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Effect of zinc oxide nanoparticles embedded NPK fertilizer on growth, yield and zinc concentration and uptake of aromatic rice (*Oryza sativa*)

KIRTTIRANJAN BARAL[1], DINESH KUMAR[2] and YASHBIR SINGH SHIVAY[3]

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

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Rice (*Oryza sativa* L.) is the staple food for half of the world's population. It not only meets daily protein and energy requirement of a large mass of people all over the world but also supports livelihood and generates employment. Almost 90% of global rice is produced in Asia and in India; it is grown on 43.79 million ha with production and productivity of 112.91 million tonnes and 2.57 tonnes/ha, respectively (DOES 2019). Although total rice production has increased over the years, India is facing plateau in rice productivity in recent years. Productivity of major food crops like rice; wheat etc should be enhanced to sustain the burgeoning population, as scope for increasing area under crops is not a viable option. According to Vision 2050 (NRRI 2013), India will require 137.3 million tonnes of rice by 2050 and there may be decline in rice area by 6–7 million ha by 2050 due to urbanization, competitive crops and industrialization etc. Decline in soil organic matter, imbalanced fertilization, micronutrients deficiencies, soil compaction due to puddling, poor resource use efficiency and climate change are the major factors behind stagnation in rice productivity. Among these, imbalanced fertilization and low nutrient use efficiency are some of the major concerns which should be addressed for sustaining productivity and enhancing profitability in major rice ecologies of India. Majority of farmers are applying NPK fertilizers without addition of organic manures and micronutrients to the soil. In addition, fertilizers application is more skewed towards N which has accelerated the problem like nutrient mining. Likewise, micronutrients particularly zinc (Zn) has emerged as one of the yield–limiting factors as nearly 36.5% of Indian soils are deficient in available Zn (Shukla and Behera 2019).

Several investigations were carried out to enhance nitrogen use efficiency like use of slow release fertilizers, nitrification inhibitors and urease inhibitors (Trenkel 2010) etc. However, application of these products has not become popular due to high cost except neem coated urea which has been popularized all over the India due to favorable Indian government policies. Similarly, phosphorus use efficiency is very low (10–20%) and mining of potassium continues in rice agroecosystem due to imbalanced fertilization. Meanwhile, India is not self–sufficient in phosphorus fertilizer production and also the entire potassic fertilizer demands of the country are met through import. Thus, balanced application of NPK along with zinc and enhancing nutrient use efficiency of applied nutrients are important concerns for enhancement of rice productivity and reduced cost of cultivation.

Nanoparticles (size <100 nm) have higher surface area, different crystal property, and unique physical, chemical and magnetic properties than that of bulk materials (Maurice and Hochella 2008). Prasad *et al*. (2012) reported that application of ZnO nanoparticles of 25 nm diameter resulted higher germination percentage, increased vigour of seedlings and higher plant growth when applied at 1000 ppm in peanut. Application of nanoscale ZnO was reported to increase the grain Zn concentration in rice (Raddy *et al*. 2017). Likewise, higher plant growth in pearl millet was observed by application of nanoparticles of ZnO (Tarafdar *et al*. 2014). Adhikari *et al*. (2015) reported beneficial effect of nanoparticles of ZnO in maize in terms of higher root dry weight, plant height, root length and root volume etc. However, most of the earlier studies were conducted in pot culture under greenhouse conditions. Limited works have been done on the field scale application of ZnO nanoparticles (ZnO Nps) in conjunction with NPK fertilizer. Thus, present investigation was carried out to evaluate the effect of ZnO nanoparticles in combination with NPK fertilizer in aromatic rice. Two aspects were investigated during this study i) the effect of ZnO nanoparticles application in conjunction with NPK fertilizer on growth parameters and yields of aromatic rice and; ii) to know about the Zn concentration and its uptake by grain and straw as influenced by ZnO nanoparticles application.

