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Synthesis of inorganic nanoparticles for the enhancement of seed quality in groundnut cv. VRI-2

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ABSTRACT : The present study was carried out to assess seed quality parameters by using inorganic nanoparticles (NPs) *viz.*, zinc oxide (ZnO), silver (Ag) and titanium dioxide (TiO_2) were synthesized by chemical method and characterized by using Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). Among the three nanoparticles, ZnO NPs possessed the least size of 35-45 nm, while the maximum of 100 nm was observed in TiO_2 NPs. Fresh seeds of groundnut were treated with NPs of ZnO, Ag and TiO_2 each @ 750, 1000 and 1250 mg kg^{-1} of seed and stored for 12 months under ambient condition. After 12 months of storage, seeds treated with ZnO NPs @ 1000 mg kg^{-1} enhanced germination (77%), vigour index (3067), electrical conductivity (0.347dSm^{-1}), catalase (0.421 µg H_2O_2 mg^{-1} min^{-1}) enzyme activity and reduced lipid peroxidation activity (0.089 OD value) against the control (66%, 2328, 0.379 dSm^{-1}, 0.385 µg H_2O_2 mg^{-1} min^{-1} and 0.112 OD value, respectively). The present investigation clearly demonstrated the effect of inorganic NPs of ZnO @ 1000 mg kg^{-1} and Ag @ 1250 mg kg^{-1} of seeds in maintaining the quality of aged groundnut seeds.

KEY **W**ORDS : Synthesis of nanoparticles, SEM, TEM, Groundnut seed quality

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Groundnut (*Arachis hypogaea* L.) believed to be "King of oilseed crops", is native of Brazil (South America). The crop is rated as 13th most important food crop, fourth important source of edible oil and third most important source of vegetable protein in the World (Reddy *et al.*, 2011). Groundnut seeds lose viability within a short period due to the accumulation of free fatty acids resulting in irreversible ageing. Unlike other oilseed crops, groundnut seeds are stored as pods and shelled at the time of sowing and cultivated mostly as rainfed crop. One of the major constraints in groundnut seed is the storability and maintenance of quality under fluctuating temperatures and relative humidity. During seed storage, reduction in germination occurs subsequent to physiological changes like delayed germination, lower tolerance to adverse storage resulting in reduced seedling growth. The biochemical changes mainly observed during storage are lipid peroxidation mediated by free radicals, inactivation of enzymes, disintegration of cell membranes and genetic damage (Murthy *et al.*, 2003). Several researchers reported that mid-term hydration-dehydration treatments performed better in improving germination and seedling vigour after storage in soybean (Mandal *et al.*, 2000). However, it can be controlled to certain extent by adopting new technologies. In order to alleviate the deterioration process in seeds, nanotechnological

approaches may offer a plausible solution. With the above considerations, the present investigation was taken up with the following objectives of to synthesize and characterization of inorganic NPs and to assess the impact of NPs on quality parameters *viz.,* seed vigour and viability of stored groundnut seeds.

RESEARCH PROCEDURE

Breeder seeds of groundnut cv.VRI-2 obtained from Oilseeds Research Station (ORS), Vridhachalam of Tamil Nadu Agricultural University were used in this study during the period between 2013-2015. The synthesis and characterization of nanoparticles were carried out at the Department of Nano Science and Technology while the storage studies were carried out at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore.

Synthesis of ZnO, Ag and TiO_2 nanoparticles :

Zinc oxide nanoparticles :

ZnO nanoparticles (NPs) were synthesized by Moghaddam *et al.* (2009) by preparing 0.45 M aqueous solution of zinc nitrate ($Zn(NO_3)_2.4H_2O$) and 0.9 M aqueous solution of sodium hydroxide (NaOH) in distilled water in two separate 250 ml glass beakers. The Zn $(NO_3)_2$ solution (100 ml) transferred to a burette was added drop wise (slowly for 40 min) to the 100 ml of NaOH contained in the beaker placed over a magnetic stirrer with hot plate set at 55^0C with high-speed stirring. The beaker after adding 100 ml $Zn(NO_3)_2$ was removed from the hot plate, sealed with aluminium foil and kept undisturbed for 2h for precipitation and settlement. The precipitated ZnO NPs were washed with Millipore water (25 ml) followed by ethanol (25 ml) five to six times until all the impurities were cleared and then vacuum dried at 60C. Nanoparticles such synthesized were transferred to air tight screw cap vial (10 ml) and stored at ambient temperature for further investigations.

