

Original Research Article

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Raise of Nano-Fertilizer Era: Effect of Nano Scale Zinc Oxide Particles on the Germination, Growth and Yield of Tomato (*Solanum lycopersicum*)

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ABSTRACT

Globally the soils are Zinc (Zn) deficient and plants are not in a position to accumulate enough Zn in edible parts that can meet the human nutrition requirement. Nanotechnology is one of the most important tools in modern agriculture. Nano agriculture involves the use of nano sized particles with unique properties (increased uptake by plants as they are small in size and have high rate of penetration through plant cell membrane) to boost crop productivity. In the present study, an attempt was made to study the effect of nano zinc oxide particle (ZnO NPs) for improving yield and Zn content in tomato plant. Initially seed priming concentration were standardized *in vitro* using ZnO NPs (400ppm) and granular zinc sulphate (ZnSO₄) (800ppm). Further standardized seed priming concentrations with different combinations of treatment such as seed priming, seed priming+ foliar spray and foliar spray were studied under field condition to evaluate their effect on biomass and Zn accumulation. The obtained results based on the physiological and yield parameters showed that the usage of ZnO NPs fertilizers through any of the method of application has significant positive effect compared to zinc sulphate. ICP-OES analysis of plant digested material revealed that uptake of ZnO NPs is higher than the granular ZnSO₄. The present study addresses the potential of nano scale particles on plant system opens an avenue for its potential use as future “nano fertilizers”. Thus nanotechnology is one of technologies where lot of scope exists to improve the plant nutrition.

Keywords

Nano zinc Oxide (ZnO NPs), Zinc sulphate (ZnSO₄)

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Introduction

Importance of agriculture to all human societies is characterized more than ever with the increasing world population. The first and most important need of every human is need to access the food, and food supply for humans is associated with agriculture directly or indirectly. The world's population will grow to an estimated 8 billion people by 2025 and 9 billion by 2050, and it is widely

recognized that global agricultural productivity must increase to feed a rapidly growing world population (FAO/WHO, 2002). Vegetables and fruits are perishables, and in the absence of effective storage, preservation and transportation, the prices are unstable and the availability uncertain in addition to the above limitation, the diets of the average Indian household did not show any significant improvement over the last few decades of the century. A challenge for global food and

nutrition security is to feed the world population with nourishing food (Quasem *et al.*, 2009; Ghaly, 2009). Hence emphasis should be laid on production of high quality food with the required level of nutrients and proteins (Pijls *et al.*, 2009; Ghaly and Alkoaik, 2010). To meet this increasing demand, researchers are trying to develop an efficient and ecofriendly production technology based on the innovative technologies.

The issue of micronutrient deficiency is related to food security (Meenakshi *et al.*, 2010; Ghaly and Alkoaik, 2010). Micronutrient deficiencies in human being as well as crop plants are difficult to diagnose and accordingly the problem is termed as 'hidden hunger' (Stein *et al.*, 2008). This hidden hunger may cause nearly 40 per cent reductions in crop productivity and also it is estimated that it affects more than a half of the global population. Micronutrient deficiency in general refers to Fe, Zn, Se, I, Cu, Ca and Mg (Zhao and Mcgrath, 2009), among them Zinc (Zn) deficiency is most wide spread next to Iron, Vitamin A and Iodine. WHO reported that Zn deficiency stands fifth risk factor for causing diseases among children's in developing countries. Based on analysis of diet composition and nutritional needs, it has been estimated that 49 per cent of the world's population (equivalent to 3 billion) are at risk of suffering from Zn deficiency. Until the recent times, soil fertilization was the only way to meet the mineral requirement of crops plants. However, several problems exist like need for large quantity of fertilizer, fixation in soil and slow uptake by plants.

Zn has a specific physiological functions in all living systems, such as i) maintenance of structural and functional integrity of biological membranes, ii) as a cofactor for more than 300 enzymes, iii) detoxification of highly toxic oxygen free radicals iv) contribution to protein synthesis and gene expression under normal

and stress conditions etc. Among all metals Zn is needed by the largest number of proteins, at least 2800 proteins are Zn dependent and make up nearly 10 per cent proteomes in eukaryotes, Zn has a vital role in several body functions such as vision, taste perception, cognition, cell reproduction, growth and immunity, resistance to some infectious diseases such as diarrhoea (Black, 1998) and immunity, (Shankar and Prasad, 1998).

