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ZnO Nanoparticles Seed Invigouration for the Maintenance of Seed Vigour and Viability in Black Gram

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ABSTRACT

Background: Nanoparticles are randomly entering into the seed may quench the reactive oxygen species and lessen the oxidative damages thereby eventually promoted viability and vigour of aged seeds. Zinc oxide (ZnO) nanoparticles to quench the free radicals that appear during seed deterioration and to increase the seed viability are being kept as the primary aim of this study.

Methods: Zinc oxide (ZnO) was synthesized and characterized with TEM, SEM, Particle Size Analyzer and Raman Spectroscope. Physiological and biochemical seed quality parameters were also observed in ZnO nano-particles treated black gram seeds.

Result: Black gram seeds were invigorated with ZnO nano-particles at 200, 400, 600, 800, 1000, 1200 and 1400 mg kg^{-1} as dry seed treatment and 100, 200, 300, 400, 500, 600 and 700 mg kg^{-1} as wet seed treatment. Dry seed treatment with nanoparticles did not give significant variation in seed germination and seedling vigour in fresh seeds. However, after accelerated ageing, nanoparticles treated seeds had recorded significantly higher values as compared to control with respect to physiological seed quality characteristics. In wet seed treatment also, nanoparticles did not give significant variation for the physiological characteristics. But after accelerated ageing, seeds treated with nanoparticles recorded higher values for seed germination, seedling length and vigour index. The improvement recorded was 26, 28, 35 and 319 per cent higher than control with ZnO @ 200 mg kg^{-1}. The positive impact of nanoparticles seed treatment was also observed for the changes in biochemical characters *viz*., electrical conductivity, free amino acid, dehydrogenase activity and lipid peroxidation. The data suggests that seeds treated with ZnO nano-particles @1000 mg kg^{-1} under dry treatment and 200-300 mg kg^{-1} under wet showed a significant increase in seed viability, seedling length and vigour besides maintenance of biochemical constituents after ageing.

Key words: Black gram, Seed viability, Seed vigour and ZnO nanoparticles.

INTRODUCTION

Seed is a basic input dictating crop stand in both rainfed and irrigated conditions. Since major parts of pulses are grown under rainfed conditions, it is quite pertinent to invigorated the seeds in order to ensure crop stand vis-a-vis productivity. Pulses are poor stored and during seed storage, seeds undergo several biochemical processes of which certain process results in production of free radicals causing lipid peroxidation leads to deterioration (Chinnasamy *et al.,* 2022; Mukiri *et al.*, 2022) Several strategies such as hydration dehydration, halogenation and antioxidant treatments have been tested that could prevent deterioration and extend the shelf life of seeds have been developed. These techniques are cumbersome and are not being adopted by farmers due to practical difficulties.

Nanotechnology is a field of convergence among life sciences, material science and information technology which is capable of manipulating at the atom level and derives solutions to unresolved field problems (Roco *et al.*, 1999). Currently, the main thrust of research in nanotechnology focuses on applications in the field of electronics (Feiner, 2006), energy (Hu *et al.*, 2007), medicine and life sciences (Caruthers *et al.*, 2007). Nanotechnology in the agricultural front has been more useful in improving the existing crop management techniques. Nano-encapsulated agrochemicals could be designed in such a way that they possess all necessary properties such as effective concentration (with high solubility, stability and effectiveness), time-controlled release in response to certain stimuli, enhanced targeted

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activity and less eco-toxicity with safe and easy mode of delivery thus avoiding repeated application (Tsuji, 2001; Boehm *et al*., 2003; Wang *et al*., 2007).

