m^{-2} s^{-1})	Hill activity (μM hr^{-1})	Chlorophyll (mg g^{-1})	Total protein (mg ml^{-1})	Shelf life(g)
Control	6.2±0.5	0.073±0.011	34.08±0.12	20.59±1.5	4126±56	38.01±5.4	1.13±0.001	54.8±0.26
$OCIMn_{ox}$	5.3±1.2	0.013±0.005	11.17±0.31	45.83±4.5	5221±48	54.6±2.5	2.28±0.05	30.1±0.24
$OCICu_{ox}$	5.3±1.5	0.008±0.001	10.85±0.08	32.76±1.5	5035±78	45.2±2.4	1.99±0.011	28.5±0.64
Fe-EDTA	5.3±1.4	0.108±0.01	31.77±0.29	26.03±2.1	4136±48	40.15±1.8	1.71±0.002	45.5±0.45
Mn-EDTA	5.3±1.4	0.134±0.005	50.15±0.83	22±1.6	3897±38	39.66±1.9	1.25±0.001	47.5±0.53
Cu-EDTA	5.3±1.1	0.167±0.008	35.41±0.11	21±1.8	3884±37	41.6±2.9	1.18±0.001	49.5±0.45
$FeSO_4$	8±1.1	0.128±0.005	21.66±0.15	23.79±2.7	4768±65	41.56±3.7	1.18±0.002	38±0.54
$MnSO_4$	6.8±0.8	0.172±0.009	27.31±2.5	20±1.4	3564±41	39.56±3.7	1.11±0.002	34.9±0.33
$CuSO_4$	6.2±1	0.144±0.012	48.97±0.86	21±1.6	3379±35	39.6±2.7	1.15±0.001	39.1±0.31
p value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
LSD	0.58	0.007	0.76	0.61	26.8	0.17	0.03	0.75

The impact of $OCIMn_{ox}$ and $OCICu_{ox}$ on useful soil microbes was assessed to ascertain their ecological compatibility in soil environment. Two well characterized beneficial soil bacterial strains [One N-fixing (*Rhizobium sp.*) and the other P solubilizing (*Serratia marcescens*)] were selected for this assessment and the results are presented in Figure 3.

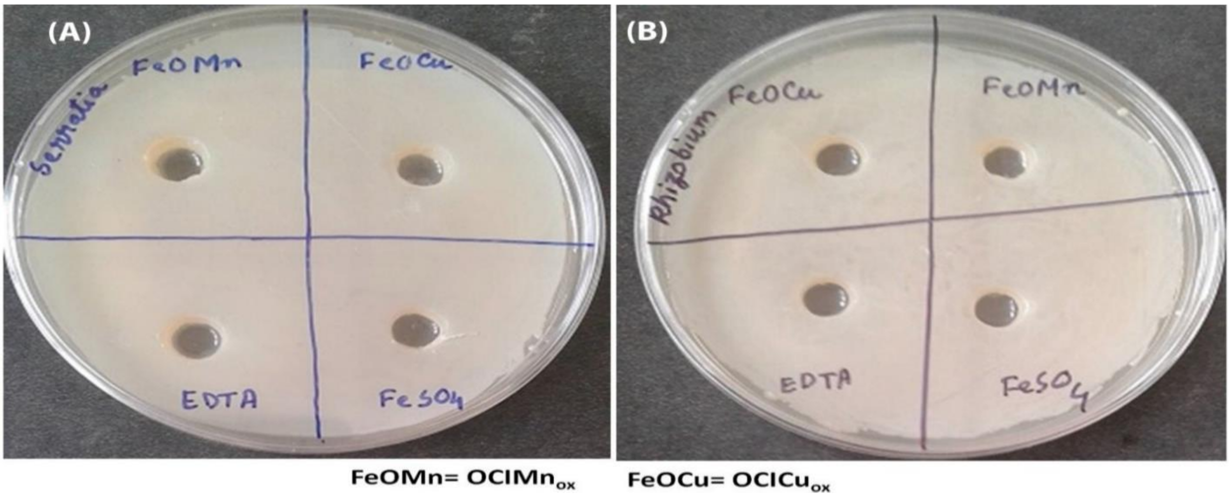


Figure 3: Evidence of non-inhibitory effects on beneficial soil microbes

Both bacterial species are well known for their efficacy as prolific soil health stimulators (Hameeda et al., 2008; Liba et al., 2006). The adopted method for this analysis relies on formation of zone of inhibition in agar wells to evaluate the inhibitory effect of any substance (in our case, the synthesized nanomaterials) on microbial growth. Interestingly, no zone of inhibition developed in $OCIMn_{ox}$ and $OCICu_{ox}$ inoculated petriplates after 3 days of incubation. As such, majority of microbial nano-toxicity assays performed in laboratory show considerable adverse effects. However, such inhibitory impacts are often lost in natural environment. Recently, Dedman et al (Dedman et al., 2021) clearly showed that detected adverse effects of TiO_2 nanoparticle on marine cyanobacterium, *Prochlorococcus*, in lab condition was absent when the exposure study was performed in seawater. However, inhibitory effects of Fe-EDTA and $FeSO_4$ were also absent in our study (Figure 3). In fact, readily available form of Fe (Fe^{2+}) facilitates the growth of N-fixing bacteria in soil (Liu et al., 2014). Moreover, benign nature of Fe-EDTA and

$FeSO_4$ in soil-plant systems is one of the major qualities for their acceptance as micronutrient fertilizer in agriculture (Kabir et al., 2016). Hence, our study reveals that $OCIMn_{ox}$ and $OCICu_{ox}$ do not induce any inhibitory impact on soil microbial health as like as their non-nanoscale counterparts.

Table 2: Effect of $OCIMn_{ox}$ and $OCICu_{ox}$ on relative seed germination (RSG), relative root growth (RRG), and germination index (GI) of tomato (Badshah *F1 hydbrid*).

Treatment	Attributes		
	RSG (%)	RRG (%)	GI (%)
Control	91±7.5	68±5	61.88±4.8
$(OCIMn_{ox})_{10}$	166.67±24.5	131±15	218.3±36
$(OCIMn_{ox})_{20}$	135±17	134±27	180.9±30
$(OCIMn_{ox})_{50}$	134±18	101±31	135.34±24
$(OCICu_{ox})_{10}$	159±21	125±12	198.75±14
$(OCICu_{ox})_{20}$	131±18.54	105±14	137.55±15
$(OCICu_{ox})_{50}$	108±14	95±4.6	102.6±13
$(FeSO_4)_{50}$	63±10	45±4.5	28.35±5.2
$(Fe\text{-}EDTA)_{50}$	45±8.5	36±3.4	16.2±5.3
p value	<0.01	<0.01	<0.01
LSD	0.86	0.82	1.08

Previous workers reported adverse impacts of nano-materials on fish and crustaceans (Zhao et al., 2011). However, we have used earthworms (*Eisenia fetida*) as test organism to assess the stress indicating attributes in nano-material treated worms as compared to $FeSO_4$ (Table 3). The CAT activity significantly reduced over time in $OCIMn_{ox}$ treated earthworms at both the concentrations whereas such reduction in CAT activity was recorded only with 10 mg kg^{-1} dose of $OCICu_{ox}$. However, CAT activity was significantly greater in $FeSO_4$ (10 and 50 mg kg^{-1}) treated earthworms. Similarly, GSH, GST, and SOD activities were significantly higher in $FeSO_4$ treated earthworms both at 10 days and 30 days after exposure. However, GST activity substantially reduced at 30 days in earthworms irrespective of treatments. Overall, the oxidative stress indicating enzyme activities were lowest in $OCICu_{ox}$ (10 mg kg^{-1}) treated worms followed by other treatments (Table 3). Eventually, a significant improvement in earthworm biomass was recorded when treated with 10 mg kg^{-1} levels ($p< 0.01$; LSD= 0.04). Effects of metal oxide nano-particles on earthworm health are largely dose dependent (Goswami et al., 2017). Recently, Adeel et al. [91] demonstrated that lanthanum and ytterbium rare earth oxides do not induce toxic effects to growth metabolism of *E. fetida* when the exposure concentrations were lower than 50 mg kg^{-1}. High dose exposure (above 400 mg kg^{-1}) induced remarkable oxidative stress in earthworms (Gomes et al., 2012; Heckmann et al., 2011). Earthworms have also been reported to be sensitive to zero-valent Fe (El-Temsah and Joner, 2012). Therefore, we did not observe earthworm toxicity in our experiment because of the Fe_3O_4 state of the iron.

Table 3: Activity of catalase, reduced glutathione (GSH), glutathione S transferase (GST), super oxide dismutase (SOD), earthworm biomass and body length in earthworms exposed to $OCIMn_{ox}$ and $OCICu_{ox}$ (mean±stdev)

Treatment	Catalase (U mg^{-1} min^{-1})		GSH (nM mg^{-1})		GST (min^{-1} mg^{-1})		SOD (min^{-1} mg^{-1})		Earthworm biomass (g)		Body length (cm)	
	10 day	30 day	10 day	30 day	10 day	30 day	10 day	30 day	10 day	30 day	10 day	30 day
$(OCIMn_{ox})_{10}$	52.01±2.8	19.29±0.95	114.08±5.9	20.88±1	121.99±6.9	43.01±4.13	43.79±2.2	24.53±1.3	0.4±0.02	0.7±0.04	5.8±0.3	10±0.5
$(OCIMn_{ox})_{50}$	94.01±4.8	24.68±1.4	138.58±6.2	21.89±1.1	190.32±9.8	62.95±3.1	56.66±2.8	30.74±1.5	0.35±0.02	0.58±0.03	5.6±0.26	8.5±0.4
$(OCICu_{ox})_{10}$	36.69±1.9	15.85±0.8	119.01±6.3	20.58±1	185.47±10	52.71±2.4	46.01±2.3	15.02±0.8	0.37±0.02	0.67±0.03	5.6±0.28	7.5±0.4
$(OCICu_{ox})_{50}$	11.09±0.58	20.42±1	122.59±6.3	26.72±1.3	169.57±8.6	82.44±4.1	67.29±3.4	33.15±1.7	0.35±0.03	0.53±0.03	5.5±0.23	7.1±0.4
$(FeSO_4)_{10}$	581.48±28.5	314.08±16.1	206.46±9.8	168.91±8.6	1428.79±68	240.38±12	381.63±20.1	472.84±23.8	0.32±0.02	0.35±0.02	5.2±0.28	5.8±0.3
$(FeSO_4)_{50}$	765.32±37.1	346.97±17.6	370.65±19.2	176.44±8.8	2617.53±110	280.39±14	473.56±23.9	366.38±18.5	0.31±0.03	0.34±0.02	5.2±0.29	5.9±0.3
Control	211.48±11.4	57.32±2.9	83.31±4.3	42.06±2.1	154.04±7.7	72.78±3.5	69.23±3.5	66.91±3.5	0.33±0.03	0.39±0.03	5.1±0.22	6±0.3
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
LSD	0.162	8.85	0.469	0.907	15.78	1.99	7.594	9.26	0.0004	0.04	0.092	0.102

3.2. Toxicity assay of synthesized nanomaterial on mammalian health

3.2.1. *Methodology*

3.2.1.1. Assessment of acute mammalian toxicity: in vivo study

The in vivo experiment was performed in Defence Research laboratory (DRDO), Tezpur following OECD (425) guideline and in accordance with standard protocols approved by the Animal Ethical Committee of the institute. Rats (strain: Wister; sex: either; and age: 8-12 weeks) were reared in favorable condition in sanitized polypropylene cages (14-34 animals per cage) with sufficient supply of water and food *ad libitum*. The total numbers of animals, cages, treatment groups, initial body weights of the rats, and randomization plan are provided in table 1.

