SEED INVIGORATION WITH INORGANIC NANOPARTICLES IN CHILLIES (*Capsicum annuum* L.)

by

GAYATHRI SATHEES

(2017-11-083)

DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

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THESIS

Submitted in partial fulfilment of the requirements for the degree of

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DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA
2019

DECLARATION

I hereby declare that this thesis entitled “**Seed invigoration with inorganic nanoparticles in chillies (*Capsicum annuum* L.)”** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellanikkara **Gayathri Sathees**

Date: (2017-11-083)

CERTIFICATE

Certified that this thesis entitled “**Seed invigoration with inorganic nanoparticles in chillies (*Capsicum annuum* L.)”** is a record of research work done independently by **Ms. Gayathri Sathees (2017-11-083)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellanikkara

Date:

Dr. Dijee Bastian

(Major Advisor, Advisory Committee)
Professor (Plant Breeding and Genetics)
Department of Seed Science and Technology
College of Horticulture
Vellanikkara

CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Gayathri Sathees (2017-11-083)**, a candidate for the degree of **Master of Science in Agriculture** with major field in Seed Science and Technology, agree that the thesis entitled **"Seed invigoration with inorganic nanoparticles in chillies (*Capsicum annuum* L.)"** may be submitted by **Ms. Gayathri Sathees,** in partial fulfilment of the requirement for the degree.

Dr. Dijee Bastian
(Chairman, Advisory Committee)
Professor (Plant Breeding and Genetics)
Dept. of Seed Science and Technology
College of Horticulture
Vellanikkara

Dr. Rose Mary Francies
(Member, Advisory Committee)
Professor and Head
Dept. of Seed Science and Technology
College of Horticulture
Vellanikkara

Dr. K. Raja
(Member, Advisory Committee)
Assistant Professor (Seed Science and Technology)
Dept. of Nano Science and Technology
Tamil Nadu Agricultural University
Coimbatore

Dr. Vimi Louis
(Member, Advisory Committee)
Professor (Plant Pathology)
Banana Research Station
Kannara

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Gayathri Sathees

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LIST OF ABBREVIATIONS

AOSA : Association of Official Seed Analysts

ISTA : International Seed Testing Association

mL : milli litre

h : hours

EC : electrical conductivity

mg : milli gram

kg : kilo gram

µS cm^{-1} : micro Siemens per centimetre

mm : milli metre

nm : nano metre

m : metre

µm : micro metre

°C : degree Celsius

Pa : Pascal

ANOVA : Analysis of Variance

ZnO : zinc oxide

nZnO : nano-zinc oxide

TiO_2 : titanium dioxide

$nTiO_2$: nano-titanium dioxide

CD : critical difference

SE$_m$: standard error of error mean sum of squares

var. : variety

cv. : cultivar

SEM : Scanning Electron Microscope

AA : accelerated ageing

NP : nano particle

IMSCS : Indian Minimum Seed Certification Standard

mg/kg : milligram per kilogram

°N : degrees north

°E : degrees east

G : gauge

ISTA : International Seed Testing Association

MAS : months after storage

% : per cent

et al. : *et alia* (Latin: ‘and others’)

V.I. : vigour index

PDA : Potato Dextrose Agar

Introduction

1. INTRODUCTION

Capsicum annuum L. is an annual crop belonging to family Solanaceae and grown in temperate and tropical regions. It is known by different names like chilli, chili, pepper, hot pepper, chile pepper, sweet pepper or bell pepper (OECD, 2006). Chilli originated in South America and has been under cultivation since 7000 B.C. It was introduced to India in the 16th centuary probably by Portuguese and Arab traders (Antonio *et al.*, 2018). India has now become one of the largest producers of chilli in the world after China, Mexico, Turkey and Indonesia (FAO, 2017). Andhra Pradesh, Maharashtra, Karnataka, Orissa and Madhya Pradesh are the leading chilli producing states in India.

One of the major constraints in chilli is its poor seed storage and quality maintenance, under fluctuating environmental conditions. Seeds from most of the agricultural crops undergo a storage phase, at the least from harvest to the next planting. It could be longer for seeds used for breeding programmes and those preserved as safeguard against natural calamities in seed banks across the world.

The seed coat of chilli is paper thin and promotes easy emergence of radicle and shoot. But the thin seed coat makes the seed highly sensitive to the external environment, and increase the chances of seed death (Manjunath *et al.*, 2008). The major source of food for chilli seed embryo is the surrounding endosperm containing proteins and lipids as the main reserves (Chen and Lott, 1992). Zou *et al.* (2015) profiled the fatty acid content in chilli seeds and found high proportion of linoleic acid (16.82 g/100g), palmitic acid (3.31g/100g), oleic acid (1.45g/100g), stearic acid (0.61g/100g) and linolenic acid (0.55g/100g). The accumulation of free fatty acids could lead to reduction in germinability and even loss of viability as a result of lipid auto oxidation during ageing. Even though the rate of seed deterioration depends on storage conditions, numerous studies have reported that the magnitude of seed ageing of different plant species stored under similar conditions varies (Walters *et al.*, 2005). The variability in seed quality of the same lot is also dependent on the genetic constitution of the seed and pre-harvest conditions to which it was exposed. It can be maintained to some extent by adopting modern technologies. However, in most of the crops, seed storage for at least one planting season is inevitable when deterioration also occurs (Soltani *et al*., 2009). Hence, it becomes necessary to adopt measures to safeguard the life of seed and maintain its vigour for extended period.

Since time immemorable numerous attempts have been made to improve the storability of seeds. Seeds can be stored in controlled environment (refrigeration) or after treating with chemicals or botanicals in ambient conditions. The seed treatment techniques commonly used for maintaining storability are priming, seed coating, pelleting, film coating and seed conditioning. Seed invigoration or seed enhancement is a series of post-harvest techniques employed to improve germination and seedling growth and thereby ensure the availability of seeds at the time of sowing (Taylor *et al.,* 1998). Nanotechnology opens a novel frontier in this regard.

Nanotechnology holds the key for many novel breakthroughs in the fields of agriculture and biotechnology due to their unique characteristics such as high surface area, reactivity and diverse pore size. Nano particles can be used as “magic bullets” for targeted delivery of pesticides, fertilizers and even genes to a particular cell organelle. In agriculture, nanotechnological applications have found their way into soil and water conservation, seed quality improvement, weed management, crop improvement, plant protection, stress tolerance, bioremediation etc. More advanced usages include insertion of genetic material into plants using functionalized nanoparticles and using nano-sensors to keep track of the temporal and seasonal changes in plant and soil system. In the field of seed technology, quantum dots (QDs) are used to separate viable seeds from non-viable and infected seed lots (Thakur and Maiti, 2016).

As Kerala experiences a typical tropical climate with high humidity (70-90 %) and high temperature (25-37°C) throughout the year, safe storage of seeds is a difficult task. The attack of insect pests and seed-borne micro-organisms are also aggravated in this situation. Even though it is not possible to completely stop senescence of seeds, use of metal oxide nanoparticles for seed treatment helps to slow down the deteriorative processes associated with ageing. One of the mechanisms of protection of nanoparticles is by the quenching of free-radicals in the seeds by oxidation-reduction reactions (Zheng *et al.,* 2005). Oxygen released during this process further promotes respiration of the germinating seeds. The most commonly used NPs are metal oxides of zinc (Zn), titanium (Ti), copper (Cu), silver (Ag), silicon (Si) and iron (Fe). Nanoparticles mixture such as nano-SiO_2 and nano-TiO_2 can promote germination and growth of soybeans (Lu *et al.,* 2002). Other modes by which NPs improve seed quality is through use the of nano-polymer for seed hardening, nano-sensors, nano-barcodes and use of magnetic NPs for aerial seeding. (Natarajan and Sivasubramanian, 2008).

Fungal growth on seeds can be detrimental to germination due to loss of carbohydrates, protein and total oil content, and increased fatty acid leading to other deteriorative biochemical changes. The particle size efficiency of NPs over its bulk counterparts can reduce the load of unnecessary plant protectant chemicals in the environment. The sole use of metal nanoparticles as anti-microbial agent is now possible with the technological advancements in nanomaterial production, making it economical (Jo and Kim, 2009) and comparatively safer option of plant pathogen management than synthetic fungicides (Min, 2009).

With the above considerations, the present investigation entitled 'Seed invigoration using inorganic nano-particles in chillies (*Capsicum annuum* L.)' was formulated with the following objectives:

i. To standardise the optimum dose of treatment chemicals to prolong seed longevity and improve viability during storage
ii. To compare the efficacy of zinc oxide and titanium dioxide based on their particle size (normal-size and nano-sized powder)
iii. To study the morphological changes occurring at cellular level in the roots of NP-treated seeds
iv. To elucidate the effect of seed treatment on chilli seeds subjected to accelerated ageing

Review of Literature

2. REVIEW OF LITERATURE

Seed is the basic unit of agriculture and quality seeds alone could improve the yield up to twenty per cent. Hence, it is imperative to take measures to prolong the quality of seeds. The efficiency of nanoparticle used for seed treatment in this regard has been reviewed in this chapter.

2.1. Factors affecting seed quality during storage

Storage of seeds begin in the field from physiological maturity. Phases of storage starts from harvest to packaging until distribution of the packed products. It is followed by in-transit storage and on-farm storage until planting in field. The significant factors affecting the seed quality during all these stages are temperature, relative humidity, oxygen pressure and seed moisture content (Bewly and Black, 1982).

Harrington's thumb rule for seed storage states that for every 5°C increase in temperature and 1 per cent increase in relative humidity surrounding the seed, its life span declines by half (Harrington, 1959). This is applicable for all orthodox seeds in the moisture range of 5-14 per cent. Below 5 per cent, the seed deterioration is caused by lipid auto oxidation, while above 14 per cent moisture content seeds become susceptible to seed-borne pathogens.

The safe moisture content for seeds depend on the crop, type of storage material used, duration of storage and storage structure. It ranges from 8-12 per cent in almost all cereals and millets and 5-8 per cent in vegetable crops. Other factors like the oil content of the seed, pre-harvest conditions experienced by the seed lot, and microbial interaction with seed also have a role to play in the maintenance of seed quality (Bewly and Black, 1982).

2.2. Seed deterioration

Seed viability is the ability of seed to maintain a state of liveliness in its embryo and is an indicator of the anabolic metabolisms taking place inside the seed. Loss of vigour is an intermediate stage in the life of seed before terminating in death. Seed deterioration has been described as a cumulative, irreversible, inexorable and degenerative process. According to Ellis *et al.* (1985), it is the loss of quality, viability and vigour either due to ageing or as the effect of adverse environment.

Morphological changes are the visible signs of seed degradation. Seed coat acts as the first level of defence against external environment and is the most assured form of protection a seed has naturally to protect its embryo. Deterioration was faster in seeds with cracked, papery and wrinkled seed coats (Yasue and Kinomura, 1984). The seed coat colour might also be indicative of the state of seed deterioration as colour changes are associated with oxidation reactions occurring in seed at high temperature and humidity.

Membrane degradation is one of the primary causes of seed deterioration. Parrish *et al.,* (1982) suggested that electrolyte leakage of aged seeds occurred as a result of this membrane damage. Auto oxidation of the lipids present in the plasma membrane due to the action of free radicals is one of the reasons for loss of membrane integrity. The loss of phospholipids could be either due to the action of phospholipase enzyme during ageing or lipid peroxidation. There is also impairment of cell organelles like tonoplast, plasmalemma and endoplasmic reticulum.

Ultra-structural changes occurring during seed deterioration are decreased membrane fluidity, variations in the folding of DNA, loss of protein elasticity and friable cellular matrix (Walters *et al.,* 2010).

Enzyme activity becomes reduced (lipase, ribonuclease, protease, catalase, amylase, dehydrogenase, peroxidase, DNase) during seed deterioration. There is an accumulation of singlet oxygen and hydrogen peroxide which could cause lipid peroxidation and produce malondialdehyde and lipid conjugates as by-products.

Cellular constituents show a reduction in proteins, oil content and total sugar, and a rise in the level of free fatty acids and reducing sugars (Verma *et al.,* 2003).

In some of the stored seeds, an increase in carbohydrate content was observed with a fall in protein content.

2.3. Role of nanoparticles in plant growth and seed quality

The term nanotechnology originated from Greek word nano meaning ‘dwarf’ and is used to express a size of 10^{-9} or one billionth of a metre. The term nanomaterial has been attributed to particles within the size range of 1-100 nm (Rai and Ingle, 2012). Their unusual properties are due to their small size, large surface area, high reactivity and tunable pore-size. Nanotechnology is defined as the study, designing, fabrication and manipulation of materials at the nanometric scale, *i.e.*, the transition scale between atoms and molecules to micro and bulk materials (Mohmmadi *et al*., 2019).

Most of the natural processes (physical and chemical) produce nanoparticles (NPs). Naturally occurring NPs are found in volcanic ashes, fine sand and dust and even as the product of bacteria and fungi (green synthesis - sulphur and selenium NPs). On the other hand, synthetic nano-particles may be generated by human activities inadvertently (mines, burning of diesel, smoke, soot) or be manufactured as per need. Engineered nanomaterials are categorized into four groups (USEPA, 2005), *viz.*, carbon-based materials (fullerene, carbon nanotube), metal based materials (quantum dots, nano-zinc, nano-aluminium, nano-metal oxides), dendrimers (nano-polymers with distinct chemical functions) and composites (a combination of nano- and bulk-particles or different nanoparticles).

Nano-863 is an agricultural product of China made by the addition of light-absorbent nanomaterials to a ceramic carrier followed by high temperature sintering (forming a compact of material by heat). When seeds of cowpea, cabbage and cucumber were incubated in nano-863 treated water, an improvement in the germination rate was observed (Huang *et al.*, 2015).

Nanoparticles can enter the plant system through seed coat, cracks or openings present on seed, root, root-shoot junctions or wounds on plant surface. The first barrier, among many (chemical and physiological), encountered by the NP is cell wall. Dietz and Herth (2011) noticed that cellulose present in cell wall allows the entry of smaller NPs (5-20nm), and not larger ones. Navarro *et al*. (2008) suggested that some nanoparticles may create larger gaps in cell wall forming a passage for the entry of bigger nano-particles.

It was found that the role NPs played was dependent on the plant species, its sensitivity, the dose of chemical used, mode of application and plant part contacting the NP. In nanoparticle-seed-treatment experiments done by Lin and Xing (2007), first plant organ to directly encounter the nano particle present on seed coat was the emerging radicle. While inhibitory effects were exhibited by the root tip at higher doses, positive outcomes were obtained at concentrations lower than the critical dose.

2.3.1. ZnO nanoparticles

In agriculture, zinc oxide nanoparticles (NPs) are used as nano-fertilizers because of their ability to alleviate zinc deficiency, and to ameliorate germination of seeds and plant growth. Being an essential micronutrient, zinc oxide is capable of increasing the dry matter production (Méndez-Argüello *et al.*, 2016) and total leaf area of plants due to its involvement in the production of carbohydrates, proteins, lipids, and nucleic acids in plants (Tarafdar *et al.*, 2013). Also, zinc oxide NP coated seeds of *Zea mays* (maize), *Glycine max* (soybean), *Cajanus cajanan* (pigeon pea) and *Abelmoschus esculentus* (lady’s finger) exhibited improved germination (Adhikari *et al.*, 2016).

Zinc oxide NPs can act as a precursor to auxin (IAA) production in plants and it help to promote germination (Kobayashi and Mizutani, 1970 and Pandey *et al.*, 2010).

2.3.2. TiO_2 nanoparticles

Yang *et al*. (2006) opined that titanium dioxide nano-particles can increase aged seeds’ vigour, accelerate chlorophyll production, hasten photosynthesis, and boost RUBISCO (ribulose 1,5-bisphosphate carboxylase) activity. It might be due to the increased activity of antioxidant enzymes (catalase, superoxide dismutase and peroxidase) defending the chloroplast from extreme light (Siddiqui *et al*., 2015). Zheng *et al.* (2004) suggested that titanium dioxide NPs re-activate aged seeds by the light-induced production of reactive oxygen species and quenching of free radicals in the seed cells by oxidation-reduction reactions. These processes could augment the water and oxygen intake by the cells, resulting in elevated metabolism and seed germination.

In the presence of ultra-violet light, titanium dioxide was found to dissociate into electrons and protons and hasten the photosynthetic rate (Zhao *et al.*, 2005). Bactericidal and sterilizing effect of titanium dioxide have also been reported.

2.4. Effect of nanoparticles on seed quality parameters

2.4.1. Germination

Crop	**Seed treatment**	**Effect on seed quality**	**Reference**
Spinach	Bulk and nano titanium dioxide	Spinach (*Spinacia oleracea*) seeds that were naturally aged for three years were soaked in nano- and non-nano-titanium dioxide solutions of 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 4.0, and 6.0% concentration for 48 h at 15°C under natural illumination. Treatment with nano-titanium dioxide (56.75 per cent) significantly increased the germination per cent of seeds compared to untreated (32.25 per cent) and non-nano-titanium dioxide (37.20 per cent) treated seeds.	Zheng *et al.*, 2005
Groundnut	Bulk zinc sulphate and nano zinc oxide	Seeds of variety K-134 with an initial germination of 85 per cent were treated with bulk zinc sulphate and nanoscale zinc oxide. Seeds treated with 1000 ppm nano zinc oxide recorded 100 per cent germination eight days after sowing.	Prasad *et al.*, 2012
Fennel	Nano and bulk titanium dioxide	Comparison of different concentrations of nanosized titanium dioxide at 0, 5, 20, 40, 60 and 80 mgL^{-1} with bulk-sized titanium dioxide was done. After 14 days of seed	Feizi *et al.*, 2013

		incubation, germination percentage highly improved following exposure to 60 ppm nanosized titanium dioxide. Final seed germination percentage was highest (76%) in seeds treated with 60 ppm titanium dioxide nanoparticles, whereas the lowest value (41%) was in seeds treated with 60 ppm bulk titanium dioxide particles. Application of 40 ppm nanosized titanium dioxide treatment improved mean germination time by 31.8 per cent in comparison to the untreated control.	
Canola	Nanoscale titanium dioxide	Canola seeds were separately treated with different concentrations of nanoscale titanium dioxide (10, 100, 1000, 1200, 1500, 1700 and 2000 mgL^{-1}). Treatment with nanoscale titanium dioxide (20 nm mean particle size) at 2000 mgL^{-1} concentration promoted both seed germination and seedling vigour. The lowest and the highest germination rate were obtained in 1500 and 2000 mgL^{-1} treatments, respectively.	Mahmoodzadeh *et al.*, 2013

Maize	Nano zinc oxide and zinc sulphate	Nano zinc oxide treated maize seeds (0.01–1000 µg/mL) were able to yield a higher germination compared to the treatment with zinc sulphate.	Pokhrel and Dubey, 2013
Rapeseed, lettuce and kidney bean	Nano-titanium dioxide	Nano-titanium dioxide did not have any significant effect on seed germination on all the three species tested.	Song *et al.*, 2013
Onion	Nano titanium dioxide	Seeds were treated with graded concentrations of titanium dioxide nanoparticles (00, 10, 20, 30, 40 and 50 g mL-1). Titanium dioxide NPs at lower concentration enhanced seed germination	Laware and Raskar, 2014
Groundnut	Zinc oxide NPs	VRI-2 seeds were treated with nano zinc oxide of 35-45 nm at various doses (750, 1000, 1250 mg kg^{-1} seeds). After 12 months of storage, seeds treated with zinc oxide NPs @ 1000 mg kg^{-1} were able to retain maximum germination (77%).	Shyla and Natarajan, 2014
Pigeon pea	Zinc and iron NPs	Seeds of pigeon pea were polymer coated with zinc and iron nanoparticles (10, 25, 50, 100, 250, 500, 750 and 1000 ppm). Seed coating with zinc NPs at 750 ppm registered significantly higher germination (96.00 %) and was on par	Korishettar *et al.*, 2016

		with zinc NPs at 500 ppm (95.30 %) and Fe NPs at 500 ppm (95.00 %).	
Chilli	Zinc oxide NPs	Chilli seeds were treated with zinc oxide NPs (0.0, 0.25,0.50 and 0.75g). Highest concentration (0.75g) of zinc oxide NPs depicted higher germination (65.7 %) whereas untreated seeds revealed to be the poorest performers (62 %).	Afrayeem and Chaurasia, 2017
Sorghum	Titanium dioxide NPs	In seeds of sorghum treated with nano titanium dioxide at various doses (750, 100, 1250 mg/kg of seed), the highest dose could exceptionally enhance the germination (97.3%).	Maity *et al*., 2018
Cowpea	Titanium dioxide NPs	Cowpea seeds treated with 1250 mg kg^{-1} of titanium oxide recorded maximum germination (97%) which was 17.3 per cent higher than control.	Maity *et al*., 2018
Okra	Titanium dioxide NPs	The seeds of okra were immersed in different concentrations of titanium dioxide NPs (25ppm, 50 ppm and 100 ppm) along with control. Seeds treated with titanium dioxide NPs at 100 ppm was able to improve the germination.	Reddy *et al.,* 2018
Maize	Nano titanium dioxide	Aged seeds were dry dressed with (200 to 800 $mgkg^{-1}$) titanium oxide nano particles. Germination was the highest	Vijayalakshmi *et al.*, 2018

		in seeds treated with 200 mgkg^{-1} titanium dioxide (88%), while lowest was in untreated control (70%).	

