

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.611.435>

Effect of Nanoparticles for Seed Quality Enhancement in Onion [*Allium cepa* (Linn) cv. CO (On)] 5

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ABSTRACT

Keywords

Onion, Seed Quality, *Allium cepa*, nano particle, Nano seed treatment, ZnO, Ag, CuO and TiO₂ Nanoparticles, SEM, TEM, Particle Size Analyzer, Raman Spectroscopy.

Article Info

Accepted:
26 September 2017
Available Online:
10 November 2017

Zinc oxide (ZnO), Silver (Ag), Copper oxide (CuO) and Titanium oxide (TiO₂) nanoparticles were synthesised using simple chemical route which were characterised using Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), Particle Size Analyzer and Raman Spectroscopy. Size of Zinc oxide (ZnO), Silver (Ag) Copper oxide (CuO) and Titanium dioxide (TiO₂) nanoparticles measured 16-50 nm, 50-100 nm, 60-150 nm and 100-120, respectively to conform the nano-size. Onion seeds when dry dressed with the synthesised nanoparticles each at 750, 1000, 1250 and 1500 mg kg⁻¹, the dose of 1000 mg kg⁻¹ outperformed in enhancing the germination (72%), shoot length (7.5 cm) root length (6.4) and thereby the vigour index (998) compared to control (60%, 6.0, 5.4 and 692) respectively.

Introduction

Onion (*Allium cepa* L.) belongs to the family Liliaceae and is one of the most important monocotyledonous and cool season vegetable crops in India. Amongst the onion producing countries in the World, India ranks second in area and production. Onion has been the largest item of export accounting to 76.2 per cent in the total export of vegetables from India. The unavailability of quality onion seed is greatly responsible for its lower yield. The seed quality parameters especially seed size and seed weight affect the final yield in onion production (Gamiely *et al.*, 1991). Furthermore, high quality seed is considered

as the critical input in onion on which all other inputs have to be managed for potential yield in onion. Onion is grown in an area of 1.01 m ha with a production of 16.8m tonnes keeping the productivity at 16.6 t ha⁻¹.The prominent onion growing states are Maharashtra, Gujarat, Uttar Pradesh, Orissa, Karnataka, Tamil Nadu and Andhra Pradesh. Perambalur district in Tamil Nadu has the highest share of production (24.6%) followed by Trichy (14.2%), Coimbatore (13.7%) and Erode (10.8%) districts. In India onion seed is getting lost quickly due to the production of free radicals by lipid peroxidation during

storage. As the current technologies available to prolong the vigour and viability of onion seed on a large scale are not satisfactorily alleviating the practical problem, an alternative simple and practicable seed treatment to control seed deterioration of onion is need of the hour.

Several researchers reported that mid-term hydration-dehydration treatments performed better in improving germinability and seedling vigour after storage in soy bean (Basu 1994; Mandal *et al.*, 2000) and okra (Kapri *et al.*, 2003). Nanoparticles can be one of the ways to retain the vigour and viability during storage by preventing the losses due to biotic and abiotic stress.

Lots of works have been done in biological system to address a wide range of field problems utilizing nanomaterials and nano-devices. (Natarajan and Sivasubramanian, 2008) elucidated various nanotechnological approaches especially in the field of agriculture including nano-polymer for seed hardening, nano-sensors, nano-barcode and use of magnetic nanoparticles for aerial seeding. (Senthil kumar, 2011) and (Sridhar, 2012) further established the use of metal oxide nano-particles in improving germination up to 30 per cent in aged seeds of black gram and tomato respectively which could be probably due to the quenching of reactive oxygen species (ROS) generated during seed storage. Applications of nanotechnology in improving 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) are few of the multifarious beneficial interventions in the field of agriculture. Hence the present investigation was made to study the effect of ZnO, Ag, CuO and TiO₂ nanoparticle on the vigour and viability of onion seed.

Materials and Methods

The first experiment synthesis of nanoparticles and characterization was carried out at Department of Nano Science and Technology and the second experiment study of seed quality enhancement was carried at Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore -03, during the year of 2012–13. The chemicals used for synthesis of nanoparticles viz., Zinc nitrate (Zn(NO₃)₂·4H₂O), AgNO₃, Trisodium citrate, copper nitrate trihydrate, TiO₂ pellets, NaOH and Ethanol were purchased from THE I.L.E. Co. Pvt. Ltd., Coimbatore, Tamil Nadu.