Most of the Indian soils are found to be Zn deficient, hence the food crops grown in those soils contain less amount of Zn in food. There has been a significant genetic variability to maintain growth and yield under Zn deficient conditions among crop species (Hacisalihoglu *et al.*, 2003). Significant variation across crop species and genotypes exists for their ability to Zn uptake, their sequestration and ability to transport to edible parts.

In order to overcome Zn disorder, several strategies are being employed including supplementation, fortification, diversification and biofortification. Among these strategies biofortification of food crops with Zn is considered to be cheaper and sustainable. The simplest of these techniques to increase Zn content of plants is through the addition of the appropriate mineral as an inorganic compound to the fertilizer. This method has been successful in many instances but depends on the crop species, cultivar, the mineral itself, quality and properties of the soil, making the strategy difficult to apply generally. The major advantages of this method are, it is simple, relatively inexpensive and enhancement can be achieved very rapidly.

However Zn being heavy metal, indiscriminate application of Zn fertilizers to soil over years will lead to accumulation in soil to the levels toxic to the plants. With the current emphasis on Zn in agriculture, care should be taken not to get over zealous with

Zn applications. Therefore, an efficient mechanism to reduce the amount of Zn fertilizer application to soil/ foliar without compromising the plant growth and yield is very essential. Hence, in recent years the application of nano scale particle of Zn is being preferred to enhance agronomic effectiveness of Zn fertilizers.

Now, after years of green revolution and decline in the ratio of agricultural products to world population growth, it is obvious that there is necessity of employing new technologies in the agriculture industry more than ever. Modern technologies such as bio and nanotechnologies can play an important role in increasing production and improving the quality of food produced by farmers. Many believe that modern technologies will secure growing world food needs as well as deliver a huge range of environmental, health and economic advantages (Wheeler, 2005). Nanotechnology is one of the most important tools in modern agriculture, and agri-food nanotechnology is anticipated to become a driving economic force in the near future. Nanoagriculture focuses currently on target farming that involves the use of nanosized particles with unique properties to boost crop and livestock productivity. The development of nano materials could open up the novel applications in plant biotechnology and soil science. It is anticipated that very soon the industrial production of manufactured nano particles will be increased by manifold and released into the market. However with significant potential benefits, there are considerable uncertainties with regards to potential risks to the environment and human health that needs to be clarified.

The current situation in nanotechnology is one in which there is great potential for benefit but an equally high uncertainty in associated risks. There are evidences for both optimism and pessimism. Pessimism is because of the huge

discrepancy between the scale of research being performed on the invention of materials such as nano particles and their associated risks. Optimism is because of the uniquely forward-looking attitude of policy makers and regulators. The unusual properties of nano particles may result in substantially different environmental fate and behaviours than their bulk counterparts but very few observations were made in higher plant growth and yield. Because nano particles are spherical or faceted metal particles typically, <100nm in size. These nanoparticles are having high surface area (30-50 m²/g), high activity, better catalytic surface, rapid chemical reaction, rapidly dispersible and adsorb abundant water. Thus the implementation of particles in nanometer range can serve as potential alternative to overcome the limitation of presently available fertilizers. So nano fertilizers may increase the efficiency of nutrient uptake, enhance yield and nutrient content in the edible parts and also minimize its accumulation in the soil. Thus present study investigates the effect of ZnO NPs on tomato plants with a view point of their potential use as future” nano fertilizers”.

Materials and Method

Preparation of particle suspension

Chelated bulk ZnSO₄ was used as a reference Zn source, the materials were suspended directly in deionised water and dispersed by ultrasonic vibration (100 W, 40 KHz) for 30 min. Different concentrations (0, 100, 200, 400, 800, 1000, 1500, 2000 ppm) of solutions were prepared. Magnetic bars were placed in the suspensions for stirring to avoid aggregation of the particles. The nano scale suspensions as expected to appeared as clear solutions. The pH of all the prepared suspensions was found to be 6.8-7.0. A control was also maintained, corresponding to pure water.

Standardization of Zn concentration for seed priming

Tomato seeds were treated with 100ml of Zn solutions /suspensions of granular ZnSO₄ and ZnO NPs for three hours. After inhibition seeds were thoroughly washed under running water and allowed for germination. Then the 10 seeds were placed in each petri dish (100 mm x15 mm) with single layer of sterilized filter paper and 5 ml of water was added (as per the recommendations of the International Seed Testing Association, 1976).

Than germination percentage, root length, shoot length were recorded using the values Seedling Vigour Index was calculated by using the formula described by Abdul-Baki and Anderson (1973).

