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Physiological Efficacy of Zinc Granular and Nano Zinc Fertilizer in the Presence of Higher Phosphorus and its Influence on Seedling Vigour Index and Zinc Content of Maize

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ABSTRACT

Micronutrient deficiency is the widespread problem in the Indian soil especially Zinc (Zn) and Iron (Fe). Fifty per cent of the Indian soils are found to be Zn deficient and is more common under the conditions of high soil pH, calcareous, light-sandy soils and high phosphorus (P) levels, which majorly impacts on crop growth and yield. However, excess use of Zn fertilizers has detrimental effect on soil and plant health. Therefore, an efficient mechanism to reduce the amount of Zn fertilizers for soil or foliar application without compromising the plant growth and yield is crucial through use of nanotechnology. Recently nano Zinc oxide (ZnO) fertilizers are upcoming for effective use of Zn fertilizers in crop productions. In this context, nano ZnO particle synthesized by green approach and chemical approach were tested on maize (*Zea mays*) germination and seedling vigour. The results indicated that, 900 ppm was optimum for good seedling vigour in ZnO treatment, whereas $ZnSO_4$ treatment showed at 1500 ppm. Further the effect of nano ZnO particle under high P conditions was studied. There was no significant difference in the plant height, root length and leaf number in nano ZnO treatment under normal and high P conditions. The chlorophyll content, relative water content and total dry matter was reduced in the high phosphorus application in all the Zn treatments. Leaf Zn content of ZnO treated plants showed little reduction under high P condition but compared to $ZnSO_4$ treated plants maintained higher levels of Zn under high P condition. Overall, the results indicated that the positive effect of nano Zn improved seedling vigour under high P conditions, resulting in improved crop growth.

Keywords: Nano ZnO, Green synthesis (GS) nano ZnO, $ZnSO_4$, Phosphorus, Maize

GLOBAL population is expected to reach 9 billion by 2050. Agricultural scientists are exploring the ways to meet the demands for food without increasing the stress on natural resources. In addition, the budding challenges such as world wide spread of micronutrient deficiencies primarily of Iron (Fe) and Zinc (Zn) in humans is alarming. Zn deficiency is a global problem, almost half of the world soils are deficient in Zn (Adhikari *et al*., 2016) and in India, the same is projected to increase to 63 per cent by 2025. The availability of Zn to the crops is limited due to the Zn deficiency induced by high soil pH, low organic matter, calcareous, light and sandy soils, and high P levels (Adhikari *et al.,* 2016). As Zn is a trace element, found in varying concentration in different soils, plants and animals and it is essential for normal healthy growth of the plants, animals and human beings (Alloway., 2008). In plants Zn plays a crucial role in structure and function of various proteins, such as enzymes, transcription factors (TFs), hormonal receptor site and biological membranes. It also has role in DNA, RNA metabolism and involved in cellular proliferation, differentiation and growth. Therefore, Zn deficiency not only reduces the crop quality and nutritive value but also critically affects crop growth and yield (Sinclair and Kramer, 2012), which in turn has put about 49 per cent of the world's population at risk of Zn deficiency (Anon., 2012). There is a need to develop sustainable functional innovations to enhance crop productivity to meet the food and nutritional security of exponentially exploding population.

To overcome Zn deficiencies in humans several strategies are being employed including supplementation, food fortification, dietary diversification and biofortification (Perez-Massot *et al*., 2013). Among these strategy, biofortification of food crops with Zn is considered as cheaper and sustainable. Biofortification of crop plants can be achieved through agronomic approaches like direct fertilizer application, breeding and transgenic approaches. Of these, addition of the appropriate mineral as inorganic fertilizer is the simplest and easiest way to achieve the target (Prasad *et al*., 2013). But Zn being a heavy metal, application of Zn fertilizers indiscriminately for every crop season has a considerable impact on the soil properties and ultimately to the crop plants (Baran., 2013).

In this perspective, nanotechnology plays a significant role in agriculture by reducing the size of fertilizer particles to nano dimensions (1-100 nm). Owing to their high surface area to volume and high reactivity and high surface energy, nano particles offer potential advantages over conventional fertilizers, which not only reduces the cost of fertilizers but also have significant impact on plant growth stimulation at trace amount of their application. The major emphasis of the work to plant nutritional scientists is toward the specific delivery of nutrients and improved nutrient uptake. Chemical fertilizer uptake efficiency in plants is less due to addiction of nutrients with other soil complexes or run off due to precipitation (Hautier *et al*., 2014).

