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Inorganic metal oxide nanoparticles seed invigouration for extended storability of sunflower (*Helianthus annus*) under ambient environment

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Abstract
An experiment was conducted to enhance viability of sunflower seeds during ageing under ambient environment. In this study, sunflower seeds were treated with Zinc oxide (ZnO NPs), Titanium oxide (TiO_2NPs) and Copper oxide (CuONPs) at 1000 mg kg^{-1},750 mg kg^{-1} and 1250 mg kg^{-1}, packed in moisture impervious containers and stored under ambient conditions along with untreated seeds. Seed quality parameters such as physiology and biochemical attributes besides volatile organic compounds (VOCs) emanation were evaluated at monthly interval, and the results demonstrated that ZnONPs seed invigouration at 1000 mg kg^{-1} excelled the other metaloxides in maintaining the seed storability in a period of six month of storage, which was 5 percent increase in germination over control (80% germination and seedling vigour of 2362). Similarly the emanation VOCs (aldehydes, ketones, alcohols, acids, esters and ethers were found to be less (14 compounds) in ZnONPs invigoured seeds than control where about 26 VOCs compounds were emanated. This study conclude that ZnONPs seed dressing could be a viable seed invigorant for extending the storability of sunflower under ambient conditions.

Keywords: Nanoparticles, sunflower, volatile emission, seed quality

Introduction
Sunflower (*Helianthus annus*) is one of the important oilseed crop being cultivated in an area of 26 million hactares with the production of 45 million metric tonnes/annum in the world. Similarly in India sunflower occupies 1.48 million hactare with 200 metric tonnes of production, In Tamil Nadu 10 metric tonnes production. Sunflower seeds rich in nutrient components such as vitamin, minerals, antioxidants, diatary fibre, phytochemicals and poly unsaturated fatty acid like linoleic and oleic constituted about 80%. In many parts of India and world, sunflower seeds are usually stored for a period of about one year before sowing. Neverthless, seed germinability deteriorates quickly due to lipid peroxidation (Kibinza *et al.*, 2006) [15] during storage. As the current technologies available to prolong the vigour and viability of sunflower seed on a large scale are not satisfactorily alleviating the practical problem, an alternative simple and practicable seed invigouration treatment to control seed deterioration of sunflower is need of the hour. These are scientific reports evidenced the nanomaterials invigouration on seed quality enhancement in wide array of crops (Raja *et al.*, 2019; Dhileepkumar *et al.*, 2015) [6]. Natarajan and Sivasubramanian (2008) [10] stated various Nano technological approaches that can be employed in Seed Science. The approaches include nano-polymer for seed Harding, Nano Particles (NPs) for seed quality enhancement, nano-sensors, nano-barcodes, use of nano-magnetic particles for aerial seeding etc. Successful attempts were made in China to counteract free radicals in spinach by using titanium nano-particles and improve the germination (Lu *et al.*, 2002) [7]. Krishnashyla *et al.*, (2016) reported that. Krishnashyla *et al.*, 2016 reported nanoparticle treated seeds performed well in improving the seed germination, shoot length, root length and seedling vigour and also it posses higher catalase, peroxidase and dehydrogenase activity with less amount of electrical conductivity volatile organic compounds emanated in groundnut under ambient environment. ZnO and ZVI nanoparticles seed dry dressing found to be enhanced the quality of blackgram seeds under storage (Senthil Kumar, 2011) [14]. Sridhar (2012) [17] has shown that dry dressing of ZnO nanorods @ 1000 and 1250 mg kg^{-1} had improved germination and vigour index and lipid peroxidase activity of tomato seeds stored under natural ageing. Therefore, this study focused to reveal the impact of metaloxide nanoparticles such as ZnO, CuO and TiO_2 NPs on the vigour and viability of sunflower seed.

Material and Methods

The present study was carried out at Department of Seed Science and Technology and Department of Nano Science and Technology in Tamil Nadu Agricultural University, Coimbatore during 2019-2020. Sunflower hybrid seeds (COH3) obtained from Department of Oilseeds, Tamil Nadu Agricultural University, Coimbatore-3 constituted as study material.