Silver nanoparticles :

The Ag NPs were prepared by using chemical reduction method according to the description outlined by Lee and Meisel (2005). Fifty ml of $AgNO_3$ 0.005 M taken in a beaker was boiled on a magnetic stirrer with hot plate. To this solution, 5ml of 1 per cent trisodium citrate was added drop by drop from 10 ml measuring cylinder with vigorous mixing of the stirrer until pale yellow colour appeared. Then the beaker was removed and kept at ambient temperature where the chemical reaction expected is :

$$4Ag^+ + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^0 + C_6H_5O_7H_3 + 3Na^+ + H^+ + O_2\uparrow$$

Titatium dioxide nanoparticles :

TiO_2 NPs were synthesized by dissolving 0.5 g TiO_2 pellets in 30 ml of NaOH solution (10 M) under vigorous stirring at room temperature for 2 h (Arami *et al.*, 2007). The obtained yellow solution was irradiated in an ultra sonicator (Soncis, VCX 1500, 20 kHz and 350 W) for 2 h at ambient temperature. The resultant precipitate was then centrifuged, washed and decanted with deionized water several times (five to six times) until all the impurities were cleared and dried at 60 C for 24 h to obtain the nanoparticles.

Characterization of synthesized nanoparticles: Characterization of the synthesized nanoparticles was performed by the techniques described below.

Scanning Electron Microscope (*SEM*) :

The SEM model FEI QUANTA 250 was used to characterize the size and morphology of the nanoparticles. Sample of test nanoparticles (0.5 to 1.0 mg) was dusted on one side of the double sided adhesive carbon conducting tape, and then mounted on the 8mm diameter aluminium stub. Sample surface were observed at different magnification and the images were recorded.

Transmission Electron Microscope (*TEM*) :

In this study, TEM FEI TECHNAI SPRIT, make or source was used to analyze the sample. Dilute suspensions of nanoparticles (0.50 mg) in pure ethanol (15 ml) were prepared by ultrasonication. A drop of the suspension placed on 300-mesh lacy carbon coated copper grid, dried and the images were recorded at different magnification.

Seed treatment :

Groundnut seeds were dry dressed with synthesized nanoparticles of ZnO, Ag and TiO_2 @ 750, 1000 and . . . . . $^{-1}$ using screw capped glass bottles at room temperature. The glass bottles containing seeds and nanoparticles were manually shaken gently for 3 min., 5 times at an interval of 3h. Seeds shaken without nanoparticles served as control. After dry dressing with the nano particles, the seeds were packed in cloth bag and stored under ambient conditions (25 ± 3°C

temperature and 95 ± 3% RH). Seed samples were drawn at monthly intervals upto 12 months and evaluated for the following seed quality parameters.

Germination (%) (ISTA, 2010) :

The germination of the seeds was assessed with 50 kernels in three replications on sand medium. The test conditions of 25±2ºC and 95±3 per cent RH were maintained in a germination room. At the end of tenth day, the number of normal seedlings was counted and the mean was expressed as percentage.

Vigour index :

The vigour index was computed using the following formula as per Abdul-Baki and Anderson (1973) and the mean was expressed as whole number.

Vigour index = Germination per cent × Seedling length (cm)

Electrical conductivity (dSm^{-1}) :

Each of ten (25 seeds in each) pre-washed samples were soaked in 50 ml of distilled water for 8 h at room temperature. The seed leachate was collected by decanting and electrical conductivity was measured in conductivity meter (Elicotype Cm-82). The mean value was expressed as dSm^{-1} (Presley, 1958).

