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Influence of seed treatment with nanoparticles on seed quality and storability of pigeonpea cv. BRG-2

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Abstract
A laboratory experiment was conducted to study the influence of seed treatment with ZnO, Ag and SiO2 nanoparticles and their bulk form on seed quality and storage potential of pigeonpea seeds. Results revealed significant effect of nanoparticles on seed quality and storability in pigeonpea. Among the treatment combinations, seeds treated with SiO2 NPs @ 250 mg found to be superior for all the tested seed quality parameters *viz.*, germination (98, 89 and 80%), mean seedling dry weight (46.45, 44.95 and 43.98mg), seedling vigour index-II (4553, 4016 and 3518), field emergence (94, 78 and 63%), total dehydrogenase activity (1.549, 1.322 and 1.196), lower electrical conductivity (11.90, 15.46 and 41.29 µS/cm/g) and reduced seed moisture content (9.16, 9.63 and 10.09%) at 0, 6 and 10 months of storage respectively. However, they were statistically on par with ZnO NPs @ 500 mg. The findings suggest the possibility of application of nanotechnology in enhancing seed quality and storability of pigeonpea.

Keywords: Nanoparticles, pigeonpea, seed quality and storability

Introduction

Pulses are an important group of crops that provide major source of dietary proteins complementing cereal proteins in human diets with essential amino acids, vitamins and minerals. Pulses are considered as 'Smart Food' as they are essential for food basket, containing 20-25 per cent of protein which is double the protein content of wheat and thrice that of rice thus providing nutritional and health benefit by addressing obesity, diabetes and malnutrition. Pigeonpea is one of the most important leguminous crops and is a rich source of protein and also rich in iron, iodine, essential amino acids like lycine, tyrocene, cystine and arginine (Tiwari and Shivhare 2018) [24]. Seed is a key input which decides the crop stand and yield under varied conditions. Poor of seed viability leads to storage loss in pulses which accounts to 30 to 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. The storage potential of any seed depends on several factors like type of seed, period of storage, chemical composition, seed treatment, seed moisture, temperature and storage containers (Delouche and Baskin, 1973) [5]. The seed deterioration is inevitable, irreversible and inexorable but it can be delayed to some extent by proper management practices like preservation under controlled conditions and by advocating some seed invigouration trestments (Basu, 1994) [2]. Several strategies such as hydration and dehydration, halogenations, antioxidant treatments and seed disinfection technologies available to prolong the vigour and viability of seeds but does not allow storing of seeds for longer period and require immediate sowing and also may cause damage to seed coat. Thus, an improved seed invigouration treatments in both fresh and old seeds to improve seed viability and vigour during storage is essential. Nanotechnology is an emerging and fascinating field of science which permits novel applications in the field of agriculture and other disciplines. Preliminary studies showed the potential of nanoparticles (NPs) in improving seed germination and growth, plant protection, pathogen detection, and pesticide/herbicide residue detection (Khot *et al.*, 2012) [8]. In seed science research, nanotechnology tools offers application of various nanoparticles for improvement of seed germination and related physiological parameters, nano membranes and nano polymer coating to enhance the storability of the seeds by incorporation of pesticides, nanosensors for better management of seed infestation during storage and incorporation of novel genes into seeds for specific trait (Chinnamuthu and Boopathi, 2009) [4]. Efficacy of nanoparticles is determined by their chemical composition, size, surface covering, reactivity and dose at which they are effective. The NPs are also known to donate electrons that pairs with the free radicals and thereby smother the impact of free radicals (Zheng *et al*., 2005) [25].