2.4.2. Seedling length

Crop	**Seed treatment chemical**	**Effect on seed quality**	**Reference**
Canola	Nano titanium dioxide	Higher concentrations of nanoscale titanium dioxide (1200 and 1500 mgL^{-1}) resulted in large plumule growth of seedling.	Mahmoodzadeh *et al.*, 2013
Maize	Nano zinc oxide and zinc sulphate	In maize, the primary root cells were structurally modified in shape and/or size at the zone of elongation upon exposure to metal NPs. Cells were elongated with both NP treatments. They appeared thinner and irregular with silver nitrate ($AgNO_3$) treatment; while with the zinc sulphate treatment, cells exhibited shorter and wider morphology, compared to the control. Phenotypic root elongation measurement was also in agreement with the microscopic observations of the cells at the zone of elongation.	Pokhrel and Dubey, 2013

Onion	Nano titanium dioxide	Root growth was more significantly affected negatively due to titanium dioxide NPs at higher treatment doses as compared to shoots.	Laware and Raskar, 2014
Sesame	Zinc oxide NPs	Different doses of nano zinc oxide (0.1, 0.25, 0.5, 1 and 2 g L^{-1}) synthesized by biological and chemical methods were used to treat seeds of *Sesamum indicum*. On 15th day after sowing, the seeds treated with nano zinc oxide @ 0.5 gL^{-1} recorded higher values of root length (6.93 cm) and shoot length (4.82 cm) compared to other treatments and control.	Narendhran *et al.*, 2016
Chilli	Zinc oxide NPs	Separate doses of zinc oxide NPs (0.0, 0.25,0.50 and 0.75 g) were prepared in distilled water to treat chilli seeds. Lower concentrations of zinc oxide NPs (0.25 and 0.50g) resulted in significant reduction in shoot and root lengths, whereas higher concentrations (0.75g) elevated the root length, shoot length and total seedling height.	Afrayeem and Chaurasia, 2017
Chickpea	Zinc oxide NPs and titanium dioxide NPs	The entire root formation was accelerated when treated with titanium dioxide nanoparticles. Root length and lateral root formation increased with augmenting doses of titanium	Hajra and Mondal, 2017

		dioxide NPs. In nano zinc oxide treated seeds, the seedling shoot was greater at 500 ppm dosage	
Pigeon pea	Zinc NPs, iron NPs, zinc sulphate and iron sulphate	The roots and shoots of seedlings developed from seeds treated with Fe NPs@25ppm (12.58 cm and19.58cm) and zinc NPs@25ppm (11.30 cm and 18.48 cm) were longer than those of control (8.9cm and 15.45cm).	Raju and Rai, 2017
Maize	Nano zinc oxide	Greater root length (14.4 mm), shoot diameter (3.4mm), root elongation as well as a supplemental number of secondary roots were observed in seeds treated with nano zinc oxide on comparison with starch-coated and uncoated seeds.	Estrada-Urbina *et al.*, 2018
Sorghum	Zinc oxide NP and titanium dioxide NP	Maximum root length (23.4 cm) was obtained in seeds treated with 750 mg/kg of nano zinc oxide which was 27% higher than control. An enhancement of root length was seen for all doses of titanium dioxide (750, 100, 1250 mg/kg) compared to control. Maximum shoot length was obtained at the highest dose (8.33 cm).	Maity *et al.*, 2018
Cowpea	Nano zinc oxide, nano-titanium dioxide	Root growth increased incrementally with doses of the nanoparticle applied with respect to control.	Maity *et al.*,2018

Maize and sesame	Zinc oxide NPs	On 15 DAS, the root length (6.93 cm), shoot length (4.82 cm) was excessive in nano zinc oxide treated maize plants with respect to untreated control. Seeds of sesame treated with 50 mg/L of zinc oxide nanoparticles gave the best performance when compared to control.	Segatto *et al*., 2018
Maize	Nano titanium dioxide	Shoot and root length of 200mg/kg treated maize seeds were 11.91cm and 19.45cm, whereas, in control it was 10.78 cm and 18.78 cm respectively.	Vijayalakshmi *et al.*, 2018
Wheat	Zinc oxide NPs and iron (Fe) NPs	Seeds were primed with different concentrations of zinc oxide NPs (0, 25, 50, 75, and 100mg /L) Fe NPs (0, 5, 10, 15, and 20 mg /L). Seedling height, was higher in NP treated seeds compared to control, in particular with the higher rates of NPs.	Rizwan *et al.*, 2019

2.4.3. Seedling dry weight

Crop	**Seed treatment chemical**	**Highlights of the experiment**	**Reference**
Bean (*Phaseolus vulgaris*)	Organic and inorganic titanium (Ti)	Dry matter yield of plants supplied with titanium @ 1 ppm was found to be higher than other treatments (0.25 and 0.5 ppm).	Ram *et al.*, 1983
Spinach	Nano and bulk titanium dioxide	Application of titanium dioxide to 3 years old spinach seeds was done in varying dosages (0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 4.0, and 6.0%) for 48 h. The increase of dry weight (76%) of seedlings was significant than that of control. The best results were obtained at 2.5% nano-titanium dioxide.	Zheng *et al.*, 2004
Fennel	Nano and bulk titanium dioxide	High shoot biomass was found in 5 ppm bulk particles (1.18 mg) and 80 ppm nanoparticles (1.16 mg) of titanium dioxide. The highest root biomass was achieved from 20 ppm bulk-titanium dioxide, 5ppm and 20 ppm of nano-titanium dioxide.	Feizi *et al.*, 2013

Chickpea	Zinc oxide NPs	Seeds of gram were treated with nanoparticles of zinc oxide (100, 500 and 1000 ppm). In nano zinc oxide treated seeds, the seedling dry biomass was greater at 1000 ppm dosage	Hajra and Mondal, 2017
Pigeon pea	Zinc NPs, iron NPs, zinc sulphate and iron sulphate	Seeds were hydroprimed with polymer coating and zinc and iron NPs (10 and 25 ppm), zinc sulphate and iron sulphate for different durations (6h and 12h). The dry weight was more in seedlings developed from seeds treated with iron NPs (0.43g) followed by zinc NPs (0.38g) while it was least for control (0.34g).	Raju and Rai, 2017
Chilli	Zinc oxide NPs	Suspensions of zinc oxide NPs were prepared (0, 100, 200 and 500 ppm) using a sonicator. The seeds were allowed to imbibe the treatment chemical for 72 h by placing 100 seeds on filter paper and adding 10 mL of the treatment solution. The germinated seeds were then placed in roll towel and seed quality was ascertained on the 14th day. Seedling dry weight was found to be highest in untreated control and it declined as the treatment dosage increased.	García-López *et al.*, 2018
Sorghum, cowpea	Titanium dioxide	Treatment with 750 mg/kg of seed produced seedlings having dry weight which were at par with the control, while	Maity *et al.*, 2018

		1000 and 1250 mg/kg doses produced significantly heavier seedlings.	
Sorghum, cowpea	Zinc oxide and silver (Ag)	750 mg/kg was found to be the best treatment in terms of seedling dry weight whereas, 1000 and 1250 mg/kg treated seeds produced seedlings having lower dry weight.	Maity *et al.*, 2018
Wheat	Zinc oxide and iron (Fe) NPs	It was found that the dry weights of shoots and roots were the lowest in the control treatments and were the highest after the 100 mgL^{-1} zinc oxide and 20 mgL^{-1} Fe NPs treatments. At 100 mgL^{-1} zinc oxide NPs, the shoot and root dry weights were increased by 53% and 46% over control, respectively. Iron NPs at 20 mgL^{-1} significantly enhanced the dry weights of shoot and root, by 58, and 61 per cent, over control.	Rizwan *et al.*, 2019

2.4.4. Vigour indices

Crop	**Seed treatment chemical**	**Highlights of the experiment**	**Reference**
Groundnut	Bulk zinc sulphate and nano zinc oxide	Seeds treated with nano zinc oxide @ 1000 ppm recorded higher value of seedling vigour index-I (1701)	Prasad *et al.*, 2012
Fennel	Nano and bulk titanium dioxide	Intermediate concentrations of nanosized titanium dioxide enhanced vigour index in fennel. Application of bulk-titanium dioxide at different concentrations had a negative effect on vigor index I, but nanoparticle treatments had a stimulating effect. Use of 5 ppm nanosized titanium dioxide gave a greater value of vigour index II compared to other doses and treatments.	Feizi *et al.*, 2013
Canola	Nanoscale titanium dioxide	An increase of germination rate and the vigour indices was noted at 0.25–4% nanoscale titanium dioxide treatment.	Mahmoodzadeh *et al.*, 2013
Groundnut	Zinc oxide, silver (Ag) and titanium dioxide NPs	Seeds were dry dressed with the synthesised nanoparticles (zinc oxide, silver and titanium dioxide), each at 500, 750, 1000 and 1250 mg kg $^{-1}$. Among the treatments, seeds treated with zinc oxide NPs resulted in maximum vigour	Shyla and Natarajan, 2014

		index (2474) than other treatments and the control (1759). Highest vigour index was observed in seeds treated with zinc oxide @ 1000 mg (2949) followed by Ag @ 1250 mg (2811).	
Rice	Titanium dioxide NPs and bulk particles	Rice seeds were treated with different concentrations of nanoparticles and bulk particles, (0.01, 0.1, 1, 10 mg/mL) of titanium dioxide. Vigour index-I had a higher value (3016) when bulk particles of titanium dioxide were used at 1 mg/mL. The values of vigour index-II were directly proportional to the dose of bulk titanium dioxide used. Among titanium dioxide NPs, 0.1 mg/mL nano titanium dioxide gave better vigour indices compared to other doses.	Jalill and Yousef, 2015
Pigeon pea	Zinc and iron NPs	Seeds of pigeon pea were polymer coated with zinc and iron nanoparticles in different doses (10, 25, 50, 100, 250, 500, 750 and 1000 ppm). Among the treatments seed polymer coating with zinc NPs at 750 ppm recorded significantly higher seedling vigour index (2556) over their bulk forms and control.	Korishettar *et al.*, 2016

Groundnut	Zinc oxide NP	VRI-2 seeds were treated with nano zinc oxide particles at various doses (750,1000,1250 mg/kg of seeds). After 12 months of storage, seeds treated with zinc oxide NPs @ 1000 mg kg^{-1} registered higher vigour index (3067).	Shyla and Natarajan, 2016
Maize	Nano zinc oxide	Maize seeds treated with 1500 $mgkg^{-1}$ of nanoscale zinc oxide registered a higher seedling vigour index.	Subbaiah *et al.*, 2016
Chilli	Zinc oxide NPs	Chilli seeds were imbibed in various doses of nano zinc oxide suspension (0, 10, 200 and 500 ppm). The vigour of seedlings developed from seeds treated with 100 and 200 ppm were 123.5 % and 129.4 % higher control.	García-López *et al.*, 2018
Cowpea	Titanium dioxide NPs	Both vigour index-I and II increased in direct proportion to dosage.	Maity *et al.*, 2018
Sorghum	Titanium dioxide NPs	Vigour index -I responded significantly to seed treatment, with better performance at increasing doses, and in all the cases they were more vigorous than control.	Maity *et al.*, 2018

2.4.5. Electrical conductivity of exudates from seed

Crop	**Seed treatment chemical**	**Highlights of the experiment**	**Reference**
Groundnut	Nano zinc oxide	VRI-2 seeds were treated with nano zinc oxide at various doses (750,1000,1250 mg/kg of seeds). After 12 months of storage, seeds treated with zinc oxide NPs @ 1000 mg kg^{-1} maintained a lower electrical conductivity (0.347dSm-1) as against 0.379 dSm^{-1} of control.	Shyla and Natarajan, 2016
Tomato	Nano titanium dioxide and nano zinc oxide	Five varieties of tomato were treated with nano zinc oxide and nano titanium dioxide (25, 50, 75, 100, 150 and 200 $mgkg^{-1}$). Lower values of electrical conductivity were obtained from seeds treated with nano titanium dioxide (68.66 μScm^{-1}) and nano zinc oxide (69.13 μScm^{-1}) at 25 $mgkg^{-1}$.	Pratap *et al.*, 2018
Maize	Nano titanium dioxide	Maize seeds treated with nano titanium dioxide @ 200 mg kg^{-1} recorded the least electrical conductivity (0.278 dSm^{-1}) and untreated seeds measured the highest E.C. (0.361 dSm^{-1}).	Vijayalakshmi *et al.*, 2018

2.5. Accelerated ageing

Crocker and Groves (1915) suggested that subjecting seeds to high temperature could lead to its deterioration which may be explained by the presence of coagulated proteins in those aged seeds. Delouche (1965) devised accelerated ageing test to estimate seed storability in warehouses of commercial seed companies. The protocol currently used for accelerated ageing was developed by McDonald and Phannendranath (1978).

The accelerated ageing test exposes the seeds to extreme conditions of high temperature and high relative humidity in a controlled environment which can cause rapid decline of seed quality. Seed lots of high vigour are able to survive these conditions and tolerate the stressful state as they deteriorate much slower than the low vigour seed lots (TeKrony, 2005). The temperature, relative humidity and duration of exposure vary according to the crop, variety as well as seed lot. Sometimes seeds from the same seed lot may have high vigour and low vigour seeds. This might be due to the different pre-harvest conditions experienced by the seed-parent in field conditions.

While working with soybean seeds, TeKrony (2005) discovered that even a 0.5°C reduction in temperature in the accelerated ageing chamber could increase the germinability of the lowest vigour seed lots by 16 per cent. So, it is important to maintain a constant temperature throughout the ageing period. The accelerated ageing test for soybean is recommended by AOSA and ISTA for seed vigour testing. In addition, this test for soybean was accepted in the International Rules of Seed Testing (ISTA, 2001). Hence, accelerated ageing test has potential to be used as vigour test in many more crops after standardization.

Several workers identified free radical-mediated lipid peroxidation, enzyme inactivation or protein degradation, disruption of cellular membranes and damage to genetic (nucleic acids) components as major causes of seed ageing (McDonald, 1999). These changes were related to elevated electrical conductivity values and decreased germination and vigour of the seeds in tomato (Ramamoorthy and Karivaradharaju, 1989) and soybean (Panobianco *et al*., 2007).

2.5.1. Effect of accelerated ageing on seed quality parameters

2.5.1.1. Germination %

Crop	**Details of ageing**	**Reference**
French bean (*Phaseolus vulgaris*), Pea (*Pisum sativum*), Lentil (*Lens culinaris*) and Millet (*Panicum miliaceum*)	Accelerated ageing was conducted at 98.2 % relative humidity (maintained using 5.96% H_2SO_4) and 20±2°C. The observations regarding seedling growth was taken at 0, 7 and 14 days after accelerated ageing. Germination per cent was measured on the 7th day after the seeds were kept for germination. It was found that the seed viability reduced with rise in ageing duration.	Chhetri *et al*., 1992
Watermelon	Watermelon seeds were aged (45°C and 79% RH) for six days after which they were dried back to their initial seed moisture level (4.7%). Significant reduction in germination per cent and speed of germination was observed in aged seeds compared to unaged seeds. An increase in lipid peroxidation and decrease in peroxide scavenging enzymes was noted.	Chiu *et al.*, 1995
Cotton (*Gossypium hirsutum* L.)	The artificial ageing condition consisted of a temperature of 40-44°C and a relative humidity of 90-95 %. After accelerated ageing treatment, the seeds were dried back to 8 % moisture content, packed and heat-sealed in polythene	Basra *et al*., 2003

	bags and stored at 8±2°C in refrigerated condition until further seed quality evaluation. The germination test revealed a reduction in germination as the ageing time increased over 3 days. By the 20th day of ageing, all the seed lots had lost viability.	
Melon	Melon seeds were made to undergo traditional and salt saturated accelerated ageing for 48, 72 and 96 hrs at 38 or 41°C. They were then tested for germination and seedling emergence (in green house). Both the ageing procedure (traditional and salt saturated) at 38 or 41°C for 72 or 96 hrs were equally effective in determining the vigour of the seed lots.	Torres and Filho, 2003
Chilli	Chilli seeds were exposed to 42°C and 100% relative humidity for 0,5,10,15,20,25 and 30 days. The germination per cent was determined based on the per cent emergence of radicle (ER%) as ageing period increased. After 20 days, rapid decline in germination was measured, culminating in 0% after 30 days of treatment.	Kaewnare *et al*., 2011
Tomato	Six seed lots of tomato (4g sample size each) were incubated at 41°C for 48 and 72 hrs using three techniques (standard: 40 mL water, saline solution: 11g NaCl/100mL water and saturated saline solution: 40g NaCl/100mL water). Saturated salt solution method was more effective as standard accelerated ageing test presented higher moisture variation.	de Silva Almeida *et al.*, 2014

Pigeon pea	Pigeon pea seeds were kept in a single layer on a mesh tray over 40 mL water or saturated salt solution. A relative humidity of 100 % and temperature of 42 and 45 °C was maintained for 24, 48, 72 and 96 h. After evaluation of germination per cent on paper substratum, it was found that saturated salt solution at a combination of 42 °C for 48 hrs (six days after sowing), along with 45°C for 24 hrs (four days after sowing) were effective.	Sanches *et al.*, 2014
Faba beans	Accelerated ageing test at 75% relative humidity (RH) and 39-42°C for 3-5 days were effective in determining the seed quality of faba beans both in laboratory as well as field conditions. At 75% RH and 39°C, the germination was 90-93%, whereas at 100% RH it was 90-92%. At 45°C and 75% RH, germination was 74-80%, while at 45°C and 100% RH, it ranged from 47-64%. Hence, it can be concluded that higher relative humidity and higher temperature had more adverse effect on germination.	El-Abady *et al.*, 2015
Soybean	By seven days of accelerated ageing, in all the cultivars of soybean seeds tested the germination per cent had reduced to 50 per cent of the initial value. Hence, seven days of accelerated ageing is considered the optimum duration to study storability of soybean seeds and classify the seed lots based on their performance.	Vijayakumar and Vijayakumar, 2015

Cotton	Storage of cotton seeds in three containers (craft-paper, polythene and aluminium bags) under different relative humidity levels (45, 60, 75 and 90%) was carried out for 60 days. The germination was highest in unaged (control) seeds after 15 days of storage, which reduced with further advancement in storage period. After 60 days in treatment conditions, the per cent germination was observed to be 86.70 (control), 86.10 (45% R.H.), 84.30 (60% R.H.), 78.60 (75% R.H.) and 68.50 (90% R.H.) when stored in polythene bags.	Yadav and Brar, 2015
Wheat	Accelerated ageing of wheat was conducted in different conditions, *i.e.*, 44h @ 43°C (73 % germination), 48h @ 43°C (72 % germination), 72 h @ 43°C (35 % germination) and 96 h @ 41°C (20 % germination). The germination test was conducted on standard germination paper for 7 days with 8 h of illumination and 16 h of darkness. Among the naturally aged and accelerated aged samples assayed, aleurone layer exhibited no significant change while scutellum behaved differently. The reduction in germination rate of was associated with decline in scutellum nuclear content and increase in the length-to-width ratio of scutellum nuclei.	Ahmed *et al*., 2016
Rice	Eight cultivars of rice were deteriorated through accelerated ageing at 45°C and 100 % RH for 24, 48, 72, 96 and 120 h. Ageing treatments gradually	Bijanzadeh *et al*., 2016

	reduced the germination percent of all cultivars with the rise in treatment duration from 24 to 120 h.	
Blackgram	Black gram seeds were kept in accelerated ageing chamber maintained at 45°C and 100% relative humidity for different periods of time (24-192 h). On comparing the aged and unaged seeds, six days of accelerated ageing was found to be equivalent to nine months of natural ageing. Per cent germination followed a declining trend from 98% initially to 66 % as duration of ageing progressed.	Gomathi *et al.*, 2016
Maize	Hybrid maize (Arjun) seeds were incubated at 42°C and 96% RH for 0 to 7 days. In tandem with accelerated ageing, seeds were stored in cloth bag and stored for natural ageing under ambient conditions. At the end of the experiments it was revealed that seeds stored in cloth bag for seven months in ambient conditions behaved similar to seeds that were accelerated aged for two days.	Khidrapure *et al.*, 2016
Brinjal	Brinjal seeds were kept over water as well as saturated NaCl solution for 24, 48 and 72 hours at 41°C. Exposure to NaCl saturated solution for 48 and 72 h and to water for 72h were efficacious in assessing the potential of brinjal seeds. The use of standard method (water) for accelerated ageing at 41°C for	Deuner *et al.*, 2018

	72 h proved to be in compliance with the results obtained from standard emergence test.	
Sesame	Sesame seeds were subjected to high temperature (40°C) and high relative humidity (100%) for various periods of time (0-10 days). The initial germination of sesame seeds reduced from 92% to 83% after two days of accelerated ageing and to 49 % after ten days ageing. The per cent reduction in germination was higher in aged seeds compared to unaged seeds by 43%.	Kumar *et al*., 2019
Tomato	Tomato seeds were kept in an oven maintained at 40°C in 100% relative humidity for 1 to 9 days (V1, V2, V3, V4, V5, V6, V7, V8, V9). Control seeds were not subjected to deteriorative conditions of ageing (V0). After ageing, the seeds were dried back to their original moisture content using filter paper. Germination loss was accentuated with progress in ageing and ranged from 93% (unaged seeds) to 0% (9-day aged seeds).	Nigam *et al.,* 2019
Okra	Loss of germination in okra was studied using accelerated ageing test for different durations (24, 48, 96 and 192 h) by incubating the seeds at 40°C and 99% relative humidity. 24 h and 48 h of ageing improved the germination (96.4%) compared to unaged seeds (92.0 %). But, 192 h of ageing reduced the germination to below 50% (49.3 % germination).	Parmoon *et al.,* 2019

2.5.1.2. Seedling length

Crop	**Details of ageing**	**Reference**
Faba bean	Seedling length was affected by the temperature and relative humidity supplied during ageing. At 39°C and 100% RH, it was 20.00 cm whereas at 45°C and 100% RH, the seedlings were only 7.00 cm long. They also found relative humidity to have a greater effect than high temperature by studying faba bean seeds exposed to 75% RH (24.00 cm) and 100 % RH (7.00 cm) for 3, 4 and 5 days.	El-Abady *et al.,* 2015
Groundnut	Groundnut seeds subjected to accelerated ageing for 7 days at 40°C and 95% RH had higher root and shoot length in the seeds maintaining higher germination per cent.	Ghosh *et al*., 2015
Soybean	Different cultivars of soybean were exposed to 100% RH and 40±1°C for various durations (3, 4, 5, 6, 7 and 8 days). The initial root length (19.7-23.9 cm) and shoot length (17.6-20.7 cm) reduced to 11.6 cm and 12.7 cm respectively, as the period of ageing increased.	Vijayakumar and Vijayakumar, 2015
Rice	Different cultivars of rice behaved differently to 120 hours of accelerated ageing. The lowest per cent reduction in root length was 54.5 % and shoot length reduction was 49.5 % compared to unaged seeds.	Bijanzadeh *et al*., 2016

Maize	One day of accelerated ageing produced roots of 21.42 cm and shoots of 20.51 cm, while one month of natural ageing in cloth bag also had similar root (22.34 cm) and shoot (21.04 cm) lengths.	Khidrapure et al., 2016
Tomato	Noticeable decline in root and shoot length occurred after ageing than in unaged seeds.	Nigam *et al.*, 2019

2.5.1.3. Seedling dry weight

Crop	**Details of ageing**	**Reference**
Pea	Seedling dry weight was influenced by the temperature (25, 35 and 45 °C) during 48, 72 and 96 hours of accelerated ageing. Initially there was an increase in the root and shoot dry biomass with increase in temperature from 25 to 35 °C, but with further increase to 45°C there was a decline.	Jatoi *et al.*, 2001
Cotton	A reduction in the root and shoot weight of cotton seedlings were observed. The accelerated ageing might have caused the deterioration of cotton seeds due to lipid peroxidation leading to membrane degradation and enzyme inactivation.	Basra *et al.*, 2003

Maize	Based on the seedling dry weight, the maize seeds which were accelerated aged for one day at 42°C and 96% RH (2.41g dry weight) were found to be similar to seeds stored in cloth bag for one month (2.42g dry weight).	Khidrapure *et al*., 2016
Tomato	Tomato seeds were aged for 1-9 days at 40°C and 100% RH. Unaged seeds were maintained as control. Seedling dry weight decreased with increase in the duration of ageing period. The dry weight of a single seedling extended from 6.18 mg (control) to 2.09 mg (8-day-aged seeds).	Nigam *et al*., 2019

2.5.1.4. Vigour indices

Crop	**Details of ageing**	**Reference**
Sunflower	Vigour index I decreased from 16.59 (control) to 3.84 (3-day ageing) and 0.0 (7-day ageing) after accelerated aging treatment.	Hussein *et al*., 2011
Physic nut (*Jatropha curcas*)	Four seed lots of *J. curcas* were placed in various accelerated ageing conditions (temperature: 42 or 45 °C; duration of treatment: 48, 72 and 96 hours). There was a decrease in seedling vigour with a rise in treatment temperature and duration. At 45°C, the incidence of fungal infection was high.	Oliveira *et al*., 2014

Groundnut	Vigour index-I of peanut seeds treated with lime and gypsum was found to range from 1715 (treated seeds) to 1414 (control seeds) after accelerated ageing (95% RH, 40°C) of 7 days.	Ghosh *et al*., 2015
Cotton	The effect of varying conditions of relative humidity (45, 60, 75 and 90 %) for different durations (15, 30, 45 and 60 days) on cotton seeds were evaluated. After 60 days, the highest vigour index obtained was 553.	Yadav and Brar, 2015
Black gram	The declining vigour of aged seeds were attributed to faulty enzymes produced due to DNA degradation from impaired transcription mechanism as a result ageing.	Gomathi *et al*., 2016
Tomato	Greater the value of vigour index stronger is the vigour of a seed. Maximum vigour and germination index were noted in unaged seeds, while it decreased drastically with ageing.	Nigam *et al*., 2019

2.5.1.5. Electrical conductivity

Crop	**Details of ageing**	**Reference**
Sunflower	Electrical conductivity increased with storage and loss of viability under ambient conditions. Contrary to this, sunflower seeds artificial aged at 95 and 100 % R.H. recorded lower conductivity despite the complete loss of	Powell, 1986

	germination than seeds aged at 50 and 75 % R.H. which were able to retain viability.	
Carrot	Seed exudates were reported to be more in aged seeds of carrot. It was caused by seed deterioration and membrane damage.	Al-Maskri *et al*., 2003
Pea	The increased seed electrolyte content accounted for loss in viability and vigour of pea seeds when subjected to accelerated ageing.	Khan *et al*. 2003
Sunflower	Seeds of sunflower subjected to 3 and 7 days of accelerated ageing (45°C and 100% RH) revealed significantly higher electrical conductivity compared to unaged seeds.	Hussein *et al*., 2011
Chilli	The electrical conductivity, concentration of K^+, Na^+, Ca^+ and Mg^{2+} were found to be lesser in the 5-10 days aged seeds than 10-30 days aged seeds.	Kaewnare *et al*., 2011
Rice	In control seeds of rice cultivars, the electrical conductivity ranged from 50-67 µScm^{-1}mg^{-1}. After 120 hours of ageing, various cultivars exhibited E.C. in the range of 272 µScm^{-1}mg^{-1} to 493 µScm^{-1}mg^{-1}.	Bijanzadeh *et al*., 2016
Black gram	As the period of accelerated ageing increased from 0 to 8 days, electrical conductivity of the seed leachate obtained from the seeds also varied from 0.082 to 0.144 dSm^{-1}. This rise might have been due to free radical chain	Gomathi *et al*., 2016

	reaction occurring in the membrane causing auto-oxidation of the poly unsaturated fatty acids (PUFAs).	
Maize	Hybrid corn seeds when stored in cloth bag for one month recorded an electrical conductivity of 0.253 dSm^{-1} whereas the same seed lot when subjected to one day of accelerated ageing measured 0.279 dSm^{-1}.	Khidrapure *et al*., 2016

2.6. Seed microflora

The involvement of fungal infection in seed aging was demonstrated by Christensen and Kaufman (1969), Christensen (1967, 1972, 1973), and Neergaard (1977), by showing that seeds infected with fungi deteriorated faster during storage than uninfected seeds.

