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Response of rice to leaf surface application of nano Zn vis-à-vis $ZnSO_4$ on productivity and grain quality

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Abstract

A field experiment was conducted in Central farm, OUAT, Bhubaneswar during 2021 and 2022, Kharif where foliar spraying of nano fertilizer (zinc) was evaluated for its suitability with conventional zinc sulphate on a cereal crop (rice). The treatments comprised of such as T_1:control (no zinc), T_2: Soil application of $ZnSO_4$ @ 12.5 Kg/ha, T_3: leaf surface application of $ZnSO_4$ once @ 0.5%,T_4: leaf surface application of IFFCO nanozinc once, T_5: leaf surface application of IFFCO Nanozinc twice,T_6: leaf surface application of Green synthesized nano zinc once, T_7: leaf surface application of Green synthesized nano zinc twice, T_8: leaf surface application of Chemically synthesized nanozinc once and T_9: leaf surface application of Chemically synthesized nanozinc once replicated thrice in RBD. All the treatments received common NPK at recommended dose. The results revealed that the rice crop responded to zinc by producing more yield over no zinc control with a response range of 5.8-17.06%. highest response was observed in the soil application of $ZnSO_4$ and lowest in the chemically synthesized nano zinc. Leaf surface application of green synthesized nanozinc twice recorded higher value Zn content (41.5) along with other quality parameters than that of leaf application of nano zinc once. Among three nano sources green synthesized performed best followed by IFFCO and chemically synthesized one. However nano zinc can be applied as supplemental dose for rice crop.

Keywords: Nano Zn, leaf surface, rice, Inceptisols

Introduction

Zinc deficiency in soils has been recognized as a global issue and is increasing in arid and semiarid regions. According to Singh *et al.* (2005) [1], Zn deficiency affects 60% of the cultivable land in India and results in a 25-35% loss in crop output and quality. Only 1 to 4% of applied $ZnSO_4$ is effective, while the majority of applied zinc is inaccessible to plants as a result of leaching and fixation (Nair *et al.*, 2010) [7]. Therefore, it is crucial to reduce nutrient losses during fertiliser application and boost crop output by utilising novel applications made possible by nanotechnology and nanomaterials. Nanoparticles (NPs) exhibit special physicochemical characteristics, such as high surface area, high reactivity, variable pore size, and particle shape, according to Prasad *et al.* (2012) [9]. They can act as "magic bullets," releasing herbicides, nanopesticides, fertilisers, or genes that specifically target and affect certain plant cellular organelles. ZnO NPs, or zinc oxide nanoparticles, promote plant growth and development. Up to 35 to 40% of the productivity in agriculture is contributed by fertiliser. Nano fertiliser may be the most effective option to increase nutrient utilisation efficiency. In an effort to control the release of zinc depending on the needs of the crops, attempts have been made to synthesize nano nutrients, specifically for zinc. It has also been stated that nano nutrients are far more effective than conventional fertiliser. Foliar fertiliser applications of nanoparticles have been found to increase yield (Raliya *et al.*, 2013) [10]. Green synthesis, a new advancement in nanotechnology, employs biological systems like plants and microbes to synthesize nanoparticles faster than physical and chemical processes. Green synthesis of nanoparticles is an eco-friendly process that uses no toxic or expensive chemicals. Metal nanoparticle synthesis utilising plant extracts is regarded as cost-effective and thus employed as an economically viable technology for large-scale production. The mechanism behind the development of nanoparticles, as stated by multiple researchers, suggested that bio-reduction of nanoparticles was caused by numerous biomolecules (vitamins, amino acids, proteins, phenolic acids, alkaloids, etc.) in the plant and microbes.

Phenolic acids have hydroxyl and carboxyl groups that can bind metals, making them effective anti-oxidants. The reduction of metal ions into the creation of metal nanoparticles may be caused by the active hydrogen. When the precursor is introduced to the leaf extract, the colour changes, signifying the development of ananocatalyst, making the overview of greensynthesis simple to understand (Yuvaraj *et al.*, 2014) [13]. (Kandasamy and Prema, 2015) [4]. Zinc can be applied to crops in a variety of ways, including fertigation, foliar spraying, seed treatment, and soil application. Foliar or combined soil+foliar application of fertilizers under field conditions has proved to be highly effective and can be a practical way to maximize the zinc accumulation and uptake in grains (Cakmak, 2008) [2]. Only 1 to 4% of applied ZnSO4 is effective, while the majority of applied zinc is inaccessible to plants as a result of leaching and fixation (Nair *et al.*, 2010) [7]. Therefore, it is crucial to reduce nutrient losses during fertiliser application and boost crop output by utilising novel applications made possible by nanotechnology and nanomaterials.