Seed Vigour Index = Germination per cent × (root length + shoot length)

Field experimentation

Experiment was conducted in the field of Department of Crop Physiology, UAS, GKVK. The experiment was conducted with three different treatments based on method of application of Zn sources, such as only seed priming, seed priming + foliar application and only foliar application. The standardized Zn concentrations in nano ZnO and granular ZnSO₄ under lab condition were used as initial seed priming concentrations. And foliar spray was given at 30 DAS (Days after Sowing) Tomato. The concentration maintained for foliar spray was 1 per cent in granular ZnSO₄ and 0.5 per cent in nano ZnO.

Physiological parameters were measured at 45 DAS. The following parameters were measured: number of branches, plant height, root length SCMR (Spad Chlorophyll Meter Reading), Relative Water Content (RWC), Specific Leaf Area (SLA),

Relative Water Content (RWC)

To determine the effect of different treatment in field experiment, RWC was measured. The leaf discs were obtained from plants from replicated treatments, and the fresh weight was determined. Discs were then floated on deionised water for 5hr under low irradiance, 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 48hr. The relative water content was calculated using the formula (Gui-Rui *et al.*, 2000).

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

Specific Leaf Area (SLA)

SLA was determined using the formula

$$\text{SLA} = \frac{\text{Leaf Area}}{\text{Dry weight}} \text{ Cm}^2/\text{ gm}$$

Yield and estimation of zinc

Yield per plant (gm) was recorded. Zinc content was analyzed in different plant parts like leaf, root and fruit by using Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES).

Results and Discussion

Standardization of Zn concentration for seed priming

Tomato seeds responded variably towards the treatment at various concentrations of both bulk ZnSO₄ and nano scale ZnO particles. Seed treated with 400ppm Nano ZnO recorded significant germination (93.33 %) and Seedling Vigour Index (919.80).

Table.1 Effect of seed priming with different concentrations of nano ZNO and bulk ZnSO₄ on germination, shoot length, root length and Seedling Vigour Index (SVI) in tomato

Concentration (ppm)	Germination (%)	Root length (cm)	Shoot length (cm)	SVI
Zn source				
Control	63.33	2.16	1.30	249.97
Nano ZnO	88.56	4.57	2.73	469.21
ZnSO ₄	82.66	4.77	2.66	497.66
SEM±	1.60	0.04	0.06	4.89
CD(0.05)	3.93	0.13	0.19	14.56
Interaction (Zn source * concentration)				
Nano ZnO 100	66.67	4.12	1.89	344.67
Nano ZnO 200	66.67	4.7	3.43	524.40
Nano ZnO 400	93.33	6.97	3.22	919.80
Nano ZnO 600	73.33	6.52	3.65	856.97
Nano ZnO 800	70.00	6.20	3.33	750.57
Nano ZnO 1000	53.33	3.63	3.27	375.50
Nano ZnO 1500	46.67	3.92	2.40	324.94
Nano ZnO 2000	40.00	2.99	2.07	176.07
ZnSO ₄ 100	66.00	4.13	1.69	395.8
ZnSO ₄ 200	70.67	4.79	2.67	509.87
ZnSO ₄ 400	73.33	5.44	4.13	612.67
ZnSO ₄ 600	78.00	5.51	2.63	718.75
ZnSO ₄ 800	80.00	6.17	4.39	834.71
ZnSO ₄ 1000	56.67	4.63	2.63	425.11
ZnSO ₄ 1500	50.00	4.317	2.17	346.31
ZnSO ₄ 2000	46.67	2.69	2.10	199.07
CD (P=0.05)	6.37	0.22	0.31	24.64
SEM ±	2.33	0.094	0.13	10.37
CV (%)	4.70	4.90	1.40	5.25

Table.2 Performance of tomato, influenced by different sources of Zn and method of application at 45 days after sowing