Synthesis of ZnO, Ag, CuO and TiO₂ Nanoparticles

Zinc oxide nanoparticles

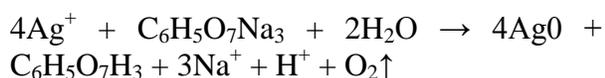
ZnO NPs were synthesized by adding 0.45 M aqueous solution of zinc nitrate (Zn(NO₃)₂·4H₂O) and 0.9 M aqueous solution of sodium hydroxide (NaOH) in distilled water taken in two separate 250 ml glass beakers.

The Zn(NO₃)₂ solution (100 ml) transferred to a burette was added drop wise (slowly for 40 min.) to the 100 ml of NaOH contained in the beaker placed over a magnetic stirrer with hot plate set at 55°C with high-speed stirring. The beaker after adding 100 ml Zn(NO₃)₂ was removed from the hot plate, sealed with aluminium foil and kept undisturbed for 2h for precipitation and settlement.

The precipitated ZnO NPs were washed with millipore water followed by ethanol and then vacuum dried at 60°C (Moghaddam *et al.*, 2009). Nanoparticles such synthesized were transferred to air tight screw cap vial (10 ml) and stored at ambient temperature for further investigations.

Silver nanoparticles

The Ag NPs were prepared by using chemical reduction method according to the description outlined by (Lee and Meisel, 2005). Fifty milliliter of AgNO₃ 0.005 M taken in a beaker was boiled on a magnetic stirrer with hot plate. To this solution, 5ml of 1% trisodium citrate was added drop by drop from 10 ml measuring cylinder with vigorous mixing on the stirrer until pale yellow colour appeared. Then the beaker was removed and kept at ambient temperature where the chemical reaction occurred would have been



Copper oxide Nanoparticles

CuO NPs were synthesised using copper nitrate trihydrate (CuN₂O₆.3H₂O, Sigma-Aldrich), and sodium hydroxide anhydrous pellets (NaOH, Carlo erba) in the presence of polyvinyl alcohol (PVA, Sigma Aldrich) as starting precursor (Wongpisutpaisan *et al.*, 2011). Sodium hydroxide was dissolved in deionized water and thus obtained solution (0.5M, 50 ml) was added drop wise to an aqueous CuN₂O₆.3H₂O solution (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 600⁰C for 2 h to obtain the nanoparticles.

Titatum oxide nanoparticles

TiO₂ NPs were synthesized by dissolving 0.5 g TiO₂ pellets in 30 ml of NaOH solution (10 M) under vigorous stirring at room temperature for 2 h. Thus obtained yellow solution was irradiated in an ultra sonicator (Soncis, VCX 1500, 20 kHz and 350 W) for 2h in ambient temperature. The resultant precipitate was then centrifuged, washed and

decanted with deionized water several times and dried at 60⁰ C for 24 h to obtain the nanoparticles (Arami *et al.*, 2007).

Characterization of synthesized nanoparticles

Characterization of the synthesized nanoparticles was performed by using Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), Particle Size Analyzer and Raman Spectroscopy.

Scanning Electron Microscope (SEM)

FEI QUANTA 250 was used to characterize the size and morphology of the nanoparticles. Sample of test nanoparticles (0.5 to 1.0 mg) was dusted on one side of the double sided adhesive carbon conducting tape, and then mounted on the 8mm diameter aluminum stub. Sample surface were observed at different magnification and the images were recorded.

Transmission Electron Microscope (TEM)

FEI TECHNAI SPRIT make was used to analyze the sample. Dilute suspensions of NPs (0.50 mg) in pure ethanol (15 ml) were prepared by ultrasonication. A drop of the suspension placed on 300-mesh lacy carbon coated copper grid upon drying, was examined and the images were recorded at different magnification.

Particle size analyzer

The particle size analyzer was used to determine the particle size and the distribution pattern of synthesized ZnO, Ag, CuO and TiO₂ nanoparticles. The particle size distribution (PSD) of a powder indicates a list of values or a mathematical function that defines the relative amount of particles

present, sorted according to size. In the present study, HORIBA nanoparticle size analyser SZ 100 was used. Accurately, 0.5 mg of sample was dispersed in 10 ml pure water through ultrasonication and the measurements were taken.

