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Standardization of nano particles for enhancing groundnut seed quality Cv. ICGV-91114

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Abstract
A laboratory study was carried out using different nano micro nutrients (Iron NPs, Zinc NPs, Al NPs, $CaCo_3$ NPs) and same nutrients as a bulk of different concentration *viz*., 100, 250, 500, 750 and 1000 ppm. Nano seed treatment improved the seed germination of aged groundnut seeds variably towards the treatment at different concentrations. Among the treatment maximum germination percentage, root length, shoot length and seedling vigour index II was promoted by ZnO nanoparticles @ 1000 ppm (84 %, 8.59 cm,18.84 cm and 2326) which was followed by TiO_2 NPs @ 500 ppm (82 %, 8.28 cm, 17.85 cm and 2135) and Nano Iron @250 ppm (80.7 % 8.07 cm, 17.73 cm and 2100) and respectively.

Keywords: Nano scale micro nutrients, groundnut, seed germination

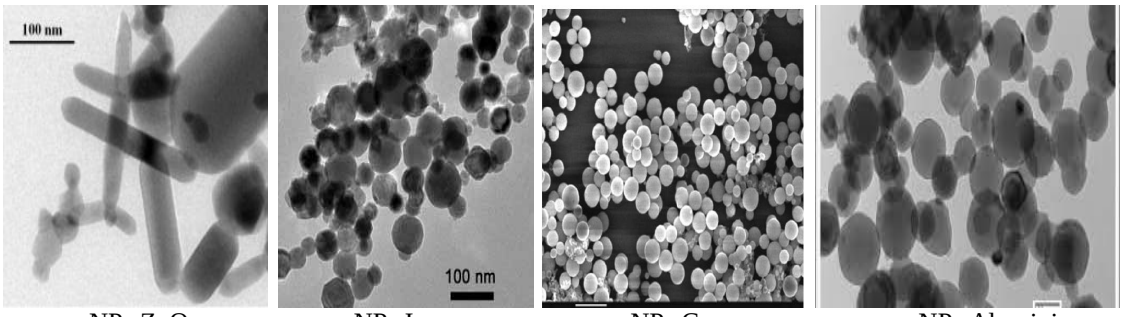
Introduction
In India groundnut constitutes roughly about 50 per cent of the total oilseed production. The productivity level in India is very low mainly because about 80 per cent of the crop is grown under rain fed conditions with minimal inputs. In many parts of India groundnut seed is usually stored for a period of about 9 to 12 months before sowing. However, seed viability is getting lost quickly due to the production of free radicals by lipid peroxidation during storage. As the current technologies available to prolong the vigour and viability of groundnut seed on a large scale are not satisfactorily alleviating the practical problem, an alternative simple and practicable seed treatment to control seed deterioration of groundnut is need of the hour. During the past decade, lots of work has been done in biological system to address a wide range of field problems utilizing nanomaterials and nano-devices. Elucidated various Nanotechnological approaches that can be employed in Seed Science.

Materials 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 medium vigour seeds having germination of 67 per cent. However, 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
The Nanoparticles 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) particles and nanoscale micro nutrient suspensions were prepared at the concentrations of 100, 250, and 500, 1000 ppm. The pH of all the prepared suspensions was from 6.8 to 7.0 and hydro-priming was considered as control (Senthil kumar, 2011 and Natarajan, 2008) [6, 5].

Laboratory Experiments
Experiment was conducted in the lab with four replications were used to determine the effect of treatment on seed germination, root length, shoot length and seedling vigor index (ISTA, 2014) [2]

Characterization of the Nanoparticles
The TEM 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.



NPs ZnO NPs Iron NPs $Caco_3$ NPs Aluminium

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

Results and Discussion

Seed Germination Study

Nano seed treatment improved the germination of aged groundnut seeds variably towards the treatment at different concentrations. Among the treatment maximum germination percentage was promoted by ZnO @ 1000 ppm nanoparticle (84 %), which was 9 per cent higher than the bulk $ZnSo_4$ (75.5 %) and 2 per cent higher than TiO_2 NPs (82 %) respectively (Table 1). Among the dosages, seeds treated ZnO @1000, $Feso_4$ @ 250 ppm, $CaCo_3$ @ 100 ppm, Tio_2 @ 500 ppm (84 %, 80.7 %, 77.27 %, 82.5 %) found to register maximum germination than other dosages. among the NPs and dosage revealed that ZnO NPs @ 1000 mg kg^- The beneficial effect improving the germination could be ascribed to higher precursor activity of nanoscale zinc in auxin production. Apart from this, zinc is one of the essential nutrients required for plant growth. It is an important component of various enzymes that are responsible for driving many metabolic reactions in all crops. Zinc oxide NPs are reported to also exhibit positive effect on the reactivity of phytohormones especially Indole Acetic Acid (IAA) facilitating in the phytostimulatory actions. Zinc-rich ZnO NPs could increase the level of IAA in roots (sprouts), which in turn can increase growth rate of seedlings.

