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Poornima R
Ph.D Scholar, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India

Koti RV
Professor, Department of Crop Physiology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India

Correspondence
Poornima R
Ph.D Scholar, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India

Effect of nano zinc oxide on growth, yield and grain zinc content of sorghum (*Sorghum bicolor*)

Poornima R and Koti RV

Abstract
An investigation was initiated at Department of Crop Physiology, UAS, Dharwad, Karnataka to examine the effects of nano zinc oxide on sorghum (*Sorghum bicolor* var M-35-1) growth, yield and grain Zn content. A pot experiment consisted of twelve treatments comprised of seed priming (200, 500 and 1000 ppm of nano ZnO and bulk $ZnSO_4$), foliar application (200, 500, 1000, and 1500 ppm of nano Zno) and 1000 ppm of $ZnSO_4$ (package of practice) with six replications was carried out. Nano ZnO 500 ppm spray recorded significantly higher plant height (186.7), leaf area (34), leaf area index (2.7) and TDM (143.7) as compared to 1000 ppm bulk $ZnSO_4$ (185, 31.7, 2.52 and 128.5) respectively. TDM is 16 percent higher in 500 ppm of nano foliar spray as compared to 1000 ppm of bulk foliar spray. Grain yield was 9.5 percent higher in 500 ppm of NFS and 1000 ppm NFS as compared to 1000 ppm of BFS. The gain zinc content was 5.6 % higher in 500 ppm of NFS as compare with 1000 ppm of BFS. Compared to control both nano ZnO (18 %) and Bulk $ZnSO_4$ (11%) have higher zinc accumulation in their grains. The inhibitory effect was observed in nano ZnO concentration >1000 ppm reveals the toxicity and need for judicious usage of nano particles in foliar applications. This is the first report on the effect of nano ZnO particles on sorghum growth, yield and grain Zn.

Keywords: Nano particles, zinc oxide, sorghum, seed priming, foliar application

Introduction
The major challenge for global food and nutrition security is to feed the increasing world population with nutritious food. Therefore in the future, it is essential to increase not only production but also of high quality food with the required level of nutrients and protein is the main challenge. Zinc is an essential micronutrient for humans, animals and plants. Indian soils are deficient in zinc and food crops grown on these soils and human beings living in this area are suffering from zinc deficiency. Sorghum is a major cereal grown in Maharastra and Northern Karnataka and bulk of the food consumed by the people in these regions contains sorghum. Cereals production in general is affected due to low soil Zn in the calcareous soils of these states and the grain Zn content has also found to be very low in the sorghum grown on these soils. To overcome this nutritional problem and crop production, conventionally bulk form of $ZnSO_4$ is applied to soil or to foliage as exogenous source, but most of the time the bulk forms are going to be fixed in the soil and making it not available to the rhizosphere and becomes toxic to the soil microorganisms and plants. Sustainable agriculture mainly aims to reduce application of chemical fertilizer and minimize nutrient losses in fertilization and increase yields through optimized nutrient management. Recently, nanotechnology is coming into focus because nano particles (NPs) are small in size (<100 nm) having high surface area and reactivity. Recent studies revealed that powder or nano sized particles are found to be effective in absorption and translocation. But, physiological aspects of nano zinc application and its accumulation in grains crops are meagre. Hence, the present study was carried out to investigate the effects of various concentrations of Zinc Oxide (ZnO) NPs on growth, yield and grain Zn content in *rabi* sorghum (*Sorghum bicolor*) *var* M-35-1.

Material and methods
A pot experiment was carried out in sorghum *var* M-35-1 *rabi* 2015 at Department of Crop Physiology, UAS, Dharwad. The pots were filled with black calcareous soil. Zinc oxide nano particle with average particle size (APS) of ≈ 30 nm and bulk $ZnSO_4\ 7H_2O$ were used in the experiment as source of nano and bulk form of zinc respectively.

Characterization of ZnO nanoparticles
Size of the nano particles were confirmed initially by UV-visible spectrophotometer (Perkin-Elmer UV-VIS spectrophotometer, Lambda-19) to know the kinetic behaviour of ZnO nanoparticles.

The scanning range for the samples was 200-850 nm at a scan speed of 480 nm min^{-1}. Base line correction of the spectrophotometer was carried out by using a blank reference. The curve excitation peak was at 300 nm indicating the presence of ZnO nanoparticle (Fig. 1). Further the purity of ZnO nano particles was examined and size of nano particles is confirmed by field emission scanning electron microscope (FE-SEM) at 1 µm and 500 nm (Fig. 2).

