Indian Journal of Science and Technology, *Vol 7(9), 1376–1381, September 2014*

ISSN (Print) : 0974-6846
ISSN (Online) : 0974-5645

Customizing Zinc Oxide, Silver and Titanium Dioxide Nanoparticles for enhancing Groundnut Seed Quality

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Abstract

Zinc oxide (ZnO), Silver (Ag) and Titanium dioxide (TiO_2) nanoparticles were synthesised using template-free aqueous solution based on simple chemical route which were characterised through SEM, TEM and XRD. Size of Zinc oxide (ZnO), Silver (Ag) and Titanium dioxide (TiO_2) nanoparticles measured 35-45nm, 20-80 nm and 85-100 nm, respectively to conform the nano-size. Groundnut seeds when dry dressed with the synthesised nanoparticles each at 500, 750, 1000 and 1250 mg kg $^{-1}$, the dose of 1000 mg kg^{-1} outperformed in enhancing the germination (75%), shoot length (20.97 cm) root length (17.98) and thereby the vigour index (2949) compared to control (55%, 16.92, 15.21 and 1759) respectively.

Keywords: Ag, Groundnut Seed Quality Enhancement, SEM, Synthesis of ZnO, TEM, TiO_2 Nanoparticles, XRD

1. 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. Natarajan and Sivasubramanian[10] elucidated various nanotechnological approaches that can be employed in Seed Science. The approaches include nano-polymer for seed hardening, Nano Particles (NPs) for seed quality enhancement, nano-sensors, nano-barcodes, use of nano-magnetic particles for aerial seeding etc. Successful attempts were made in China to counteract free radicals in spinach by using titanium nano-particles and improve the germination[8]. Kumar[12] also observed positive impact of ZnO and Zero Valent Ion (ZVI) nanoparticles as wet and dry treatments on black gram seeds for physiological characters (germination, seedling growth and vigour index) and biochemical traits (electrical conductivity, free amino acids, dehydrogenase activity and lipid peroxidation). Hence the present investigation was made to study the effect of ZnO, Ag, and TiO_2 nanopartilcle on the vigour and viability of groundnut seed.

2. Materials and Methods

The first experiment synthesis of nanoparticles and characterization was carried out at Department of Nano Science and Technology and the second experiment study of seed quality enhancement was carried at Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore -03, during the year of 2013–14.

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The chemicals used for synthesis of nanoparticles *viz.*, Zinc nitrate (Zn $(NO_3)_2.4H_2O$), $AgNO_3$, Trisodium citrate, TiO_2 pellets, NaOH and Ethanol were purchased from THE I.L.E. Co. Pvt. Ltd., Coimbatore, Tamil Nadu.

2.1 Synthesis of ZnO, Ag and TiO_2 Nanoparticles

2.1.1 Zinc Oxide Nanoparticles

ZnO NPs were synthesized[9] 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 $(NO_3)_2$ solutions (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° C 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 60° C. NPs such synthesized were transferred to air tight screw cap vial (10 ml) and stored at ambient temperature for further investigations.

2.1.2 Silver Nanoparticles

The Ag NPs were prepared based on chemical reduction method according to the description outlined by Lee and Meisel[6]. 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% 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:

$$4Ag^+ + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag0 + C_6H_5O_7H_3 + 3Na^+ + H^+ + O_2\uparrow$$

2.1.3 Titatium dioxide Nanoparticles

TiO_2 NPs were synthesized[2] 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. Thus obtained yellow solution was irradiated in an ultra sonicator (Soncis, VCX 1500, 20 kHz and 350 W) for 2 h in 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.

2.2 Characterization of Synthesized Nanoparticles

Characterization of the synthesized nanoparticles was performed by the techniques such as SEM, TEM and X-ray diffraction.

2.2.1 Scanning Electron Microscope (SEM)

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 aluminum stub. Sample surface were observed at different magnification and the images were recorded.

2.2.2 Transmission Electron Microscope (TEM)

FEI TECHNAI SPRIT make was used to analyze the sample. Dilute suspensions of NPs (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 upon drying, was examined and the images were recorded at different magnification.