Materials and Methods

The current study was carried out during the Kharif season of 2021 at E Block, Central Research Station (RRTTS), OUAT, Bhubaneswar, India. E Block, Central Research Station (RRTTS), OUAT, Bhubaneswar, is located about 2 km away from the College of Agriculture, BBSR, OUAT. Bhubaneswar falls under the East and South Eastern Coastal Plain agro-climatic zone and lies at 20°15' N latitude and 85°55' E longitude, at an altitude of 25.9 meters above mean sea level.

The landtype of experimental site was medium land and land was irrigated through tubewell. The experiment was laidout in a randomized blockdesign (RBD) with three replications. The main field was divided into three plots for three replications, and each plot was subdivided into nine subplots based on the sources of zinc treatment. The T_1 plot of each replication was the control, with only the soil test dose of fertilizers and no zinc application. In plot T_2, soil application of zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) @ 12.5 kg ha^{-1} was done before transplanting. T_3 plot was treated with foliarspray of 0.5% $ZnSO_4.7H_2O$ after 25 days after transplanting. T_4 and T_5 plot were treated with IFFCO liquid nano zinc @ 2 ml/litre after 30 days after transplanting. T_5 plot was again treated with second foliar spray of IFFCO liquid nano zinc @ 2 ml/litre at 60 days after transplanting. $T_6$and T_7 plot were treated with green synthesized nano zinc @ 2 ml/litre after 30 days after transplanting. T_7 plot was again treated with second foliarspray of green synthesized nano zinc @ 2ml/litre at 60 DAT. T_8 & T_9 plot were treated with chemically synthesized nano zinc @ 2 ml/litre at 30 DAT. T_9 plot was again treated with second foliar spray of chemically synthesized nano zinc @ 2 ml/litre at 60 DAT.

Rice variety Moti (IET-9170) was raised as test crop following standard POPs. The soil test doses of N, P_2O_5 and K_2O were 100 kg/ha, 40 kg/ha and 50 kg/ha respectively. All treatments received common NPK. Nitrogen was applied in the form of urea & DAP with 50% as basal and rest nitrogen was top dressed at 2 splits i.e., at 40 DAT and at PI stage. Required amount of phosphorus was applied as 100% basal in each plot and total amount of potassium in form of Muriate of potash (MOP) was applied in two splits i.e. 50% at basal and 50% at PI stage. Zinc was applied in two mode-soil application and foliar spray. Zinc @ 2.5 kg/ha in the form of 12.5 kg $ZnSO_4$ at the time of transplanting as basal, foliar spray of Zn in form of ZnSO4 @ 0.5% applied at 25 DAT. Nano formulation were applied @ 2 ml/L at 30 and 60 DAT as foliar spray.

Measurement of stomatal diameter

Leaf samples were collected from the rice plant from plots. Thin sections of leaf were prepared and stained with cotton blue. The samples were observed under compound microscope in 20X magnification to observe the size of stomata. Photographs of stomatas were taken in Compound Microscope (Labovision) and diameter were also measured by the software provided by Labovision. (Fig 1)

NPs were prepared in the laboratory. The solution was then centrifuged at 10,000 rpm for 10 minutes, and the substrate that settled at the bottom was collected in a crucible and dried in an oven before calcination. Sufficient calcination of these substrates was carried out in a muffle furnace at 500 °C for 1.5 hours to produce greyish-white ash-coloured ZnO nanoparticles. The nanoparticles were then stored at 4 °C in the laboratory for further study and application.

The ZnO powder thus obtained can be sent for analysis by transmission electron microscopy (TEM), X-ray diffraction (XRD), ultraviolet-visible spectrophotometric spectroscopy (UV-VIS), and Fourier transform infrared spectroscopy (FTIR).

