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□ *An investigation was initiated to examine the effects of nanoscale zinc oxide particles on plant growth and development. In view of the widespread cultivation of peanut in India and in other parts of the globe and in view of the potential influence of zinc on its growth, this plant was chosen as the model system. Peanut seeds were separately treated with different concentrations of nanoscale zinc oxide (ZnO) and chelated bulk zinc sulfate ($ZnSO_4$) suspensions (a common zinc supplement), respectively and the effect this treatment had on seed germination, seedling vigor, plant growth, flowering, chlorophyll content, pod yield and root growth were studied. Treatment of nanoscale ZnO (25 nm mean particle size) at 1000 ppm concentration promoted both seed germination and seedling vigor and in turn showed early establishment in soil manifested by early flowering and higher leaf chlorophyll content. These particles proved effective in increasing stem and root growth. Pod yield per plant was 34% higher compared to chelated bulk $ZnSO_4$. Consequently, a field experiment was conducted during* Rabi *seasons of 2008–2009 and 2009–2010 with the foliar application of nanoscale ZnO particles at 15 times lower dose compared to the chelated $ZnSO_4$ recommended and we recorded 29.5% and 26.3% higher pod yield, respectively, compared to chelated $ZnSO_4$. The inhibitory effect with higher nanoparticle concentration (2000 ppm) reveals the need for judicious usage of these particles in such applications. This is the first report on the effect of nanoscale particles on peanut growth and yield.*

Keywords: nanoscale ZnO, peanut, zinc uptake, seed germination, pod yield

INTRODUCTION

Zinc (Zn) is typically the second most abundant transition metal in organisms after iron and the only metal represented in all six enzyme classes

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(oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases) (Auld, 2001). Zinc is an essential micronutrient for humans, animals and plants. Higher plants generally absorb Zn as a divalent cation (Zn^{2+}), which acts either as the metal component of enzymes or as a functional structural or a regulatory co-factor of a large number of enzymes. A number of researchers have reported the essentiality and role of zinc for plant growth and yield (Camp and Fudge, 1945; Chapman, 1966; Viets, 1966; Anderson, 1972; Mengel and Kirkby, 1978; Marschner, 1993; Brown et al., 1993; Fageria et al., 2002). Based on analysis of 298 soil samples collected from different countries in the world, Zn deficiency has been found to be the most widespread micronutrient deficiency (Sillanpaa, 1990; Sillanpaa and Vlek, 1985). In India, Zn is now considered the fourth most important yield-limiting nutrient after nitrogen (N), phosphorus (P), and potassium (K). In India alone, 50% of the soils that groundnut is grown in show Zn deficiency, which is causing considerable yield loss (Singh, 1999). Half of the cultivated soils in Turkey have Zn deficiency (Eyupoglu et al., 1993). Considerable increases in grain yield by Zn application was also demonstrated in India (Tandon, 1995, 1998) and in Australia (Graham et al., 1992). Zinc is required for chlorophyll production, pollen function, fertilization and germination (Kaya and Higgs, 2002; Pandey et al., 2006; Cakmak, 2008). Zinc plays an important role in biomass production (Kaya and Higgs 2002). Among the micronutrients, Zn and manganese (Mn) can affect the susceptibility of plants to drought stress (Khan et al., 2003). A number of mechanisms may underlie Zn efficiency (Rengel, 2001). Depending on experimental conditions and the plant species, the most important mechanisms may be Zn utilization in tissues, called internal efficiency (Hacisalihoglu et al., 2003) and Zn uptake, called external efficiency (Genc et al., 2006). Zinc is intermediate in its mobility or phloem export. Longnecker and Robson (1993) suggested that zinc efficiency depends on the amount supplied and the nature of plant species. Zinc moves from leaves to roots, stem and developing grain and from one root to another (Rengel, 2001). Higher uptakes of other nutrients are also known to increase the demand of Zn.

Graham et al. (2001) reported that over 3 billion people across the world suffer from micronutrient deficiencies and suggested that a considerable amount of research in the 21st century should be devoted to develop technologies for enhanced uptake and accumulation of micronutrients in edible plant parts. Groundnut is an important legume food crop of India grown in about 8 million ha of land. Groundnut cultivation occurs in 108 countries around the world. The average productivity of groundnut in India is around 1178 kg ha^{-1}, which is far less than the world's average 1400 kg ha^{-1} (Directorate of Groundnut Research, 2008). The low productivity is mainly due to the fact that the crop is mostly grown in rain-fed, low fertility soils. Micronutrients, particularly Zn, will play an important role in stepping up the productivity of groundnut. In a field experiment on groundnut nutrition, the yield losses due to Zn deficiency were found to be 13.3% to 20% (Singh et al., 2004). The soil application of zinc sulfate showed a positive response with good germination and increased pod yield, pod number and oil content (Singh et al., 2004). Seed dressing with zinc oxide increase the pod yield (Gopala Gowda et al., 1994). A significant increase in number of pods/plant (14.97%), shelling percentage (3.56%), and pod yield (22%) due to the application of P and Zn were reported by Majumdar et al. (2001). Based on the data obtained from field experiment, Geeta et al. (1996), reported that the main root length, root dry weight and leaf area in groundnut were significantly influenced by the seed treatment with calcium (Ca)+Zn+Mn. More recently, substantial arable crop responses to Zn fertilization have been reported in Australia, India and Turkey, where wheat grain yields have increased by over 600% since the mid 1990s with the concomitant annual economic benefit of US $100 million (Cakmak, 2004). Particle size may affect agronomic effectiveness of Zn fertilizers. Decreased particle size results in increased number of particles per unit weight of applied Zn. Decreased particle size also increases the specific surface area of a fertilizer, which should increase the dissolution rate of fertilizers with low solubility in water such as zinc oxide (ZnO) (Mortvedt, 1992). Granular zinc sulfate ($ZnSO_4$) (1.4 to 2 mm) was somewhat less effective than fine $ZnSO_4$ (0.8 to 1.2 mm) whereas granular ZnO was completely ineffective (Allen and Terman, 1966). Gradual increase in Zn uptake could be observed with decreasing granule size and only the powder form produced plants with Zn concentrations in the sufficient range. Since granules of 1.5 mm weigh less than granules of 2.0 or 2.5 mm, smaller granules were used for the same weight, resulting in a better distribution of Zn, and the higher surface area of contact of Zn fertilizer resulted in better Zn uptake (Liscano et al., 2000). Therefore, ample work has been done and emphasis was made on the particle size to increase the efficiency of the fertilizers for better uptake and higher yields.

