

Original Research Article

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ZnO Nanoparticle Improves Maize Growth, Yield and Seed Zinc under High Soil pH Condition

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ABSTRACT

Zinc (Zn) is a micronutrient, which is involved in activating many physiological and biochemical processes. Inadequate supply of Zn affects crop growth, development and in turn yields. Most of us depend on agriculture based foods which are grown under inadequate Zn supply and hence we get less of Zn as recommended. A few strategic approaches are being adapted to overcome inadequacy of Zn in food. Biofortification is one of the best strategies to enhance the Zn content in the food grain. Among these, direct fertilizer application to the plant is the easiest way to enhance the Zn content in the food grain. Perhaps, excess use of Zn is not recommended because of heavy metal and di cationic characteristic properties. In this context application of the nano fertilizer enhances efficacy of targeted delivery and nutrient use efficiency. Zn deficiency is more common under conditions of high soil pH. In this regard a study was conducted to understand the efficacy of ZnO nanoparticle synthesised through biological approach under the high soil pH condition. Nano Zn treatment showed higher chlorophyll content, yield and shoot biomass as compared to ZnSO₄ treatment. Further there was no significant difference were observed in plant height, number of leaf per plant, leaf area, cob length, number of seeds/row, cob weight and test weight compared to ZnSO₄ treatment. Higher leaf Zn was found in the ZnO nano treatment with Seed priming + foliar application. Whereas, seed Zn content was high in biologically synthesized ZnO nanoparticle with seed priming treatment. Overall, the results indicated that the positive effect of nano Zn improved the growth, yield and Zn content under high pH conditions.

Keywords

Zinc, Biologically synthesised Nano, ZnSO₄, pH, Maize

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Introduction

Zinc (Zn) is fourth important micronutrient deficiency in human followed by Vitamin A, Iron (Fe) and iodine (I) globally. Zn deficiency leads to diarrhoea and pneumonia in children. Childhood dwarfism is considered as indication of Zn deficiency and globally

about 61 million children under the age of 15 years are reported to be dwarf (Hotz and Brown, 2004). Zn also plays an important role in synthesis of proteins and thus helps in wound healing, blood formation, growth and maintenance of tissue (Bell and dell, 2008). Stein *et al.*, (2007) indicated that using DALYs (Disability Adjusted Life Years)

showed that Zn deficiency is a highly relevant health problem and is responsible for a loss of 2.8 million DALYs per annum in India. Biofortification of cereals (Rice and Wheat) might reduce this burden by 20–51 per cent and save 0.6–1.4 million DALYs each year. Importance of Zn in human nutrition and health has received tremendous attention in India.

Plants play a major role in human nutrition it provides all essential nutrients required for the better health. However, not in a position to meet the daily required mineral nutrients, most of the essential micronutrients are not present in required concentrations in the food grain. Most of the edible food crops have lower amount of Zn. Therefore, value addition of food crops is essential for micronutrient fortification especially Zn. Globally 50 per cent soils are Zn deficient and hence plants are not able to take up, translocate and accumulate enough amount in edible parts that can meet the human nutrition requirement (Adhikari *et al.*, 2016). To overcome this problem the prior information on physiological, biochemical and molecular mechanisms that contribute to the uptake, transport and accumulation of Zn in plants is very essential. Based on this information it will be easy to develop hypothesis to manipulate the crops for their nutritional status.

Biofortification of food crop is essential in order to increase Zn content in the seeds and to mitigate malnutrition problem (Zhao and McGrath, 2009; Salunke *et al.*, 2011; Rehman *et al.*, 2015) and this can be achieved through adopting breeding and molecular approaches or by direct application of Zn fertilizers to standing crops. Direct application of Zn fertilizers to the crop at appropriate stage showed increased Zn content in the edible parts (Prasad *et al.*, 2012). However, Zn is a heavy metal, excess application leads to toxic to the plants (Baran, 2013). A few strategic

approaches to increase Zn content in plants per say has been successful, however with low input of Zn fertilizers / Zn nutrients has been improved. An emerging innovative nano technology is extensively being used in industrial and service industry. However, this technology rightly exploited in agriculture industry to fetch enumerable advantages. The application of these nano Zn fertilizers will be having high surface area and also required in very less quantity and promotes better absorption, translocation and hence reduces the Zn toxicity. In this regard, the present investigation is to check the efficacy of zinc oxide (ZnO) synthesised through biological approach under high soil pH condition. The soil pH is the major factor that influencing the mobility and bioavailability of metal elements to plants (Cheng, 2003; Domanska and Filipek, 2011; Ginocchio *et al.*, 2002; Kukier *et al.*, 2004; Piłkuła and Stępien, 2007; Wang *et al.*, 2006). Generally, the mobility of metals in alkaline soils decreases in the order Cd>Ni>Zn>Mn>Cu>Pb (Fijałkowski *et al.*, 2012) and is highly variable and strongly dependent on the content and type of organic ingredients as present in the soil (Badaway *et al.*, 2002; Du Laing *et al.*, 2008; Wang *et al.*, 2006). Present study with the emphasis of increased availability and efficacy of ZnO nanoparticles evaluated under high soil pH condition. Chemically synthesized nano were used as check along with ZnSO₄ standard.