The application of nanotechnology in the area of agriculture has been widely studied in recent years (Peng *et al*., 2012; Chen *et al*., 2014; Rameshraddy *et al*., 2017a and Rameshraddy *et al.,* 2017b). In India, supplementing the Zn requirement of agricultural crops through water-soluble Zinc sulphate ($ZnSO_4$) fertilizer, whereas, utilization of ZnO as a source of Zn could be an alternative cost effective option to encourage farmers for wider adoption. Slaton *et al.,* (2005) demonstrated that, finer the size of ZnO particles, more was the Zn uptake in plants. Therefore, more emphasis is needed on utilization of nano-scale ZnO particles to increase the efficiency of ZnO as fertilizer for better uptake and crop yield (Adhikari *et al*., 2016). In this regard, the present study has been focused on the impact of nano ZnO particle synthesized by chemical and green approaches on maize seedlings compared to $ZnSO_4$.

Material and Methods

Preparation of Nano ZnO suspension and standardization of concentration for seed priming

Zinc oxide (ZnO) nano particles synthesized by chemical approach (procured from SRL Pvt. Ltd. Company) and Green synthesis (GS) nano particle synthesized by using *Cassia fistula* leaf with little modification of protocol adopted by Suresh *et al*., (2015), was used in the study and $ZnSO_4$ was used as a reference. Nano ZnO particles were suspended directly in deionized milli Q water and dispersed by ultrasonic vibration (100 W, 40 kHz) for 30 min to dissolve nano particles. The nano scale suspensions appearing as clear solutions at pH of 6.8–7 were used. Magnetic bars were placed in the suspensions for stirring to avoid aggregation of particles. To standardize Zn concentration for seed priming, the solutions of nano ZnO suspensions synthesized by chemical and green approach and $ZnSO_4$ with 50 to 2000 ppm concentrations were prepared. The maize seeds were procured from Syngenta Pvt. Ltd. Company. The seeds were soaked in different concentrations of Zn solutions for five hours. Then washed with distilled water and seeds were placed in petri plate with a sterilized filter paper added with 10 ml of water for seed germination. Petri plates were covered and placed in an incubator at 26 ± 1 ºC. Three replications were maintained and observations on the germination percentage, root length and shoot length were recorded. SVI was calculated using the formula (Oyun., 2006).

SVI = Germination % X (Root length + Shoot length)

Preparation of different levels of Zn and altering the phosphorus levels in Hoagland solution

Generally the uptake of Zn is inhibited by the phosphorus. Seedling were exposed with sub optimal level of Zn by altering the phosphorus in the Hoagland solution. Different levels of phosphorus were provided in the Hoagland solution for creating the suboptimal condition for Zn. One set of seedlings with normal phosphorus concentration (31 ppm) and another set with double phosphorus concentration (62 ppm) were exposed. Two week old seedling were exposed to high P condition for three week and observations on the plant height, root length, number of leaf, chlorophyll content (using chlorophyll meter -MC 100), total dry weight and estimated the Zn content in the leaf tissue were recorded.

Estimation of Zinc content

Zn content in leaf samples was estimated by using Atomic Absorption Spectrometer (AAS). The leaf samples collected from different treatments were oven dried. Samples were ground to a fine powder using liquid nitrogen with pestle and mortar. 250 mg of sample was digested with 5 ml of concentrated nitric acid overnight. The next day, 5 ml of diacid mixture (nitric: per chloric acid in the ratio of 10:4) was added and placed on a hot plate until a thick white residue was left in the flask. The volume was made up to 25 ml using deionized, triple distille water and filtered using 125 mm filter paper, and the filtrate was used for Zn estimation using AAS. A standard graph was developed with standard solution. Leaf sample Zn content is expressed in mg/100g.