Nanoparticle treated germinated seeds exhibited higher root and shoot length and SVII than control. ZnO NPs @ 1000 ppm treated seeds induced maximum root length and shoot length and SVII (8.59 cm 18.84 cm, 2326) followed by NPs Tio_2 @ 500 ppm (8.28 cm, 17.85 cm, 2135) and were higher than control respectively (6.9 cm, 14.64 cm, 1238) (Table 2, 3 &4). Such promoting effect of nanoscale TiO_2 on germination was reported in soya bean, in which authors noticed increased nitrate reductase enzyme activity and enhanced antioxidant system (K. Prasad *et al*, 2009) [3]. Similar results were observed by Zheng, 2005 [7]. M. Koizumi *et al*, 2008 [4], Zhu H, *et al*, 2008 [8] in ZnO NPs.

Control Tio_2 NPs @ 500 ppm

$ZnSO_4$ @1000 ppm Tio_2 @1000 ppm

ZnO NPs@ 1000 ppm $Caco_3$ NPs @ 100 ppm

$FeSo_4$@1000 ppm $Caco_3$ @1000 ppm

Plate 2: Effect of nano micronutrient and bulk micro nutrient on germination in groundnut

Table 1: Influence of seed treatment with nano particles and bulk particles on germination per cent

Treatments	ZnO	FeO	$CaCo_3$	TiO_2
T_1	75.43	72.71	70.79	73.36
T_2	68.89	69.11	68.46	68.57
S.Em±	0.47	0.42	0.33	0.47
C.D (0.05P)	1.78	1.61	1.26	1.80
CV (%)				
Concentration				
N_1	64.13	64.63	64.13	64.13
N_2	69.63	69.38	69.13	70.00
N_3	73.00	70.75	71.13	70.13
N_4	72.63	73.25	71.75	72.88
N_5	74.38	72.38	70.75	73.38
N_6	76.13	73.38	70.25	74.13
N_7	75.25	72.63	70.25	72.13
S.Em ±	0.88	0.79	0.62	0.88
C.D (0.05P)	3.34	3.02	2.35	3.36
CV (%)				
Interaction (T x N)				
T_1N_1	64.00	64.75	64.00	64.00
T_1N_2	71.75	72.00	71.75	73.00
T_1N_3	76.25	73.25	75.00	73.00
T_1N_4	78.00	77.00	74.00	77.25
T_1N_5	79.00	75.25	71.75	78.25
T_1N_6	82.00	74.75	70.00	76.50
T_1N_7	77.00	72.00	69.00	71.50
T_2N_1	64.25	64.50	64.25	64.25
T_2N_2	67.50	66.75	66.50	67.00
T_2N_3	69.75	68.25	67.25	67.25
T_2N_4	67.25	69.50	69.50	68.50
T_2N_5	69.75	69.50	69.75	68.50
T_2N_6	70.25	72.00	70.50	71.75
T_2N_7	73.50	73.25	71.50	72.75
S.Em ±	1.24	1.12	0.87	1.25
C.D (0.05P)	4.72	4.27	3.33	4.75
CV (%)	3.43	3.16	2.50	3.51

T_1:Nano particle
T_2:Bulk particle

N_1: Control
N_2: 50 ppm
N_3: 100 ppm
N_4: 250 ppm
N_5: 500 ppm
N_6: 1000 ppm
N_7: 2000 ppm

Table 2: Influence of seed treatment with nano particles and bulk particles on root length (cm)

Treatments	ZnO	FeO	$CaCo_3$	TiO_2
T_1	8.14	7.44	7.44	7.63
T_2	7.69	7.22	7.22	7.29
S.Em ±	0.05	0.04	0.05	0.05
C.D (0.05P)	0.18	0.14	0.20	0.17
CV (%)				
Concentration				
N_1	6.77	6.79	6.75	6.75
N_2	7.40	7.16	7.33	7.30
N_3	7.89	7.36	7.58	7.51
N_4	8.08	7.61	7.49	7.66
N_5	8.26	7.52	7.43	7.82
N_6	8.57	7.41	7.38	7.57
N_7	8.43	7.46	7.38	7.59
S.Em ±	0.09	0.07	0.10	0.08
C.D (0.05P)	0.34	0.26	0.38	0.32
CV (%)				
Interaction (T x N)				
T_1N_1	6.77	6.79	6.79	6.78
T_1N_2	7.83	7.10	7.68	7.35
T_1N_3	8.09	7.45	7.95	7.68
T_1N_4	8.16	7.98	7.63	8.00
T_1N_5	8.49	7.76	7.53	8.28
T_1N_6	9.02	7.53	7.33	7.65
T_1N_7	8.63	7.48	7.23	7.65
T_2N_1	6.76	6.78	6.71	6.73
T_2N_2	6.98	7.23	6.98	7.26
T_2N_3	7.70	7.27	7.20	7.35
T_2N_4	8.01	7.25	7.35	7.33
T_2N_5	8.03	7.28	7.33	7.35
T_2N_6	8.11	7.30	7.43	7.50
T_2N_7	8.23	7.45	7.53	7.53
S.Em ±	0.13	0.10	0.14	0.12
C.D (0.05P)	0.48	0.37	0.54	0.46
CV (%)	3.20	2.66	3.84	3.22

