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Effect of Nanoscale Zinc Oxide on Plant Growth, Seed Yield and Quality in Groundnut

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ABSTRACT

An investigation was carried out to examine the influence of nanoscale zinc oxide on plant growth, seed yield and quality during 2014-15 and 2015-16. Seed treatment with nanoscale ZnO (25 nm) @1000 ppm significantly promoted seed quality attributes like germination (83 %), root length (8.9 cm), shoot length (18.9 cm) and SVI (2354). Besides, plant growth parameters *viz*., field emergence (82.95 %), plant height (37 cm), days to initiation of flowering (25 days) and yield parameters like, pod yield per plant (29.9 g), number of pods per plant (23.5) and haulm yield per plant (192 g) also differed significantly due to treatments compared to traditional source of Zn application with $ZnSO_4$ @ 30 gm / 15 liter of water + RDF with the foliar application and seed application with these elements. The inhibitory effect with higher nano-particle (2000 ppm) suggested the need for judicious usage of these for enhanced crop performance.

Keywords : Nanoscale zinc oxide, plant growth, seed yield, quality attributes

GROUNDNUT (*Arachis hypogaea* L.) is the third most important source for edible oil in the world. It provides high quality edible oil (48-50 %) with easily digestible protein (26-28 %) and nearly half of the 13 essential vitamins and 7 of the 20 essential minerals which are necessary for normal human growth, besides providing high quality fodder for livestock. It thus plays a significant role in the livelihood security of marginal farmers by generating higher income and nutritional security to their livestock. Groundnut is grown in an area of 31.3 m. ha worldwide with a total production of 42.9 m. t. (FAO, 2014). In India, groundnut is grown in an area of 6.16 m. ha with a total production of 7.17 million tonnes and constitutes about 50 per cent of total oilseed production (CMIE, 2009). In Karnataka, it is one of the major oilseed crops grown over an area of 6.57 lakh ha with a production of 5.78 lakh tonnes and productivity 926 kg ha^{-1} (Annon., 2014).

Zinc (Zn) is the second most abundant transition metal in organisms after iron and the only metal represented in all six enzyme classes like oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. It is an essential micronutrient for human, 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. 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 per cent of the soils where groundnut is grown exhibit Zn deûciency, which is causing considerable yield loss (Pandey *et al*., 2006). Zinc is required for chlorophyll production, pollen function, fertilization and germination (Senthil kumar, 2011) and also play an important role in biomass production (Cakmak, 2008).

MATERIAL AND METHODS

Seeds: Freshly harvested groundnut pods of variety ICGV-91114 were procured from the National Seed Project (Crops), University of Agricultural Sciences, GKVK Bengaluru, Karnataka, India. A laboratory study was conducted with seeds having germination of 63 per cent. However, for field study, seed lot having germination of 85 per cent was used. The seeds were shelled from pod and graded to obtain uniform size to minimize errors in seed germination, seedling vigor and field emergence.

Preparation of Particle Suspensions for Seed Treatment

Chelated bulk $ZnSO_4$ was used as a reference Zn source. Since bulk ZnO would not dissolve in water and plants cannot absorb it, therefore, farmers are widely using chelated $ZnSO_4$. The seeds were suspended directly in de-ionized 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 the concentrations of 100, 250, 500, 1000 and 2000 ppm. The pH of all the prepared suspensions was from 6.8 to 7.0 and hydro-priming was considered as control.

Laboratory Experiments

Two sets of seed treatment experiments were conducted in the lab. One set of treated seeds with four replications were used to determine the effect on seed germination, root length, shoot length and seedling vigor index and another set of seeds were used for conducting the pot culture experiment to analyze the growth parameters.

Pot Culture Experiment

Groundnut seeds / kernels treated with nano-particles were sown in pots 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, excess plants were removed and one plant per pot was maintained throughout. Proper agronomic and plant protection measures were taken during experimentation for better expression and to minimize the experimental error. The following observations were recorded on ten plants maintained in three replications.

a) Days to complete emergence of seedlings.

b) Plant height (cm) measured from ground node to shoot growing apex (after 30 DAS).

c) Number of leaves (after 30 DAS).

d) Days to initiation of flowering calculated based on the days taken from sowing to the appearance of first flower.