Preparation of particle suspensions for seed priming and foliar treatment

Required concentration of ZnO nano particle was suspended directly in deionised water and dispersed by ultrasonic vibration (100 W, 40 KHz) for 30 min. Magnetic bars were placed in the suspensions for stirring before use to avoid aggregation of the particles. The nano scale suspensions as expected appeared as clear solutions. The pH of all the suspensions were measured and adjusted to 6.8 to 7.0. Bulk $ZnSO_4$ was used as a reference Zn source and it dissolves easily in water.

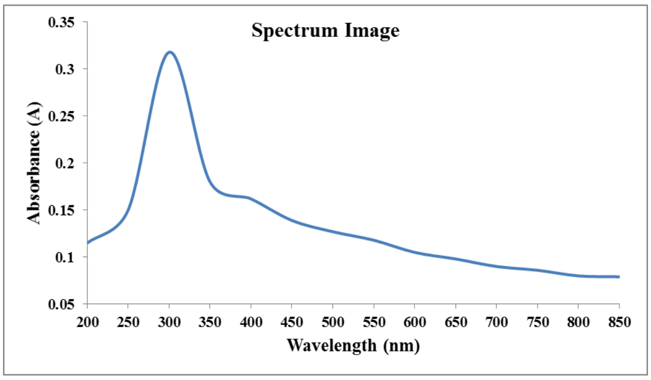

Fig 1: The UV- visible spectra of ZnO nanoparticles formulations shows a strong absorption at 300 nm

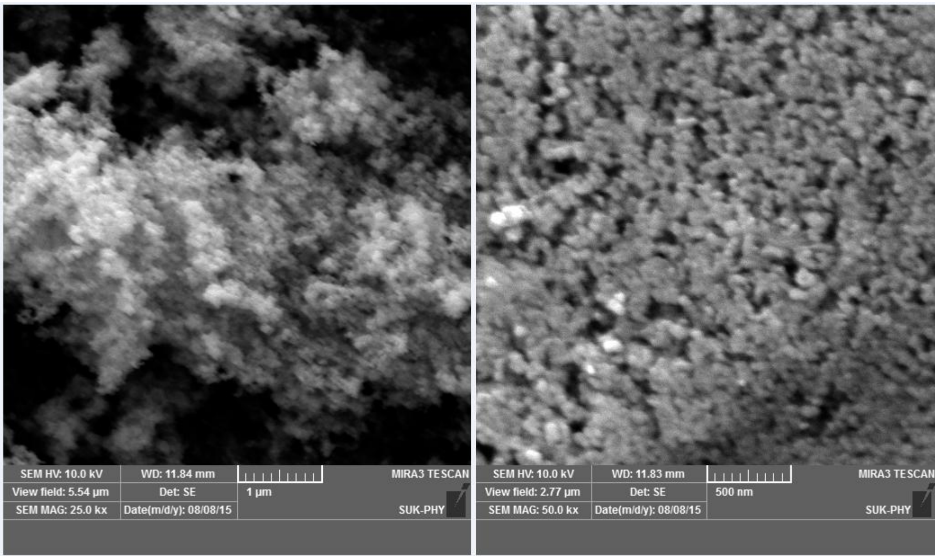

Fig 2: Field Emission Transmission Electron Microscope (FE-SEM) image of ZnO nanoparticles at 1 µm and 500 nm

Seed priming and foliar application of nano ZnO and bulk $ZnSO_4$

The bulk $ZnSO_4$ solution and nano ZnO suspensions were prepared with concentrations of 200, 500, 1000 ppm (concentrations in terms of Zn content). Seeds were soaked in 100 ml of these solution of bulk $ZnSO_4$ (bulk seed priming: BSP) and suspensions of nano ZnO (nano seed priming: NSP) for six hours and shade dried till it reaches initial weight. Simultaneously, two sets of seeds were soaked in water and shade dried for foliar treatments (200, 500, 1000 and 1500 ppm of nano ZnO and 1000 ppm bulk $ZnSO_4$) along with control (without zinc). For each treatment six replications were maintained. Both nano ZnO foliar spray (NFS) and bulk $ZnSO_4$ foliar spray (BFS) were carried out at 35 and 75 days after sowing. The morphological parameters like plant height, leaf area and leaf area index, total dry matter (TDM) are measured at 85 DAS and yield parameters like earhead length, harvest index (HI), test weight and grain yield were measured at harvest. After harvest, sorghum grains were powdered and digested using diacid mixture (nitric acid: perchloric acid at 10:4 ratio) and then the zinc content was estimated using atomic absorption spectrophotometer (GBC Avanta Ver 2.02 Model). For data analysis the level of significance used in 'F' and 't' test was P = 0.01. The least significant differences (LSD) values were calculated wherever the 'F' test was significant by Duncan's Multiple Range Test (DMRT).