Raman spectroscopy

Raman spectroscopy is a spectroscopic technique based on inelastic scattering of monochromatic light, usually from a laser source. Inelastic scattering means that the frequency of photons in monochromatic light changes upon interaction with the sample. Photons of the laser light are absorbed by the sample and then reemitted. Frequency of the reemitted photons can be shifted either up or down in comparison to the original monochromatic frequency which is called the Raman Effect. This shift provides information about vibrational, rotational and other low frequency transitions happening in the molecules. Raman spectroscopy can be used to study solid, liquid and gaseous samples.

Raman spectrum is a spectral “fingerprint”. If number of different compounds is present in a mixture, the resulting Raman spectra will be a superposition of the spectrum of each of the components. The relative intensities of the peaks can be used to give quantitative information on the composition of mixture of known compounds. The Raman spectrum was measured for the synthesized nanoparticles using Raman spectrum Model- R- 3000- QE. The powdered, dried NPs kept in a polythene bag were spread to an extent of 1 cm² and Raman probe was placed on the sample packets without exposing the sample directly to the probe (Fig. 2).

Seed treatment

Fresh seeds of onion (CO 5) obtained from the Department of Vegetable Crops, Horticultural College and Research Institute,

Coimbatore were dry dressed with each of the synthesized nanoparticles viz., ZnO, Ag, CuO and TiO₂ @ 750, 1000, 1250, and 1500 mg kg⁻¹ in screw capped glass bottles at room temperature. The glass bottles containing seeds and nanoparticles were manually shaken gently for 3 min., 5 times in a span of 3h. Seeds shaken without nanoparticles served as control. After dry dressing with the nanoparticles, the seeds were packed in cloth bag and stored under ambient condition (25 ± 3^oC temperature and 95 ± 3% RH).

Seed samples were drawn at monthly intervals up to six months and evaluated for the following seed quality parameters. viz., germination percentage, shoot length, root length, and vigour index.

Germination test in quadruplicate using 100 seeds each with four replicates of 25 seeds was carried out in paper medium. The test conditions of 25 ± 2 ^oC and 95 ± 3 per cent RH were maintained in the germination room. At the end of 14 days, the number of normal seedlings was counted and the mean was expressed as percentage (ISTA, 2005).

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

Vigour index was computed by adopting the method suggested by (Abdul-Baki and Anderson, 1973) and expressed as whole number.

Vigour index = Germination percentage ×
Seedling length in cm.

Results and Discussion

Characterization of nanoparticles (ZnO, Ag, CuO and TiO₂)

The surface morphology of Zinc Oxide (ZnO), Silver (Ag), Copper Oxide (CuO), and Titanium Oxide (TiO₂) nanoparticles were examined under SEM, TEM, Particle Size Analyzer and Raman Spectroscopy. The morphology of different nanoparticles observed are presented below.

The particle size analyzer was used to analyze the size of the particle using laser scattering principle for estimating the average particle size and distribution pattern for synthesized ZnO, Ag, CuO, and TiO₂ nanoparticles. The particle size distribution of ZnO, Ag, CuO and TiO₂ was found to be 16, 53.7 nm, 183 nm and 387 nm respectively (Fig. 1).

Raman spectroscopy was employed to identify the chemical composition and to confirm the four different nanoparticles

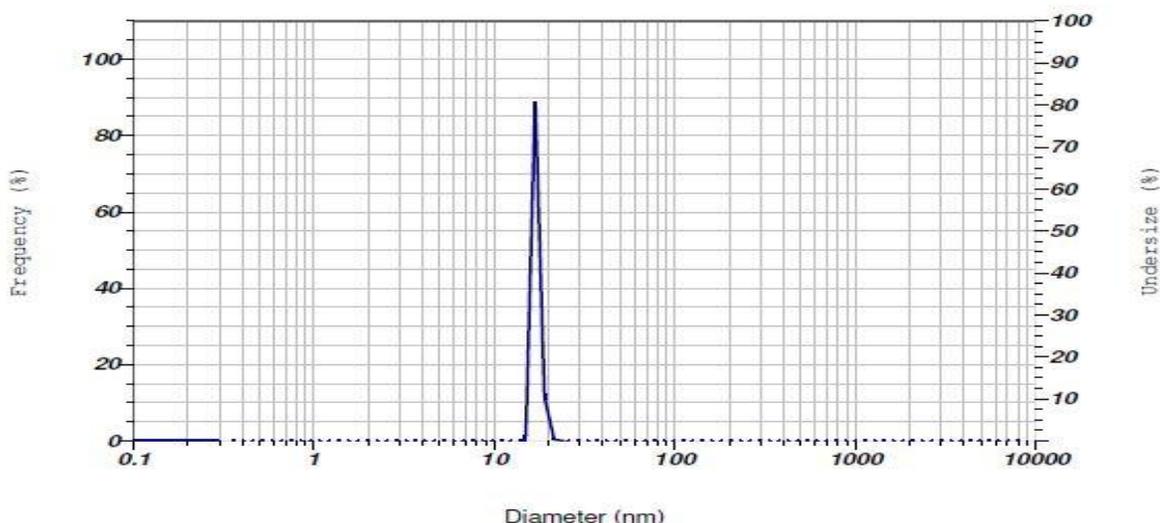
synthesized by observing the peaks. The peaks were observed at 308, 908, 1152 and 1280 cm⁻¹ for CuO while at 528, 871, 945 and 1411 cm⁻¹ for Ag, 276, 637, 1327 and 1458 cm⁻¹ for TiO₂ and 366, 723, 1066 and 1219 cm⁻¹ for ZnO nanoparticle confirming the respective chemical compounds (Fig. 2).