Table 1: The total numbers of animals, cages, treatment groups, initial body weights of the rats, and randomization plan

Group	Cage no	Animal no	Body weight	Group mean	Treatment
group-1	C-1, F	2	180	168.5	Control
		3	157		
group-2		4	128	122	Control R1
		5	116		
group-3	C-2, F	6	166	184	Control R2
		7	202		
group-4		8	180	170	Control R3
		9	160		
group-5	C-3, M	10	240	241.5	FeO N
		11	243		
group-6		16	118	123	FeO R1
		17	128		
group-7	C-4, M	28	187	231.5	FeO R2
		13	251		
group-8		14	263	219	FeO R3
		18	200		
group-9	C-5, M	20	248	240	CuO N
		19	232		
group-10		24	120	113	CuO R1
		25	106		
group-11	C-6, F	22	160	138.5	CuO R2
		23	117		
group-12		15	219	232	CuO R3
		21	245		
group-13	C-7, M	29	131	245.5	MnO N
		30	147		
group-14		29	131	139	MnO R1
		30	147		
group-15	C-8, M	26	138	144	MnO R2
		27	150		
group-16		31	205	222	MnO R3
		32	239		

The rats were acclimated for five days prior use. Randomly selected groups were separately administered with OCIO, $OCICu_{ox}$, and $OCIMn_{ox}$ (@ 2000 mg kg^{-1} dose). In addition, previously harvested tomato fruits, grown in OCIO, $OCICu_{ox}$, and $OCIMn_{ox}$ added soil (hereafter, OCIO-treated tomato, $OCICu_{ox}$-treated tomato, and $OCIMn_{ox}$-treated tomato) were also orally administered in the rats. For this, 2 g tomato was thoroughly ground in mortar pestle and dissolved in 10 ml deionized water. The control groups were fed with 10 ml pure deionized water. The details of treatment dose formulations are furnished in table 2.

Table 2: The details of treatment dose formulations

Group	Treatment	Cage no	Animal no	Body weight	Dose amount (mg)	Dose volume (ml)
group-1	Control	C-1	2	180	360	1.8
			3	157	314	1.6
group-2	Control R1		4	128	256	1.3
			5	116	232	1.2
group-3	Control R2	C-2	6	166	332	1.7
			7	202	404	2.0
group-4	Control R3		8	180	360	1.8
			9	160	320	1.6
group-5	FeO N	C-3	10	240	480	2.4
			11	243	486	2.4
group-6	FeO R1		16	118	236	1.2
			17	128	256	1.3
group-7	FeO R2	C-4	28	187	374	1.9
			13	251	502	2.5
group-8	FeO R3		14	263	526	2.6
			18	200	400	2.0
group-9	CuO N	C-5	20	248	496	2.5
			19	232	464	2.3
group-10	CuO R1		24	120	240	1.2
			25	106	212	1.1
group-11	CuO R2	C-6	22	160	320	1.6
			23	117	234	1.2
group-12	CuO R3		15	219	438	2.2
			21	245	490	2.5
group-13	MnO N	C-7	33	223	446	2.2
			34	268	536	2.7
group-14	MnO R1		29	131	262	1.3
			30	147	294	1.5
group-15	MnO R2	C-8	26	138	276	1.4
			27	150	300	1.5
group-16	MnO R3		31	205	410	2.1
			32	239	478	2.4

The rats were subjected to fasting overnight before dosing and also for four hours prior sacrificing them for various biochemical analyses. The incubation was conducted for two weeks.

3.2.1.2. Behavioral and biochemical attributes

The behavioral changes (body weight and feed consumption) and clinical signs in the rats were observed twice and once in a week respectively. The biochemical responses in treated and control rats were enumerated by following standard protocols. In brief, anticoagulant free blood was collected from the orbital sinus of rats using a gel separation technique. Eventually, the serum was separated within 30 minutes via centrifugation and assayed for glucose (Gl), total cholesterol (TC), triglyceride (TG), creatinine (Cre), glutamic oxaloacetic transaminase (SGOT), and glutamic pyruvic transaminase (SGPT). Gl, TC, and TG were analyzed using kits from Sigma Aldrich. Creatinine content in serum was estimated using Rat Creatinine ELISA Kit following manufacturer's instruction. The SGOT and SGPT levels were estimated using kits from Span Diagnostics Ltd., India by following the manufacturer's instructions.

***2.2.1.3. Statistical analysis*:** All of the data were reported as means ± standard errors. One way ANOVA was performed in SPSS 16.0 software for the animal experiment data and the least significant difference (LSD) test was performed to evaluate the impact of the nanomaterials and the nano-treated tomato on the health of the treated animals. As the field experiment is yet to be completed the results of the data analyses will be provided in the next (i.e., final) report.

3.2.2. Results and Discussion:

3.2.2.1. Changes in body weight and feed consumption in rats under in vivo experiment

The data on changes in body weight and feed consumption rate of control, nanomaterial administered, and nanomaterial-treated tomato administered rats have been presented in table 3 and table 4 respectively. The body weights of the rats in control group increased by 1.01-1.26 folds and their food consumption rates were satisfactory. In general, food consumption was higher in the first week as compared to the second week in most of the animals irrespective of treatments.

Such behavioral responses are considered as normal for Wister rats (Saikia et al., 2012).

Normal body weight increment was also recorded for OCIO-administered rats. On the other hand, about 1.14-1.26 folds increment in body weights was recorded in rats treated by OCIO grown tomato. Similar pattern of body weight gain and food habit was also recorded in rats administered with $OCICu_{ox}$ and $OCIMn_{ox}$ grown tomatoes. This indicates that high nutritional value of nano-treated tomato could be the reason for such gain in body weights of the rats. However, direct administration of $OCICu_{ox}$ caused mortality in some rats within 2-3 hours of dosing. In fact, the $OCICu_{ox}$ exposure caused hematuria, nasal bleeding, and darkening of organs due to deposition of particles as detected during necropsy.

Table 3: Changes in body weight in rats under in vivo experiment

Group	cage no	Animal no	Body weight (gm)				
			30-03-2021	02-04-2021	06-04-2021	09-04-2021	13-04-2021
group-1	C-1, F	2	180	214	217	220	224
		3	157	193	190	192	198
group-2		4	128	164	154	167	172
		5	116	144	142	151	156
group-3	C-2, F	6	166	168	172	176	171
		7	202	199	203	204	200
group-4		8	180	191	196	190	185
		9	160	170	174	169	165
group-5	C-3, M	10	240	260	263	260	259
		11	243	262	260	264	267
group-6		16	118	141	139	144	149
		17	128	145	152	149	150
group-7	C-4, M	28	187	202	207	210	216
		13	251	270	274	282	286
group-8		14	263	297	296	279	267
		18	200	213	228	220	218
group-9	C-5, M	20	248	257	Died	Died	Died
		19	232	249	Died	Died	Died
group-10		24	120	132	135	139	141
		25	106	123	130	131	133
group-11	C-6, F	22	160	182	182	180	182
		23	117	135	129	135	138
group-12		15	219	245	240	242	239
		21	245	257	245	246	243
group-13	C-7, M	33	223	242	249	250	252
		34	268	185*	187	195	205
group-14		29	131	145	152	160	163
		30	147	165	165	173	180
group-15	C-8, M	26	138	158	153	169	177
		27	150	167	164	170	176
group-16		31	205	228	231	232	234
		32	239	256	244	255	258