Field experiment

The field experiment were conducted during *Rabi* and *Kharif* seasons of 2014–15 to 2015-16 at the farmer field, Chintamani Taluk, Chickballapur district (Eastern dry zone). The experiment was laid out in Factorial Randomized Block Design. The gross plot size was 4×5 m^2. Two treatments *viz.*, A_1: Zn NPs @ 6 gm / 6 liter H_2O (1000 ppm) + RDF, A_2: $ZnSO_4$ @ 30 gm / 15 liter H_2O + RDF, A_3: control and three spray B_1: Seed application, B_2: Foliar application (30 and 75 DAS), B_3: Seed application + Foliar application were imposed. The initial soil (red sandy loam) parameters like pH 6.8, electrical conductivity (0.1 dSm^{-1}), organic carbon (0.71%), available phosphorus (44.82 kg ha^{-1}), potassium oxide (387 kg ha^{-1}), Zinc 2.94 ppm; Copper 0.50 ppm; Iron 6.58 ppm, Manganese 5.56 ppm, Magnesium 2.0, and Sulphur 11.32 ppm were also recorded.

Statistical Analysis: Each experiment was conducted with three replicates and the results were presented as mean ± standard deviation (SD). The statistical analysis of experimental data was carried out as per ANOVA Program. Each experimental value was compared to its corresponding control. Statistical signiûcance was accepted when the probability of the result assuming the null hypothesis (p) is less than 0.05 (level of probability).

Results and Discussion

Characterization of the Nanoparticles

The Transmission Electronics Microscope image of the Zn nano-particles showed that the mean particle diameter was 25 nm and particles looked slightly aggregated as there were no protecting ligands on the surface (Plate 1.)

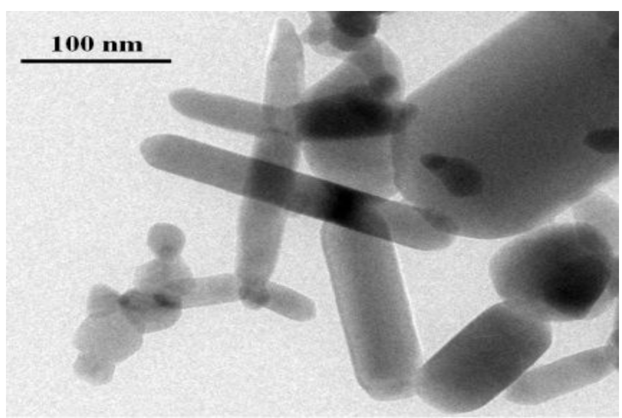

Plate 1: TEM micrograph of nano-ZnO particles after dispersed in Milli-Q water.

Seed Germination Study

Groundnut seeds responded variably towards the treatment at various concentrations of both bulk $ZnSO_4$ and nanoscale ZnO particles. Seed treated with 1000

TABLE I

Effect of nanoscale ZnO and bulk $ZnSO_4$ on germination and seedling vigour index in groundnut cv. ICGV-91114

Concentration	Germination (%)		Mean root length (cm)		Mean Soot length (cm)		Seedling Vigour Index (SVI)	
	$ZnSo_4$	Nano ZnO	$ZnSo_4$	Nano ZnO	$ZnSo_4$	Nano ZnO	$ZnSo_4$	Nano ZnO
Control	65	66.33	7.0	7.5	15.4	15.2	1447	1504
50 ppm	66	73.33	7.9	7.7	15.8	17.7	1570	1861
100 ppm	68	76.33	8.1	8.0	17.2	17.4	1727	1943
250 ppm	66	76.67	8.0	8.2	17.1	17.5	1665	1969
500 ppm	69	78.67	8.0	8.4	16.3	18.4	1687	2105
1000 ppm	74	83.00	8.0	8.9	16.9	18.9	1855	2302
2000 ppm	68	74.33	7.3	8.1	15.2	14.9	1528	1707
Mean	68	75.52	7.8	8.1	16.3	17.1	1638	1913
S.Em±	1.8	1.76	0.1	0.2	0.5	0.5	41.22	36.5
CD (0.05P)	7.5	7.41	0.4	1.0	2.2	2.0	173.54	153
CV (%)	4.5	4.03	2.0	4.9	5.50	4.90	4.35	3.30

ppm nanoscale ZnO recorded signficantly higher germination (83 %) and seedling vigor index I (2354). The root growth was also very good that can be seen in Plate 2. However, the results of bulk $ZnSO_4$ treated seeds were not promising (Table I). Among the different nanoscale ZnO concentrations, 1000 ppm showed higher Seed Vigour Index (SVI) but increased concentration (2000 ppm) resulted in decreased seedling vigor index. Such inhibitory effects of nanoparticles were also reported by Lin and Xing (2007) on radish, rape and ryegrass. However, performance of the bulk material is better than the untreated control. Nanoscale ZnO showed treated seeds better root growth compared to bulk $ZnSO_4$ and control (Plate2).

