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Effect of soil and foliar applied ZnO nano particles on soil fertility status of rice soil

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Abstract
In order to investigate the effect of nano source of Zn along with $ZnSO_4$ foliar and soil application on physiological performance of rice (*Oryza sativa L.*), a pot experiment was conducted in the calcareous soil of Bihar with completely randomized design with three replications during seasons of 2013-2014. In this study, different concentrations of nano ZnO and $ZnSO_4$ were taken @ 0.1% ZnO nano, 0.02% Nano and 0.5% $ZnSO_4$ as foliar spray; 1 kg Zn (ZnO NPs), 0.2 kg Zn (ZnO NPs) and 5 kg Zn ($ZnSO_4$) as soil application in which nano concentrations are equivalent to $1/6^{th}$ of the normal recommendations. The results shows that the soil parameters like pH, EC and organic carbon were does not altered much over control in all treated pots. Whereas soil amended ZnO nano and $ZnSO_4$ increased the DTPA extractable Zn and Fe content in calcareous soil. Therefore, results can be indicated that the application of nano ZnO via soil enhance the availability of DTPA extractable micronutrient content in calcareous soil.

Keywords: ZnO, soil application, foliar application

Introduction
Calcareous soils cover over 30 per cent of the earth's land surface. Nutrient management in calcareous soils is very difficult, because its pH on soil nutrient availability and chemical reactions that affect the loss or fixation of almost all nutrients especially zinc deficiency due to reduced solubility of zinc at alkaline pH values by formation of carbonate compounds. Soil samples collected across the country shows 61% samples tested deficient in available Zn (Shukla and Pakhare, 2015). Zinc deficiency in extreme cases may lead to complete crop failure, which highlights the importance of iron nutrition in calcareous soils. Therefore, efforts need to be made to find out effective remedy to overcome Zn deficiency in crop plants. In this context, nanotechnology has been described as the next great frontier of agricultural science and could open up the novel applications in agriculture, including soil science. Nano particles are particles with dimensions between 1 to 100 nm. Nanotechnology is gradually marching from the experimental stage to the stage of operational and practical. This will lead to a more tangible presence of the technology in the agricultural sector (Baruah and Dutta, 2009). For instance, altering micronutrients to become nano fertilizers may help to enhance the use efficiency of applied fertilizers besides quality and yield of the produce. The use of nano fertilizer to control release of nutrients can be an effective step towards achieving sustainable agriculture and environment. Uses of chelated forms which are stabilized by reaction of the metal salts with natural and synthetic complexes are the most important ways to protect iron from the precipitation at increased soil pH. Keeping in view these facts, present study was undertaken to determine the effect of nano ZnO on rice.

Materials and methods
Experimental soil description
The pot culture study was conducted during the *kharif* season to see the effect of nano ZnO particles on rice crop grown on calcareous soil at Division of Soil Science and Agricultural Chemistry, Indian Agricultural Research Institute (IARI), New Delhi. Zn-deficient calcareous soil was brought from Central Agricultural University, Samastipur, Bihar for research work. Seven treatments *viz.*, T_1: control (NPK alone), T_2: 0.5% $ZnSO_4$, T_3: 0.1% ZnO, T_4: 0.02% ZnO, T_5: 5 Kg Zn through $ZnSO_4$, T_6: 1 Kg Zn through nano ZnO and T_7: 0.2 Kg Zn through nano ZnO. T_2, T_3 and T_4 are applied as foliar spray; T_5, T_6 and T_7 are soil amended. Treatments were allocated in completely randomized block design (CRD) and replicated thrice. Nano ZnO was synthesised as per the suggested method Prasad *et al*. (2012) with addition to this we were added 0.02 M Cetyl trimethylammonium bromide (CTAB) to maintain the stability of nano particles and synthesised nano particles were characterised for its crystalinity, size by XRD and TEM. The rice variety PUSA-1509 was used for this evaluation

investigation. Mat nursery was raised for producing healthy rice seedlings. After 15 days, 4 rice seedlings were transferred to pots and kept for 30 DAT, 60 DAT and maturity periods. Recommended dose of N (150 kg ha^{-1}), phosphorus (60 kg P_2O_5 ha^{-1}) and potash (60 kg ha^{-1}) were applied through the urea, single super phosphate and muriate of potash. The soil of experimental soil was sandy loam in nature having pH of 8.56 (1.2.5), electrical conductivity (EC) 0.36 dSm^{-1}. The initial soil was low in DTPA-Zn (0.48 mg kg^{-1}), so this soil serves the purpose of interest to see the effect of nano ZnO on rice crop grown under calcareous soil.

