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Nanoparticles influence seed germination traits and seed pathogen infection rate in forage sorghum (*Sorghum bicolour*) and cowpea (*Vigna unguiculata*)

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Nanoparticles (NPs) influence germination and growth of plants and also reported to have antimicrobial effect on seed. In the present study, effect of four metal/metal oxide NPs *viz.* Zinc oxide (ZnO), Titanium oxide (TiO_2), Copper oxide (CuO) and Silver (Ag) on seed germination traits and seed pathogenicity of forage sorghum [*Sorghum bicolour* (L.)] and cowpea [*Vigna unguiculata* (L.)] was assessed. NPs were synthesized before seed treatment and characterized for size and chemical property by particle size analyzer (PSA), scanning electron microscope (SEM), transmission electron microscope (TEM) and X-ray diffraction (XRD) following standard procedure. All NPs were pure and confirmed as under nano-size (>90% of particles below 100 nm). Seed surfaces were observed under SEM for natural openings that apparently acted as entry points for NPs. Seeds were treated with NPs at 0 mg (D0), 750 mg (D1), 1000 mg (D2) and 1250 mg/kg of seed (D3). Except TiO_2, all other NPs enhanced germination at lower dose (D1), but germination was reduced at higher dose (D3) (p=0.05) as compared to control (D0). Seed vigour traits (germination, shoot length, root length and seedling dryweight in this experiment) were also influenced by NPs. Ag NP was proved to be strong antimicrobial agent in this study.

Keywords: Antimicrobial, Nanotechnology, Shoot and root length

The nanotechnology is a tool of basic science, has now grown more relevant in our day-to-day life. The agricultural sector too has incepted experimentation on this technology for betterment of agricultural inputs and tools[1,2]. Nanoparticles (NPs) literally range from 1–100 nanometre and fall in the transition zone between individual molecules and the corresponding bulk materials, which generate both positive and negative biological effects in living cell[3]. They acquire a wide array of advanced physical, chemical and electrical properties as compared to their bulk materials by means of enhanced surface area and high surface reactivity compared to otherwise identical larger particles. It has advantages as well as disadvantages. There is increasing amount of research on the biological effects of NPs on higher plants; and carbon-based and metal-based nanoparticles (NPs) have been most commonly studied for plant response[4,5]. Though the underlying mode of impacting the plant system by NPs is under study throughout the world[6], limited studies have been reported on the promotional effects of NPs on plants in low concentrations. Maity *et al.*[7] reported that germination traits of berseem and oat seeds responded differentially to different doses of Ag, CuO, ZnO and TiO_2 nanoparticles. Treatments with zinc oxide nanoparticles improved the germination percentage and seedling growth in groundnut[8], garden tomato[9] and in maize[10]. Lower doses of alumina NPs enhanced seedling growth, pigments, sugar and protein contents of cabbage seedlings[11]. It has been shown that a mixture of nanoscale SiO_2 and TiO_2 can increase nitrate reductase activity in soybean (*Glycine max*), enhance its ability to absorb and utilize water and fertilizer, stimulate its antioxidant system, and apparently hasten its germination and growth[12]. Nano-TiO_2 has further been reported to promote photosynthesis and nitrogen metabolism, and thus greatly improve growth of spinach at certain concentrations[13]. Some of the negative effects of nano-scale materials on plant growth have been identified as inhibition in root growth and seed germination[14,15] which varies greatly depending on NPs composition, size, concentration and also plants

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species. It was reported that root elongation of 5-day-old *Zea mays* seedlings reduced with 2000 mg/L of 60 nm alumina NPs (nAl_2O_3) while under the same conditions no negative effects were observed on the growth of *Raphanus sativus*, *Brassica napus*, *Cucumis sativus*, *Lolium perenne* and *Lactuca sativa*[16]. Lin and Zhing[14] reported 2000 mg/L of nano-Zn and nano-ZnO suspensions significantly inhibiting root growth in *Zea mays* and terminating the root development of the other five plant species, whereas, nAl_2O_3 (13 nm) at 2 mg/mL concentration inhibited the root length of five plants, namely, *Zea mays*, *Cucumis sativus*, *Glycine max*, *Brassica oleracea* and *Daucus carota*[17].