A major way in which microorganisms damage seeds is by the production of exocellular enzymes and toxins. Among the enzymes produced, cellulases, pectinase, amylase, lipase, protease, and nuclease are of major importance. Aflatoxins and mycotoxins, produced by fungi in the *Aspergillus* group, reduce seedling elongation, inhibit chlorophyll synthesis, inhibit various enzymes, and degenerate the endoplasmic reticulum (Halloin, 1986). Microorganism infection may also cause an increase in electrolyte leakage, which is apparently due to the damage of the cell membrane and seed integuments.

Microorganisms that infect seeds can enter through natural openings, such as the micropyle, or through wounds or cracks. Some fungi penetrate directly through thin seed coats (Neergaard, 1977 and Herman, 1983).

Crop	**Micro-organisms observed**	**Reference**
Chilli	*lternaria alternata, Aspergillus flavus, A. niger, Chaetomium bostrychodes, Drechslera tetramera, Fusarium moniliforme, F. pallidoroseum, Paecilomyces* spp., and *Rhizopus stolonifera.*	Sharfun-Nahar *et al.*, 2004)
	Alternaria alternata, Botrytis cinerea, and *Myrothecium verrucaria*.	Nishikawa *et al.*, 2006
	Aspergillus niger, Aspergillus flavus, Colletotrichum capsica, Alternaria alternate, Fusarium oxysporum, Curvularia lunata, Macrophomina phaseolina, Penicillium citrinum, and *Rhizopus nigricans.*	Jogi *et al.*, 2010

	Colletotrichum capsica (54.75%,), *Aspergillus niger* (44.00%) and *A. flavus* (29.75 %)	Chigoziri and Ekefan, 2013
	Curvularia lunata, Rhizopus stolonifer, Colletotrichum capsici, Fusarium moniliforme and *Aspergillus flavus.*	Alam *et al.*, 2014
	Aspergillus niger, Aspergillus flavus, Pencillium spp., and *Alternaria* spp.	Navya, 2016
	Aspergillus spp., *Pencillium* spp. and *Alternaria* spp.	Sandhya,2016
	Aspergillus niger, A. flavus, A. nidulans, Fusarium oxysporum, Alternaria alternate, Curvularia lunata and *Colletotrichum capsica.*	Guldekar *et al.,* 2017
	Blotter paper method: *Aspergillus niger* (8.40%), *A. flavus* (6.34%), *Penicillium* spp. (2.65%) and *Rhizopus* spp. (0.32%) in sterilized seeds. In unsterilized seeds, *A. niger* (16.18%), *A. flavus* (15.75%), *Fusarium solani* (10.50%), *Rhizopus* spp. (7.43%), *Colletotrichum capsici* (6.81%) and *Penicillium* spp. (5.75%) were present. **Agar plate method**: *A. niger* (27.54%) and *A. flavus* (20.62%), *Fusarium solani* (7.26%), *Penicillium* spp. (3.50%), *Rhizopus* spp. (3.20%) and *Colletotrichum capsica* (2.59%) in unsterilized seeds.	Chauhan *et al.,* 2018
Tomato	*Fusarium* spp. (23%), *Cladosporium* spp. (21%), *Aspergillus flavus* (19%) and *Aspergillus niger* (13%).	Hamim *et al.,* 2014
	Fusarium moniliforme, Curvularia lunata, Alternariasolani, Helminthosporium solani, Aspergillus flavus.	Raju, 2017

	Alternaria solani, *Fusarium oxysporum, F. solani*, *Botrytis cineria*, *A. alternata*, *Chaetomium globosum*, *Curvularia lunata*, *Aspergillus niger*, *Drechslera specifer* and *Rhizoctonia solani.*	Chohan *et al.*, 2017
Tomato and brinjal	*Aspergillus*, *Rhizopus*, *Fusarium*, *Cladosporium* and *Monilia* spp.	Patekar, 2017
Brinjal	*Cladosporium sphaerospermum* and *Arthrinium* spp. (germination was suppressed), *Penicillium variabile* (seminal root elongation was inhibited).	Nishikawa *et al.*, 2006
	Alternaria alternata, Aspergillus flavus, Curvularia lunata, Fusarium oxysporum, Fusarium solani and saprophytic non-disease-causing organisms such as *Epicoccum, Mucor* and *Penicillium*.	Habib *et al.,* 2007
	Fusarium spp. (19%), *Phomopsis vexans* (15%), *Aspergillus flavus* (13%), *Curvularia* spp. and *Aspergillus niger* (9%)	Hamim *et al.*, 2014
Potato	*Fusarium sambucinum, Helminthosporium solani*, and *Verticillium dahlia.*	Gore, 2017
Amaranth	*Alternaria* spp., *Aspergillus flavus, Aspergillus niger, Curvularia* spp., *Fusarium* spp. and *Penicillium* spp.	Begum, 2000
Okra	*Aspergillus flavus* (23%), *Fusarium* spp. (20%), *Colletotrichum dematium* (19%), *Aspergillus niger* (18%), *Macrophomina phaseolina* and *Penicillium* spp.	Hamim *et al.*, 2014
	Aspergillus flavus, Aspergillus niger and Rhizopus spp.	Reshma, 2018
Cucumber	*Fusarium* spp., *Curvularia* spp., *Alternaria* spp. and *Penicillium* spp.	Hamim *et al.*, 2014

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled 'Seed invigoration with inorganic nanoparticles in chillies (*Capsicum annuum* L.)' was conducted in the Department of Seed Science and Technology, College of Horticulture, Kerala Agricultural University, Vellanikkara from February 2018 to May 2019. The study aimed to assess the seed quality in fresh and accelerated aged seeds of chilli treated with inorganic nanoparticles. The details of the experiment are described below.

3.1 Experimental site

The laboratory experiments were conducted in the Department of Seed Science and Technology, College of Horticulture, Vellanikkara. The facilities at Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore and Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore were also availed for seed treatment.

3.2 Climatic conditions

Vellanikkara, a suburban region of Thrissur is located 22.25 m above mean sea level between 10.5452 °N and 76.2740 °E co-ordinates and it experiences a hot and humid climate. The weather parameters (Appendix I) encountered in the locale of seed storage during February 2018 to May 2019 ranged from 20.4 to 36.7 °C temperature and 47 to 89 % relative humidity.

3.3 Experimental material

The seeds of chilli variety Anugraha were procured from the Department of Vegetable Science, College of Horticulture, Vellanikkara immediately after harvest in February 2018.

Experimental method

The present study was organized into four experiments as listed below:

Experiment 1: Effect of zinc oxide on seed quality of fresh seeds

Experiment 2 - Effect of titanium dioxide on seed quality of fresh seeds

Experiment 3 - Effect of zinc oxide on seed quality after accelerated ageing

Experiment 4 - Effect of titanium dioxide on seed quality after accelerated ageing

Each of these experiments were carried out in completely randomized design (CRD) in three replications each of the seven treatments of chilli variety Anugraha.

3.4 Treatment details

Nano and bulk grade zinc oxide (ZnO) and titanium dioxide (TiO_2) were used for the treatment of seeds. The invigoration treatment the seeds were subjected to in various experiments is detailed in the Table 1 to 4. The untreated seeds served as control.

Table 1: Treatment details of experiment 1 - Effect of zinc oxide on seed quality of fresh seeds

Treatment	Details
T_1	Control
T_2	500 mg ZnO/kg of seed
T_3	900 mg ZnO/kg of seed
T_4	1300 mg ZnO/kg of seed
T_5	100 mg nano ZnO/kg of seed
T_6	250 mg nano ZnO/kg of seed
T_7	500 mg nano ZnO/kg of seed

Table 2: Treatment details of experiment 2 - Effect of titanium dioxide on seed quality of fresh seeds

Treatment	Details
T_1	Control
T_2	500 mg TiO_2/kg of seed
T_3	900 mg TiO_2/kg of seed

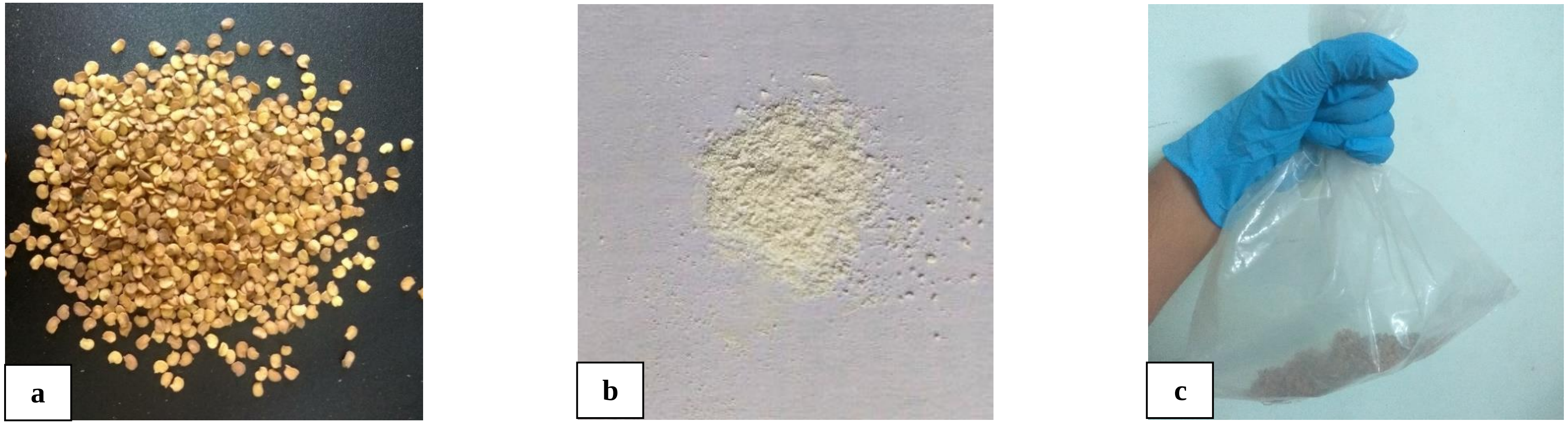
T_4	1300 mg TiO_2/kg of seed
T_5	100 mg nano TiO_2/kg of seed
T_6	250 mg nano TiO_2/kg of seed
T_7	500 mg nano TiO_2/kg of seed

Table 3: Treatment details of experiment 3 - Effect of zinc oxide on seed quality after accelerated ageing

Treatment	Details
T_1	Control
T_2	500 mg ZnO/kg of seed
T_3	900 mg ZnO/kg of seed
T_4	1300 mg ZnO/kg of seed
T_5	100 mg nano ZnO/kg of seed
T_6	250 mg nano ZnO/kg of seed
T_7	500 mg nano ZnO/kg of seed

Table 4: Treatment details of experiment 4 - Effect of titanium dioxide on seed quality after accelerated ageing

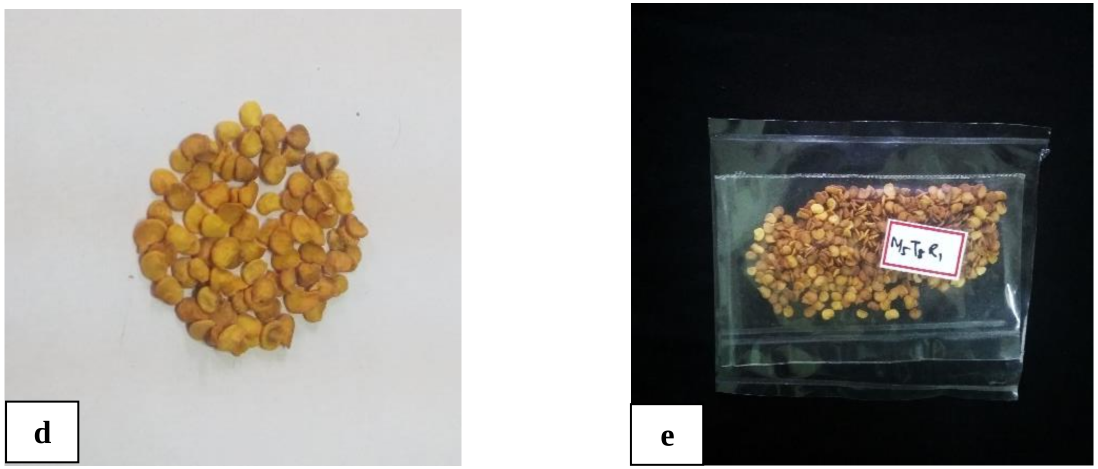
Treatment	Details
T_1	Control
T_2	500 mg TiO_2/kg of seed
T_3	900 mg TiO_2/kg of seed
T_4	1300 mg TiO_2/kg of seed
T_5	100 mg nano TiO_2/kg of seed
T_6	250 mg nano TiO_2/kg of seed
T_7	500 mg nano TiO_2/kg of seed



Untreated seeds

Nano titanium dioxide powder (500 mg)

Dry dressing of seed with treatment chemical



Treated seeds

Treated seeds packed in 700-G polythene bag

Plate 1: Seed treatment of chilli seeds

3.4.1 Seed treatment procedures

The seeds were shade dried to reduce the moisture content to below 8 per cent before administering the seed invigoration treatments. The seeds were dry dressed with the required quantity of ZnO, ZnO NPs, TiO_2, and TiO_2 NPs as detailed in Tables 1, 2, 3 and 4. The seeds were manually shaken with the required dose of chemicals in a polythene bag for 3 minutes, 5 times in a span of 3 hours. For experiments 1 and 2, the treated seeds were packed separately in 700-gauge polythene bag for storage. In experiments 3 and 4, the treated seeds were subjected to accelerated aging before packing.

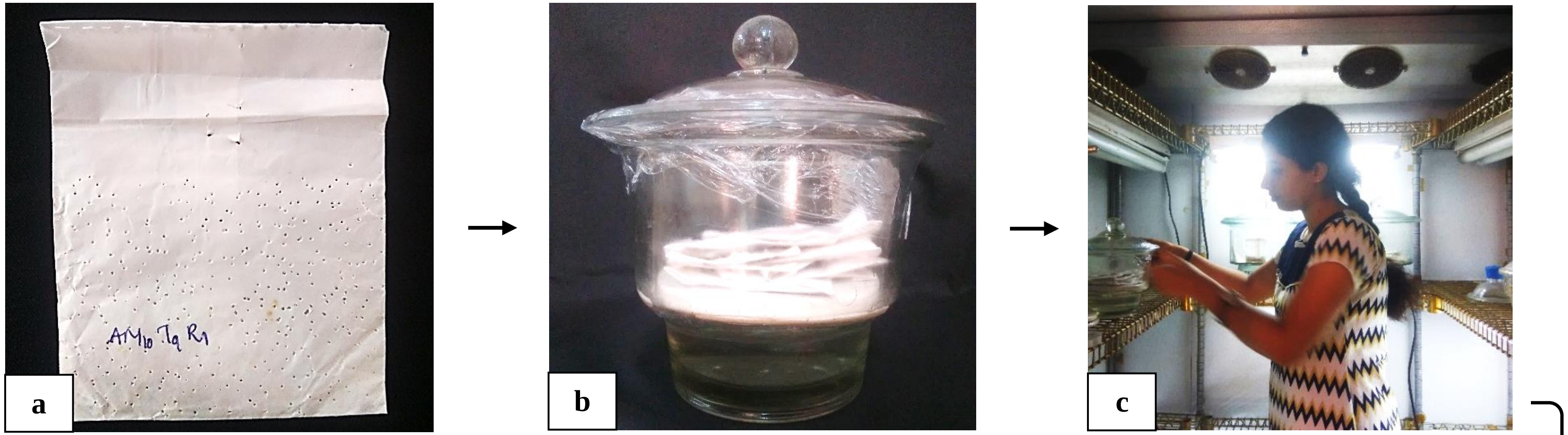
3.4.1.1 Accelerated ageing procedure

The treated seeds and control seeds were subjected to accelerated ageing as detailed below.

Perforated butter paper bags (containing equal number of holes in each packet) were filled with equal quantity of the seeds. The perforations ensured exposure of the seeds to the high temperature and humidity. The seed-filled bags were placed in a glass desiccator jar containing water to maintain 100 per cent relative humidity. The glass jars were sealed shut and was maintained at 40 ± 1° C for one day in accelerated ageing chamber. During the ageing period, the perforated bags were frequently shuffled to ensure adequate exposure of the seeds to the ageing conditions. The seeds were taken out after accelerated ageing and tested for seed quality parameters such as germination, root length, shoot length, seedling dry weight, electrical conductivity of seed leachates and vigour indices.

3.4.2 Method of storage

After dry dressing with the treatment chemicals, the seeds were packed in 700-gauge polythene bags, each replication of the treatments separately, for ease of obtaining monthly observations and also to avoid fluctuations in moisture content of the seeds over the period of storage. The bags were then heat sealed and stored under ambient conditions. The storage study period spanned from February 2018 to May 2019.



Perforated butter paper bags filled with seeds

Water-filled desiccator containing seeds

Placement of desiccator in the accelerated ageing chamber

Seeds after one day of accelerated ageing

Butter paper bag containing seeds after ageing

Accelerated ageing chamber set at 40° C

Plate 2: Accelerated ageing of chilli seeds

3.5 Observations recorded

Observations such as germination per cent, root and shoot length of the seedling, seedling dry weight and electrical conductivity (E.C.), were recorded at monthly intervals, while seed moisture content and seed microflora infections were assessed at the start and end of storage. The seeds required for each observation were drawn randomly from the seed lot. The seed quality parameters were determined as per the procedures enumerated below.

3.5.1 Germination (%)

Germination test was conducted by using between paper method according to the ISTA (1995) recommendation. Roll paper towel was used as the substratum. Four replications of hundred seeds were taken from each replication of each treatment. The seeds arranged on moistened roll paper towel was covered by rolling another layer of wet paper towel over it. The rolled towels were placed in a slanting position in a bucket containing water. The buckets were maintained at 25 ± 2 °C and 95 ± 2 per cent relative humidity in seed germinator. The water level in the bucket was maintained only high enough to promote the capillary uptake of water by the germination paper to keep the paper moist, and also to prevent the submergence of seeds. The number of normal seedlings were counted on the 14th day and expressed in per cent.

3.5.2 Seedling shoot length (cm)

At the end of germination test (14th day), ten normal seedlings were randomly selected from each replication of the treatments for obtaining the shoot length. The length between tip of the shoot and collar region was measured using ruler and expressed in centimetre.

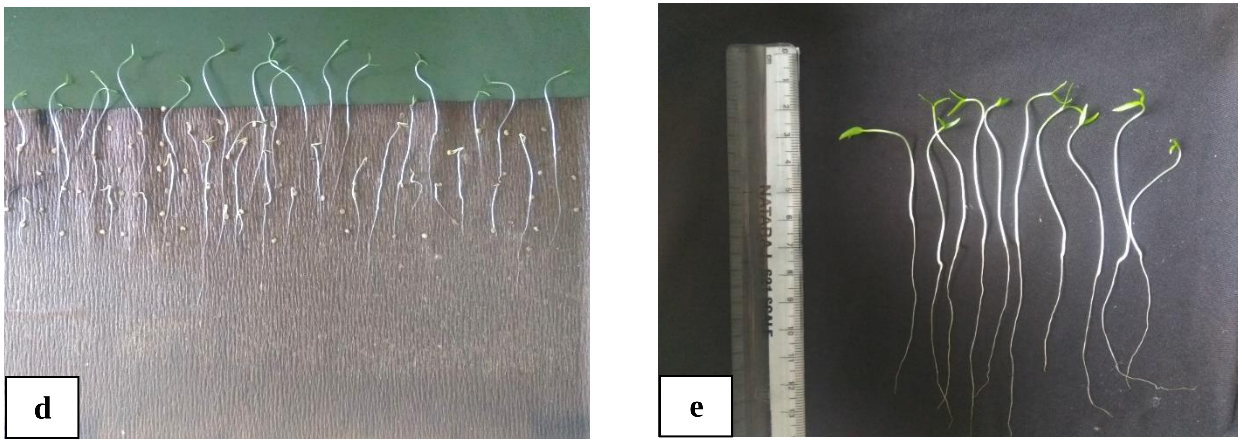
3.5.3 Seedling root length (cm)

The same seedlings used for measuring shoot length were used to measure the root length. The length between tip of the root and the collar region was measured and recorded in centimetre.

Day 1: Moistened roll towel paper containing 100 seeds

Rolling of paper towel

Rolled towels kept for germination



Day 14: Germinated seedlings on opening the roll towel

10 seedlings taken for root and shoot length measurement

Plate 3: Germination test by between paper method using roll towel paper as substratum

3.5.4 Seedling dry weight (mg)

The seedling dry weight estimation was done with the same ten seedlings used for the measurement of shoot and root length. They were taken in properly labelled butter paper bags and kept in hot air oven maintained at 80° C temperature for 24 h. The butter paper bags were then transferred to a desiccator to cool for 30 minutes. The weight of ten seedlings was recorded using a digital weighing balance and expressed in milli gram (mg).

3.5.5 Vigour indices

Vigour index-I and II were computed using the formula given by Abdul-Baki and Anderson (1973) and expressed as a whole number:

Vigour index-I = Germination (%) x Seedling length (cm)

Vigour index-II = Germination (%) x Seedling dry weight (mg)

3.5.6 Electrical conductivity of seed leachate ($\mu S\ cm^{-1}$)

Three replications of five grams of seeds from each treatment were taken at random in 50 mL beakers. The seeds were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for one minute and then thoroughly washed with distilled water several times to ensure the removal of treatment chemicals. The washed seeds were soaked in 50 mL of distilled water for 24 h (Presley, 1958). The seed leachate was collected after decanting and the electrical conductivity (EC) was measured using EUTECH CON-510 digital conductivity meter with a cell constant of 0.1. The mean value of three replications were expressed in micro Siemens per centimetre ($\mu S\ cm^{-1}$).

