Influence of zinc oxide nano particle on biophysical parameters, yield and grain zinc content of rabi sorghum (*Sorghum bicolor*)

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Abstract

A pot experiment was conducted to find out the influence of zinc oxide nano particle on photosynthesis, shoot and root growth and yield in *rabi* sorghum (*Sorghum bicolor var M-35-1*) at Department of Crop Physiology, UAS, Dharwad, Karnataka. Experiment consisted of twelve treatments comprised of seed priming (200, 500 and 1000 ppm of nano ZnO and bulk ZnSO₄), foliar application (200, 500, 1000, and 1500 ppm of nano Zno) and 1000 ppm of ZnSO₄ (package of practice) with six replications was carried out. Nano ZnO 500 ppm of foliar spray recorded significantly higher shoot length (186.67), shoot dry weight (108.6), root length (83.33), root volume (310) and root dry weight (57.23) as compared to 1000 ppm bulk ZnSO₄. Nano ZnO 500 ppm foliar spray recorded significantly higher chlorophyll content (1.39), SPAD values (49.57), photosynthetic rate (41.23), transpiration rate (0.19) and stomatal conductance (7.27) as compared to 1000 ppm bulk ZnSO₄. Grain yield was 9.5 percent higher in 500 ppm NFS and 1000 ppm NFS as compared to 1000 ppm BFS. The gain zinc content was 5.6% higher in 500 ppm of NFS as compared to 1000 ppm of BFS. Compared to control both nano ZnO (18%) and Bulk ZnSO₄ (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, photosynthesis, grain yield and zinc content.

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

Introduction

Among abiotic stresses nutritional stress is the third most important stress limiting plant productivity. Among different nutrients, Zn is considered as the fourth most important yield-limiting nutrient after nitrogen (N), phosphorus (P) and potassium (K). 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. Therefore the zinc content in grains, such as rice, wheat and sorghum is of major importance which is consumed in bulk worldwide and Karnataka (Anand et al., 2008) [2]. 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 Maharashtra and Northern Karnataka and bulk of the food consumed by the people in these regions contains sorghum. To overcome this nutritional problem and crop production, conventionally bulk form of ZnSO₄ is applied to soil or to foliages as exogenous source, but most of the time the bulk forms are going to be fixed in the soil and making it non-available to the rhizosphere and becomes toxic to the soil microorganisms and plants. 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 (White Plastic bags) experiment was carried out in sorghum var M-35-1 *rabi* 2015 at Department of Crop Physiology, UAS, Dharwad. The bags were filled with calcareous soil. Zinc oxide nano particle (ZnO NPs) with average particle size (APS) of ≈ 30 nm and bulk ZnSO₄•H₂O were used in the experiment as source of nano and bulk form of zinc respectively (Plate 1).

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. Bulk ZnSO₄ was used as a reference Zn source and it dissolves easily in water.

Seed priming and foliar application of nano ZnO and bulk ZnSO₄
The bulk ZnSO₄ 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 ZnSO₄ (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 ZnSO₄) along with control (without zinc). For each treatment two replications were maintained. Both nano ZnO foliar spray (NFS) and bulk ZnSO₄ foliar spray (BFS) were carried out at 35 and 75 days after sowing. The morphological parameters like shoot length, shoot dry weight, root length, root volume, root dry weight and shoot chlorophyll content, SPAD values, Photosynthetic rate, transpiration rate and stomatal conductance are measured at 85 DAS. Grain yield and grain zinc content was recorded at harvest. 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 chlorophyll content, SPAD values, Photosynthetic rate, transpiration rate, stomatal conductance, shoot length, shoot dry weight, root length, root volume, root dry weight, grain yield and grain zinc content as compared to bulk ZnSO₄ application.

Influence of zinc oxide nano particles on chlorophyll content and photosynthesis
The leaf chlorophyll content is often used as an indicator of plant growth for evaluating assimilation and vigour and is also a major factor determining the solar radiation interception, canopy photosynthesis. All the essential mineral nutrients directly or indirectly have role to play in photosynthetic process. Zinc is one among them, proved that external supply would bring about changes in physiology and growth of plants (Anand et al., 2008 in sorghum; Dore et al., 2016 in rice; Raghavendra et al., 2015 in wheat) [2, 8, 17]. In the experiment nano zinc oxide is adopted because of its unique properties like small size and high reactivity as compared to bulk ZnSO₄.

Nano ZnO at concentration of 500 ppm NFS and followed by 1000 ppm NFS proved to be optimum and effective in improving chlorophyll content (1.39 and 1.28), SPAD values (49.57 and 47.23), Photosynthetic rate (41.23 and 40.97), Transpiration rate (0.19 and 0.18) and stomatal conductance (7.27 and 6.93) as compared to 1000 ppm BFS and control (Table 1) respectively. Similar results were reported by Zayed et al. (2011) [24] and Sarwar et al. (2013) [20] in rice crop but no reports are available in sorghum.