On the other side, forage crops and grasses which ensure the better availability of milk and other animal products are often blamed for their low seed germinability and other qualities, which hinders the higher productivity in field condition. Technology transfer and adoption in farmers' field remain low due to unavailability of high quality seeds along with other socioeconomic causes. Bailly[18] stated that accumulation of reactive oxygen species (ROS) and free radicals during storage leads to lipid peroxidation and damage of the cell organelles, micro and macromolecules within and surface of the cell generating several cracks and lesions on seed surface. These changes by chain reaction of free radicals are generally considered as the major contributors to seed deterioration. Development of appropriate technological interventions to slow down the deterioration process so as to maintain the seed viability for a longer period will be of immense help to seed producers as well as seed industry.

Nanoparticles (NPs) can be one of the ways to retain the vigour and viability during storage by preventing the losses due to biotic and abiotic stress. NPs of different elements, compounds and organic products are being reported now-a-days to enhance the seed germinability and quality in many crops by altering the deteriorating physiological processes through scavenging of free radicle as discussed earlier[7,19]. Several crops are being explored worldwide for the same line of work. To test the contrasting reports in the literature about influence of NPs on seed germination traits and seed borne microbes, we chose one poaceae crop, sorghum and one leguminosae crop, cowpea to see the effects of NPs in diverse plant systems.

Materials and Methods

Seeds of popular varieties of fodder sorghum (cv MP Chari) and cowpea (cv Kohinoor) were collected from Division of Seed Technology, Indian Grassland and Fodder Research Institute, Jhansi, Uttar Pradesh. Seed surfaces were examined for the cracks generated by deterioration process during storage under SEM (Fig. 1). The cracks were conceptualized to act as the entry point of NPs into the seeds during treatment of seeds with NPs.

Synthesis and characterization of NPs

Synthesis and characterization of NPs were done in Department of Nanoscience and Technology, Tamil Nadu Agricultural University, Coimbatore.

Synthesis of ZnO NPs

A 0.45 M $Zn(NO_3)_2$ solution (100 ml) transferred to a burette was added drop wise (slowly for 40 min) to a 0.9 M aqueous solution of sodium hydroxide (NaOH) 100 mL 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 2 h for precipitation and settlement. The precipitated ZnO NPs were washed with millipore water followed by ethanol and then vacuum dried at 60°C[20]. NPs such synthesized were transferred to air tight screw cap vial (10 mL) and stored for further investigations.

Synthesis of Ag NPs

The Ag NPs were prepared according to the method of Lee and Meisel[21]. Fifty milliliter of $AgNO_3$ 0.005 M taken in a beaker was boiled on a magnetic stirrer with hot plate. 5 mL of 1% trisodium citrate was added drop by drop to this solution from 10 mL measuring cylinder with vigorous mixing on the stirrer until pale yellow colour appeared. After removal the beaker was kept at ambient temperature where the chemical reaction took place

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

Synthesis of CuO NPs

CuO NPs were synthesised using, and sodium hydroxide in the presence of polyvinyl alcohol (PVA, Sigma Aldrich) as starting precursor[22]. Sodium hydroxide anhydrous pellets (NaOH, Carlo erba) was dissolved in deionized water and thus obtained solution (0.5M, 50 mL) was added drop wise to an aqueous solution of copper nitrate trihydrate ($CuN_2O_6.3H_20$, Sigma-Aldrich) (0.1 M, 50 mL) for 30 min. Sonication of the solution was performed using Sonics Model VCX 1500 until complete precipitation. Finally, precipitated powder was calcined at 600℃ for 2 h to obtain the NPs.

Synthesis of TiO_2 NPs

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. Thus, the 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 and dried at 60℃ for 24 h to obtain the NPs[23].

2,2-Diphenyl-1-picrylhydrazyl (DPPH) test for radical scavenging activity

NPs are reported to exhibit strong antioxidant activity *i.e.* they can quench free radicals, and thus can reduce the ageing process in biological system through upregulation of antioxidant enzymes[24]. Free radical scavenging activity of the NPs based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by Braca *et al.*[25]. Sonicated nanoparticle solution (0.1 mL) was added to 3 mL of 0.004% methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min and the percentage inhibition activity was calculated as follows

$$\%\ \text{Inhibition} = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100$$

Where,

Ac = Control absorbance and As = Sample absorbance

NPs were characterized by particle size analyzer (PSA), scanning electron microscope (SEM), transmission electron microscope (TEM) and X-ray diffraction (XRD) following standard procedure.

Scanning electron microscope (SEM)

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 of FEI QUANTA 250 to assess the size and morphology of the nanoparticles. Sample surface were observed at different magnification and the images were recorded.

Transmission electron microscope (TEM)

Sample (NPs 0.50 mg) was diluted in suspensions by pure ethanol (15 mL) through 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 in FEI TECHNAI SPRIT make.