3.5.7 Seed moisture content (%)

Moisture content of the seed was measured at the start and end of storage by using the low constant temperature procedure given by ISTA (1985). Five gram seeds from each replication of the treatments were drawn from the sample and evenly distributed over the surface of the container. Care was taken to ensure that the container used was made of non-corrosive metal or glass of approximately 0.5

mm thickness. Both the container and its cover were weighed before and after filling. It was then placed rapidly in an oven maintained at 103±2 °C and dried for 17±1 h. The drying period was considered to have begun from the time when oven reaches 103 °C. At the completion of the prescribed time, the container was removed from the hot air oven and placed in a desiccator to cool for 30-45 minutes. After cooling, the container along with its cover was weighed and the seed moisture content was calculated using the following formula:

$$\text{Moisture content (\%)} = \frac{\text{M2-M3}}{\text{M2-M1}} \times 100$$

where,

M1: weight of container with lid

M2: weight of container with lid + seeds before drying

M3: weight of container with lid + seeds after drying

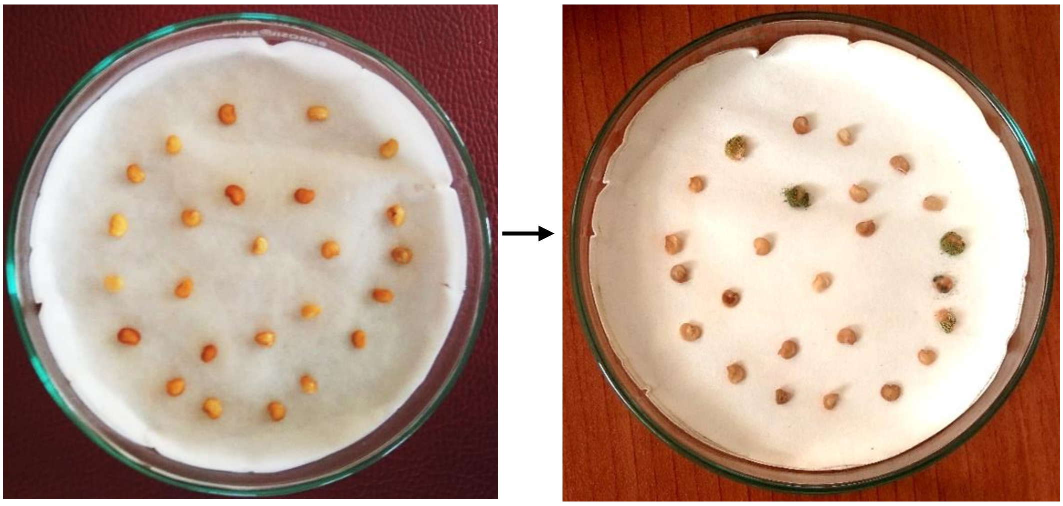
3.5.8 Seed infection

Seed health test was conducted by blotter paper method and agar plate method as recommended by ISTA (1999).

3.5.8.1 Blotter paper method

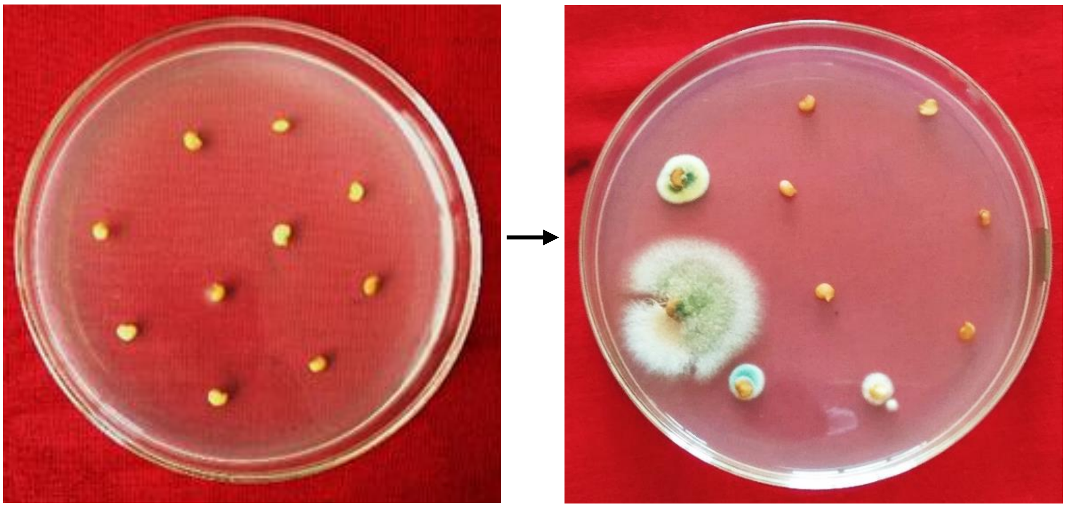
The detection of seed microflora by using blotter paper was carried out by adopting ISTA’s standard blotter test described by Neergaard (1979). Three layers of sterilized blotter papers were kept in sterilized petri plates. Sterilized water was added in the plates to soak the filter paper and the excess water was removed. Twenty-five seeds of chilli were kept on the blotter paper equidistantly under aseptic conditions maintained by laminar air flow. The outer most layer consisted of 16 seeds, 8 in the middle and 1 seed at the centre. After plating the seeds, they were incubated at 20±2 °C for seven days in an alternate cycle of 12 h darkness and 12 h light. On the eighth day, the plates were observed for the presence of seed microflora under stereo binocular microscope. The number of infected seeds were counted and recorded in per cent. They were also identified based on the

Blotter paper method



Initial Final

Agar plate method



Initial Final

Plate 4: Testing chilli seeds for presence seed microflora

morphological characters of the conidia, conidiophores and fruiting bodies by making slides of the same.

3.5.8.2 Agar plate method

The agar plate method for detection of seed microflora requires surface sterilization. Potato Dextrose Agar (20 mL) was poured into sterilized petri plates in aseptic conditions. The seeds were surface sterilized with 0.1% sodium hypochlorite and subsequently washed with sterile distilled water thrice. The washed seeds were then placed on sterile filter paper to remove the excessive water on its surface. After the media had set, the seeds were kept equidistantly in PDA plate. The petri plates were incubated under bell jar for six days. After incubation, the number of infected seeds were observed and recorded as per cent. The identification of the infection causing pathogen was also done by preparation of slide.

3.6 Scanning Electron Microscope (SEM)

The details of radicle morphology of treated and control seeds were examined using Fei Quanta 250 SEM (scanning electron microscope) that uses Everhart Thornley Detector and Tungsten as the electron source. The sample was prepared by placing freshly emerged radicle of 3-5 mm length in deep freezer at -20 °C. A minimum of five radicles per sample is required. It was ensured that all the samples were at the same growth stage. The samples were taken out only just before the time for observation and was transported in ice box to prevent damage to the radicles. To take SEM pictures, the radicle samples were spread on double sided conductive carbon tape fixed on 12 mm diameter aluminium stub and the same was placed in the sample chamber of ESEM (Environmental Scanning Electron Microscope). After attaining high vacuum ($3.99e^{-4}$ Pa), the filament was switched on and various parameters like electron beam, intensity, spot size, voltage and emission current were adjusted and the images were captured.

Plate 5: Quanta 250 - scanning electron microscope (SEM)

3.7 Statistical analysis

The statistical analysis of the data obtained from the laboratory experiments was carried out using the statistical package for agricultural analysis WASP (Web Agri Stat Package 2.0) developed by Jangam, A.K. and Wadekar, P.N. at the ICAR Research Complex for Goa. The critical difference was calculated at 5 per cent probability for all the F-tests which exhibited significance. The ranking of the significant treatments was done using Duncan's Multiple Range Test (DMRT). The treatment efficacy parameters consisting of percentage data (germination per cent) were subjected to arcsine transformation. The zero values present in the data were converted to $\frac{1}{n}$ value, where 'n' is the number of observations.

The data thus obtained were analysed using ANOVA table as depicted below:

Source of variation	**Degree of freedom (*df*)**	**Sum of Squares (SS)**	**Mean square MS = SS/*df***	**Computed F**
Treatment	t-1	SST	MST	MST/MSE
Error	n-t	SSE	MSE	
Total	N-1	SST_0		

where,

t: Treatments

n: Number of observations

MST: Treatment mean sum of squares

MSE: Error mean sum of squares

3.7.1 DMRT test for ranking

Duncan's Multiple Range Test (DMRT) is applied for the evaluation and ranking of all possible pairs of treatment means, particularly for experiments having big numbers of treatment. Numerical boundaries are created by DMRT which helps to classify the difference between any two treatments as significant or non-significant. DMRT value calculation depends primarily on the specific standard error (SE_m) of the pair of treatments being compared. The following procedure has been suggested by Gomez and Gomez (1976) for ranking of data:

i. Treatment means are ranked in the increasing or decreasing order (as per the order of preference).

ii. The standard error of error mean sum of squares (SE_m) was calculated by using the formula

$$SE_m = \sqrt{\frac{2MSE}{r}}$$

where, 'MSE' is the error mean sum of squares and 'r' is the number of replications.

iii. The (t-1) values of shortest significant ranges was calculated as follows:

$$R_p = \frac{(r_p)(SE_m)}{\sqrt{2}} \qquad \text{for } p = 2, 3, \ldots, t$$

where, 't' is the number of treatments, 'SE_m' is the standard error obtained in step ii, 'r_p' refers to the tabulated value of ranges which are significant and 'p' is the difference in rank between the pairs of treatment means to be compared ($p = t$ for the highest and lowest means).

iv. Identification and grouping of all treatment means which do not differ significantly from each other was done.

v. The results hence obtained were presented using alphabetical notation such that same alphabet is allotted to all treatments connected by the same vertical line.

Results

4. RESULTS

The present study on 'Seed invigoration with inorganic nanoparticles in chillies' was carried out in the Department of Seed Science and Technology, College of Horticulture, Vellanikkara. The results obtained with respect to seed quality parameters such as germination, shoot length, root length, seedling dry weight, vigour index - I and II, electrical conductivity of seed leachates, seed moisture content, seed micro flora and scanning electron microscope (SEM) study of seedling radicle are presented in this chapter.

4.1. Initial seed quality

The initial quality of the seeds prior to seed treatment was determined at the start of the experiment and presented in Table 5. The seeds had an initial germination of 83 per cent and recorded a shoot length of 4.93 cm and a root length of 6.95 cm. The dry weight, vigour index-I and vigour index-II were found to be 24.1 mg, 986, and 2000 respectively. The electrical conductivity of the seed leachate was 153 μScm^{-1}. The initial moisture content of the seed was 6.72 per cent and the seed infection with microflora was 3.00 per cent.

4.2. Analysis of variance

The analysis of variance of the observations recorded at monthly intervals revealed that there existed significant differences among the various treatments for seed quality parameters like germination, shoot length, root length, seedling dry weight, vigour index – I and II, electrical conductivity of seed leachates and seed moisture content during most of the storage period.

Table 5: Initial seed quality of chilli var. Anugraha

Seed quality parameter	Value
Germination (per cent)	83.00
Seedling shoot length (cm)	4.93
Seedling root length (cm)	6.95
Seedling dry weight (mg 10 seedlings^{-1})	24.1
Vigour index-I	986
Vigour index-II	2000
Electrical conductivity (µS cm^{-1})	153
Seed infection (per cent)	3.00
Seed moisture content (per cent)	6.72

4.3. Effect of zinc oxide (ZnO) on seed quality during natural ageing

4.3.1. Germination per cent

Chilli seeds treated with various doses of bulk and nano grades of zinc oxide exhibited significant differences in the germination per cent from six months of storage (MAS) only (Table 6). Irrespective of the treatment, the germinability of the seeds were found to decline over the period of storage. The quality of untreated seeds (Control) was found to be poor compared to the treated seeds.

The highest germination was observed in the second month of storage. Germination at 2 MAS ranged from 89 per cent in T4 (ZnO @ 1300 mg kg^{-1} seed) and T7 (nano ZnO @ 500 mg kg $^{-1}$ of seed) to 83 per cent in untreated seeds.

All the treatments except the control had maintained the IMSCS (Indian Minimum Seed Certification Standards) prescribed standard of seed germination for seed certification (60.00 per cent for chilli) till the end of the nine months of storage. The germination fell below IMSCS at nine MAS in the control. T4 (ZnO @ 1300 mg kg^{-1} seed) and T7 (nano ZnO @ 500 mg kg $^{-1}$ of seed) retained IMSCS for germination up to 10th month after storage. These treatments were found to be on par with each other.

At the end of the storage period (twelve months after storage), germination in all the treatments were found to ebb below 60 per cent. Treatments T7 (ZnO NP @ 500 mg kg^{-1} of seed) recorded the highest estimate of 36.67 per cent germination, and was found to be on par with T6 (ZnO NP @ 250 mg kg $^{-1}$ seed) at 35.00 per cent and T4 (ZnO @ 1300 mg kg^{-1} seed) at 31.00 per cent.

Considering the above, seed quality at 10 MAS will henceforth be discussed upon.

4.3.2. Seedling shoot length (cm)

Zinc oxide particles exerted a significant influence on the shoot length (Table 7) during the storage period except at fourth, seventh, tenth and twelfth months of storage. At eleven months of storage, T7 (nano ZnO @ 500 mg kg $^{-1}$ of seed) registered the highest shoot length (4.43 cm). T7 was found to be on par with T4 (ZnO @ 1300 mg kg^{-1} seed) at 4.10 cm.

Table 6: Influence of ZnO (bulk and nano) particles on germination (per cent) of chilli during natural ageing

Germination per cent												
Treatment (T)	**Month of storage (M)**											
	MI	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
T1 Control	82.33 (55.48)	83.00 (56.16)	82.00 (55.29)	80.33 (53.84)	78.67 (52.52)	73.33^{c} (47.20)	70.00^{b} (44.46)	67.33^{c} (42.37)	58.33^{c} (22.96)	47.67^{d} (28.50)	41.00^{d} (24.22)	20.00^{bc} (11.54)
T2 ZnO@500	82.00 (55.54)	86.66 (60.14)	85.66 (59.10)	85.33 (58.86)	83.00 (56.76)	79.00^{abc} (52.31)	69.00^{b} (43.75)	70.33^{bc} (44.73)	69.33^{ab} (41.09)	58.33^{c} (35.70)	46.67^{bcd} (27.84)	21.33^{bc} (12.35)
T3 ZnO@900	81.67 (56.22)	86.66 (60.52)	84.66 (58.40)	86.00 (60.53)	84.00 (57.26)	82.33^{ab} (55.51)	73.00^{ab} (47.13)	71.00^{bc} (45.26)	68.33^{ab} (45.55)	61.00^{bc} (37.62)	44.00^{cd} (26.12)	29.00^{ab} (16.89)
T4 ZnO@1300	83.67 (56.84)	89.00 (63.34)	87.00 (61.14)	87.33 (60.88)	86.00 (60.00)	84.66^{a} (58.17)	80.33^{a} (53.75)	79.33^{a} (52.81)	73.33^{a} (45.15)	67.67^{a} (42.61)	53.00^{a} (32.05)	31.00^{a} (18.08)
T5 nZnO@100	80.33 (53.56)	86.00 (59.35)	84.66 (57.98)	84.00 (57.27)	82.00 (55.25)	76.67^{bc} (50.10)	65.33^{b} (40.93)	69.67^{c} (44.22)	64.00^{bc} (41.16)	58.33^{c} (35.72)	44.67^{cd} (26.55)	15.00^{c} (8.63)
T6 nZnO@250	81.33 (54.65)	87.00 (61.51)	86.00 (59.88)	85.00 (58.24)	83.00 (56.17)	79.33^{abc} (52.54)	75.67^{ab} (49.31)	76.33^{ab} (49.82)	71.66^{a} (42.63)	62.33^{bc} (38.60)	52.00^{ab} (31.40)	35.00^{a} (20.55)
T7 nZnO@500	84.33 (57.70)	89.00 (64.88)	87.33 (61.52)	88.33 (63.69)	87.00 (61.51)	83.33^{a} (56.59)	81.67^{a} (55.05)	79.00^{a} (52.26)	74.33^{a} (49.31)	64.67^{ab} (40.37)	49.00^{abc} (29.36)	36.67^{a} (21.54)
SE_m	5.70	5.94	5.15	5.69	5.75	2.64	4.04	2.51	6.73	1.67	1.90	2.61
CD	NS	NS	NS	NS	NS	5.67	8.67	5.38	14.44	3.59	4.07	5.60

*Values within parenthesis are arc sine transformed values **NS – Non-significant

Table 7: Influence of ZnO (bulk and nano) particles on shoot length of chilli under natural ageing

Treatment (T)	Shoot length (cm)											
	Month of storage (M)											
	MI	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
T1 Control	6.35^{e}	6.28^{c}	6.22^{d}	6.04	5.92^{c}	5.57^{b}	5.21	5.18^{b}	4.82c	4.34	2.54^{c}	2.58
T2 ZnO@500	6.45^{de}	6.36^{bc}	6.30^{cd}	6.248	6.22^{b}	6.19^{a}	6.144	6.35^{a}	5.32^{abc}	4.49	2.92^{c}	1.89
T3 ZnO@900	6.67^{cd}	6.51^{bc}	6.45^{bcd}	6.419	6.36^{ab}	6.30^{a}	6.28	6.17^{a}	5.50^{ab}	4.51	3.62^{b}	2.96
T4 ZnO@1300	7.14^{a}	6.96^{a}	6.84^{a}	6.61	6.58^{a}	6.46^{a}	6.34	5.92^{a}	5.69^{ab}	4.70	4.10^{ab}	4.01
T5 nZnO@100	6.87^{abc}	6.61^{abc}	6.53^{abcd}	6.51	6.40^{ab}	6.38^{a}	6.31	6.30^{a}	5.11^{bc}	4.42	2.57^{c}	2.93
T6 nZnO@250	6.72^{bc}	6.68^{ab}	6.59^{abc}	6.55	6.47^{ab}	6.43^{a}	6.39	6.31^{a}	5.30^{abc}	4.46	2.88^{c}	2.86
T7 nZnO@500	7.01^{ab}	6.87^{a}	6.72^{ab}	6.62	6.51^{ab}	6.47^{a}	6.43	6.48^{a}	5.77^{a}	4.61	4.43^{a}	3.02
SE_m	0.12	0.18	0.18	0.24	0.15	0.14	0.38	0.34	0.28	0.45	0.32	0.75
CD	0.29	0.36	0.36	NS	0.33	0.33	NS	0.73	0.58	NS	0.69	NS

*NS - Non-significant

4.3.3. Seedling root length (cm)

The effect of zinc oxide on root length of chilli over storage was recorded and found to be significant in the fourth, seventh, ninth, tenth, eleventh and twelfth month of storage (Table 8). A progressive fall in the length of roots was observed over the period of storage. The initial root length of 8.11 cm (T4) and 7.26 cm (T1) declined to 6.36 cm in T4 (ZnO @ 1300 mg kg^{-1} seed) and 4.50 cm in T1 (control) at the tenth month of storage.

4.3.4. Seedling dry weight (mg)

There existed a variation in the dry matter production of the seedlings during the experiment span with the general trend showing a decline with the progression of storage period (Table 9).

At the start of the evaluation, there was no significant differences between the treatments and control. At the tenth month of storage, high dry matter production (20.70 mg 10^{-1} seedlings) could be discerned in seeds treated with nano-ZnO @ 100 mg kg^{-1} (T5) and the least was in control (T1) @ 15.87 mg 10^{-1} seedlings.

4.3.5. Vigour index I

Zinc oxide treatments of chilli seeds in different doses manifested significant differences among the treatments in terms of vigour index-I from sixth month of storage (Table 10). Vigour index-I declined over the period of storage. The highest value (1315) was observed in seeds coated with ZnO @ 1300 mg kg^{-1} followed by nano-ZnO @ 500 mg kg^{-1} seed (1303) in the second month of storage.

At the tenth month of storage, dry dressing of seeds with ZnO @ 1300 mg kg^{-1} gave the highest vigour index-I (743), which was on par with that of nano-ZnO @ 500 mg kg-1 treated seeds (675) and ZnO @ 900 mg kg^{-1} treated seeds (641). At 10 MAS, the control recorded a vigour index of 493.

Table 8: Influence of ZnO (bulk and nano) particles on root length of chilli during natural ageing

Root length (cm)												
Treatment (T)	Month of storage (M)											
	MI	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
T1 Control	7.26	6.94	6.68	6.15^{c}	5.73	5.40	5.15^{c}	4.79	4.66^{c}	4.50^{c}	2.93^{c}	2.32^{c}
T2 ZnO@500	7.49	7.28	6.95	6.72^{b}	6.45	6.23	6.08^{bc}	5.39	5.74^{b}	5.62^{ab}	3.47^{c}	3.00^{bc}
T3 ZnO@900	7.73	7.51	7.26	7.11^{ab}	6.74	6.66	6.43^{ab}	6.29	6.01^{ab}	6.04^{a}	4.34^{ab}	3.23^{b}
T4 ZnO@1300	8.11	7.81	7.62	7.30^{a}	7.15	6.95	6.64^{ab}	6.55	6.47^{ab}	6.36^{a}	4.64^{a}	4.35^{a}
T5 nZnO@100	7.38	7.20	7.11	6.85^{ab}	6.71	6.47	6.18^{ab}	5.63	5.74^{bc}	5.97^{bc}	3.61^{bc}	3.65^{ab}
T6 nZnO@250	7.56	7.39	7.16	7.08^{ab}	6.99	6.68	6.30^{ab}	6.28	6.27^{ab}	5.68^{ab}	4.37^{ab}	4.12^{a}
T7 nZnO@500	7.89	7.75	7.59	7.33^{a}	7.29	7.12	7.07^{a}	6.93	6.74^{a}	6.14^{a}	4.93^{a}	4.43^{a}
SE_m	0.70	0.62	0.45	0.26	0.91	0.92	0.48	0.75	0.41	0.35	0.39	0.41
CD	NS	NS	NS	0.56	NS	NS	0.97	NS	0.88	0.74	0.99	0.88

*NS - Non-significant

Table 9: Influence of ZnO (bulk and nano) particles on seedling dry weight of chilli during natural ageing

Seedling dry weight (mg 10 seedlings^{-1})												
Treatments (T)	**Months of storage (M)**											
	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
T1 Control	27.73	29.95	25.28	23.39^{b}	21.45^{b}	20.67^{b}	18.78^{b}	17.42^{d}	16.24^{c}	15.87^{b}	15.37^{d}	12.34^{d}
T2 ZnO@500	30.51	31.50	28.14	27.59^{a}	25.46^{a}	24.16^{a}	23.28^{a}	21.93^{abc}	19.30^{abc}	18.3^{ab}	21.45^{a}	16.40^{bc}
T3 ZnO@900	27.42	28.80	26.49	25.84^{a}	24.10^{a}	23.89^{a}	23.34^{a}	22.61^{abc}	20.30^{ab}	19.70^{a}	17.93^{bc}	18.73^{a}
T4 ZnO@1300	29.37	30.90	28.64	27.81^{a}	26.18^{a}	25.41^{a}	24.07^{a}	22.84^{ab}	17.65^{bc}	19.73^{a}	19.63^{ab}	18.10^{abc}
T5 nZnO@100	28.51	29.30	26.46	26.22^{a}	25.98^{a}	24.53^{a}	22.43^{a}	21.70^{bc}	20.30^{ab}	20.70^{a}	18.13^{bc}	16.33^{c}
T6 nZnO@250	28.55	29.40	26.94	26.54^{a}	25.67^{a}	24.97^{a}	23.11^{a}	20.57^{c}	16.37^{c}	18.20^{ab}	19.30^{abc}	18.60^{ab}
T7 nZnO@500	28.87	29.53	27.21	26.87^{a}	26.25^{a}	25.57^{a}	24.87^{a}	23.96^{a}	21.70^{a}	19.85^{a}	17.23^{cd}	17.63^{ab}
SE$_{m}$	1.62	1.64	1.34	1.11	1.14	1.09	1.36	1.02	4.61	1.09	1.11	1.04
CD	NS	NS	NS	2.37	2.45	2.34	2.91	2.18	5.76	NS	2.36	2.23

*NS - Non-significant

Table 10: Influence of ZnO (bulk and nano) particles on vigour index - I of chilli during natural ageing

Vigour index – I												
Treatments (T)	**Months of storage (M)**											
	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
T1 Control	1056	1124	1070	1007	908	824^{b}	710^{d}	722^{d}	612^{e}	493^{c}	234^{d}	99^{c}
T2 ZnO@500	1144	1182	1135	1106	1062	982^{ab}	842^{cd}	826^{cd}	766^{cd}	602^{b}	297^{cd}	103^{c}
T3 ZnO@900	1179	1214	1161	1165	1104	1066^{a}	932^{bc}	886^{bc}	785^{bcd}	641^{ab}	351^{bc}	180^{bc}
T4 ZnO@1300	1308	1315	1257	1215	1184	1131^{a}	1048^{ab}	988^{ab}	890^{ab}	743^{a}	463^{a}	260^{ab}
T5 nZnO@100	1146	1188	1156	1122	1078	985^{ab}	819^{cd}	832^{cd}	695de	608^{b}	278^{cd}	106^{c}
T6 nZnO@250	1163	1228	1181	1159	1118	1040^{a}	958^{abc}	962^{abc}	831^{abc}	634^{b}	377^{b}	233^{ab}
T7 nZnO@500	1272	1303	1250	1232	1198	1132^{a}	1101^{a}	1058^{a}	930^{a}	675^{ab}	459^{a}	273^{a}
SE$_{m}$	123.31	100.59	73.83	76.86	127.52	78.49	73.28	65.54	50.64	49.94	35.47	39.39
CD	NS	NS	NS	NS	NS	168.36	157.18	140.59	108.62	107.12	76.09	84.49

*NS - Non-significant

4.3.6. Vigour index II

Significant differences in vigour index-II were observed among the treatments from the fifth month onwards (Table 11).