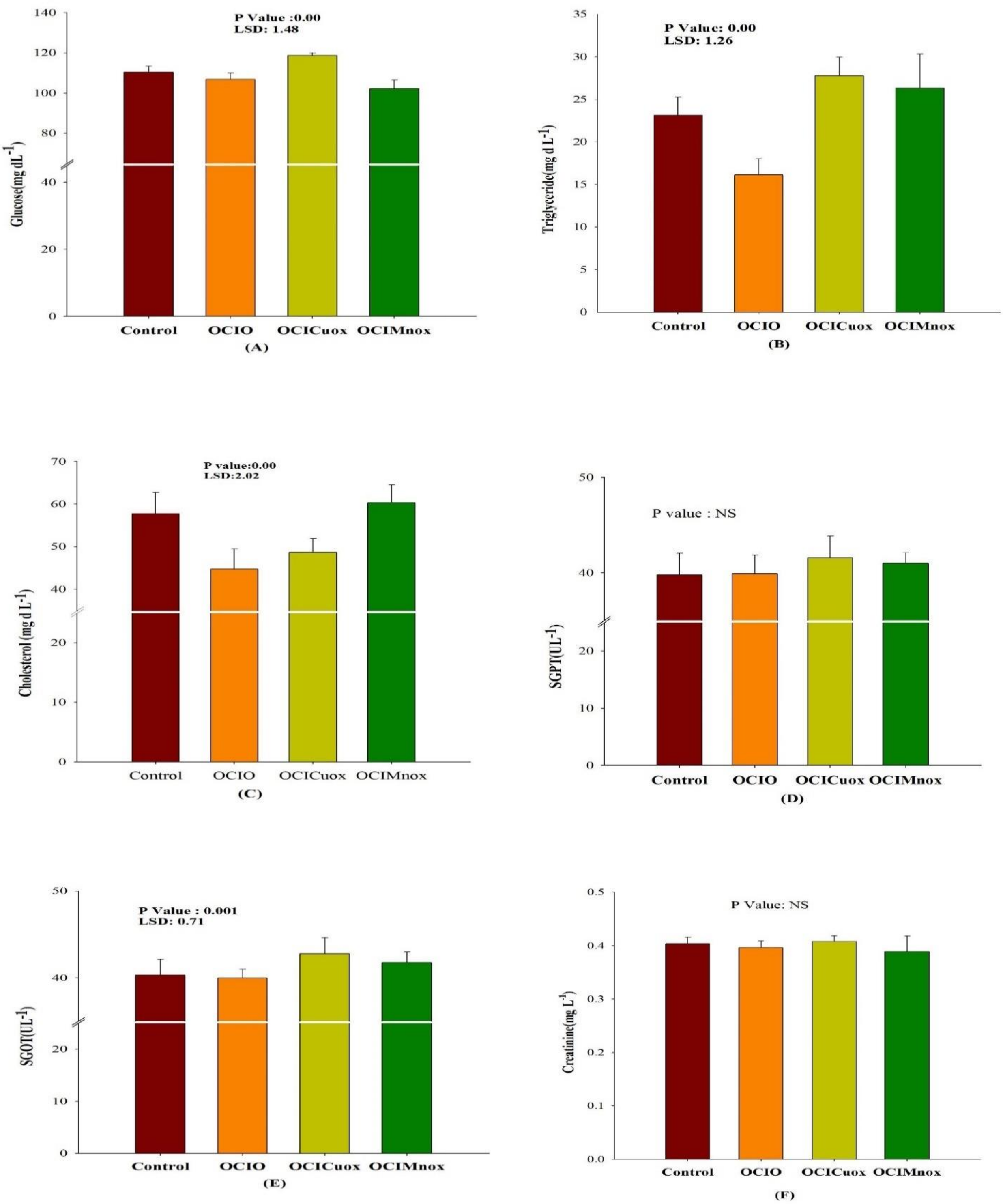
Table 4: Changes in feed consumption rate in rats under in vivo experiment

Cage no	02-04-2021	06-04-2021		09-04-2021		13-04-2021		15-04-2021
	Input	left over	Input	left over	Input	left over	Input	left over
C-1	200	69	200	68	200	56	200	137
C-2	200	45	200	63	200	81	200	129
C-3	200	103	200	79	200	46	200	107
C-4	200	74	200	81	200	72	200	114
C-5*	200	132	100	32	100	29	100	59
C-6	200	69	200	84	200	62	200	119
C-7	200	72	200	51	200	67	200	132
C-8	200	91	200	49	200	84	200	103

Some animals were also found to be sick and moribund within 72 hours of direct $OCIMn_{ox}$ exposure. Overall, these results imply that although high dose exposure of $OCICu_{ox}$ and $OCIMn_{ox}$ induced lethal impacts on the rats, the crop grown in these nanomaterials treated soil was beneficial for them. In contrast, OCIO exposure was not only harmless, but also facilitated growth and development of the animals.

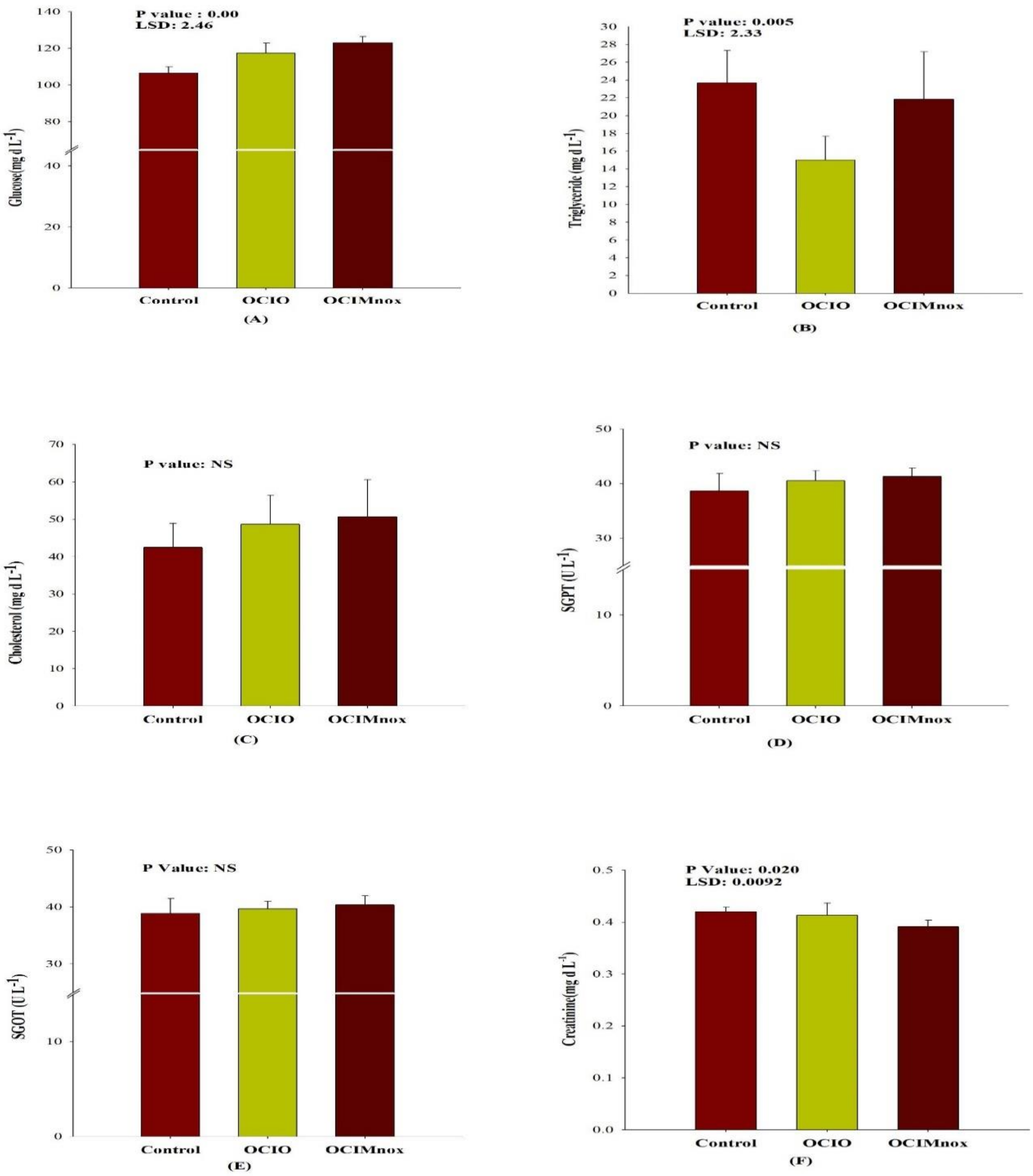
3.2 Effects on lipid profile, creatinine, and liver enzyme secretion in rats

The effects of the synthesized nanomaterials on lipid profile (Gl, TG, and TC), creatinine, and secretion of liver enzymes (SGOT and SGPT) were studied in rat serum figure 1. It is noteworthy to mention that these attributes were not studied in $OCICu_{ox}$ administered animals because of nano-induced mortality. The serum concentrations of Gl, TG, and TC in the control group were 110.44 mg d L^{-1}, 23.11 mg d L^{-1}, and 57.8 mg d L^{-1} respectively. The Gl level in OCIO and $OCIMn_{ox}$ administered rats were significantly higher than the control group ($p < 0.05$). However, TG and TC levels were significantly lower in OCIO and $OCIMn_{ox}$ administered rats as compared to control group ($P < 0.05$). Moreover, Gl, TG, and TC concentrations were dramatically lower when the rats were fed with OCIO and $OCIMn_{ox}$ grown tomatoes. These results imply that OCIO and $OCIMn_{ox}$ treated fruits might play a vital role in abatement of diabetes. Interestingly, the results also indicate that direct administration of OCIO can be a useful proposition for treating diabetic patients. Bilirubin accumulation in blood leads to hepatitis (WHO, 2016). The concentrations of SGOT and SGPT in blood indicate the possibility of development of hepatitis in the liver (S. Das et al., 2018). Therefore, reduction in SGOT and SGPT activities signifies solubilization of bilirubin. In the present, SGOT activity was noticeably lower in OCIO-treated tomato administered rats as compared to the control group (figure 4: (E)). On the other hand, levels of these enzymes were marginally greater in $OCICu_{ox}$ and $OCIMn_{ox}$ treated tomato administered rats as compared to the control groups. However, no significant difference was found in SGPT activity in the nano-treated tomato administered rats as compared to the control group (figure 4: (D)). Interestingly, direct exposure of OCIO, $OCICu_{ox}$, and $OCIMn_{ox}$ had no impact on activity of SGOT and SGPT in serum as the difference with the control group was not significant (figure 5: (D) and (E)). This indicates high dose (2000 ppm) administration of the nano materials have not induced any detrimental effect in regard to bilirubin accumulation in such animals and thus it can be implicated that OCIO, $OCICu_{ox}$, and $OCIMn_{ox}$ exposure had little adverse impact on liver function of the treated rats (figure 5). High creatinine content in serum indicates malfunctioning of kidney (Rahbari-Oskoui et al., 2014). The kidneys purify blood through excretion of waste materials like creatinine and thus creatinine elevation in blood denotes kidney failure (Kim and Takayama, 2015). In the present study, we found comparatively lower creatinine contents in OCIO and $OCIMn_{ox}$ administered rats than the control group. However, administration of OCIO, $OCICu_{ox}$, and $OCIMn_{ox}$ treated tomato fruits has not significantly enhanced creatinine concentrations, which implied that all the three nanomaterials were safe for agricultural application (figure 4: (F)). A previous finding suggests that Fe-nanoparticles have strong binding affinity for creatinine and urea (Banerji and Pramanik, 2015). Therefore, our results indicate that careful administration of OCIO and OCIO-treated food crops can facilitate in cure of kidney failure. Similar role of $OCICu_{ox}$ and $OCIMn_{ox}$ grown foods might also be found.



NS- Not significant

Figure 4: The effects of the synthesized nanomaterials on lipid profile (Gl, TG, and TC), creatinine, and secretion of liver enzymes (SGOT and SGPT)



NS- Not significant

Figure 5: Direct exposure of synthesized nanomaterials ($OCICu_{ox}$ and $OCIMn_{ox}$) on rat and their effect on lipid profile (Gl, TG, and TC), creatinine, and secretion of liver enzymes (SGOT and SGPT).