[1]Ph D Scholar (e mail: kirttiagroiari111@gmail.com), [2]Principal Scientist (e mail: dineshctt@yahoo.com), [3]Ex–Professor and Principal Scientist (e mail: ysshivay@iari.res.in), Division of Agronomy

A field experiment was carried during rainy (*kharif*) season (July–November) of 2018 at ICAR–Indian Agricultural Research Institute, New Delhi. The climate of Delhi is sub–tropical and semi–arid type. The summer is hot and dry and winter is cold. The mean annual rainfall was 853.8 mm during crop period. Rice–wheat cropping system was adopted for previous many years at the particular location before undertaking present study. The texture of the experimental site was sandy clay loam (typic *Ustochrept*) with sand, silt and clay of 50.7%, 22.1% and 27.2%, respectively. The soil (0–15 cm) had a *p*H 7.5 in soil water suspension (1:2.5) and bulk density of 1.48 Mg/m^3. The oxidizable soil organic carbon, available N, available P, available K and DTPA–extractable Zn were found to be 0.53%, 210 kg/ha, 12.3 kg/ha, 210.4 kg/ha and 0.61 mg/kg soil, respectively as per the procedures described by Prasad *et al.* (2006).

Ten treatments, viz. absolute control (0:0:0 N:P_2O_5:K_2O kg/ha), 7.5 g ZnO Nps/ha, 100% NPK, 100% NPK + 5 kg Zn/ha through $ZnSO_4.7H_2O$, 100% NPK + 5 kg Zn/ha through ZnO (bulk), 100% NPK+ 7.5 g ZnO Nps/ha, 75% NPK+ 7.5 g ZnO Nps/ha, 50% NPK + 7.5 g ZnO Nps/ha, 25% NPK + 7.5 g ZnO Nps/ha and 12.5% NPK + 7.5 g ZnO Nps/ha were tested in a randomized block with three replications. The 100% NPK used was 120:60:40 N:P_2O_5:K_2O kg/ha and ZnO nanoparticles (mean particle size 38 nm) used at 7.5 g/ha. The aromatic rice variety 'Pusa 1612 was the test crop with a seed rate of 25 kg/ha to raise the nursery before transplanting. The gross plot size was 5 m × 4 m. The whole amount of P and K were applied at basal and required amount of nitrogen as per the treatments was applied in three splits, i.e. 50% at transplanting and remaining 50% in two equal splits at 22 and 45 days after transplanting (DAT). The $ZnSO_4.7H_2O$ and ZnO (bulk) were applied at transplanting as per the treatments. Required amount of nano Zn for the gross plot area was dissolved uniformly in one litre of water and suspension was made and required amount of NPK fertilizer for basal application as per treatment was added to it. Then the suspension was added to the soil uniformly at transplanting. The 24–days old seedlings were transplanted at 20 cm × 10 cm spacing in the first fortnight of July, 2018. Other standard agronomic packages of practices were followed for cultivation of rice.

The leaf area index was calculated as per Evans (1972). For dry matter determination samples were dried in shade for 5–7 days followed by oven drying (~60±2° C) for 24 hr. Grain yield was calculated from the area (leaving 2 border rows on each side) and reported at 14% moisture content. The 0.5 g of dry matter of grain and straw were taken and digested by di–acid mixture followed by analysis using atomic absorption spectrophotometry (Prasad *et al*. 2006). Zinc uptake was calculated by multiplying Zn concentration with the respective dry weight. Singh *et al.* (2014) method was used for computation of Zn harvest index (ZnHI), i.e.

$$\text{ZnHI (\%)} = \frac{\text{Zinc uptake by grain (g/ha)}}{\text{Zinc uptake by both grain + straw (g/ha)}} \times 100$$

The data was analyzed with the help of analysis of variance (ANOVA) using the F–test following Gomez and Gomez (1984). LSD values at P=0.05 were used to determine the significance of difference between treatment means.