X-ray diffraction (XRD)

The phase formation of powder samples was confirmed by X-ray diffraction (XRD) technique using an X-ray powder diffractometer (Rigaku Corporation Japan, Smart Lab 3kW) with CuKα radiation (λ = 1.5405Å) in slow scan in the 2θ range of 20-80°.

Seed treatment and Germination test

Seeds were treated with these inorganic NPs with 750, 1000 and 1250 mg per kg of seed. Standard germination test was followed[26]. About 400 seeds in 4 replicates of 100 seeds were put in germinator as per Between Paper method. Seedling shoot and root length, and dry weight were measured at the end of the germination test. The germination has been reported on percent basis. After taking the final count of germination, seedlings were measured for their height and then dried at temperature of 90°C for 18-24 h and their weights were taken[27]. Vigour index I was calculated as [seedling length (cm) × per cent germination] and Vigour index II as [seedling dry-weight (g) × per cent germination]. All data were analysed using SAS software.

Blotter test

Thereafter, the treated seeds were kept in Blotter Test (at 20±2°C in 12/12 h alternating cycles under UV light and darkness for 7 days) to detect pathogen infection. The result has been reported on percent basis.

Statistical analysis

The obtained data were statistically analysed using analysis of variance (ANOVA) and means were compared and grouped by using the Least Significant Difference test (LSD 0.05) with the SAS 9.1.3 programme[28].

Results

Seed surface

After harvesting, seeds were kept for few days, it generates natural openings and/or cracks on seed coat possibly due to gradual drying/desiccation and universal deteriorative processes like lipid peroxidation in seed coat, which later on act as entry point for microorganisms. Openings on seed surfaces under SEM ranged from 1–35 µm in length and 1–5 µm in width (Fig. 1A).

NPs: size and characterization

NPs tested by the particle size analyser were under nano size: ZnO 20-50 nm, TiO_2 30-60 nm, CuO 50-80 nm and Ag 5-15 nm (Table 1). The size of NPs was further confirmed by the SEM (Fig. 2A) and TEM (Fig. 2B) and its chemical identity was confirmed by XRD (Fig. 3).

The phase formation of powder samples was confirmed by X-ray diffraction (XRD) technique using an X-ray powder diffractometer (Rigaku Corporation Japan, Smart Lab 3kW) with CuKα radiation (λ = 1.5405Å) in slow scan in the 2θ range of 20-80°. Fig. 3 (A-C) depicts indexed

Fig. 1 — (A) Sorghum and Cowpea seed coat surface under SEM; and (B) Germinated seedlings of sorghum and cowpea on treatment with ZnO nanoparticles

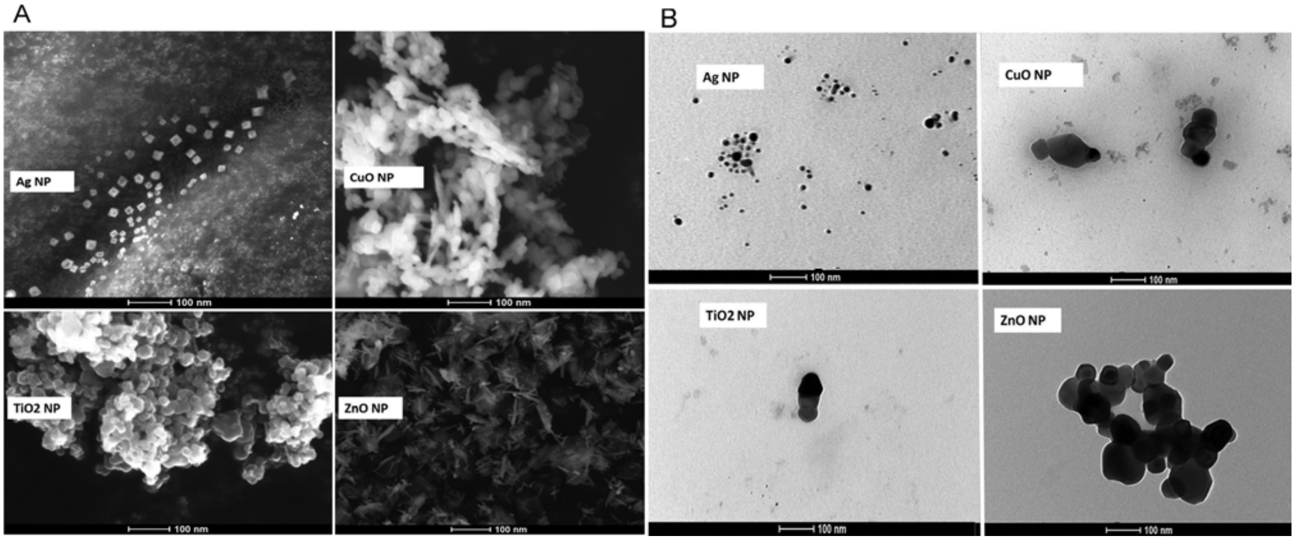

Fig. 2 — Nanoparticles under (A) scanning electron microscope (SEM); and (B) transmission electron microscope (TEM) confirming the nanosize