1. Metal oxides nanoparticles seed invigouration

Freshly harvested seeds were obtained dried to a moisture content of 6% by sun drying and graded by specific gravity separator. After grading seeds were dry dressed with ZnO, TiO_2, CuO @ 1000,750 and 1250 mg kg^{-1}, respectively as follow.

2. Nanoparticles seed invigouration (dry dressing)

Sunflower seeds and required dose of respective NPs were kept in screw capped glass bottles at room temperature. The glass bottles were gently shaken for 5 times each at 3 times at an interval of 3h, manually. Seeds shaken without nps considered as control. Then treated seeds were stored under ambient storage condition with (25±3 ºC) temperature and 95% RH. Seed samples were taken at monthly intervals up to 6 months and evaluated for the following seed quality attributes. The experiment was designed in Factorial Completely Randomized Design (FCRD) with four replication.

Germination (%)

The germination test was conducted in germination room with a temperature of 25±3 ºC and 4 replication of 100 seeds were tested. At the end of the 10^{th} day (Final count) the normal, abnormal seedlings were counted and mean data values were expressed as percentage.

Root length (cm)

From germination test, at the final count, ten normal seedlings were randomly selected and then root length was measured from root tip region to collar region and expressed in centimeter.

Shoot length (cm)

The seedlings selected for measuring the root length were also used for taking shoot length and expressed in centimeter.

Vigour Index (Abdul-Anderson Bakiand (1973)

It was calculated using a formula of germination % and seedling length (cm) and expressed as a whole number.
(Eqn.1)
Vigour Index = Germination % × Seedling length (cm) (1)

Electrical conductivity (dSm^1) (Presley 1958) [12]

25 seeds of each replication samples were soaked in 25 ml of distilled water for 6 h at normal room temperature. Then the seed leachate was decanted and electrical conductivity was measured in electrical conductivity meter. Finally the mean value was expressed in dSm^{-1}.

Dehydrogenase activity (OD) (Kittock and Law. 1968) [4]

The 5 embryonic axis were soaked in 0.1% of Tetrazolium solution incubated at 40 ºC at dark condition for 2h. Then the Stained embroyonic axis wash with water and soaked in 5ml of 2 methoxy ethanol overnight period. Methyl cellosolve used as a blank and reading were taken using spectrophotometer @ 470nm.

Catalase Activity (μ $H_2O_2mg^{-1}$ min^{-1}) (Aebi 1984) and *Peroxidase Activity* (Malik and Singh 1980). Moreover, volatile aldehyde profile was determined using Gas Chromatography Mass Spectrometry(GC-MS) @ DSQII GC model with a carrier gas of Helium (99.99%) using source temperature of 200°C and injector temperature of 250°C in order to find out the relationship with seed quality.

Statistical analysis

The data recorded from different laboratory experiments were analyzed statistically by adopting the procedure described by Panse and Sukatme (1985) [11]. Whenever required the percent values was transformed to angular values. At 5% probability level critical difference (CD) were computed. Volatiles emitted from the stored seeds were correlated with germination as per the procedure given by Dewey and Lu. (1959) [7].