Objective 4: Impact assessment of the synthesized products in different agroclimatic and cropping system

4.1. Large-scale application of the three nanomaterials in different agro-climatic zone.

4.1.1. Field Study with Rice (*Oryza sativa*) crops

A field experiment was performed in two different locations, i.e., Sonitpur and Birbhum. Figure 6. shows the experiment area of two different locations. In the Sonitpur district, the field experiment was conducted near the Tezpur university campus, located approximately 13 km away from Tezpur town, Assam. The average temperature throughout the experimental period (June 2021 to November 2021) ranged from 13.44°C (minimum) to 36.26°C (maximum), and relative humidity varied between 74.5 to 83%. The soil samples of this area are generally typical alluvial in nature with sandy loam texture and acidic nature. On the other hand, another experiment is conducted in Birbhum near the Visva-Bharati, university. The average temperature throughout the experimental period (June 2021 to November 2021) ranged from 11.69°C (minimum) to 33.56°C (maximum), and relative humidity varied between 80.90 to 89.5%. And in both the locations, the nursery was bred with standard techniques, and well-grown seedlings at the 2-3 leaf stage were transplanted to the main field approximately 21-25 days after sowing. The details of the agronomic operations have been presented in table 2.1.

Table 2.1. Agronomic operation details

SL NO.	Agronomic operations in two different locations		
	Particulars	Sonitpur, Assam	Birbhum, West Bengal
		Dates	Dates
1	Nursery preparation	02-06-2021	07-06-2021
2	Seed sowing	04-06-2021	10-06-2021
3	Land preparation Plot size 6m×6m, demarcation of plots was done by bunding	20-06-2021	27-06-2021
4	Transplanting of nursery-grown seedlings	03-07-2021	11-07-2021
5	Application of nanomaterials	10-07-2021	18-07-2021
6	Start-up light irrigation	20-07-2021	27-07-2021
7	Application of basal NPK (1/3rd N, full P & K)	20-07-2021	27-07-2021
8	Weeding, cleaning, gap-filling, etc.	05-08-2021	11-08-2021
9	Second light irrigation	06-08-2021	12-08-2021
10	Application of 2/3 N as a split dose	10-08-2021	16-08-2021
11	Weeding, cleaning	20-08-2021	27-08-2021
12	Leaf sample collection	15-09-2021	22-09-2021
13	Harvesting	20-11-2021	28-08-2021

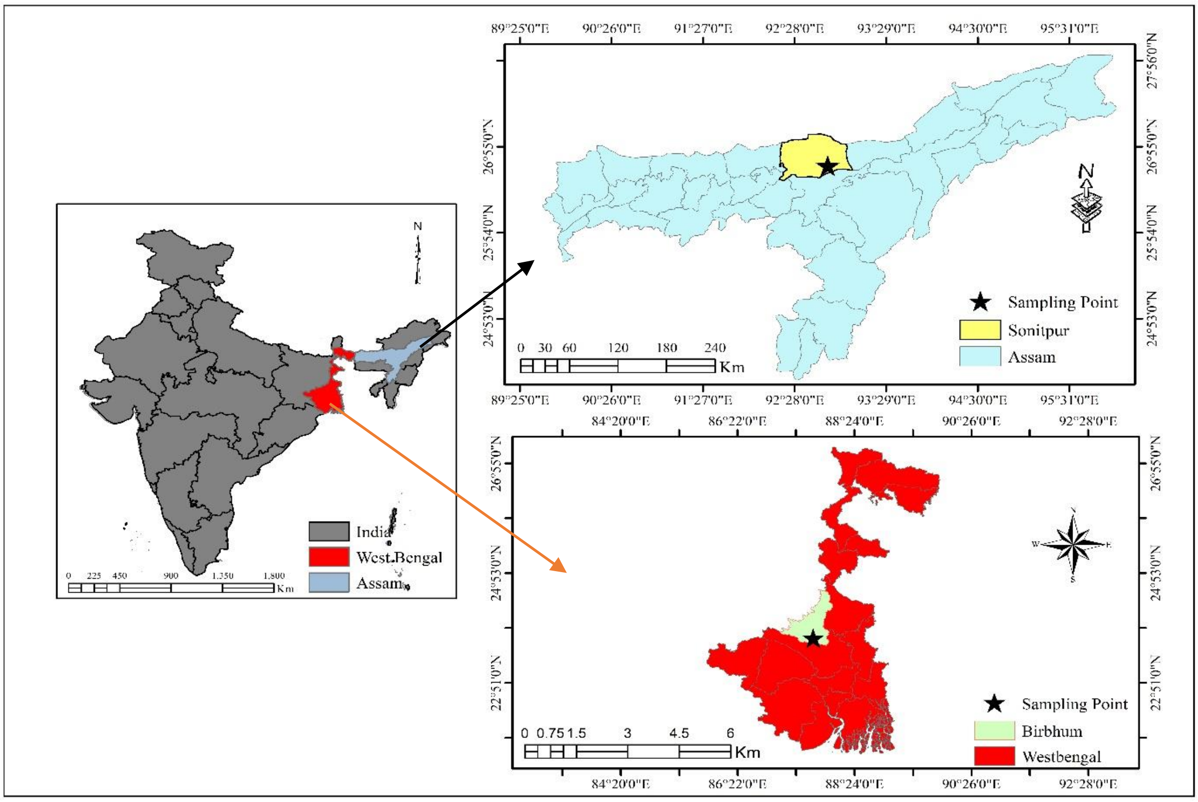


Figure 6. Experimental location

2.2.1. Treatment combination

The treatment combination for the field study is detailed below (for both two locations treatment combinations are the same):

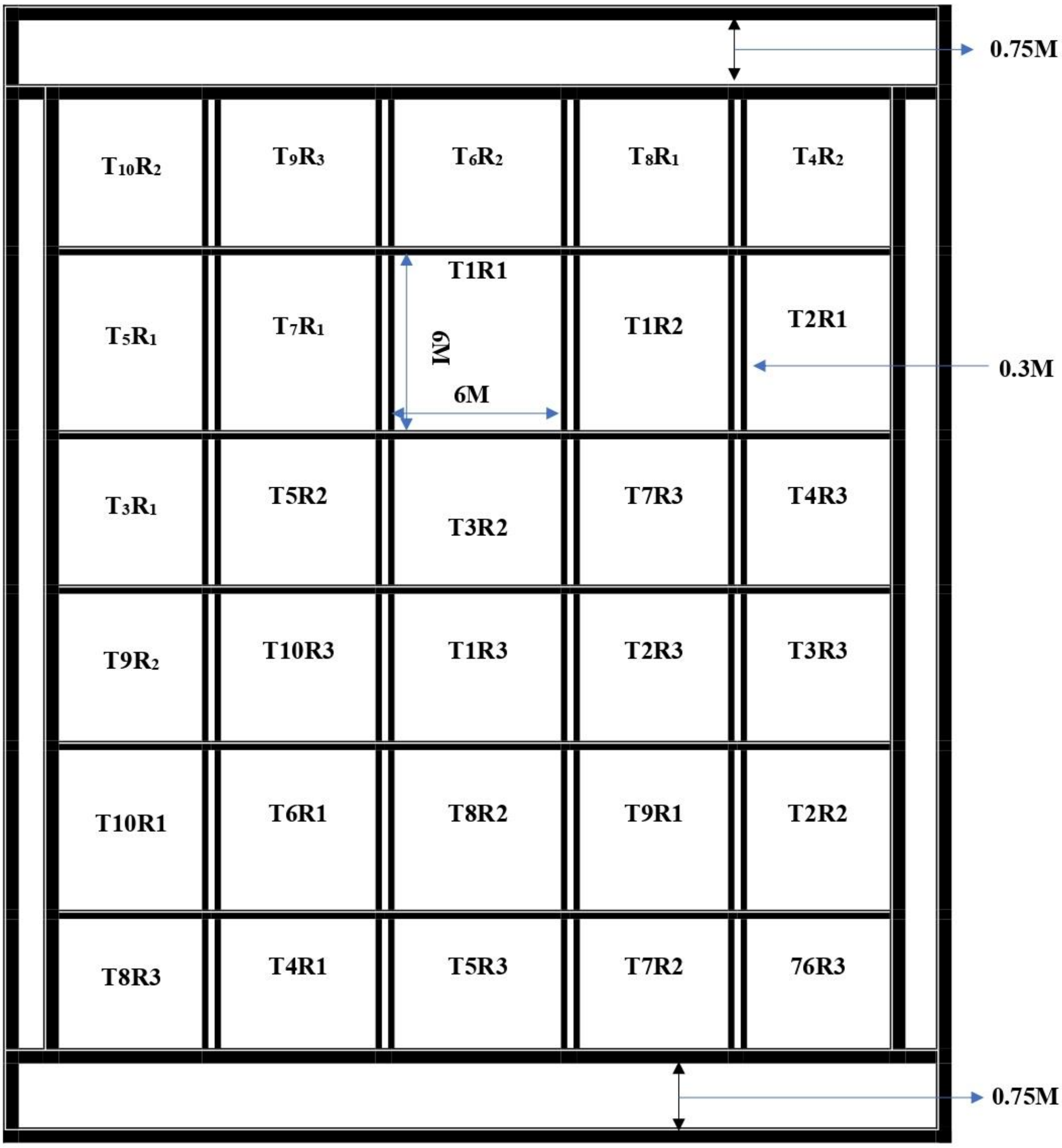
T1	**NPK+FYM (Recommended dose of NPK and FYM)**
T2	**NPK+FYM+OCIO (2 kg/ha)**
T3	**NPK+FYM+OCI-Cuox (2 kg/ha)**
T4	**NPK+FYM+OCI-Mnox (2 kg/ha)**
T5	**NPK+FYM+OCIO (5 kg/ha)**
T6	**NPK+FYM+OCI-Cuox (5 kg/ha)**
T7	**NPK+FYM+OCI-Mnox (5 kg/ha)**
T8	**NPK+FYM+$FeSO_4$**
T9	**NPK+FYM+$CuSO_4$:$FeSO_4$ (1:1)**
T10	**NPK+FYM+$MnSO_4$:$FeSO_4$ (1:1)**

*** Doses for N, P, K, $FeSO_4$, $CuSO_4$, and $MnSO_4$ will be based on local recommendations**

The recommended dose of NPK for rice (*Oryza sativa*) was: N- 140 kg ha^{-1}; P_2O_5- 40 kg ha^{-1}; K_2O- 40 kg ha^{-1}. Urea, single super phosphate (SSP), and Muriate of Potash (MOP) were used as N, P_2O_5, and K_2O sources, respectively. As per the prescribed package of practice for rice the NPK was uniformly applied in each plot.