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 chemicals as an eco-friendly approach. Zinc (Zn) is essential micronutrients, required for the normal plant growth and development, important components of various enzymes responsible for many metabolic reactions. Increasing evidence suggests that zinc oxide nanoparticles increase plant growth and development in soybean (Sedghi *et al*., 2013) [15]. Silver (Ag) nanoparticles are among the most potential candidates for modulating the redox status of plants because of their ability to support electron exchange with $Fe2+$ and $Co3+$, the two elements that participate in several biological redox reactions (Sharma *et al*., 2012) [17]. Silicon is one of the most widespread macro elements, numerous field studies have shown that supplying crops with adequate plant available silicon can suppress plant disease, reduce insect attack, improve environmental stress tolerance and increase crop productivity. Nano silicon dioxide (SiO2) results in increased seed germination by providing better nutrients availability with adequate pH and conductivity to the growing medium in maize seeds (Suriyaprabha *et al*., 2012) [21]. However, from our earlier study on standardization of seed treatment protocol with nanoparticles for enhancing seed quality in pigeonpea (Surabhi *et al*., 2018) [20] indicated dry seed treatment with ZnO, Ag and SiO2 NPs found to have positive effect on seed quality in pigeonpea. Therefore, an effort was made to adopt an advanced third generation seed treatment with nanoparticles to enhance seed quality parameters and to prolong seed viability/shelf life in pigeonpea during storage under ambient conditions.

Material and Methods

The storage study was carried out at the Seed Technology Research Laboratory, All India Coordinated Research Project on Seed Technology Research Unit, National Seed Project (Crops), University of Agricultural Sciences, Gandhi Krishi Vignan Kendra, Bangalore during 2017-2018. Seeds of pigeonpea cv. BRG-2 and cloth bags were obtained from Seed Production Unit of National Seed Project (Crops), GKVK, Bangalore and dried to safe and uniform moisture level (<9%). Zinc oxide (ZnO), Silver (Ag) and Silicon dioxide (SiO2) both bulk and nano forms were obtained from the Sigma-Aldrich whose particle size was less than 100 nm. The treatment combination of both nanoparticles (NPs) and bulk (B) *viz*., ZnO NPs @ 500 and 750 mg; ZnO bulk @ 750 and 1000 mg; Ag NPs @ 250 and 500 mg; Ag bulk @ 500 and 750 mg; SiO2 NPs @ 250 and 500 mg; SiO2 bulk @ 500 and 750 mg were prepared for the experiment by thoroughly mixing the chemicals and seeds in a glass jar for even and uniform coating and the treated seeds were incubated for a short period to achieve equilibration. Thereafter these seeds were packed in cloth bag and stored under ambient conditions where the mean temperature was 23.42°C and mean relative humidity of 87.55% and 52.27% at 07.00hr and 14.00hr, respectively for a period of ten months (June, 2017 - April, 2018). Further, various seed quality tests were conducted on monthly basis.

The seed moisture content (%), germination (%), mean seedling dry weight (mg) and electrical conductivity (µS/cm/g) were recorded as per ISTA (2013); the total dehydrogenase activity (A480 nm) as per Kittock and Law (1968) [9]; field emergence (%) was also recorded by planting 100 seeds in three replications on a well prepared seed bed with adequate moisture and final count was taken on 12th day taking into account emergence of normal seedlings and expressed in percentage. The seedling vigour index-II was calculated as per Abdul-Baki and Anderson (1973) [1].

Seedling vigour index - II = Germination (%) x Mean seedling dry weight (mg)

The mean data of the storage experiment were statistically analyzed adopting completely randomized design with suitable ANOVA as outlined by Panse and Sukhatme (1985) [13]. The critical differences were calculated at five per cent level of probability wherever 'F' test was found significant for various seed quality parameters under the study.

Results and Discussion

Results of the present investigation showed that seed treatment with nanoparticles had significant influence on seed quality parameters. At the initial month of storage, no significant difference were observed in seed moisture content with SiO2 NPs @ 500 mg recording lowest seed moisture content (9.08%) followed by SiO2 B @ 750 mg (9.12%) and SiO2 NPs @ 250 mg (9.16%) while the highest was recorded in ZnO NPs @ 750 mg (9.28%). However, at the end of the storage period, SiO2 NPs @ 250 mg recorded significantly lowest seed moisture content (10.09%) followed by SiO2 NPs @ 500 mg (10.19%), while the highest was recorded in control (12.04%) which was significantly higher but not in safer limit (Table 1).