2.2.3 X-ray Diffraction

The X-ray diffractograms have been recorded on Powder XRD (Bruker D8 Advance Powder X-ray Diffractometer, Germany). The machine exploits Cu-Kα radiation (0.154 nm) for measuring the crystalline nature of atoms in the material[7]. The diffractograms were recorded in the range of $2\theta = 10 - 80$ degrees at a scanning speed of 0.080 and step times 1s at room temperature 25° C.

2.3 Seed Treatment

Groundnut pods of cv. VRI 2 stored under ambient conditions (25 ± 3°C temperature and 95 ± 3% RH) for 13 months at the Department of Seed Science and Technology, TNAU were shelled and the kernels were dry dressed with nanoparticles of ZnO, Ag and TiO2 each at 500, 750, 1000 and1250 mg kg-1 in screw capped glass bottles at room temperature. These glass bottles were gently shaken for 3 min about 5 times at an interval of 3h, manually. Seeds shaken without NPs served as control. Seeds subjected to the treatments were evaluated after seven days for their physiological seed quality parameters viz., germination percentage, shoot length, root length, dry matter production and vigour index.

The germination test was conducted with 50 kernels in five replications in sand medium. The test conditions of 25 ± 2° C and 95 ± 3% 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[4].

Root length of all the normal seedlings from the germination test was measured from collar region to the root tip and the mean was expressed in centimetre.

Shoot length of all the normal seedlings from the germination test was measured from collar region to the shoot apex and the mean was expressed in centimetre.

Vigour index was computed by adopting the method suggested by Abdul-Baki and Anderson[1] and expressed as whole number.

Vigour index = Germination percentage × Seedling length in cm.

3. Results and Discussion

3.1 Characterization of Nanoparticles (ZnO, Ag and TiO_2)

The surface morphology of the nanoparticles synthesized and examined under SEM revealed that ZnO nanorods appeared like a petals radiating from a flower (Figure 1a) measuring 50–80 nm diameter supporting the study reported by Moghaddam[9]; while Ag nanoparticles appeared like a bundle of needle each having a diameter of 400–450 nm (Figure 1b), a similar observation made by Sileikaite[14]. As against above, TiO_2 NPs were in spherical shape with size ranging from 85–100 nm (Figure 1c) and confirmed with earlier report[2].

To confirm our results of SEM, the same NPs were characterized under TEM. Rod shaped particles that fused at centre to form a radiating structure was observed for ZnO nanorods (Figure 2a) which scaled only to 35–40 nm as against 50–80 nm. TEM micrographs of Ag NPs 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 times (Figure 2b). TiO_2 NPs were diagnosed primarily to be cylindrical in shape as against spherical under SEM but measured 100 nm is in conformity (Figure 2c).

The obtained X-ray diffraction peaks are in agreement with precursors of zinc oxide (ZnO), silver (Ag) and titanium oxide (TiO_2) micro-sized materials, respectively. It confirms that the synthesised particles are crystalline and in nano dimension with different shapes (rod, needle and spherical) regardless of metal. The XRD pattern showed the intense peaks (Figure 3a, 3b, 3c) ranging from 10 to 80° and the corresponding 2θ and d-spacing value as