Table 1: Initial soil parameters

Soil Parameter	Value	Method/Instrument	Reference
Soil texture	Sandy loam	Bouyoucos hydrometer	Bouyoucos (9162)
pH (1:2.5)	8.56	Soil-water suspension	Jackson (1973)
Electrical Conductivity (dSm^{-1})	1.36	Soil-water suspension	Jackson (1973)
Organic carbon	0.50	Wet Digestion	Walkley and Black (1934)
DTPA-Zn Fe (mg kg^{-1})	0.48	DTPA-$CaCl_2$-TEA	Lindsay and Norvell (1978)

Results and discussion:

Characterization of ZnO nano particles

The XRD pattern of ZnO nano particles were analysed for its XRD peaks to analyse for its crystalinity and size of the particle. Figure 1 clearly shown the X ray diffraction peaks were obtained at 2 theta ranges 31.73, 34.9, 36.19 and 47.73. These peaks were attributed to the planes of 100, 002, 101 and 102. These closely matched with the spacing pattern of ZnO (Sun *et al*., 2003; Arefi and Zarchi, 2012). Particle size was calculated by the Dubye- Schrerrer equation

$$D=K\lambda/(\beta \cos \theta)$$

Where, D is the diameter of the crystalline size, K is the shape factor (0.89 used as a typical value), λ is the wavelength of incident beam, β is the broadening of the diffraction line measured in radians at half of its maximum intensity (full width half maxima-FWHM) and θ is the Bragg's angle. With these four main diffraction peaks the average crystalline size of the particle was 38 nm. Size of the synthesised ZnO nanoparticles was observed through transmission electron microscope (TEM) Figure 1. Mixed morphologies of nano spherical ZnO were found to be present in synthesised ZnO material. It is interesting to know that the size derived calculated (schrerr equation) is accordance with measured image of TEM that these particles are single crystals. Our results supports with the findings of Sahu *et al*., (2011) and Wang *et al*., (2013).

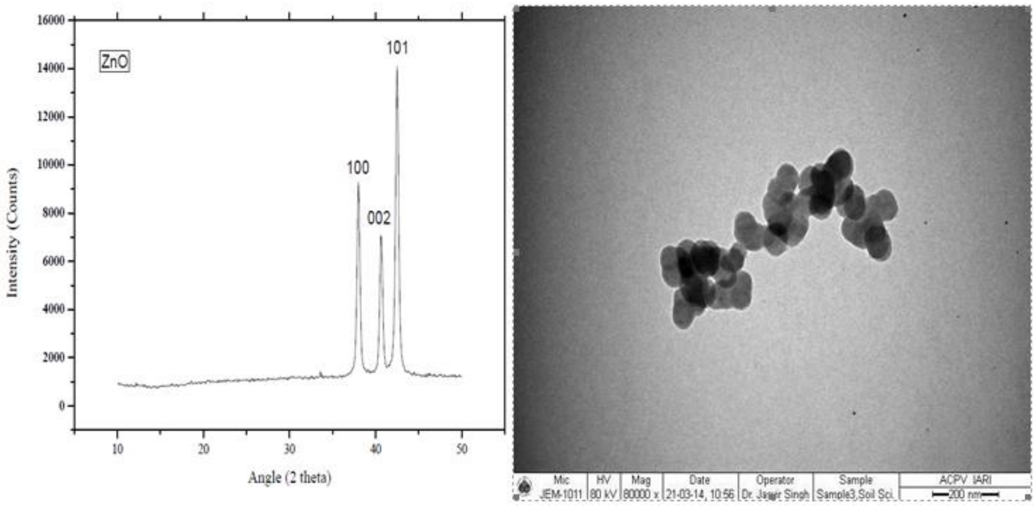

Fig 1: X-ray diffraction (XRD) pattern and Transmission Electron Microscopy (TEM) pattern of ZnO nano particles.

Soil fertility status

pH, EC and organic carbon

Use of nano particles either in foliar or soil amendment did not cause any significant changes or improvement in the soil reaction (pH and EC) of calcareous soil (Table 2). The soil pH of post-harvest soil was ranged from 7.72-8.37 (irrespective of treatments). However, mode of application and doses ZnO nano was a little influence on the soil pH of the rice soil. While, the soil amended sulphide form of Zn ($ZnSO_4$) consistently decreased the soil pH by 0.2 unit over the initial status and nano ZnO had negligible effects on the soil pH. A similar result was observed by Rousk *et al*., (2012) and Smolders *et al*., (2009) in nano ZnO. Contrary to the pH, EC data shown that change in soil EC was found to be significant among the stages and treatments as well. Significantly higher EC (overall) was found in the control treatment (0.85 dSm^{-1}), foliar (0.66 dSm^{-1}) and soil (0.74 dSm^{-1}), respectively. However, pooled (mode of application) results indicated that the soil EC decreased significantly in foliar over soil application. Likewise, organic carbon content of the soil, which varied between 0.41-0.56% which did not change significantly due to external addition of ZnO NPs in both soil and foliar application. Form this overall results it is clearly seen that nano ZnO and $ZnSO_4$ addition with lower dose level through foliar and soil amendment behaves similarly and build-up same amount of organic carbon (0.51 and 0.52% foliar and soil, respectively) in rice soil. The increase in above said treatments is attributed to the direct secretions due to Zn addition in the soil which stimulated the growth and activity of microorganisms, subsequent better root growth and resulting in higher biomass production.