T_1:Nano particle
T_2:Bulk particle

N_1: Control
N_2: 50 ppm
N_3: 100 ppm
N_4: 250 ppm
N_5: 500 ppm
N_6: 1000 ppm
N_7: 2000 ppm

Table 3: Influence of seed treatment with nano particles and bulk particles on shoot length (cm)

Treatments	ZnO	FeO	$CaCO_3$	TiO_2
T_1	16.77	16.33	15.73	16.44
T_2	14.95	15.12	15.46	15.44
S.Em ±	0.22	0.28	0.18	0.21
C.D (0.05P)	0.85	1.06	0.69	0.80
CV (%)				
Concentration				
N_1	14.03	14.13	14.07	14.33
N_2	15.59	16.11	15.17	15.61
N_3	15.66	15.93	15.39	16.08
N_4	16.25	16.60	16.27	16.36
N_5	16.49	16.42	16.42	16.88
N_6	17.18	16.30	16.34	16.51
N_7	15.80	14.56	15.49	15.80
S.Em ±	0.41	0.52	0.34	0.39
C.D (0.05P)	1.58	1.99	1.29	1.49
CV (%)				
Interaction (T x N)				
T_1N_1	14.17	14.11	14.02	14.28
T_1N_2	16.99	16.89	15.92	16.33
T_1N_3	17.07	16.79	15.83	16.95
T_1N_4	17.25	17.73	16.55	17.33
T_1N_5	17.69	17.31	16.85	17.77
T_1N_6	18.84	16.72	16.43	17.03
T_1N_7	15.38	14.75	14.50	15.38
T_2N_1	13.90	14.15	14.12	14.38
T_2N_2	14.18	15.33	14.43	14.89
T_2N_3	14.25	15.08	14.95	15.21
T_2N_4	15.25	15.47	15.98	15.39
T_2N_5	15.30	15.52	16.00	16.00
T_2N_6	15.52	15.89	16.25	16.00
T_2N_7	16.23	14.38	16.48	16.23
S.Em ±	0.59	0.74	0.48	0.55
C.D (0.05P)	2.24	2.81	1.82	2.11
CV (%)	7.39	9.36	6.13	6.94

T_1:Nano particle
T_2:Bulk particle

N_1: Control
N_2: 50 ppm
N_3: 100 ppm
N_4: 250 ppm
N_5: 500 ppm
N_6: 1000 ppm
N_7: 2000 ppm

Table 4: Influence of seed treatment with nano particles and bulk particles on seed vigour index –I

Treatments	ZnO	FeO	$CaCO_3$	TiO_2
T_1	1527	1393	1272	1400
T_2	1236	1228	1266	1225
S.Em ±	16.22	26.53	18.29	15.17
C.D (0.05P))	61.88	101.22	69.81	57.8
CV (%)				
Concentration				
N_1	1115	1122	1114	1118
N_2	1393	1293	1301	1218
N_3	1417	1346	1426	1243
N_4	1454	1513	1332	1372
N_5	1498	1406	1308	1519
N_6	1604	1438	1304	1491
N_7	1188	1057	1098	1225
S.Em ±	30.34	49.63	34.23	28.38
C.D (0.05P)	115.76	189.36	130.5	108.3
CV (%)				
Interaction (T x N)				

T_1N_1	1113	1126	1113	1115
T_1N_2	1517	1403	1427	1268
T_1N_3	1588	1488	1567	1301
T_1N_4	1656	1740	1325	1573
T_1N_5	1727	1522	1267	1794
T_1N_6	1903	1413	1190	1649
T_1N_7	1184	1062	1018	1098
T_2N_1	1116	1117	1115	112
T_2N_2	1269	1183	1176	1168
T_2N_3	1246	1204	1286	1184
T_2N_4	1252	1286	1339	1172
T_2N_5	1270	1289	1349	1244
T_2N_6	1305	1463	1418	1334
T_2N_7	1193	1052	1177	1353
S.Em ±	42.90	70.18	48.40	40.14
C.D (0.05P)	163.7	267.7	184.69	153.18

T_1:Nano particle
T_2:Bulk particle

N_1: Control
N_2: 50 ppm
N_3: 100 ppm
N_4: 250 ppm
N_5: 500 ppm
N_6: 1000 ppm
N_7: 2000 ppm

Conclusion

Study indicated that, Nanoparticles tested in the investigation were supportive in enhancing the seed germination and seedling vigour of the groundnut seeds which are supposed to be highly prone for deterioration in storage. Application of nanoparticles especially ZnO @ 1000 mg kg^{-1} seed improved germination and related physiological parameters. However, the findings are to be verified under large scale field condition before recommending to farmer for adoption.

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