Plants treated with nano ZnO had significantly higher chlorophyll content than the bulk Zn. Zinc is known to have primary role in leaf photosynthesis and may probably keep up chlorophyll synthesis through sulphhydryl bunch protection (Cakmak 2000) [8] and plays major role in chlorophyll synthesis and activation of some proteins (Broadley et al., 2007) [4]. Earlier works also confirm that zinc application improves chlorophyll content (Chaab et al., 2011; Weisany et al., 2014; Kotti et al., 2009 and Gurmani et al., 2012) [7, 23, 12]. Taiz and Zeiger (2010) [21] reported that many enzymes require Zn⁺ ion for their activity, and Zn may be required for chlorophyll biosynthesis in plants. Besides that, Zn plays a key role in photosynthesis, affecting the activity of enzymes such as carbonic anhydrase (Rengel, 1995a) [19], as well as affecting chlorophyll concentration and stomatal conductance (Hu and Sparks, 1991). In this context, Bergmann (1992) [3] reported that Zn is believed to be involved in chlorophyll synthesis through its influence on protein, carbohydrate, and energy metabolism. Supportive to the present work, bioengineered nano ZnO in cotton increased the rate of photosynthetic pigments significantly even at higher doses, and this might be due to the presence of phyto molecule ligands on the nano ZnO and level of total soluble protein (Priyanka and Venkatachalam, 2016) [10]. Results of Adhikari et al. (2015) indicated that seed coating of maize, soybean, pigeon pea and ladies finger with micron and nano scale zinc through different sources increased the total chlorophyll content in the leaves and IAA production by root and subsequently increases the overall growth of the plant.

Influence of zinc oxide nano particles on shoot and root growth
Root is the most important and deciding plant part for uptake of water and nutrients from soil and translocation to the above ground parts, similarly shoot is the deciding part of the plant in assimilation and photosynthesis leading to crop growth and yield. Results revealed that nano ZnO at concentration of 500 ppm proved to be optimum and effective in improving shoot length (186.67), shoot dry weight (108.60) root length (83.33), root dry weight (57.23), root volume (310), grain yield (63) and grain zinc content (19.73) respectively as compared to 1000 ppm of bulk ZnSO₄ and control (Table 2 and Plate 2).

Although the root to shoot ratios are the main indicators of understanding the division of photosynthates and nutrients to above and below ground parts and helps to know the source to sink ratio (Upadhyaya et al., 2015) [22]. Zinc acts as an activator of enzymes in plants and is directly involved in the biosynthesis of auxin, which produces more cells and leads to high shoot and root growth in studies of Rahman et al. (2001) [18] and Nathan et al. (2001) [15] in rice, Genç et al. (2006) [10] and Faruk et al. (2006) [9] in bread wheat, 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 helps in enhancing the vegetative growth and reproductive growth of plants.

Similar results were obtained by Burmana et al. (2013) [5] and Mahajan et al. (2011) [14] in chickpea seedling supplied with nano ZnO. Similarly, Laware and Raskar, 2014 also reported significant increase in root growth and dry weight in case of onion after zinc oxide nano-particles application. Higher uptake of other mineral nutrients are also known to increase the demand of Zn and also by the complementary effect of other inherent nutrients like magnesium, iron and sulphur (Koti et al., 2009) [12]. The results revealed that nano zinc oxide particle application has promotory effect at optimum concentration but has inhibitory/toxic effects at higher concentration on root and shoot growth. 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 ZnSO₄ (1000 ppm BFS) has significantly increased the growth and photosynthesis 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 ZnSO₄ 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 sized particles.

Table 1: Effect of nano ZnO and bulk ZnSO₄ application on chlorophyll content, SPAD values, photosynthetic rate, transpiration rate and stomatal conductance in sorghum at 85 DAS.

| Treatments | Total Chl content (mg g⁻¹ fwt) | SPAD values | Photosynthetic rate (µmole of CO₂ m⁻² sec⁻¹) | Transpiration rate (µmole of H₂O m⁻² sec⁻¹) | Stomatal conductance (µmol H₂O m⁻² sec⁻¹) |
|------------|-----------------------------|-------------|---------------------------------|---------------------------------|---------------------------------|
| 200 ppm BSP | 0.51bc | 46.03ec | 32.63b | 0.15bc | 5.20c |
| 500 ppm BSP | 0.55bc | 46.80ab | 35.53bc | 0.17bc | 5.74bd |
| 1000 ppm BSP | 0.55bc | 46.83ab | 38.50a | 0.18ab | 6.25ac |
| 200 ppm NSP | 0.47c | 42.83bc | 31.40d | 0.12a | 4.80bc |
| 500 ppm NSP | 0.57bc | 45.03ec | 32.27d | 0.14c | 5.17c |
| 1000 ppm NSP | 0.74a | 43.57ab | 32.07d | 0.13a | 5.14c |
| 200 ppm NFS | 0.57bc | 46.07ab | 33.47d | 0.16d | 5.29c |
| 500 ppm NFS | 1.39b | 49.57b | 41.23a | 0.19a | 7.23a |
| 1000 ppm NFS | 1.28a | 47.23ab | 40.97b | 0.18ab | 6.93a |
| 1500 ppm NFS | 0.74a | 46.97ab | 39.40a | 0.18a | 6.47ab |
| 1000 ppm BFS | 0.90a | 47.50ab | 41.17a | 0.18a | 7.11a |
| Control | 0.64st | 40.40b | 29.77c | 0.11b | 4.40b |
| S.Em.± | 0.03 | 1.31 | 0.64 | 0.01 | 0.26 |
| LSD @1% | 0.12 | 5.24 | 2.55 | 0.03 | 1.03 |