Table 1 — Calculated lattice parameters, type of structure and space group

Compound	Lattice parameters			Structure	Space group	DB card no.	Size (nm)	*Free radicalscavenging activity* (%)
	a(A^o)	b(A^o)	c(A^o)					
CuO	4.68193	3.41979	5.12789	Orthorhombic	15 : C12/c1	01-080-1268	20-50	78
ZnO	3.23847	3.23847	5.18517	Tetragonal	186 : P63mc	01-074-9940	30-60	53
TiO_2	3.77627	3.77627	9.48739	Tetragonal	141 : I41/amd	01-073-1764	50-80	39
Ag	4.088666	4.088666	4.088666	Cubic	225 : Fm-3m	00-004-0783	5-15	72

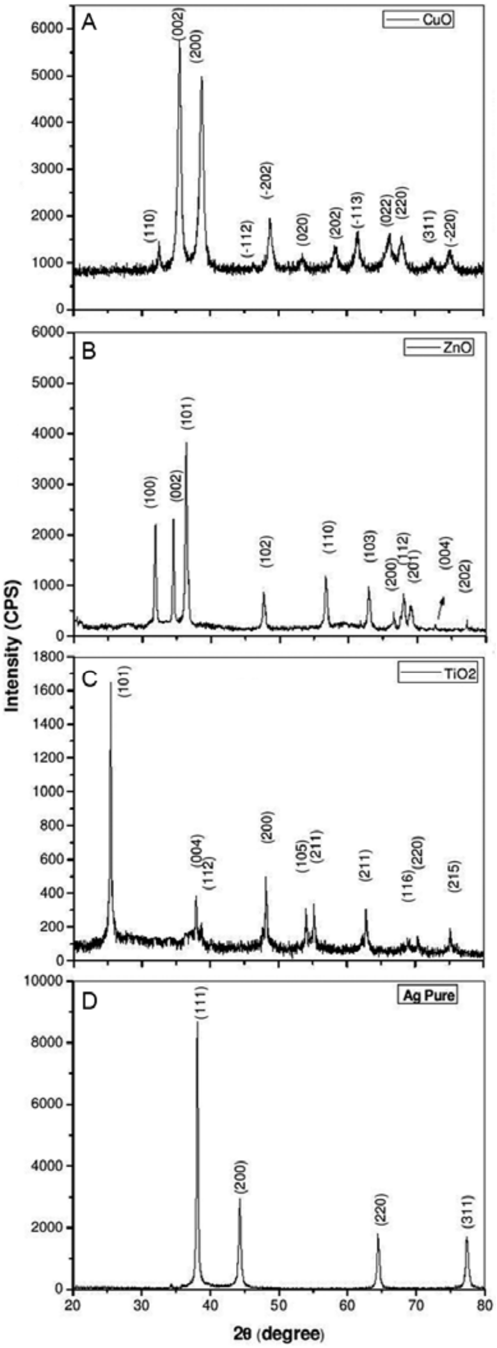

Fig. 3 — Powder XRD patterns of synthesised (A) nano copper oxide (CuO); (B) nano zinc oxide (ZnO); (C) nano titanium oxide (TiO_2); and (D) nano silver (Ag)

X-ray diffraction pattern of CuO, ZnO and TiO_2, respectively. All the reflections are clearly visible, sharp and pointed which indicate the formation of the compounds with high purity and good homogeneity. XRD peaks were indexed by using X-ray diffraction interpretation and analysis in computer software PDXL2 (ICDD (PDF-2/Release 2013)). The diffraction peaks indicate good homogeneity with high purity. Formation of almost pure phase is confirmed in all four materials. All the reflections are well matched with the XRD intensities of CuO (DB card No. 01-080-1268) (space group 15 : C12/c1,unique-b,cell-1), ZnO (DB card No. 01-074-9940) (space group 186 : P63mc), TiO_2 (DB card No. 01-073-1764) (space group 141:I41/amd,choice-2) and Ag (DB card No. 00-004-0783) (space group 225:Fm-3m), respectively.