Catalase activity (µg H_2O_2 mg^{-1} min^{-1}) :

Five hundred mg of pregerminated seed samples were homogenised in a 0.066M sodium phosphate buffer (pH 6.8) and centrifuged at 2000 rpm for 20 min. at 5ºC to extract enzymes. To that, 0.2 ml of enzyme extract, five ml of phosphate buffer (pH 6.8) and four ml of 0.3N hydrogen peroxide (substrate) were added. The reaction was stopped after 15 min. of incubation by adding ten ml of 2N H_2SO_4. The blank was maintained for each set which contained 0.2 ml enzyme extract with 2N H_2SO_4. The contents were titrated against 0.1N $KMnO_4$ and the titre values were noted down. Differences between the titre values give the volume of permanganate equivalent to enzyme activity. The activity was expressed as µg H_2O_2 mg^{-1} min^{-1} (Povolotskaya and Sedenka, 1956).

$$\text{Catalase activity} = \frac{\text{Difference in titre value}}{\text{Vol. of sample pipetted out}} \times \frac{1}{15 \text{ min}}$$

Lipid peroxidation (OD value) :

Lipid peroxide formation was studied by thiobarbituric acid (TBA) colour reaction (Bernheim *et al*., 1948) with minor modification. Five ml of 0.5 per cent TBA solution and 2 ml of 1N H_2SO_4 were added to 25 numbers of embryo in a hard glass tube with close fitting glass lid. The mixture was thoroughly shaken and placed in an oven at 100ºC for 1h. After cooling, 5ml of methyl cellosolve (2 methoxy ethanol) was added and centrifuged at 2500 rpm for 10min. and the absorbance of the clear supernatant was measured in a spectrophotometer at 520 nm. Total lipid peroxidation activity was expressed in terms of absorbance.

Statistical analysis :

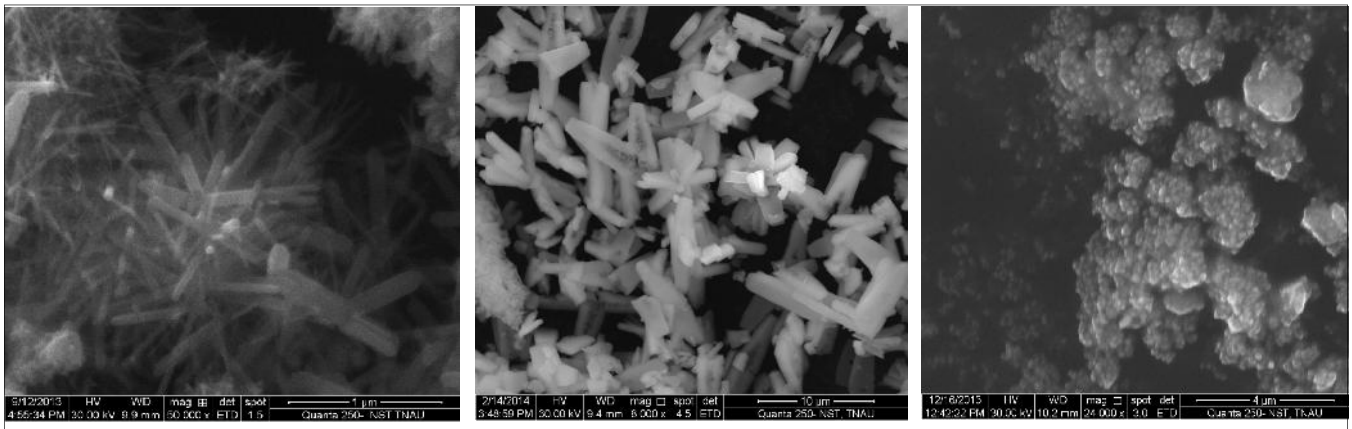
The data obtained from different laboratory experiments were analyzed statistically by adopting techniques described by Panse and Sukhatme (1985). The critical differences (CD) were calculated at 5 per cent probability level. Wherever necessary, the percentage values were transformed into arcsine values. Volatiles emitted from the preconditioned seeds were correlated with germination as per the preconditioned seeds by Dewey and Lu (1959).