Measurement of Relative water content, chlorophyll and Total dry matter

Relative water content : The leaf discs were obtained from plants, and the fresh weight was recorded. Discs were then floated on deionised water for 5 hrs under the turgid tissue was then quickly blot dried with tissue paper prior to determining turgid weight. Dry weight was then determined after oven drying at 70 °C for 48 hrs. The relative water content was calculated using the formula.

$$RWC\ (\%) = \frac{\text{Fresh weight - dry weight}}{\text{Turgid weight - dry weight}} \times 100$$

Chlorophyll measurement: chlorophyll was measures using the chlorophyll meter MC 100 portable device which will measure the chlorophyll content in the leaf tissue and expressed in µmol/m^2.

Total dry matter (g/plant): forty day old plants were harvested and dried in hot air oven for five days, recorded the dry weight of the sample and expressed as g /plant.

Statistical analysis

Experimental data were analysed using MSTAT-C statistical programme at 5% significance level in a CRD two factorial design. The analysis of variance (ANOVA) test was carried out to demonstrate the effect of Zn treatments on the growth and Zn content in maize.

RESULTS AND DISCUSSION

Effect of Nano ZnO on seed germination and seedling vigour

Seeds soaked in different concentration of the nano ZnO particle synthesized from both the chemical and green approaches showed significantly improved seed germination and Seedling Vigour Index (SVI). Response was quite significant when seeds were treated with nano ZnO particles. Significant improvement in germination percent, root length and shoot length was observed at 900 ppm both in nano particle synthesized from chemical and green approaches (Fig. 1). However positive response was

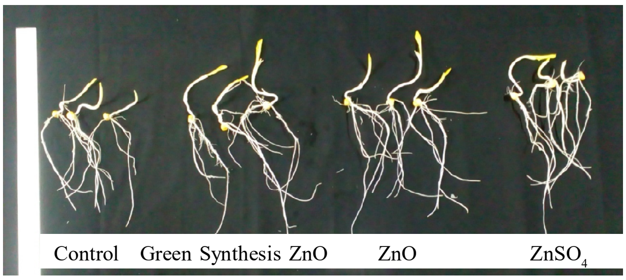

Fig. 1 : Response of maize seedlings exposed to different Zn sources at 900 ppm

seen up to 1500 ppm of $ZnSO_4$. The result showed that seed priming with 900 ppm nano ZnO was optimum to substantially increase the SVI compared to control (Table 1). The seed treatment with ZnO at 900 ppm showed 38.70 and 37.12 per cent increase in SVI in nano ZnO particle from chemical and green approach, respectively compared to control. The significant reduction in SVI was seen at 1500 ppm in ZnO particles synthesised from both approaches.

TABLE 1
Effect of nano ZnO particle synthesized from chemical and green approach and $ZnSO_4$ on seedling vigour index (SVI)

Concentration (ppm)	GS ZnO	ZnO	ZnSO4	Control
50	2350	2245	2110	
100	2326	2119	2137	
200	1802	2192	1916	
300	2066	2158	1892	
400	2406	2620	2430	
500	2460	2456	1800	
600	2308	2020	1850	1802
700	2422	2325	2110	
800	2390	2330	1933	
900	2940	2866	2400	
1000	2286	2504	2712	
1500	1993	1904	2464	
2000	1632	1689	1692	
Sem±		72.29		
CD (0.05%)		201.54		
CV		7.65		

(Note: GS- Green synthesis ZnO - Zinc oxide nano particle $ZnSO_4$ - zinc sulphate)

Whereas, $ZnSO_4$ showed increasing SVI up to 1500 ppm but at 2000 ppm concentration toxic effect on the seedling was observed and hence showed reduction in seedling vigour. Similar results were found in rice and groundnut using nano ZnO particle at 1000 ppm showed highest germination percent and SVI as compared to $ZnSO_4$ (Rameshraddy *et al*., 2017a and Prasad *et al*., 2012). Seed priming with Zn in barley and rice seeds was effective in improving seed germination and seedling development (Ajouri *et al*., 2004 and Prom *et at*., 2012)