Table 1: Influence of seed treatment with nanoparticles on seed moisture content (%) in pigeonpea cv. BRG-2 during storage

Treatments	Storage period in months (June, 2017 to April, 2018)											
	0	1	2	3	4	5	6	7	8	9	10	Mean
T1	9.20	9.28	9.57	9.88	10.04	10.10	10.45	10.90	11.05	11.71	12.04	10.38
T2	9.17	9.18	9.27	9.45	9.53	9.56	9.74	9.88	10.06	10.18	10.35	9.67
T3	9.28	9.30	9.40	9.68	9.80	9.84	10.00	10.45	10.77	10.88	11.03	10.04
T4	9.22	9.24	9.33	9.42	9.52	9.57	9.86	9.98	10.11	10.25	10.52	9.73
T5	9.25	9.26	9.35	9.45	9.58	9.61	9.98	10.09	10.18	10.30	10.59	9.79
T6	9.18	9.20	9.30	9.40	9.50	9.55	9.71	9.88	10.04	10.16	10.43	9.67
T7	9.22	9.26	9.37	9.46	9.57	9.59	9.92	10.02	10.14	10.28	10.56	9.76
T8	9.16	9.18	9.30	9.41	9.53	9.56	9.88	9.98	10.13	10.27	10.54	9.72
T9	9.16	9.20	9.32	9.42	9.51	9.54	9.85	9.95	10.08	10.21	10.50	9.70
T10	9.16	9.18	9.20	9.32	9.39	9.42	9.63	9.77	9.89	9.99	10.09	9.55
T11	9.08	9.10	9.18	9.23	9.31	9.35	9.55	9.83	9.97	10.08	10.19	9.53

T12	9.17	9.18	9.20	9.28	9.32	9.36	9.58	9.80	9.95	10.05	10.20	9.55
T13	9.12	9.16	9.19	9.27	9.30	9.33	9.59	9.84	9.99	10.10	10.24	9.56
Mean	9.18	9.21	9.31	9.44	9.53	9.57	9.83	10.03	10.18	10.34	10.55	9.74
S.Em ±	0.040	0.007	0.010	0.003	0.006	0.004	0.005	0.005	0.005	0.005	0.003	
CD (0.05P)	NS	0.020	0.029	0.010	0.017	0.069	0.015	0.016	0.015	0.015	0.009	
CV (%)	0.750	0.131	0.188	0.061	0.108	0.011	0.094	0.094	0.088	0.086	0.053	

T1 – Control, T2 - ZnO NPs @ 500 mg, T3 - ZnO NPs @ 750 mg, T4 - ZnO B @ 750 mg, T5 - ZnO B @ 1000 mg, T6 - Ag NPs @ 250 mg, T7 - Ag NPs @ 500 mg, T8 - Ag B @ 500 mg, T9 - Ag B @ 750 mg, T10 - SiO2 NPs @ 250 mg, T11 - SiO2 NPs @ 500 mg, T12 - SiO2 B@ 500 mg, T13 - SiO2 B @ 750 mg

The moisture content of seed increased as the storage period advanced which may be due fluctuations in relative humidity and temperature in the surrounding environmental conditions since the seeds were stored in the cloth bag that might have result in and metabolic release of water during respiration. The fluctuation of moisture content was significantly higher in untreated seeds compared to seed treatment with nanoparticles. Probably the nanoparticles might have acted as physical barrier, which is assumed to restrict the movement of water vapour in and out of the treated seeds and hence, reduced the fluctuation of moisture content during the storage. Another major beneficial effect of silicon dioxide might be related to its hydrophilicity thus maintaining seed moisture content in safer limit throughout the storage period. Similar observations were also reported by Korishettar *et al*. (2017) [10] on Zn and Fe nanoparticles (NPs) in pigeonpea.

A significant influence on seed germination percentage by seed treatment with nanoparticles was observed during storage period of ten months (Table 2). In the initial month of storage, highest germination was recorded in SiO2 NPs @ 250 mg (98%) and it was statistically on par with Ag NPs @ 250 mg (97%), SiO2 B @ 500 mg (97%) and ZnO NPs @ 500 mg (96%), while the lowest germination was recorded in control (93%). At the end of ten months of storage, highest germination was recorded in SiO2 NPs @ 250 mg (80%) which was on par with ZnO NPs @ 500 mg (79%) followed by SiO2 NPs @ 500 mg (77%) and lowest germination was recorded in control (61%). The reason might be that nanoparticles would induce oxidation-reduction reactions via superoxide ion radicals during germination, resulting the quenching of free radicals in the aged seeds. In turn, oxygen produced in such process could also be used for respiration, which would further promote germination (Zheng *et al*., 2005) [25]. Exogenous application of silicon may improve seed germination and seedling growth by enhancing antioxidant defense and improvement in Fe nutrition (Shi *et al*., 2014) [18]. Similar findings were reported by Tejaswini *et al*. (2019) [23] in groundnut.