Nanomaterials are proposed to be the materials for the new millennium. Carbon-based and metal-based nanoparticles are most the commonly engineered and are often studied. Nanoparticles of size below 100 nm fall in the transition zone between individual molecules and the corresponding bulk materials, which generate both positive and negative biological effects in living cell (Nel et al., 2006). There is increasing amount of research on the biological effects of nanoparticles on higher plants. Several studies are concerned with the synthesis of nanomaterials using biological routes. Only limited studies have been reported on the promotory effects of nanoparticles on plants in low concentrations. Nanoscale titanium dioxide (TiO_2) was reported to promote photosynthesis, and growth of spinach (Hong et al., 2005; Yang et al., 2006). Similarly, mixture of nanoscale SiO_2 and TiO_2 hasten germination and growth in soya bean (Lu et al., 2002). The presence of nanoscale aluminum (Al) particles did not have a negative effect on the growth of *Phaseolus vulgaris* and *Lolium Perenne* in the tested concentration range (Doshi et al., 2008). Zhu et al., (2008) reported that *Cucurbita maxima* growing in an aqueous medium containing magnetic nanoparticles can absorb, move and accumulate the particles in the plant tissues, whereas *Phaseolus limensis* is not able to absorb and move particles. It indicates that different plants have different response to the same nanoparticles. Phytotoxicities of nanoparticles on plants were reported by Yang and Watts (2005) (in cabbage and carrot) and Lin and Xing (2007) (radish, rape, and rye grass) at concentrations greater than 2000 ppm. The experiments on application of aqueous TiO_2 (size 25 nm and 100 nm) to willow cuttings did not show any significant toxic effects and also it was observed that amount of aggregate formation and sedimentation seemed to be higher with the larger particles (Seeger et al., 2009). Compared to NPK chemical fertilizer, the application of slow/controlled release fertilizer coated and felted by nanomaterials were reported to improve grain yield with an insignificant increase in protein content and a decrease in soluble sugar content in wheat (Qiang et al., 2008). Phytotoxicity of commercially available ZnO nanoparticles to rye grass was reported by Lin and Xing (2008) and developmental phytotoxicity of commercially available metal oxide nanoparticles to *Arabidopsis Thaliana* was reported by Lee et al. (2010).

The present study was taken up to investigate the promotory or inhibitory effects of various concentrations of ZnO nanoparticles on growth, development and final yield of groundnut (*Arachis hypogaea* L). Nanoparticles with small size and large surface area are expected to be the ideal candidates for use as a Zn fertilizer in plants. Farmers are using both sulfates and chelated Zn (with ethylenediamminetetraacetic acid, EDTA) for soil and foliar applications; however, the efficacy is low. Therefore, this study was initiated to generate new information on the efficacy of nanoscale zinc oxide on the growth and development of groundnut. Four aspects were studied in this investigation: 1) the synthesis of nanoscale ZnO (mean particle size 25 nm); 2) the seed treatment with nanoscale ZnO and study of seed germination; 3) pot culture experiment; and 4) field experiment with foliar application of $ZnSO_4$ and nanoscale ZnO. Several experiments were conducted to optimize the dose of application of nanoscale ZnO and an optimum dose of 2g 15 L^{-1} (or 0.13 g L^{-1}) was arrived at for foliar spray, which has been compared to the recommended dose of chelated zinc sulfate (30 g 15 L^{-1}, or 2 g L^{-1}).

MATERIALS AND METHODS

ZnO Nanoparticles

ZnO nanoparticles of mean size of 25 nm diameter were used in the study. Nanocrystalline zinc oxide has been prepared by using the oxalate decomposition technique. Zinc oxalate was prepared by mixing equimolar

(0.2 M) solutions of zinc acetate and oxalic acid. The resultant precipitate was collected and rinsed extensively with double deionized water (DI-water) and dried in air. The oxalate was then ground and decomposed in air by placing it in a pre-heated furnace for 45 minutes at 500°C. The characterization of the samples was done by transmission electron microscopy (HRTEM, JEOL 3010; Jeol Ltd, Peabody, MA, USA), scanning electron microscopy (SEM, FEI Quanta 200; FEI, Malvern, UK) and energy dispersive analysis of X-rays (EDAX, FEI Quanta 200; FEI). The TEM samples were prepared by drop casting the suspensions on carbon coated Cu grids.

Seeds

Peanut seeds of variety 'K-134' were procured from Agricultural Research Station, Kadiri, Acharya N. G. Ranga Agricultural University, Andhra Pradesh, India. The average germination rate of the seeds was 85% as shown by a preliminary study. The seeds selected were of uniform size to minimize errors in seed germination and seedling vigor.

Preparation of Particle Suspensions and Seed Treatment

Chelated bulk $ZnSO_4$ was used as a reference Zn source. Because bulk ZnO will not dissolve in water and plants cannot absorb it, farmers are widely using chelated $ZnSO_4$. The materials were suspended directly in deionized 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. Both bulk (chelated) $ZnSO_4$ and nanoscale ZnO suspensions were prepared at concentrations of 400, 1000 and 2000 ppm (concentrations referred to in terms of zinc content). Five peanut seeds were soaked in 100 mL of these solutions/suspensions of both bulk $ZnSO_4$ and nanoscale ZnO for three hours. Four replicates were maintained. All references to bulk $ZnSO_4$ later in the text is for chelated $ZnSO_4$

The suspensions are labeled such that B and N refer to bulk $ZnSO_4$ and nanoscale ZnO, respectively. For example, 400B and 400N refer to suspensions of 400 ppm bulk $ZnSO_4$ and nanoscale ZnO, respectively. The nanoscale suspensions, as expected, appear 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.

Lab Experiments

Two sets of seed treatment experiments were conducted in the lab. One set of treated seeds (four replicates) was used for conducting lab experiments to determine the effect of treatment on seed germination and seedling vigor index and another set of seeds was used for conducting the pot culture experiment.

Seedling Vigor Index

Treated peanut seeds were shade-dried for 1 hour. Then the seeds were placed in a Petri dish (100 mm x15 mm) with one piece of sterilized filter paper and 5 mL of water was added (as per the recommendations of the International Seed Testing Association (1976). Petri dishes were covered and placed in an incubator at 26 ± 1°C for eight days. Watering was given to all Petri plates. After eight days, maximum seeds were germinated and developed into normal seedlings. Germination was calculated based on the number of seeds germinated in a Petri plate having five seeds and expressed as germination percentage. Seedling Vigor Index (SVI) was calculated by the formula described by Abdul-Baki and Anderson (1973).

$$\text{Seed Vigor Index} = \text{Germination\%} \times (\text{root length} + \text{shoot length})$$

Pot Culture Experiments

Another set of treated peanut seeds (four replicates) were sown in pots (20 cm × 40 cm) filled with equal quantity of soil and watered to field capacity. Proper care was taken to use similar soil in all the pots to minimize soil heterogeneity effects. After germination, one plant per pot was maintained throughout. Proper agronomic and plant protection management was done to all the treated plants for their maximum growth expression. The following data were collected on all the plants of four replications.

a) Plant height was measured from ground node to shoot growing apex and expressed in cm before harvest.
b) Days to flowering were calculated based on the days taken from sowing to the appearance of first flower.
c) The procedure developed by Witham et al. (1971) was followed for estimation of chlorophyll content of leaves.

Harvesting

After 110 days from sowing, plants were uprooted gently along with the whole soil mass. Plants with whole root were recovered by spraying fine water on the soil mass. Roots were separated and used for recording the parameters. Similarly, matured, filled and unfilled pods were dried to the moisture level of 12% and dry weight per plant was recorded.

Roots were thoroughly washed and their volume was measured by water replacement method and expressed as mL and total length was measured and expressed in cm. Then the roots were dried for two days at 80°C in an oven and dry weight was taken and expressed in grams.