Vigour index II was the highest recorded in T4 (2751) at the second month of storage followed by T7 (2618) in the same month. At the tenth month of storage, T4 (ZnO@ 1300 mg kg^{-1}) maintained high vigour (1335), and it was on par with T7 (ZnO NP@ 500 mg kg^{-1}) at 1285 and T3 (ZnO@ 900 mg kg^{-1}) at 1199.

4.3.7. Electrical conductivity of seed leachates (µS cm^{-1})

Table 12 depicts the electrical conductivity of leachate from seeds dry dressed with varying concentration of zinc oxide, both bulk-sized and nano-sized. The electrical conductivity value depicted a significantly increasing trend throughout the storage period regardless of the treatment.

At ten MAS, T7 - nano-ZnO @ 900 mg kg^{-1} (246.33 µScm^{-1}) registered the least electrical conductivity of seed leachate followed by T6 - nano-ZnO @ 250 mg kg^{-1} (265.23 µScm^{-1}). The untreated seeds produced the highest electrical conductivity for their seed leachates (415.00 µScm^{-1}).

4.3.8. Seed moisture content (per cent)

Variation in seed moisture content was found to be insignificant in zinc oxide-treated and untreated seeds with the advancement of storage period (Table 13).

4.3.9. Seed microflora

Seed infection percent of the seeds at the end of storage was found to be minimal (Table 14). In blotter paper method, none of the treatments except control (T1; 1.33% infection) and nano-ZnO @ 250 mgkg^{-1} treated seeds (T6; 1.33% infection) were found to be infected by fungi. In agar plate method also control (T1) seeds expressed 3.33 per cent infection along with T2 (ZnO@500 mg kg^{-1}), while all other treatments tested negative for pathogen detection. Occurrence of *Aspergillus niger* and *Aspergillus flavus* was recorded.

Table 11: Influence of ZnO (bulk and nano) particles on vigour index – II of chilli during natural ageing

Vigour index – II												
Treatments (T)	**Months of storage (M)**											
	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
T1 Control	2149	2545	2101	1940	1687^{b}	1549^{b}	1286^{d}	1261^{d}	1049^{c}	867^{d}	678^{c}	245^{b}
T2 ZnO@500	2496	2731	2411	2353	2117^{a}	1910^{a}	1602^{c}	1543^{c}	1338^{b}	1068^{c}	1000^{ab}	354^{b}
T3 ZnO@900	2248	2501	2244	2224	2022^{a}	1968^{a}	1723^{bc}	1606^{bc}	1390^{ab}	1199^{abc}	790^{c}	542^{a}
T4 ZnO@1300	2519	2751	2491	2429	2243^{a}	2154^{a}	1932^{ab}	1814^{ab}	1360^{ab}	1335^{a}	1043^{a}	557^{a}
T5 nZnO@100	2293	2520	2242	2204	2135^{a}	1881^{a}	1545^{c}	1510^{c}	1297^{bc}	1210^{abc}	809^{c}	243^{b}
T6 nZnO@250	2319	2551	2314	2256	2129^{a}	1957^{a}	1750^{bc}	1569^{c}	1185^{bc}	1137^{bc}	1001^{ab}	652^{a}
T7 nZnO@500	2446	2613	2378	2365	2288^{a}	2133^{a}	2024^{a}	1894^{a}	1616^{a}	1285^{ab}	843^{bc}	649^{a}
SE_m	223.77	165.88	166.11	160.06	143.33	128.49	112.46	105.87	123.92	90.71	87.68	81.82
CD	NS	NS	NS	NS	307.44	275.62	241.22	227.09	265.80	194.59	188.07	175.50

*NS - Non-significant

Table 12: Influence of ZnO (bulk and nano) particles on electrical conductivity of seed leachates of chilli during natural ageing

E.C. (µScm^{-1})												
Treatment (T)	**Month of storage (M)**											
	MI	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
T1 Control	147.62^{a}	163.94^{a}	172.15^{a}	198.31^{a}	225.57^{a}	254.32^{a}	336.50^{a}	350.46^{a}	383.76^{a}	415.00^{a}	436.81^{a}	462.34^{a}
T2 ZnO@500	129.71^{b}	135.00^{c}	149.25^{b}	157.19^{c}	165.87^{cd}	203.75^{bc}	231.67^{b}	232.00^{c}	290.29^{b}	311.45^{b}	326.67^{bc}	373.46^{b}
T3 ZnO@900	122.79^{bc}	128.00^{d}	136.05^{cd}	142.30^{e}	168.67^{c}	197.50^{c}	226.33^{b}	249.67^{bc}	266.25^{d}	288.25^{bcd}	303.67^{cd}	336.33^{d}
T4 ZnO@1300	119.46^{cd}	124.00^{d}	132.25^{d}	145.21^{de}	192.53^{b}	209.10^{bc}	229.33^{b}	234.67^{c}	274.57^{c}	301.18^{bc}	339.00^{b}	357.02^{c}
T5 nZnO@100	127.44^{b}	141.00^{b}	153.49^{b}	162.32^{c}	168.80^{c}	214.43^{b}	239.00^{b}	270.00^{b}	281.36^{c}	283.42^{cd}	292.33^{de}	345.05^{d}
T6 nZnO@250	112.54^{de}	129.00^{d}	139.38^{c}	147.53^{d}	163.83^{cd}	204.67^{bc}	242.33^{b}	229.00^{c}	246.18^{e}	265.23^{de}	273.67^{e}	324.21^{e}
T7 nZnO@500	106.55^{e}	117.00^{e}	125.26^{e}	137.40^{f}	153.09^{d}	169.41^{d}	182.00^{c}	204.45^{d}	224.00^{f}	246.33^{e}	268.67^{e}	316.49^{e}
SE$_{m}$	3.66	2.62	2.47	2.61	6.24	7.24	9.05	10.23	3.40	11.78	11.07	4.15
CD	7.87	5.64	5.30	3.96	13.38	15.53	19.42	21.94	7.30	25.26	23.75	8.90

*NS - Non-significant

Table 13: Influence of ZnO (bulk and nano) particles on seed moisture content of chilli at the end of storage during natural ageing

Treatment	Seed moisture content (per cent)
T1: Control	6.94
T2: ZnO – 500 mg/kg	6.98
T3: ZnO – 900 mg/kg	7.04
T4: ZnO – 1300 mg/kg	6.96
T5: nanoZnO – 100 mg/kg	7.00
T6: nanoZnO – 250 mg/kg	6.94
T7: nanoZnO – 500 mg/kg	6.97
SE_m	0.27
CD	NS

Table 14: Influence of ZnO (bulk and nano) particles on seed infection per cent of chilli at the end of storage on natural ageing

Treatments	Seed infection (per cent)	
	Blotter paper method	Agar plate method
T1: Control	1.33	3.33
T2: ZnO – 500 mg/kg	0.00	3.33
T3: ZnO – 900 mg/kg	0.00	0.00
T4: ZnO – 1300 mg/kg	0.00	0.00
T5: nanoZnO – 100 mg/kg	0.00	0.00
T6: nanoZnO – 250 mg/kg	1.33	0.00
T7: nanoZnO – 500 mg/kg	0.00	0.00

4.3.10. SEM analysis of radicle

Scanning electron microscope observations of the radicle cells of seeds treated with ZnO NP @ 500 mg kg^{-1} and untreated control seeds were carried to study the anatomical changes. The root tip of the nanoparticle treated seeds exhibited faster cell proliferation compared to the untreated seeds (Plate 6). In the region of elongation (Plate 7), measurements of the cell revealed the presence of larger cell (cell length: 19.08 – 28.43 µm, cell width: 6.61 – 14.28 µm) when seeds were treated with nano-ZnO compared to the radicle anatomy of control seeds (cell length: 9.07 – 16.49 µm, cell width: 5.77 – 5.80 µm).

4.4. Effect of titanium dioxide (TiO_2) on seed quality during natural ageing

4.4.1. Germination per cent

A gradual decline in germination was observed in control seeds as well as seeds treated with TiO_2 and nano-TiO_2 throughout the storage period during natural ageing. All seeds including control were able to maintain 60.00 per cent germination up to eight months of storage (Table 15). At the end of tenth month of storage only treated seeds were able to retain the minimum germination standard of 60 per cent set for chilli as per the IMSCS (Indian Minimum Seed Certification Standards).

At ten months after storage the highest germination of 63.00 per cent was seen in seeds treated T7 (nTiO_2 @ 500 mg kg^{-1} of seed), which was on par with seeds treated with TiO_2 @ 900 mg kg^{-1} (T3) at 61.33 per cent, TiO_2 @ 1300 mg kg^{-1} (T4) at 61.00 per cent and nTiO_2 @ 250 mg kg^{-1} (T6) at 60.00 per cent germination.

Table 15: Influence of TiO_2 (bulk and nano) particles on germination of chilli during natural ageing

Treatment (T)	Germination per cent											
	Month of storage (M)											
	MI	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
T1 **Control**	82.33 (55.48)	83.00 (56.16)	82.00 (55.30)	80.33 (53.84)	78.67 (52.52)	73.33^{c} (47.20)	70.00^{c} (44.46)	67.33^{b} (42.37)	58.33^{d} (35.72)	47.66^{d} (28.50)	41.00 (24.22)	20.00^{b} (11.54)
T2 **TiO_2@500**	81.33 (54.80)	87.00 (60.78)	84.67 (58.27)	84.33 (58.08)	83.00 (56.60)	78.33^{abc} (51.72)	72.67^{bc} (46.65)	72.33^{a} (46.39)	64.00^{c} (39.83)	54.00^{cd} (32.72)	46.00 (27.42)	28.66^{a} (16.67)
T3 **TiO_2@900**	84.67 (58.07)	86.67 (60.61)	85.67 (59.28)	84.67 (58.10)	85.00 (59.03)	82.67^{ab} (55.83)	74.33^{abc} (48.10)	71.00^{ab} (45.27)	66.00^{bc} (41.33)	61.33^{ab} (37.86)	43.33 (25.71)	30.33^{a} (17.67)
T4 **TiO_2@1300**	82.00 (56.05)	87.33 (61.42)	86.33 (59.89)	84.00 (57.62)	83.00 (56.21)	84.33^{a} (57.75)	79.33^{ab} (52.73)	73.67^{a} (47.50)	69.33^{ab} (43.92)	61.00^{ab} (37.67)	44.00 (26.24)	24.66^{ab} (14.30)
T5 **$nTiO_2$@100**	80.00 (53.92)	86.00 (60.35)	85.00 (59.39)	84.67 (58.27)	80.67 (54.86)	75.00^{bc} (48.80)	72.33^{c} (46.39)	73.33^{a} (47.20)	65.67^{bc} (41.08)	56.33^{bc} (34.31)	44.00 (26.14)	26.33^{ab} (15.28)
T6 **$nTiO_2$@250**	81.33 (55.24)	86.33 (59.99)	86.33 (60.12)	82.00 (55.37)	82.00 (55.25)	77.67^{abc} (51.21)	75.33^{abc} (49.13)	70.33^{ab} (44.73)	68.33^{abc} (43.20)	60.00^{abc} (36.94)	45.00 (26.78)	27.33^{a} (15.87)
T7 **$nTiO_2$@500**	83.33 (57.06)	87.66 (61.46)	87.33 (61.16)	85.66 (59.12)	86.00 (59.46)	83.67^{a} (56.91)	81.00^{a} (54.15)	73.67^{a} (47.52)	70.67^{a} (45.01)	63.00^{a} (39.08)	46.33 (27.70)	30.66^{a} (17.88)
SE_m	6.28	5.33	3.83	5.03	6.04	3.36	2.87	1.51	3.43	2.04	3.00	1.74
CD	NS	NS	NS	NS	NS	7.21	6.17	3.25	1.59	4.39	NS	3.75

*Values within parenthesis are arc sine transformed values **NS – Non-significant

Significant differences among the treatments were observed from 6 MAS. Untreated seeds maintained 60 per cent germination only up to eighth month of storage. Germination in the treated seeds, T2 (TiO_2 @ 500 mg kg $^{-1}$) and T5 (nano-TiO_2 @ 100 mg kg $^{-1}$) fell below the IMSCS standards of germination at ten months after storage. They recorded 54.00 per cent and 56.33 per cent respectively. Treatment T6 and T7 (nano-TiO_2 @ 500 mg kg^{-1}) retained IMSCS standards at ten months of storage.

4.4.2. Seedling shoot length (cm)

Seedling shoot length did not show any significant difference among the treatments during the initial part of the storage study (Table 16). The effect of seed treatment on shoot length was found to be non-significant at 10 MAS. At the end of storage in the twelfth month, T6 (nano-TiO_2 @ 250 mg kg^{-1}) was superior over the rest of the treatments and was on par with T7 (nano-TiO_2 @ 500 mg kg^{-1}) at 4.16 cm, whereas control developed 2.58 cm long shoots.

4.4.3. Seedling root length (cm)

No significant effect of dry dressing of chilli seeds with titanium dioxide was seen on the growth of root (Table 17) until seventh month of storage. At 7 MAS, T7 (nano-TiO_2 @ 500 mg kg^{-1} seed) registered the highest root length (6.58 cm) and T1 (control) produced the smallest root (5.15 cm). At the tenth month of storage also the longest roots were produced by T7 (6.44 cm) while the untreated seeds developed 4.5 cm long roots.

4.4.4. Seedling dry weight (mg)

The data pertaining to the influence of titanium dioxide particle on the seedling dry weight during natural ageing in ambient conditions is presented in Table 18.

Significant variability in the dry matter production of seedlings was noticed from the third month of storage except at ninth and eleventh months of storage. At the tenth month of storage, highest dry biomass weight was exhibited by T7 (nano-TiO_2 @ 500 mg kg^{-1} seed) at 19.37 mg 10^{-1} seedlings and the least dry weight was obtained from T1 (control) @ 15.87 mg 10^{-1} seedlings.

Table 16: Influence of TiO_2 (bulk and nano) particles on shoot length of chilli during natural ageing

Shoot length (cm)												
Treatment (T)	**Month of storage (M)**											
	MI	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
T1 Control	6.39	6.35	6.33	6.28	6.22	6.14	6.05^{b}	6.03^{b}	5.00^{bc}	4.51	2.54^{d}	2.58^{c}
T2 TiO_2@500	6.51	6.48	6.42	6.37	6.35	6.30	6.23^{b}	6.12^{b}	5.67^{a}	5.00	3.67^{c}	2.67^{c}
T3 TiO_2@900	6.63	6.61	6.59	6.53	6.41	6.38	6.24^{b}	6.15^{b}	5.46^{ab}	4.62	4.30^{a}	3.64^{ab}
T4 TiO_2@1300	7.23	7.15	7.06	6.82	6.67	6.53	6.42^{ab}	6.24^{b}	5.59^{a}	4.56	4.19^{ab}	2.92^{bc}
T5 $nTiO_2$@100	6.38	6.31	6.28	6.26	6.24	6.17	6.10^{b}	5.96^{b}	4.59^{c}	4.46	3.72^{c}	3.20^{bc}
T6 $nTiO_2$@250	6.72	6.68	6.54	6.48	6.37	6.33	6.29^{b}	6.25^{b}	5.49^{ab}	4.61	4.39^{a}	4.28^{a}
T7 $nTiO_2$@500	6.92	6.90	6.87	6.85	6.82	6.79	6.68^{a}	6.76^{a}	5.77^{a}	4.77	3.84^{bc}	4.16^{a}
SE_m	0.61	0.48	0.82	0.61	0.23	0.61	0.15	0.14	0.24	0.21	0.18	0.41
CD	NS	NS	NS	NS	NS	NS	0.34	0.34	0.54	NS	0.41	0.88

*NS – Non-significant

Table 17: Influence of TiO_2 (bulk and nano) particles on root length of chilli during natural ageing

Root length (cm)												
Treatment (T)	**Month of storage (M)**											
	MI	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
T1 Control	7.26	6.94	6.68	6.15	5.73	5.40	5.15^{c}	4.79^{c}	4.66	4.50^{c}	2.93^{d}	2.32^{c}
T2 TiO_2@500	7.36	7.26	6.89	6.65	6.32	6.12	5.65^{bc}	5.65^{b}	5.26	5.44^{bc}	3.57^{cd}	3.14^{bc}
T3 TiO_2@900	7.68	7.42	7.22	6.84	6.53	6.58	6.50^{ab}	6.25^{ab}	6.22	6.05^{ab}	4.03^{bc}	3.53^{b}
T4 TiO_2@1300	7.92	7.73	7.54	7.29	7.03	6.85	6.54^{ab}	6.42^{a}	6.35	6.16^{ab}	4.85^{ab}	3.99^{ab}
T5 $nTiO_2$@100	7.23	7.05	6.92	6.53	6.50	6.31	6.18^{ab}	6.13^{ab}	5.54	5.73^{ab}	3.82^{cd}	3.22^{bc}
T6 $nTiO_2$@250	7.47	7.29	7.00	6.88	6.76	6.49	6.27^{ab}	6.18^{ab}	5.95	6.08^{ab}	5.01^{a}	4.62^{a}
T7 $nTiO_2$@500	7.81	7.65	7.48	7.27	7.18	6.92	6.58^{a}	6.32^{ab}	6.31	6.44^{a}	5.60^{a}	4.94^{a}
SE_m	0.51	0.50	0.47	0.56	0.18	0.94	0.93	0.84	0.69	0.44	0.41	0.48
CD	NS	NS	NS	NS	NS	NS	0.44	0.70	NS	0.96	0.90	1.04

*NS – Non-significant

Table 18: Influence of TiO$_2$ (bulk and nano) particles on seedling dry weight of chilli during natural ageing

Treatments (T)	Seedling dry weight (mg 10 seedlings^{-1})											
	Months of storage (M)											
	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
T1 Control	28.67	29.95	26.28^{c}	24.39^{c}	22.57^{b}	21.46^{c}	19.86^{c}	18.30^{b}	17.15	15.87^{d}	15.37	12.34^{d}
T2 TiO$_2$@500	30.58	32.15	29.67^{ab}	28.62^{a}	25.64^{ab}	26.21^{a}	24.71^{a}	22.46^{a}	20.20	18.57^{abc}	17.03	14.88^{c}
T3 TiO$_2$@900	28.21	29.10	27.78^{bc}	26.51^{abc}	25.21^{ab}	23.68^{abc}	21.89^{abc}	20.40^{ab}	18.13	18.93^{ab}	17.22	15.43^{bc}
T4 TiO$_2$@1300	28.36	29.73	26.64^{c}	25.84^{bc}	24.05^{b}	23.87^{abc}	22.78^{abc}	21.29^{a}	19.93	16.90^{cd}	17.52	17.77^{a}
T5 nTiO$_2$@100	29.35	30.10	28.33^{abc}	26.41^{abc}	24.75^{ab}	22.14^{bc}	21.53^{bc}	20.37^{ab}	19.10	18.29^{abc}	17.48	17.19^{ab}
T6 nTiO$_2$@250	31.43	32.70	30.85^{a}	28.61^{a}	27.38^{a}	25.37^{ab}	24.42^{ab}	21.56^{a}	19.15	17.07^{bcd}	17.24	16.97^{abc}
T7 nTiO$_2$@500	29.52	30.77	29.34^{ab}	28.45^{ab}	27.67^{a}	26.32^{a}	24.85^{a}	22.21^{a}	20.40	19.37^{a}	18.77	17.47^{ab}
SE$_m$	1.16	1.83	1.23	1.27	1.46	1.57	1.44	1.09	1.78	0.89	1.11	1.03
CD	NS	NS	2.65	2.74	3.14	3.37	3.09	2.35	NS	1.91	NS	2.22

*NS – Non-significant

4.4.5. Vigour index I

Seedling length and germination per cent were used to calculate vigour index-I and the relevant data are shown in Table 19. Significant differences in VI-I among the treatments were observed from the sixth month of storage. At the end of tenth month, seeds that were treated with nano-TiO_2 @ 500 mg kg^{-1} recorded the highest vigour index I (683) which was on par with T4 (634), T3 (633) and T6 (623), whereas control (T1) registered the least (431) value.

4.4.6. Vigour index II

Vigour index-II declined over the period of study irrespective of the treatment given with TiO_2 (Table 20). For the initial three months of storage, no significant difference could be detected among the treatments. Ten months after storage, T7 (nano-TiO_2 @ 500 mg kg^{-1}) was the best treatment by maintaining a vigour index of 1221 and was on par with T3 (1162).

4.4.7. Electrical conductivity of seed leachates (µS cm^{-1})

The electrical conductivity of the seed leachate was found to be significantly influenced by the seed invigoration using titanium dioxide bulk and nano particles during natural ageing (Table 21).

Notwithstanding the treatment given, electrical conductivity of the seed leachate increased over the time period indicating seed deterioration. At ten months of storage the least electrical conductivity was measured in the seed leachate of T7 (331.33 µS cm^{-1}), closely followed by T4 (332.67 µS cm^{-1}) and T3 (342.33 µS cm^{-1}), when the control seed registered a value of 415 µScm^{-1}.

4.4.8. Seed moisture content (per cent)

Dry dressing of chilli seeds with the treatment chemicals did not exhibit any significant influence on seed moisture content (Table 22).