The result of DPPH radical scavenging activity showed that ZnO had the highest scavenging activity of 78% while the least was observed with CuO NPs (39%) whereas TiO_2 and Ag had 53 and 72%, respectively.

Germination percentage

All the four NPs *viz*. ZnO, TiO_2, CuO and Ag improved germination percentage significantly as compared to control in both crops. In sorghum, TiO_2 exceptionally enhanced germination at higher doses and maximum value was recorded at D3 (97.3%). Otherwise, in case of other three NPs germination was promoted at lower doses and reduced subsequently. Like in case of CuO, highest germination (97%) was observed at D1of CuO and it was reduced by 6% at D3 (Fig. 4A). Lowest germination (89%) was recorded at D3 of Ag. In cowpea, the same trend was observed. D3 of TiO_2 stimulated maximum germination (97%, 17.3% higher than the control) compared to others. Ag at D3 showed minimum germination (78.7%) which was 6% lower than the control.

Shoot length

In sorghum, Ag and TiO_2 promoted shoot length at higher doses and TiO_2stimulated maximum shoot length at D3 (8.33 cm) (Fig. 4). ZnO promoted the trait at D1 (7.53 cm), but D2 and D3 were at par with

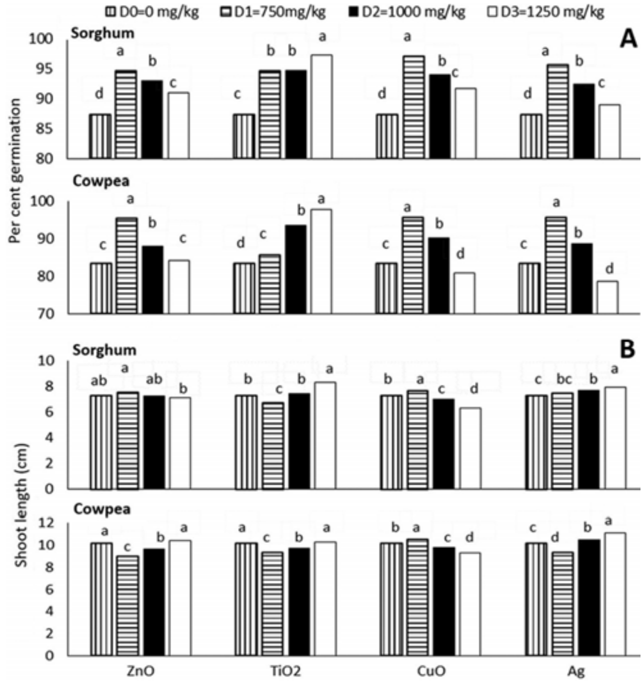

Fig 4 — Effect of nanoparticles on (A) germination (%); and (B) shoot length (cm) of sorghum and cowpea

control. CuO enhanced shoot length at D1 (7.68 cm) but reduced it significantly at D2 and D3 by the magnitude of 4 and 13%, respectively as compared to control (Fig. 4B). In cowpea, Ag at D3 promoted maximum shoot length (11.1 cm) by 9.4% as compared to control; whereas ZnO at D1 showed minimum value (9.01 cm) which was 11.2% lower than the control. All NPs promoted the trait except CuO.

Root length

In case of sorghum, ZnO and CuO stimulated root length significantly at D1, but at subsequent doses it reduced the root length as compared to D1 (Fig. 5A). Maximum root length was found at ZnO D1 (23.40 cm), 27% higher than the control and it was reduced by 9.4% at D3. TiO_2 and Ag enhanced sorghum root length at higher doses and all doses showed significantly higher root length as compared to control. In cowpea, root growth increased incrementally with dose as compared to control except CuO where root growth decreased at subsequent doses. Ag at D3 showed maximum (20.28 cm) and CuO at D3 showed minimum (15.7 cm) root growth.

Seedling dry-weight

In sorghum, except TiO_2, all NPs reduced seedling dry weight as compared to control and the result showed significant variation even among doses (Fig. 5B). TiO_2 disfavoured the seedling dry weight at D1 (3.27 mg) and favoured it a little bit at higher doses, D3 was at par with control. Least dry weight was found at D3 of Ag (1.39 mg). Same trend was in case of cowpea where D1 of TiO_2 was at par with control and seedling dry weight was enhanced at higher doses. Highest seedling dry weight was observed at D3 of TiO_2 (3.75 mg) and lowest was at D3 of CuO (1.74 mg). Whereas, CuO reduced seedling dry weight significantly at D1 itself, ZnO and Ag maintained it at D1 but reduced at D2 and D3.