Materials and Methods

Saline soil

Obtained the high pH (saline) soil from farm land of College of Agriculture, VC Farm, Mandya, Karnataka. The pH of the soil was 9.0, mixed with the vermicompost in 2:1 proportion and filled in 10 kg capacity pots. The pH of the soil was measured at 15 days interval during the crop stage.

Nano ZnO synthesis

Synthesised nano ZnO through biological approach by using *Cassia fistula* leaf extract. Commercial nano ZnO procured from SRL Pvt. Ltd. Company and ZnSO₄ was procured from SRL laboratory used as reference. Nano ZnO particles were suspended directly in deionized milli Q water and dispersed by ultrasonic for 30 min (Prasad *et al.*, 2012).

Seed and plant material

The leading hybrid maize seeds were procured from Syngenta Pvt. Ltd. Company. Treatment includes seed priming (T1), seed priming and foliar application (T2) and control (T3). The seeds were sown with recommended dose of fertilizer application. Plants were grown till harvest and recorded the observations.

Seed priming

The seed priming was done with biologically synthesised nano ZnO and commercial ZnO (900 ppm) and ZnSO₄ (1000 ppm) by soaking in the respective solutions for five hours. Seeds were washed with distilled water to remove excess ZnO and ZnSO₄ particles.

Foliar application

The plants were sprayed with biologically synthesised (BS) and Chemically synthesised (CS) nano ZnO (150 ppm) and ZnSO₄ (300 ppm) at 30 and 60 days after sowing (DAS).

Results and Discussion

Effect of ZnO nanoparticle under high soil pH on plant growth

Ten days after the second foliar application, recorded the growth and development parameters of plants. Non-significant difference was observed for plant height

among Zn treatments of seed priming, however plant height was higher in ZnO nano treated (both BS and CS) plants compared to control plants. Treatment seed priming + foliar application with nano ZnO showed significantly higher plant height (160.3 cm) than ZnSO₄ treatment (Table 1). Further BS ZnO nano treated plants maintained higher chlorophyll content (276.8 $\mu\text{mol/m}^2$) followed by ZnO nano (248.7 $\mu\text{mol/m}^2$) compared to ZnSO₄ (219.5 $\mu\text{mol/m}^2$) treatment. However, higher chlorophyll was observed in Zn treated leaves (both nano ZnO and ZnSO₄) than control plants (199.3 $\mu\text{mol/m}^2$). Seed priming + foliar application treatment showed significantly higher chlorophyll (241 $\mu\text{mol/m}^2$) content when compared with seed priming alone (231.2 $\mu\text{mol/m}^2$). Similar results were found with the use of nano Zn oxide in groundnut that higher leaf chlorophyll content was manifested by early flowering and effective in increasing stem and root growth (Prasad *et al.*, 2012). In *Vigna radiate* and *Cicer arietinum* absorbed more of ZnO nanoparticles and promoted the root and shoot length biomass (Prasad *et al.*, 2011).

In *Triticum aestivum* observed that increase in leaf chlorophyll and protein content with nano-ZnO treatment (Ramesh *et al.*, 2014). ZnO nano at 10 mg⁻¹ concentrations were optimum for inducing the growth response in *Brasica napus* (Rahmani, 2016).

Non-significant difference was observed in number of leaves per plants with different Zn sources and also with method of applications. Higher leaf area (3524 cm²/plant) was observed in ZnO nano treated plants compared to BS nano ZnO (3410 cm²/plant) and ZnSO₄ (3330 cm²/plant) treatments. Compared to control all Zn treated plants showed higher leaf area. Method of application did not influence the total leaf area significantly (Table 1).