At harvest, the rice plants were tallest (~108.8 cm) with application of 100% NPK + 7.5 g ZnO Nps/ha (Table 1) which was statistically at par with 75% NPK + 7.5 g ZnO Nps/ha, 100% NPK + 5 kg Zn/ha through $ZnSO_4.7H_2O$ and 100% NPK + 5 kg Zn/ha through ZnO bulk and these treatments were significantly taller than control. The shortest plant height was recorded in control (~ 93.1 cm) at harvest. The treatments, viz. 100% NPK + 7.5 g ZnO Nps/ha and 75% NPK + 7.5 g ZnO Nps/ha resulted 16.8% and 14.9%

Table 1 Effect of ZnO nanoparticles embedded NPK fertilizer on plant height, leaf area index (LAI), dry matter accumulation, tillers/m^2 and grain yield of aromatic rice

Treatment	Plant height at harvest (cm)	LAI at 90 DAT	Dry matter accumulation (g/hill) at 90 DAT	Tillers/m^2 (Nos.)			Grain yield (t/ha)
				30 DAT	60 DAT	90 DAT	
Absolute control	93.1	1.71	25.3	253	260	249	2.40
7.5 g ZnO Nps/ha	97.3	1.72	27.8	258	271	260	2.68
100% NPK	103.8	1.94	39.0	360	390	375	4.15
100% NPK + 5 kg Zn/ha through $ZnSO_4.7H_2O$	107.9	1.97	40.9	400	441	432	5.10
100% NPK + 5 kg Zn/ha through ZnO bulk	106.3	1.95	40.9	390	432	420	4.85
100% NPK+ 7.5 g ZnO Nps/ha	108.8	2.05	45.8	460	494	481	5.82
75% NPK + 7.5 g ZnO Nps/ha	107.0	2.00	45.6	440	485	475	5.53
50% NPK + 7.5 g ZnO Nps/ha	103.5	1.83	37.2	331	355	343	3.65
25% NPK + 7.5 g ZnO Nps/ha	102.1	1.79	33.3	308	340	325	3.10
12.5% NPK +7.5 g ZnO Nps/ha	100.7	1.70	31.5	273	301	281	2.84
SEm±	1.03	0.03	0.59	8.8	10.58	10.24	0.10
LSD (P=0.05)	3.07	0.10	1.75	26.13	31.43	30.44	0.30

ZnO Nps, ZnO nanoparticles; 100% NPK, 120:60:40 (N:P_2O_5:K_2O) kg/ha; DAT, Days after transplanting greater plant height, respectively over control. The lowest leaf area index (LAI) (Table 1) was recorded in control at 90 DAT which remained statistically at par with 7.5 g ZnO Nps/ha, 12.5% NPK + 7.5 g ZnO Nps/ha and 25% NPK + 7.5 g ZnO Nps/ha. The LAI was at par in 100% NPK + 7.5 g ZnO Nps/ha and 75% NPK + 7.5 g ZnO Nps/ha. At 90 DAT, both 100% NPK + 7.5 g ZnO Nps/ha and 75% NPK + 7.5 g ZnO Nps/ha gave highest dry weight (Table 1) and the control resulted in lowest dry weight (~25.33 g /hill)· The dry matter accumulation was comparable with application of 100% NPK + 5 kg Zn/ha through $ZnSO_4.7H_2O$ and 100% NPK + 5 kg Zn/ha through ZnO bulk which were significantly different from the application of 100% NPK.

At 30 DAT, the highest tillers/m^2 (Table 1) was recorded with application of 100% NPK + 7.5 g ZnO Nps/ha (~460 nos.) and 75% NPK + 7.5 g ZnO Nps/ha (~440 nos.) which remained significantly higher than rest of the treatments. Application of 100% NPK resulted in ~360 tillers/m^2. At 60 DAT, the highest number of tillers/ m^2 was recorded with application of 100% NPK + 7.5 g ZnO Nps/ha (~494 nos.) and 75% NPK + 7.5 g ZnO Nps/ ha (~485 nos.) which remained significantly different from rest of the treatments. Application of 100% NPK + 5 kg Zn/ha through $ZnSO_4.7H_2O$ and 100% NPK + 5 kg Zn/ ha through ZnO bulk were comparable. Due to very small size, nanoparticles possess high surface area. Nanoparticles coating on fertilizers help in sustained release of nutrients and prevent loss of nutrient due to higher surface tension than bulk surface (Duhan *et al*. 2017). It could be possible that nanoparticles help in taking nutrients throughout entire crop life span and thereby reduces losses (Chen and Yada 2011). Although zeolites don't belong to nanoscale, the high surface area and cation exchange capacity is attributed to arrangement of silicon (Si) and aluminium (Al) in making crystal which help in creating nanoscale channels and voids within 0.3–10 nm, thereby aid in slow release of nutrients augmented with zeolite (Liu and Lal 2015).