RESEARCH ANALYSIS AND REASONING

The findings of the present study as well as relevant discussion have been presented under following heads :

Characterization of nano particles (ZnO, Ag and TiO_2) :

The surface morphology of the synthesized nanoparticles were examined under SEM revealed that ZnO nanorods appeared like a petals radiating from a flower (Fig. 1a) measuring 50-80 nm diameter. Similar results were reported by Moghaddam *et al.* (2009) while Ag nanoparticles appeared like a bundle of needle each having a diameter of 400-450 nm (Fig. 1b) and also by Sileikaite *et al*. (2006). As against above, TiO_2 nanoparticles was in spherical shape with size ranging from 85-100 nm (Fig. 1c) compared with earlier report (Arami *et al.*, 2007). To confirm our results of SEM, the same nanoparticles were characterised under TEM. Rod-shaped particles that fused at centre to form a radiating structure was observed for ZnO nanorods (Fig. 2a) which scaled only to 35-40 nm as against 50-80 nm. TEM micrographs of Ag nanoparticles were found to be spherical shaped scattered without clumping with a size ranging from 25 to 85 nm accounting for an average of 40 nm whereas the SEM measurements got enlarged 10



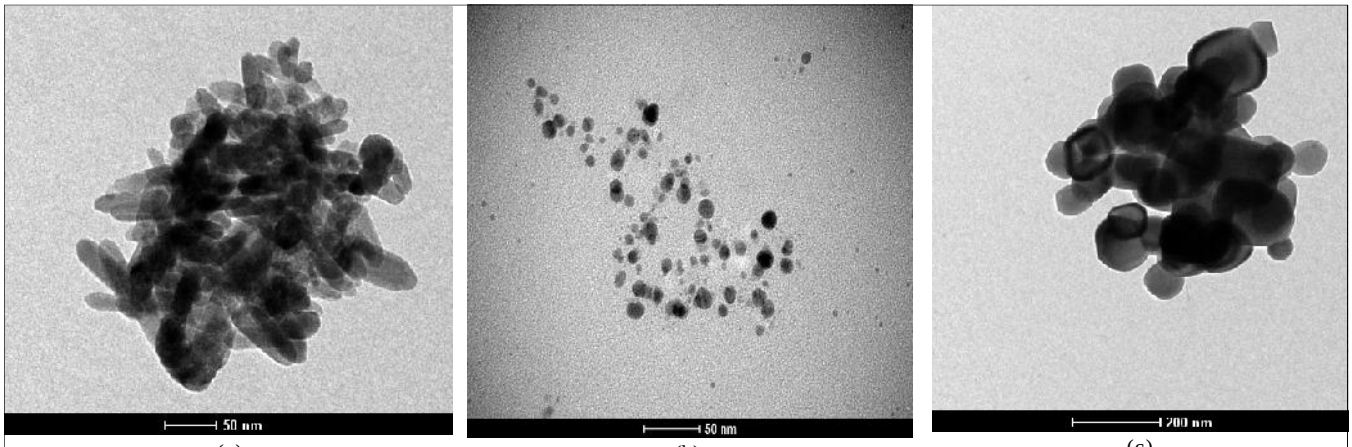
(a) (b) (c)

Fig. 1: SEM images of (a) ZnO, (b) Ag and (c) TiO_2 nanoparticles

times (Fig. 2b). TiO_2 nanoparticles were diagnosed primarily to be cylindrical in shape as against spherical under SEM but measured 100 nm in conformity (Fig. 2c).

Effect of inorganic nanoparticles on seed quality and storability of groundnut cv. VRI 2 seeds :

Nanoparticle treated seeds significantly outperformed than control in terms of germination, shoot length, root length, dry matter production and vigour index even after 12 months of storage. Significant differences were also observed between the nanoparticle treatments, storage period and their interaction. Among the tested NPs, ZnO @ 1000 mg kg^{-1} followed by Ag @ 1250mg kg^{-1} significantly increased the germination of treated fresh seeds, allowing for slow natural ageing upto 12 months (Table 1) compared to the remaining treatments including control. Prasad *et al.* (2012) also observed that ZnO nanoparticles at a concentration of 1000 ppm improved the germination, root growth, shoot growth, dry weight and pod yield in groundnut. The beneficial effect of the ZnO NPs in improving the germination could be ascribed to higher precursor activity of nanoscale zinc in auxin production (Kobayashi and Mizutani, 1970). ZnO NPs could increase the level of IAA in roots (sprouts) thereby increasing growth rate of seedlings in *Cicer arietinum* (Pandey *et al.*, 2010). Among the treatments, seeds that received ZnO@1000mg kg^{-1} had the highest vigour index (3067) followed by Ag @ 1250mg kg^{-1} (3006) which was 24 and 23 per cent increase respectively over the control (2328). This reveals the property of NPs in improving the germination and for increased shoot length of germinated seeds. Enhanced physiological performance due to nano particles treatment could be attributed to the