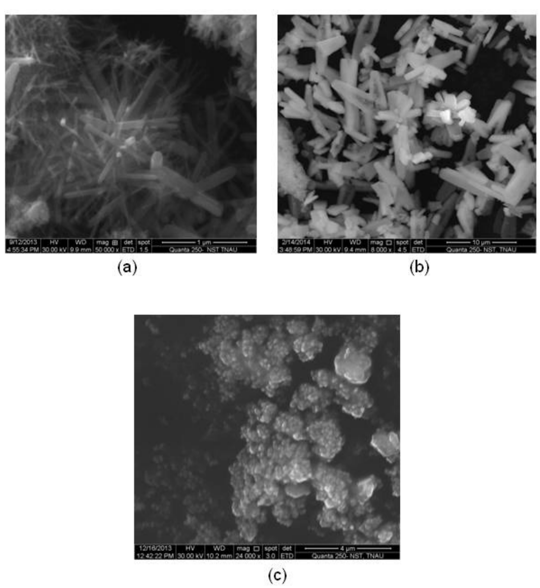

Figure 1. SEM images of (a) Zno, (b) silver and (c) TiO_2 nanoparticles

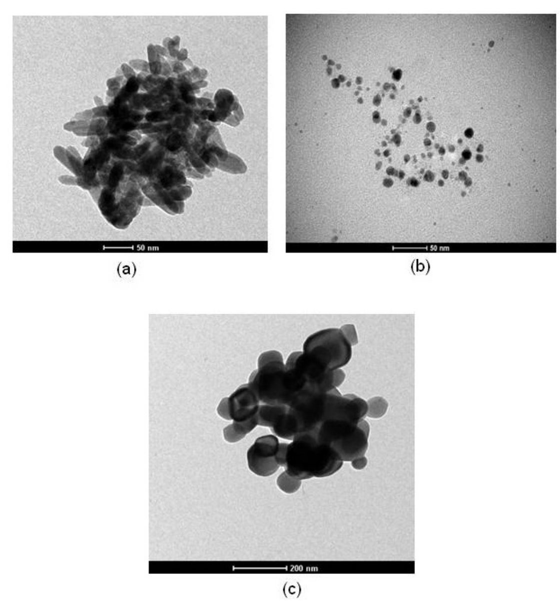

Figure 2. TEM images of (a) Zno, (b) silver and (c) TiO_2 nanoparticles

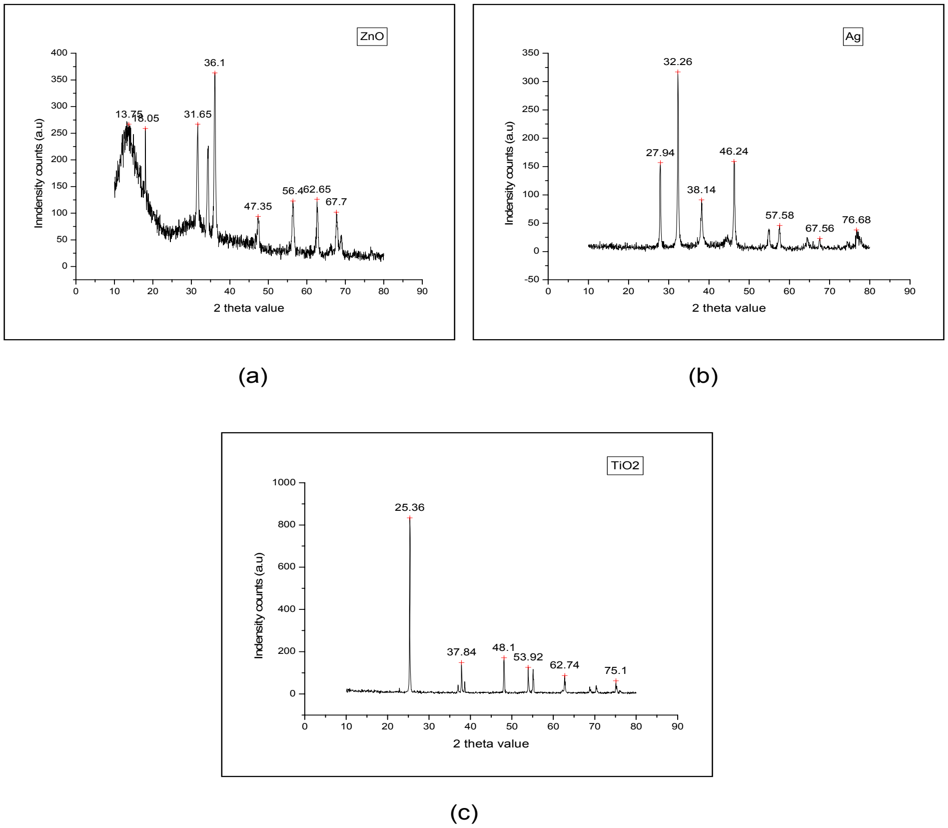

Figure 3. Powder XRD patterns of Synthesised (a) Zinc Oxide Nanorods (Zno) (b) silver nanoparticles (Ag-NPs) (c) Nano Titanium Oxide (TiO_2)