Field Experiment

The field experiment was conducted during *Rabi* seasons, 2008–2009 and 2009–2010 at Regional Agricultural Research Station, Acharya N. G. Ranga Agricultural University, Tirupati. The experiment was laid out (Var. Narayani) in randomized block design replicated seven times. The gross plot size was 4×5 m^2. Three treatments viz, T_1: NPK (30-40-50), T_2: NPK + chelated zinc at 30 g 15 L^{-1} foliar spray (at 35 days and 70 days) and T_3: NPK + nanoscale ZnO (size 25 nm) at 2 g 15 L^{-1} foliar spray (at 35 days andand 70 days) were imposed. The initial soil (red sandy loam) parameters were pH 6.85; electrical conductivity (E.C.; dS m^{-1}) 0.132; organic carbon (O.C,%) 0.485; available phosphorus pentoxide (P_2O_5) 14.43 kg ha^{-1}; potassium oxide (K_2O) 172 kg ha^{-1}; zinc (Zn) 0.74 ppm; copper (Cu) 1.55 ppm; iron (Fe) 9.93 ppm; and manganese (Mn) 28.06 ppm. Plant physiological parameters *viz* plant height and number of branches were recorded in all the treatments.

Statistical Analysis

Each treatment was conducted with seven replicates and the results were presented as mean ± standard deviation (SD). The statistical analysis of experimental data utilized the ANOVA program. Each experimental value was compared to its corresponding control. Statistical significance was accepted when the probability of the result assuming the null hypothesis (p) is less than 0.05 (level of probability).

RESULTS

Characterization of the Nanoparticle

Figure 1 shows an HRTEM image of the nanoparticle sample. The image shows ZnO nanoparticles with mean particle diameter of 25 nm and they looked slightly aggregated as there were no protecting ligands on the surface. The particles are crystalline as revealed by the high magnification image and the lattice of ZnO is clearly seen. Nanoparticles showed lattice spacing of 0.26 nm and 0.28 nm corresponding to (0002) and ($10\bar{1}0$) planes of wurtzite ZnO (Lin et al., 2009, Zhu et al., 2009).

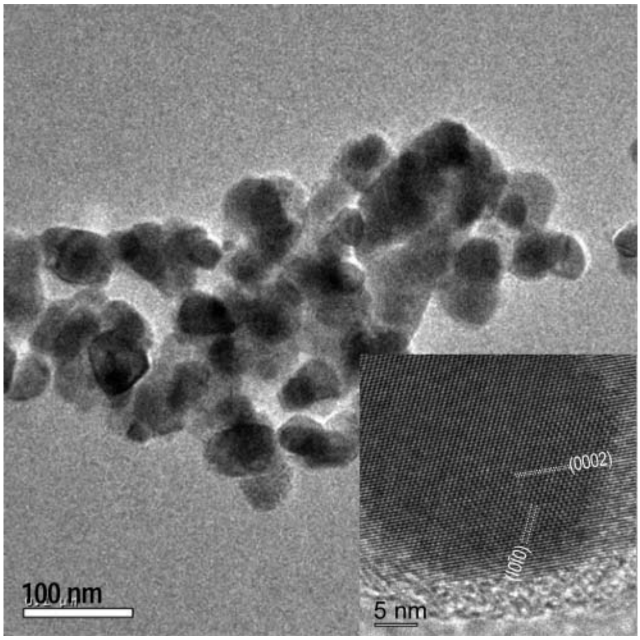

FIGURE 1 Large area TEM image of ZnO nanoparticles. Inset shows the high resolution image of a single particle.

Confirmation of Zn Uptake by Seeds

The uptake of Zn by the seeds was confirmed by SEM-EDAX measurements (Figure 2). SEM of thin sections of the peanut embryo was examined by sectioning the seed. Although the concentration of Zn was low as expected, it could be observed in EDAX spectra and the EDAX images confirmed the presence of higher amounts of Zn in regions where C and N concentrations are higher in the seeds treated with nanoscale ZnO. The post harvest leaf and kernel samples were analyzed to estimate the zinc content by using Atomic Absorption Spectrophotometer (AAS).

Seed Germination and Seedling Vigor

Peanut seeds responded variably towards the treatment at various concentrations of both bulk $ZnSO_4$ and nanoscale ZnO particles. Seed treated with 1000 ppm nanoscale ZnO recorded significant germination (100%) and seedling vigor index (1701.3). Root growth was also very good as can be observed from the picture (Figure 3). The results from the bulk $ZnSO_4$ treated seeds were not promising (Table 1). Among the different nanoscale ZnO concentrations, 1000 ppm showed the maximum and increased concentration (2000 ppm) showed decreased seedling vigor index. Such inhibitory effects of nanoparticles were also reported by Lin and Xing (2007) on radish,

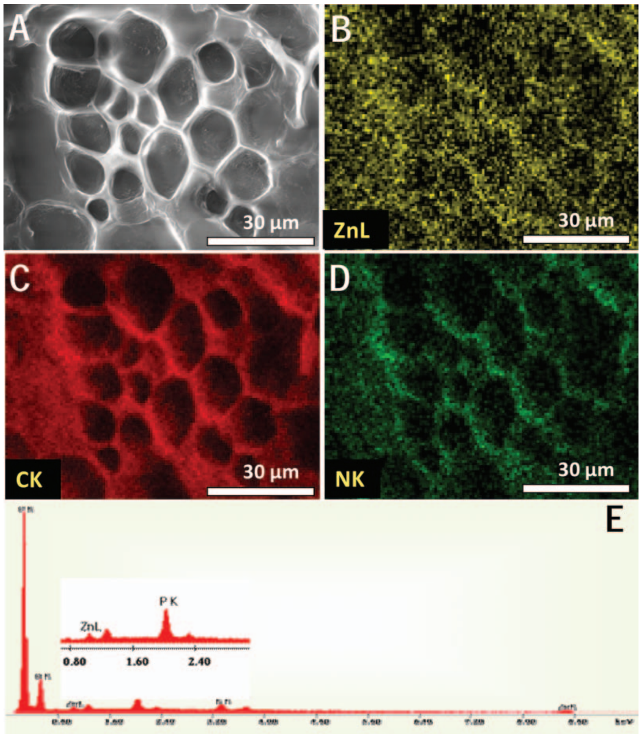

FIGURE 2 A) SEM image of the peanut seed embryo after soaking in nanoscale ZnO (1000 ppm) for 3 h. B, C, D) EDAX images of the region in A using Zn Lα, C Kα and N Kα lines. E) EDAX spectrum from the region in A (Color figure available online).

rape, and rye grass. However, performance of the bulk material is better than the control.

Nanoscale ZnO showed large root growth of seedling compared to bulk $ZnSO_4$ and control. Such promotory effect of nanoscale SiO_2 and TiO_2 on germination was reported in soya bean (Lu et al., 2002), in which authors noticed increased nitrate reductase enzyme activity and enhanced antioxidant system. Plant growth, in terms of plant height was significantly increased with 400 and 1000 ppm nanoscale ZnO compared to control and the respective bulk $ZnSO_4$ concentrations (Table 2). Seeds treated with 1000 ppm concentration of nanoscale ZnO recorded highest plant growth (15.4 cm)

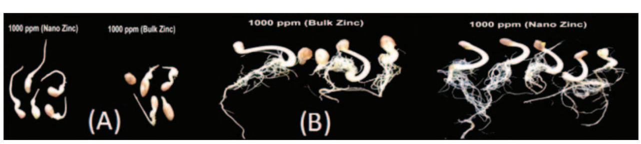

FIGURE 3 Photographs of the seeds showing differences in germination and root growth A) after three days and B) nine days after the treatment (Color figure available online).

