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Standardization of Seed Treatment Protocol with Nanoparticles for Enhancing Seed Quality in Pigeonpea

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Abstract

A study was conducted to standardize the wet and dry seed treatment protocol with different nanoparticles (NPs) and their bulk form for enhancing seed quality in pigeonpea. Among the treatments, maximum germination (%), field emergence (%), mean seedling length (cm), seedling vigour index and lower electrical conductivity was observed by SiO_2NP @ 250 mg (98%, 95%, 43.68 cm, 4526 and 11.93 µS/cm/g, respectively) which was closely followed by ZnO NP @ 500 mg (97%, 94%, 42.57 cm, 4499 and 11.96 µS/cm/g). The seeds treated with Ag NP @ 250 mg also recorded slightly better quality attributes (96%, 92%, 41.96 cm, 4206 and 12.41 µS/cm/g, respectively) compared to untreated control (87%, 71%, 37.31cm, 3306 and 16.49µS/cm/g). The findings suggested that seed treatment of nanoparticle in dry form improved seed quality of pigeonpea variably at different concentrations.

Keywords: Pigeonpea, Nanoparticle, Seed quality

Pigeonpea (*Cajanus cajan* [L.] Millsp) commonly known as red gram or tur, is the second most important pulse crop in the country. It accounts for about 11.80 per cent of the total pulse area and 17 per cent of total pulse production in the country. It is a rich source of protein and also rich in iron, iodine, essential amino acids like lycine, tyrocene, cystine and arginine. It is one of the most important leguminous crops in the Indian diet and possesses valuable properties as restorer of nitrogen to the soil. Globally, it is grown on an area of 6.22 million ha with an annual production of 4.742 milliontones and productivity of 762 kg/ha. India is one of the major pigeonpea growing country in the world, accounting for 46.5 lakh ha area, 30.22 lakh tones of production and productivity of 650 kg/ ha. The state-wise trend shows that Maharashtra ranks first in area (29.19%) and production (29.68%) followed by Karnataka (19.23% and 15.96%, respectively) according to Tiwari and Shivhare (2016). Storage loss in pulses has been reported form 30-70 per cent due to storage moulds, insect infestation especially bruchids and lipid peroxidation due to the production of reactive oxygen species leading to rapid deterioration in storage. Several strategies such as hydration and dehydration, halogenations and antioxidant treatments does not allow storing of seeds and require immediate sowing and also may cause damage to seed coat. Therefore, an effort was made to adopt seed treatment with nanoparticles and refinement of methodology to enhance and prolong the seed quality in pigeonpea.

Nanotechnology, a new emerging and fascinating field of science, permits advanced research in many areas, and nano technological discoveries could open up novel applications in the field of agriculture. Preliminary studies showed the potential of nanoparticles in improving seed germination and growth, plant protection, pathogen detection, and pesticide / herbicide residue detection (Khot *et al.*, 2012). Efficacy of nanoparticles is determined by their chemical composition, size, surface covering, reactivity and most importantly the dose at which they are effective. Hence, standardizing the concentration for particular crop plant is much more important in obtaining affirmative result. Plants require micro elements in minute quantity for their growth and development, application of these elements in nano form can be cost effective, besides reducing the usage of pesticides drastically as an eco-friendly approach. Increasing evidence suggests that zinc oxide nanoparticles increase plant growth and development in soybean (Sedghi *et al.,* 2013). Silver nanoparticles are among the most potential candidates for modulating the redox status of plants, because of their ability to support electron exchange with Fe^{2+} and Co^{3+}, the two elements that participate in several biological redox reactions (Sharma *et al.,* 2012). Nano-SiO_2 increased seed germination by providing better nutrients availability with adequate pH and conductivity to the growing medium in maize seeds (Suriyaprabha *et al.,* 2012).

Material and Methods

Seeds: Seeds of pigeonpea variety BRG-2 was obtained from the National Seed Project, University of Agricultural Sciences, Bengaluru and dried to safer and uniform moisture level (8-9%).

