Indian Journal of Agricultural Sciences **94** (10): 1136–1139, October 2024/Short Communication
https://doi.org/10.56093/ijas.v94i10.151163

Nano-sized ZnO enhances photosynthetic parameters, yield and Zn content in rice (*Oryza sativa*)

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Received: 02 May 2024; Accepted: 23 July 2024

Keywords: Rice, Zinc fertilizer, ZnO nanoparticles, Zinc content

Zinc (Zn) is an essential micronutrient for plants and plays a major role in plant metabolism. It is crucial for the growth and development of rice (*Oryza sativa* L.), affecting its yield and quality (Prasad *et al.* 2013). In the Indian scenario, 56% of soil samples were shown deficient in Zn content which is below 0.5 ppm (Shukla *et al.* 2021). Zn deficiency condition prevailing for a longer period will impact rice growth through poor tillering, limiting root growth, decreasing the rate of seed set, and leading to insufficient zinc accumulation in the plant. Boosting zinc (Zn) levels in crops can be done through specific management practices like the use of a correct source of fertilizers with the correct dose and mode of application in the field crops and following the fortified varieties (Vijayakumar *et al.* 2021, Senguttuvel *et al.* 2023). Foliar Zn applications are promising, although environmental conditions influence their effectiveness. The advancement in the use of new materials (i.e. nanoparticles) in the fertilizer sector and plant nutrition has increased to the next level by enhancing plant growth and resilience in agriculture. Due to its small size, it has more surface area and is exposed to more active sites than the macro/microparticles (Gobinath *et al.* 2021). Zinc oxide nanoparticles (ZnO NPs), outperform traditional Zn fertilizer's by being more readily absorbed by plants due to their large surface area and reactivity. Studies have shown that ZnO NPs increase rice yields and grain Zn levels and contribute to better growth (Singh *et al.* 2022). Foliar application of ZnO NPs swiftly changes into zinc ions, which then move through the plant and ascend to the aerial parts via the xylem (Du *et al.* 2019). The dissolution rate of ZnO NPs is dependent on soil characteristics, especially *p*H and the concentration of the nanoparticles. Importantly, ZnO NPs have been found to enhance the zinc content in crops without posing a higher risk associated with nano-sized particles. But, the knowledge gap on the mode of application, and the rate of their impact on the rice yield and nutrient content are not clear. Keeping these gaps in view, the current investigation was taken with different sources of Zn along with nano-scale ZnO to evaluate the effect on yield attributes, Zn content, and physiological changes in rice crops and soil.

Present study was carried out during rainy (*kharif*) season of 2021 at ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana. Seeds of rice variety MTU 1001 were used for the study and were cleaned with 2% hypochlorite solution to remove dead seeds and control the seed-borne diseases and further washed thoroughly with distilled water to remove excess hypochlorite solution on the seeds. Experiment was conducted in a completely randomized design (CRD) to evaluate the influence of different sources of Zn i.e. bulk ZnO (500 mg/litre, 1000 mg/litre, 1500 mg/litre), nano ZnO (50 mg/litre, 100 mg/litre, 150 mg/litre), and $ZnSO_4$ (0.5% spray) on rice crop growth and yield attributes. The experimental soil was sandy loam in texture, with a *p*H of 8.81, electrical conductivity (EC) of 0.31 dS/m, organic content (OC) of 0.19%, and DTPA-Zn content of 0.48 mg/kg. The nanoparticle suspension was prepared using an ultrasonicator as prescribed by Gobinath *et al.* (2023). Plant samples (shoot and grain) were collected at harvest stages, to assess the overall impact of added nanoparticles on the rice plants. The Zn content in the plant samples was estimated using the di-acid extract method via Atomic Absorption Spectroscopy (AAS). The chlorophyll content was estimated using a DMSO solution. Further chlorophyll content was estimated as suggested by Arnon (1949) as follows:

$$\text{Chlorophyll A} = 12.7 \times D_{663} - 2.69 \times D_{645} \times V/(1000 \times W)$$

$$\text{Chlorophyll B} = 22.9 \times D_{645} - 4.68 \times D_{663} \times V/(1000 \times W)$$

After harvesting, soil samples were air-dried, sieved through a 2 mm sieve, and subsequently stored for chemical analysis (DTPA-Zn). The collected data were subjected to a

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two-way analysis of variance (ANOVA) using the general linear model procedures within the Statistical Analysis System (SAS Institute, NC). The F-test was employed to determine the significant effects of iron sources and for comparing means, the least significant difference (LSD) method was used.