Effect of ZnO nanoparticle under high soil pH on yield attributes of maize

Highest cob length was observed in BS nano ZnO treatment (9.5 cm) followed by CS ZnO nano treatment (9.0 cm) and ZnSO₄ (7.7 cm) treatment (Table 2). However, compared to ZnSO₄ treatment, a significant increase in cob length was observed in nano ZnO treated plants. Biologically synthesised nano ZnO treatment showed 13 rows per cob whereas ZnSO₄ treatment showed 12 rows and control was 11 rows per cob. BS nano ZnO and CS nano ZnO treated plants showed significantly higher number of seeds per row (15 seeds/row) compared to ZnSO₄ treatment (12 seeds/row) followed by control (11 seeds/row). BS nano ZnO treated plants showed higher cob weight (6.7g) compared to CS ZnO nano treatment (5.5 g) followed by ZnSO₄ (5.5 g) treatment. The CS nano ZnO treated plants showed significantly higher seed weight (44 g) followed by BS nano ZnO treated plants (41.3 g) and ZnSO₄ treated plants (35.7 g) when compared to control (30.0 g). The CS nano ZnO treated plants showed 23 % and BS nano ZnO treated plants showed 16% increase in the seed yield compared to ZnSO₄ treatment. Supporting to obtained results, similar study by Prasad *et al.*, (2012) and Rameshraddy *et al.*, (2017) showed application of nano Zn enhances yield in groundnut and rice. ZnO nanoparticle treated plants (both BS and CS) did not show any significant difference in shoot biomass. However, compared to the ZnSO₄ treatment nano ZnO treated plants showed significantly higher shoot biomass. In CS nano ZnO treated plants showed 32 % increased and BS nano ZnO treated plants showed 29 % increase in shoot biomass compared to ZnSO₄ (Table 2) treated plants. Similarly Raliya and Tarafdar (2013) reported that nano ZnO particles induced a significant improvement in *Cyamopsis tetragonoloba* plant biomass. However, method of application (Seed

priming with foliar or alone application) showed non-significant difference between yield attributes. Similarly Tarafdar *et al.*, (2012) observed significant increase in yield due to foliar application of nanoparticles as fertilizer.

Interaction effect between the different Zn sources and method of applications on grain yield of maize under high pH condition

Highest seed yield found in the ZnO nano treatment with seed priming and foliar application treatment (44.66 g), followed by ZnO nano treatment with seed priming treatment (43.33 g). Non-significant difference was observed in both chemical and biologically synthesised nano ZnO and method of application (Table 3). However, significant difference observed in the nano ZnO treated plants compared to ZnSO₄ treatment. Similar results were found with Tarafdar *et al.*, (2014), the grain yield was increased by 37.7 % due to application of zinc nanofertilizer in pearl millet and also Raliya *et al.*, (2013) observed the cluster bean seeds increase by 7.5 per cent due to ZnO nano application.

Leaf Zn content

Significant increase in the leaf Zn content (Fig. 1) was found in both chemically and biologically synthesised nano ZnO treated plants with seed priming + foliar application treatment (4.84 mg/100 g and 5.73 mg/100 g) respectively followed by ZnSO₄ (2.69 mg/100 g) when compared to control (1.63 mg/100 g).

Seed Zn content

BS nano ZnO treated plants showed significant increase in the seed Zn content (3.21 mg/100 g) in seed priming treatment compared to CS nano ZnO treatment (2.06 mg/100 g) in seed priming + foliar application treatment (Fig. 2).

Table.1 Growth of maize influenced by high soil pH with different sources of zinc and method of applications at vegetative stage

Treatments	Plant height (cm)	Chlorophyll ($\mu\text{ mol/m}^2$)	No. of leaves/plant	Leaf area (cm^2/plant)
Zinc sources				
BS ZnO	157.2	276.8	11.33	3410
ZnO	170.5	248.7	11.00	3524
ZnSO₄	156.0	219.5	11.33	3330
Control	137.7	199.3	11.00	2755
SEm\pm	5.01	7.75	0.36	163.9
CD (P=0.05)	15.03	23.23	NS	491.3
Method of Applications				
SP	150.4	231.2	11.08	3177
SP + F	160.3	241.0	11.25	3333
SEm\pm	3.54	5.48	0.25	115.9
CD (P=0.05)	NS	NS	NS	NS
CV (%)	7.9	8.04	7.97	12.3

(Note: BS - Biological synthesis; ZnO - Zinc oxide nanoparticle; ZnSO₄ - zinc sulphate; SP – Seed priming; SP + F – Seed priming and foliar application).

Table.2 Yield attributes of maize influenced by high soil pH with different sources of zinc and application methods at harvest

Treatments	Cob length (cm)	No. of rows/cob	No. of seeds/row	Cob weight (g)	Seed weight (g/plant)	Test weight (g/100 seeds)	Shoot biomass / plant (g)
Zinc sources							
BSZnO	9.50	13.16	14.66	6.67	41.33	27.41	63.33
ZnO	9.00	12.16	14.66	5.50	44.00	28.33	65.16
ZnSO₄	7.66	11.83	11.83	5.50	35.66	27.00	49.16
Control	6.00	10.66	11.33	4.33	30.00	26.00	43.33
SEm \pm	0.38	0.43	0.81	0.26	0.74	0.52	1.17
CD (P=0.05)	1.14	1.29	2.43	0.78	2.22	1.56	3.51
Method of Applications							
SP	7.75	11.66	12.91	5.41	37.33	26.62	53.91
SP+F	8.33	12.25	13.33	5.58	38.16	27.75	54.58
SEm \pm	0.27	0.3	0.57	0.18	0.52	0.36	0.83
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS
CV (%)	11.63	8.87	15.16	11.74	4.81	4.7	5.31

(Note: BS - Biological synthesis ZnO - Zinc oxide nanoparticle; ZnSO₄ - zinc sulphate; SP – Seed priming; SP + F – Seed priming and foliar application).