2.2.2. Layout of the field experiment

The layout of the field experiment is given below (for both two locations field layout is the same):



2.3. Sample Collection

Composite and representative soil sampling was done thrice; first, before application of any treatment, second from each plot after fertilizer and nanomaterial application, and third after harvesting of the crop. The collected soil samples were air-dried and then ground, sieved through 5 mm wire mesh, and stored in clean, airtight plastic containers with the proper name and collection date accordingly for further analyses. Sample collection, preparation (air drying, grinding, and sieving), and preservation were done as per the recommendations of AOAC.

2.3.1. Soil sample analyses

Most of the sample analysis was done within 2-3 days after the collection of samples. The collected soil samples were analysed for water holding capacity (WHC), bulk density (BD), pH, Total Organic Carbon (TOC), Total Nitrogen (TKN), and Avl. phosphorous (Av. P), Avl. potassium (Av. K), DTPA extractable Fe, Cu, and Mn following standard methods (Lindsay and Norvell, 1978; Page et al., 1982). And for plant samples

2.3.2. Plant sample analyses

Plant leaves samples were collected at the panicle initiation stage for estimation of chlorophyll contents and during the harvesting time plant samples is collected and dried for estimation of total organic carbon, total Kjeldahl nitrogen, available potassium, available phosphorous, metal (iron, manganese and copper) uptake by following the standard protocol. After harvesting the crop, the yield per plot was measured.

2.4.Statistical Analysis

MANOVA was executed for the at 95% level of significance in SPSS (version 16.0). Moreover, the least significant difference (LSD) post hoc test was conducted to identify the significant differences among the treatment in between two locations.

3. Result and Discussion

3.1.Basic properties of experimental soil and climatic attributes of the two locations

The climatic and soil Physico-chemical characteristics of the two locations are presented in Table 3.1. The soil of Sonitpur was an inceptisol, while Birbhum possessed red lateritic soil (Jha and Kapat, 2011; Raychaudhuri et al., 2014). Both the soils were acidic in nature (Table 3.1). K and P availability in soil was higher in Sonitpur than in Birbhum, whereas TOC and TKN availability were higher in Birbhum than in Sonitpur.

The acidic nature of Birbhum soil is due to lateritic parent material. While the acidic nature of Sonitpur was could be attributed to the fact that, with an increase in rainfall, more soluble bases are lost from the soil due to run-off and leaching, resulting in a low pH. The higher K concentration in Sonitpur soil may be attributed to the K-rich parent material.

The climates of the two locations are generally similar as both areas fall in the humid subtropical climate region of the country (Das et al., 2018). Although the amount of precipitation and relative humidity were substantially more significant in Birbhum than in Sonitpur, the rainfall pattern of the two locations was similar in 2021. Therefore, the data suggests that rainfall pattern, humidity, and temperature regimes of both the locations were quite similar during the study period but the soil properties of the locations under study were noticeably different. These observations corroborate the finding of Das et al. (2018).

Table 3.1: Soil and climatic characteristics of Sonitpur and Birbhum

Parameter	Soil				Air temperature (^oC) 2021	Precipitation (mm) 2021	Relative humidity (%) 2021
	Sonitpur	Birbhum					
pH	5.06±0.06	4.45±0.11	Sonitpur	June	24.26-35.08	8.45	79.19
Electrical Conductivity (EC)	0.003±0.002	0.0024±0.002		July	25.23-35.68	7.63	80.81
Bulk Density (g cc^{-1})	1.15±0.001	1.31±0.0012		August	24.52-36.26	4.73	83
Water Holding Capacity (%)	55.42±0.01	36.82±0.57		September	23.91-34.67	5.72	81
Total N (%)	0.69±0.008	0.52±0.30		October	18.3-35.69	2.03	78.44
TOC (%)	0.72±0.06	0.11±0.27		November	13.44-29.07	0.03	74.5
Available P (mg kg^{-1})	55.78±0.53	36.36±0.84	Birbhum	June	24.4-39.4	13.29	80.94
Available K (mgkg^{-1})	47.33±3.2	94.67±2.52		July	25.15-33.35	10.08	87.12
				August	24.34-33.56	6.61	88.75
				September	23.74-32.65	9.82	89.5
				October	16.94-31.98	5.37	85.19
				November	11.69-27.5	1.01	82.75

3.2. Changes in the soil pH, electric conductivity, bulk density, and water holding capacity under various treatments

3.2.1. Changes in pH

Table 3.2. Changes in soil pH

Treatment	**pH**	
	Sonitpur (Mean ± SD)	**Birbhum** (Mean ± SD)
T1 (NPK+FYM)	5.18±0.35	4.46±0.06
T2 (NPK+FYM+OCIO (2 kg/ha))	5.31±0.03	4.72±0.09
T3 (NPK+FYM+OCI-Cuox (2 kg/ha))	5.29±0.05	4.65±0.05
T4 (NPK+FYM+OCI-Mnox (2 kg/ha))	5.40±0.18	4.52±0.03
T5 (NPK+FYM+OCIO (5 kg/ha))	5.53±0.23	4.58±0.01
T6 (NPK+FYM+OCI-Cuox (5 kg/ha))	5.45±0.13	4.68±0.03
T7 (NPK+FYM+OCI-Mnox (5 kg/ha))	5.52±0.03	4.51±0.05
T8 (NPK+FYM+FeSO4)	4.67±0.25	4.43±0.06
T9 (NPK+FYM+CuSO4:FeSO4 $_{(1:1)}$)	4.45±0.22	4.42±0.09
T10 (NPK+FYM+MnSO4:FeSO4 $_{(1:1)}$)	4.42±0.26	4.43±0.03
$P_{treatment}$= < 0.001 $P_{location}$ =<0.001 $P_{treatment \times location}$ =<0.001 $LSD_{treatment}$ =0.085		

The field experiment was performed in a typical alluvial soil in Assam and red laterite soil in West Bengal. Both soils are inherently acidic in nature (Table 4.1). In the alluvial soil of Assam, we found that the soil's acidity sharply increased by ~7-12% due to the use of $FeSO_4$, $CuSO_4$, and $MnSO_4$, while such increase in acidity was nominal in red and lateritic soil of West Bengal. In contrast, approximately a 9% increase in soil pH was recorded due to the application of OCIO @ 5 kg ha$^{-1,}$ followed by the others in Assam. soil and in case of West Bengal soil approximately 6% pH increases due to application of OCIO @ 2 kg ha^{-1} followed by OCI-Cuox @ 5 kg ha^{-1}, OCI-Cuox @ 2 kg ha^{-1}, OCIO @ 5 kg ha^{-1}, OCI-Mnox @ 2 kg ha^{-1}, and OCI-Mnox @ 5 kg ha^{-1} doses ($P_{treatment}$=>0.001; LSD=0.085) (Table 3.2). Such buffering capacity of OCIO, OCIMnox, and OCICuox was evidenced in our lab-scale and miniature plants experiments. This may be due to the hydrogen scavenging (H+) property of the nanomaterial, which probably facilitated the pH increment in soil (Das et al., 2018).

3.2.2. Changes in soil bulk density

Table 3.3. Changes in soil bulk density

Treatment	Bulk Density (g cc^{-1})	
	Sonitpur (Mean ± SD)	**Birbhum** (Mean ± SD)
T1 (NPK+FYM)	0.957±0.036	1.25±0.006
T2 (NPK+FYM+OCIO (2 kg/ha))	0.947±0.024	1.22±0.002
T3 (NPK+FYM+OCI-Cuox (2 kg/ha))	0.947±0.012	1.23±0.022
T4 (NPK+FYM+OCI-Mnox (2 kg/ha))	0.941±0.035	1.22±0.010
T5 (NPK+FYM+OCIO (5 kg/ha))	0.954±0.026	1.23±0.010
T6 (NPK+FYM+OCI-Cuox (5 kg/ha))	0.957±0.023	1.23±0.013
T7 (NPK+FYM+OCI-Mnox (5 kg/ha))	0.957±0.015	1.23±0.012
T8 (NPK+FYM+FeSO4)	0.967±0.041	1.26±0.002
T9 (NPK+FYM+CuSO4:FeSO4 $_{(1:1)}$)	0.962±0.030	1.27±0.007
T10 (NPK+FYM+MnSO4:FeSO4 $_{(1:1)}$)	0.963±0.012	1.25±0.005
$P_{treatment}$=<0.001 $P_{location}$ =< 0.001 $P_{treatment \times location}$=<0.001 $LSD_{treatment}$ = 0.36		

The field experiment was performed in a typical alluvial and red laterite soil with medium to high bulk density (BD) (Table 3.1). While conducting field experiments with OCIO, OCIMnox and OCICuox, $FeSO_4$, $MnSO_4$, and $CuSO_4$, we found soil bulk density significantly reduced under OCIO, OCIMnox, and OCICuox treated plots irrespective of the treatment combinations. While comparing among the various doses for OCIO, OCIMnox, and OCICuox, we found that the 2 kg ha^{-1} dose was the most effective among all with respect to the lowering of soil bulk density, followed by 5 kg ha^{-1} doses in both locations (Table 3.3). These results are in good agreement with a previous finding (Das et al., 2018). Contrarily, such gainful impact on soil bulk density was not evidenced in non-nano scale conventional fertilizer (i.e., $FeSO_4$, CuSO4, and MnSO4) treated plots. As such, a reduction in bulk density indicates an improvement soil porosity and structural stability (Brady and Weil, 2004). The result thus indicates that the low dose application of OCIO, OCIMnox, and OCICuox could induce the beneficial effect of the nanoforms by rendering structural stability and improving porosity to arable soil.