Table 19: Influence of TiO_2 (bulk and nano) particles on vigour index - I of chilli during natural ageing

Vigour index – I												
Treatments (T)	Months of storage (M)											
	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
T1 Control	1051	1129	1066	995	947	846^{c}	793^{d}	729^{d}	584^{d}	431^{c}	223^{b}	99^{c}
T2 TiO_2@500	1128	1202	1124	1097	1057	970^{bc}	862^{cd}	860^{c}	699^{bc}	543^{b}	335^{a}	168^{b}
T3 TiO_2@900	1208	1217	1179	1132	1092	1059^{ab}	955^{bc}	880^{bc}	771^{ab}	633^{ab}	360^{a}	231^{a}
T4 TiO_2@1300	1253	1299	1261	1188	1136	1130^{a}	1027^{ab}	932^{ab}	827^{a}	634^{ab}	400^{a}	150^{bc}
T5 $nTiO_2$@100	1069	1150	1128	1081	1026	937^{bc}	887^{cd}	887^{bc}	665^{cd}	573^{b}	332^{a}	177^{b}
T6 $nTiO_2$@250	1164	1208	1166	1099	1078	989^{b}	948^{bc}	865^{c}	782^{ab}	623^{ab}	422^{a}	242^{a}
T7 $nTiO_2$@500	1231	1276	1254	1210	1204	1154^{a}	1074^{a}	963^{a}	853^{a}	683^{a}	436^{a}	278^{a}
SE_m	107.87	108.19	82.09	77.48	99.45	58.10	49.15	29.31	46.48	47.41	49.71	24.30
CD	NS	NS	NS	NS	NS	124.65	105.44	62.88	99.71	101.69	106.63	52.14

*NS – Non-significant

Table 20: Influence of TiO_2 (bulk and nano) particles on vigour index – II of chilli during natural ageing

Vigour index – II												
Treatments (T)	**Months of storage (M)**											
	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
T1 Control	2210	2547	2154	1955^{d}	1788	1577^{c}	1388^{d}	1234^{c}	1001^{d}	756^{d}	629^{c}	245^{b}
T2 TiO_2@500	2484	2795	2517	2411^{ab}	2138	2058^{ab}	1797^{abc}	1625^{a}	1292^{abc}	1003^{c}	788^{ab}	425^{a}
T3 TiO_2@900	2384	2529	2382	2242^{abc}	2153	1960^{abc}	1630^{bcd}	1449^{b}	1197^{c}	1162^{ab}	742^{b}	471^{a}
T4 TiO_2@1300	2338	2595	2298	2163^{c}	1999	2018^{ab}	1804^{abc}	1568^{ab}	1381^{ab}	1030^{bc}	773^{ab}	440^{a}
T5 $nTiO_2$@100	2316	2591	2419	2234^{bc}	2008	1667^{bc}	1556^{cd}	1492^{ab}	1254^{bc}	1030^{bc}	772^{ab}	451^{a}
T6 $nTiO_2$@250	2562	2821	2658	2340^{abc}	2241	1963^{abc}	1842^{ab}	1516^{ab}	1309^{abc}	1025^{bc}	773^{ab}	464^{a}
T7 $nTiO_2$@500	2449	2692	2567	2435^{a}	2376	2206^{a}	2014^{a}	1634^{a}	1441^{a}	1221^{a}	862^{a}	534^{a}
SE_m	202.84	211.09	195.39	93.54	239.92	184.40	132.87	82.04	72.91	73.92	52.38	53.73
CD	NS	NS	NS	200.66	NS	395.55	285.01	175.98	156.39	158.57	112.37	115.26

*NS – Non-significant

Table 21: Influence of TiO_2 (bulk and nano) particles on electrical conductivity of seed leachate of chilli during natural ageing

E.C. (µScm^{-1})												
Treatment (T)	**Month of storage (M)**											
	MI	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
T1 Control	147.62^{a}	163.94^{a}	172.15^{a}	198.31^{a}	225.57^{a}	254.32^{a}	336.50^{a}	350.46^{a}	383.76^{a}	415.00^{a}	436.81^{a}	462.34^{a}
T2 TiO_2@500	131.52^{b}	154.34^{b}	156.12^{b}	164.44^{b}	170.97^{b}	231.12^{b}	239.00^{c}	275.33^{b}	304.33^{b}	355.33^{b}	372.53^{b}	398.07^{b}
T3 TiO_2@900	125.16^{bcd}	140.51^{cd}	139.79^{c}	150.36^{d}	160.60^{c}	200.47^{d}	274.00^{b}	294.00^{b}	240.33^{d}	342.33^{b}	353.32^{cd}	374.27^{c}
T4 TiO_2@1300	121.44^{cd}	134.73^{d}	152.26^{b}	168.17^{b}	166.57^{bc}	208.62^{cd}	278.67^{b}	286.33^{b}	250.67^{c}	332.67^{b}	340.58^{e}	351.50^{d}
T5 $nTiO_2$@100	128.30^{bc}	147.94^{bc}	155.46^{b}	162.18^{bc}	164.50^{bc}	208.58^{cd}	241.33^{c}	259.33^{b}	252.67^{cd}	363.00^{b}	358.43^{c}	367.36^{c}
T6 $nTiO_2$@250	118.08^{de}	126.48^{e}	134.98^{cd}	153.65^{cd}	164.93^{bc}	219.63^{bc}	229.67^{c}	291.67^{b}	274.33^{c}	358.33^{b}	345.23^{de}	371.15^{c}
T7 $nTiO_2$@500	109.35^{e}	115.32^{f}	127.46^{d}	139.66^{e}	157.90^{c}	205.32^{cd}	232.00^{c}	264.67^{b}	239.67^{d}	331.33^{b}	324.63^{f}	336.35^{e}
SE_m	4.25	3.48	3.82	4.56	4.64	6.55	11.90	16.16	13.80	20.80	5.53	3.46
CD	9.11	7.47	8.20	9.78	9.96	14.06	25.54	34.67	29.61	44.62	11.86	7.44

*NS – Non-significant

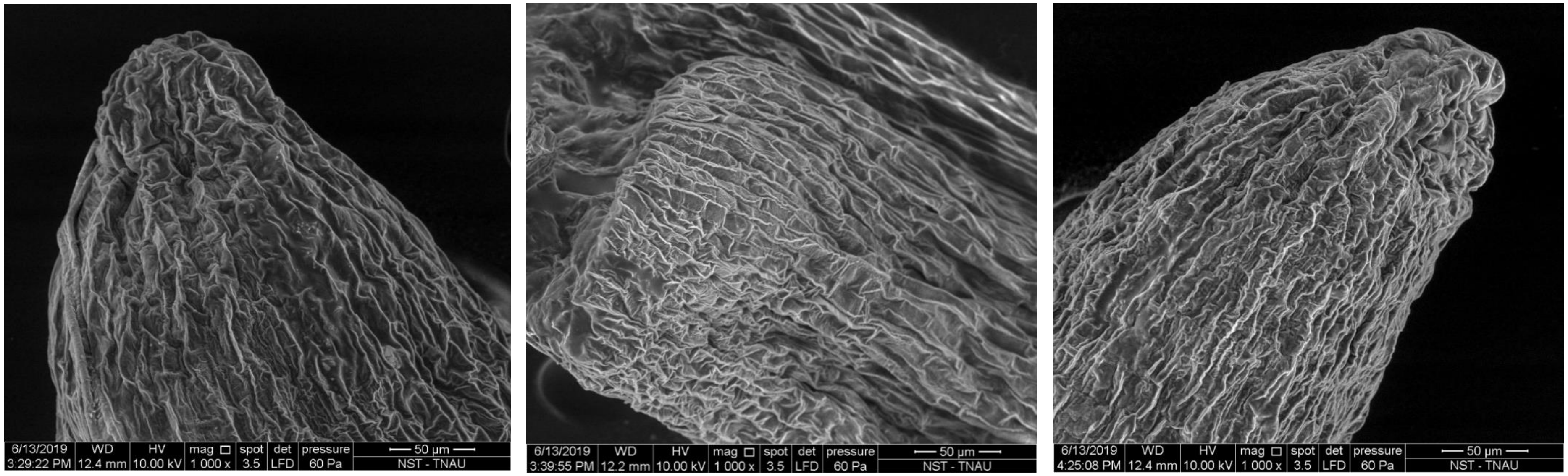
4.4.9. Seed microflora

The effect of seed treatment on the occurrence of seed microflora (Table 23) among the treatments was found to be minimal in both blotter and agar plate method.

A higher per cent of infection was seen in agar plate method compared to blotter method. At the end of the storage period, 1.33 per cent infection was observed in control seeds (T1), while none of the treated seeds showed any infection whatsoever using blotter paper method. In agar plate method, T1 (control) and T2 (TiO_2 @ 500 $mgkg^{-1}$) showed 3.33 per cent infection. The micro-organisms detected were *Aspergillus niger* and *Aspergillus flavus*.

4.4.10. SEM analysis of radicle

Changes in the cell anatomy of the radicle of cells treated with TiO_2 @ 500 mg kg^{-1} (T7) and control (T1) seeds were studied using scanning electron microscope micrographs (Plate 8). Micrographs of the root tip and zone of elongation were taken (Plates 6 and 7). At the tip of the radicle, a higher number of cells were observed indicating higher rate of cell proliferation. In the zone of elongation, roots developed from T7 seeds showed larger cells (cell length: 21.59 – 28.55 µm, cell width: 6.98 – 17.13 µm) compared to T1 (cell length: 9.07 – 16.49 µm, cell width: 5.77 – 5.80 µm).

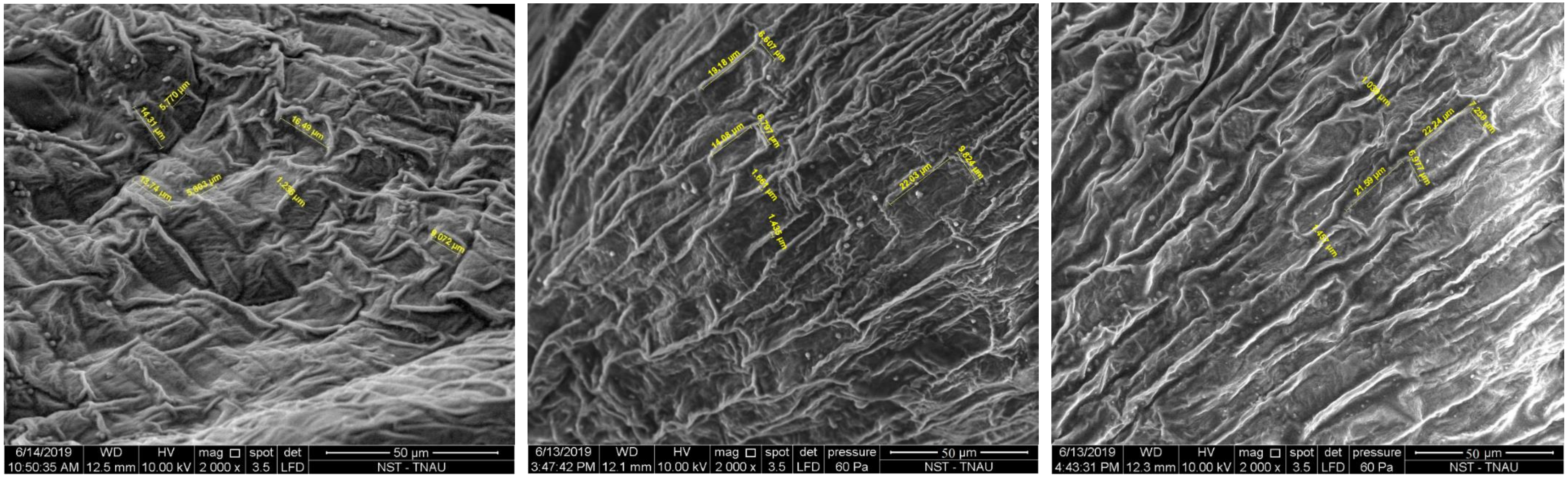


Cell proliferation at the root tip of untreated control

Cell proliferation of seeds treated with nano-ZnO @ 500 mg/kg

Cell proliferation of seeds treated with nano-TiO_2 @ 500 mg/kg

Plate 6: Cell proliferation at the tip of radicle as observed under SEM



Cells in the zone of elongation of untreated control

Enhanced elongation of root cells treated with nano-ZnO @ 500 mg/kg

Enhanced elongation of root cells treated with nano-TiO_2 @ 500 mg/kg

Plate 7: Variation in cell length in the zone of elongation of radicle as observed under SEM

Table 22: Influence of TiO_2 (bulk and nano) particles on seed moisture content of chilli at the end of storage during natural ageing

Treatment	Seed moisture content (per cent)
T1: Control	7.07
T2: TiO_2 – 500 mg/kg	6.93
T3: TiO_2 – 900 mg/kg	7.02
T4: TiO_2 – 1300 mg/kg	6.99
T5: nanoTiO_2 – 100 mg/kg	6.86
T6: nanoTiO_2 – 250 mg/kg	7.02
T7: nanoTiO_2 – 500 mg/kg	7.09
SE_m	0.34
CD	NS

Table 23: Influence of TiO_2 (bulk and nano) particles on seed infection per cent of chilli at the end of storage on natural ageing

Treatments	Seed infection (per cent)	
	Blotter paper method	**Agar plate method**
T1: Control	1.33	3.33
T2: TiO_2 – 500mg/kg	0.00	3.33
T3: TiO_2 – 900mg/kg	0.00	0.00
T4: TiO_2 – 1300mg/kg	0.00	0.00
T5: nanoTiO_2 – 100 mg/kg	0.00	0.00
T6: nanoTiO_2 – 250 mg/kg	0.00	0.00
T7: nanoTiO_2 – 500 mg/kg	0.00	0.00

4.5. Effect of zinc oxide on seed quality on accelerated ageing

4.5.1. Germination per cent

Seeds of chilli accelerated aged for twenty-four hours revealed significant differences among the treatments (Table 24). One month after ageing, it was observed that the germination per cent fell below fifty per cent (< than IMSCS). At 1 MAS, the germination per cent was found to be the highest in seeds treated with nano-ZnO @ 500 mgkg^{-1} seed (49.00 %), followed by T4 (ZnO @ 1300mgkg^{-1} seed) at 43 per cent. The germination was the least in untreated seeds (12.00 %) followed by seeds treated with nano-ZnO @ 100 mg kg^{-1}of seed (14.67 %).

4.5.2. Seedling shoot length (cm)

The shoot length of ZnO treated seeds were observed to be higher than that of the control (Table 25). The longest shoots (4.8 cm) were produced by T4 (ZnO @ 1300 mg kg^{-1} of seed). T4 was on par with T7 (ZnO NP @ 500 mg kg $^{-1}$ of seed; 4.36 cm). The least value was found in control seeds (2.64 cm) which was found to be on par with ZnO NP @ 100 mg kg^{-1} treated seeds (2.87cm).

4.5.3. Seedling root length (cm)

Significant difference was observed in the root length of zinc oxide treated and control seeds (Table 25). Root was the longest in T7 - nano ZnO @ 500 mg kg^{-1} at 5.78 cm. It was on par with T6 (nano-ZnO @ 250 mg kg^{-1}) at 5.53 cm and T4 (ZnO @ 1300 mg kg^{-1}) at 5.48 cm. The smallest roots were recorded in T1 (control) having 3.56 cm length.

4.5.4. Seedling dry weight (mg)

The dry matter producing capability of treated seeds were found to vary significantly when they were accelerated aged before storing (Table 26). The dry matter production was highest in seedlings developed from T4 (26.15 mg). It was on par with T7 (21.65 mg). The least dry weight was observed in T1 (Control; 6.00 mg), followed by T5 (15.95 mg).

Table 24: Influence of ZnO (bulk and nano) particles on germination of chilli on accelerated ageing condition

Treatment (T)	Germination per cent		
	Month of storage (M)		
	M1	M2	M3
T1: Control	12.00^{c} (6.89)	4.00^{e} (2.30)	0.66^{bc} (0.38)
T2: ZnO – 500 mg/kg	17.33^{c} (9.99)	4.00^{e} (2.30)	0.00^{c} (0.00)
T3: ZnO – 900 mg/kg	41.00^{b} (24.22)	9.66^{d} (5.45)	0.00^{c} (0.00)
T4: ZnO – 1300 mg/kg	43.00^{b} (25.49)	13.00^{c} (7.47)	3.33^{a} (1.92)
T5: nZnO – 100 mg/kg	14.66^{c} (8.43)	13.66^{c} (7.76)	1.00^{bc} (0.57)
T6: nZnO – 250 mg/kg	41.33^{b} (24.44)	16.00^{b} (9.22)	2.00^{ab} (1.15)
T7: nZnO – 500 mg/kg	49.00^{a} (29.36)	20.66^{a} (11.83)	3.00^{a} (1.72)
SE_m	1.57	0.19	0.90
CD	3.38	0.39	1.94

*Values within parenthesis are arc sine transformed values

Table 25: Influence of ZnO (bulk and nano) particles on shoot length (cm) and root length (cm) of chilli on accelerated ageing condition

Treatment (T)	Shoot length (cm)			Root length (cm)		
	Month of storage (M)			Month of storage (M)		
	M1	M2	M3	M1	M2	M3
T1: Control	2.64^{c}	0.83^{b}	0.00	3.56^{c}	1.35^{b}	0.00
T2: ZnO – 500 mg/kg	2.94^{c}	3.42^{a}	0.00	5.04^{ab}	4.31^{a}	0.00
T3: ZnO – 900 mg/kg	4.18^{b}	3.63^{a}	0.00	3.58^{c}	4.65^{a}	0.00
T4: ZnO – 1300 mg/kg	4.80^{a}	4.22^{a}	0.60	5.48^{a}	4.78^{a}	0.93
T5: nZnO – 100 mg/kg	2.87^{c}	3.12^{a}	0.00	4.23^{bc}	3.98^{a}	0.00
T6: nZnO – 250 mg/kg	4.12^{b}	3.04^{a}	0.00	5.53^{a}	4.12^{a}	0.00
T7: nZnO – 500 mg/kg	4.36^{a}	3.28^{a}	0.00	5.78^{a}	4.42^{a}	0.100
SE$_m$	0.27	0.59	0.31	0.44	0.49	0.29
CD	0.58	1.27	NS	0.95	1.06	NS

*NS - Non-significant

Table 26: Influence of ZnO (bulk and nano) particles on seedling dry weight of chilli on accelerated ageing condition

Treatment (T)	Seedling dry weight (mg 10 seedlings^{-1})		
	Month of storage (M)		
	M1	M2	M3
T1: Control	6.00^{d}	4.80^{d}	0.00
T2: ZnO – 500 mg/kg	17.05^{bc}	14.40^{c}	0.00
T3: ZnO – 900 mg/kg	19.00^{bc}	15.65^{abc}	0.00
T4: ZnO – 1300 mg/kg	26.15^{a}	18.05^{ab}	5.56
T5: nZnO – 100 mg/kg	15.95^{c}	13.30^{c}	0.00
T6: nZnO – 250 mg/kg	17.00^{bc}	14.87^{bc}	0.00
T7: nZnO – 500 mg/kg	21.65^{ab}	18.85^{a}	1.67
SE$_m$	2.53	1.67	0.32
CD	5.43	3.58	NS

*NS - Non-significant

4.5.5. Vigour index I

The vigour index-I differed significantly among the treatments in accelerated aged condition also (Table 27). Higher doses of zinc oxide nano particles proved to be more effective in retaining vigour of the seedling even after stressful conditions. T7 (nano-ZnO @ 500 mg kg $^{-1}$) registered the highest vigour index-I (497) and was on par with T4 - 1300 ZnO @ 1300 mg kg^{-1} (441), followed by T6 – nano-ZnO @ 400 mg kg^{-1} (400). Untreated seeds produced the least vigorous seedlings (76) and were on par with T5 (103) and T2 (139).

4.5.6. Vigour index II

Vigour index-II showed significant differences among the treatments (Table 27). The seedlings of seeds dry dressed with ZnO @ 1300 mg kg^{-1} registered the highest vigour index-II (1125) and was on par with T7 (ZnO NP @ 500 mg kg $^{-1}$; 1064), followed by T6 (ZnO NP @ 250 mg kg $^{-1}$; 701). The least value was observed in control (72).

4.5.7. Electrical conductivity of seed leachates (µS cm^{-1})

The seed leachates significantly differed in their conductivity (Table 28). The highest conductivity was recorded in control seeds (T1: 299.67 µScm^{-1}). T1 was found to be on par with T5 (298.67 µScm^{-1}). The least value was observed in T7 (266.67 µScm^{-1}) which was on par with T4 (268 µScm^{-1}).

4.5.8. Seed moisture content (per cent)

Seed moisture content of the seeds at the end of storage period is presented in Table 29. It was found that the least moisture content was in seeds treated with T4 (ZnO @ 1300 mg/kg; 5.95 %). All the other treatments were on par with each other.

Table 27: Influence of ZnO (bulk and nano) particles on vigour index - I and vigour index - II of chilli on accelerated ageing condition

Treatment (T)	Vigour index - I			Vigour index - II		
	Month of storage (M)			Month of storage (M)		
	M1	M2	M3	M1	M2	M3
T1: Control	76^{d}	7^{d}	0	72^{d}	16^{e}	0
T2: ZnO – 500 mg/kg	139^{d}	31^{d}	0	297^{c}	57^{d}	0
T3: ZnO – 900 mg/kg	318^{c}	79^{c}	0	775^{b}	149^{c}	0
T4: ZnO – 1300 mg/kg	441^{ab}	117^{b}	8	1125^{a}	235^{b}	28
T5: nZnO – 100 mg/kg	103^{d}	97^{bc}	0	236^{cd}	178^{c}	0
T6: nZnO – 250 mg/kg	400^{b}	115^{b}	0	701^{b}	239^{b}	0
T7: nZnO – 500 mg/kg	497^{a}	158^{a}	0	1064^{a}	385^{a}	5
SEm	32.25	13.70	2.94	9.93	18.69	15.08
CD	69.18	29.38	NS	197.19	40.09	NS

*NS - Non-significant

Table 28: Influence of ZnO (bulk and nano) particles on electrical conductivity of seed leachate of chilli on accelerated ageing condition

Treatment (T)	E.C. (µScm^{-1})		
	Month of storage (M)		
	M1	M2	M3
T1: Control	299.67^{a}	402.67^{a}	551.67^{a}
T2: ZnO – 500 mg/kg	271.33^{b}	399.00^{a}	513.67^{bc}
T3: ZnO – 900 mg/kg	269.00^{b}	376.00^{b}	512.67^{bc}
T4: ZnO – 1300 mg/kg	268.00^{b}	322.00^{c}	434.67^{d}
T5: nZnO – 100 mg/kg	298.67^{a}	397.00^{a}	518.67^{ab}
T6: nZnO – 250 mg/kg	277.67^{b}	358.00^{b}	488.67^{bc}
T7: nZnO – 500 mg/kg	266.67^{b}	334.67^{c}	484.67^{c}
SEm	5.75	8.55	15.60
CD	12.34	18.34	33.48

Table 29: Influence of ZnO (bulk and nano) particles on seed moisture content (per cent) chilli on accelerated ageing

Treatment	Seed moisture content (per cent)
T1: Control	6.97^{a}
T2: ZnO – 500 mg/kg	6.50^{a}
T3: ZnO – 900 mg/kg	6.70^{a}
T4: ZnO – 1300 mg/kg	5.95^{b}
T5: nanoZnO – 100 mg/kg	6.47^{a}
T6: nanoZnO – 250 mg/kg	6.49^{a}
T7: nanoZnO – 500 mg/kg	6.72^{a}
SE$_m$	0.24
CD	0.50

Table 30: Influence of ZnO (bulk and nano) particles on seed infection (per cent) of chilli on accelerated ageing

Treatments	Seed infection per cent	
	Blotter paper method	Agar plate method
T1: Control	17.33^{c}	26.67
T2: ZnO – 500 mg/kg	53.33^{c}	43.33
T3: ZnO – 900 mg/kg	37.33^{ab}	30.00
T4: ZnO – 1300 mg/kg	20.00^{bc}	23.33
T5: nanoZnO – 100 mg/kg	17.33^{c}	33.33
T6: nanoZnO – 250 mg/kg	9.33^{c}	13.33
T7: nanoZnO – 500 mg/kg	5.33^{c}	10.00
SE$_m$	5.92	8.07
CD	17.95	NS

*NS - Non-significant

4.5.9. Seed microflora

The seed infection significantly varied with the treatments in both blotter paper and agar plate method (Table 30).