Similarly 1000 ppm nanoscale ZnO produced days to field emergence (17.67) compared to control (20 days) and bulk $ZnSO_4$ (19 days) and same trend in number of leaves (15.7, 14, and 15.7). Such effects can be due to higher seedling vigour and early vegetative growth. Nanoscale ZnO increased Plant height and days to initiation of flowering irrespective of concentrations compared to bulk $ZnSO_4$ and control (Table II). Higher plant height may be due to

Plate 2. Effect of ZnO and $ZnSO_4$ on seedling growth in groundnut

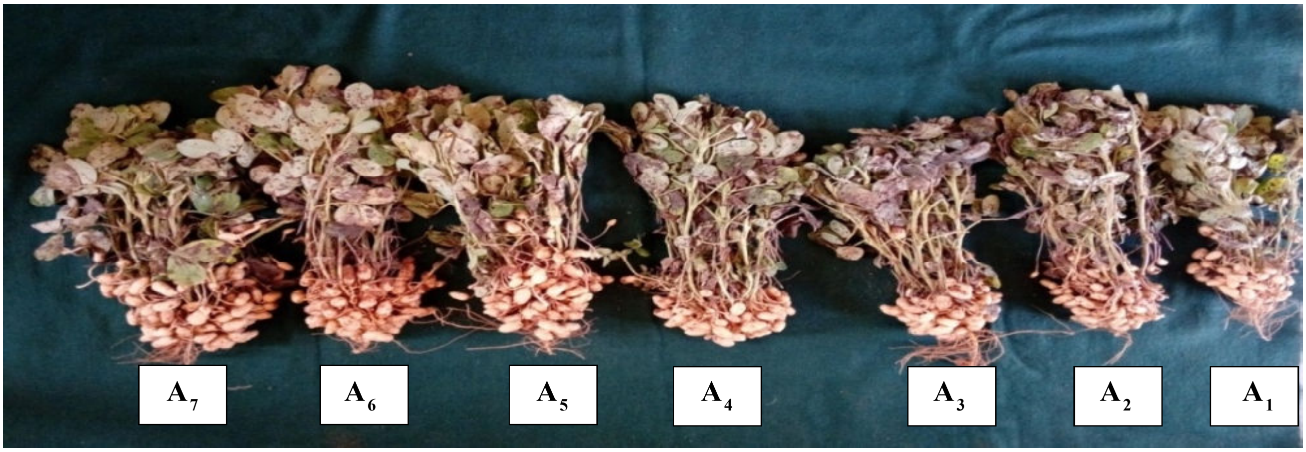

Plate 3. Effect of ZnO and $ZnSO_4$ on pod yield in groundnut

A_1:Control
A_2: $Znso_4$ foliar application
A_3: $Znso_4$ seed treatment
A_4: $Znso_4$ Foliar + seed treatment
A_5: ZnO foliar application
A_6: ZnO seed treatment
A_7: ZnO seed treatment + foliar application

complementary effect of other inherent nutrients like magnesium, iron and sulfur. Similar results were observed by Zhang *et al.,* 2005 when *Spinacia oleracea* (Spinach) seeds were treated with nanoscale TiO_2 particles.

Plant growth and Seed Yield Attributes

The experimental results revealed that the response of groundnut to lower dose of nanoscale ZnO was found to be highly significant. The dry pod yield of groundnut was greatly influenced (Plate 3) by nanoscale zinc application @ 6 gm / 6 liter H_2O (1000 ppm). The experimental data indicated significant increase in field emergence (80.20 per cent), plant height at harvest (37 cm), initiation of flowering (24.82 days) with the application of nano scale ZnO @ 6gm / 6 L H_2O in combination of seed treatment and foliar application (Table III).

TABLE II

Effect of nanoscale ZnO and bulk $ZnSO_4$ on plant growth and flowering in pot culture experiment of groundnut under green house conditions.