During the one past decade, lot of works has been done in improving seed quality by using metal-based nanoparticles (*e.g*., AgNPs, AuNPs, CuNPs, FeNPs, FeS_2NPs, TiO_2NPs, ZnNPs, ZnONPs) and carbon-based nanoparticles (*e.g*., fullerene and carbon nanotubes) of various agricultural and horticultural crops (Raja *et al.,* 2019; Pragathi *et al.,* 2022; Raja *et al.,* 2023). Nanoparticles improved wheat seed germination, emergence and growth of seedlings (Zhang *et al*., 2006), thwarting pest attack (Nair *et al*., 2010) and for early pathogen detection (Alocilja and Radke, 2003). Engineered nanomaterials such as carbon nanotubes, quantum dots, Nano gold, Nano zinc, Nano aluminium, Titanium oxide (TiO_2) and Zinc oxide (ZnO) have received particular attention for their positive impact in Seed Science and Technology. Among the nanoparticles, ZnO (Li and Haneda *et al*., 2003) and TiO_2 (Bhatkhande *et al*., 2001) are best examples for photocatalysis properties. In the presence of UV light, the valancy electron in the nanoparticles is excited to form electron hole pairs. Therefore, customizing Zinc oxide (ZnO) nanoparticles to quench those free radicals that appear during seed deterioration and to increase the seed viability are being kept as the primary aim of this study.

MATERIALS AND METHODS

Genetically pure black gram seeds (*Vigna mungo* (L) Hepper) cv. VBN 4 obtained from National Pulse Research Station, Vamban, were used for this study. The zinc oxide (ZnO) nanoparticles synthesized at the Department of Nano Science and Technology, TNAU, Coimbatore were used for seed treatment.

Synthesis and characterization of nanoparticles

Synthesis of ZnO nanoparticles

ZnO nanoparticles (NPs) were synthesized by dissolving 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. The Zn $(NO_3)_2$ solution was added drop-wise (slowly for 40 min.) to the NaOH heated solution at 55°C under high-speed stirring. The beaker sealed at this condition was kept as such for 2 h. The precipitated ZnO NPs were cleaned with Millipore water followed by ethanol and then, dried in vacuum drier at 60°C (Moghaddam *et al.,* 2009).

Characterizing the nanoparticles

The synthesized ZnO and ZVI nanoparticles were characterized by using the following instruments.

Scanning electron microscope (SEM)

For taking images of samples, about 0.5 to 1.0 mg of sample was dusted on the carbon conducting tape. Then the tap was mounted on sample stage and the images were taken in 24,000X magnification and 15 KV using FET SEM Model "QUANTA 250".

Transmission electron microscope (TEM)

Accurately 0.5 mg of sample was dispersed in 10 ml pure ethanol and sonicated for 5 minutes at 1500 rpm. About 0.1 ml of this sample was taken in the sample holder and images were taken using FEI TEM Model "TECHNI SPIRIT".

Particle size analyzer

The particle size analyzer was used to determine the particle size and size distribution pattern for synthesized ZnO nanoparticles. MALVERN, Zetasizer Ver.6.01 Particle size analyser was used. Accurately 0.5 mg of sample was dispersed in 10 ml pure water and sonicated for 5 minutes at 1500 rpm. Then measurements were taken using particle size analyser.

Raman spectroscopy

The Raman spectrum was measured by Raman spectrum Model-R-3000-QE. The powder dried sample were kept in a polythene bag were spread to an extent of $1cm^2$ and Raman probe was placed on the sample pockets without exposing the sample directly to the probe.

Energy dispersive X-ray spectroscopy (EDAX)

For taking images of samples, about 0.5 to 1.0 mg of sample was dusted on the carbon conducting tape. Then the tape was mounted on sample stage and the images were taken using FET SEM Model "QUANTA 250".

Standardization of dosage for ZnO and ZVI nanoparticles to control seed deterioration

Standardization of dosage for dry treatment of ZnO nanoparticles

Black gram seeds were dry dressed with ZnO nanoparticles @ 200, 400, 600, 800, 1000, 1200 and 1400mg kg^{-1} in screw capped glass bottles at room temperature. The glass bottles containing seed and nanoparticles were shaken gently for 3 min., 5 times at an interval of 3 h. Seeds shaken without nanoparticles served as control.