Table.3 Interaction effect of different Zn sources and method of applications on grain yield of maize under high soil pH

Zinc Sources	Method of Applications	
	SP	SP+F
BSZnO	40.66	42.00
ZnO	43.33	44.66
ZnSO₄	35.33	36.00
Control	30.00	30.00
SEm±	1.04	
CD (P=0.05)	3.11	
CV (%)	4.81	

(Note: BS - Biological synthesis; ZnO - Zinc oxide nanoparticle; ZnSO₄ - zinc sulphate; SP – Seed priming; SP + F – Seed priming and foliar application).

Fig.1 Maize leaf zinc content of different Zn sources treated plants. (SP- seed priming; SP + F- Seed priming and foliar application)

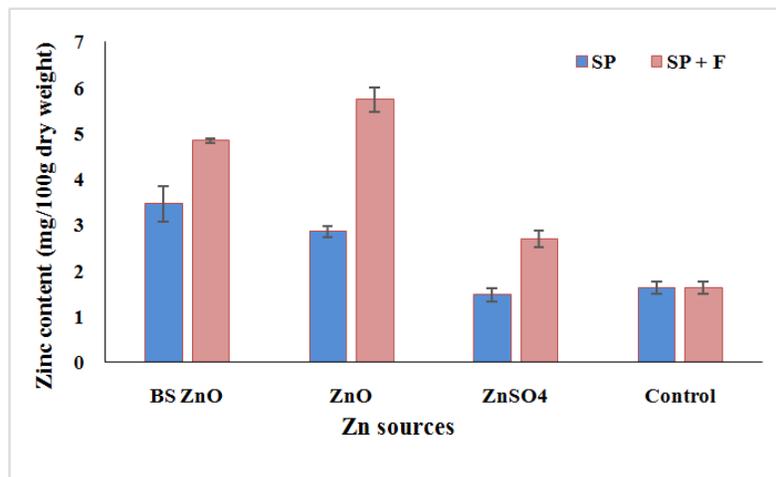
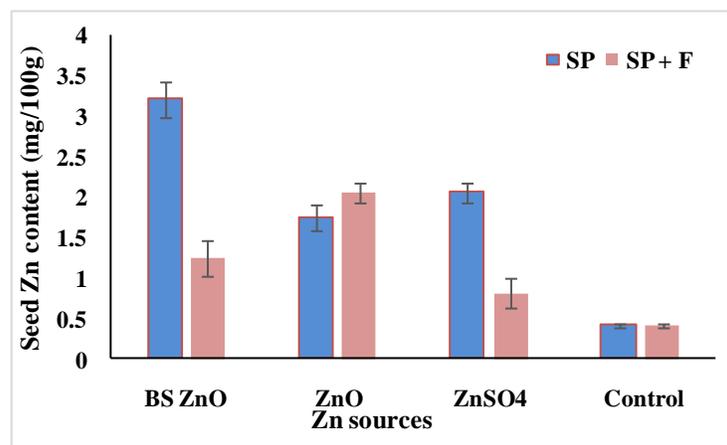


Fig.2 Maize seed zinc content in different Zn sources treated plants. (SP- seed priming; SP + F- Seed priming and foliar application)



Similar results found with Dapkekar *et al.*, (2018) Rameshraddy *et al.*, (2017) and Prasad *et al.*, (2012) experiments wherein application of the ZnO nanoparticle enhanced the leaf and seed Zn content of groundnut, Rice and wheat respectively.

Soil pH play a major role in altering the availability of the micronutrients mainly Fe, Mn, Cu, and Zn, higher soil pH reduces the uptake. Zn is also one such metal ion which availability is decreased 100 folds every unit increase in soil pH. In this experiment also found that high pH affects the Zn availability to the plants which results in poor growth and yield. This has been proved by Sims, 1986 in wheat the availability of the micronutrient like Cu, Mn and Zn altered with the soil pH. Narwal *et al.*, (1983) showed that higher soil pH leads to lower availability of Zn. ZnO nanoparticles absorbed by plants to larger extent unlike bulk ZnSO₄. These particles proved to be effective in enhancing plant growth, development and yield in maize. Seed priming and lower dose of foliar application is proved significantly productive in enhancing the yield under high soil pH condition compared to ZnSO₄ treatment. Further leaf and seed sample analysis, revealed a significant increment in zinc content when supplied with Zn oxide nano scale compared to chelated ZnSO₄.

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