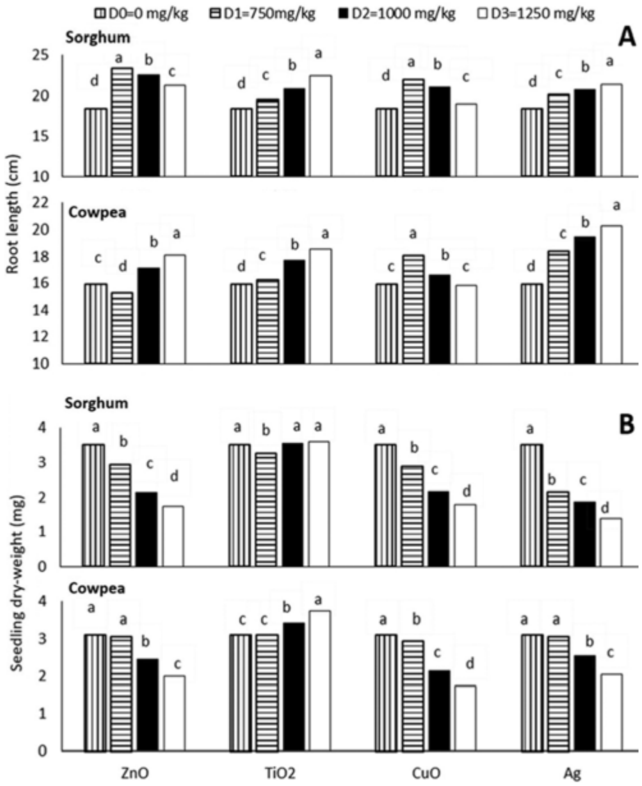

Fig 5 — Effect of nanoparticles on (A) root length (cm); and seedling dry weight (mg) (B) of sorghum and cowpea

Root/shoot ratio and seed vigour index

ZnO and CuO NPs significantly influenced root/shoot ratio in sorghum, whereas TiO_2 and Ag had no effect (Table 2). ZnO exhibited highest R/T at D1 (3.12) and reduced at higher dose (D3=2.96), but could maintain higher than control (D0=2.50). CuO showed a reverse trend— it exhibited highest R/T ratio at D2 (3.00) with insignificant difference with D3 (2.99). In case of cowpea, ZnO and CuO NPs had insignificant influence on R/T, whereas TiO_2 and Ag NPs had significant effect. In all the cases (ZnO, CuO, TiO_2 and Ag NPs), D1 enhanced R/T ratio (1.69, 1.73, 1.72 and 1.97, respectively) as compared to control (1.56) (Table 3).

Table 2 — Effect of NPs on root/shoot ratio (R/T), vigour index – I (V1) and vigour index – II (V2) of sorghum

Dose (D)	ZnO			TiO_2			CuO			Ag		
	R/T	V1	V2	R/T	V1	V2	R/T	V1	V2	R/T	V1	V2
Control	2.50	637.21	0.3066	2.50	637.21	0.3066	2.50	637.21	0.3066	2.50	637.21	0.3066
D1	3.12	712.95	0.2783	2.87	640.28	0.3096	2.86	745.71	0.2798	2.69	714.88	0.2066
D2	3.08	679.00	0.1995	2.80	705.44	0.3362	3.00	659.55	0.2044	2.69	713.40	0.1731
D3	2.96	651.11	0.1584	2.70	811.66	0.3518	2.99	584.05	0.1648	2.69	707.81	0.1236
CD (p=0.05)	0.310	NS	0.017	NS	54.96	NS	0.207	51.47	0.036	NS	NS	0.035

[R/T= Root/shoot ratio; V1= Vigour index – I; V2= Vigour index – II]

Table 3 — Effect of NPs on root/shoot ratio (R/T), vigour index – I (V1) and vigour index – II (V2) of cowpea

Dose (D)	ZnO			TiO_2			CuO			Ag		
	R/T	V1	V2	R/T	V1	V2	R/T	V1	V2	R/T	V1	V2
Control	1.56	845.29	0.2587	1.56	845.29	0.2587	1.56	845.29	0.2587	1.56	845.29	0.2587
D1	1.69	860.26	0.2918	1.73	802.19	0.2665	1.72	1005.90	0.2814	1.97	895.92	0.2937
D2	1.77	850.22	0.2172	1.82	909.38	0.3203	1.70	882.90	0.1932	1.86	929.62	0.2263
D3	1.74	877.88	0.1680	1.80	1003.00	0.3664	1.69	754.45	0.1406	1.83	873.30	0.1624
CD (p=0.05)	NS	NS	0.014	0.123	49.61	0.020	NS	49.59	0.021	0.133	NS	0.032