TABLE 1 Effect of nanoscale ZnO and bulk $ZnSO_4$ on peanut mean germination and vigor

S. No.	Concentration (ppm)	Germination (%)		Shoot length (cm)		Root length (cm)		SVI	
		$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO
1.	400	84.01 ± 0.94	90.33* ± 1.40	3.80 ± 0.20	6.60* ± 0.18	5.84 ± 0.12	11.52** ± 0.23	793.02* ± 6.83	1522.61** ± 12.32
2.	1000	90.32* ± 1.26	99.02** ± 1.41	4.32 ± 0.15	8.71* ± 0.20	6.72* ± 0.19	11.81** ± 0.19	910.36* ± 8.56	1701.33** ± 9.89
3.	2000	88.75 ± 1.29	96.04* ± 1.49	3.76 ± 0.18	4.94 ± 0.11	8.06* ± 0.21	9.42* ± 0.14	1195.72* ± 10.90	1321.74** ± 10.54
4.	Control	85.30		3.11		5.02		693.60	
	CD@5%	2.80		1.93		1.16		15.82	

Each value is the mean± SE of seven replicates.
*Significant at p (level of probability) less than 0.05.
**Highly significant at p (level of probability) less than 0.05.

TABLE 2 Effect of nanoscale ZnO and bulk $ZnSO_4$ on peanut mean plant growth, flowering and leaf chlorophyll content

S. No.	Concentration (ppm)	Plant height (cm)		Initiation of flowering (days)		Chlorophyll content (mg/g fresh wt.)	
		$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO
1.	400	9.38* ± 0.12	13.46** ± 0.10	29.12 ± 2.67	29.96 ± 2.32	1.44* ± 0.01	1.66* ± 0.02
2.	1000	12.42** ± 0.09	15.40** ± 0.02	29.01 ± 1.94	27.24 ± 1.65	1.74** ± 0.02	1.97** ± 0.02
3.	2000	9.54* ± 0.10	10.41** ± 0.05	30.42 ± 2.85	30.09 ± 2.96	1.52* ± 0.01	1.76** ± 0.01
4.	Control	8.22		29.00		1.39	
	CD@5%	0.16		NS		0.015	

Each value is the mean± SE of seven replicates.
*Significant at p (level of probability) less than 0.05.
**Highly significant at p (level of probability) less than 0.05.

due to extended inter-nodal length. Such increase can be ascribed to higher precursor activity of nanoscale zinc in auxin production (Kobayashi and Mizutani, 1970). Similarly 1000 ppm nanoscale ZnO produced early flowers compared to control and bulk $ZnSO_4$. Such effects can be due to higher seedling vigour and early vegetative growth. Nanoscale ZnO increased leaf chlorophyll content irrespective of concentrations compared to bulk $ZnSO_4$ and control. Nanoscale ZnO at 1000 ppm recorded the highest chlorophyll content (1.97 mg/g/rt.wt). Higher chlorophyll accumulation may be due to complementary effect of other inherent nutrients like magnesium, iron and sulfur. Similar results were observed by Zhang et al., 2005 when *Spinacia oleracea* seeds were treated with nanoscale TiO_2 particles. An increase of germination rate and the vigor indices was noted at 0.25–4% nanoscale TiO_2 treatment. During the growth period, the plant dry weight increased. These results confirmed that the physiological effects were related to the nanometer sized particles.

Root Growth and Yield

Plants were harvested after 110 days from sowing. The results revealed the promotory effect of nanoscale ZnO at optimum concentrations and inhibitory effect at high concentrations on root and shoot growth (Figure 4) and pod yield (Table 3). Nanoscale ZnO at 1000 ppm proved to be effective in improving both root volume and root dry weight, as it was also noticed in the seedling stage (Figure 5). An increase of the shoot/root ratio compared to that of the control was reported by Shah and Belozerova (2009) while analyzing the influence of metal nanoparticles on germination of *Lactuca* seeds. Due to its promotory effects on plant growth, pod yields were significantly increased over control and $ZnSO_4$. At higher concentration of nanoscale ZnO, at 2000 ppm, both plant growth and pod yield decreased and these results were in accordance with the reports on radish, rape, corn, lettuce and cucumber by Lin and Xing, 2007.

Yield and Yield Attributes

The results revealed that the response of groundnut to lower dose of nanoscale ZnO was highly significant. The dry pod yield of groundnut was greatly influenced by nanoscale zinc (Figure 6). Figures 6 A and B show increased pod yield upon application of nanoscale ZnO at 2 g 15 L^{-1}. The data in Table 4 indicate significant increase in number of pods per plant, number of filled pods per plant, and also plant height with the application of nanoscale ZnO at 2 g 15 L^{-1}. From the data in Tables 5 and 6 it is observed that 30.5% and 38.8% higher pod yield was recorded with the application of nanoscale ZnO at 2 g 15 L^{-1} + NPK compared to NPK alone and 29.5% and 26.3% higher pod yield compared to chelated zinc at 30 g 15 L^{-1} + NPK.

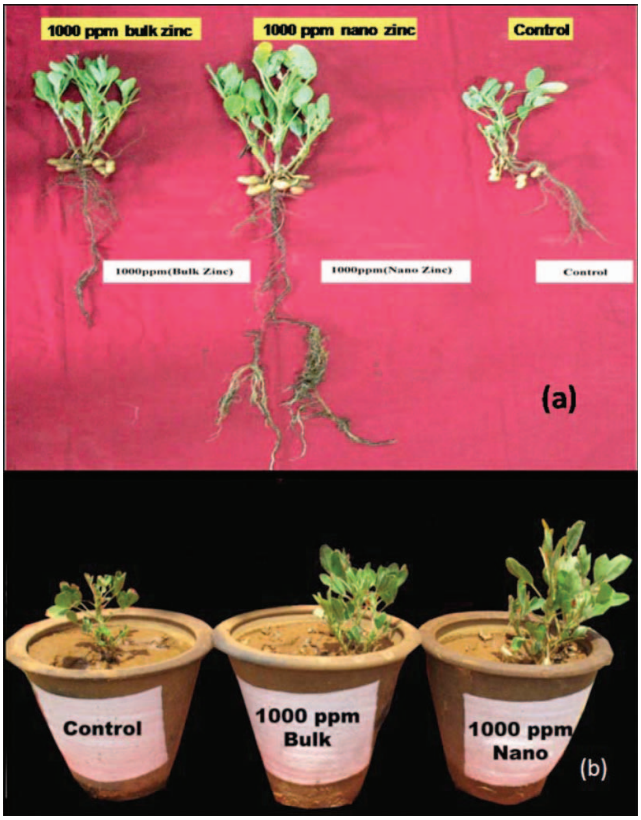

FIGURE 4 A) Higher root growth of peanut plant after nanoscale ZnO treatment (1000 ppm). The plants were uprooted after 110 days. B) Pot culture experiment showing higher plant growth after nanoscale ZnO treatment (1000 ppm), after 110 days (Color figure available online).

In general, foliar application of nanoscale ZnO at 2 g 15 L^{-1} significantly increased pod yield and shelling percent and other biometric parameters. Figure 6C shows the control experiment at similar conditions showing lower yield.