provided in parenthesis (2θ, d) for ZnO - (18.05, 4.90), (31.65, 2.82), (36.1, 2.48), (47.6, 1.91), (56.4, 1.62), (62.65, 1.48), (67.7, 1.38)), Ag- ((27.94, 3.19), (32.26, 2.77), (38.14, 2.35), (46.24, 1.96), (57.58, 1.67), (67.56, 1.60)) and TiO_2- ((25.36, 3.51), (37.84, 2.36), (48.1, 1.88), (53.92, 1.69), (62.74, 1.48), (75.1, 1.26)), respectively. The typical XRD pattern revealed that the sample contained rod shaped ZnO which compared with earlier report of Babu and Narayanan[3] and needle structure of Ag nanoparticle support the results of Thiel[15] and spherical TiO_2 nanoparticles also support the observations of Arami[2].

3.2 Seed Germination and Seedling Vigour

Nanoparticles of ZnO, Ag and TiO_2 when treated in different concentrations viz., 500, 750, 1000 and 1250 mg kg^{-1} had significantly outperformed control in terms of germination, shoot length, root length and vigour index. Significant differences were also observed between the nano particles and doses.

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 nanoparticle (67 %), which was 2 percent and 3 percent higher than the Ag (65%) and TiO_2 NPs (63%) respectively (Table 1). Among the dosages, seeds treated @1000 (72%) and 1250 mg kg^{-1} (70%) found to register maximum germination than other dosages. Interaction among the NPs and dosage revealed that ZnONPs @ 1000 mg $kg^{-1}$and Ag NPs @ 1250 mg kg^{-1} recorded in the maximum germination of 75 percent while the minimum in the seed treated with TiO_2 at the 500 mg kg^{-1}.

The beneficial effect of the ZnO NPs in improving the germination could be ascribed to higher precursor activity of nanoscale zinc in auxin production[5]. 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[11]. Enhanced

Table 1. Effect of nanoparticles on germination % of stored (13 month old) seeds of groundnut

Treatments (mg/kg^{-1})	Germination (%)			
	ZnO	Ag	TiO_2	Mean
500	58 (49.60)	56 (48.44)	55 (47.87)	56 (48.44)
750	63 (52.53)	62 (51.94)	60 (50.76)	62 (51.94)
1000	75 (60.00)	69 (56.16)	71 (57.41)	72 (58.05)
1250	71 (57.41)	73 (58.69)	67 (54.94)	70 (56.79)
Mean	67 (54.94)	65 (53.73)	63 (52.53)	65 (53.73)
Control	55 (47.87)			

	Treatment	Dosage	Treatment × Dosage
SEd	0.66	0.66	1.32**
CD (P = 0.05)	1.34**	1.34**	2.69**

Table 2. Effect of nanoparticles on shoot length of stored (13 month old) seeds of groundnut

Treatments (mg/kg^{-1})	Shoot length (cm)			
	ZnO	Ag	TiO_2	Mean
500	18.40	17.60	17.07	17.69
750	19.88	19.35	18.47	19.23
1000	20.97	20.24	20.75	20.65
1250	20.28	20.86	20.06	20.40
Mean	19.88	19.51	19.09	19.49
Control	16.92			

	Treatment	Dosage	Treatment × Dosage
SEd	0.15	0.15	0.23
CD (P = 0.05)	0.31**	0.31**	0.46*

Table 3. Effect of nanoparticles on root length (cm) of stored (13 month old) seeds of groundnut VRI 2

Treatments (mg/kg^{-1})	Root length (cm)			
	ZnO	Ag	TiO_2	Mean
500	15.67	15.54	15.34	15.52
750	16.76	16.05	15.84	16.22
1000	17.98	17.18	17.46	17.54
1250	17.20	17.61	16.51	17.11
Mean	16.90	16.60	16.29	16.60
Control	15.21			

	Treatment	Dosage	Treatment × Dosage
SEd	0.13	0.13	0.21
CD (P = 0.05)	0.26**	0.26**	0.42*

physiological parameters could be attributed and quenching of free radicals by nanoparticles which could entered through cracks present seed coat, reached into free radicals resulting in enhanced seed vigour.