3.2.3. Changes in electrical conductivity

Table 3.4. Changes in electric conductivity

Treatment	Electric conductivity (dS)	
	Sonitpur (Mean ± SD)	Birbhum (Mean ± SD)
T1 (NPK+FYM)	0.005±0.0007	0.0027±0.00001
T2 (NPK+FYM+OCIO (2 kg/ha))	0.004±0.001	0.0031±0.00004
T3 (NPK+FYM+OCI-Cuox (2 kg/ha))	0.004±0.0010	0.0029±0.0002
T4 (NPK+FYM+OCI-Mnox (2 kg/ha))	0.004±0.0007	0.0028±0.000005
T5 (NPK+FYM+OCIO (5 kg/ha))	0.005±0.0005	0.0028±0.00049
T6 (NPK+FYM+OCI-Cuox (5 kg/ha))	0.004±0.0010	0.0027±0.00049
T7 (NPK+FYM+OCI-Mnox (5 kg/ha))	0.004±0.0017	0.0028±0.00003
T8 (NPK+FYM+FeSO4)	0.004±0.0002	0.0025±0.0004
T9 (NPK+FYM+CuSO4:FeSO4 $_{(1:1)}$)	0.004±0.0008	0.0025±0.0003
T10 (NPK+FYM+MnSO4:FeSO4 $_{(1:1)}$)	0.006±0.0030	0.0024±0.0001
$P_{treatment}$ = <0.001 $P_{location}$ = < 0.001 $P_{treatment \times location}$ =<0.001 $LSD_{treatment}$ = 0.18		

The field experiment was performed in typical alluvial and red laterite soil with low electric conductivity (Table 3.1). While conducting our field experiment with OCIO, OCIMnox and OCICuox, $FeSO_4$, $MnSO_4$, and $CuSO_4$, we found the remarkable increase of electrical conductivity due to the use of OCIO, OCI-Cuox, and OCI-Mnox. While comparing among the OCIO, OCIMnox and OCICuox we found that the @ 2 kg ha^{-1} doses is most effective in case of electrical conductivity increment followed by other in Assam and West Bengal soil (Table 3.4). But, such a type of effect is not seen in non-nano scale convention fertilizer i.e., $FeSO_4$, $MnSO_4$, and $CuSO_4$. This may be due to the hydrogen scavenging (H+) property of the three nanomaterial.

3.2.4. Changes in water holding capacity

A significant augmentation of water holding capacity (WHC) in soil could be achieved under all the OCIO, OCIMnox, and OCICuox treated plots, while a similar impact was absent with their non- nanoscale counterparts (i.e., $FeSO_4$, $MnSO_4$, and $CuSO_4$). For instance, WHC increased by 3% due to the use of OCIO @ 2 kg ha^{-1} followed by others in Assam soil, and in West Bengal soil ~ 9% increases due to application of OCIO @ 2 kg ha^{-1} followed by others (Table 3.5). This may be due to variation of physico-chemical properties of the soil and climatic variation of two locations.

Table 3.5. Changes in the water holding capacity in soil

Treatment	Water Holding Capacity (%)	
	Sonitpur (Mean ± SD)	**Birbhum** (Mean ± SD)
T1 (NPK+FYM)	52.85±2.17	36.81±0.58
T2 (NPK+FYM+OCIO (2 kg/ha))	57.23±3.33	38.62±0.98
T3 (NPK+FYM+OCI-Cuox (2 kg/ha))	55.51±2.24	37.22±0.39
T4 (NPK+FYM+OCI-Mnox (2 kg/ha))	56.66±1.93	37.49±0.40
T5 (NPK+FYM+OCIO (5 kg/ha))	56.28±1.99	37.29±0.40
T6 (NPK+FYM+OCI-Cuox (5 kg/ha))	55.98±2.48	37.14±1.00
T7 (NPK+FYM+OCI-Mnox (5 kg/ha))	55.59±0.74	37.02±0.45
T8 (NPK+FYM+FeSO4)	53.71±2.68	35.24±1.03
T9 (NPK+FYM+CuSO4:FeSO4 $_{(1:1)}$)	54.77±2.10	35.03±0.21
T10 (NPK+FYM+MnSO4:FeSO4 $_{(1:1)}$)	54.14±0.71	36.88±0.76
$P_{treatment}$ = <0.010 $P_{location}$ = < 0.001 $P_{treatment \times location}$ = < 0.001 $LSD_{treatment}$ = 0.93		

3.3.Changes in Total Organic Carbon, Total N, Available P & K under various treatments

The field experiment was carried out in soil with medium Total organic carbon (TOC) and total nitrogen availability, but low P and K availability (Table 3.1).

3.3.1. Changes in Total Organic Carbon in soil

A significant increase of TOC was recorded in OCIO, OCIMnox and OCICuox treated plots irrespective of their doses of application (Table 3.6). Treatment wise recorded order is OCIO @ 2 kg ha^{-1} > OCIMnox @ 2 kg ha^{-1} > OCICuox @ 2 kg ha^{-1} > OCIO @ 5 kg ha^{-1} > OCIMnox @ 5 kg ha^{-1} and OCICuox 5 kg ha^{-1}. While comparing among the various doses of OCIO, OCIMnox and OCICuox used in this study we found that the 2 kg ha^{-1} dose was the most prolific among all with respect to TOC improvement in soil. Such increment of total organic carbon was not found in non-nano scale conventional fertilizer (i.e., FeSO4, MnSO4, and CuSO4) in treated plots. This indicated that the OCIO, OCIMnox and OCICuox application probably inhibited TOC loss by virtue of their unique oxalate–Fe-oxide combination (Das et al., 2018). The previous report suggests that Fe-oxide nanoparticles readily release Fe2+ or Fe3+ in soil owing to their reactive nature (Zhou et al., 2012). Eventually, accelerated Fe release probably enhanced TOC levels in soil via complexation with the natural organic matter of the soil (Jansen et al., 2005).

Table 3.6. Changes of Total Organic Carbon in soil

Treatment	TOC (%)	
	Sonitpur (Mean ± SD)	**Birbhum** (Mean ± SD)
T1 (NPK+FYM)	1.02±0.07	0.21±0.09
T2 (NPK+FYM+OCIO (2 kg/ha))	1.11±0.55	0.36±0.14
T3 (NPK+FYM+OCI-Cuox (2 kg/ha))	1.15±0.56	0.35±0.21
T4 (NPK+FYM+OCI-Mnox (2 kg/ha))	1.18±0.12	0.37±0.12
T5 (NPK+FYM+OCIO (5 kg/ha))	1.16±0.17	0.31±0.09
T6 (NPK+FYM+OCI-Cuox (5 kg/ha))	1.16±0.022	0.37±0.03
T7 (NPK+FYM+OCI-Mnox (5 kg/ha))	1.10±0.20	0.31±0.07
T8 (NPK+FYM+FeSO4)	1.07±0.12	0.29±0.09
T9 (NPK+FYM+CuSO4:FeSO4 $_{(1:1)}$)	1.09±0.38	0.22±0.17
T10 (NPK+FYM+MnSO4:FeSO4 $_{(1:1)}$)	1.10±0.47	0.26±0.12
$P_{treatment} = <0.05$ $P_{location} = < 0.001$ $P_{treatment \times location} = <0.05$ $LSD_{treatment} = 0.56$		

3.3.2. Changes in Total Kjeldahl Nitrogen

The N significantly increased under OCIO, OCIMnox and OCICuox treated plots in both locations (Table 3.1). Treatment wise recorded order is OCIO @ 2 kg ha^{-1} > OCIMnox @ 2 kg ha^{-1} > OCICuox @ 2 kg ha^{-1} > OCIO @ 5 kg ha^{-1} > OCIMnox @ 5 kg ha^{-1} and OCICuox 5 kg ha^{-1} ($P_{treatment}$ = > 0.001 and LSD =0.98) (Table 4.7). While comparing among the various doses of OCIO, OCIMnox and OCICuox used in this study we found that the 2 kg ha^{-1} dose was the most prolific among all with respect to TKN improvement in soil (Table 3.7). Contrarily, a similar benefit in regard to N availability was not achieved by $FeSO_4$, $MnSO_4$, and $CuSO_4$ application (Table 3.1). This is because the abundance of Fe^{2+} greatly accelerates denitrification by augmenting NO_3- reduction, which is highly possible due to FeSO4 application in soil (Klueglein et al., 2014). In contrast, we presume that the oxalate covering in OCIO, OCIMnox and OCICuox could greatly inhibit NO_3- reduction through controlled release of Fe. For that reason, total nitrogen is recorded higher in nano-scale fertilizer than non-nano scale conventional fertilizer.

Table 3.7. Changes in Total Kjeldahl Nitrogen in the soil

Treatment	**TKN (%)**	
	Sonitpur (Mean ± SD)	**Birbhum** (Mean ± SD)
T1 (NPK+FYM)	0.703±0.040	0.52±0.23
T2 (NPK+FYM+OCIO (2 kg/ha))	0.787±0.069	0.99±0.19
T3 (NPK+FYM+OCI-Cuox (2 kg/ha))	0.780±0.028	0.81±0.30
T4 (NPK+FYM+OCI-Mnox (2 kg/ha))	0.789±0.069	0.97±0.09
T5 (NPK+FYM+OCIO (5 kg/ha))	0.761±0.008	0.65±0.26
T6 (NPK+FYM+OCI-Cuox (5 kg/ha))	0.757±0.045	0.30±0.17
T7 (NPK+FYM+OCI-Mnox (5 kg/ha))	0.757±0.021	0.65±0.16
T8 (NPK+FYM+FeSO4)	0.447±0.069	0.18±0.35
T9 (NPK+FYM+CuSO4:FeSO4 $_{(1:1)}$)	0.438±0.024	0.29±0.33
T10 (NPK+FYM+MnSO4:FeSO4 $_{(1:1)}$)	0.552±0.037	0.15±0.11
$P_{treatment}$ = <0.001 $P_{location}$ = <0.001 $P_{treatment \times location}$ = <0.001 $LSD_{treatment}$ = 0.98		

3.3.3. Changes in available potassium (K)

The experiment was performed at acidic soil and at that pH low available potassium was recorded (Table 3.1). Because of increased H+ concentration and soluble aluminium, K has higher competition for CEC sites at low soil pH. Other exchangeable cations (K+) are displaced by H+ and Al, which moves them into soil solution and increases the potential for leaching.

After the adding of nano fertilizer, a significant increase of available potassium and pH is recoded under all the OCIO, OCIMnox and OCICuox treated plots in both locations. When the pH of the soil rises, the CEC rises with it, allowing for higher potassium concentrations on the CEC. Therefore, after adding the nano fertilizer a slight increment of available potassium was recorded (Table 3.8). However, such improvement in K availability was not observed with FeSO4, MnSO4, and CuSO4 because of low pH.