Application of 100% NPK + 7.5 g ZnO Nps/ha (~5.82 t/ ha) and 75% NPK + 7.5 g ZnO Nps/ha (~5.53 t/ha) recorded at par grain yield (Table 1) which was significantly higher over rest of the treatments. Grain yield was comparable under 100% NPK + 5 kg Zn/ha through $ZnSO_4.7H_2O$ and 100% NPK + 5 kg Zn/ha through ZnO bulk and remained significantly different from rest of the treatments. Application of 100% NPK resulted in the grain yield of ~4.15 t/ha which remained significantly lower than 100% NPK + 5 kg Zn/ha and 100% NPK + 5 kg Zn/ha through ZnO bulk. The lowest grain yield was recorded in control (~2.40 t/ha) which remained statistically at par with 7.5 g ZnO Nps/ha (~2.68 t/ha). Improvement in economic yield was reported by Tarafdar *et al*. (2014) who found 37.7% higher grain yield over the control by foliar application of zinc nano–fertilizer in pearl millet.

Fertilizer treatments, viz 100% NPK + 7.5 g ZnO Nps/ha and 75% NPK + 7.5 g ZnO Nps/ha recorded at par Zn concentration in grain and these treatments were significantly higher from rest of the treatments (Table 2). Similarly, Zn concentration in grain was comparable in treatments, viz 100% NPK + 5 kg Zn/ha through $ZnSO_4.7H_2O$ and 100% NPK + 5 kg Zn/ha through ZnO bulk. The highest Zn concentration in straw was recorded with application of 100% NPK + 7.5 g ZnO Nps/ha which were significantly different from rest of the treatments. The fertilizer treatments, viz 100% NPK + 5 kg Zn/ha through ZnO bulk and 100% NPK + $ZnSO_4.7H_2O$ were comparable. The highest Zn uptake in both grain and straw were recorded with application of 100% NPK + 7.5 g ZnO Nps/ ha which was significantly higher than rest of the fertilizer treatments. Application of 75% NPK + 7.5 g ZnO Nps/ha resulted in Zn uptake in grain and straw ~179.6 g/ha and

Table 2 Effect of ZnO nanoparticles embedded NPK fertilizer on Zn concentration in rice grain, straw and their uptake and zinc harvest index of aromatic rice

Treatment	Zn concentration in grain (mg/kg)	Zn concentration in straw (mg/kg)	Zn uptake by grain (g/ha)	Zn uptake by straw (g/ha)	Total Zn uptake (g/ ha)	Zinc harvest index (%)
Absolute control	27.2	44.2	65.4	156.0	221.4	29.5
7.5 g ZnO Nps/ha	27.4	45.2	73.5	178.7	252.2	29.2
100% NPK	28.5	53.0	118.3	285.7	404.0	29.3
100% NPK + 5 kg Zn/ha through $ZnSO_4.7H_2O$	30.9	56.0	157.9	350.5	508.4	31.1
100% NPK + 5 kg Zn/ha through ZnO bulk	30.7	56.0	149.0	335.7	484.6	30.8
100% NPK+ 7.5 g ZnO Nps/ha	33.0	61.5	192.0	413.9	605.9	31.7
75% NPK + 7.5 g ZnO Nps/ha	32.5	59.3	179.6	382.8	562.4	32.0
50% NPK + 7.5 g ZnO Nps/ha	28.1	52.5	102.8	260.8	363.6	28.3
25% NPK + 7.5 g ZnO Nps/ha	27.6	49.8	85.4	216.5	301.9	28.3
12.5% NPK + 7.5 g ZnO Nps/ha	27.3	48.5	77.5	198.4	275.8	28.2
SEm±	0.38	0.69	4.00	10.28	13.34	0.54
LSD (P=0.05)	1.14	2.07	11.88	30.55	39.63	1.60