Wet treatment: Nanoparticle solutions were obtained by dissolving the nanopowder in distill water using the ultra sonicator. The seeds were soaked with zinc oxide, silver, silicon dioxide nano and bulk particles at different concentrations *viz*., 100, 250, 500, 750 and 1000 ppm for 3 hrs. Hydro-primed seeds were taken as control. The treated seeds were then subjected to shade drying for moisture equilibration and uniform absorption of nanoparticles and were evaluated for various seed quality attributes.

Dry treatment: Seeds were treated with nanoparticles and their bulk form as dry treatment at different concentration *viz*., 100, 250, 500, 750 and 1000 mg in a glass jar and mixed thoroughly for even coating distribution, the bulk form of the chemicals used as control. Then the seeds were incubated for a short period to achieve equilibration and evaluated for various seed quality parameters.

Characterizing the nanoparticles

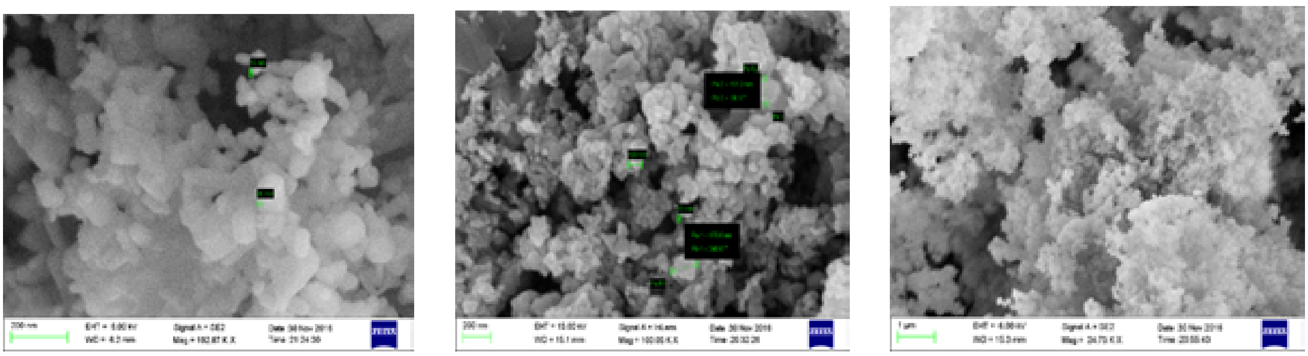
To confirm the particle size of the nanoparticles like Zinc oxide, Silver, Silicon dioxide (both nano and bulk form) which were obtained from the Sigma-Aldrich whose particle size was less than 100 nm were characterized using Scanning Electron Microscope (SEM) at the Indian Institute of Science (IISc.), Bengaluru (Fig. 1).

Seed quality study in the ISTA Member Lab

Treated seeds with three replications were used to determine various quality aspects like seed germination (%) as per ISTA (2013), field emergence (%), seedling length (cm), seedling vigour index and electrical conductivity (µS/cm/g). The mean data obtained were statistically analyzed by using suitable ANOVA and the results were presented as mean ± standard deviation (SD) and by comparing each experimental value with its corresponding control. The critical differences were calculated at five per cent level of probability, wherever 'F' test was significant.

Results and Discussion

All the wet treatment combinations showed reduced seed germination and seedling vigour index *i.e.,* ZnO bulk @ 1000 ppm (89% and 2766, respectively) compared to control (92% and 3013, respectively) (Table 1, 2 and Fig. 2). This may be due to the soaking injury *i.e.,* rupture of pores by nanoparticles and leach



Zinc oxide nanoparticle Silver nanoparticle Silicon dioxide nanoparticle

Fig. 1 : SEM micrograph of nanoparticles

TABLE 1

Influence of wet seed treatment with ZnO, silver and silicon dioxide (both NP and bulk form) on germination (%) in Pigeonpea