Table 2: Influence of seed treatment with nanoparticles on germination (%) in pigeonpea cv. BRG-2 during storage

Treatments	**Storage period in months (June, 2017 to April, 2018)**											
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**
T1	93	91	89	87	84	82	79	77	73	68	61	80
T2	96	95	93	92	91	90	87	85	83	81	79	88
T3	95	94	92	91	89	87	85	82	80	77	74	86
T4	96	94	91	89	87	85	83	80	79	74	71	84
T5	95	92	89	87	85	84	81	78	75	72	69	82
T6	97	94	91	90	88	86	83	81	78	76	73	85
T7	94	94	90	88	84	83	81	79	77	74	70	83
T8	95	94	91	89	86	84	82	80	76	72	68	83
T9	93	91	89	86	85	82	80	78	74	71	68	82
T10	98	96	94	93	92	91	89	86	83	82	80	89
T11	95	95	93	91	90	89	86	84	82	80	77	87
T12	97	94	92	90	89	87	84	83	81	78	75	86
T13	94	93	91	89	88	86	82	80	77	75	73	84
Mean	95	94	91	89	87	86	83	81	78	75	72	85
S.Em ±	0.64	0.65	0.51	0.42	0.37	0.32	0.33	0.38	0.40	0.38	0.32	
CD (0.05P)	1.86	1.88	1.47	1.23	1.07	0.93	0.97	1.11	1.17	1.11	0.93	
CV (%)	1.16	1.20	0.96	0.82	0.73	0.65	0.69	0.81	0.89	0.88	0.77	

T1 – Control, T2 - ZnO NPs @ 500 mg, T3 - ZnO NPs @ 750 mg, T4 - ZnO B @ 750 mg, T5 - ZnO B @ 1000 mg, T6 - Ag NPs @ 250 mg, T7 - Ag NPs @ 500 mg, T8 - Ag B @ 500 mg, T9 - Ag B @ 750 mg, T10 - SiO2 NPs @ 250 mg, T11 - SiO2 NPs @ 500 mg, T12 - SiO2 B @ 500 mg, T13 - SiO2 B @ 750 mg

The results on mean seedling dry weight (mg) were significantly influenced by seed treatment with nanoparticles during storage. Irrespective of the seed treatments, mean seedling dry weight decreased gradually with the storage period. At the end of the storage period, SiO2 NPs @ 250 mg and ZnO NPs @ 500 mg recorded higher seedling dry weight (43.98 and 43.93 mg, respectively) compared to control (36.25 mg) (Table 3). Pandey *et al*. (2010) [12] reported that ZnO NPs increased the level of IAA in the roots (sprouts) and thereby an increase in the growth rate in Cicer arietinum. Besides increased synthesis and activity of hydrolytic enzymes resulted in increased dry matter production. Similar observations were also made by Tahir *et al*. (2010) [22] where wheat biomass increased with the application of silicon.

Table 3: Influence of seed treatment with nanoparticles on mean seedling dry weight (mg/seedling) in pigeonpea cv. BRG-2 during storage