The blotter paper test revealed the occurrence of the highest infection (53.33 per cent) in seeds treated with ZnO @ 500 mg kg^{-1}, while nanoparticle treated seeds developed low infection. The lowest infection was found in T7 (5.33 per cent). The microorganisms observed include *Aspergillus niger* and *Aspergillus flavus*.

Agar plate method tested seeds showed a lower infection per cent as compared to the blotter paper method. T2 developed the highest infection (43.33 per cent), followed by T5 (33.33 per cent). Higher doses of zinc oxide NPs (nanoparticles) were effective in controlling the seed infection. The storage fungi detected include *Aspergillus niger, A. flavus* and *Mucor* spp.

4.6. Effect of titanium dioxide on seed quality after accelerated ageing

4.6.1. Germination per cent

Seeds dry dressed with titanium dioxide were able to retain more than 50 % of IMSCS germination even after accelerated ageing (Table 31) in the first month. Germination in seeds treated with nano-TiO_2 @ 500 mg kg^{-1} (T7) was 51.67 per cent. It was on par with the effect produced by T4 (47.33 %) and T3 (44.67 %). All the treated seeds performed better than the untreated seeds (5.67 %).

4.6.2. Seedling shoot length (cm)

The seedling shoot length significantly differed with the various treatments given (Table 32). The highest shoot length was observed in chilli seeds treated with $nTiO_2$ @ 500 mg kg^{-1} (T7; 4.34 cm), which was on par with the treatment efficacy of TiO_2 @ 1300 mg kg^{-1} (T4; 3.96 cm), $nTiO_2$ @ 250 mg kg^{-1} (T6; 3.84 cm), and TiO_2 @ 900 mg kg^{-1} (T3; 3.74 cm). The lowest performance was by control seeds (T1; 1.80 cm).

Table 31: Influence of TiO_2 (bulk and nano) particles on germination of chilli on accelerated ageing condition

Treatment (T)	Germination per cent		
	Month of storage (M)		
	M1	M2	M3
T1: Control	5.67^{c} (3.16)	4.00^{d} (2.30)	0.00^{c} (0.00)
T2: TiO_2 – 500 mg/kg	12.00^{c} (6.89)	13.00^{c} (7.48)	1.00^{bc} (0.57)
T3: TiO_2 – 900 mg/kg	44.67^{a} (26.60)	15.66^{b} (8.92)	1.33^{b} (0.76)
T4: TiO_2 – 1300 mg/kg	47.33^{a} (28.24)	19.00^{b} (10.96)	2.00^{ab} (1.15)
T5: $nTiO_2$ – 100 mg/kg	13.00^{c} (7.47)	8.00^{d} (4.59)	0.00^{c} (0.00)
T6: nTiO2 – 250 mg/kg	32.33^{b} (18.84)	20.00^{b} (11.54)	1.00^{bc} (0.57)
T7: $nTiO_2$ – 500 mg/kg	51.67^{a} (31.12)	27.66^{a} (15.97)	3.00^{a} (1.72)
SE_m	2.86	1.26	0.18
CD	6.15	2.70	0.39

*Values within parenthesis are arc sine transformed values

Table 32: Influence of TiO_2 (bulk and nano) particles on shoot length (cm) and root length (cm) of chilli on accelerated ageing condition

Treatment (T)	Shoot length (cm)			Root length (cm)		
	Month of storage (M)			Month of storage (M)		
	M1	M2	M3	M1	M2	M3
T1: Control	1.80^{d}	0.83^{d}	0.00	1.68^{c}	1.35^{d}	0.00
T2: TiO_2 – 500 mg/kg	2.87^{bc}	2.46^{bc}	0.00	2.02^{c}	3.3^{bc}	0.00
T3: TiO_2 – 900 mg/kg	3.74^{ab}	3.32^{abc}	0.00	5.26^{ab}	4.66^{ab}	0.00
T4: TiO_2 – 1300 mg/kg	3.96^{a}	3.92^{ab}	0.00	5.28^{ab}	4.64^{ab}	0.00
T5: $nTiO_2$ – 100 mg/kg	1.93^{cd}	1.84^{cd}	0.00	4.23^{b}	2.22^{cd}	0.00
T6: nTiO2 – 250 mg/kg	3.84^{ab}	2.40^{bcd}	0.00	5.16^{ab}	3.46^{bc}	0.00
T7: $nTiO_2$ – 500 mg/kg	4.34^{a}	4.20^{a}	0.00	5.72^{a}	5.52^{a}	0.00
SE_m	0.48	0.73	-	0.64	0.64	-
CD	1.03	1.58	-	1.36	1.38	-

4.6.3. Seedling root length (cm)

The root length of the seedlings among treatments was found to be significantly different after accelerated ageing (Table 32). Among the treatments, seeds treated with $nTiO_2$ @ 500 mg kg^{-1} (T7) had the longest root (5.72 cm). It was found to be on par with T4 (5.28 cm), T3 (5.26 cm) and T6 (5.16 cm). T1 produced the smallest root (1.68 cm) followed by T2 (2.02 cm).

4.6.4. **Seedling dry weight (mg)**

Dry weight of seedlings varied with the treatments (Table 33). T3 (TiO_2 @ 900 mg kg^{-1}) produced seedlings having the highest dry weight (19.31 mg). It was on par with T7 ($nTiO_2$ @ 500 mg kg^{-1}; 18.45 mg), T4 (TiO_2 @ 1300 mg kg^{-1}; 17.15 mg) and T6 ($nTiO_2$ @ 250 mg kg^{-1}; 17.00 mg).

4.6.5. Vigour index I

Zinc oxide treatment exhibited significant influence on vigour index-I (Table 34). Among the treatments, T7 ($nTiO_2$ @ 500 mg kg^{-1}; 520) was on par with T4 (TiO_2 @ 1300 mg kg^{-1}; 437) followed by T3 (TiO_2 @ 900 mg kg^{-1}; 403).

4.6.6. Vigour index II

Vigour index-II varied significantly due to seed treatment (Table 34). All the treated seeds performed better than the control (42). Among the different titanium dioxide treatments, 500 mg kg^{-1} of TiO_2 nanoparticles resulted in higher vigour index (T7; 953) followed by 900 $mgkg^{-1}$ of TiO_2 (T3; 863) and 1300 mg kg^{-1} of TiO_2 (T4; 813), while the least VI-II was observed in seeds treated with 100 mg kg^{-1} (T5; 170) of TiO_2 NPs.

Table 33: Influence of TiO_2 (bulk and nano) particles on seedling dry weight of chilli on accelerated ageing condition

Treatment (T)	Seedling dry weight (mg 10 seedlings^{-1})		
	Month of storage (M)		
	M1	M2	M3
T1: Control	6.95^{d}	6.00^{c}	0.00
T2: TiO_2 – 500 mg/kg	15.43^{bc}	15.20^{ab}	0.00
T3: TiO_2 – 900 mg/kg	19.31^{a}	17.45^{a}	0.00
T4: TiO_2 – 1300 mg/kg	17.15^{ab}	16.35^{ab}	0.00
T5: $nTiO_2$ – 100 mg/kg	13.13^{c}	11.45^{bc}	0.00
T6: nTiO2 – 250 mg/kg	17.00^{ab}	13.19^{ab}	0.00
T7: $nTiO_2$ – 500 mg/kg	18.45^{ab}	16.47^{ab}	0.00
SE_m	1.68	2.56	-
CD	3.61	5.50	-

Table 34: Influence of TiO_2 (bulk and nano) particles on vigour index - I and vigour index - II of chilli on accelerated ageing condition

Treatment (T)	Vigour index - I			Vigour index - II		
	Month of storage (M)			Month of storage (M)		
	M1	M2	M3	M1	M2	M3
T1: Control	19^{d}	7^{d}	0	42^{c}	24^{d}	0
T2: TiO_2 – 500 mg/kg	60^{d}	81^{c}	0	182^{c}	204^{bc}	5
T3: TiO_2 – 900 mg/kg	403^{bc}	122^{bc}	2	863^{a}	274^{b}	0
T4: TiO_2 – 1300 mg/kg	437^{ab}	164^{b}	0	813^{a}	311^{b}	0
T5: $nTiO_2$ – 100 mg/kg	80^{d}	33^{d}	0	170^{c}	90^{cd}	0
T6: nTiO2 – 250 mg/kg	294^{c}	117^{c}	0	553^{b}	262^{b}	0
T7: $nTiO_2$ – 500 mg/kg	520^{a}	266^{a}	0	953^{a}	450^{a}	0
SE_m	52.44	21.80	0.78	100.75	53.66	2.67
CD	112.48	46.76	NS	216.12	115.11	NS

*NS – Non-significant

4.6.7. Electrical conductivity of seed leachates (µS cm^{-1})

Dry dressing with titanium dioxide considerably decreased the electrical conductivity in the leachate of aged chilli seeds irrespective of its concentrations compared to control (Table 35). Significantly lower electrical conductivity was recorded by TiO_2 NPs @ 500 mg kg^{-1} (T7: 245.67 µScm^{-1}), followed by TiO_2 @ 1300 mg kg^{-1} (T4: 269.67 µScm^{-1}) and TiO_2 @ 900 mg kg^{-1} (T3: 276.67 µScm^{-1}). Meanwhile, the highest E.C. was recorded in control seeds (T1: 308 µScm^{-1}).

4.6.8. Seed moisture content (per cent)

The variation in seed moisture content was found to be statistically significant after accelerated ageing (Table 36). Control seeds registered the highest seed moisture content (6.96 per cent). Among the TiO_2 treatments, TiO_2 NPs@ 250 mg kg^{-1} (T6) registered the least seed moisture content (5.98 per cent). All other treatments were found to be at par with each other.

4.6.9. Seed microflora

Significant differences with respect to the occurrence of pathogens in the seeds were observed due to titanium dioxide seed treatments (Table 37).

Blotter test for detection of seed-borne fungi revealed that highest infection was found in seeds treated with TiO_2 @ 900 mg kg^{-1} (T3: 32 %) followed by TiO_2 @ 1300 mg kg^{-1} (T4: 28 %). The least infection was found in seeds that were treated with TiO_2 NPs @ 500 mg kg^{-1} (T7: 12.00 %).

A similar trend was also observed in agar plate method. The highest infection was recorded in T3 (43.33 %), followed by T1 (26.67 %). As seen in blotter paper method, the least seed infection per cent was observed in seeds treated with TiO_2 NPs @ 500 mg kg^{-1} (T7: 6.67 %). *Alternaria* spp., *Aspergillus flavus* and *Aspergillus niger* were detected in both the methods.

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Table 35: Influence of TiO_2 (bulk and nano) particles on electrical conductivity of seed leachate of chilli on accelerated ageing condition

Treatment (T)	E.C. (μScm^{-1}) Month of storage (M) M1	 M2	 M3
T1: Control	308.00^{a}	379.00^{a}	512.67^{a}
T2: TiO_2 – 500 mg/kg	299.67^{ab}	343.67^{b}	483.67^{b}
T3: TiO_2 – 900 mg/kg	276.67^{c}	337.67^{b}	453.00^{c}
T4: TiO_2 – 1300 mg/kg	269.67^{c}	322.33^{bc}	434.67^{cd}
T5: $nTiO_2$ – 100 mg/kg	298.67^{ab}	375.00^{a}	431.34^{cde}
T6: nTiO2 – 250 mg/kg	287.00^{bc}	333.00^{bc}	422.00^{de}
T7: $nTiO_2$ – 500 mg/kg	245.67^{d}	313.00^{c}	406.67^{e}
SE_m	9.58	10.76	11.59
CD	20.54	23.09	24.88

Table 36: Influence of TiO_2 (bulk and nano) particles on seed moisture content (per cent) chilli after accelerated ageing

Treatment	Seed moisture content (per cent)
T1: Control	6.97^{a}
T2: TiO_2 – 500 mg/kg	6.47^{b}
T3: TiO_2 – 900 mg/kg	6.23^{bc}
T4: TiO_2 – 1300 mg/kg	6.47^{b}
T5: nanoTiO_2 – 100 mg/kg	6.28^{bc}
T6: nanoTiO_2 – 250 mg/kg	5.99^{c}
T7: nanoTiO_2 – 500 mg/kg	6.44^{b}
SE_m	0.14
CD	0.34

Table 37: Influence of TiO_2 (bulk and nano) particles on seed infection (per cent) of chilli on accelerated ageing

Treatments	Seed infection per cent	
	Blotter paper method	Agar plate method
T1: Control	17.33^{bc}	26.67
T2: TiO_2 – 500 mg/kg	20.00^{abc}	10.00
T3: TiO_2 – 900 mg/kg	32.00^{a}	43.33
T4: TiO_2 – 1300 mg/kg	28.00^{ab}	10.00
T5: nanoTiO_2 – 100 mg/kg	25.33^{ab}	10.00
T6: nanoTiO_2 – 250 mg/kg	20.00^{abc}	23.33
T7: nanoTiO_2 – 500 mg/kg	12.00^{c}	6.67
SE_m	3.97	8.26
CD	12.03	NS

*NS – Non-significant

Discussion

5. DISCUSSION

Storing of seeds until the next cropping season is an inevitable process and also serves as a safeguard against environmental insecurities. The climatic conditions of Kerala are particularly not conducive for long term seed storage due to its high temperature and high relative humidity. So, an endeavour has been made to derive a meaningful and accurate interpretation of the results obtained in the study.

5.1. Effect of seed invigoration with zinc oxide on seed quality in the course of natural ageing during storage in ambient condition

5.1.1. Germination

Regardless of the treatment dose, germination capacity of seeds decreased as the period of storage increased. But the rate of deterioration was faster in control seeds. This was in agreement with the studies conducted by Sandhya (2016) and Navya (2016) in chilli.

In the initial months of storage, an increase in the rate of germination was observed after seed treatment. Increased viability after the first month of storage could be attributed to the primary dormancy observed in chilli during the initial development stages. Fresh seeds of chilli (Evans, 1984) and tomato (Hilhorst *et al.*, 1998) undergo further development after harvest resulting in a decline in abscisic acid content and hence improving their ability to germinate. Goodrazi *et al.* (2017) discovered in their experiments with *Prunus mahaleb* that when dormant seeds were immersed in titanium dioxide NPs and zinc oxide NPs their germination could be improved. After this initial rise, the germination per cent declined progressively with storage.

Chilli seeds dry dressed with different quantities of zinc oxide were able to retain the IMSCS set standards of germination (minimum 60 per cent) up to tenth month after seed treatment (Table 6). The best dosage was found to be ZnO @ 1300 mg/kg of seed (67.67 per cent), which was on par with nano ZnO @ 500 mg/kg seed (64.67 per cent). It was followed by nano ZnO @ 250 mg/kg (62.33 per cent) and ZnO @ 900 mg/kg seed (61.00 per cent). The ability of even lower doses of nano zinc oxide to improve germination render T7 (nano-ZnO @ 500 mg/kg of seed) a good treatment. On comparing the same dose (500 mg/kg) of nanoparticle (T7) and bulk-particle (T2) of zinc oxide, the nano zinc oxide displayed better influence on promoting germination by 9.8 per cent.

This might probably be due to the penetration of nanoparticles into cellwall and plasmamembrane of root and shoot epidermal cells and their subsequent accumulation in the vascular tissues which could have resulted in increased cell division and cell elongation (Raghu *et al.,* 2017). Segatto *et al*. (2018) ascribed the increased germination of nano zinc oxide treated seeds to the presence of zinc oxide nanoparticles inside the seed. According to Pandey *et al.* (2010), zinc oxide NPs have a phyto-stimulatory effect and can improve the growth of seedlings by increased reactivity of plant hormones such as IAA (indole acetic acid) in seedling shoot and radicle.

Nanoparticles of zinc oxide was found to have a positive effect on germination in maize and cabbage (Pokhrel and Dubey, 2013), groundnut (Shyla and Natarajan, 2014 and Prasad, 2012), chilli (Afrayeem and Chaurasia, 2017), pigeon pea (Korishettar *et al.*, 2016) and sesame (Segatto *et al*., 2018).

5.1.2. Seedling shoot length

The shoot length showed a decreasing trend as the storage period advanced. The decline occurred at a faster rate in untreated seeds compared to all the other treatments. Similar trend was observed by Navya (2016) and Sandhya (2016) in chilli and Reshma (2018) in okra and oriental pickling melon.

After eleven months of storage of zinc-oxide treated seeds, nano-ZnO @ 500 mg/kg (T7) and ZnO @ 1300 mg/kg (T3) were able to develop seedlings with longer shoots (Table 7). Seeds invigorated with 500 mg/kg of nano zinc-oxide (T7) had 34 per cent longer shoot length (4.43cm) compared to bulk-grade zinc oxide (T2) of the same concentration (2.92 cm).

Likewise, the seedlings grown from zinc-oxide nanoparticle coated seeds manifested higher values of shoot length compared to untreated controls and their bulk-scale equivalents in maize (Segatto *et al*., 2018), sesame (Narendhran *et al.*, 2016) and maize (Tiwari, 2017). Studies conducted by Maity *et al.* (2018) revealed that lower doses of nano scale zinc oxide (750 mg/kg) were beneficial to shoot length in sorghum seeds than higher doses (1000 and 1250 mg/kg).

5.1.3. Seedling root length

Analysis of the effect of zinc oxide treatment on root length of chilli seeds over the period of twelve months of storage revealed a slower rate of decline in the root length of treated seeds (Table 8). Chilli seeds treated with T4 - ZnO @ 1300 mg/kg (6.36 cm) developed longer roots and was on par with T7 - nano-ZnO @ 500 mg/kg (6.14 cm), T3 - ZnO @ 900 mg/kg (6.04 cm), T6 - nano-ZnO @ 250 mg/kg (5.68 cm) and T2 - ZnO @ 500 mg/kg (5.62 cm).

While comparing the particle size efficiency of zinc oxide treated seeds, it was noticed that seeds treated with 500 mg/kg of nano-zincoxide (T7) recorded 6.14 cm root length while bulk-zincoxide (T2) registered 5.62 cm. In this study, treatment with the lowest dose of nano zinc oxide (100 mg/kg) was found to have no effect on root length and was on par with control. Concurrent results were reported by Afrayeem and Chaurasia (2017) in chilli, and Bagawade and Jagtap (2018) in wheat.

5.1.4. Seedling dry weight

The dry matter content of seedlings is an indication of its physiological vigour and is often regulated by metabolites, plant growth hormones and the enzymatic activities (Qualls and Cooper, 1968).

Zinc oxide had a significant effect on seedling dry matter production from the fourth month after storage and was found to decrease with advancing storage period irrespective of the treatments (Table 9). After ten months of storage, the lowest dose of nano-ZnO (100 mg/kg) recorded greater seedling dry matter production (20.70 mg 10 seedlings^{-1}) and the least value was obtained in control (15.87 mg 10 seedlings^{-1}). This was in agreement with the findings of Tiwari (2017) in maize and Maity *et al.* (2018) in jowar and cowpea. Seed treatment with zinc oxide NPs was able to promote the biomass production in sesame (Narendhran *et al.*, 2016) at intermediate doses, in wheat (Rizwan *et al.*, 2019) at highest and intermediate doses and in chickpea (Hajra and Mondal, 2017) at highest doses. By the end of twelfth month after storage, bulk-TiO_2 @ 500 mg/kg (T2) produced 14.88 mg dry biomass and nano-TiO_2 @ 500 mg/kg (T7) gave 17.47 mg.

5.1.5. Vigour indices

Seed vigour is the sum of those properties which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence. Abdul-Baki and Anderson (1973) formulated the equation for computing vigour index I and II as the product of germination percent with seedling length (cm) or seedling dry weight (mg), respectively.

Vigour index-I (Table 10) and vigour index-II (Table 11) of zinc oxide treated seeds declined with advance of time during the twelve months of storage indicating the irreversible, inexorable and inevitable nature of seed deterioration.

Significant differences among the treatments were observed from sixth month (vigour index-I) and fifth month (vigour index-II) onwards. After ten months of storage, vigour index-I values of seeds treated with T4 - ZnO @ 1300 mg/kg (743) was on par with T7 - nano-ZnO @ 500 mg/kg (675) and T3 - ZnO @ 900 mg/kg (641), while vigour index-II recorded higher values in T4 - ZnO @ 1300 mg/kg (1335) which was on par with T7 - nano-ZnO @ 500 mg/kg (1285), T5 - nano-ZnO @ 100 mg/kg (1210) and T3 - ZnO @ 900 mg/kg (1199). Contrasting results were divulged from the studies of García-López *et al*. (2018), who found 500 ppm nano zinc oxide in chilli to produce less vigorous seedlings than lower doses. But, seedling vigour was found to increase proportionate to the doses of zinc oxide NPs in groundnut (Prasad *et al.,* 2012) up to 1000 ppm.

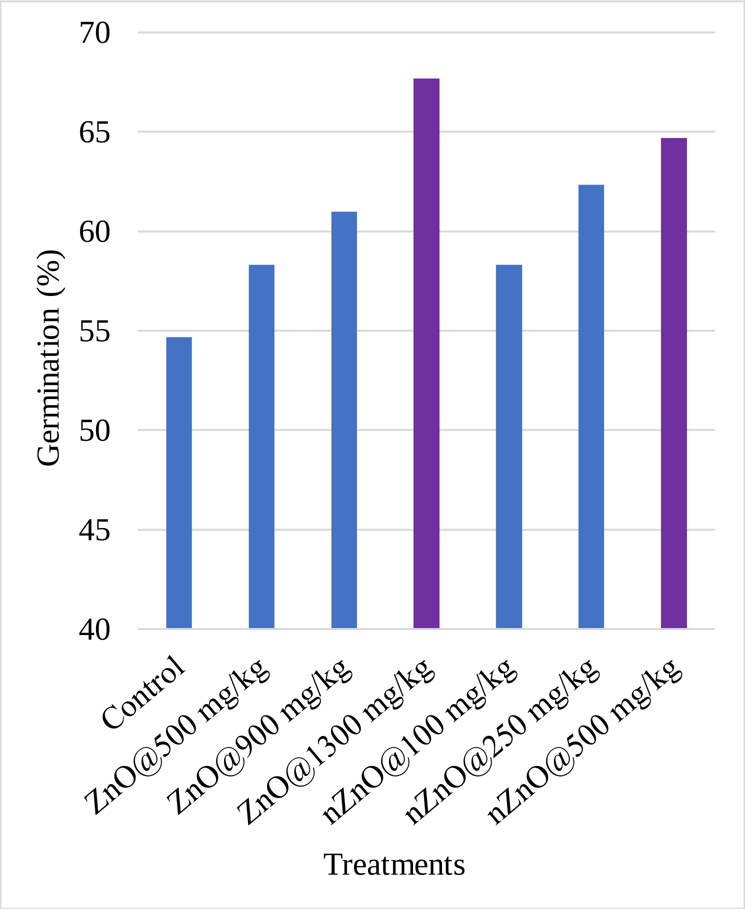


Fig. 1: Effect of zinc oxide on maintaining germination of seeds ten months after storage

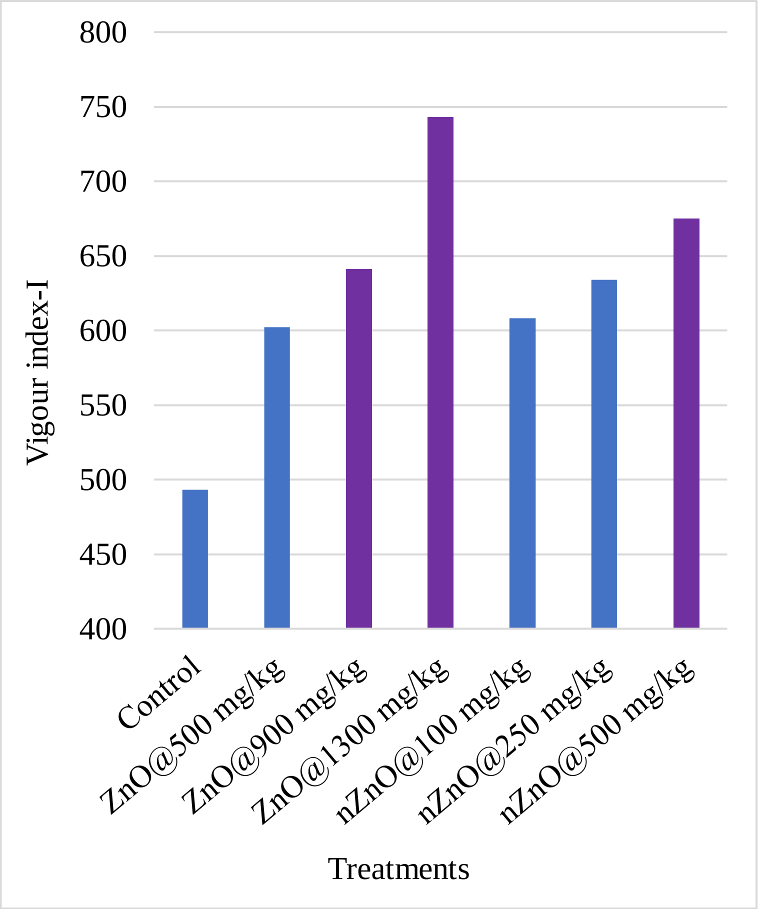


Fig. 2: Effect of zinc oxide treatment on vigour index-I after ten months of storage

5.1.6. Electrical conductivity

Electrical conductivity was found to increase as storage time elapsed in zinc oxide (Table 12) treated seeds.