Application of 150 mg/litre ZnO nanoparticles and 0.5% $ZnSO_4$ through foliar mode registered significantly higher plant height as 55.1, 53.7 cm, and 83.3 and 73.7 cm, at 45 DAT and maturity, respectively over control (36.1 and 69.3 cm) (Table 1). Less improvement in plant height was observed with the bulk ZnO-treated plants i.e. 39.6, 36.7, and 29.6 cm with 500, 1000, and 1500 mg/litre concentrations, respectively. Whereas, ZnO NP application exhibited a positive trend with respect to the plant height and graded level of ZnO NP increased the height by 18% and 52% with 100 and 150 mg ZnO NP and 20% with 150 mg/litre ZnO NPs, respectively at 45 DAT and Harvest. This scenario with bulk ZnO could be a size-restricted entry into the plants causing less improvement in the rice plant height. However, on-par results were observed with the nano 150 mg/litre and ZnSO4 (0.5%) spray. A similar report was recorded by El-Dahshouri *et al.* (2017), foliar application of 0.5% $ZnSO_4$ during the tillering stage increased shoot length across wheat's growth stages compared to the control. The highest chlorophyll 'a' and 'b' was observed in $ZnSO_4$ treated plants as 5.33 and 4.83 µg/ml which was on par with the ZnO NP (150 mg/litre) treatment 5.10 and 4.56 µg/ml, respectively (Table 2). Added Zn is more actively involved in the chlorophyll development mechanism than other physiological mechanisms in rice crop. Gobinath *et al.* (2023), recorded the influence of nano Fe_2O_3 in rice crops on plant height under irrigated conditions. Similar findings were reported by Zhang *et al.* (2021) in rice with ZnO NPs, which enhanced chlorophyll synthesis by controlling the activities of glutamyl RNA reductase in rice plants which showed an evident-based promotion in chlorophyll content in rice plants via foliar application. The $ZnSO_4$ treatment also had higher chlorophyll content than nano and control treatments. Singh *et al.* (2022), demonstrated that lower doses of ZnO i.e. 150 ppm are valuable for increasing the photosynthetic activity in rice crops.

Application of Zn sources via bulk, nano ZnO and $ZnSO_4$, registered a significant effect in both grain and straw yield of rice crops (Fig. 1). It is evident from the results that foliar application of Zn NPs significantly improves the grain yield over bulk ZnO treatments, being the values of 4.99, 5.27 and 5.97 g/pot which were above the control plants (4.36 g/pot). However, the highest grain yield was noticed in $ZnSO_4$ (6.03 g/pot) which was on par with the Zn NPs (150 mg/litre) spray. Nanoparticle application exhibited the grain yield to the tune of 15, 21 and 37% over the control whereas bulk ZnO registered improvement to the level of 9, 3 and 1% with 500 mg/litre, 1000 mg/litre, 1500 mg/litre, respectively. This favourable response to the added Zn NPs can be attributed to the deficiency of Zn in the experimental soil. Similar findings were observed by Gobinath *et al.* (2023) with Fe_2O_3 nanoparticles application in rice crops. The plants grown on deficient soil will give a positive response to the external application of fertilizer. Various responses of grain and straw yield to added nanoparticles were due to the dissolution behaviour of added sources of nutrients i.e. bulk, nano and sulphate sources of Zn in the study.