Seed germination and seedling vigour

Nanoparticles of ZnO, Ag, CuO and TiO₂ when treated in different concentrations viz., 750, 1000, 1250 and 1500 mg kg⁻¹ had significantly outperformed control in terms of germination, shoot length, root length and vigour index. Significant differences were also observed between the nanoparticles and doses.

Nano seed treatment improved the germination of aged onion seeds variably towards the treatment at different concentrations.

Fig.1 Particle analyzer average size and intensity distribution of ZnO nanoparticles



Peak No	S.P. Area Ratio	Mean	S.D	Mode
1	1.00	16.1nm	0.7 nm	16.0 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	16.1 nm	0.7 nm	16.0 nm

Fig.2 Raman spectra of (a) ZnO, (b) Ag, (c) CuO and (d) TiO₂ nanoparticles

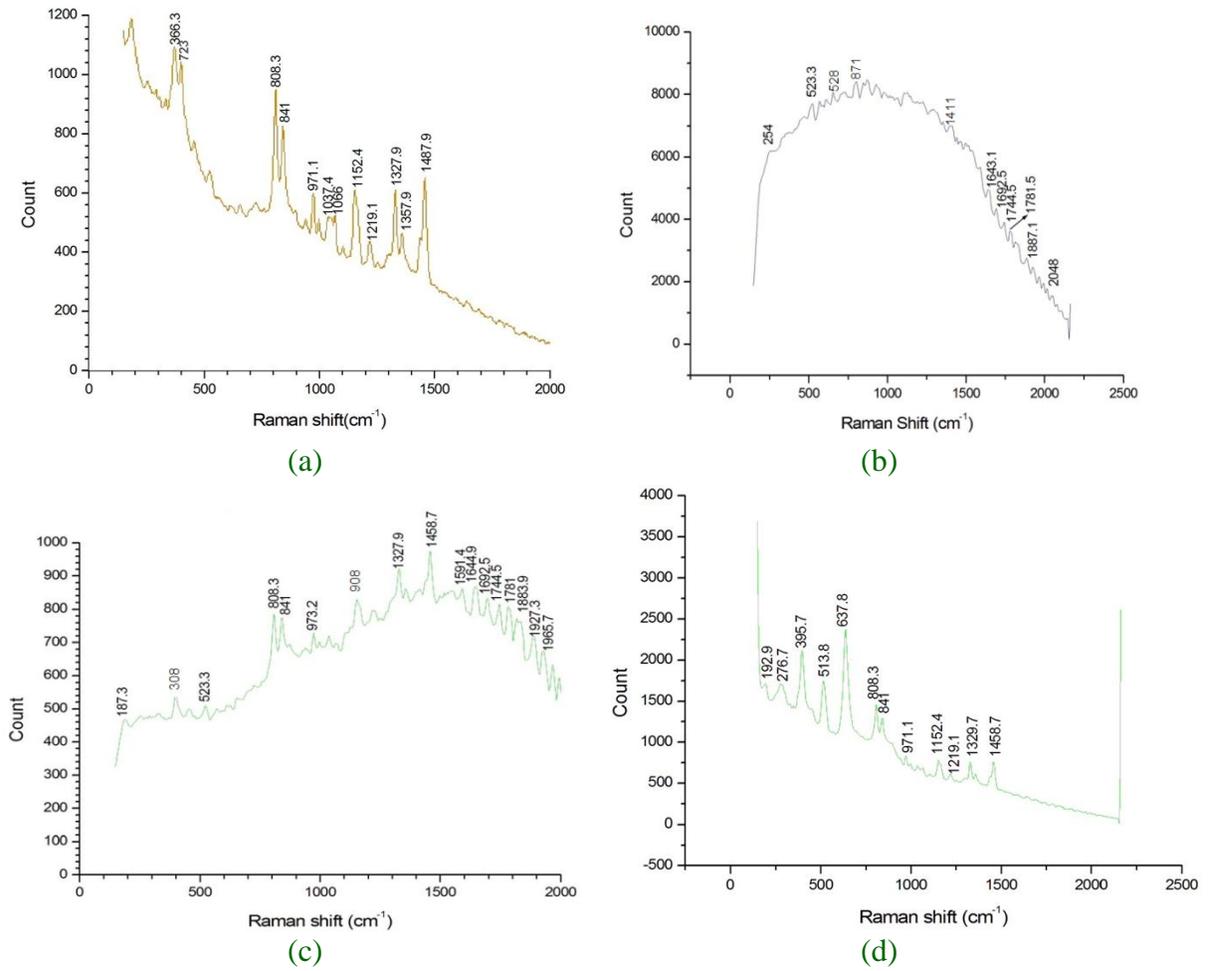
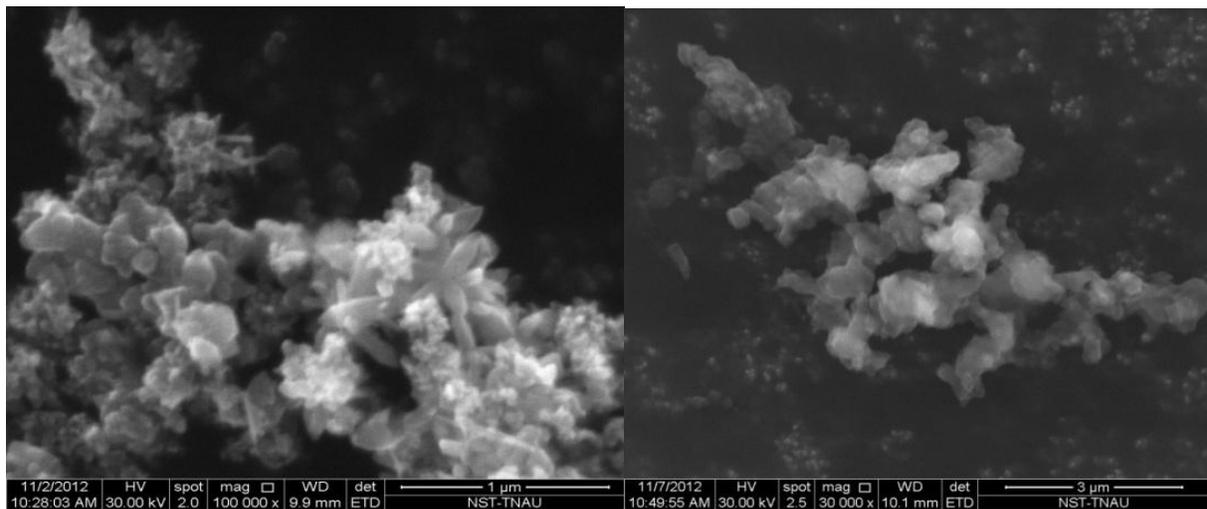