DISCUSSION

Zinc plays a fundamental role in protecting and maintaining structural stability of cell membranes (Welch et al., 1982; Cakmak, 2000). Zn is used for protein synthesis, membrane function, cell elongation and tolerance to environmental stresses (Cakmak, 2000). Plants emerging from seeds with low Zn have poor seedling vigor and field establishment on Zn-deficient soils (Yilmaz et al., 1998). Rengel and Graham (1995) 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

TABLE 3 Effect of nanoscale ZnO and bulk $ZnSO_4$ on mean root growth, shoot growth, dry weight and pod yield in peanut

S. No	Concentration (ppm)	Root volume		Root dry wt (g)		Stem dry wt (g)		No.of filled pods/pl		Pod dry wt (g)	
		$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO
1.	400	2.20 ± 0.10	3.20 ± 0.08	0.72* ± 0.05	1.21** ± 0.02	3.84* ± 0.21	6.64* ± 0.26	1.93 ± 0.01	1.96 ± 0.07	2.70* ± 0.09	3.04* ± 0.16
2.	1000	2.10 ± 0.09	4.22 ± 0.10	0.54 ± 0.01	1.20** ± 0.06	4.29* ± 0.15	8.72** ± 0.18	5.96** ± 0.04	6.59 ± **0.01	3.97* ± 0.07	5.39** ± 0.11
3.	2000	3.21 ± 0.16	2.16 ± 0.06	0.47 ± 0.02	0.92* ± 0.03	3.75* ± 0.20	4.96* ± 0.22	3.05* ± 0.03	2.04 ± 0.02	1.70 ± 0.02	1.09 ± 0.04
4.	Control	2.10		0.47		1.91		2.00		1.18	
	CD@5%	NS		0.07		0.01		0.08		0.60	

Each value is the mean± SE of seven replicates.
*Significant at p (level of probability) less than 0.05.
**Highly significant at p (level of probability) less than 0.05.

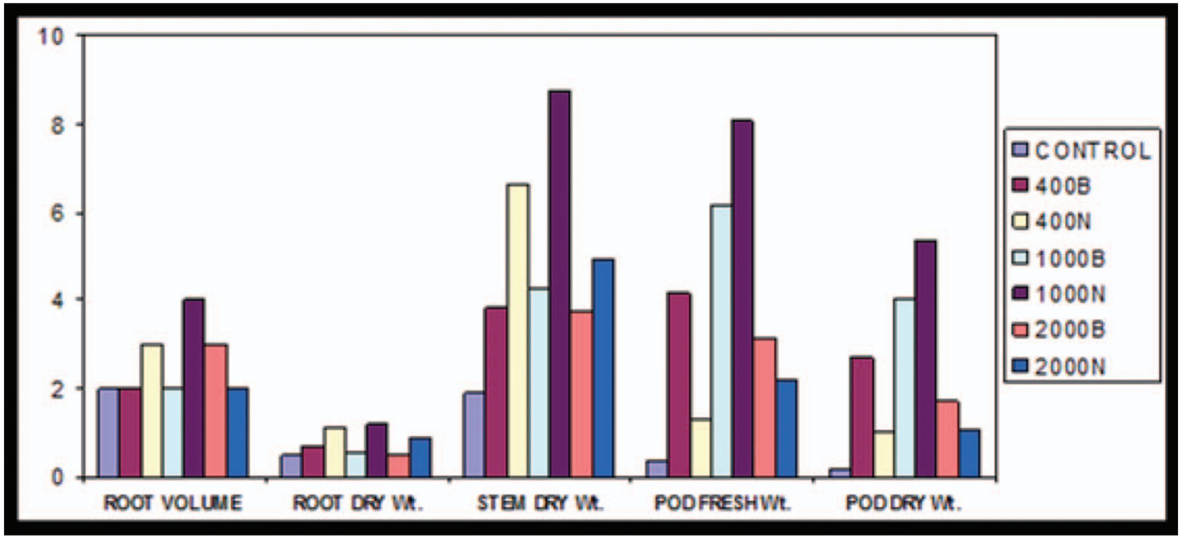

FIGURE 5 Bar chart showing the effect of nanoscale ZnO (N) and bulk $ZnSO_4$ (B) concentrations on peanut plant root, stem growth and pod yield (Color figure available online).

FIGURE 6 Photograph showing the effect of foliar application of lower dosage of nanoscale ZnO on the pod yield. A and B) nanoscale ZnO @ 2 g/ 15 L and C) control (Color figure available online).

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. Slaton et al. (2001) reported that treating rice seeds with Zn greatly increased grain yield and concluded that this type of Zn application method is a very economical alternative to more expensive broadcast Zn fertilizer applications. In the present study, treating groundnut seeds with nanoscale ZnO particles with a concentration of 1000 ppm has shown significant increment in germination, shoot length, root length and vigor index over other concentrations of the same material and varying concentrations of another material (chelated zinc sulfate) tested. The exact reason for these effects is not known but it is likely to be due to the higher concentrations of zinc in the seed when treated with nanoscale ZnO particles.

Foliar fertilization is more effective than soil application. Foliar Zn application significantly increased grain Zn concentrations of wheat, indicating high mobility of Zn within plants. Spraying with 0.5% $ZnSO_4$ gave

TABLE 4 Response of peanut to application of nanoscale zinc oxide

S. No.	Treatment	Plant height (cm)	No. of branches per plant	No. of pods per per plant	No. of filled pods per plant	No. of ill filled pods per plant
1.	T1 = NPK (Control)	36.50 ± 1.20	3.85 ± 0.42	9.20 ± 1.89	8.20 ± 1.80	1.00 ± 0.04
2.	T2 = NPK + $ZnSO_4$ (Chelated)@30g/15 L	37.10 ± 1.98	3.85 ± 0.78	10.10 ± 2.42	9.10 ± 2.01	1.00 ± 0.02
3.	T3 = NPK + ZnO (Nano)@2g/15 L	43.80* ± 2.10	4.57 ± 0.65	16.80* ± 2.01	15.00* ± 1.98	1.80 ± 0.05
	CD@5%	4.47	NS	3.76	2.99	0.92

Each value is the mean± SE of seven replicates.
*Significant at p (level of probability) less than 0.05.
**Highly significant at p (level of probability) less than 0.05.

TABLE 5 Effect of nanoscale zinc oxide on yield and yield attributes of peanut (*Rabi* season 2008–2009)

S. No.	Treatment	Pod yield (kg/ha)	100 pod weight (g)	100 kernel weight (g)	Shelling percentage
1.	T1 = NPK (Control)	2391.56 ± 38.40	77.27 ± 1.52	31.50 ± 2.08	63.81 ± 2.26
2.	T2 = NPK + $ZnSO_4$ (Chelated)@30g/15 L	2410.82 ± 72.86	74.82 ± 0.58	30.92 ± 1.96	64.62 ± 2.17
3.	T3 = NPK + ZnO (Nano)@2g/15 L	3121.54** ± 115..23	83.90** ± 0.46	36.25* ± 2.14	67.50** ± 1.45
	CD@5%	199.92	2.89	2.52	2.68

Each value is the mean± SE of seven replicates.
*Significant at p (level of probability) less than 0.05.
**Highly significant at p (level of probability) less than 0.05.