Results and Discussion

The dissertation was conducted to assess the influence of metaloxide NPs on seed storability of sunflower seeds (COH3) under natural ageing. Dry dressing of sunflower seeds with ZnO, TiO_2 and CuO NPs @ 1000,750 and 1250 mg kg^{-1} performed well as compared to control during ageing. Significance difference observed among NPs seed treatments. Seeds invigourated with ZnO at 1000 mg kg^{-1} of seeds recorded higher germination percentage (85%),root length (25.9)cm & shoot length (14)cm, vigour index (2544) and dry matter production (0.300) (Table1,2 & 3). This was 5 percentage higher in germination, root & shoot length, vigour index and dry matter production over untreated seeds where the minimum germination of 80%,root length (23.8)cm, shoot length (13.3)cm, vigour index (2362) and dry matter production (0.261) were observed at the end of six month of storage. Similarly the ZnO NPs treated seeds registered low electrical conductivity of seed leachate (1.003) at six months of ageing while it was higher in untreated seeds (1.175) (Table 3). The seeds treated with metaloxide NPs have outperformed in recording dehydrogenase and anti-oxidant enzymes activity over a period of six month storage. Seeds treated with ZnO NPs had higher dehydrogenase activities than other metaloxide NPs and control which recorded the minimum dehydrogenase (1.280), catalase (1.273) and peroxidase (0.366) enzyme activities (Figure 1). The outperformance of ZnO NPs in maintaining the viability under ambient environment is by quenching of free radicals by donating free electrons to pair with unpaired electrons that protect the cell membrane integrity and avoid cell collapse (Krishnashyla *et al.*, 2016) and this was also evidenced in observing less number of VOCs emanated from stored seeds in present study (Figure 2). The higher germination and seedling vigour in ZnO NPs invigourated seeds is due to higher precursor activity of nanoscale zinc in auxin production (Kobayashi and Mizutani, 1970) [5]. Sagili *et al.*, 2017 reported ZnO nanoparticle improved seed quality parameters over control seeds in spinach. Anandaraj (2017) stated that ZnO NPs seed dry dressing at 1000 mg kg^{-1} increased the seedling growth attributes and maintained the quality of the paddy seeds. Pandey *et al.*, 2010 observed increased level of Indole Acetic Acid (IAA) in roots, which can increase growth rate of seedlings in *Cicer arietinum* treated ZnO NPs. Moreover, ageing was also associated with a decrease in the activity of superoxide dismutase, catalase, peroxidase enzyme activity (Kibinza *et al.*, 2006) [15]. Seeds treated with nanoparticles had low electrical conductivity in the seed leachate implying the probable role of nanoparticles in curing the damaged membranes and recording more of anti-oxidant enzymes such as catalase and peroxidase enzymes activities (Krishnashyla *et al.*, 2016).

Table 1: Effect of nanoparticles on Germination (%) and vigour index of sunflower COH3 under natural ageing

Treatment	Germination (%) Period of storage							Mean A	Vigour index Period of storage							Mean A
	P0	P1	P2	P3	P4	P5	P6		P0	P1	P2	P3	P4	P5	P6	
Control	97 (80.06)	95 (77.61)	94 (76.02)	90 (72.33)	89 (70.63)	83 (65.66)	80 (63.42)	89 (72.25)	3846	3732	3541	3199	2664	2537	2362	3126
ZnO1000mg	97 (80.32)	96 (78.59)	94 (76.73)	93 (74.81)	91 (72.55)	89 (72.55)	85 (67.79)	92 (74.49)	3882	3952	3735	3517	2812	2759	2544	3314
TiO750mg	97 (79.99)	94 (76.16)	92 (73.91)	90 (71.55)	88 (69.70)	83 (65.64)	81 (64.32)	89 (71.61)	3752	3541	3418	2930	2673	2430	2309	3008
CuO1250mg	96 (79.60)	95 (77.14)	93 (74.65)	91 (73.28)	90 (71.64)	86 (68.66)	84 (66.39)	91 (73.05)	3866	3801	3663	2946	2733	2674	2473	3165
Mean B	96 (79.99)	95 (77.37)	93 (75.33)	91 (72.99)	89 (71.13)	85 (67.65)	82 (65.48)		3836	3756	3589	3148	2720	2600	2422	
	T			P			T×P		T			P			T×P	
SEd	0.442			0.585			1.170		16.765			22.179			44.357	
CD	0.881			1.165			2.331		33.400			44.184			88.368	

Table 2: Effect of nanoparticles on shoot length (cm) and root length (cm) of sunflower COH3 under natural ageing

Treatment	Shoot length(cm) Period of storage							Mean A	Root length(cm) Period of storage							Mean A
	P0	P1	P2	P3	P4	P5	P6		P0	P1	P2	P3	P4	P5	P6	
Control	16.1	15.3	15.1	14.6	14.2	13.8	13.3	14.6	29.7	28.9	28.2	27.5	25.7	25.2	23.8	27.0
ZnO1000mg	16.5	16.2	15.9	15.6	14.9	14.5	14.0	15.4	30.1	29.3	29.0	28.6	26.8	26.4	25.9	28.0
TiO750mg	15.9	15.4	15.1	14.5	14.1	13.6	13.2	14.6	29.7	28.6	28.2	27.4	25.5	25.5	23.5	26.9
CuO1250mg	16.3	15.8	15.6	14.9	14.5	14.0	13.8	15.0	30.0	29.0	28.8	27.9	26.4	26.0	24.9	27.5
Mean B	16.2	15.7	15.4	14.9	14.4	13.9	13.6		29.9	28.9	28.5	27.8	26.1	25.7	24.5	
	T			P			T×P		T			P			T×P	
SEd	0.075			0.099			0.198		0.143			0.189			0.377	
CD	0.149			0.198			0.395		0.284			0.376			0.752	