Response of Nano ZnO under high phosphorus condition

The increased levels of P in the soil leads to inhibition of Zn uptake by the plants. To understand the uptake of nano ZnO particles under high phosphorus condition, maize seedlings were grown in the Hoagland solution supplied with normal and high P concentrations. There was no significant difference in the plant height, root length and number of leaves in nano ZnO treated plants in both normal and high P conditions. Whereas, $ZnSO_4$ treated plants showed significant reduction in plant height, root length as compared to nano ZnO treatment under both normal and high P conditions (Table 2). Total chlorophyll content and Relative Water Content (RWC) was high in nano ZnO treated plants under normal condition as compared to $ZnSO_4$. But significant reduction of chlorophyll and RWC in high P condition of nano ZnO and $ZnSO_4$ treatment was observed compared to normal condition. Plants were harvested at 40 days after sowing and measured the total dry matter (TDM). The nano ZnO treated plants showed higher TDM as compared to $ZnSO_4$ and control under both normal and high P conditions. But in high P condition there was a reduction in the TDM in all the treatment as compare to normal condition. The application of nano ZnO particles under normal and high P condition increased the plant growth and development as compared to $ZnSO_4$. However, was no much change in the growth of the plants under the nano ZnO treatment under normal and high P conditions. Similarly high P application leading to Zn deficiency has been showen in stevia plant (Das *et al.,* 2005).

Estimation of Zn content

The leaf Zn content was analysed and the results showed that improved Zn content was obsreved in leaf of all treatments compared to control plants. Under normal P condition there was an increase in the Zn content 5.21 mg/100 g and 4.47 mg/100 g in ZnO and GS ZnO nano treatment respectively compared to $ZnSO_4$ treatment. Similarly in high P conditions also nano ZnO treatment showed higher Zn content of 5.16 mg/100g and 4.11 mg/100g in ZnO and GS ZnO treatment respectively. Whereas, $ZnSO_4$ treatment showed significant reduction in the Zn content in High P condition about 2.87 mg/100 g compared to normal condition about 3.72 mg / 100 g. Similarly Sayed Roholla Mousavi (2011) showed that zinc absorption capacity is reduced by high phosphorus utilization in plant. The interaction effect of the

TABLE 2

Influence of Zn treatment under normal and high phosphorus application on maize plant

Treatments	Plant height (cm)	Root length (cm)	Number of leaves	Chlorophyll µmol/m^2	RWC (%)	TDM (g)
			Normal condition			
GS ZnO	122.00	52.33	7.67	558.00	91.34	9.63
ZnO	120.00	50.00	7.67	557.33	89.43	8.26
$ZnSO_4$	117.00	46.67	7.33	510.00	84.64	7.86
Control	103.33	36.33	6.33	448.00	81.96	6.10
			High Phosphorus (62 ppm) condition			
GS ZnO	122.00	50.33	7.66	548.33	88.11	9.10
ZnO	121.00	50.66	7.66	546.33	80.91	8.16
$ZnSO_4$	112.67	42.00	7.33	489.33	85.41	7.16
Control	98.67	29.00	6.66	440.00	80.00	6.16
Sem±	1.15	1.11	0.33	1.4	0.75	0.28
CD (0.05%)	3.44	3.32	0.98	4.19	2.24	0.83
CV	1.75	4.31	7.92	0.47	1.51	6.36

(Note: GS- Green synthesis ZnO - Zinc oxide nano particle $ZnSO_4$ - zinc sulphate)

TABLE 3

Interaction effect of different Zn sources and Phosphorus levels on Zn content

Treatments	Zn content (mg/ 100g) Normal condition	Zn content (mg/100g) High Phosphorus condition
GS ZnO	4.47	4.11
ZnO	5.21	5.16
$ZnSO_4$	3.72	2.87
Control	1.56	0.6
Sem±	0.053	
CD (0.05 %)	0.16	
CV	2.69	

(Note: GS- Green synthesis ZnO - Zinc oxide nano particle $ZnSO_4$ - zinc sulphate)

different Zn concentrations with normal and high P supplied was observed. Nano ZnO treated plants showed little reduction in Zn content under high P conditions but compared to $ZnSO_4$ treated plants maintained higher levels of Zn under high P conditions (Table 3).

The role of micronutrient during the germination stage is very essential to get good seedling vigour. The application of nano ZnO particle directly influenced the seedling vigour as compared to normal $ZnSO_4$ treatment. In the high P condition the availability of the Zn was inhibited. When nano ZnO particle exposed to the high P condition it does not influence the plant height, root length and number of leaf but it reduced the chlorophyll content, RWC and TDM under high P condition. The higher Zn content was found in nano ZnO treated plants under both normal and high P conditions as compared to $ZnSO_4$ treated plants. Overall, nano ZnO treated plants are able to maintain higher Zn level even under high P condition.

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