Concentration	Days to field emergence		Number of leaves		Plant height (cm)		Days to initiation of flowering	
	$ZnSo_4$	Nano ZnO	$ZnSo_4$	Nano ZnO	$ZnSo_4$	Nano ZnO	$ZnSo_4$	Nano ZnO
Control	21	20.67	11.3	14.0	8.0	9.9	28.7	30.7
50 ppm	19	19.00	13.3	17.7	8.5	9.7	28.0	30.7
100 ppm	20	20.33	13.3	17.0	8.9	10.1	28.0	29.3
250 ppm	18	18.00	13.7	18.0	9.3	9.9	28.0	28.7
500 ppm	17	17.00	14.0	18.7	9.7	11.5	27.0	27.0
1000 ppm	18	17.67	15.7	15.7	10.1	9.0	27.3	28.3
2000 ppm	19	18.67	10.0	12.0	11.1	9.0	26.3	31.0
Mean	19	18.76	13.0	16.1	9.4	9.9	27.6	29.4
S.Em±	1.0	1.01	0.9	0.8	0.7	0.4	0.8	1.0
CD (0.05P)	4.2	4.24	3.6	3.6	2.8	1.7	3.2	4.1
CV (%)	9.3	9.30	11.5	9.1	12.1	7.3	4.7	5.7

TABLE III

Effect of ZnO nanoparticles and bulk $ZnSO_4$ on field emergence, plant height and initiation of flowering in groundnut cv. ICGV-91114 (pooled data)

Concentration	Field emergence (%)			Plant height at harvest (cm)			Initiation of flowering (days)		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
A1	82.70	83.20	82.95	37.00	37.67	37.33	25.26	25.78	25.52
A2	73.93	75.63	74.78	33.56	33.00	33.28	26.30	27.28	26.79
S.Em+	1.611	1.090	0.891	1.029	1.058	0.902	0.512	0.618	0.428
CD (0.05P)	5.076	3.436	2.807	3.241	3.333	2.844	NS	NS	NS
Spray									
B1	77.85	78.65	78.25	35.83	34.33	35.08	26.45	27.68	27.07
B2	78.05	78.25	78.15	32.50	35.17	33.83	26.55	26.60	26.58
B3	79.05	81.35	80.20	37.50	36.50	37.00	24.33	25.30	24.82
S.Em+	1.973	1.335	1.091	1.260	1.295	1.105	0.627	0.757	0.524
CD (0.05P)	NS	NS	NS	NS	NS	NS	NS	NS	1.653
Interaction									
A1B1	82.3	82.8	82.6	37.7	36.7	37.2	24.3	26.5	25.4
A1B2	82.4	82.2	82.3	34.0	37.0	35.5	26.4	26.2	26.3
A1B3	83.3	84.7	84.0	39.3	39.3	39.3	25.0	24.6	24.8
A2B1	73.4	74.5	74.0	34.0	32.0	33.0	28.6	28.8	28.7
A2B2	73.7	74.3	74.0	31.0	33.3	32.2	26.7	27.0	26.8
A2B3	74.8	78.0	76.4	35.7	33.7	34.7	23.7	26.0	24.8
S.Em+	2.79	1.89	1.543	1.78	1.83	1.56	0.89	1.07	0.74
CD (0.05P)	NS	NS	NS	NS	NS	NS	2.79	NS	NS
Control	64.00	58.33	61.17	30.67	31.00	30.83	30.13	29.73	29.93
S.Em+	3.444	2.27	1.73	1.741	1.800	1.449	0.925	1.42	0.74
CD (0.05P)	10.61	7.00	5.33	5.37	NS	4.47	2.85	NS	2.29
CV (%)	7.71	6.27	6.07	11.12	10.46	9.41	8.49	7.34	6.82

NS : Non significant

Concentration
A_1: Zn NPs @ 6 gm/6 Litre H_2O (1000 ppm)+RDF
A_2: $ZnSO_4$ @ 30 gm/15 Litre H_2O +RDF

Spray
B_1: Seed treatmentB_2: Foliar application (30 and 75 DAS)
B_3: Seed treatment + Foliar application

TABLE IV

Effect of ZnO nanoparticles and bulk $ZnSO_4$ on number of pods per plant, pod yield per plant and haulm yield per plant in groundnut cv. ICGV-91114 (Pooled data)