Standardization of dosage for wet treatment of ZnO and ZVI nanoparticles

For wet seed treatment, 100, 200, 300, 400, 500, 600 and 700mg kg^{-1} of ZnO nanoparticles were dispersed in distilled water by sonicating for 5 min. The black gram seeds were soaked in that solution for 3h. Soaked seeds were then removed and dried back to original moisture content. The seeds soaked in water served as control.

Accelerated ageing of seeds

The dry and wet nanoparticles treated black gram seeds were placed in 15×10 cm cotton net bags and subjected to a relative humidity of 95±1% and temperature of 40°±1°C for 10 days (Delouche and Baskin, 1973, with modification).

During this period, the seeds were shuffled daily. The relative humidity, temperature and duration in respect of ageing test was standardized through pilot studies using progressively decreasing RH from 100 to 90 %, temperature 40-35°C and duration from 15 to 10 days. The treated seeds along with the control were tested for vigour and viability parameters such as germination, root and shoot length (ISTA, 2010), Vigour index (Abdul Baki and Anderson, 1973) immediately and after accelerated ageing.

Effect of nanoparticles on physiological and biochemical characters during deterioration of black gram seeds

From the standardization study, the best performing treatments in each method of treatments were forwarded to next experiment to confirm the optimum dosage and suitable method of nano treatment. In dry dressing, ZnO NPs at 400, 600, 800 and 1000 mg kg^{-1} and in wet treatment, 100, 200, 300 and 400 mg kg^{-1} were taken. For dry treatment, black gram seeds were mixed with the nanoparticles as mentioned above in a screw capped bottle described earlier. For wet treatment, the nanoparticles were dispersed in distilled water and the seeds were soaked in the solution for 3 hrs then removed and dry back to original moisture content. The untreated and water-soaked seeds served as control for dry and wet treatment respectively. Part of the treated and control seeds were subjected to accelerated ageing as described earlier. The fresh and aged seeds were evaluated for physiological seed quality parameters as explained earlier. In addition, the following biochemical quality attributes namely electrical conductivity (Presley, 1958).Free amino acid (Ching and Ching, 1964), Dehydrogenase activity (Kittock and Law, 1968) and Protein content (Ali-Khan and Youngs, 1973) were analyzed.

Statistical analysis

The data obtained from different laboratory experiments were analyzed statistically adopting techniques described by Panse and Sukhatme (1985). The critical differences (CD) were calculated at 5 per cent probability level.

RESULTS AND DISCUSSION

Characterization of synthesized nanoparticles

The surface morphology of ZnO (Fig 1) obtained by SEM micrograph showed that they were in the shape of bunches of flowers. Each bunch was gathered of closely packed

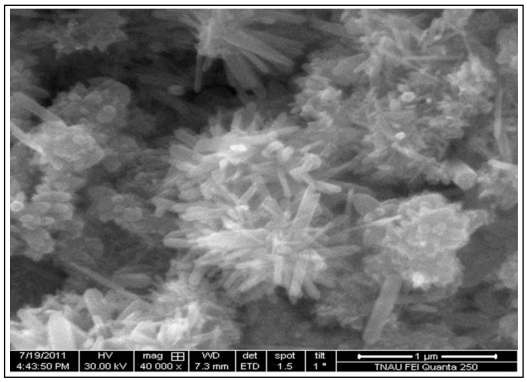

Fig 1: SEM image of ZnO nanoparicle.

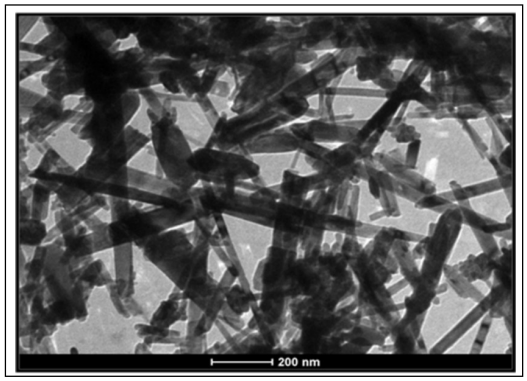

Fig 2: TEM image of ZnO nanoparticle.