	B_1	B_2	B_3	B_4	B_5	B_6	Mean A
A_1	91	72	79	81	77	83	80
A_2	92	65	77	75	78	89	79
A_3	90	73	80	70	68	69	75
A_4	92	77	67	66	62	71	72
A_5	91	66	77	72	71	77	75
A_6	96	68	77	76	75	76	78
Mean B	92	70	76	73	71	77	

Interactions	A	B	AxB
SEm±	1.04	1.12	2.95
CD (0.01P)	3.91	4.18	11.05
CV (%)		6.65	

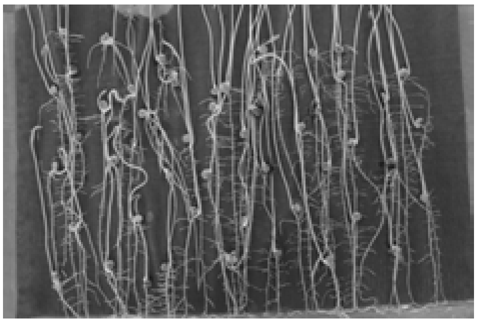
TABLE 2

Influence of wet seed treatment with ZnO, Silver and Silicon dioxide (both NP and bulk form) on seedling vigour index in Pigeonpea

	B_1	B_2	B_3	B_4	B_5	B_6	Mean A
A_1	2916	2134	2369	2392	2401	2538	2458
A_2	2976	2012	2405	2440	2389	2766	2498
A_3	2833	1914	2319	2067	1883	2248	2211
A_4	2993	2321	2102	1838	2072	2069	2233
A_5	3145	1987	2279	2225	2041	2007	2281
A_6	3216	2045	2345	2383	2317	2134	2407
Mean B	3013	2069	2303	2224	2184	2294	

Interactions	A	B	AxB
SEm±	42.99	45.96	121.59
CD (0.01P)	160.8	172	455
CV (%)		8.97	

A_1: Zinc oxide NP
A_2: Zinc oxide bulk
A_3: Silver NP
A_4: Silver bulk
A_5: Silicon dioxide NP
A_6: Silicon dioxide bulk
B_1: Control
B_2: 100 ppm
B_3: 250 ppm
B_4: 500 ppm
B_5: 750 ppm
B_6: 1000 ppm



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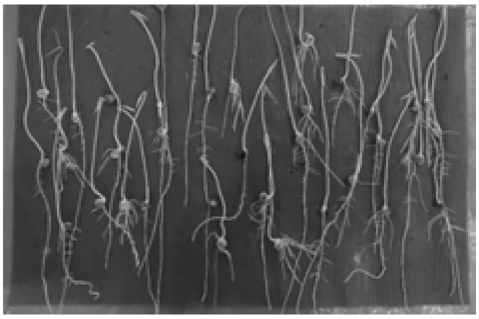
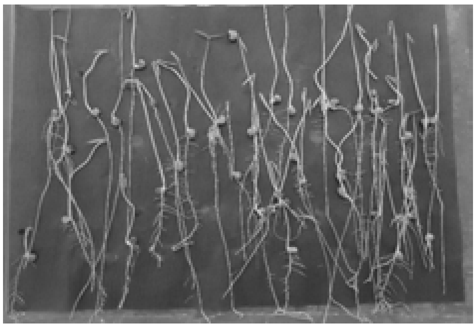
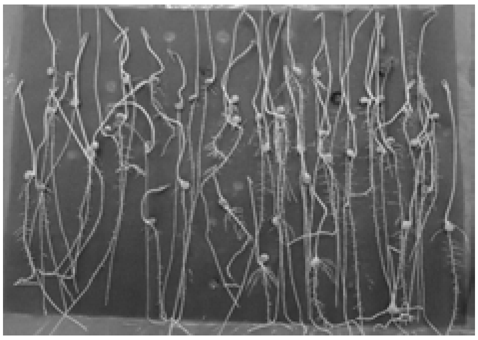


SiO_2 NP 100 ppm



ZnO NP 100 ppm



Ag NP 100 ppm

Fig. 2 : Effect of wet seed treatment of nanoparticles on germination (%) in pegionpea

out of the electrolytes in the seed coat leading to shriveling of the seeds. This reduces the overall seed germination and seedling vigour index in pegionpea.