(a) (b) (c)

Fig. 2: TEM images of (a) ZnO, (b) Ag and (c) TiO_2 nanoparticles

Table 1 : Effect of inorganic nanoparticles on germination (%) of fresh seeds of groundnut cv. VRI-2 under natural ageing

Treatments	Period of storage (p)												
	P 0	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10	P 11	P 12	Mean
Control	91(72.54)	89(70.63)	86(68.02)	81(64.15)	75(60.00)	68(55.55)	64(53.13)	61(51.35)	57(49.02)	52(46.14)	49(44.42)	44(41.55)	42(40.39)	66(54.33)
ZnO 750 mg	89(70.63)	88(69.73)	86(68.02)	83(65.65)	75(60.00)	68(55.55)	65(53.73)	62(51.94)	59(50.18)	53(46.72)	50(45.00)	46(42.70)	44(41.55)	67(54.94)
ZnO 1000 mg	91(72.54)	90(71.57)	88(69.73)	86(68.02)	86(68.02)	82(64.89)	79(62.72)	77(61.34)	74(59.34)	69(56.16)	65(53.73)	62(51.94)	58(49.60)	77(61.34)
ZnO 1250 mg	92(73.57)	91(72.54)	87(68.86)	85(67.21)	83(65.65)	80(63.43)	77(61.34)	74(59.34)	71(57.41)	65(53.73)	62(51.94)	58(49.64)	56(48.44)	75(60.00)
Ag 750 mg	90(71.57)	88(69.73)	86(68.02)	82(64.89)	75(60.00)	68(55.55)	65(53.73)	61(51.35)	59(50.18)	56(48.44)	52(46.14)	48(43.85)	44(41.55)	67(54.94)
Ag 1000 mg	91(72.54)	89(70.63)	87(68.86)	85(67.21)	82(64.89)	78(62.02)	75(60.00)	68(55.55)	66(54.33)	63(52.53)	59(50.18)	57(49.02)	55(47.87)	73(58.69)
Ag 1250 mg	91(72.54)	89(70.63)	88(69.73)	86(68.02)	84(66.42)	80(63.43)	77(61.34)	74(59.34)	71(57.41)	68(55.55)	64(53.13)	61(51.35)	58(49.60)	76(60.66)
TiO_2 750 mg	89(70.63)	87(68.86)	85(67.21)	83(65.65)	76(60.66)	68(55.55)	64(53.13)	61(51.35)	58(49.60)	52(46.14)	49(44.42)	45(42.13)	43(40.97)	66(54.33)
$TiO_2$1000 mg	90(71.57)	89(70.63)	87(68.86)	85(67.21)	83(65.65)	78(62.02)	76(60.66)	72(58.05)	68(55.55)	65(53.73)	62(51.94)	59(50.18)	56(48.44)	75(60.00)
$TiO_2$1250 mg	89(70.63)	88(69.73)	86(68.02)	84(66.42)	79(62.72)	74(59.34)	71(57.41)	68(55.55)	65(53.73)	59(50.18)	56(48.44)	52(46.14)	50(45.00)	71(57.41)
Mean	90(71.57)	89(70.63)	87(68.86)	84(66.42)	80(63.43)	74(59.34)	71(57.41)	68(55.55)	65(53.73)	60(50.76)	57(49.02)	53(46.72)	51(45.57)	71(57.41)
					Treatments			Period				Treatments x period		
S.E.±					0.78			0.89				2.83		
C.D. (P=0.5)					1.55**			1.76**				5.58**		

** indicate significance of value at P=0.01

quenching of free radicals by the nano particles. Smaller size of the nanoparticles would have easily entered through the cracks present on the outer seed surface, reacted with free radicals resulting in enhanced seed vigour and viability.