ZnO Nps, ZnO nanoparticles; 100% NPK, 120:60:40 ($N:P_2O_5:K_2O$) kg/ha

~382.8 g/ha, respectively which was significantly different from rest of the fertilizer treatments. Similarly, Zn uptake by both grain and straw were comparable with application of 100% NPK + 5 kg Zn/ha through $ZnSO_4.7H_2O$ and 100% NPK + 5 kg Zn/ha through ZnO bulk. The lowest Zn uptake by grain was recorded in control which remained on par with 7.5 g ZnO Nps/ha and 12.5% NPK + 7.5 g ZnO Nps/ha. Likewise, the lowest Zn uptake by straw was obtained in control (~156.0 g/ha) which remained on par with 7.5 g ZnO Nps/ha. The total Zn uptake (Table 2) was recorded highest with application of 100% NPK + 7.5 g ZnO Nps/ha which were significantly higher from rest of the fertilizer treatments. Application of 75% NPK + 7.5 g ZnO Nps/ha registered total Zn uptake ~562.4 g/ha. Total Zn uptake were comparable with application of 100% NPK + 5 kg Zn/ha through $ZnSO_4.7H_2O$ and 100% NPK + 5 kg Zn/ha through ZnO bulk. Application of 100% NPK resulted in total Zn uptake ~404.0 g/ha which remained significantly different from rest of the treatments. The lowest total Zn uptake was obtained in control which remained on par with 7.5 g ZnO Nps/ha and 12.5% NPK + 7.5 g ZnO Nps/ha. Application of 100% NPK + 5 kg Zn/ha through $ZnSO_4.7H_2O$, 100% NPK + 5 kg Zn/ha through ZnO bulk, 100% NPK + 7.5 g ZnO Nps/ha and 75% NPK + 7.5 g ZnO Nps/ha were statistically at par with 12.5% NPK+ 7.5 g ZnO Nps/ha and 25% NPK + 7.5 g ZnO Nps/ha with respect to ZnHI (%) (Table 2). Moreover, the dissolution kinetics of nanoparticles is different from than that of bulk particles (Liu and Lal 2015), which help in effective use of nutrients. Significant improvements in root Zn concentration of gram and mungbean seedlings were reported by nanoscale treatment of ZnO (Mahajan *et al*. 2011). Several findings are reported pertinent to enhancement in concentration of nutrients by application of nanoscale particles like silver nanoparticles in *Lolium multiform* (Yin *et al*. 2011), nano–silica powder in maize (Yuvakkumar *et al*. 2011), nano iron (Fe) particles in cowpea (Afshar *et al*. 2012), nano–ZnO in groundnut (Prasad *et al*. 2012) and nano–chelated iron particles in spinach (Vattani *et al*. 2012) etc.

SUMMARY

Application of ZnO nanoparticles in conjunction with NPK fertilizers helped in increasing growth parameters, enhancement in Zn concentration in rice grain and straw and also grain yield and could save a significant amount of nutrients. As highest grain yield was recorded with application of 100% NPK + 7.5 g ZnO Nps/ha which was statistically at par with 75% NPK + 7.5 g ZnO Nps/ha, hence it can be concluded that application of 75% NPK + 7.5 g ZnO Nps/ha is best as it required 25% less NPK. Likewise, ZnO nanoparticles application had a significant effect on Zn concentrations. Therefore, it can be inferred that ZnO nanoparticles application embedded with NPK fertilizer can save nutrients, increase nutrient concentration and also profit can be accrued due to less requirement of nutrients than recommended dose of fertilizers.

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