Treatments	Storage period in months (June, 2017 to April, 2018)											
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**
T1	38.52	38.07	37.88	37.43	37.36	37.05	36.93	36.76	36.60	36.44	36.25	37.21
T2	46.91	46.70	45.88	45.52	45.45	45.13	44.94	44.75	44.46	44.20	43.93	45.26
T3	43.67	43.42	43.10	42.58	42.48	42.17	42.98	42.75	42.54	42.35	42.20	42.75
T4	41.75	40.07	39.93	39.67	39.60	39.45	39.17	38.95	38.76	38.55	38.33	39.48
T5	42.00	41.65	41.32	41.11	41.04	40.78	40.52	40.32	40.16	39.93	39.76	40.78
T6	42.10	41.82	41.53	41.20	41.12	40.90	40.76	40.55	40.21	40.04	39.86	40.92
T7	43.23	42.92	42.76	42.46	42.38	42.15	41.90	41.78	41.54	41.32	41.16	42.15
T8	41.30	40.75	40.51	40.31	40.27	40.03	39.86	39.63	39.50	39.29	39.12	40.05
T9	41.05	40.25	40.09	39.86	39.82	39.58	39.36	39.20	38.93	38.76	38.53	39.58
T10	46.45	46.15	45.97	45.60	45.47	45.17	44.95	44.77	44.48	44.23	43.98	45.20
T11	44.00	43.85	43.66	43.34	43.28	43.01	42.83	42.78	42.56	42.39	42.26	43.09
T12	42.57	42.13	41.95	41.73	41.65	41.33	41.15	40.96	40.77	40.51	40.31	41.37
T13	42.60	42.41	42.25	41.09	40.98	40.66	40.46	40.29	40.06	39.85	39.68	40.94
Mean	42.78	42.32	42.06	41.68	41.61	41.34	41.22	41.04	40.81	40.61	40.41	41.44
S.Em ±	1.02	0.05	0.02	0.09	0.01	0.01	0.01	0.01	0.03	0.01	0.01	
CD (0.05P)	2.97	0.15	0.06	0.27	0.02	0.02	0.02	0.02	0.09	0.03	0.02	
CV (%)	4.14	0.21	0.09	0.38	0.02	0.02	0.02	0.02	0.13	0.02	0.02	

T1 – Control, T2 - ZnO NPs @ 500 mg, T3 - ZnO NPs @ 750 mg, T4 - ZnO B @ 750 mg, T5 - ZnO B @ 1000 mg, T6 - Ag NPs @ 250 mg, T7 - Ag NPs @ 500 mg, T8 - Ag B @ 500 mg, T9 - Ag B @ 750 mg, T10 - SiO2 NPs @ 250 mg, T11 - SiO2 NPs @ 500 mg, T12 - SiO2 B @ 500 mg, T13 - SiO2 B @ 750 mg

Irrespective of the treatments, seedling vigour index-II and field emergence percentage gradually declined with the storage period advancement. At the end of ten months of storage, highest seedling vigour index-II and field emergence (3518 and 63%, respectively) recorded in SiO2 NPs @ 250 mg followed by ZnO NPs @ 500 mg (3485 and 61%, respectively) compared to control (2224 and 45%, respectively) (Table 4 & 5). This could be ascribed to the beneficial effects of these NPs in repairing of damaged vital cell organelles, synthesis and activation of essential enzymes during germination and counteraction of lipid peroxidation and minimization of free radical reactions. Senthil Kumar *et al*. (2011) [16] reported 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. Similar results were reported by Krishna Shyla and Natarajan (2014) in groundnut; Korishettar *et al*. (2017) [10] on Zn and Fe nanoparticles (NPs) in pigeonpea.

Table 4: Influence of seed treatment with nanoparticles on seedling vigour index-II in pigeonpea cv. BRG-2 during storage

Treatments	Storage period in months (June, 2017 to April, 2018)											
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**
T1	3569	3464	3371	3269	3138	3026	2930	2831	2684	2466	2224	2997
T2	4519	4436	4252	4188	4121	4047	3925	3819	3690	3595	3485	4007
T3	4133	4082	3965	3861	3795	3683	3639	3519	3389	3247	3109	3675
T4	4004	3766	3633	3531	3445	3353	3238	3129	3049	2852	2709	3337
T5	3991	3832	3691	3591	3475	3439	3269	3159	3025	2862	2730	3369
T6	4084	3945	3779	3694	3605	3504	3397	3271	3150	3056	2923	3492
T7	4064	4035	3834	3750	3574	3485	3380	3315	3185	3058	2895	3507
T8	3937	3817	3687	3588	3450	3376	3282	3171	3002	2816	2673	3345
T9	3818	3663	3581	3441	3371	3259	3136	3071	2881	2765	2607	3236
T10	4553	4446	4321	4226	4183	4096	4016	3865	3707	3642	3518	4052
T11	4165	4165	4075	3944	3881	3842	3669	3608	3504	3406	3240	3773
T12	4131	3960	3859	3769	3721	3609	3443	3386	3289	3174	3010	3577
T13	4018	3958	3831	3671	3593	3511	3331	3236	3098	2975	2884	3464
Mean	4076	3967	3837	3732	3642	3556	3435	3337	3204	3070	2924	3525
S.Em ±	96.79	29.39	21.51	21.13	15.13	13.16	13.81	15.43	16.03	15.49	12.74	
CD (0.05P)	281.37	85.45	62.51	61.43	43.99	38.27	40.15	44.85	46.60	45.03	37.04	
CV (%)	4.11	1.28	0.97	0.98	0.72	0.64	0.70	0.80	0.87	0.87	0.75	