significantly higher peanut pod yield compared to no spraying. However, soil application of 10 kg ha^{-1} $ZnSO_4$ at sowing gave yield on par with no $ZnSO_4$ 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 sulfate, 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 $ZnSO_4$. If the plants are soft or sensitive and the conditions are harsh like high temperatures, $ZnSO_4$ has a large salt index, which may burn the plant. Moreover, the zinc content in the mixture is usually very low (9–12%). Our study suggests that ZnO in the nanoscale form is absorbed by plants to a larger extent unlike bulk $ZnSO_4$. These particles proved effective in enhancing plant growth, development and yield. A

TABLE 6 Effect of nanoscale zinc oxide on yield and yield attributes of peanut (*Rabi* season 2009–2010)

S. No.	Treatment	Pod yield (kg/ha)	100 pod weight (g)	100 kernel weight (g)	Shelling percentage
1.	T1 = NPK (Control)	2711.78 ± 25.34	83.26 ± 1.11	31.60 ± 0.56	60.22 ± 0.21
2.	T2 = NPK + $ZnSO_4$ (Chelated)@30g/15 L	2978.42* ± 39.71	112.14* ± 1.78	37.82* ± 0.22	66.97* ± 0..72
3.	T3 = NPK + ZnO (Nano) @2g/15 L	3763.65** ± 56.09	117.80** ± 2.43	47.91** ± 0.34	69.30** ± 0.65
	CD@5%	90.78	3.49	0.98	1.03

Each value is the mean± SE of seven replicates.
*Significant at p (level of probability) less than 0.05.
**Highly significant at p (level of probability) less than 0.05.

TABLE 7 Effect of nanoscale zinc oxide on uptake of zinc by leaf and kernel of peanut

S. No.	Treatment	Zinc content (ppm) 2008–2009 (*Rabi* season)		Zinc content (ppm) 2009–2010 (*Rabi* season)	
		Leaf (post harvest)	Kernel	Leaf (post harvest)	Kernel
1.	T1 = NPK (Control)	22.31 ± 1.08	21.84 ± 0.67	22.81 ± 1.31	20.46 ± 0.56
2.	T2 = NPK + $ZnSO_4$ (Chelated)@30g/15 L	31.46* ± 1.05	28.32* ± 0.84	32.36* ± 0.93	29.21* ± 0.76
3.	T3 = NPK + ZnO (Nano)@2g/15 L	44.80** ± 1.08	40.20** ± 0.31	41.83** ± 1.06	39.90** ± 0.89
	CD@5%	1.50	1.36	1.46	1.35

Each value is the mean± SE of seven replicates.
*Significant at p (level of probability) less than 0.05.
**Highly significant at p (level of probability) less than 0.05.

lower dose of foliar application is proved to be significantly productive. The post harvest leaf and kernel samples analysis (Table 7) revealed a significant increment in zinc content in leaves (42%, 29%) and kernels (42%, 36.6%) when supplied with nanoscale ZnO compared to chelated $ZnSO_4$ (in *Rabi* seasons 2009 and 2010, respectively). Similarly, nanoscale nutrients at high concentrations are detrimental just as the bulk nutrients. Similar results were observed by Racuciu and Creanga (2007) when they analyzed the influence of magnetic nanoparticles coated with tetramethylammonium hydroxide on the growth of *Zea mays* plant in early ontogenetic stages. Small concentrations of aqueous ferrofluid added in culture medium had a stimulating effect on the growth of plantlets while higher concentrations of aqueous ferrofluid induced an inhibitory effect.

The mechanism of foliar uptake pathway for aqueous solutes and water–suspended nanoparticles was well discussed by Eichert et al. (2008) in the context of *Allium porrum* and *Vicia faba* (L). The results suggest that the stomatal pathway differ fundamentally from the cuticular foliar uptake pathway. Low penetration rates in thick leaves, rapid drying of spray solution, limited translocation within the plant, and leaf damage are the problems of concern (Marschner, 1995) and most foliar applied micronutrients are not efficiently transported towards the roots. Concentrated liquid suspensions of ZnO are used for foliar application but their performance is strongly determined by the size range specification of the ZnO particles present in the formulation (Moran, 2004). Leaf water repellency of adaxial or abaxial surface is a main limiting factor, which can affect the Zn uptake through spray application processes (Watanabe and Yamaguchi, 1991; Holder, 2007). The permeability of the cuticle to water and to lipophilic organic molecules increases with mobility (distribution co-efficients) and solubility (partition co-efficients) of these compounds within the transport-limiting barrier of the cuticles. Ions being highly water soluble might have some hindrance in penetrating the lipophilic cuticle. This may be acting as a limiting factor in the case of chelated $ZnSO_4$. But our custom-made nanoscale ZnO, which is having less hydrophilicity and being more dispersible in lypophilic substances compared to the ions, can penetrate through the leaf surface (Da Silva et al., 2006) compared to $ZnSO_4$. Also the mobility of the nanoparticles is known to be very high which ensures the phloem transport and ensures the nutrient to reach all parts of the plant. The presence of nanoparticles both in the extracellular space and within some cells in the living plant *Cucurbita pepo* was reported (Gonzalez-Melendi et al., 2008). The bioavailability of the nanoparticle because of its size and lower water solubility (which inhibit rapid falling off compared to ionic supplements) can also be higher compared to chelated $ZnSO_4$. The inherent small size and the associated large surface area of nanoscale ZnO fertilizer may increase the uptake as reported earlier. This enhanced uptake of Zn was seen in the EDAX analysis of the seeds also. All these factors may be responsible to give higher yields for nanoscale ZnO compared to chelated $ZnSO_4$. The promotory effects of nanoscale ZnO at cellular level has to be understood by further in depth investigations.

In addition, most of the research conducted on the micronutrient nutrition of plants deals with correcting the deficiencies and thereby increasing the grain yield. But research on enhancing micronutrient concentrations in grain or other parts of the plant is very limited. More research is required on improving the bioavailability levels of micronutrients in grains.

Properties of engineered nanoparticles depend on the size, shape, surface functionalization, etc. The results presented in this study used a specific kind of nanoparticle, which were made in view of agricultural applications. These results may not be extrapolated to the same nanomaterial or any other nanomaterial prepared using other routes.

CONCLUSIONS

In order to understand the possible benefits of applying nanomaterials in agriculture, it is important to analyze penetration and transport of nanoparticles in the plants. Size plays an important role in behavior, in reactivity and in toxicity. Considering these aspects, both positive and negative effects of nanoparticles are observed in living plants.

The results suggest that the micronutriuent, Zn can be delivered into peanut seeds through ZnO nanoparticles. A higher amount of Zn was present in the seed when treated with nanoscale ZnO. This improves the germination, root growth, shoot growth dry weight and pod yield of the treated seeds. Significant zinc uptake by the leaf and kernel was observed with the foliar application of nanoscale ZnO compared to chelated zinc sulfate (Table 7). The results point to the use of nanomaterials in agriculture, especially in peanut, one of the main sources of livelihood in certain parts of the world. The results emphasize that nanoscale nutrients can be supplied to the crops either through seed dressing or by foliar application with much decreased doses to get the desired results. Detailed studies have to be performed to understand the mechanism of action of nanoscale materials. Further, the most efficient method of application has to be evaluated.

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REFERENCES

Abdul-Baki, A. A., and J. D. Anderson. 1973. Vigor determination in soybean seed by multiple criteria. *Crop Science* 13: 630–633.