Biochemical constituent of seeds is an important factor which influences the physiological soundness of seed. Ageing induces progressive seed deterioration leading to lethal damage and inability of the seeds to germinate (Bouteau *et al.*, 2011). Seeds treated with ZnO nano rods and Zero valent iron (ZVI) nanoparticles enhanced the physiological and biochemical properties resulting in improved vigour and viability of aged seeds (Senthil Kumar, 2011) in black gram and tomato (Sridhar, 2012). In the present investigation, the nano particles treated seeds maintained the vigour and viability during natural ageing as evidenced from reduced electrical conductivity (Table 3), the lowest being due to ZnO @ 1000mg ($0.347 dSm^{-1}$) and Ag @ 1250mg kg^{-1} ($0.347 dS m^{-1}$).

Damage to the membrane is one of causes for the loss of viability during storage, which under normal condition could have repaired by itself (Kaewnaree *et al.*, 2011). Seeds with the reduced activity of this repair system make the seeds to germinate slowly than the normal untreated seeds which can undergo self-repair rapidly. If the capacity for repairing is below a critical level, damage would continue to accumulate resulting in the death of seeds. The electrical conductivity of the seed leachate in groundnut increased with increasing storage period. The mean highest value recorded with untreated control seeds ($0.379 dSm^{-1}$), might be due to the fact that this high oil containing seed, have free fatty acids which leach out with increase in storage period due to loss of membrane integrity and due to peroxidation of unsaturated fatty acids. The present results are in conformity with the earlier reports of Kalappa (2002) in sunflower and Anuja and Aneja (2004) in soybean. Similar results were also obtained by Paramasivam (2005) in groundnut.

Groundnut seeds up on treatment with ZnO@ 1000mg kg^{-1} and Ag@ 1250mg kg^{-1} exhibited higher catalase activity of 0.421 µg H_2O_2 mg^{-1} min^{-1} and 0.421 µg H_2O_2 mg^{-1} min^{-1} over control seeds (Table. 4) followed by ZnO@ 1250mg kg^{-1} (0.418 µg H_2O_2 mg^{-1} min^{-1}) which had been 8.5, 8.5 and 7.8 per cent increase over the control, respectively (0.385 µg H_2O_2 mg^{-1} min^{-1}). Catalase activity gets reduced with increasing storage period, but significant reduction was observed from fourth month of

storage irrespective of the treatments. Catalases are good scavenging enzymes involved in free radical mechanism on lipid peroxidation and protects the mitochondrial components from oxidative damage (Chander and Kapoor, 1990). Decreased catalase activity was found associated with ageing, accompanied by an increase in lipid peroxidation and loss of vigour and viability in sunflower (Bailly *et al.*, 2002). Lipid peroxidation creates profound damage to membranes and changes in oil quality as well. As a result, long chain fatty acids are broken into smaller and smaller compounds, some of these being released as volatile hydrocarbons. The final consequence is loss of membrane structure, leakiness resulting in inability to complete the normal metabolism. In the present study, nano seed treatment in naturally aged seeds had minimum lipid peroxidation value than the control. After twelve months of storage, the overall mean of nanoparticles treatment (0.174 OD value) was significantly lower than the control (0.195 OD value). Seeds treated with ZnO @ 1000 mg kg^{-1} had the lowest mean value of

Table 2 : Effect of nanoparticles on vigour index of fresh seeds of groundnut cv. VRI-2 under natural ageing

Treatments	Period of storage (p)													
	P 0	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10	P 11	P 12	Mean
Control	3804	3667	3483	3159	2760	2373	2048	1861	1716	1539	1421	1258	1176	2328
ZnO 750 mg	3667	3599	3492	3287	2805	2407	2106	1934	1800	1606	1490	1348	1258	2369
ZnO 1000 mg	3795	3726	3626	3517	3474	3280	3113	3003	2842	2629	2438	2300	2123	3067
ZnO 1250 mg	3855	3777	3567	3468	3328	3184	3003	2842	2698	2444	2306	2129	2027	2971
Ag 750 mg	3744	3608	3492	3231	2783	2394	2093	1891	1788	1663	1524	1382	1250	2372
Ag 1000 mg	3749	3649	3524	3383	3182	2909	2678	2407	2290	2161	2006	1904	1826	2744
Ag 1250 mg	3804	3694	3626	3500	3385	3200	3011	2864	2712	2570	2374	2239	2105	3006
TiO_2 750 mg	3694	3567	3434	3262	2812	2380	2061	1873	1746	1544	1426	1287	1213	2331
$TiO_2$1000 mg	3744	3667	3532	3400	3212	2933	2721	2556	2387	2249	2127	1994	1859	2799
$TiO_2$1250 mg	3711	3634	3509	3343	3042	2753	2528	2394	2249	2024	1898	1737	1640	2651
Mean	3757	3659	3528	3355	3078	2781	2536	2362	2223	2043	1901	1758	1648	2664
				Treatments				Period				Treatments x period		
S.E.±				41.48				47.29				149.57		
C.D. (P=0.5)				81.69**				93.14**				294.54**		