Table 3.8. Changes in soil available potassium (K)

Treatment	Av. K. (mg/kg)	
	Sonitpur (Mean ± SD)	**Birbhum** (Mean ± SD)
T1 (NPK+FYM)	49.43±15.79	147.27±2.67
T2 (NPK+FYM+OCIO (2 kg/ha))	63.43±29.34	171.97±1.85
T3 (NPK+FYM+OCI-Cuox (2 kg/ha))	61.67±16.44	158.17±3.95
T4 (NPK+FYM+OCI-Mnox (2 kg/ha))	63.40±5.55	164.40±6.26
T5 (NPK+FYM+OCIO (5 kg/ha))	59.37±20.90	156.50±4.21
T6 (NPK+FYM+OCI-Cuox (5 kg/ha))	54.17±20.60	149.63±2.15
T7 (NPK+FYM+OCI-Mnox (5 kg/ha))	57.43±21.61	150.17±2.84
T8 (NPK+FYM+FeSO4)	51.90±8.88	144.00±5.94
T9 (NPK+FYM+CuSO4:FeSO4 $_{(1:1)}$)	39.73±6.44	144.97±5.8
T10 (NPK+FYM+MnSO4:FeSO4 $_{(1:1)}$)	49.23±11.91	144.93±3.33
$P_{treatment}$ = <0.001 $P_{location}$= NS $P_{treatment \times location}$= <0.001 $LSD_{treatment}$=4.57		

3.3.4. Changes in soil Available Phosphorous (Av. P.)

The low acidic nature of the soil under study often leads to P deficiency. Usually, P availability in soil is a pH-dependent phenomenon and the nutrient is readily soluble at the pH range of 5.5 to 7.0 (Tisdale and Nelson, 1966). After the adding of nano fertilizer, it's recorded that the pH increases 7-12% from the initial value. Interestingly, in our experiment P availability remarkably increased over time due to low dose application of OCIO, OCIMnox and OCICuox (Table 3.9). In OCIO treated plotes available phosphorous increases from 1.3 to1.6 folds and in OCIMnox treated plots increases from ~1.1 to1.3 fold and in OCICuox treated plots 1.4 folds increases as compare to the initial value in both locations. On the other hand, the use of non-nano-scale fertilizers (i.e., FeSO4, MnSO4, and CuSO4) sharply reduced soil pH and thereby greatly affected P availability.

Table 3.9. Changes in soil Available Phosphorous (Av. P.)

Treatment	Av. P. (mg/kg)	
	Sonitpur (Mean ± SD)	**Birbhum** (Mean ± SD)
T1 (NPK+FYM)	50.45±14.20	43.18±3.64
T2 (NPK+FYM+OCIO (2 kg/ha))	74.09±17.61	55.00±13.00
T3 (NPK+FYM+OCI-Cuox (2 kg/ha))	63.18±3.18	34.09±3.43
T4 (NPK+FYM+OCI-Mnox (2 kg/ha))	63.94±16.50	49.70±5.23
T5 (NPK+FYM+OCIO (5 kg/ha))	60.45±8.52	42.88±8.98
T6 (NPK+FYM+OCI-Cuox (5 kg/ha))	63.64±0.79	38.03±6.82
T7 (NPK+FYM+OCI-Mnox (5 kg/ha))	56.06±2.50	40.61±5.03
T8 (NPK+FYM+FeSO4)	59.24±20.46	44.85±4.55
T9 (NPK+FYM+CuSO4:FeSO4 $_{(1:1)}$)	45.76±4.73	33.64±9.58
T10 (NPK+FYM+MnSO4:FeSO4 $_{(1:1)}$)	56.67±12.63	31.21±4.32
$P_{treatment}$ = <0.001 $P_{location}$= <0.001 $P_{treatment \times location}$= <0.034 $LSD_{treatment}$ = 1.7		

3.4.Effect of OCIO, OCI-Mnox, and OCI-Cuox on plant growth and yield

The data on plant height, panicle number, and yield of rice plants are show in table 3.10. When we compared conventional non-nano fertilizer and synthesized nano fertilizer it was observed that the synthesized nano material treated plants show higher plant height, panicle number, and yield than the non-nano fertilizer in both locations. Moreover, the grain yield was significantly greater in Sonitpur than in Birbhum soil. The grain yield under OCIO @ 2 kg ha^{-1} followed by OCIO @ 5 kg ha^{-1} treatments was significantly greater than the other treatments in Sonitpur. In contrast, the OCIMnox @ 5 kg ha^{-1} followed by OCIO @ 2 kg ha^{-1} resulted in greater yield as compared to the others in Birbhum soil. However, the productivity of the nano-treated plots was significantly higher than the average yield data of Birbhum of monsoon rice (Chakraborty, 2015). While comparing among the various doses of OCIO, OCIMnox and OCICuox used in this study we found that the 2 kg ha^{-1} dose was the most prolific among all with respect to plant height improvement. This type improvement is not recorded in non- nanoscale conventional fertilizer (i.e., FeSO4, MnSO4, and CuSO4) treated plots. This might be due to the fact that nano fertilizers have a high potential for supplying nutrients to plants (Liu and Lal, 2015), which enhanced production capacity by stimulating the plant's metabolic system. Another possible explanation for improved plant growth and production using Nano fertilizer is that the gradual release of Fe promoted chlorophyll biosynthesis and the redox mechanism in plants (Briat et al., 2007).

Table 3.10. Effect of OCIO, OCI-Cuox, and OCI-Mnox on plant height, panicle number and yield

Treatment	Sonitpur (Mean ± SD)			Birbhum (Mean ± SD)		
	Plant height	**Panicles no**	**Yield (kg/ha)**	**Plant height**	**Panicles no**	**Yield (kg/ha)**
T1(NPK+FYM)	87.00±8.19	431.0±40.95	5666.67	91.97±2.0	322.67±18.0	3550
T2 (NPK+FYM+OCIO (2kg/ha))	91.33±5.51	558.3±59.65	6166.67	105.00±3	366.67±24.4	4775
T3 (NPK+FYM+OCI-Cuox (2 kg/ha))	91.00±9.54	451.3±48.01	5833.33	103.67±2.1	344.00±58.1	4275
T4 (NPK+FYM+OCI-Mnox (2 kg/ha)0	91.00±5.29	529.7±39.37	5666.67	104.33±4.0	378.67±34.0	4175
T5 (NPK+FYM+OCIO (5 kg/ha))	88.00±10.54	469±89.37	6000.00	98.00±2	357.33±44.6	4625
T6 (NPK+FYM+OCI-Cuox (5 kg/ha))	86.33±6.81	437.3±28.54	5833.33	101.83±3.5	338.67±26.0	4800
T7 (NPK+FYM+OCI-Mnox (5 kg/ha))	89.00±1015	436.7±47.43	5500.00	103.73±3.4	366.67±36.6	5150
T8 (NPK+FYM+FeSO4)	84.67±15.18	433±22.52	5166.67	97.63±1.1	332.00±22.3	4735
T9 (NPK+FYM+CuSO4:FeSO4 $_{(11)}$	78.33±6.43	447.7±24.09	4833.33	99.67±4.5	352.00±49.2	3003
T10 (NPK+FYM+OCI-	80.00±8.72	369±42.32	5166.67	96.43±4.8	344.00±28.8	2675
$P_{treatment =}$ <0.05 $P_{location}$= <0.05 $P_{treatment \times location}$= <0.034 $LSD_{treatment}$ = 5.7						

3.5.Effect of OCIO, OCI-Mnox, and OCI-Cuox on NPK uptake on rice grain

The field experiment was performed in a typical alluvial soil in Assam and red laterite soil in West Bengal with low nitrogen content, low available phosphorous, and low available potassium.

3.5.1. Effect of OCIO, OCI-Mnox, and OCI-Cuox on rice grain nitrogen uptake

Table 3.11 represents the nitrogen uptake data in rice grain. From the table it is observed that nanomaterial treated plots has high nitrogen content compare to other treated plots. When we compare among non-nanoscale conventional fertilizer and synthesised nanomaterial it is recorded that the synthesized nanomaterial treated plant shows the higher nitrogen content in their grain in both locations. As found earlier, that the grain yield was significantly greater in Sonitpur in Assam as compared to Birbhum in West Bengal. Interestingly, such prolific impact of nano-aided fertilization scheme was probably due to greater uptake of N in Sonitpur soil as compared to the Birbhum soil.

Table 3.11. Effect of OCIO, OCI-Mnox, and OCI-Cuox on N uptake

Treatment	**TKN (%)**	
	Sonitpur (Mean ± SD)	**Birbhum** (Mean ± SD)
T1 (NPK+FYM)	1.84±0.09	0.537±0.252
T2 (NPK+FYM+OCIO (2 kg/ha))	1.93±0.07	0.877±0.175
T3 (NPK+FYM+OCI-Cuox (2 kg/ha))	1.89±0.16	0.705±0.194
T4 (NPK+FYM+OCI-Mnox (2 kg/ha))	1.91±0.10	0.770±0.216
T5 (NPK+FYM+OCIO (5 kg/ha))	1.89±0.13	0.756±0.206
T6 (NPK+FYM+OCI-Cuox (5 kg/ha))	1.80±0.19	0.630±0.087
T7 (NPK+FYM+OCI-Mnox (5 kg/ha))	1.84±0.08	0.644±0.326
T8 (NPK+FYM+FeSO4)	1.78±0.11	0.518±0.120
T9 (NPK+FYM+CuSO4:FeSO4 $_{(1:1)}$)	1.76±0.13	0.490±0.61
T10 (NPK+FYM+MnSO4:FeSO4 $_{(1:1)}$)	1.66±0.16	0.495±0.119

$P_{treatment} = <0.05$
$P_{location} = < 0.001$
$P_{treatment \times location} =$ NS
LSD = 0.08

3.5.2. Effect of OCIO, OCI-Mnox, and OCI-Cuox on K uptake in rice

Table 3.12 demonstrate the potassium concentration in rice grain. From the table it is easily recognised that the potassium concentration is higher in nano material treated plots than the non-nanoscale treated plots and in Birbhum soil such benefit was highly pronounced probably due to inherently high K content in the soil. Overall, K uptake was significantly higher in OCIO @ 2 kg ha^{-1} treated plots as compared to the other treatments in both locations. In addition, the results suggest that the OCI-Mnox @ 2 kg ha^{-1} was also efficient in facilitating the K uptake in rice. This is because when the pH of the soil rises, the CEC rises with it, allowing for higher potassium concentrations on the CEC. Therefore, after adding the nano fertilizer in soil increment of available potassium was recorded. That is the reason in rice grain maximum K concentration is found under nanomaterial treated plots.