T1 – Control, T2 - ZnO NPs @ 500 mg, T3 - ZnO NPs @ 750 mg, T4 - ZnO B @ 750 mg, T5 - ZnO B @ 1000 mg, T6 - Ag NPs @ 250 mg, T7 - Ag NPs @ 500 mg, T8 - Ag B @ 500 mg, T9 - Ag B @ 750 mg, T10 - SiO2 NPs @ 250 mg, T11 - SiO2 NPs @ 500 mg, T12 - SiO2 B @ 500 mg, T13 - SiO2 B @ 750 mg

Table 5: Influence of seed treatment with nanoparticles on field emergence (%) in pigeonpea cv. BRG-2 during storage

Treatments	Storage period in months (June, 2017 to April, 2018)											
	0	1	2	3	4	5	6	7	8	9	10	Mean
T1	90	89	85	82	80	77	69	63	57	53	45	72
T2	94	92	90	88	85	80	78	74	69	65	61	80
T3	88	86	84	84	82	78	76	74	68	64	58	77
T4	90	87	86	83	81	76	76	73	66	63	57	76
T5	87	86	83	81	79	75	72	66	62	57	52	73
T6	92	90	88	85	83	78	75	69	65	60	54	76
T7	86	85	84	81	78	74	72	67	64	58	53	73
T8	91	89	87	83	81	77	74	69	65	59	55	75
T9	86	84	81	80	78	74	73	68	62	56	52	72
T10	94	93	91	89	87	82	78	75	70	67	63	81
T11	89	89	86	84	82	77	76	74	69	64	60	77
T12	92	90	88	85	83	76	74	70	67	63	56	77
T13	88	87	85	82	80	75	72	68	61	56	52	73
Mean	90	88	86	83	81	77	74	70	65	60	55	76
S.Em ±	0.89	0.55	0.56	0.42	0.33	0.38	0.36	0.38	0.40	0.32	0.33	
CD (0.05P)	2.58	1.59	1.62	1.23	0.97	1.11	1.04	1.11	1.17	0.93	0.97	
CV (%)	1.71	1.07	1.13	0.88	0.71	0.86	0.84	0.94	1.07	0.92	1.05	

T1 – Control, T2 - ZnO NPs @ 500 mg, T3 - ZnO NPs @ 750 mg, T4 - ZnO B @ 750 mg, T5 - ZnO B @ 1000 mg, T6 - Ag NPs @ 250 mg, T7 - Ag NPs @ 500 mg, T8 - Ag B @ 500 mg, T9 - Ag B @ 750 mg, T10 - SiO2 NPs @ 250 mg, T11 - SiO2 NPs @ 500 mg, T12 - SiO2 B @ 500 mg, T13 - SiO2 B @ 750 mg

A linear increase in the electrical conductivity of seed leachate (µS/cm/g) with increasing storage period was observed (Table 6). At the end of storage period, control recorded highest electrical conductivity (55.42 µS/cm/g) while, SiO2 NPs @ 250 mg recorded lowest electrical conductivity (41.29 µS/cm/g) followed by ZnO NPs @ 500 mg (41.78 µS/cm/g). The results depicts that the thin layer of nanoparticles over seeds surface maintains the seed coat integrity thereby reduces the leakage of solutes from the seeds. Sahebi *et al*. (2015) [14] reported that silicon increases the plasma membrane integrity by providing more stable lipids involved in their cell membrane. However, the EC of untreated seeds of pegionpea was enormous at the end of seed storage which is an indicative factor of seed deterioration.