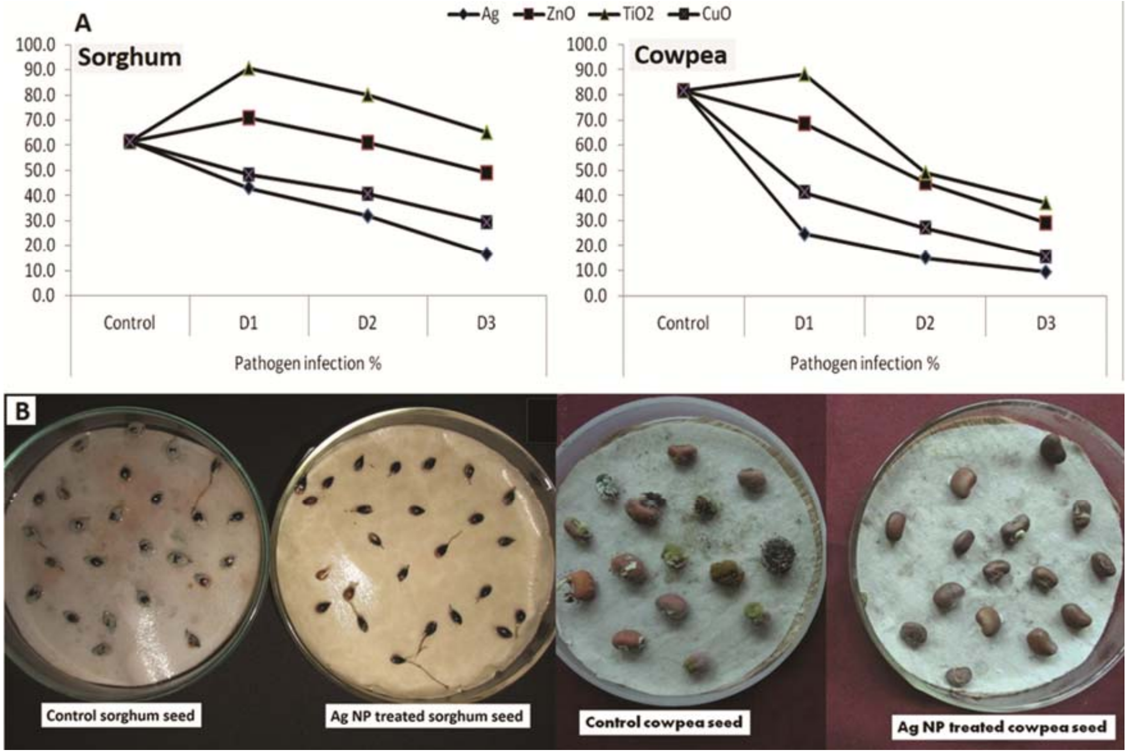

Fig 6 — Effect of nanoparticles on (A) seed pathogen infection (%) in sorghum and cowpea at different dose; and (B) fungal infection of seed under blotter test

In sorghum, vigour index-I (V1) responded insignificantly to ZnO and Ag NPs, but significantly to TiO_2 and CuO NPs. TiO_2 promoted V1 with increasing dose, whereas CuO had detrimental effect at higher dose (Table 2). However, in both the cases the value was higher than control (637.21). V1 in cowpea responded in similar trend as in sorghum (Table 3). Vigour index-II (V2) in sorghum responded significantly to ZnO, CuO and Ag NPs with a reducing trend (Table 2), but TiO_2 had no effect. But, in cowpea TiO_2 significantly enhanced V2 with increase in dose, whereas other three NPs significantly reduced V2 with increasing dose (Table 3).

Pathogen infection (%) in blotter test

AgNPs inhibited the fungal attack on seed at highest degree (16.6% in sorghum & 9.3% in cowpea) followed by CuO NPs in both the crops (Fig. 6). TiO_2

treated seeds showed maximum pathogen attack (90.7% in sorghum & 88.2% in cowpea). Remarkably, at higher doses the NPs inhibited the infection more and D3 showed maximum inhibition.