Results and Discussion

In the present experiment application of nano zinc oxide as foliar spray recorded significantly higher growth, yield and grain zinc content as compared to bulk $ZnSO_4$ application.

Morphological parameters

Variation in the morphology of the plant is the interaction of plant metabolism and environmental effect Plant height and leaf area are two important and easily noticeable form of growth changes in the plant which ultimately leads to the increase in total dry matter of the plant and which has been observed with the application of different type and concentrations of zinc. The plant height (cm) varied significantly (Table 1) among the treatments and foliar application of 500 ppm NFS recorded significantly higher plant height (186.8) and is on par with 1000 ppm BFS (185), whereas lowest plant height was recorded in control (112.3). The results indicated that leaf area (dm^2) and leaf area index (LAI) (Table 1) was significantly higher in 500 ppm NFS (33.95 and 2.7) followed by 1000 ppm BFS (31.72 and 2.52), whereas significantly lower leaf area were recorded in control (20.56 and 1.64) respectively. Total dry matter (g $plant^{-1}$) production was significantly higher in nano foliar treatments as compared to bulk foliar and seed treatments. The TDM (Table 1) at harvest was significantly higher in 500 ppm NFS (143.7) followed by 1000 ppm BFS (128.77), whereas significantly lower TDM was recorded in control (89.5). TDM is 16 percent higher in 500 ppm of nano foliar spray as compared to 1000 ppm of bulk foliar spray. A coupled increase in plant height and photosynthetically active leaf area due to nano ZnO might be the reason for increased dry matter accumulation and also might be due to the complementary effect of other inherent nutrients like magnesium, iron and sulphur with zinc (Koti *et al*., 2009) [7]. Zinc acts as an activator of enzymes in plants and is directly involved in the biosynthesis of auxin, which produces more cells and dry matter that in turn will be stored in seeds as sink. Rahman *et al.* (2001) [15] and Nathan *et al.* (2001) [12] in rice, Genc *et al.* (2006) [5] and Faruk *et al.* (2006) [4] in bread wheat, Anand *et al*., (2008) [1] in *rabi* sorghum found similar increase in total dry matter, yield and seed zinc content due to external zinc application. Probable reason might be due to favourable effect of zinc on the proliferation of roots and thereby increasing the uptake of other plant nutrients from the soil, supplying it to the aerial parts of the plant and ultimately enhancing the vegetative growth of plants. Plant height, leaf area, LAI and TDM were found highest in 1000 ppm BSP (174, 28.9, 2.3 and 125.6) as compared to 1000 ppm NSP (163, 26.5, 2.11 and 95.1). Seed priming with bulk form of zinc is efficient as compared to nano form of zinc in sorghum. Comparatively lower growth in nano treated seeds might be because of the toxic effect of nano particles, so the concentration and soaking time needs to be reduced. Similar results were obtained in the studies of Zafar *et al*. (2016) [18], where the concentration of 1500 mg L^{-1} ZnO NPs adversely influences the *Brassica nigra* seed germination and seedling development. Yang *et al*. (2015) [17] also observed phytotoxic effect of seven metal oxide nanoparticles (NPs) on maize and rice and concluded that phytotoxicity is mainly concentration dependent, but not brought about by the relating metal ion nano particles

Yield and grain zinc content

The overall increase in the growth of the plant has been reflected ultimately in the yield and yield related parameters. The results (Table 2) indicated that there is a significant variation among seed treatment and foliar applications of nano ZnO and $ZnSO_4$ along with control. Yield attributes like ear head length (cm), harvest index percent (HI %), test weight (g) and grain yield (g $^{-1}$ plant) varied significantly among the treatments whereas higher grain yield was recorded in 500 ppm NFS (63) as compared to 1000 ppm BFS (57.5). Test weight was significantly higher in 500 ppm NFS (45.69) as compared to 1000 ppm BFS (42.23). The ear head length at harvest was significantly higher in 500 ppm NFS (19.33) followed by 1000 ppm NFS (19), whereas significantly lowest was recorded in control (12) which was on par with 200 ppm NSP (12.17), 500 ppm NSP (14).