Further, the seed treatment with dry form of zinc oxide, silver, silicon dioxide (both nano and bulk) was carried out to know its effect on seed quality parameters in pigeon pea. Dry seed treatment with nanoparticles and their bulk form showed improved seed quality parameters at various concentrations. Among the treatments maximum germination (98%) and field emergence (95%) was observed in SiO_2 NP @ 250 mg which is on par with ZnO NP @ 500 mg (97 and 94%, respectively), SiO_2 bulk @ 500 mg (97 and 93%, respectively), ZnO bulk @ 750 mg (96 and 93%, respectively), Ag NP @ 250 mg (96 and 92%, respectively) and followed by treatment combination Ag bulk @ 500 mg (95 and 91%, respectively) when compared to control (87 and 71%, respectively) (Table 3 and 4). Nanoparticles have large surface area resulting in enhanced activity. They have increased permeability through biological barriers (cell membrane) and thus create pores on the seed coat which helps in easy water absorption. Nanoparticle acts as a passage to channelize the water from the substrate into the seeds and fasten germination rates. Silicon is a critical element for a number of metabolic and physiological plant activities. It plays an important role as a physicomechanical barrier, and is deposited on the walls of epidermis and vascular tissues of the stem, leaf sheath and hull in most plants especially monocots (Parven and Ashraf, 2010). It also regulates physiological activities in plants. Presence of silicon has been shown to stimulate activity of active compounds such as chitinase, peroxidase, polyphenol oxidases, and flavonoid phytoalexinsall of which can protect against fungal pathogens (Heckman, 2013). SiO_2 NP as protective agent or abrasive was used to coat various seeds of pulses against infestation by stored product pests which controlled the pest by desiccation or causing abrasion on the elytra (Arumugam *et al.,* 2016). The increase in germination percentage due to SiO_2 NP noticed in the present study is in conformity with the report of Siddiqui and Al-Whaibi (2014) in tomato.

Higher mean seedling length, seedling vigour index (product of mean seedling dry weight and mean

TABLE 3

Influence of dry seed treatment with ZnO, Silver and Silicon dioxide (both NP and bulk form) on germination (%) in Pigeonpea

	B_1	B_2	B_3	B_4	B_5	B_6	Mean A
A_1	89	94	94	97	92	94	93
A_2	89	94	95	92	96	92	93
A_3	91	93	96	91	92	93	93
A_4	83	92	91	95	90	90	90
A_5	90	95	98	93	90	92	93
A_6	85	94	91	97	91	90	91
Mean B	87	94	94	94	92	92	

Interactions	A	B	A x B
SEm±	0.59	0.63	1.66
CD (0.01P)	2.19	2.35	6.21
CV (%)		3.10	

TABLE 4

Influence of dry seed treatment with ZnO, Silver and Silicon dioxide (both NP and bulk form) on field emergence (%) in pigeonpea

	B_1	B_2	B_3	B_4	B_5	B_6	Mean A
A_1	74	82	79	94	78	79	81
A_2	72	86	86	89	93	78	84
A_3	71	85	92	74	78	86	81
A_4	63	81	76	91	86	84	80
A_5	79	87	95	80	89	82	85
A_6	69	88	87	93	76	71	80
Mean B	71	84	85	86	83	80	

Interactions	A	B	A x B
SEm±	1.17	1.25	3.30
CD (0.01P)	4.37	4.66	12.35
CV (%)		7.02	

A_1: Zinc oxide NP A_4: Silver bulk B_1: Control B_4: 500 ppm
A_2: Zinc oxide bulk A_5: Silicon dioxide NP B_2: 100 ppm B_5: 750 ppm
A_3: Silver NP A_6: Silicon dioxide bulk B_3: 250 ppm B_6: 1000 ppm

TABLE 5

Influence of dry seed treatment with ZnO, Silver and Silicon dioxide (both NP and bulk form) on mean seedling length (cm) in pigeonpea