Treatments	no. of branches	Specific Leaf area (cm ² /gm)	Shoot length (cm)	Root length (cm)	SCMR	RWC (%)	Shoot Dry weight (gm)	Root dry weight (gm)
Zn Sources								
Nano ZnO	5.00	138.42	49.45	37.21	52.92	86.50	46.00	8.40
ZnSO ₄	5.08	121.76	41.34	33.18	47.07	76.97	38.09	6.95
Control	5.00	111.43	38.77	24.87	45.98	63.90	29.76	4.33
SEm±	0.32	0.94	0.77	0.56	0.94	0.73	0.67	0.07
CD(P=0.05)	0.80	2.36	1.93	1.41	2.36	1.83	1.68	0.18
Method of application × Zn sources								
Nano ZnO control(T1)	4.33	109.01	35.50	26.10	43.47	66.96	30.66	6.10
Nano ZnO Seed priming (T2)	5.33	152.39	59.5	46.07	57.07	71.16	43.30	8.03
Nano ZnO Seed priming +foliar spray (T3)	5.00	169.43	57.13	36.80	68.30	80.07	54.48	8.57
Nano ZnO Foliar Spray (T4)	5.33	142.85	45.67	29.00	52.83	71.89	35.56	6.90
ZnSO ₄ Control (T1)	4.33	109.01	35.50	28.10	43.47	66.96	30.66	6.10
ZnSO ₄ seed priming (T2)	5.33	136.34	51.83	31.87	49.33	73.83	39.23	7.47
ZnSO ₄ seed priming + Foliar spray (T3)	5.00	144.00	61.87	33.87	54.33	72.87	49.52	8.00
ZnSO ₄ Foliar spray (T4)	5.67	127.70	36.13	27.00	46.90	66.163	32.91	6.23
SEm ±	0.65	1.87	1.55	1.13	1.88	1.47	1.34	0.134
CD (P=0.05)	2.16	6.21	5.15	3.75	6.24	4.88	4.45	0.43
CV (%)	22.28	2.40	5.59	6.16	6.40	3.57	5.85	3.33

Table.3 Interaction effect of different Zn sources and method of application on yield and Zn content in tomato

Treatment	Yield (kg/plant)	Fruit Zn (mg/100gm)	Leaf Zn (mg/100gm)	Root Zn (mg/100gm)
Nano ZnO control(T1)	3.54	2.60	2.80	2.20
Nano ZnO Seed priming (T2)	4.30	4.91	4.24	3.03
Nano ZnO Seed priming +foliar spray (T3)	4.67	6.93	6.87	3.67
Nano ZnO Foliar Spray (T4)	3.97	4.69	5.63	3.76
ZnSO ₄ Control (T1)	3.54	2.60	2.80	2.20
ZnSO ₄ seed priming (T2)	3.54	2.60	2.80	2.20
ZnSO ₄ Seed priming + Foliar spray (T3)	4.23	2.96	3.00	2.98
ZnSO ₄ Foliar spray (T4)	4.43	5.19	5.19	3.47
Nano ZnO control (T1)	3.83	3.60	4.27	3.10
SEm ±	0.08	0.13	0.11	0.21
CD(P=0.05)	0.27	0.41	0.37	0.70
CV (%)	3.62	5.58	4.46	12.03

The results from the bulk ZnSO₄ treated seeds were not promising (Table 1). Among the different nano scale ZnO concentrations, 400ppm showed the maximum and increased concentration showed decreased seedling vigour index

Field experiment

The observations recorded at 45 DAS reveals the promotory effect of nano scale ZnO. The result shows that there is a significant difference between both the Zn sources and also the method of application. The high root length was observed in nano ZnO T₂ (46.07cm) treatment followed by nano ZnO T₃ (36.80 cm). In ZnSO₄ all the treatments had comparatively less root length than nano ZnO. But all the Zn treated plants showed significantly highest root length compared to control (26.10 cm). ZnO NPs at all the methods of application proved to be effective in improving both root length and root dry weight it is represented in Table 2. These results confirmed that the physiological

effects were related to the nano meter sized particles.

Yield and Zn content

The results revealed that the response of tomato to nanoscale ZnO was highly significant. In comparison with granular zinc sulphates and method of application, ZnO NPs with Seed Priming + Foliar spray recorded highest yield with highest Zn accumulation in fruit (6.93mg/100gm), leaf (6.87mg/100gm) and root (3.67mg/100gm). Table 3 indicates the significant increase in yield and Zinc content by nano ZnO over granular ZnSO₄ and control. Zn Sources and method of application on yield and Zn content in tomato. Due to promotory effects of nano ZnO on plant growth, yield and zinc content significantly increased over ZnSO₄ and control. Such effects can be due to higher seedling vigour and early vegetative growth.

Nano particles (NPs) with small size and large surface area are expected to be the ideal

material for use as a Zn fertilizer in plants. Currently use of nano materials has been expanded in every fields of science including agriculture. It has been stated that application of micronutrient fertilizers in the form of NPs is an important route to release required nutrients gradually and in a controlled way, which is essential to mitigate the problems of fertilizer pollutions (Naderi and Abedi, 2012). It is because of that when materials are transformed to a nano, they change their physical, chemical and biological characteristics as well as catalytic properties and even more increase the chemical and biological activities (Mazaherinia *et al.*, 2010). The micronutrients in the form of NPs can be used in crop production to increase yield (Reynolds, 2002).