Table 3: Effect of nanoparticles on dry matter production (g $10 seedling^{-1}$) and electrical conductivity of sunflower COH3 under natural ageing:

Treatment	Dry matter production(g $10seedling^{-1}$) Period of storage							Mean A	Electrical conductivity Period of storage							Mean A
	P0	P1	P2	P3	P4	P5	P6		P0	P1	P2	P3	P4	P5	P6	
Control	0.537	0.413	0.368	0.352	0.292	0.285	0.261	0.358	0.594	0.631	0.698	0.744	0.896	0.988	1.175	0.818
ZnO1000mg	0.584	0.460	0.395	0.354	0.328	0.309	0.300	0.390	0.530	0.628	0.661	0.702	0.831	0.927	1.003	0.755
TiO750 mg	0.529	0.416	0.353	0.328	0.280	0.266	0.245	0.345	0.581	0.679	0.688	0.716	0.863	0.983	1.182	0.813
CuO1250mg	0.542	0.435	0.379	0.350	0.320	0.300	0.284	0.373	0.562	0.641	0.673	0.721	0.875	0.945	1.163	0.797
Mean B	0.548	0.431	0.374	0.346	0.305	0.290	0.273		0.567	0.645	0.680	0.721	0.866	0.961	1.131	
	T			P			T×P		T			P			T×P	
SEd	0.002			0.003			0.005		0.004			0.006			0.012	
CD	0.004			0.005			0.011		0.009			0.012			0.023	

Effect of Nanoparticles treatment on seed volatile emission

A different types of volatile compounds was produced by seeds during storage suggesting that several reactions like (maillared reaction and lipid peroxidation) occur during deterioration in seeds, some of which reactions are detectable by volatile analysis (Zhang *et al.*, 1997, Taylorson *et al.*, 1999) [19, 18]. Different types of volatiles molecules produced from lipid peroxidation process can act as a indicator for detecting seed viability. This investigation was made to find out the influence of metaloxide nanoparticles on volatile emission of six months stored seeds. Nanoparticle treated seeds emanated less number of volatile compounds as compared to untreated seeds under natural ageing. In this study, in ZnO NPs invigourated seeds only 14 volatile compounds (Alcohols, acids, ether, aldehydes, ketones) were found to be emanated at six months of storage whereas about 26 VOCs were observed in untreated seeds (Figure 2). Akimoto *et al.*, 2004 [2] and Mira *et al.*, 2010 [9] who reported on the release of acetaldehyde, ethanol and methanol as major volatile constituents of the seeds. Krishnashyla *et al.*, 2016 reported that volatiles emanated from the seeds treated with NPs revealed that the number of compound emitted under aldehydes, ketone, acids, ether, ester and carboxyl groups was less (20) while the control had (36) compounds, expressing a negative correlation to the germination.