	B_1	B_2	B_3	B_4	B_5	B_6	Mean A
A_1	37.41	39.33	35.92	42.57	41.35	39.37	39.33
A_2	36.45	39.39	41.22	38.40	42.21	40.34	39.67
A_3	39.19	40.49	41.96	41.12	40.98	38.36	40.35
A_4	37.58	41.74	40.57	41.92	41.38	39.69	40.48
A_5	36.66	39.08	43.68	39.87	40.75	39.29	39.89
A_6	36.54	38.92	38.44	42.37	40.53	38.96	39.29
Mean B	37.31	39.83	40.30	41.04	41.20	39.34	

Interactions	A	B	A x B
SEm±	0.57	0.61	1.63
CD (0.01P)	NS	NS	NS
CV (%)		7.19	

TABLE 6

Influence of dry seed treatment with ZnO, Silver and Silicon dioxide (both NP and bulk form) on seedling vigour index in pigeonpea

	B_1	B_2	B_3	B_4	B_5	B_6	Mean A
A_1	3250	3259	3589	4499	3586	3447	3605
A_2	3227	3601	3939	3243	4221	3692	3653
A_3	3401	3642	4206	3493	3767	3544	3675
A_4	3307	3809	3347	4139	3602	3334	3589
A_5	3369	3433	4526	4062	3840	4045	3879
A_6	3287	4139	3572	4264	3875	3508	3774
Mean B	3306	3647	3863	3950	3815	3595	

Interactions	A	B	A x B
SEm±	70.40	75.26	199.13
CD (0.01P)	263.4	281.6	745.1
CV (%)	10.8		

A_1: Zinc oxide NP
A_2: Zinc oxide bulk
A_3: Silver NP
A_4: Silver bulk
A_5: Silicon dioxide NP
A_6: Silicon dioxide bulk
B_1: Control
B_2: 100 ppm
B_3: 250 ppm
B_4: 500 ppm
B_5: 750 ppm
B_6: 1000 ppm

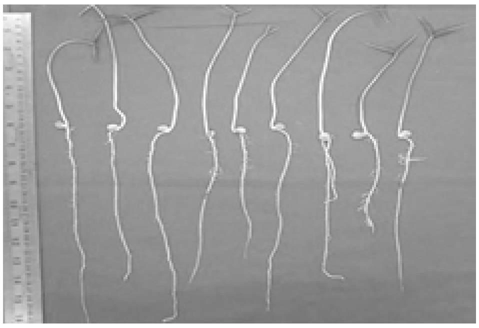
TABLE 7

Influence of dry seed treatment with ZnO, Silver and Silicon dioxide (both NP and bulk form) on electrical conductivity (µS/cm/g) in pigeonpea

	B_1	B_2	B_3	B_4	B_5	B_6	Mean A
A_1	14.88	13.02	12.70	11.96	13.08	13.50	13.19
A_2	16.02	13.19	14.12	15.27	12.29	14.48	14.22
A_3	16.83	14.42	12.41	13.75	14.15	14.37	14.32
A_4	17.09	15.17	13.79	12.65	15.49	15.51	14.95
A_5	17.20	13.27	11.93	13.05	14.32	14.45	14.03
A_6	16.93	13.62	13.38	12.04	12.65	13.71	13.72
Mean B	16.49	13.78	13.06	13.12	13.66	14.34	

Interactions	A	B	A x B
SEm±	0.18	0.19	0.51
CD (0.01P)	0.67	0.72	1.90
CV (%)		6.26	

A_1: Zinc oxide NP
A_2: Zinc oxide bulk
A_3: Silver NP
A_4: Silver bulk
A_5: Silicon dioxide NP
A_6: Silicon dioxide bulk
B_1: Control
B_2: 100 ppm
B_3: 250 ppm
B_4: 500 ppm
B_5: 750 ppm
B_6: 1000 ppm