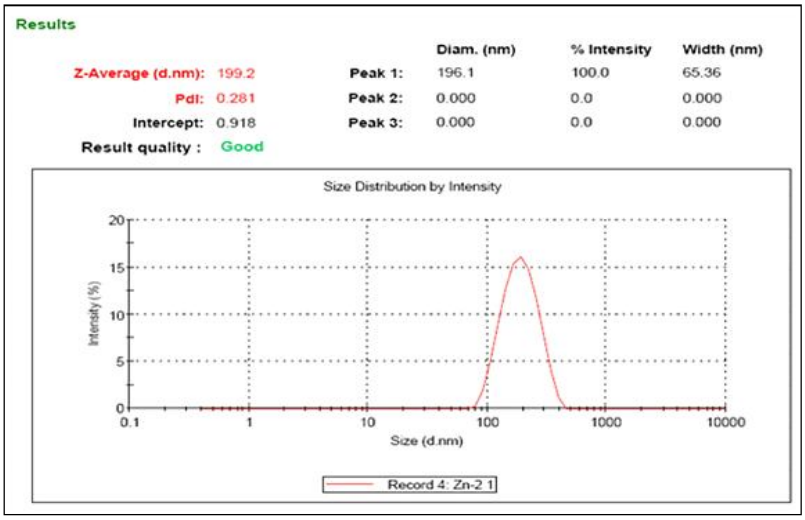

Fig 3: Particle size distribution of ZnO nanoparticles.

nanometer scale rods and forms radiating structures. The results are consistent with Moghaddam *et al*. (2009). TEM for ZnO nanoparicle exhibited rod shape structures (Fig 2). The morphology of ZnO from the individual crystalline nucleus was further confirmed by the TEM image.A similar morphology of ZnO was previously reported by Gopikrishnan *et al*. (2010). The particle size distribution of ZnO nanoparticles indicated that the average size of the ZnO nanoparticles measured a diameter of 199.2 nm (Fig 3). The ZnO nanoparticles exhibiting the minimum size showed high surface area to volume ratio and high surface reactivity. The results of Raman spectrum showed that the intensity of peaks for ZnO were 437.1, 1062, 1128.5, 1294 and 1438 cm^{-1} which conformed with reference peaks at 437.1 and 1062 $cm^{-1}$of ZnO nanoparticles (Fig 4). EDAX spectrum confirmed the elemental composition of ZnO NPs and resulted 82.7 per cent Zn and 17.3 per cent oxygen on weight basis in the K shell (Fig 5).

Standardization of best dosages of nanoparticles for dry and wet seed treatments

An invigouration treatment should bring about a qualitative improvement in the seed which should persist after the treatment is removed and the treatments are basically physiological in nature. Pre-sowing seed treatments in various crops have been standardized for improving vigour and viability of seed (Sengupta and Mandal 2005; Jerlin *et al.,* 2010; Rathinalvel and Raja, 2007). In order to address the issues existing seeds treatments, experiments were taken up to treat the seeds with ZnO NPs nanoparticles both in dry as well as wet conditions. The treatment of nanoparticles either in wet or dry condition influenced the