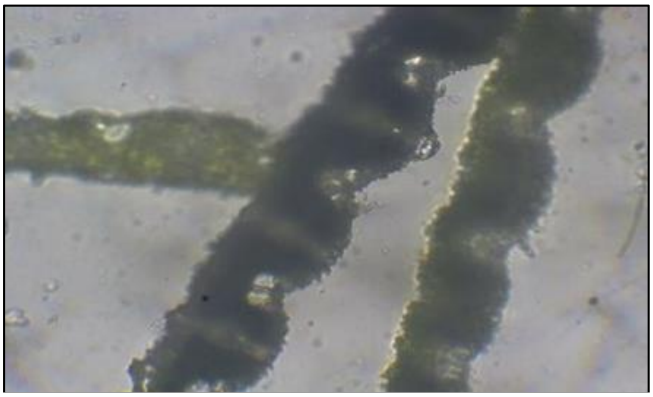

Fig 1: Stomata diameter measurement under compound microscope

Collection of leaf, root & shoot samples for analysis

Collection and processing of plant samples

For the tissue nutrient analysis of the harvested crop, the labelled grain and straw samples were collected from the field and brought to the laboratory. After proper cleaning, the samples were kept in a hot-air oven for 24 hours at 65 °C. The oven-dried samples were then ground using a Willey mill and sieved through a 0.5 mm sieve. The samples were then ready for analysis.

Physico-chemical properties were analysed as per the method of Jackson (1973) [3]. Estimation of protein was done using the method of Lowry *et al.* (1951) [6] from leaf and grain separately. Total carbohydrate content of grain was estimated by the anthrone method given by Yoshida *et al.* (2005) [7]. Total Zn in grain and the rice plant was estimated by the diacid digestion method.

DTPA (Diethylene Triamine Pentaacetic Acid)-extractable Zn in soil was estimated as per the method of Lindsay and Norvell (1978) [5], and the concentration of elements was determined using AAS (Page *et al.*, 1982) [8].

Results and Discussion

Initial Soil characteristics of experimental soil

Sl. No.	Parameters	Value
1	Texturalclass	Sandyloam
2	pH (1:2.5)	5.7
3	EC (dS m^{-1})	0.39
4	O.C (%)	0.63
5	Available N($Kgha^{-1}$)	147
6	Available P ($Kgha^{-1}$)	9.2
7	Available K ($Kgha^{-1}$)	89
8	DTPAZn (mg Kg^{-1})	0.45

It was revealed from the table that soil reaction (pH) was acidic and electrical conductivity was low, containing less soluble salts which was non saline. The organic carbon content was found to be medium (i.e. 0.63). Then available nitrogen, phosphorus and potassium found to be 147 Kg ha^{-1}, 9.2 Kg ha^{-1}, 89 Kg ha^{-1}. The available zinc extracted by DTPA solution & analysed by using AAS was found to be 0.45 mg kg-^{1}whichwas categorized a slow as it was less than critical limit which is 0.60.

Table 1: Size of stomata diameter of rice plant

Sl. No	Treatments	Before1st foliar spray (nm)	Before 2nd foliar spray (nm)
T_1	control(no zinc)	14347	14525
T_2	Soil application of $ZnSO_4$ @ 12.5 Kg/ha,	18432	18550
T_3	leaf surface application of $ZnSO_4$ once	23467	23558
T_4	leaf surface application of IFFCO nanozinc once	15098	15167
T_5	leaf surface application of IFFCO Nanozinc twice	16141	16261
T_6	leaf surface application of Green synthesized nano zinc once	16556	16781
T_7	leaf surface application of Green synthesized nano zinc twice	17627	17889
T_8	leaf surface application of Chemically synthesized nanozinc once	19109	19214
T_9	leaf surface application of Chemically synthesized nanozinc twice	21012	21037

Grain yield

Effect of different Nano Zn sources on grain and straw yield of Rice is presented in table 2.

Table 2: Effect of foliar application of nanozinc on grain yield of rice

Treatment Details	Grain Yield (q/ha)	Increase over control (%)	Straw yield (q/ha)
control(no zinc)	38.11	-	60.98
Soil application of $ZnSO_4$ @ 12.5 Kg/ha,	44.62	17.08	61.58
leaf surface application of $ZnSO_4$ once	43.85	15.06	60.95
leaf surface application of IFFCO nanozinc once	40.32	5.79	58.87
leaf surface application of IFFCO Nanozinc twice	43.10	13.09	61.20
leaf surface application of Green synthesized nano zinc once,	41.37	8.55	59.99
leaf surface application of Green synthesized nano zinc twice	43.31	13.6	60.63
leaf surface application of Chemically synthesized nanozinc once	40.29	5.7	59.63
leaf surface application of Chemically synthesized nanozinc twice	43.08	13.04	61.39
Sem (±)	7.60		11.9
CD (0.05)	2.13		3.19
CV (%)	11.29		11.13

Grain yield of rice was recorded from different plots and expressed in kg/ha presented in table 2 and it revealed that the grain yield ranged from 38.11 q/ha to 44.62 q/ha. The highest grain yield was recorded in T_2 (STD + Soil application of Zn @ 2.5 Kg/ha ($ZnSO_4$)) i.e 44.62 q/ha, where as the lowest yield was observed in T_1 (Control) which was found to be 17% more than that of control. Twice foliar spray of nano zinc was recorded more yield than once foliar spray of any nano zinc sources. All the treatments were found producing higher grain yield over control significantly.