Plate.1 SEM images of (a) ZnO, (b) silver, (c) CuO and (d) TiO₂ nanoparticles



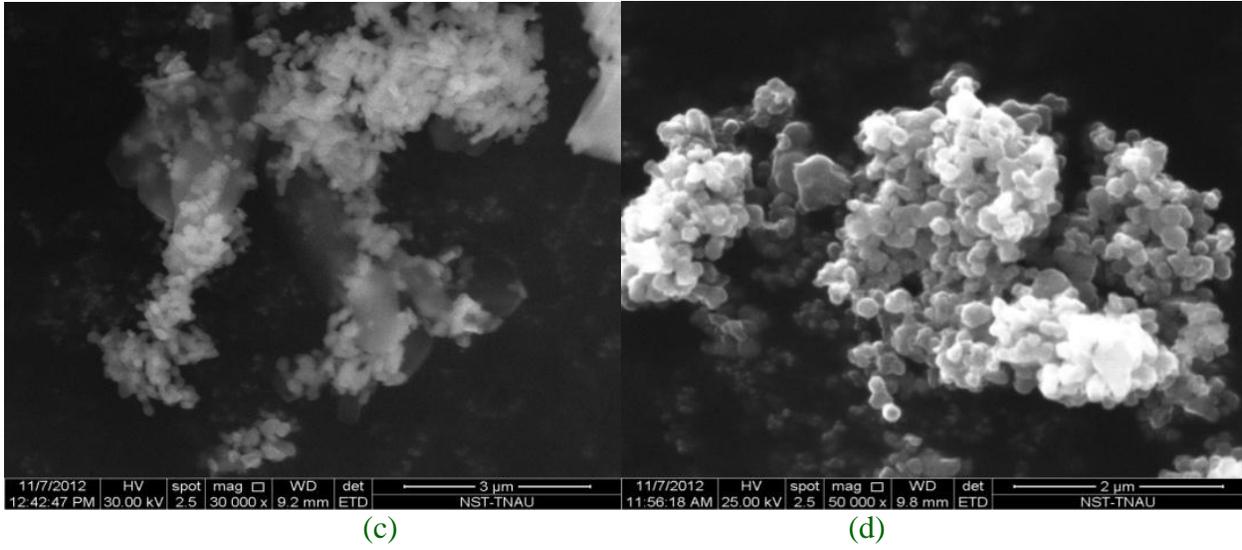


Plate.2 TEM images of (a) ZnO, (b) silver, (c) CuO and (d) TiO₂ nanoparticles

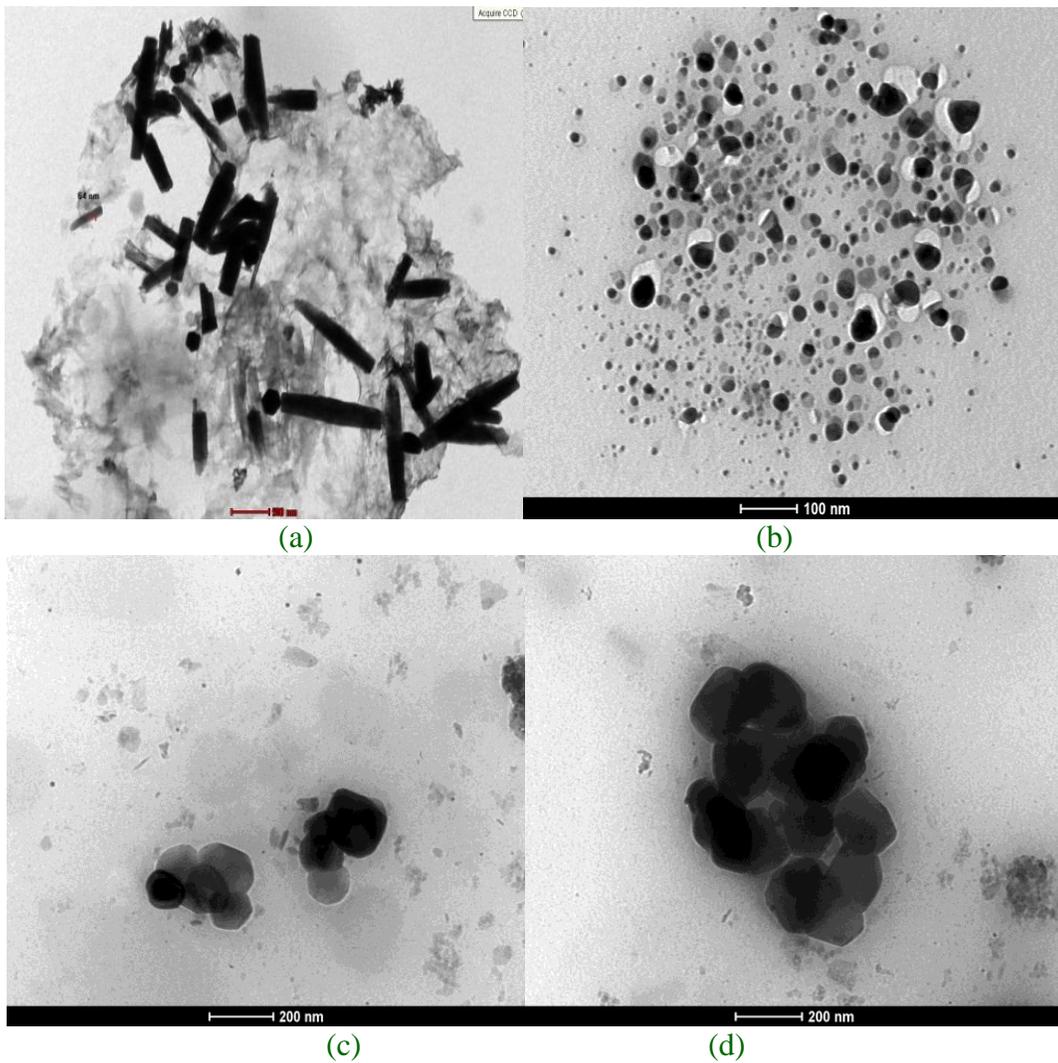


Table.1 Effect of nanoparticles on germination % of stored (6 months old) seeds of onion (CO 5)

Treatments (mg /kg-1)	Germination %				Mean
	ZnO	Ag	CuO	TiO ₂	
750	64(53.13)	65(53.73)	60(50.76)	60(50.76)	62(51.94)
1000	72(58.05)	69(56.18)	66(54.33)	65(53.73)	68(55.55)
1250	70(56.79)	67(54.94)	66(54.33)	60(50.76)	66(54.33)
1500	66(54.33)	66(54.33)	61(51.35)	62(51.94)	64(53.13)
Mean	68(55.55)	67(54.94)	63(52.53)	62(51.94)	65(53.73)
Control	60(50.76)				
	T	D	TD		
SEd	0.84	0.75	1.68		
CD	1.66**	1.48**	NS		

Table.2 Effect of nanoparticles on shoot length (cm) of stored (6 months old) seeds of onion (CO 5)

Treatments (mg /kg-1)	Shoot length (cm)				Mean
	ZnO	Ag	CuO	TiO ₂	
750	7.2	6.7	6.5	6.5	6.725
1000	7.5	7.5	6.4	6.7	7.025
1250	7.3	7.4	6.7	6.7	7.025
1500	7.4	7.5	6.5	6.4	6.95
Mean	7.3	7.3	6.5	6.6	6.925
Control	6.0				
	T	D	TD		
SEd	0.09	0.08	0.18		
CD	0.17**	NS	NS		

Table.3 Effect of nanoparticles on root length (cm) of stored (6 months old) seeds of onion (CO 5)

Treatments (mg /kg-1)	Root length (cm)				Mean
	ZnO	Ag	CuO	TiO ₂	
750	6.2	5.6	5.2	5.3	5.575