** indicate significance value at P=0.01

Table 3 : Effect of inorganic nanoparticles on electrical conductivity (dsm^{-1}) of fresh seeds of groundnut cv. VRI -2 under natural ageing

Treatments	Period of storage (p)													
	P 0	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10	P 11	P 12	Mean
Control	0.314	0.317	0.322	0.331	0.345	0.368	0.385	0.405	0.411	0.423	0.428	0.434	0.442	0.379
ZnO 750 mg	0.317	0.319	0.325	0.330	0.343	0.361	0.383	0.402	0.407	0.421	0.426	0.431	0.438	0.377
ZnO 1000 mg	0.315	0.318	0.321	0.324	0.329	0.335	0.344	0.352	0.361	0.368	0.376	0.381	0.387	0.347
ZnO 1250 mg	0.316	0.319	0.323	0.327	0.333	0.338	0.347	0.359	0.364	0.373	0.379	0.385	0.391	0.350
Ag 750 mg	0.315	0.318	0.325	0.330	0.344	0.363	0.383	0.402	0.408	0.422	0.426	0.432	0.438	0.377
Ag 1000 mg	0.312	0.315	0.323	0.328	0.338	0.345	0.360	0.377	0.383	0.392	0.398	0.405	0.411	0.361
Ag 1250 mg	0.313	0.316	0.319	0.325	0.331	0.337	0.346	0.354	0.362	0.369	0.376	0.382	0.387	0.347
TiO_2 750 mg	0.316	0.319	0.328	0.335	0.345	0.365	0.384	0.405	0.409	0.421	0.427	0.434	0.440	0.379
$TiO_2$1000 mg	0.316	0.318	0.327	0.330	0.339	0.345	0.359	0.377	0.381	0.390	0.397	0.403	0.409	0.361
$TiO_2$1250 mg	0.315	0.319	0.327	0.332	0.340	0.347	0.361	0.379	0.382	0.398	0.403	0.409	0.415	0.364
Mean	0.315	0.318	0.324	0.329	0.339	0.350	0.365	0.381	0.387	0.398	0.404	0.410	0.416	0.364
				Treatments				Period				Treatments x period		
S.E.±				0.003				0.003				0.011		
C.D. (P=0.5)				0.006**				0.006**				0.022*		

* and** indicate significance of value at P=0.05 and 0.01, respectively

Table 4 : Effect of nanoparticles on catalase (µg H_2O_2 mg^{-1} min^{-1}) of fresh seeds of groundnut cv. VRI-2 under natural ageing