Table 6: Influence of seed treatment with nanoparticles on electrical conductivity of seed leachate (µS/cm/g) in pigeonpea cv. BRG-2 during storage

Treatments	Storage period in months (June, 2017 to April, 2018)											
	0	1	2	3	4	5	6	7	8	9	10	Mean
T1	13.52	14.12	14.82	15.05	15.60	16.18	22.44	29.87	38.41	49.21	55.42	25.88
T2	12.12	12.84	13.03	13.44	13.68	13.77	16.66	20.55	26.89	35.43	41.78	20.02
T3	13.63	13.91	14.10	14.47	14.92	14.98	18.74	22.43	30.68	40.33	47.34	22.32
T4	13.49	14.01	14.23	14.57	15.03	15.16	19.55	25.62	32.98	41.57	47.98	23.11
T5	14.35	14.82	15.06	15.37	15.58	15.78	19.87	25.77	33.27	42.18	48.24	23.66
T6	13.74	14.33	14.68	14.98	15.26	15.56	18.61	24.59	33.97	42.78	48.66	23.38
T7	13.15	13.74	13.98	14.25	14.56	14.67	17.41	24.36	34.13	43.05	49.43	22.98
T8	12.43	13.08	13.45	13.79	14.05	14.18	17.29	24.23	34.10	43.44	49.77	22.71
T9	13.51	13.73	13.87	14.07	14.29	14.39	17.45	23.98	32.93	41.98	48.13	22.58
T10	11.90	11.96	12.08	12.44	12.66	12.77	15.46	20.26	26.45	35.24	41.29	19.32
T11	12.32	12.46	12.78	12.97	13.11	13.25	16.98	20.77	27.02	36.15	46.98	20.44
T12	12.17	12.58	12.88	13.05	13.42	13.55	17.24	23.56	32.45	41.28	47.45	21.78
T13	12.67	12.76	12.91	13.11	13.53	13.64	17.39	23.77	32.77	41.96	48.09	22.05
Mean	13.00	13.41	13.68	13.97	14.28	14.45	18.08	23.83	32.00	41.12	47.74	22.32
S.Em ±	0.16	0.04	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	
CD (0.05P)	0.46	0.13	0.05	0.02	0.02	0.02	0.05	0.02	0.02	0.02	0.02	
CV (%)	2.09	0.56	0.20	0.07	0.09	0.06	0.15	0.04	0.03	0.02	0.01	

T1 – Control, T2 - ZnO NPs @ 500 mg, T3 - ZnO NPs @ 750 mg, T4 - ZnO B @ 750 mg, T5 - ZnO B @ 1000 mg, T6 - Ag NPs @ 250 mg, T7 - Ag NPs @ 500 mg, T8 - Ag B @ 500 mg, T9 - Ag B @ 750 mg, T10 - SiO2 NPs @ 250 mg, T11 - SiO2 NPs @ 500 mg, T12 - SiO2 B @ 500 mg, T13 - SiO2 B @ 750 mg

Seed treatment with nanoparticles on physiological parameter like total dehydrogenase activity (A480 nm) showed elevated effect. A significant variation in dehydrogenase activity (TDH) was observed in seeds treated with ZnO, Ag and SiO2 nanoparticles during the initial months of storage and gradual decline in the activity was also observed with advancement of storage period irrespective of the treatments (Table 7). Results showed that SiO2 NPs @ 250 mg recorded significantly higher dehydrogenase activity (1.549) followed by SiO2 NPs @ 500 mg (1.434) and ZnO NPs @ 500 mg (1.432) while the lowest was recorded in ZnO B @ 1000 mg (1.138). At the end of the storage period, SiO2 NPs @ 250 mg recorded significantly higher dehydrogenase activity (1.196) followed by SiO2 NPs @ 500 mg (1.191) and ZnO NPs @ 500 mg (1.164) while the lowest was recorded in bulk form of ZnO @ 1000 mg (0.924) followed by control (0.925).