Discussion

There is a lot of ambiguity regarding the effect of NPs on seed germination. Although in many of the cases NPs have shown to enhance the seed germination or to have no effect on it[12,4,29,30], few researchers have reported the negative effect of NPs on seed[31,32]. Our study, in most of the cases, is as per with the first group. Raliya and Tarafdar[33] in clusterbean and Raliya *et al.*[34] in tomato reported that ZnO NPs increased the seedling growth. Similar results are reported by many researchers[8-10]. In the present experiment, CuO improved germination at D1 (750 mg/kg of seed) and reduced the germination and growth at higher doses, which is supported by many studies[35-37]. There are limited literature available on influence of Ag NPs on plant growth[7,38], limited to the antimicrobial property[39,40]. Our study shows that Ag NPs enhanced germination at lower dose (750 mg/kg of seed) whereas reduced at higher doses and seedling growth responded contrariwise with germination. In case of TiO_2, maximum researchers report that it promotes plant growth and metabolite production. In the present study also, it improved germination and seedling growth, which is supported by many studies. Singh *et al.*[41] and Rezaei *et al.*[42] reported enhancement in germination and growth by TiO_2 and the promotion of plant growth may be due to altered enzyme activity[13,43].

NPs by virtue of their nano sizes (10^{-9}m) possess larger surface area resulting in increased catalytic activity and are highly reactive[44]. It is also observed that NPs are known to donate electrons that pairs with the free radicals and smother the impact of these radicals. Zheng *et al.*[45] observed a similar phenomenon in spinach where TiO_2 NPs could accelerate the germination of aged seeds and increase its vigour by scavenging the free radicals that otherwise accelerate the seed deterioration process[46] and sometimes increasing the concentration of free radical scavenging enzymes[12]. It also helps the seed to imbibe more water, thus triggering the germination phenomenon[4]. Previous studies have provided similar reports of the positive effects of NPs on germination and growth of plants. For example, TiO_2 and SiO_2 NPs were found to enhance both the germination and growth of soybean seeds by increasing nitrate reductase and enhancing its ability to absorb and utilize water and fertilizer, stimulate its antioxidant system, and apparently hasten its germination and growth[12]. Carbon nanotubes (CNT) were discovered to improve germination and root elongation of tomato seeds[4] and Nano-Al were shown to augment root elongation of radish and rape seedlin[14]. In the present study also, NPs showed antioxidant activity or radical scavenging activity in the biochemical test and as a result it might have influenced the seed germination traits by reducing the deteriorative process induced by reactive oxygen species. Zheng *et al.*[46] stated that the significant effect of nanosized TiO_2 on spinach germination in tests was probably because of small particle size, which allowed NPs to penetrate the seed during the treatment period, exerting its enhancing functions during growth. In blotter test, the age-old concept of antimicrobial effect of NPs was revealed and Ag NPs were proved to be strongest antimicrobial agent. Plenty of literature is found in this direction.

All metal oxide nanoparticles are reported to influence the growth and development of plants. They generally boost or retard seed germination, shoot/root elongation, biomass accumulation and physiological and biochemical reactions. Some plant species show no physiological change, whereas some species exhibit substantial changes in antioxidant enzyme activity and upregulation of heat shock protein. Plants have evolved antioxidant defence mechanism which involves enzymatic as well as non-enzymatic components to prevent oxidative damage and enhance plant resistance to metal oxide toxicity. The exact mechanism of plant defence against the toxicity of nanomaterials has not been fully explored. Therefore, mechanism of nanotoxicity remains unknown; however, it would be closely related to the chemical composition, chemical structure, particle size and surface area of the NPs. Attentions to appropriate experimental design and interpretation are needed to provide a defensible scientific understanding of the biological effects of NPs[16]. In order to understand the possible benefits of applying nanotechnology to agriculture, the first step must be to analyze penetration and transport of NPs in plants, which is under intensive and extensive research.

Conclusion

NPs tested in the investigation were supportive in enhancing the germination and seedling vigour of the sorghum and cowpea seeds which is supposed to be highly prone for deterioration in storage.

In conclusion, our experiment confirmed the dose-specific effect of NPs on seeds of these crops. All the four NPs *viz*. ZnO, TiO_2, CuO and Ag at lower dose significantly enhanced germination percentage and vigour traits as compared to control in both the crops. But the crops responded differentially to the concentration of NPs regarding germination percentage and seedling traits. Ag NP has shown good fungicidal property. Thus the technology holds immense potential in various sectors if all the issues with NPs are addressed properly. However, the findings are to be verified under large scale field condition before recommending to farmer for adoption.

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