Ajouri, A., H. Asgedom, and M. Becker. 2004. Seed priming enhances germination and seedling growth of barley under conditions of P and Zn deficiency. *Journal of Plant Nutrition and Soil Science* 167: 630–636.

Allen, S. E, and G. L. Terman. 1966. Response of maize and sudangrass to zinc in granular micronutrients. *Trans Comm. II and IV*: 255–266.

Alvarez, J. M, and D. Gonzalez. 2006. Zinc transformations in neutral soil and zinc efficiency in maize fertilization. *Journal of Agricultural and Food Chemistry* 54: 9488–9495.

Anderson, W. B. 1972. Zinc in soils and plant nutrition. *Advances in Agronomy* 24: 147–186.

Auld, D. S. 2001. Zinc coordination sphere in biochemical zinc sites. *Biometals* 14: 271–313.

Brown, P. H., I. Cakmak, and Q. Zhang. 1993. Forms and function of zinc in plants. In: *Zinc in Soil and Plants*, ed. A. D. Robson, pp. 93–106. Dordrecht, the Netherlands: Kluwer Academic Publishers.

Cakmak, I. 2000. Role of zinc in protecting plant cells from reactive oxygen species. *New Phytologist* 146: 185–205.

Cakmak, I. 2004. Identification and correction of widespread zinc deficiency in Turkey – A success story (A NATO-Science For Stability Project). *Proceedings of the International Fertilizer Society* 552: 1–26.

Cakmak, I. 2008. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant and Soil* 302: 1–17.

Camp, A. F., and B. R. Fudge. 1945. Zinc as a nutrient in plant growth. *Soil Science* 60: 157–164.

Channabasavanna, A. S, and R. A. Setty. 1993. Effect of nitrogen, ferrous sulfate and zinc sulfate on groundnut yield in deep black soils. *Indian Journal of Agronomy* 38: 329–330.

Chapman, H. D. 1966. Zinc. In: *Diagnostic Criteria for Plant and Soils*, ed. H. D. Chapman, pp. 484–499. Riverside, CA: University of California.

Da Silva, L. C., M. A. Oliva, A. A. Azevedo, and M. J. De Araujo. 2006. Response of resting plant species to pollution from an iron pelletization factory. *Water, Air and Soil Pollution* 175: 241–256.

Directorate of Groundnut Research. 2008. AICRP on Groundnut. Junagadh, India: Directorate of Groundnut Research.

Doshi, R., W. Braida, C. Christodoulatos, M. Wazne, and G. O'connor. 2008. Nano aluminum: Transport through sand columns and environmental effects on plant and soil communities. *Environmental Research* 106: 296–303.

Eichert, T., A. Kurtz, U. Steiner, and H. E. Goldbach. 2008. Size exclusion limits and lateral heterogeneity of the stomatal foliar uptake pathway for aqueous solutes and water suspended nanoparticles. *Physiologia Plantarum* 134: 151–160.

Eyupoglu, F., N. Kurucu, and U. Sanisa. 1993. Status of plant available micronutrients in Turkish soils. In: *Annual Report. Report No. R-118*, pp.25–32. Ankara, Turkey: Soil and Fertilizer Research Institute.

Fageria, N. K., V. C. Baligar, and R. B. Clark. 2002. Micronutrients in crop production. *Advances in Agronomy* 77: 189–272.

Geeta, K. N., A. G. Shankar, and K. Shiva Shankar. 1996. Effect of molybdenum, zinc and calcium on productivity of groundnut (*Arachis hypogaea* Gaertu). *Journal of Oilseeds Research* 13: 167–172.

Genc, Y., G. K. McDonald, and R. D. Graham. 2006. Contribution of different mechanisms to zinc efficiency in bread wheat during early vegetative stage. *Plant and Soil* 281: 353–367.

Gonzalez, D., A. Obrador, and J. M. Alvarez. 2007. Behavior of zinc from six organic fertilizers applied to a navy bean crop grown in a calcareous soil. *Journal of Agricultural and Food Chemistry* 55: 7084–7092.

Gonzalez-Melendi, P., R. Fernandez Pacheco, M. J. Coronado, E. Corredor, P. S. Testillano, M. C. Risueno, C. Marquina, M. R. Ibarra, D. Rubiales, and A. Perez-De-Luque. 2008. Nanoparticles as smart treatment-delivery systems in plants: Assessment of different techniques of microscopy for their visualization in plant tissues. *Annals of Botany* 101: 187–195.

Gopala Gowda, N., B. Shivaraj, and A. Gowda. 1994. Effect of zinc and molybdenum application on yield and uptake of zinc by groundnut. *Journal of Research, Andhra Pradesh Agricultural University* 22: 40–42.

Graham, R. D., J. S. Ascher, and S. C. Hynes. 1992. Selecting zinc efficient cereal genotypes for soils of low zinc status. *Plant and Soil* 146: 241–250.

Graham R. D., R. M. Welch, and H. E. Bouis. 2001. Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: Principles, perspectives and knowledge gaps. *Advances in Agronomy* 70: 77–142.

Hacisalihoglu, G., J. J. Hart, Y. H. Wang, I. Cakmak, and L. V. Kochian. 2003. Zinc deficiency is correlated with enhanced expression and activity of zinc-requiring enzymes in wheat. *Plant Physiology* 131: 595–602.

Holder, C. D. 2007. Leaf water repellency of species in Guatemala and Colorado (USA) and its significance to forest hydrology studies. *Journal of Hydrology* 336: 147–154.

Hong, F. S, F. Yang, Z. N. Ma, J. Zhou, C. Liu, C. Wu, and P. Yang. 2005. Influences of nano-TiO_2 on the chloroplast ageing of spinach under light. *Biological Trace Element Research* 104: 249–260.

International Seed Testing Association. 1976. International rules for seed testing. *Seed Science and Technology* 4: 3–49.

Kaya, C., and D. Higgs. 2002. Response of tomato (*Lycopersicon esculentum* L) culture at low zinc. *Scientific Horticulture* 93: 53–64.

Khan, H. R., G. K. McDonald, and Z. Rengel. 2003. Zinc fertilization improves water use efficiency, grain yield and seed Zn content in chickpea. *Plant and Soil* 249: 389–400.

Kobayashi, Y., and S. Mizutani. 1970. Studies on the wilting treatment of corn plant: 3. The influence of the artificial auxin control in nodes on the behavior of rooting. *Proceedings of the Crop Science Society of Japan* 39: 213–220.

Lee, C. W., S. Mahendra, K. Zodrow, D. Li, Y. C. Tsai, J. Braam, and P. J. Alvarez. 2010. Developmental phytotoxicity of metal oxide nanoparticles to *Arabidopsis thaliana*. *Environmental Toxicology and Chemistry* 29: 669–75.

Lin, D., and B. Xing. 2007. Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. *Environmental Pollution* 150: 243–250.

Lin, D., and B. Xing. 2008. Root uptake and phytotoxicity of ZnO nanoparticles. *Environmental Science Technology* 42: 5580–5585.

Lin, X. X, Y. F. Zhu, and W. Z. Shen. 2009. Synthesis and optical and magnetic properties of diluted magnetic semiconductor Zn-MnO hollow spherical structures. *Journal of Physical Chemistry C* 113: 1812–1817.