Nanoparticle treated germinated seeds exhibited higher root and shoot length than control. ZnO NPs treated seeds induced maximum shoot length (19.88 cm) followed by Ag NPs (19.51 cm) and were 14.90 and 13.28 percent higher than control respectively (16.92 cm) (Table 2). NPs treatment and dosage interaction revealed that ZnO NPs @ 1000 mg $kg^{-1}$and Ag NPs @1250 mg kg^{-1} promoted maximum shoot length and the minimum was observed in seed treated with TiO_2 NPs @ 500 mg kg^{-1} (17.07 cm). In case of root length also the, ZnO NPs treated seeds had the maximum root length (16.90 cm) while the minimum (16.29 cm) in TiO_2 NPs treated seeds than the control (Table 3). Interaction between the nano seed treatment and dosage revealed that ZnO @ 1000 mg kg^{-1}(17.98 cm) and Ag NPs @ 1250 mg kg^{-1} (17.61) produced maximum root length than others. Such promoting effect of nano-scale SiO_2 and TiO_2 on germination was reported in soya bean[8], in which authors noticed increased nitrate reductase enzyme activity and enhanced antioxidant system. Similar results were observed by Zheng[16] when *Spinacia oleracea* seeds were treated with nanoscale TiO_2 particles.

The results revealed the promotory effect of ZnO nanoparticles at optimum concentrations and inhibitory effect at high concentrations on root and shoot growth. An increase of the shoot/root ratio compared to that of the control was reported by Shah and Belozerova[13] while analyzing the influence of metal nanoparticles on germination of *Lactuca* seeds.

Significant variation was observed for vigour index due to nano seed treatment, their dosages and their interactions (Table 4). Among the nano seed treatments, seeds treated with ZnO NPs resulted in maximum vigour index (2474) than other treatments and the control (1759). Interaction between nano seed treatment and its dosages revealed that highest vigour Index was observed in seeds treated with ZnO @ 1000 mg (2949) which was followed by Ag @ 1250 mg (2811).

The beneficial effect of nanoparticle in improving the seed quality may be attributed that nano particles would

Table 4. Effect of nanoparticles on vigour index of stored (13 month old) seeds of groundnut VRI 2

Treatments (mg /kg^{-1})	Vigour index			
	ZnO	Ag	TiO_2	Mean
500	1976	1856	1787	1873
750	2308	2227	2072	2203
1000	2949	2584	2711	2748
1250	2661	2811	2474	2649
Mean	2474	2370	2261	2368
Control	1759			

	Treatment	Dosage	Treatment × Dosage
SEd	23.36**	23.36	46.72
CD (P = 0.05)	47.59**	47.59**	95.18**

induce oxidation-reduction reactions via the superoxide ion radical during germination, resulting the quenching of free radicals in the germinating seeds. In turn, oxygen produced in such process could also be used for respiration, which would further promote germination[16]. The experiments carried out by Kumar[12] revealed that black gram seeds treated with ZnO nano rods and ZVI NPs enhanced the physiological and biochemical properties resulting in improved vigour and viability of aged seeds. The reason attributed was the donation of electrons by the nano particles in scavenging the free radicals in the aged seeds.

4. Conclusion

Nanoparticles tested in the investigation were supportive in enhancing the germination and seedling vigour of the groundnut seeds which are supposed to be highly prone for deterioration in storage. Application of nanoparticles especially ZnO @ 1000mg 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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