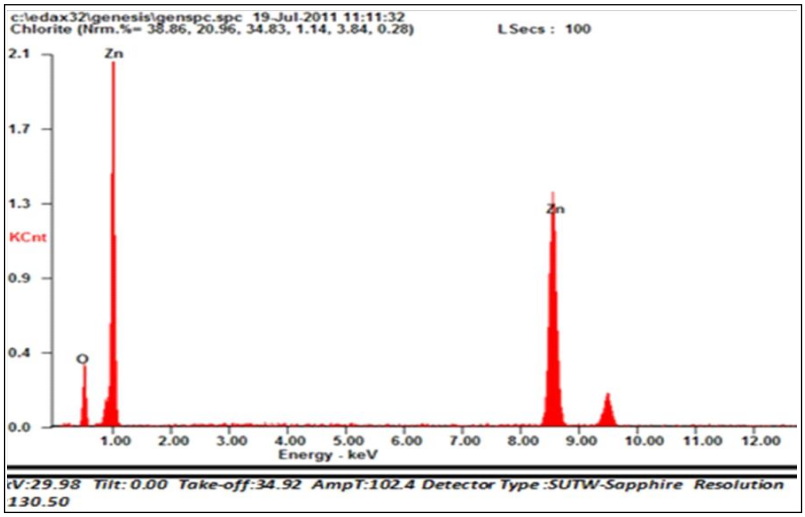

Fig 4: EDAX spectrum of ZnO nanoparticles.

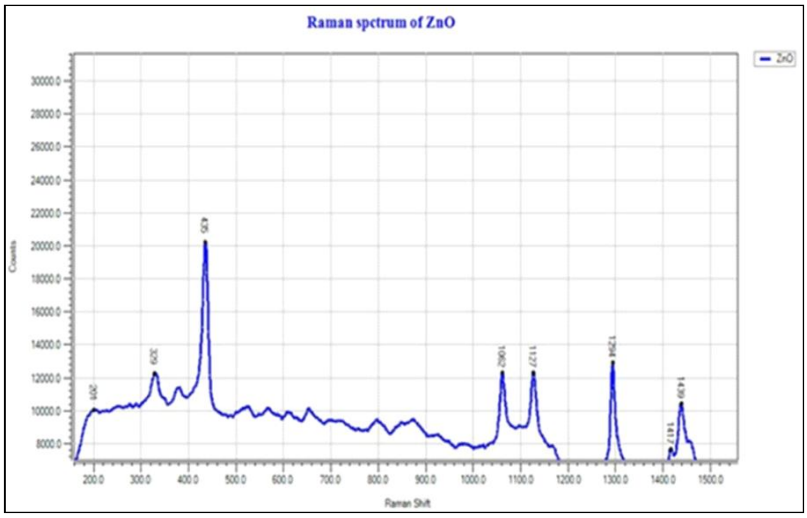

Fig 5: Raman spectrum of ZnO nanoparticles.

seed vigour and viability which leads to the proposition that wet condition nanoparticles seed treatment can be employed in high value low volume crops of commercial importance as dry treatment is generally practiced. In dry treatment, ZnO NPs were treated with seeds from 200 to 1400 mg with an increment of 200 mg kg^{-1}. Similarly for wet treatment, the above nanoparticles were treated with 100 to 700 mg kg^{-1} with an increment of 100 mg kg^{-1}. The treated seeds were artificially aged by accelerated ageing process to assess the effect of different dosage of nanoparticles.

Table 1a: Effect of dry treatment of ZnO nanoparticles on germination, seedling length and vigour index of black gram seeds immediately after treatment.

Dosage of ZnO (mg kg^{-1})	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index
Control	94 (76.36)	13.3	20.8	3205
200	94 (76.03)	13.2	19.4	3064
400	96 (79.06)	13.4	19.3	3139
600	95 (77.89)	13.9	20.3	3249
800	95 (76.74)	13.8	20.2	3230
1000	96 (78.47)	13.6	20.6	3286
1200	93 (74.97)	13.1	19.8	3060
1400	94 (75.57)	13.4	20.1	3149
Mean	95 (77.89)	13.5	20.1	3173
SEd	-	-	-	-
CD (P=0.05)	NS	NS	NS	NS

(Figures in parentheses indicate arcsine values).

Table 1b: Effect of dry treatment of ZnO nanoparticles on germination, seedling length and vigour index of black gram seeds after ageing.