Zinc content

Zn content in rice grain and other parts were estimated treatment wise and presented in Table 3.

Table 3: Effect of nano zinc on zinc content in rice

Sl. No.	Treatment details	Zinc concentration (mg/kg)		
		Grain	Husk	Straw
T_1	control(no zinc)	23.1	31.3	29.1
T_2	Soil application of $ZnSO_4$ @ 12.5 Kg/ha,	43.1	54.5	50.2
T_3	leaf surface application of $ZnSO_4$ once	42.4	53.2	49.3
T_4	leaf surface application of IFFCO nanozinc once,	29.3	40.3	36.2
T_5	leaf surface application of IFFCO Nanozinc twice	40.3	51.1	46.2
T_6	leaf surface application of Green synthesized nano zinc once,	30.1	41.2	37.3
T_7	leaf surface application of Green synthesized nano zinc twice	41.5	52.1	47.1
T_8	leaf surface application of Chemically synthesized nanozinc once	28.2	39.2	35.1
T_9	leaf surface application of Chemically synthesized nanozinc twice	39.6	50.3	45.3
	Sem (±)	1.7	1.9	2.2
	CD (0.05)	4.7	5.4	6.3
	CV (%)	12.1	11.2	10.7

Zinc content in rice grain ranged from 23.1 in control to highest of 43.1 ppm in T_2 receiving soil application. Significantly highest mean zinc content was obtained in treatment T_2 (RDF+ Soil application of $ZnSO_4.7H_20$ @ 25 kg/ha) followed by STD + Leaf surface application of $ZnSO_4$ once @ 0.5%. Higher content of zinc in grain, husk and straw in twice leaf surface application of liquid nano zinc at 25 DAT and 50 DAT due to higher surface: volume ratio of nano fertilizers which is mainly due to very less size of particles which provide more site to facilitate different metabolic process in the plant system resulting production of more photosynthates. Zinc content of rice straw was higher than that of rice grain and husk, irrespective of treatments.

Table 4: Quality attributes as effected by Nano Zn Foliar spraying

Sl. No.	Treatment details	Protein	Carbohydrate
		mg/gm	
T_1	control(no zinc)	48.64	404.89
T_2	Soil application of $ZnSO_4$ @ 12.5 Kg/ha	78.49	654.75
T_3	leaf surface application of $ZnSO_4$ once	76.41	634.06
T_4	leaf surface application of IFFCO nanozinc once,	61.54	510.64
T_5	leaf surface application of IFFCO Nanozinc twice	75.10	623.20
T_6	leaf surface application of Green synthesized nano zinc once,	61.63	511.42
T_7	leaf surface application of Green synthesized nano zinc twice	75.47	626.26
T_8	leaf surface application of Chemically synthesized nanozinc once	61.49	510.28
T_9	leaf surface application of Chemically synthesized nanozinc twice	75.06	622.84
	Sem (±)	4.373	36.356
	CD (0.05)	12.24	101.788
	CV (%)	11.1	11.12

Effect of soil and foliar application of Zn on quality parametrs like carbohydrate and protein are presented in Table 4.Which indicated that Protein and carbohydrate content was highest in Soil application of Zn which was at par with foliar nano Zn sources. Among the three nano Zn sources tested green synthesized nano Zn source was better as compared to IFFCO and chemically synthesised source in improving quality parameters.

Conclusion

From the above experiment the conclusion may be drawn as follows Twice foliar spray of nano zinc sources produced higher grain yield with better agronomic parameters, biochemical quality and zinc content in rice plant which were comparable with soil application of Zn @ 2.5 kg ha^{-1} or foliar spray of ZnS04 @ 0.5%.

Among three nano sources green synthesized performed best followed by IFFCO and chemically synthesized one. However nano zinc can be applied as supplemental dose for rice crop.

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