1000	6.4	6.3	5.3	5.4	5.85
1250	6.4	6.2	5.5	6.4	6.125
1500	6.3	5.8	5.6	6.3	6
Mean	6.3	6.0	5.4	5.9	5.9
Control	5.4				
	T	D	TD		
SEd	0.07	0.06	0.15		
CD	0.15**	0.13**	0.30**		

Table.4 Effect of nanoparticles on vigour index of stored (6 months old) seeds of onion (CO 5)

Treatments (mg /kg-1)	Vigour index				
	ZnO	Ag	CuO	TiO ₂	Mean
750	858	796	704	707	766.25
1000	998	958	775	795	881.5
1250	954	918	798	779	862.25
1500	909	887	735	791	830.5
Mean	929	890	753	768	835
Control	692				
	T	D	TD		
SEd	11.50	10.28	23.00		
CD	22.64**	20.25**	NS		

Characterization of Nanoparticles (ZnO, Ag, CuO and TiO₂)

Nanoparticles	Morphological Descriptions	
	SEM	TEM
ZnO	Lanceolated nanoscaled rods measuring 50-80 nm diameter; appeared to be radiating from a central core (Plate 1a)	Rod shaped fused at centre to form a radiating structure as observed in SEM (Plate 2a)
Ag	Appeared as a bundle of spheres measuring for 400-450 nm (Plate 1b)	Spherical in shape with a size ranging from 50 -100 nm (Plate 2b)
CuO	Uniform spherical to oval sized particle measuring 60 – 150 nm (Plate 1c)	Uniform crystalline particles measuring 80-140 nm (Plate 2c)
TiO₂	Irregular spherical shaped peanut like particle with an average diameter of 120 nm (Plate 1d)	Rutile nano particle, primarily tetragonal in shape with an average size of 100 nm (Plate 2d).