Dosage of ZnO (mg kg^{-1})	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index
Control	30 (33.21)	9.8	11.9	651
200	42 (40.40)	11.3	13.2	1029
400	54 (47.30)	11.4	14.8	1415
600	68 (55.56)	12.8	13.9	1816
800	58 (49.61)	11.3	13.8	1456
1000	62 (51.75)	12.2	14.3	1643
1200	52 (46.15)	11.8	13.6	1321
1400	54 (47.30)	11.1	13.8	1345
Mean	54 (47.30)	11.5	13.7	1335
SEd	1.05	0.26	0.26	21.27
CD (P=0.05)	2.23**	0.55**	0.56*	45.09**

(Figures in parentheses indicate arcsine values).

Table 2a: Effect of wet treatment of ZnO nanoparticles on germination, seedling length and vigour index of black gram seeds immediately after treatment.

Dosage of ZnO (mg kg^{-1})	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index
Control	95 (77.12)	14.3	23.1	3553
100	96 (78.52)	14.8	23.4	3667
200	95 (76.74)	14.6	23.9	3658
300	93 (75.10)	14.9	23.8	3599
400	97 (81.07)	14.2	23.6	3667
500	94 (75.57)	13.9	22.9	3459
600	93 (74.69)	14.1	23.1	3460
700	94 (75.86)	14.2	23.3	3525
Mean	95 (76.74)	14.4	23.4	3574
SEd	-	-	-	-
CD (P=0.05)	NS	NS	NS	NS

(Figures in parentheses indicate arcsine values).

Immediately after treatment, no noticeable improvement in vigour or germination of seedling was detectable due to the nanoparticle treatments. But after ageing, significant differences were observed. From the observations made, treating the seeds with 400 to1000 mg kg^{-1} of nanoparticles was found to be optimum in dry conditions while 100 to 400 mg kg^{-1} in wet condition (Table 1a, 1b, 2a and 2b).

Effect of nanoparticles on physiological and biochemical characters of blackgram seeds

In the present investigation the germination, seedling length and vigour index values were higher in ZnO nanoparticles treated seeds of wet and dry treatment after accelerated ageing (Table 3, 4, 5, 6). Similar findings were reported by Lei *et al.* (2005) in aged seeds of spinach on improvement in seed vigour. The improvement in treated seed may be due to the quenching of free radicles by the ZnO nanoparticles (Table 7 and 8). The beneficial effect of the nanoparticle treatment observed in the present investigation could be due to repair of damage to vital cell organelles (Villiers, 1973; Villiers and Edgcumbe, 1975; Burgass and Powell, 1984) and counteraction of lipid peroxidation and minimization of free radical reactions

Table 2b: Effect of wet treatment of ZnO nanoparticles on germination, seedling length and vigour index of black gram seeds after ageing.

Dosage of ZnO (mg kg^{-1})	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index
Control	26 (30.66)	12.1	19.2	814
100	42 (40.40)	13.6	22.6	1520
200	40 (39.47)	13.2	21.9	1404
300	43 (41.17)	14.1	22.8	1587
400	48 (43.86)	13.4	23.1	1752
500	32 (37.45)	13.1	21.9	1120
600	40 (39.23)	12.4	20.8	1328
700	30 (33.21)	12.8	21.1	1017
Mean	38 (39.17)	13.1	21.7	1318
SEd	0.62	0.17	0.41	25.98
CD (P=0.05)	1.32**	0.37*	0.88*	55.07*

(Figures in parentheses indicate arcsine values)

Table 3: Influence of dry treatment of nanoparticles on germination (%) of black gram seeds immediately and after ageing.

Dosage (mg-kg^{-1})	Age of seed		
	Fresh	Aged	Mean
0	97 (79.60)	33 (31.26)	65 (57.43)
400	90 (71.92)	55 (47.68)	73 (59.80)
600	93 (75.22)	52 (47.10)	73 (61.16)
800	91 (72.90)	63 (52.93)	73 (62.92)
1000	97 (81.52)	75 (59.57)	86 (70.55)
Mean	92 (74.23)	56 (48.51)	74 (62.37)
	Ageing	Dosage	Ageing×Dosage
SEd	0.67	1.06	1.51
CD (P=0.05)	1.41**	2.23**	3.15**

(Figures in parentheses indicate arcsine values).