Treatments	Period of storage (p)													
	P 0	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10	P 11	P 12	Mean
Control	0.446	0.443	0.435	0.428	0.418	0.411	0.397	0.382	0.366	0.348	0.328	0.308	0.289	0.385
ZnO 750 mg	0.445	0.442	0.436	0.435	0.425	0.417	0.405	0.387	0.372	0.354	0.334	0.316	0.302	0.390
ZnO 1000 mg	0.447	0.445	0.445	0.443	0.440	0.438	0.435	0.435	0.426	0.409	0.386	0.371	0.359	0.421
ZnO 1250 mg	0.446	0.442	0.439	0.436	0.436	0.435	0.433	0.433	0.424	0.406	0.384	0.369	0.357	0.418
Ag 750 mg	0.444	0.441	0.437	0.433	0.422	0.413	0.401	0.384	0.368	0.352	0.332	0.312	0.296	0.387
Ag 1000 mg	0.447	0.446	0.442	0.438	0.433	0.430	0.429	0.415	0.397	0.381	0.359	0.342	0.329	0.407
Ag 1250 mg	0.449	0.447	0.445	0.442	0.439	0.435	0.435	0.433	0.426	0.407	0.386	0.370	0.357	0.421
TiO_2 750 mg	0.445	0.442	0.434	0.427	0.419	0.411	0.399	0.384	0.366	0.350	0.330	0.310	0.294	0.385
$TiO_2$1000 mg	0.448	0.446	0.442	0.438	0.434	0.430	0.429	0.416	0.399	0.381	0.359	0.343	0.330	0.407
$TiO_2$1250 mg	0.447	0.444	0.441	0.438	0.433	0.427	0.427	0.413	0.397	0.380	0.359	0.342	0.328	0.406
Mean	0.446	0.444	0.440	0.436	0.430	0.425	0.419	0.408	0.394	0.377	0.356	0.338	0.324	0.403
				Treatments				Period				Treatments x period		
S.E.±				0.003				0.003				0.011		
C.D. (P=0.5)				0.006**				0.007**				0.022**		

** indicate significance of value at P=0.01

Table 5 : Effect of nanoparticles on lipid peroxidase (OD value) of fresh seeds of groundnut cv. VRI-2 under natural ageing

Treatments	Period of storage (p)													
	P 0	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10	P 11	P 12	Mean
Control	0.048	0.053	0.059	0.068	0.080	0.092	0.106	0.119	0.137	0.152	0.169	0.182	0.195	0.112
ZnO 750 mg	0.050	0.054	0.058	0.061	0.076	0.086	0.097	0.108	0.125	0.141	0.155	0.169	0.189	0.105
ZnO 1000 mg	0.048	0.052	0.056	0.062	0.063	0.072	0.081	0.088	0.102	0.113	0.124	0.140	0.155	0.089
ZnO 1250 mg	0.049	0.053	0.055	0.060	0.067	0.078	0.087	0.097	0.108	0.121	0.135	0.149	0.163	0.094
Ag 750 mg	0.052	0.056	0.062	0.069	0.077	0.087	0.099	0.110	0.128	0.145	0.160	0.171	0.186	0.108
Ag 1000 mg	0.047	0.052	0.057	0.066	0.073	0.083	0.095	0.105	0.117	0.130	0.145	0.160	0.177	0.101
Ag 1250 mg	0.048	0.053	0.056	0.061	0.066	0.075	0.084	0.091	0.102	0.115	0.127	0.143	0.159	0.091
TiO_2 750 mg	0.054	0.057	0.061	0.072	0.077	0.089	0.101	0.114	0.131	0.147	0.163	0.175	0.190	0.110
$TiO_2$1000 mg	0.046	0.049	0.054	0.065	0.070	0.079	0.089	0.101	0.113	0.131	0.145	0.152	0.168	0.097
$TiO_2$1250 mg	0.051	0.053	0.058	0.065	0.072	0.083	0.093	0.104	0.121	0.136	0.149	0.162	0.180	0.102
Mean	0.049	0.053	0.058	0.065	0.072	0.082	0.093	0.104	0.118	0.133	0.147	0.160	0.176	0.101
				Treatments				Period				Treatments x period		
S.E.±				0.0017				0.0019				0.006		
C.D. (P=0.5)				0.0033**				0.0038**				0.012**		

** indicate significance of value at P=0.01

0.089 OD value whereas control had maximum mean OD value of 0.112 (Table 5). The reason attributed was the donation of electrons by the nano particles in scavenging the free radicals in the aged seeds.

Conclusion :

Application of nanoparticles are especially ZnO @ 1000mg kg^{-1}seed favored germination and related physiological and biochemical parameters. Hence, ZnO may be considered in the crop production as one of the inputs for treating the seeds upon confirming the performance under field condition and subjecting to human safety tests before recommending for seed treatment in nano particles for adoption by farmers. This outcome is valuable both for farmers as well as seed industries because nanoparticles utilization may be a feasible approach to increase the germination, vigour, storability and also to reduce consumption of chemicals substance in agriculture that would reduces environmental pollution.

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