BSP-Bulk Zn Seed priming, NSP-Nano Zn Seed priming, NFS-Nano Zn Foliar Spray, BFS-Bulk Zn Foliar Spray

Table 2: Effect of nano ZnO and bulk ZnSO₄ application on shoot length, shoot dry weight, root length, root volume, root dry weight, grain yield and grain zinc in sorghum.

| Treatment | Shoot length (cm plant⁻¹) | Shoot dry weight (g plant⁻¹) | Root length (cm plant⁻¹) | Root volume (cm³) | Root dry weight (g plant⁻¹) | Grain yield (g plant⁻¹) | Grain Zinc (ppm) |
|----------|---------------------------|--------------------------------|--------------------------|-----------------|----------------------------|-------------------------|-----------------|
| 200 ppm BSP | 164.67a | 66.07cd | 67.33cd | 142.33d | 40.10c | 52.4bc | 14.40bc |
| 500 ppm BSP | 171.00a | 76.30c | 73.00bc | 163.33bc | 44.67b | 52.9bc | 16.58bc |
| 1000 ppm BSP | 174.00a | 80.50b | 73.67b | 213.67c | 45.17bc | 53.8bc | 13.75c |
| 200 ppm NSP | 128.67b | 58.03d | 65.00d | 230.00d | 31.27d | 46.4b | 15.63c |
| 500 ppm NSP | 164.33a | 63.40d | 67.33cd | 240.00d | 38.07c | 49.8bc | 17.60c |
| 1000 ppm NSP | 163.00a | 61.23d | 66.33d | 246.67c | 32.07d | 48.1bc | 18.40b |
| 200 ppm NFS | 166.00a | 68.00f | 72.67bc | 273.00ab | 40.70c | 51.7bc | 16.88c |
| 500 ppm NFS | 186.67a | 108.60d | 83.33a | 310.00d | 57.23a | 63.0b | 19.73a |
| 1000 ppm NFS | 185.00a | 91.20b | 80.00a | 285.00ab | 46.33b | 55.1c | 16.15c |
| 1500 ppm NFS | 175.00a | 80.50d | 78.00b | 250.00bc | 45.67b | 55.2c | 16.35c |
| 1000 ppm BFS | 185.00a | 102.87d | 81.00a | 291.67ab | 46.40b | 57.5b | 18.68a |
| Control | 112.33a | 51.33b | 62.00d | 133.33b | 28.00a | 46.2d | 16.6c |
| S.Em.± | 7.1 | 1.17 | 1.44 | 12.6 | 0.97 | 2.21 | 0.93 |
| LSD @1% | 28.31 | 4.66 | 5.72 | 50.22 | 3.88 | 8.81 | 3.72 |

BSP-Bulk Zn Seed priming, NSP-Nano Zn Seed priming, NFS-Nano Zn Foliar Spray, BFS-Bulk Zn Foliar Spray
Plate 1: General view of sorghum experiment treated with different concentrations of nano ZnO and bulk ZnSO₄

Plate 2: Variation in the root length and root volume of sorghum influenced by the application of nano ZnO and bulk ZnSO₄ (BSP- Bulk ZnSO₄ seed priming, NSP- Nano ZnO seed priming, NFS- Nano ZnO Foliar Spray, BFS- Bulk ZnSO₄ Foliar Spray, Concentrations are in ppm)

Conclusion
The study shows that application of nano ZnO recorded more photosynthetic rate, shoot, root growth, grain yield and grain zinc content of sorghum as compare to bulk ZnSO₄. Application of nano ZnO formulations may reduce the high doses of bulk fertilizers, wastage of fertilizers, environmental hazards and increase nutrient use efficiency. There is a need to standardize the optimum nano fertilizer dose for different crop and optimum stage of application to the crop to achieve better crop production. Need to understand 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. In conclusion, the nano ZnO application through foliar spray is effective to obtain the desired crop growth, yield and grain zinc content at much lower doses than bulk ZnSO₄.
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