Table 1: Plant height, Leaf area, LAI and TDM as influenced by nano ZnO and bulk $ZnSO_4$ application in sorghum

Treatments	Plant height (cm)	Leaf Area (dm^2)	Leaf Area Index	Total Dry Matter (g $plant^{-1}$)
200 ppm BSP	164.67^{a}	27.03^{a-c}	2.15^{a-c}	106.83^{bc}
500 ppm BSP	171.00^{a}	28.44^{ab}	2.26^{a-c}	124.90^{ab}
1000 ppm BSP	174.00^{a}	28.89^{ab}	2.30^{a-c}	125.60^{ab}
200 ppm NSP	128.67^{b}	24.86^{bc}	1.98^{bc}	094.77^{c}
500 ppm NSP	164.33^{a}	26.63^{bc}	2.12^{a-c}	096.23^{c}
1000 ppm NSP	163.00^{a}	26.51^{bc}	2.11^{a-c}	095.07^{c}
200 ppm NFS	166.00^{a}	27.54^{a-c}	2.19^{a-c}	110.00^{bc}
500 ppm NFS	186.67^{a}	33.95^{a}	2.70^{a}	143.70^{a}
1000 ppm NFS	185.00^{a}	30.26^{ab}	2.41^{ab}	126.77^{ab}
1500 ppm NFS	175.00^{a}	29.62^{ab}	2.36^{ab}	125.73^{ab}
1000 ppm BFS	185.00^{a}	31.72^{ab}	2.52^{ab}	128.47^{ab}
Control	112.33^{b}	20.56^{c}	1.64^{c}	089.50^{c}
Mean	164.64	28.00	2.23	113.96
S.Em.$\pm$	7.10	1.59	0.15	5.11
LSD @1 %	28.31	6.36	0.61	20.37

BSP- Bulk $ZnSO_4$ seed priming, NSP-Nano ZnO seed priming, NFS-Nano ZnO Foliar Spray, BFS-Bulk $ZnSO_4$ Foliar Spray

The harvest index was significantly higher in 1000 ppm NFS (57) on par with 1000 ppm BFS (59) indicating that HI is not much influenced by nano form of zinc application. Interestingly, there is an increase in grain yield by 9.5 percent in 500 ppm of NFS as compared to 1000 ppm of BFS. Zinc content in the grain was higher in 500 ppm NFS (19.73 ppm) as compared to 1000 ppm BFS (18.68 ppm), whereas lowest is observed in control (16.6 ppm). The gain zinc content was 5.6 % higher in 500 ppm of NFS as compare to 1000 ppm of BFS. Zinc content in grain has improved in both nano and bulk treatments as compared to control, but interestingly the percent increase over control was high in nano ZnO (18%) than Bulk $ZnSO_4$ (11%). Among seed priming, earhead length and grain yield was found highest in all the BSP treatments as compared to the NSP but as a contrary the same is not reflected in the grain zinc content in BSP treatment. This is clearly an evident from the data that even thought the yield is high in both nano and bulk forms of zinc seed priming but the grain nutritional quality is improved in the seed primed with nano zinc but not with bulk zinc. The probable reason might be because of the high uptake and translocation efficiency of nano zinc oxide than bulk zinc form. Similarly, Zhou *et al.*, (2011) also reported that ZnO NPs with high specific surface and surface reactivity cannot only be easily adsorbed on physical surface, but also react with biological proteins and even absorbed into the cell faster. Lin and Xing (2008) also investigated that ZnO NPs were primarily adsorbed onto the cell surface and then their uptake is followed further quickly and efficiently translocated to the sink.

It is worth mentioning here that higher/toxic doses of ZnO NPs *i.e.* >1000 ppm caused significant reduction in grain yield of sorghum, so it clearly indicates that nano particles are efficient and helps in increasing yield at much lower concentrations itself as compared to bulk forms of zinc and the results were in accordance with the reports on radish, rape, corn, lettuce and cucumber by Lin and Xing, 2007. Lower doses of nano ZnO is sufficient to achieve positive response and higher doses showed growth retardation.

Nano ZnO foliar application at the half (500 ppm NFS) or equal (1000 ppm NFS) concentration when compared to its counter bulk $ZnSO_4$ (1000 ppm BFS) has significantly increased the growth and yield in sorghum. Positive improvement in NFS might be due to the quick translocation and assimilation of Zn nanoparticles which further leading to the expression of growth accelerating enzymatic activity and auxin metabolism in plants. The probable reasons for inefficiency of bulk $ZnSO_4$ are its high solubility and low retention time in the plant system. So the bioavailability/ sustainable availability of bulk particle inside the plant system and also at the site of uptake are not confirmed. But as an advantage over bulk nano ZnO is quickly absorbed by leaf surface and also metabolized faster than bulk form because of its nano size, high surface to volume ratio and high reactivity.