The values recorded for control was 60 per cent. Among the nanoparticles treatment, seeds treated with ZnO NPs @ 1000 mg kg⁻¹ had the highest germination of 72 per cent which was followed by Ag NPs @ 1000 mg kg⁻¹ (69%). Control recorded the lowest germination (60%) (Table 1). Among the dosages, seeds treated @1000 (72%) found to register maximum germination than other dosages. Interaction among the NPs and dosage revealed that ZnONPs @ 1000 mg kg⁻¹ and Ag NPs @ 1000 mg kg⁻¹ recorded in the maximum germination of 72 percent while the minimum in the seed treated with TiO₂ at the 750 mg kg⁻¹. The beneficial effect of the ZnO NPs in improving the germination

could be ascribed to higher precursor activity of nanoscale zinc in auxin production. Apart from this, zinc is one of the essential nutrients required for plant growth. It is an important component of various enzymes that are responsible for driving many metabolic reactions in all crops. Zinc oxide NPs are reported to also exhibit positive effect on the reactivity of phytohormones especially Indole Acetic Acid (IAA) facilitating in the phytostimulatory actions. Zinc-rich ZnO NPs could increase the level of IAA in roots (sprouts), which in turn can increase growth rate of seedlings. Enhanced physiological parameters could be attributed and quenching of free radicals by nanoparticles which could

entered through cracks present seed coat, reached into free radicals resulting in enhanced seed vigour.

Nanoparticle treated germinated seeds exhibited higher root and shoot length than control. ZnO NPs treated seeds induced maximum shoot length (7.5 cm) compared to control (6.0 cm) after six months of storage. Among the different nano particle treatments, seeds treated with ZnO and Ag NPs @ 1000 mg kg⁻¹ produced the lengthiest shoot length (7.5 and 7.5 cm) than control (6.0cm) (Table 2). Nanoparticles treatment did not influence the root length of seedlings immediately after treatment. After six months of storage, nanoparticles treated seeds had higher root length (6.4cm) compared to control (5.4 cm). Among the different nanoparticle treatments, seed treated with ZnO NPs @ 1000 and TiO₂ @ 1250 mg kg⁻¹ produced the lengthiest root (6.4 cm) than control (5.4 cm) (Table 3). Such promoting effect of nanoscale SiO₂ and TiO₂ on germination was reported in soya bean, in which authors noticed increased nitrate reductase enzyme activity and enhanced antioxidant system. Similar results were observed by (Zheng *et al.*, 2005) when *Spinacia oleracea* seeds were treated with nanoscale TiO₂ particles.

The results revealed the promotory effect of ZnO nanoparticles at optimum concentrations and inhibitory effect at high concentrations on root and shoot growth. An increase of the shoot/root ratio compared to that of the control was reported by (Shah and Belozerovala, 2009) while analyzing the influence of metal nanoparticles on germination of *Lactuca* seeds.

Significant variation was observed for vigour index due to nano seed treatment, their dosages and their interactions (Table 4). Among the nano seed treatments, seeds treated with ZnO NPs resulted in maximum

vigour index (998) than other treatments and the control (692). Interaction between nano seed treatment and its dosages revealed that highest vigour Index was observed in seeds treated with ZnO @ 1000 mg kg⁻¹ (998) which was followed by Ag @ 1000 mg kg⁻¹ (958).

The beneficial effect of nanoparticle in improving the seed quality may be attributed that nano particles would induce oxidation-reduction reactions via the superoxide ion radical during germination, resulting the quenching of free radicals in the germinating seeds. In turn, oxygen produced in such process could also be used for respiration, which would further promote germination. The experiments carried out by (Senthil kumar, 2011) revealed that black gram seeds treated with ZnO nano rods and ZVI NPs enhanced the physiological and biochemical properties resulting in improved vigour and viability of aged seeds. The reason attributed was the donation of electrons by the nano particles in scavenging the free radicals in the aged seeds.

Nanoparticles tested in the investigation were supportive in enhancing the germination and seedling vigour of the onion seeds which are supposed to be highly prone for deterioration in storage. Application of nanoparticles especially ZnO @ 1000mg kg⁻¹ seed improved germination and related physiological parameters. However, the findings are to be verified under large scale field condition before recommending to farmer for adoption.

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How to cite this article:

Anandaraj, K. and Natarajan, N. 2017. Effect of Nanoparticles for Seed Quality Enhancement in Onion [*Allium cepa* (Linn) cv. CO (On)] 5. *Int.J.Curr.Microbiol.App.Sci.* 6(11): 3714-3724. doi: <https://doi.org/10.20546/ijcmas.2017.611.435>