Sources of Zn added to rice crops significantly influenced the Zn content of rice straw and grain (Table 2). Spray of $ZnSO_4$ recorded the highest buildup of Zn content in both straw and grain of rice at 55.3 and 20.3 mg/kg, respectively which was on par with the Zn NPs applied in the concentration of 150 mg/litre (54.3 and 19.3 mg/kg, respectively). The higher per cent improvement of Zn in rice grain was noticed with $ZnSO_4$ treatment (84%) followed by 150 mg/litre of ZnO NPs (77%). However, a reduction in Zn accumulation was recorded with bulk ZnO treatments and even negative accumulation was observed i.e. -5% was registered with the 1500 mg/litre of bulk ZnO treatment (Fig. 2). The cumulative effect of ZnO nanoparticles application for Zn enhancement rather than bulk ZnO was observed in the current study. The size-dependent translocation in the plant xylem is considered

Table 1 Effect of ZnO nanoparticles on plant height and chlorophyll parameters in rice plants

Treatment	45 DAT	90 DAT	NDVI	SPAD	Chl a (µg/ml)	Chl b (µg/ml)
Control	36.1	69.3	0.37	34.7	4.86	3.86
Bulk ZnO (500 mg/litre)	36.7	64.0	0.34	34.0	4.86	4.60
Bulk ZnO (1000 mg/litre)	39.6	54.3	0.44	34.6	4.00	3.20
Bulk ZnO (1500 mg/litre)	29.6	50.6	0.36	31.5	3.83	3.43
Nano ZnO (50 mg/litre)	36.1	62.3	0.46	32.9	4.50	4.56
Nano ZnO (100 mg/litre)	42.6	70.3	0.44	31.9	4.73	4.60
Nano ZnO (150 mg/litre)	55.1	83.3	0.63	35.9	5.10	4.56
$ZnSO_4$ (0.5% spray)	53.7	73.7	0.51	35.3	5.33	4.83
CD (P=0.05)	10.2	8.1	0.13	NS	NS	0.79
SEm	3.0	2.7	0.04	1.3	0.79	0.26

DAT, Days after transplanting.

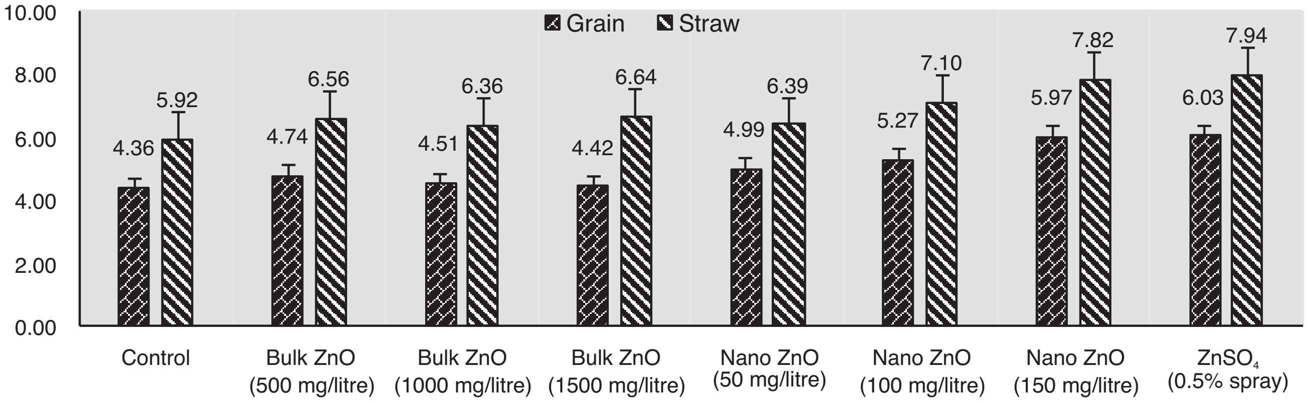

Fig. 1 Effect of Zn nanoparticles application on grain and straw yield of rice. Error bars represent the critical difference at 0.05 between the treatments.