Table 4: Influence of dry treatment of nanoparticles on vigour index of black gram seeds immediately and after ageing.

Dosage (mg-kg^{-1})	Age of seed		
	Fresh	Aged	Mean
0	3550	677	2113
400	3276	1485	2380
600	3348	1435	2391
800	3176	1714	2445
1000	3463	2078	2770
Mean	3362	1477	2420
	Ageing	**Dosage**	**Ageing×Dosage**
SEd	27.646	43.712	61.818
CD (P=0.05)	57.669**	91.183**	128.952**

(Tappel, 1973; Pammenter *et al*., 1974; Berjak, 1978; McDonald, 1999; Hsu *et al*.2003; Span *et al*., 2009; Khanahmadi *et al*., 2010). One of the reasons attributed for the loss of viability during storage is due to the damage to the membrane (Roberts, 1972; Murthy *et al*., 2003; Khan *et al*., 2003; Maskri *et al*., 2003; Kaewnareea *et al*., 2011) which under normal condition could have repaired by itself (Cuming and Osborne, 1978). Seeds with the reduced activity of this repair system make the seed 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. Deteriorated seeds were found to leach out more solutes (Abdul Baki and Anderson, 1970; Mandal *et al*., 2000; Krishnaveni, 2003; Kaewnareea *et al*., 2011) with higher electrical conductivity compared to vigourous and healthy seeds (Ramamoorthy *et al*., 1990; Saha *et al*., 1990; Maskri *et al*., 2003). The electrical conductivity wasthe highest in control than the nanoparticles treated seed (De *et al*., 1998; Mandal *et al*., 1999). This is mainly attributed to the membrane damage by alteration in the structure of membrane resulting in more

Table 5: Influence of wet treatment of nanoparticles on germination (%) of black gram seeds immediately and after ageing.

Dosage (mg-kg^{-1})	Age of seed		Mean
	Fresh	Aged	
0	93 (75.15)	12 (20.23)	53 (47.69)
100	100 (87.99)	38 (37.86)	69 (62.93)
200	98 (83.92)	38 (38.05)	68 (60.99)
300	97 (80.65)	35 (36.26)	66 (58.46)
400	98 (83.73)	37 (37.46)	68 (60.60)
Mean	97 (82.29)	32 (33.97)	65 (58.13)
	Ageing	**Dosage**	**Ageing×Dosage**
SEd	0.72	1.15	1.63
CD (P=0.05)	1.52**	2.40**	3.40*

(Figures in parentheses indicate arcsine values).

Table 6: Influence of wet treatment of nanoparticles on vigour index of black gram seeds immediately and after ageing.

Dosage (mg-kg^{-1})	Age of seed		Mean
	Fresh	Aged	
0	3581	296	1938
100	3920	1155	2538
200	3974	1243	2609
300	3783	1057	2420
400	3965	1217	2591
Mean	3845	994	2419
	Ageing	**Dosage**	**Ageing×Dosage**
SEd	30.13	47.65	67.39
CD (P=0.05)	62.86**	99.40**	140.57*

Table 7: Influence of dry treatment of nanoparticles on lipid peroxidation (OD Values) black gram seeds immediately and after ageing.

Dosage (mg-kg^{-1})	Age of seed		Mean
	Fresh	Aged	
0	0.119	0.151	0.135
400	0.118	0.144	0.131
600	0.111	0.147	0.129
800	0.116	0.136	0.126
1000	0.114	0.141	0.128
Mean	0.116	0.144	0.130
	Ageing	**Dosage**	**Ageing×Dosage**
SEd	0.0011	0.0018	0.0026
CD (P=0.05)	0.0024**	0.0039**	0.00551**

leakage of seeds (Heydecker, 1972; Villiers, 1973; Murthy *et al*., 2003; Kaewnareea *et al*., 2011; Eevera *et al*., 2024). Seeds treated with nanoparticles were found to record low electrical conductivity in the seed leachate which implies on the probable role of nanoparticles in curing the damaged membranes.