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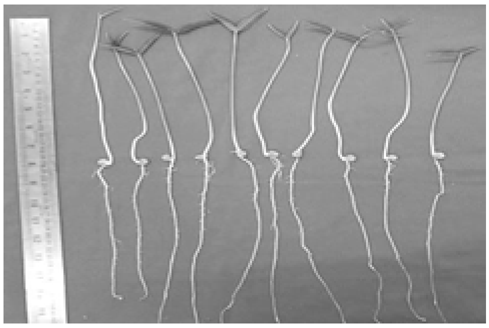
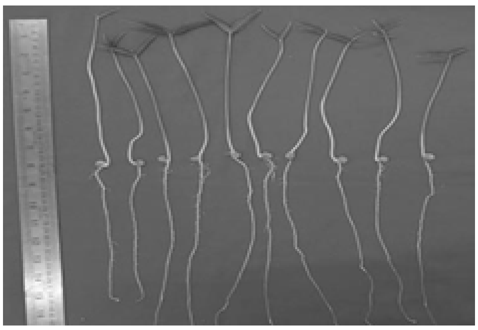
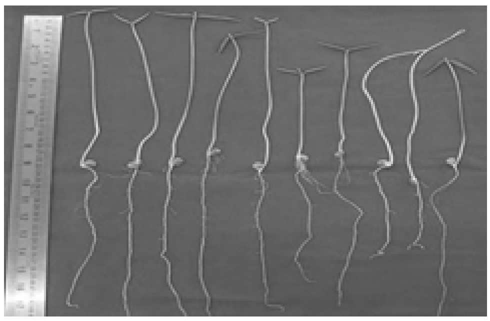


Silicon dioxide NPs @ 250 ppm



ZnO NP @ 500 ppm



Ag NP @ 250 ppm

Fig. 3 : Effect of dry seed treatment of nanoparticles on mean seedling length (cm) in pegionpea.

germination) and lower electrical conductivity (EC) was observed in SiO_2 NP @ 250 mg (43.68cm, 4526 and 11.93µS/cm/g, respectively) followed by treatment combinations of ZnO NP @ 500 mg (42.57cm, 4499 and 11.96µS/cm/g, respectively), SiO_2 bulk @ 500 mg (42.37cm, 4264 and 12.04µS/cm/g, respectively), ZnO bulk @ 750 mg (42.21 cm, 4221 and 12.29µS/cm/g, respectively), Ag NP @ 250 mg (41.96 cm, 4206 and 12.41 µS/cm/g, respectively) and Ag bulk @ 500 mg (41.92cm, 4139 and 12.65µS/cm/g, respectively) compared to control (37.31cm, 3306 and 16.49µS/cm/g, respectively) which is depicted in Table 5, 6, 7 and Fig. 3). Nanoparticle pay way for easy water absorption leading to considerable metabolic activity that is characterized by enzyme synthesis and activation which coincide with seedling growth and emergence. Zinc is one of the essential nutrients required for plant growth and is a component of numerous enzymes responsible for various metabolic activities. Nanoparticles of metal oxide are quickly transported through the plant and included in the metabolic processes. Zinc oxide NPs are reported to have enhanced the plant growth hormones and also exhibit positive effect on the reactivity of phytohormones especially Indole Acetic Acid (IAA) facilitating in the phytostimulatory actions (Jayarambabu *et al.*, 2014). Similar findings were observed by Korishettar *et al.* (2016) in pigeonpea.

Results conclude that wet seed treatment of nanoparticles caused soaking injury to the seed coat and thus reducing the overall seed quality in pigeonpea. Whereas, dry seed treatment with certain optimum concentration of nanoparticles showed improved seed quality parameters. Among the treatments, maximum seed germination, field emergence, mean seedling length, seedling vigour index and lower electrical conductivity were reported by SiO_2 NP @ 250 mg (98%, 95%, 43.68cm, 4526 and 11.93 µS/cm/g). Further investigations are required to understand the positive and negative impacts on the crop metabolism and soil health. Studies on the safe use and disposal, its impact on the environment and human health shall also be a concern although the technology found useful in enhancing quality of seeds.

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