Liscano, J. F., C. E. Wilson, R. J. Norman Jr., and N. A. Slaton. 2000. Zinc availability to rice from seven granular fertilizers. *AAES Research Bulletin* 963: 1–31.

Longnecker, N. E., and A. D. Robson. 1993. Distribution and transport of zinc in plants. In: *Zinc in Soils and Plants*, ed. A. D. Robson, pp. 79–91. Dordrecht, the Netherlands: Kluwer Academic Publishers.

Lu, C. M., C. Y. Zhang, J. Q. Wen, G. R. Wu, and M. X. Tao. 2002. Research of the effect of nanometer materials on germination and growth enhancement of *Glycine max* and its mechanism. *Soya Bean Science* 21: 168–172.

Majumdar, B., M. S. Venkatesh, B. Lal, K. Kumar, and C. S. Singh. 2001. Effect of phosphorous and zinc nutrition on groundnut in an acid hapludalf of Meghalaya. *Annals of Agricultural Research New Series* 22: 354–359.

Marschner, H. 1993. Zinc uptake from soils. In: *Zinc in Soils and Plants*, ed. A. D. Robson, pp. 59–79. Dordrecht, the Netherlands: Kluwer Academic Publishers.

Marschner, H. 1995. *Mineral Nutrition of Higher Plants*. San Diego, CA: Academic Press.

Mengel, L., and E. A. Kirkby. 1978. *Principles of Plant Nutrition*. Basel, Switzerland: International Potash Institute.

Moran, K. 2004. *Micronutrient Product Types and Their Development*. International Fertiliser Society - Proceeding 545. York, England: International Fertilizer Society.

Mortvedt, J. J. 1992. Crop response to level of water soluble zinc in granular zinc fertilizers. *Fertilizer Research* 33: 249–255.

Nel, A., T. Xia, L. Madler, and N. Li. 2006. Toxic potential of materials at the nanolevel. *Science* 311: 622–627.

Pandey, N., G. C. Pathak, and C. P. Sharma. 2006. Zinc is critically required for pollen function and fertilization in lentil. *Journal of Trace Elements in Medicine and Biology* 20: 89–96.

Prasad, B., and M. K. Sinha. 1981. The relative efficiency of zinc carriers on growth and zinc nutrition of corn. *Plant and Soil* 62: 45–52.

Qiang, X., Z. Fu-Dao, W. Yu-Jun, Z. Jian-Feng, and Z. Shuqing. 2008. Effects of slow/controlled release fertilizers felted and coated by nano-materials on crop yield and quality. *Plant Nutrition and Fertility Study News Letter* 14: 951–955.

Racuciu, M., and D. Creanga. 2007. TMA-OH coated magnetic nanoparticles internalized in vegetal tissues. *Romanian Journal of Physics* 52: 395–402.

Rengel, Z. 2001. Genotypic differences in micronutrient use efficiency in crops. *Communications in Soil Science and Plant Analysis* 32: 1163–1186.

Rengel, Z., and R. D. Graham. 1995. Importance of seed zinc content for wheat growth on zinc-deficient soil. I. Vegetative growth. *Plant and Soil* 173: 259–266.

Seeger, E. M., A. Baun, M. Kastner, and S. Trapp. 2009. Insignificant acute toxicity of TiO_2 nanoparticles to willow trees. *Journal of Soils and Sediments* 9: 46–53.

Shah, V., and I. Belozerova. 2009. Influence of metal nanoparticles on the soil microbial community and germination of lettuce seeds. *Water, Air and Soil Pollution* 97: 143–148.

Sillanpaa, M.. 1990. Micronutrients assessment at the country level: An international study. FAO Soils Bulletin 63. Rome: Food and Agriculture Organization of the United Nations.

Sillanpaa, M., and P. L. G. Vlek. 1985. Micronutrients and the agroecology of tropical and Mediterranean regions. *Fertilizer Research* 7: 151–167.

Singh, A. L. 1999. Mineral nutrition of groundnut. In: *Advances in Plant Physiology*, ed. A. Hemantha Rajan, pp. 161–200. Jodhpur, India: Scientific Publications.

Singh, A. L., M. S. Basu, and N. B. Singh. 2004. *Mineral Disorders of Groundnut*. New Delhi, India: ICAR Publications.

Slaton, N. A., C. E. Wilson, S. Ntamatungiro, R. J. Norman, and D. L. Boothe. 2001. Evaluation of zinc seed treatments for rice. *Agronomy Journal* 93: 152–157.

Tandon, H. L. S. 1995. Major nutritional constraints to crop production and the soil fertility management strategies in different agro climatic regions of Asia. In: *Proceedings of the International Potash Institute Colloquium on Potassium in Asia: Balanced Fertilization to Increase and Sustain Agricultural Production*. pp. 43–72. Basel, Switzerland: International Potash Institute.

Tandon, H. L. S. 1998. Use of external inputs and the state of efficiency of plant nutrient supplies in irrigated cropping systems in Uttar Pradesh, India. In: *Proceedings of the IFPRI/FAO Workshop on Soil Fertility, Plant Nutrient Management, and Sustainable Agriculture: The Future Through 2020*, eds. P. Gruhn, F. Goletti, and R. N. Roy, pp. 199–233. Washington, DC and Rome: International Food Policy Research Institute, and FAO.

Viets, F. G. 1966. Zinc deficiency in the soil-plant system. In: *Zn Metabolism*, ed. A. S. Prasad, pp. 90–128. Springfield, IL: Thomas.

Watanabe, T., and I. Yamaguchi. 1991. Evaluation of wettability of plant leaf surfaces. *Journal of Pesticide Science* 16: 491–498.

Welch, R. M., M. J. Webb, and J. F. Loneragan. 1982. Zinc in membrane function and its role in phosphorus toxicity. In: *Proceedings of the Ninth Plant Nutrition Colloquium*, ed. A. Scaife, pp. 710–715. Wallingford, UK: CAB International.

Witham, F. H., D. F. Blaydes, and R. M. Devlin. 1971. Chlorophyll absorption spectrum and quantitative determinations. In: *Experiments in Plant Physiology*, pp. 55–56. New York: Von Nostra and Ren FOLD Company.

Yang, F., F. S. Hong, W. J. You, C. Liu, F. Q. Gao, C. Wu, and P. Yang. 2006. Influences of nano-anatase TiO_2 on the nitrogen metabolism of growing spinach. *Biological Trace Element Research* 110: 179–190.

Yang, L., and D. J. Watts. 2005. Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. *Toxicology Letters* 158: 122–132.

Yilmaz, A., H. Ekiz, I. Gultekin, B. Torun, H. Barut, S. Karanlik, and I. Cakmak. 1998. Effect of seed zinc content on grain yield and zinc concentration of wheat growth in zinc-deficient calcareous soils. *Journal of Plant Nutrition* 21: 2257–2264.

Zhang, L., F. Hong, S. Lu, and C. Liu. 2005. Effect of nano TiO_2 on strength of naturally aged seeds and growth of spinach. *Biological Trace Element Research* 105: 83–91.

Zhu, H., J. Han, J. Q. Xiao, and Y. Jin. 2008. Uptake, translocation and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. *Journal of Environmental Monitoring* 10: 713–717.

Zhu, G., C. Xu, Y. Yang, K. Zheng, and X. Sun. 2009. Disk-capped multipod arrays of zinc oxide. *Materials Chemistry and Physics* 113: 115–118.