Concentration	Field emergence (%)			Plant height at harvest (cm)			Initiation of flowering (days)		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
A1	26.69	19.80	23.24	29.37	29.22	29.29	192.74	192.39	192.57
A2	22.13	16.13	19.13	25.55	25.80	25.67	179.99	179.87	179.93
S.Em+	0.368	0.266	0.249	0.299	0.413	0.281	2.418	2.235	1.959
CD (0.05P)	1.159	0.837	0.786	0.941	1.300	0.887	7.618	7.041	6.174
Spray									
B1	22.52	15.80	19.16	26.75	27.23	26.99	175.29	175.01	175.15
B2	24.43	18.77	21.60	27.28	26.95	27.12	178.78	178.18	178.48
B3	26.28	19.33	22.81	28.35	28.35	28.35	205.04	205.20	205.12
S.Em+	0.450	0.325	0.305	0.366	0.505	0.345	2.961	2.737	2.400
CD (0.05P)	1.42	1.02	0.962	1.15	NS	1.086	9.33	8.62	7.561
Interaction									
A1B1	23.4	16.9	20.2	28.5	28.8	28.7	170.2	167.8	169.0
A1B2	28.4	20.2	24.3	28.9	28.2	28.6	178.2	178.2	178.2
A1B3	28.3	22.3	25.3	30.7	30.7	30.7	229.8	231.1	230.5
A2B1	21.7	14.7	18.2	25.0	25.7	25.3	180.4	182.2	181.3
A2B2	20.5	17.3	18.9	25.7	25.7	25.7	179.3	178.1	178.7
A2B3	24.2	16.4	20.3	26.0	26.0	26.0	180.3	179.3	179.8
S.Em±	0.637	0.460	0.432	0.517	0.715	0.487	4.188	3.870	3.394
CD (0.05P)	2.01	1.45	1.361	NS	NS	NS	13.20	12.20	10.693
Control	15.17	9.53	12.35	19.37	19.37	19.37	164.08	159.75	161.92
S.Em+	0.735	0.46	0.45	0.518	0.72	0.51	3.859	3.54	3.10
CD (0.05P)	2.27	1.43	1.38	1.60	2.21	1.58	11.89	10.90	9.56
CV (%)	13.37	14.95	13.33	8.14	7.80	7.68	11.32	11.75	11.42

NS : Non significant

Concentration

A1 Zn NPs @ 6 gm/6 Litre H2O (1000 ppm)+RDF

A2: ZnSO4 @ 30 gm/15 Litre H2O +RDF

Spray

B1: Seed treatment

B2: Foliar application (30 and 75 DAS)

B3: Seed treatment + Foliar application

The data (Table IV) indicate signiûcant increase in number of pods per plant, pod yield per plant and also plant height with the application of nanoscale ZnO at 6 g in 6 L. It is observed that 26.69 per cent and 19.8 per cent higher pod yield with the application of nano scale ZnO @ 6gm / 6 L H_2O (1000 ppm) compared to RDF (15.17 %, 9.53 %) during 2015 and 2016, respectively.

Zinc plays a fundamental role in protecting and maintaining structural stability of cell membranes (Cakmak, 2008). Zn is used for protein synthesis, membrane function, cell elongation and tolerance to environmental stresses (Krishna Shyla and Natarajan, 2014). Hence it may be concluded that high Zn content in seed could act as a starter fertilizer. Raskar and Laware (2014) reported that seed priming with Zn was very effective in improving seed germination and seedling development in barley. These findings suggested that a high level of Zn in seeds has very important physiological roles during seed germination and early seedling growth (Prasad *et al*., 2012; Avinash, *et al*., 2010). Further, these particles also proved effective in enhancing plant growth, development and yield. The bioavailability of the nanoparticle because of its size and lower water solubility (which inhibit rapid falling off compared to ionic supplements) is also 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. All these factors may be responsible to give higher yields due to nanoscale ZnO compared to chelated $ZnSO_4$. In addition, most of the research conducted on the effect of micro nutrient in plants deals with correcting the deficiencies and thereby increasing the grain yield and biomass.

In order to understand the possible benefits of applying Nanoparticles in agriculture, it is imperative to analyze penetration and transport of nano particles in the plants. Size of particles also plays an important role in behavior, reactivity and toxicity induction. Considering these aspects, both positive and negative effects of nanoparticles are observed in living plants. The results indicated that the micro nutrient, Zn can be delivered into groundnut through ZnO nano particles. 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 fairly well. The results emphasize that nano scale nutrients can be supplied to the crops either through seed dressing or by foliar application with considerably lower doses to get the desired level of results. Detailed studies have to be performed to understand the mechanism of action of nanoscale materials including the most efficient method of application.

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