Similar to our studies nano ZnO showed significantly higher crop improvement by enhancing initial crop establishment, chlorophyll content and ultimately crop growth and yield were obtained in the studies of Pandey *et al.*, (2010) [13] in *Cicer arietinum*, Boonyanitipong *et al.*, (2011) [2] in rice, Prasad *et al.*, (2012) [14] in peanuts, Sedghi *et al.*, (2013) [16] in soybean, Jayarambabu *et al.*, (2014) [6] in mungbean and Yang *et al.*, (2015) [17] in maize and rice. Similarly, Laware and Raskar, (2014) [8] also reported significant increase in root growth and dry weight in case of onion after zinc oxide nano particles application. In chickpea seedling similar results were obtained by Burmana *et al.* (2013) [3] and Mahajan *et al.* (2011) [11] when treated with nano ZnO. Prasad *et al.* (2012) [14] found that foliar application of zinc oxide nano particle is more effective than the soil application and there was an improvement of pod yield and zinc content in peanut by zinc oxide foliar application.

Table 2: Yield components and grain zinc content as influenced by nano ZnO and bulk $ZnSO_4$ application in sorghum

Treatment	Ear head length (cm)	Harvest Index (%)	Test wt (g)	Grain yield (g plant^{-1})	Grain zinc (ppm)
200 ppm BSP	14.00^{de}	44^{de}	39.01^{b-d}	52.4^{bc}	14.40^{bc}
500 ppm BSP	16.33^{c}	38^{e}	40.20^{a-c}	52.9^{bc}	16.58^{a-c}
1000 ppm BSP	16.83^{bc}	39^{e}	40.20^{a-c}	53.8^{a-c}	13.75^{c}
200 ppm NSP	12.17^{e}	47^{cd}	34.98^{cd}	46.4^{c}	15.63^{a-c}
500 ppm NSP	14.00^{de}	44^{de}	36.93^{b-d}	49.8^{bc}	17.60^{a-c}
1000 ppm NSP	14.00^{de}	52^{a-c}	36.79^{b-d}	48.1^{bc}	18.40^{ab}
200 ppm NFS	16.00^{cd}	41^{de}	39.46^{b-d}	51.7^{bc}	16.88^{a-c}
500 ppm NFS	19.33^{a}	55^{ab}	45.69^{a}	63.0^{a}	19.73^{a}
1000 ppm NFS	19.00^{ab}	57^{a}	41.61^{ab}	55.1^{a-c}	16.15^{a-c}
1500 ppm NFS	17.70^{a-c}	48^{b-d}	40.30^{a-c}	55.2^{a-c}	16.35^{a-c}
1000 ppm BFS	19.00^{ab}	59^{a}	42.23^{ab}	57.5^{ab}	18.68^{a}
Control	12.00^{e}	42^{de}	33.70^{a}	46.2^{c}	16.6^{a-c}
Mean	15.86	47	39.26	52.68	16.7
S.Em.$\pm$	0.52	02	1.37	2.21	0.93
LSD @1 %	2.07	07	5.47	8.81	3.72

BSP- Bulk $ZnSO_4$ seed priming, NSP-Nano ZnO seed priming, NFS-Nano ZnO Foliar Spray, BFS-Bulk $ZnSO_4$ Foliar Spray

Conclusion

The study shows that application of nano ZnO recorded more yield and growth of sorghum as compare to bulk $ZnSO_4$. Hence, by using a very less quantity of fertilizers may reduce the application doses of fertilizers, wastage of fertilizers, environmental hazards and increase nutrient use efficiency. There is need to study the effects of nano zinc oxide particles on soil beneficial microorganisms and different beneficial process like nitrification, nitrogen fixation, decomposition of organic material, mineralization, and immobilization. There is also need to standardize the nano fertilizers doses for different crop and optimum stage of crop for to achieve better crop production and also need to know the intra and extra cellular mechanisms involved in uptake and translocation of nano particles. In conclusion, foliar fertilization of 500 ppm NFS was found more effective than 1000 ppm BFS. Seed treatment with bulk bulk $ZnSO_4$ has given higher TDM and grain yield but grain zinc content was highest was highest in nano ZnO treatments. So further there is a need to optimize the nano ZnO concentration and time of exposure to seed priming. Among the method of application foliar spray is found much better than seed priming in the present study.

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