Table 3.12. Effect of OCIO, OCI-Mnox, and OCI-Cuox on rice grain potassium uptake

Treatment	Av. K. (mg/kg)	
	Sonitpur (Mean ± SD)	Birbhum (Mean ± SD)
T1 (NPK+FYM)	286.47±44.09	698.1±152.33
T2 (NPK+FYM+OCIO (2 kg/ha))	293.57±40.05	975.9±15.25
T3 (NPK+FYM+OCI-Cuox (2 kg/ha))	287.27±66.66	742.2±211.48
T4 (NPK+FYM+OCI-Mnox (2 kg/ha))	292.67±35.31	759.2±233.34
T5 (NPK+FYM+OCIO (5 kg/ha))	269.17±63.51	735.2±217.01
T6 (NPK+FYM+OCI-Cuox (5 kg/ha))	246.07±29.92	709.1±23.55
T7 (NPK+FYM+OCI-Mnox (5 kg/ha))	259.37±29.65	726.5±24.81
T8 (NPK+FYM+FeSO4)	259.13±66.27	709.2±97.75
T9 (NPK+FYM+CuSO4:FeSO4 $_{(1:1)}$)	244.47±53.54	675.6±176.43
T10 (NPK+FYM+MnSO4:FeSO4 $_{(1:1)}$)	254.67±27.76	736.0±22.66
$P_{treatment} = <0.05$		

P $_{location}$= < 0.001

P $_{treatment \times location}$= NS

3.5.3. Effect of OCIO, OCI-Mnox, and OCI-Cuox on P uptake in rice

Table 3.13 shows the concentration of available phosphorous content in rice grain. From the table it is recorded that the available phosphorous content is maximum under @ 2 kg ha^{-1} nanomaterial treated plants followed by @ 5 kg ha^{-1} treated plants in both the locations. But such same gaining effect is not seen in non-nanoscale treated plants, because in non-nanoscale conventional fertilizer sharply reduce the pH concentration. But the synthesised nanomaterial increases the pH concentration in soil. Therefore, in synthesised nanomaterial treated plots rice plant has maximum P content was recorded.

Table 3.13. Effect of OCIO, OCI-Mnox, and OCI-Cuox on rice grain phosphorous (Av. P.) uptake

Treatment	**Av. P. (mg/kg)**	
	Sonitpur (Mean ± SD)	**Birbhum** (Mean ± SD)
T1 (NPK+FYM)	22.54±5.86	13.92±1.10
T2 (NPK+FYM+OCIO (2 kg/ha))	28.23±8.22	55.61±2.29
T3 (NPK+FYM+OCI-Cuox (2 kg/ha))	23.23±4.19	51.18±0.88
T4 (NPK+FYM+OCI-Mnox (2 kg/ha))	26.67±7.17	55.40±1.47
T5 (NPK+FYM+OCIO (5 kg/ha))	23.16±12.87	50.57±0.30
T6 (NPK+FYM+OCI-Cuox (5 kg/ha))	21.13±6.66	33.84±0.12
T7 (NPK+FYM+OCI-Mnox (5 kg/ha))	22.14±0.79	38.93±0.46
T8 (NPK+FYM+FeSO4)	19.58±2.55	31.42±0.32
T9 (NPK+FYM+CuSO4:FeSO4 $_{(1:1)}$)	15.95±8.92	26.56±0.20
T10 (NPK+FYM+MnSO4:FeSO4 $_{(1:1)}$)	18.98±0.88	12.77±0.47

P $_{treatment}$ = < 0.001

P $_{location}$= < 0.001

P $_{treatment \times location}$=< 0.001

LSD $_{treatment}$ = 2.82

3.6.Conclusion

From the above result and discussion, it is recorded that the application of the three nanomaterials (i.e., OCIO, OCIMnox, and OCICuox) significantly influenced soil physical and chemical compositions, and there were also differential impacts of the applied nanomaterials depending on the soil types. We also found that the soil organic carbon and N availability in soil substantially increased by 2 kg ha^{-1} application dose of OCIO as compared to the non-nano scale controls in alluvial soil, and the 2 kg ha^{-1} dose of OCIMnox and OCICuox doses were prolific in augmenting organic C and N status of the red and lateritic soil compared to non-nano scale conventional fertilizers. The soil's inherent acidic nature often leads to P deficiency; fascinatingly, OCIO, OCICuox, and OCIMnox incorporation corrected soil acidity, thereby promoting P availability. Eventually, the beneficial influence of nano-form micronutrients (Fe, Mn, and Cu) resulted in promoting rice productivity in both the locations; however, the yield advantage was highly beneficial in Birbhum as compared to the Assam soil as the average productivity of monsoon rice is 1.5-2 times lower in Birhbum than the result of the present experiment. Hence, we conclude that the three nanomaterials (i.e., OCIO, OCIMnox, and OCICuox) could be effectively used as an alternative source of micronutrients and as soil conditioners in acidic soils.

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Memo No: TU/Env/Pro/3865 dated 12/07/2023.

GYTI PROJECT

STATEMENT OF EXPENDITURE (2 copies)

(For the Financial year of 1st December 2021 to 31st July 2022)

1. Name of Host Institute/ University/ College: **Tezpur University**
2. Name of Candidate with Project Registration Number: **Ratul Pegu. DoRd/Chem Sc./KS/2-409**
3. Previous Sanction order No. and Date: **BIRAC SRISTI PMU 2018 dated 19th November 2018**
4. Previous SANCTION Amount (in Rupees): **Rupees Three Lakhs Seventy-Five Thousand**
5. Date of Commencement of the GYTI Project: **14/03/ 2019**
6. Previous Grants Received in each Installment as per sanction orders issued (in Rupees)

1st Installment	2nd Installment	3rd Installment	4th Installment	Total Amount
3,75,000/- (27679/- +299215/- +48106/-)	3,75,000/-	3,75,000/-	3,75,000	15,00,000/-

7. **Statement of the Expenditure (in Rupees)**

Sl. No.	Sanction Budget Heads	Expenditure incurred (Financial year-wise) (in Rs.)							Balance as on 31/07/2022	Remarks if any
		1st Installment			2nd Installment	3rd Installment	4th Installment	Total	Rs. 2185	
		1st UC	2nd UC	3rd UC						
1	Scholarship	0		0	2,75,000	2,00000	1,25,000	6,00,000		
2	Consumables and Materials	0	2,95,563	11,483	31,935	78,618	2,63,430	6,81,029		
3	Internal Travel	27,679	0	26,846	0	0	0	54,525		
4	Field Work	0	0	0	62,160	25,640	70809	1,58,609		
5	Outsourcing	0	3,652	0	0	0	0	3,652		
6	Interest Accumulated	0	0	0	0	0	0	0		
7	Total	27,679	2,99,215	38,329	3,69,095	3,04,258	4,59,239	14,97,815		

Ratul Pegu Name & Signature Of PI Date: 20/03/2023	20/3/23 Name & Signature of Supervisor Date: Seal: Assistant Professor Dept.of Environmental Science Tezpur University,	Name & Signature of the head of the Department Date: 20/03/2023 Seal: Head Deptt. of Environmental Science Tezpur University Napaam : Tezpur	Name & Signature of Competent Financial Authority 31/3/2023 Date: Seal: Finance Officer Tezpur University

Utilization Certificate

(For the Financial year of 1st December 2021 to 31st July 2022)

1. Name of Host Institute/ University/ College: **Tezpur University**
2. Name of Candidate with Project Registration Number: **Ratul Pegu. DoRd/Chem Sc./KS/2-409**
3. Name of the Department of the Institute/University/College: **Environmental Science, Tezpur University**
4. Name of the Ph.D. Supervisor: **Dr. Satya Sundar Bhattacharya**
5. Sanction Order No. & Date: **BIRAC SRISTI PMU 2018 dated 19th November 2018**
6. Head of Account as given in original Sanction Order**: GYTI Award 2018**
7. Amount brought forward from the previous financial year: **Rs. 86424/-**
8. Amount received during the financial year: **3,75,000/-**
9. Total amount available for expenditure (excluding commitments): **3,75,000/-**
10. Actual expenditure (excluding commitments) incurred during 1/12/2021 to 31/07/2022: Rs. **4,59,239/-**
11. Balance amount available at the end of the financial year: **Rs. 2185/-**
12. Amount to be carried forward to the financial year (if applicable): **Rs. 2185/-**

Certificate

Certified that out of Rs. 3,75,000/- of grant-in-aid sanction during the year 1/12/2021 to 31/07/2022 in favour of BIRAC SRISTI funded GYTI Award 2018, order No. BIRAC SRISTI PMU 2018 dated 19th November 2018; a sum of Rs.4,59,239 has been utilized for the purpose of Nano Based Soil Conditioner for Agricultural Application for which it was sanctioned and the balance amount of Rs.2185/- remaining unutilized will be adjusted towards the grant-in-aid payable during the next year.

Ratul Pegu

Signature of PI

Date: 20/03/23

Bhattacharya 20/3/23		31/3/23	31/3/23
Signature of the Supervisor	Signature of the Head of Department	Signature of Accounts officer	Signature of Head of Institute/University
Date & Seal: Assistant Professor, Dept. of Environmental Science, Tezpur University	Date & Seal: Head, Deptt. of Environmental Science, Tezpur University, Napaam : Tezpur	Date & Seal: Finance Officer, Tezpur University	Date & Seal: Registrar, Tezpur University

(To be filled in at DST)

Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned has been fulfilled/ are being fulfilled and I have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned: -

Kind of Checks exercised:

1.

2.

3.

Signature/ Designation/ Date

A Voice of Creative Grassroots Innovators and Traditional Knowledge Holders

June 03, 2022

Extension of project period sanctioned on 19th November 2018; No. BIRAC SRISTI PMU-2018/013

To,

AWARDEE NAME: Ms. Pallabi Das / Ms. Kasturi Sarmah / Ratul Pegu

SUPERVISOR: Dr. Satya Sundar Bhattacharya

INSTITUTE: Tezpur University

Dear Awardee,

This is regarding extension of your project "Nano based soil conditioner for agricultural application". As per your request regarding the extension of project duration to complete the work and fund utilisation in accordance with the award letter agreement (ALA), we hereby give extension till June, 2022.

You have already been released all the phases and last 4th tranche was released on 31.03.2022.

Best of luck and Hope to get good outcomes for **Nano based soil conditioner for agricultural application.**

Thanking you.

Niwas Kumar

BIRAC SRISTI PMU

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