Recently it has been studied that nano ZnO positive impact on germination, growth and yield of peanut (Prasad *et al.*, 2012). It has been reported from pot culture experiments on wheat plants that increasing seed zinc content from 0.25 μg per seed to 0.70 μg per seed significantly improved root and shoot growth under Zn deficiency. Hence it may be concluded that high Zn content in seed could act as a starter fertilizer. Ajouri *et al.*, (2004) reported that seed priming with Zn was very effective in improving seed germination and seedling development in barley. These results may indicate that high Zn concentration in seeds has very important physiological roles during seed germination and early seedling growth. In our study we have standardized the Zn concentration for Tomato seeds using nano ZnO. At 400 ppm nano ZnO tomato seeds showed significantly highest germination percentage, root length, shoot length and seedling vigour index (Table 1). A significant increment in the germination, shoot length, root length and seedling vigour index was observed in the nano ZnO standardized concentration compare to the common ZnSO₄ at the same concentrations.

In one of the study on groundnut seeds with nano ZnO particles with a concentration of 1000 ppm also reported the significant increase in germination; shoot length, root length and vigour index (Prasad *et al.*, 2012). In one of the study on mung (*Vigna Radiata*) similar results were found with nano ZnO seed priming and also been observed that beyond optimum concentration growth was inhibited (Pramod *et al.*, 2011). Another report showed that effect of ZnO nanoparticle on the seed germination and root growth in black gram (*Cicer arietinum*) seeds on the reactivity of phytohormones especially indole acetic acid (IAA) involved in the phyto stimulatory actions. Due to oxygen vacancies, the oxygen deficient, i.e. zinc-rich ZnO nano particle increased the level of IAA in roots (sprouts) which in turn indicate the increase in the growth rate of plants (Avinash and Pandey *et al.*, 2010). Reports on mung (*Vigna radiate*) and in gram (*Cicer arietinum*) by using nano ZnO particle in agar method, which found to affect the growth of mung and gram seedlings at different concentrations. The maximum effect was found at 20 ppm for mung and 1 ppm for gram (Pramod Mahajan *et al.*, 2011).

Method of application of fertilizer is the most important concerned to the uptake and translocation onto the different parts of plant. Foliar fertilization is an important tool for the sustainable and productive management of crops. The ability of plant leaves to absorb water and nutrients was recognized approximately three centuries ago (Fernández and Eichert, 2009). The application of nutrient solutions to the foliage of plants as an alternative means of fertilize to crops such as grass. Spraying with 0.5 per cent ZnSO₄ gave significantly higher peanut pod yield compared to no spraying. However, soil application of 10 kg /ha ZnSO₄ during sowing gave yield on par with control plants without ZnSO₄ application. This indicates that

groundnut responds to foliar spray but not to soil application (Channabasavanna and Setty, 1993). The effectiveness of various synthetic and natural chelates has been widely investigated (Alvarez and Gonzalez, 2006; Gonzalez *et al.*, 2007; Prasad and Sinha, 1981). Apart from their effectiveness, application of chelates is generally expensive and may result in potential leaching risk because the more mobile the chelate, or the less biodegradable the carrier, the greater the risk of leaching (Gonzalez *et al.*, 2007). Zinc sulphate, which is highly soluble, can easily be taken up by plants but is known to fall off quickly. The retention time in the plant system is low. So the bioavailability of nutrients for long period was not sure with the use of ZnSO₄. If the plants are soft or sensitive and if the conditions are harsh like high temperatures, ZnSO₄ has a large salt index, which may burn the plant. Moreover, the zinc content in the mixture is usually very low (9–12 %). (Brown *et al.*, 1993; Fageria *et al.*, 2002)

In this study different treatments with nano Zn oxide particle and ZnSO₄ such as seed priming, foliar application and seed priming with foliar application were imposed to examine the treatment effect. Results suggest that nano ZnO form is absorbed by plants to a larger extent unlike bulk ZnSO₄. Nano ZnO has proved to be more effective in enhancing productivity and absorption of Zn because of high surface area to volume ratio. Better growth and 12-14 days early flowering was observed in nano ZnO treated onion plants at 20 and 30 µg/ml compared to control onion plants (Laware, 2014).

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