Table 2 Effect of Zn sources on Zn content in plant and soil of rice crop

Treatment	Straw	Grain	DTPA-Zn
Control	40.9^{d}	10.9^{d}	0.42^{b}
Bulk ZnO (500 mg/litre)	47.2^{c}	12.2^{c}	0.43^{b}
Bulk ZnO (1000 mg/litre)	47.0^{c}	11.9^{c}	0.44^{b}
Bulk ZnO (1500 mg/litre)	45.4^{c}	10.4^{c}	0.44^{b}
Nano ZnO (50 mg/litre)	50.7^{b}	15.6^{b}	0.42^{b}
Nano ZnO (100 mg/litre)	50.3^{b}	15.3^{b}	0.44^{b}
Nano ZnO (150 mg/litre)	54.3^{a}	19.3^{a}	0.51^{a}
$ZnSO_4$ (0.5% spray)	55.3^{a}	20.3^{a}	0.53^{a}
CD (*P*=0.05)	2.3	2.2	0.06

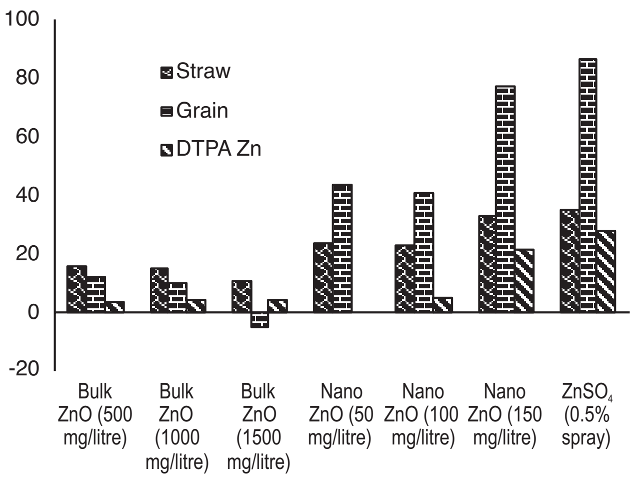

Fig. 2 Percent improvement over control treatment (Straw, grain and DTPA Zn content).

a critical factor for Zn enrichment in rice grain with bulk ZnO, nano ZnO and $ZnSO_4$ sources of Zn. The application of macro-sized bulk ZnO exhibited negative effects such as less dissolutions, and rapid aggregation on the rice foliage and these interactions hindered the Zn transport in the plant cells i.e. straw and grain (Monreal *et al.* 2016). The soil zinc (Zn) level is notably affected by the application of various Zn sources in rice soil. It is worth mentioning that a higher concentration of ZnO NPs (150 mg/litre) (0.51 mg/kg) and recommended 0.5% $ZnSO_4$ spray (0.53 mg/kg) registered on par and significantly higher DTPA Zn content in soil over other treatments. Supplement of Zn either through nano ZnO 150 mg/litre and 0.5% $ZnSO_4$ could enhance the Zn content to a level of 33,35; 77,87 and 21,28% in straw, grain and soil, respectively over control plants.

SUMMARY

A pot culture experiment was conducted during rainy (*kharif*) season of 2021 at ICAR-Indian Institute of Rice Research, Hyderabad, Telangana to study the influence of the foliar application of various Zn sources such as nano ZnO, bulk ZnO, and $ZnSO_4$ on rice crop. The experiment was laid out in a completely randomized design (CRD) with the following treatments, containing 3 concentrations of bulk ZnO (500 mg/litre, 1000 mg/litre, and 1500 mg/litre), 3 concentrations of nano ZnO (50 mg/litre, 100 mg/litre, and 150 mg/litre), 0.5% $ZnSO_4$ and control with 3 replications. The findings revealed that ZnO nanoparticles (NPs) positively impacted rice grain yield, dry matter accumulation, and Zn content. Compared to the control (no zinc), ZnO NPs (150 mg/litre) and standard $ZnSO_4$ treatments exhibited the highest plant height (83.3 cm and 73.7 cm, respectively), grain yield (5.97 g/pot and 6.03 g/pot), and straw yield (7.82 g/pot and 7.94 g/pot). The increased photosynthetic substances and higher dry matter accumulation throughout the rice growth stage contributed to the enhanced grain yield. Furthermore, supplementing Zn *via* nano ZnO (150 mg/litre) and 0.5% $ZnSO_4$ resulted in significant Zn enrichment: 33,35; 77,87 and 21, 28% in straw, grain and soil, respectively over control plants. This study effectively demonstrates that ZnO nanoparticles have the potential to serve as high-performance fertilizers, benefiting both rice yield and quality.

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