Table 7: Influence of seed treatment with nanoparticles on total dehydrogenase activity (A480nm) in pigeonpea cv. BRG-2 during storage

Treatments	Storage period in months (June, 2017 to April, 2018)											
	0	1	2	3	4	5	6	7	8	9	10	Mean
T1	1.288	1.259	1.228	1.194	1.172	1.145	1.120	1.077	1.025	0.984	0.925	1.129
T2	1.432	1.417	1.395	1.352	1.322	1.316	1.298	1.254	1.228	1.197	1.164	1.307
T3	1.407	1.397	1.367	1.346	1.320	1.306	1.254	1.228	1.198	1.155	1.138	1.283
T4	1.176	1.152	1.140	1.113	1.094	1.076	1.054	1.039	1.025	0.994	0.967	1.075
T5	1.138	1.108	1.097	1.057	1.036	1.022	1.011	0.996	0.973	0.943	0.924	1.028
T6	1.368	1.338	1.323	1.298	1.256	1.233	1.214	1.196	1.170	1.157	1.134	1.244
T7	1.237	1.203	1.197	1.165	1.122	1.110	1.096	1.063	1.032	1.011	0.990	1.111
T8	1.153	1.127	1.103	1.076	1.046	1.029	1.006	0.984	0.971	0.954	0.936	1.035
T9	1.212	1.193	1.176	1.134	1.108	1.094	1.056	1.026	0.997	0.974	0.954	1.084
T10	1.549	1.434	1.415	1.398	1.356	1.345	1.322	1.298	1.256	1.228	1.196	1.345
T11	1.434	1.407	1.397	1.366	1.340	1.321	1.295	1.261	1.243	1.219	1.191	1.316
T12	1.171	1.147	1.132	1.104	1.085	1.066	1.038	1.011	0.987	0.964	0.944	1.059
T13	1.156	1.135	1.120	1.098	1.055	1.043	1.018	0.988	0.963	0.941	0.926	1.040
Mean	1.286	1.255	1.238	1.208	1.178	1.162	1.137	1.109	1.082	1.055	1.030	1.158
S.Em ±	0.023	0.006	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	
CD (0.05P)	0.066	0.016	0.005	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	
CV (%)	3.058	0.772	0.229	0.091	0.104	0.077	0.070	0.080	0.080	0.082	0.084	

T1 – Control, T2 - ZnO NPs @ 500 mg, T3 - ZnO NPs @ 750 mg, T4 - ZnO B @ 750 mg, T5 - ZnO B @ 1000 mg, T6 - Ag NPs @ 250 mg, T7 - Ag NPs @ 500 mg, T8 - Ag B @ 500 mg, T9 - Ag B @ 750 mg, T10 - SiO2 NPs @ 250 mg, T11 - SiO2 NPs @ 500 mg, T12 - SiO2 B @ 500 mg, T13 - SiO2 B @ 750 mg

Reduction in glucose utilization occurs in the deteriorating seeds which are reflected through lower dehydrogenase activity. From this point of view, NPs treated seeds were found to have higher TDH activity and thus might have protected seeds in improving their vigour and viability. The increased availability of micronutrients at nanoscale with increased chemical reactivity resulted in the increase in synthesis and activity of the enzymes coupled with repair of damaged vital cell organelles (Burgass and Powell, 1984) [3] and reduced counteraction of lipid peroxidation and minimization of free radical reactions (Khanahmadi *et al*., 2010) [7]. These beneficial effects of nano size powders were also observed by Zheng *et al*. (2005) [25] in aged seeds of spinach on improving the seed vigour. Thus, the present investigation leads to the assumption that the deterioration in the enzymatic system of seed with ageing might have been protected by the NPs by quenching free radicles (Sridhar, 2012) [19].

Conclusion

The results of our study clearly demonstrate that seed treatment with nanoparticles is a promising technology which improves the seeds quality and storability in pigeonpea. The nanoparticles are capable of entering into seeds utilizing the cracks and crevices available on the seed coat during the imbibition. This improves the enzymatic activity and free radical scavenging system by quenching the free radicals thereby lowering the oxidative damages, eventually promoting viability and vigour of seeds. Among the treatment combination, SiO_2 NPs @ 250 mg was found to be superior in maintaining or enhancing the storability of pigeonpea seeds.

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