Carbohydrate metabolism, an important process that occurs inside the seed and it acts as an indicator for as certaining the seed deterioration. Reduction in glucose utilization occurs in the deteriorated seeds which are reflected through the improved dehydrogenase activity. Thus, measured from the point of dehydrogenase activity, nanoparticles treated seeds were found to have lesser dehydrogenase activity (Table 9 and 10). This implies the positive role of nanoparticles in improving the seed vigour and viability. In general, higher the leakage of amino acids, higher will be the damage to the membranes during ageing (Kavitha *et al*., 2023; Dey and Basu, 1982; Jeng and Sung, 1994; Krishnaveni, 2003; Kaewnareea *et al*., 2011). Hence, treating the seeds with nanoparticles could reduce the free amino acid pool in the leachate which may delay the deterioration of the membrane. Thus, the present investigation clearly established the deterioration in membrane system of seed with ageing while nanoparticle

Table 8: Influence of wet treatment of nanoparticles on lipid peroxidation (OD value) of black gram seeds immediately and after ageing.

Dosage (mg-kg^{-1})	Age of seed		Mean
	Fresh	Aged	
0	0.114	0.144	0.129
100	0.111	0.138	0.123
200	0.110	0.130	0.116
300	0.108	0.138	0.129
400	0.111	0.135	0.123
Mean	0.110	0.137	0.124
	Ageing	**Dosage**	**Ageing×Dosage**
SEd	0.001	0.0016	0.0022
CD (P=0.05)	0.002 **	0.0033*	0.0047*

Table 9: Influence of wet treatment of nanoparticles on dehydrogenase activity (OD value) of black gram seeds immediately and after ageing.

Dosage (mg-kg^{-1})	Age of seed		Mean
	Fresh	Aged	
0	1.348	0.210	0.779
100	1.361	0.216	0.788
200	1.331	0.227	0.779
300	1.425	0.242	0.833
400	1.432	0.239	0.835
Mean	1.379	0.226	0.803
	Ageing	**Dosage**	**Ageing×Dosage**
SEd	0.0081	0.0128	0.0182
CD (P=0.05)	0.0170**	0.0269**	0.0380**

Table 10: Influence of dry treatment of nanoparticles on dehydrogenase activity (OD value) of black gram seeds immediately and after ageing.

Dosage (mg-kg^{-1})	Age of seed		Mean
	Fresh	Aged	
0	1.450	0.337	0.894
400	1.472	0.321	0.897
600	1.431	0.373	0.902
800	1.481	0.415	0.948
1000	1.493	0.401	0.947
Mean	1.465	0.369	0.917
	Ageing	**Dosage**	**Ageing×Dosage**
SEd	0.0026	0.0041	0.0058
CD (P=0.05)	0.0054**	0.0086**	0.0122**

treatment could circumvent such damages by quenching reactive oxygen species.

CONCLUSION

The present study concluded that the pulses seeds artificially aged get deteriorated and loses its viability and vigour as a consequence of production of free radicals and associates impacts of lipid peroxidation. Current results clearly demonstrated that nanoparticles are capable of entering into seeds utilizing the cracks and crevices available on the seed coat as dry mixing. The positive impact of nanoparticles seed treatment was also observed for the changes in biochemical characters *viz*., electrical conductivity, free amino acid, dehydrogenase activity and lipid peroxidation. The data suggests that seeds treated with ZnO nano-particles @1000 mg kg^{-1} under dry treatment and 200-300 mg kg^{-1} under wet showed a significant increase in seed viability, seedling length and vigour besides maintenance of biochemical constituents after ageing. The nanoparticles randomly entering into the seeds may quench the reactive oxygen species and lessen the oxidative damages thereby eventually promoted viability and vigour of aged seeds of pulses.

Conflict of interest

All authors declare that they have no conflict of interest.

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