Soil Micronutrients (Zn, Fe, Mn and Cu)

The content of DTPA-extractable Zn and Fe ranged from

0.43-1.31 and 3.16 to 5.50 mg kg^{-1} respectively in across the stages of rice crop growth (Table). A scrutiny of represented data of DTPA extractable Zn and Fe were also not affected by any of the foliar ZnO nano and $ZnSO_4$ application significantly in rice crop. However, soil amended Zn nano and $ZnSO_4$ sources increases the availability of DTPA extractable Zn significantly and did not alter the DTAP-Fe in rice crop. Higher values of DTPA-Zn 1.31, 1.17 were noticed in the treatment of soil amended with $ZnSO_4$ and Zn nano. The same kind of results were noticed in the work of Kher (1993), where organic acid secretion from rice roots form organic chelates of higher stability which decreased their susceptibility to fixation and precipitation resulting enhancing it availability. Wang *et al*., (2010) reported that ZnO Nps and bulk particles have higher solubility in soil environment.

Conclusion

In conclusion, derived results can be stated that as used ZnO nanoparticles for the experiment were in the range of less than 100 nm and amendment of ZnO nano particles in via soil application has the ability to enhance the DTPA extractable Zn and Fe content in rice soil, via dissolution of Zn through amended ZnO nano particles. However, addition of ZnO nano particles did not alter the soil properties like pH, EC and organic carbon in rice grown calcareous soil as well different growth stages of rice did not altered the soil fertility status with respect to ZnO nanoparticle addition.

Table 2: Effect of nano ZnO application on shoot growth and shoot growth rate of rice crop grown under calcareous soil

Treatments		pH			EC (dSm^{-1})			Org.C (%)		
		30 DAS	60 DAS	Maturity	30 DAS	60 DAS	Maturity	30 DAS	60 DAS	Maturity
Control		8.14	8.02	7.72	1.33	0.64	0.59	0.42	0.48	0.42
Foliar	0.5% $ZnSO_4$	8.35	7.96	7.78	0.79	0.51	0.62	0.47	0.52	0.53
	0.1% ZnO NPs	8.24	7.94	7.70	0.76	0.41	0.88	0.40	0.51	0.51
	0.02% ZnO NPs	8.34	7.96	7.75	0.69	0.56	0.76	0.48	0.56	0.50
Soil	5 Kg Zn through $ZnSO_4$	8.19	7.87	7.81	0.95	0.61	0.75	0.55	0.47	0.53
	1 Kg Zn through nano ZnO	8.30	7.95	7.82	0.72	0.71	0.76	0.48	0.49	0.51
	0.2 Kg Zn through nano ZnO	8.37	8.00	7.75	0.84	0.51	0.80	0.53	0.50	0.53
	Mean	8.27^{A}	7.95^{B}	7.65^{C}	0.86^{A}	0.56^{C}	0.73^{B}	0.47^{A}	0.51^{A}	0.51^{A}

Table 3: Effect of ZnO nano particle application on DTPA Zn and Fe (mg kg^{-1}) content in calcareous soil

Treatments		DTPA-Zn			Mean	DTPA-Fe			Mean
		30 DAS	60 DAS	Maturity		30 DAS	60 DAS	Maturity	
Control		0.43	0.54	0.52	0.49^{c}	4.50	4.44	4.06	4.34^{a}
Foliar	0.5% $ZnSO_4$	0.73	0.58	0.74	0.68^{b}	4.76	4.96	4.26	4.66^{a}
	0.1% ZnO NPs	0.50	0.48	0.65	0.54^{cb}	4.76	5.00	4.70	4.82^{a}
	0.02% ZnO NPs	0.47	0.68	0.64	0.60^{cb}	3.93	5.50	4.63	4.68^{a}
Soil	5 Kg Zn through $ZnSO_4$	1.17	0.83	1.13	1.04^{a}	4.50	5.00	4.30	4.60^{a}
	1 Kg Zn through nano ZnO	1.02	1.31	0.49	0.94^{a}	4.96	5.60	4.50	5.02^{a}
	0.2 Kg Zn through nano ZnO	0.49	0.66	0.47	0.54^{c}	4.90	4.10	3.16	4.05^{a}
Mean		0.68^{A}	0.72^{A}	0.66^{A}		4.61^{A}	4.94^{A}	4.23^{A}	

Values followed by common letters are not significantly different at $P \leq 0.05$. Lowercase letters are used to show the statistical significance in case of DTPA-Zn and Fe content with respect to the treatments, while uppercase letters are used to show the statistical significance in case of stages.

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