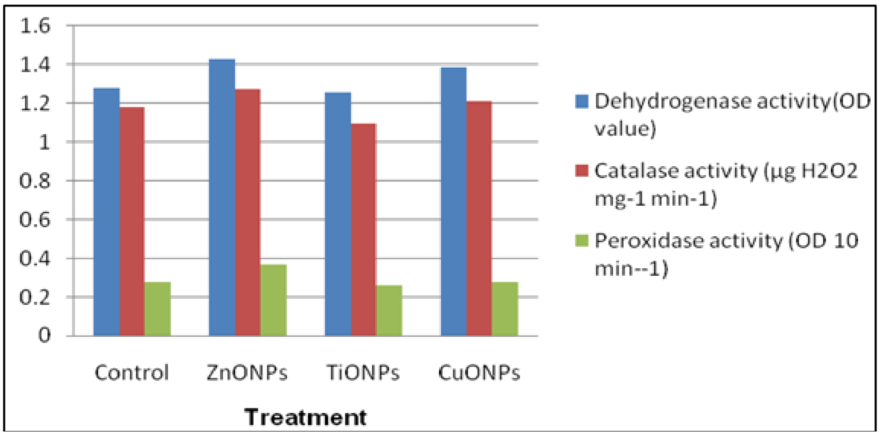

Fig 1: Effect of nanoparticles on dehydrogenase, catalase and peroxidase activity

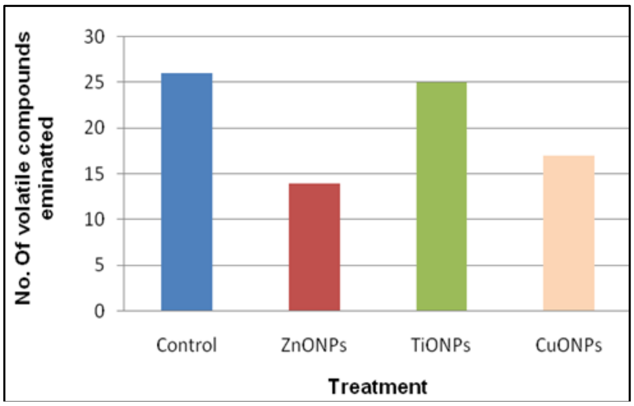

Fig 2: Effect of nanoparticles on volatile emission

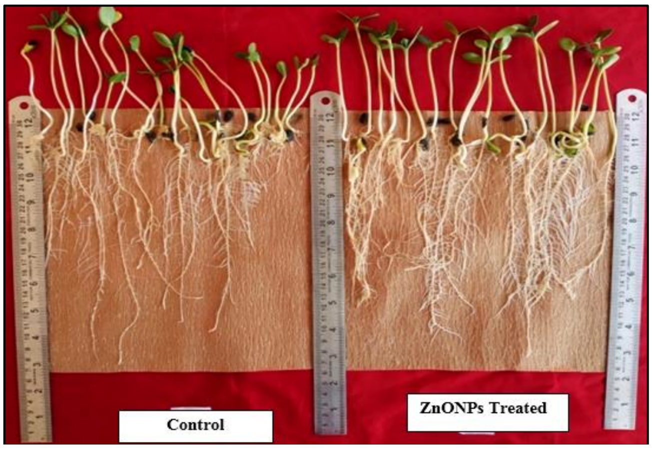

Fig 3: Effect of nanoparticles on seed germination

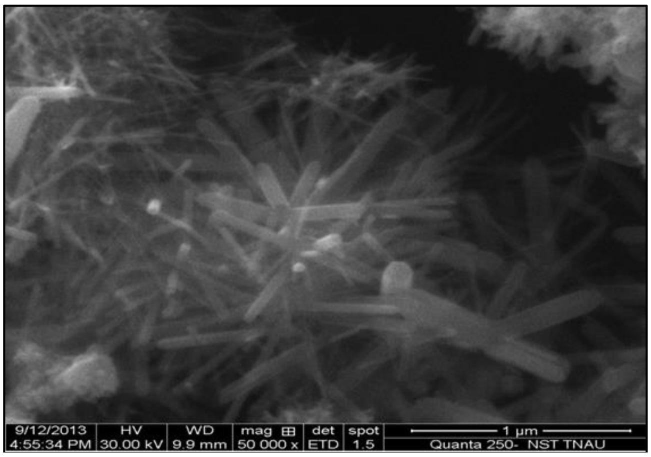

Fig 4: SEM image of zinc oxide nanoparticles (ZnONPs)

Conclusion

Finally it concluded that inorganic metal oxide nanoparticles particularly Zinc oxide @ 1000 mg kg^{-1} excelled other nps and performed better over control and also NPs increase the seedling growth characters *viz*., germination, root length, shoot length and vigour index, dry matter production while control has less germination. And also ZnONPs maintains the quality attributes than control.

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