Electrical conductivity (EC) test is a measure of the integrity of cell membrane. It has been well correlated with plant vigour and field emergence in some crops. As deterioration occurs, the seed membrane degrades and it causes seeds to exude electrolytes such as amino acids, organic acids and sugar. The principle of the EC test is that less vigorous or more deteriorated seeds show a lower speed of cell-membrane repair during seed water uptake for germination and therefore release greater amounts of solutes to the external environment (Marcos-Filho, 2015).

Good quality seeds as well as treated seeds are able to retain membrane integrity for longer period and hence recorded lower values of electrical conductivity from the seed leachates.

After ten months of storage of zinc oxide treated seeds, nanoparticle treated seeds revealed lower EC values compared to its bulk-grade counterparts. Least electrical conductivity was measured in T7 - nano-ZnO @ 500 mg/kg (246µS/cm) and was on par with T6 - nano-ZnO @ 250 mg/kg (265µS/cm). Shyla and Natarajan (2016) also reported lower electrical conductivity from seeds treated with higher doses of zinc oxide nanoparticles up to 1000 mg/kg in peanut.

5.1.7. Seed moisture content (per cent)

No significant difference in seed moisture content was observed among the treatment (Table 13) at the end of storage period. This might due to the water and moisture impervious packaging (700-gauge polyethylene bags) material used.

5.1.8. Seed microflora

The seed infection detected at end of storage period in ZnO (Table 14) treated seeds were found to be minimal.

In ZnO treated seeds, in both blotter paper and agar plate methods, infection was detected only in control and lower doses of the treatment, which indicates that

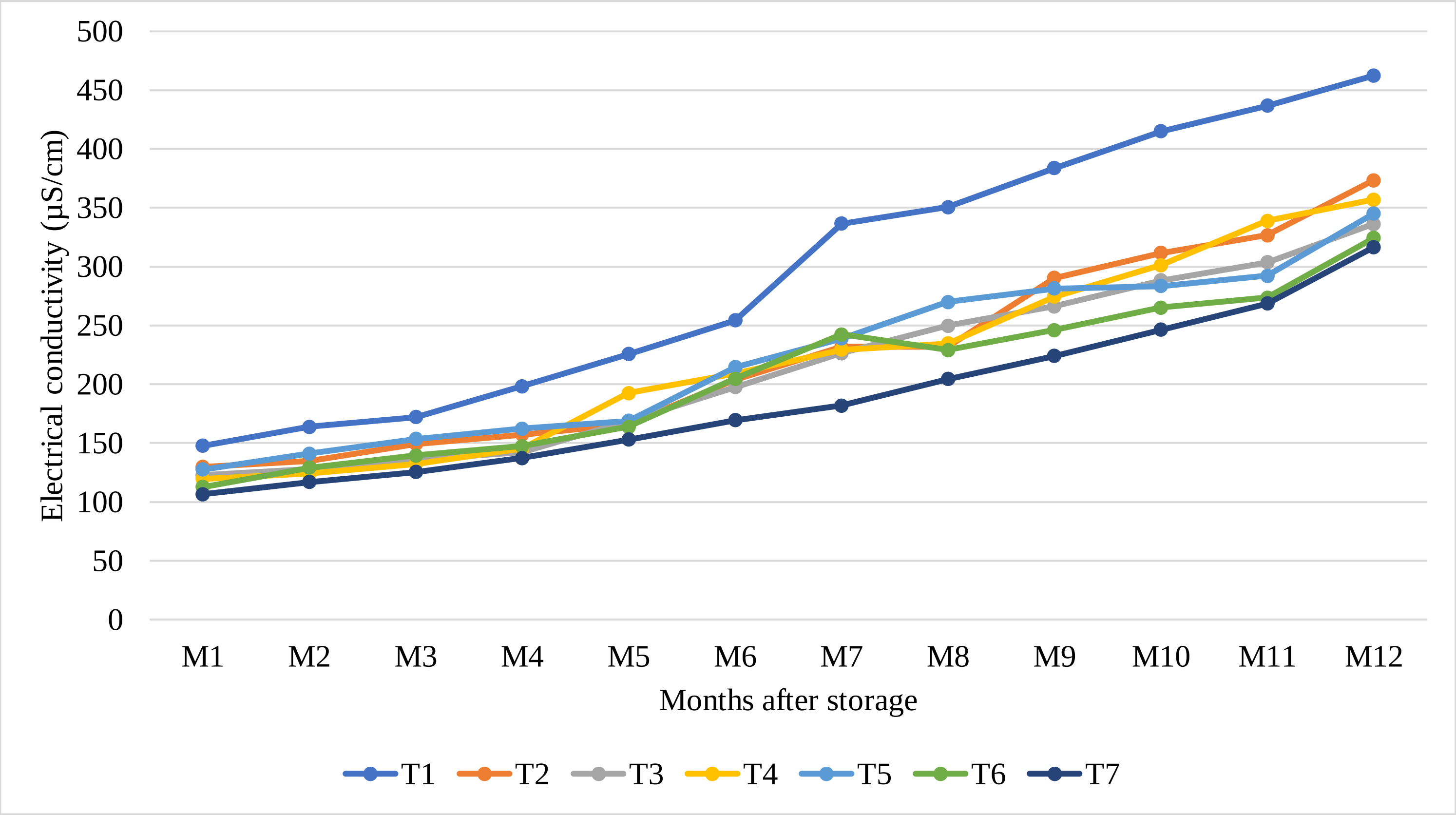


Fig. 3: Effect of zinc oxide on electrical conductivity of seed leachate during storage

ZnO is beneficial in protection against seed-borne pathogens. Antifungal activity of zinc oxide nanoparticles has been reported by Jasim (2015) against *Aspergillus fumigatus* and *Candida albicans.* In avocado, nano-zincoxide was able to inhibit the radial growth of fungal mycelium (*Colletotrichum gloeosporioides*) by 80 per cent (de la Rosa-García, 2018). It was inferred to be due to the inhibition or deformed sporulation, swelling of hyphae, structural malformation and melanisation of hyphae caused by zinc oxide NP.

The antimicrobial action of nanoparticles against fungi, bacteria and viruses could be due to the interaction of nanoparticles with the outer membrane of these microbes, leading to inhibition of respiration and other metabolic processes which might result in fatality (Kumar *et al.,* 2015). Allahverdiyev (2011) hypothesised about the ability of NPs to penetrate the cell wall of micro-organisms and inactivate microbial enzymes leading to the production of reactive oxygen species and subsequent death of the cell.

5.1.9. SEM analysis of root

The scanning electron microscope analysis of radicles of chilli seeds treated with zinc oxide and untreated control revealed significant differences.

The root tips of the seedlings grown from treated seeds (T7 - nano-ZnO @ 500 mg/kg) had higher cell proliferation compared to the untreated seeds. In cell elongation zone, the treated seeds exhibited higher cell elongation than control. This result was in agreement with the observations of other scientists such as Helaly *et al.* (2014), who found that somatic embryogenesis could be boosted by providing nano zinc-oxide in MS (Murashige and Skoog) media. Raliya and Tarafdar (2013) observed that the radicles of *Vigna radiata* and *Cicer arietinum* could absorb zinc oxide NPs which might have improved the growth of root and shoot in terms of length and biomass. Similar observations were also made by Saranya *et al.* (2017) in onion seeds primed with zinc sulphate ($ZnSO_4$). Ahmed *et al.,* (2016) found that the variation in cell diameter of aleurone layer of wheat did not have any significant effect on rate of germination, while length did.

5.2. Effect of seed invigoration with titanium dioxide on seed quality in the course of natural ageing during storage in ambient conditions

5.2.1. Germination

Regardless of the treatment dose, germination capacity of seeds decreased as the period of storage increased. But the rate of deterioration was faster in control seeds. This was in agreement with the studies conducted by Reshma (2018) in okra and oriental picking melon.

The seeds treated with various doses of bulk and nano grade titanium dioxide was also able to maintain the IMSCS up to ten months after storage in four of the treatments (Table 15). All the four treatments were found to be on par with each other, *i.e.*, T7 - nano-TiO_2 @ 500 mg/kg (63.00 %), T3 - TiO_2 @ 900 mg/kg (61.33 %), T4 - TiO_2 @ 1300 mg/kg (61.00 %) and T6 - nano-TiO_2 @ 250 mg/kg (60.00 %). Meanwhile at the same doses, T7 (nano-TiO_2 @ 500 mg/kg) had 7.4% higher germination per cent than its bulk counterpart (T2 - TiO_2 @ 500 mg/kg). T2 (58.33 % germination) lost its IMSCS germination standard by ninth month after storage, whereas T7 could retain viability for an additional month up to the tenth month.

A mixture of nano- SiO_2 and nano-TiO_2 could increase the nitrate reductase in soybean (*Glycine max*) that enhanced its abilities of absorbing and utilizing water and fertilizer, stimulate its SOD, CAT, POX and apparently hastened its germination and growth (Lu *et al.*, 2002). Song *et al.* (2012) opined that duckweed (*Lemna minor*) exposed to *n*TiO_2 (10–2,000 ppm) suspensions for 7 days did not show lipid peroxidation at $\leq$ 200 ppm.

Investigations conducted by Zheng *et al.* (2005) in spinach using nano and non-nano titanium oxide revealed that nano-titanium oxide at higher doses was the better treatment chemical in terms of germination per cent. Similar outcomes were obtained in sorghum and cowpea (Maity *et al*., 2018), canola (Mahmoodzadeh *et al.,* 2013), okra (Reddy *et al.,* 2018), fennel (Feizi *et al.*, 2013) and maize (Vijayalakshmi *et al.,* 2018). Whereas, contrasting results were obtained in onion where lower doses of titanium dioxide NPs were found to be beneficial (Laware and Raskar, 2014).

The remarkable effect of nano titanium dioxide compared to the same dose of its bulk counterpart is possibly due to its small particle size enabling penetration into the seed during the treatment period and enhancing the growth , whereas the non-nano titanium dioxide might not have been able to enter the seed as effectively resulting in a less pronounced effect (Zheng *et al.*, 2005). Hong *et al.* (2005) reported the ability of titanium oxide to promote light absorption, quicken the transport of light energy and ameliorate the activity of antioxidant enzymes such as catalase, peroxidase, superoxide dismutase which could help prolong the life of seed during storage.

5.2.2. Seedling shoot length

Significant differences among the titanium dioxide treatments in terms of seedling shoot length were observed from the seventh month after storage (Table 16). At the end of the storage period (12 months), the treatments T6 - nano-TiO_2 @ 250 mg/kg (4.28 cm), T7 - nano-TiO_2 @ 500 mg/kg (4.16 cm) and T3 - TiO_2 @ 900 mg/kg (3.64 cm) developed longer seedling shoots.

Higher concentrations of nano titanium dioxide resulted in improved plumule growth of canola (Mahmoodzadeh *et al.*, 2013), chickpea (Hajra and Mondal, 2017) and sorghum (Maity *et al.*, 2018).

After twelve months of storage, seed dressing with nano-TiO_2 @ 500 mg/kg (T7) produced 4.16 cm long shoots, while the same dose of bulk particles of titanium dioxide (T2 - TiO_2 @ 500 mg/kg) produced only 2.67 cm shoot length. The ability of nano-titanium dioxide to enhance chlorophyll formation and increase RUBISCO activity have been documented by Zheng *et al.* (2005) and Yang *et al.* (2006) which could have aided the improved growth.

Many studies were found to support the positive effect of nano titanium dioxide on growth of shoots viz., maize (Vijayalakshmi *et al.*, 2018), cowpea and sorghum (Maity *et al.*, 2018) and wheat (Ramesh *et al.*, 2014).

5.2.3. Seedling root length

The root length of seeds treated with titanium dioxide declined gradually with the advancement of storage time, however, the control seeds deteriorated faster (Table 17). Seed invigoration with nano-TiO_2 @ 500 mg/kg (T7), nano-TiO_2 @ 250 mg/kg (T6) and TiO_2 @ 1300 mg/kg (T4) prompted higher seedling root growth at the end of twelve months of storage. The root growth was 3.14 cm in bulk titanium dioxide treated seeds at 500 mg/kg (T2), whereas in nano treatment at same dose (T7), the roots developed were 4.94 cm long (36 per cent higher).

The intake of titanium nanoparticles through seed coat helps in water absorption which is important as mature seeds contain less seed moisture and cellular metabolisms begin only after the availability of sufficient water (Feizi *et al.*, 2011). During the later stages of seedling growth, these absorbed nanoparticles display more intense functioning and promote plant growth (Zheng *et al.*, 2005).

Analogous findings were also reported in crops such as maize (Vijayalakshmi *et al.*, 2018), cowpea and sorghum (Maity *et al.*, 2018), pigeon pea (Raju and Rai, 2017), wheat (Ramesh *et al.*, 2014), onion (Andersen *et al.*, 2016) and cucumber (Servin *et al.*, 2012). Lettuce seeds impregnated with nano-TiO_2 showed a better root growth with increasing dose up to 2500 mg/L (Song *et al.*, 2013).

5.2.4. Seedling dry weight

Influence of titanium dioxide treatment on seedling dry matter production also followed a trend similar to the zinc oxide application (Table 18). After ten months of storage, the best treatments were T7 - nano-TiO_2 (500 mg/kg) on par with bulk-TiO_2 @ 500 mg/kg (T2) and 900 mg/kg (T3). A negative impact on seedling dry weight was seen in seeds treated with 1300 mg/kg of normal grade titanium dioxide (T4), whereas nano-TiO_2 @ 500 mg/kg (T7) gave the best result. This was in concurrence with the outcomes of the study conducted by Feizi *et al.* (2013) in fennel using nano and bulk titanium dioxide. Maity *et al.* (2018) noticed a dose-dependent increase in seedling dry weight of cowpea and sorghum seeds treated with nano-titanium dioxide.

A probable mechanism by which nano titanium dioxide improved seedling dry weight has been described by Mishra and co-workers (2014). Titanium dioxide NPs can regulate the action of enzyme like nitrate reductase, glutamate dehydrogenase, glutamine synthase and glutamic-pyruvic transaminase which are involved in nitrogen metabolism. They promote nitrate absorption and formation of chlorophyll and proteins which could increase the fresh and dry biomass of the plants. Another explanation was attributed to the increased levels of Fe^{2+}ions in leaves of titanium dioxide supplemented plants which could improve chlorophyll synthesis and uptake of nutrients (P, Fe, Mn and Zn) (Wojcik and Wojcik, 2001; Dumon and Ernst, 1988).

Titanium dioxide nanoparticles were able to promote the hydrolytic enzymes' (amylase, protease etc.) functioning. When amylase activity increases, starch molecules are transformed to soluble sugars which are used for the growth of embryonic axis during the initial phases of germination (Laware and Raskar, 2014).

5.2.5. Vigour indices

In titanium dioxide treatments, both vigour index I and II declined during the duration of storage, but treated seeds presented better performance on comparison to untreated control (Table 19 and 20).

Vigour index-I of the titanium dioxide treated seeds were significantly different after ten months of storage. Dry dressing of seeds with T7 - nano-TiO_2 @ 500 mg/kg of seeds (683), T4 - TiO_2 @ 1300 mg/kg (634), T3 - TiO_2 @ 900 mg/kg (633) and T6 - nanoTiO_2 @ 250 mg/kg (623) produced higher vigour index-I compared to control. Vigour index-II of seeds treated with T7 - nanoTiO_2 @ 500 mg/kg (1221) and T4 - TiO_2 @ 1300 mg/kg (1162) performed better than other treatments. So, vigour index-II could be used as a reliable tool to assess the quality of seeds. In a similar study, vigour index-I of sorghum seeds treated with titanium oxide NPs increased with increase in dosage, while vigour index-II did not show any effect (Maity *et al.*, 2018).

Seeds absorb nanoparticles by penetration through seed coat resulting in increased water or nutrient absorption through the vascular system and improved

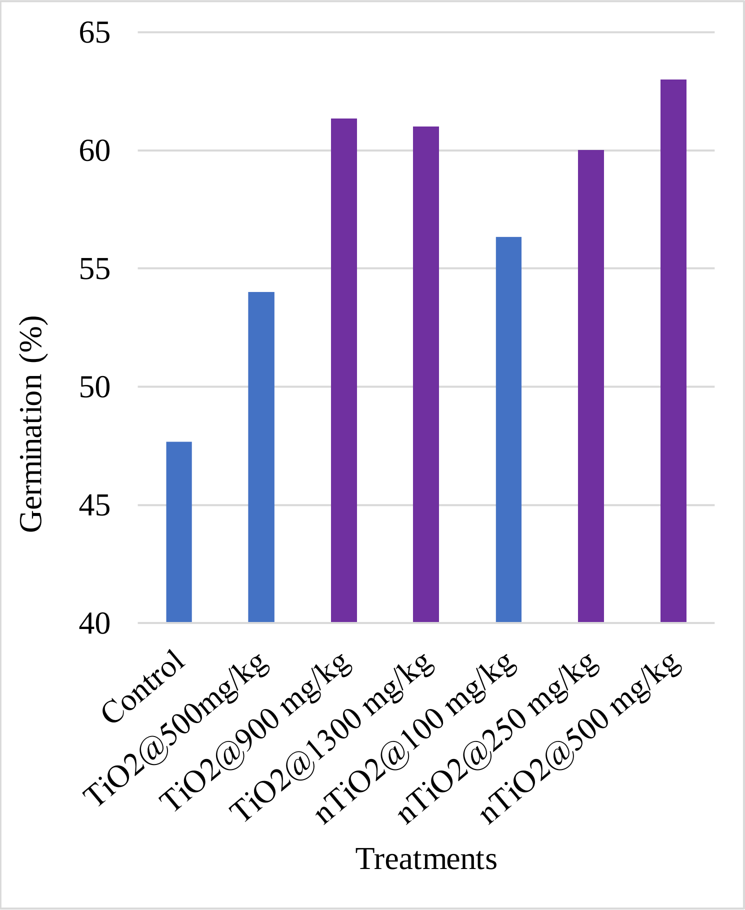


Fig. 4: Effect of titanium dioxide on maintaining germination of seeds ten months after storage

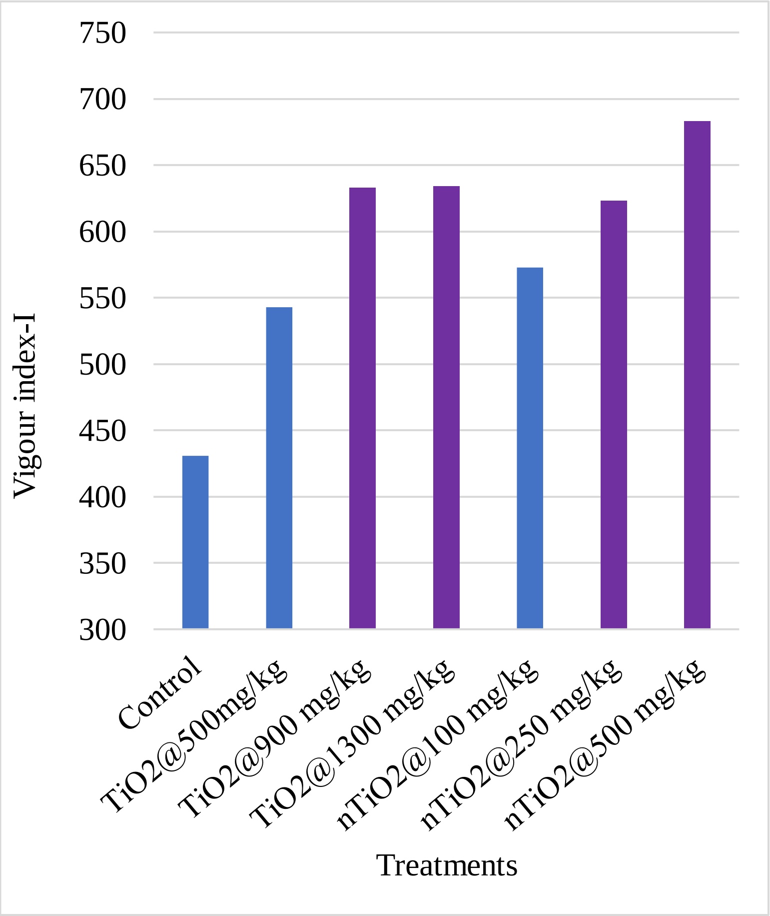


Fig. 5: Effect of titanium dioxide treatment on vigour index-I of chilli after ten months of storage

seed germination (Lyu *et al.,* 2017). After the formation of these nano-sized holes in seed coat, transfer of oxygen and water into the cell may increase, accelerating the metabolism of the seed (Laware and Raskar, 2014). The physiochemical properties of titanium dioxide nanoparticles were found to rely on the NP size, morphology and surface area (Dietz and Herth, 2011).

5.2.6. Electrical conductivity

Electrical conductivity was found to increase as storage time elapsed in titanium dioxide (Table 21) treated seeds.

The results regarding electrical conductivity of seed leachates depend not only on the treatment provided, but also on preharvest environment conditions such as high humidity and warm temperatures, seed mechanical damage induced by harvesting or conditioning equipment as well as the genetic constitution of seed.

After twelve months of storage of titanium oxide treated seeds, the least values of electrical conductivity of seed leachates was in T7 - nano-TiO_2 @ 500 mg/kg (336 µS/cm), followed by T4 - TiO_2 @ 1300 mg/kg (351 µS/cm), while control seeds recorded highest value (462 µS/cm). This was parallel to the results obtained by Vijayalakshmi *et al.* (2018) in maize. But, Pratap *et al.* (2018) noticed lower EC values in seed treatment with lower doses of nano-particles titanium dioxide which increased with dosage.

5.2.7. Seed moisture content (per cent)

No significant difference in seed moisture content was observed among the treatment (Table 22) at the end of storage period. This might due to the water and moisture impervious packaging (700-gauge polyethylene bags) material used.

5.2.8. Seed microflora

The seed infection detected at end of storage period in TiO_2 (Table 23) treated seeds were found to be minimal.

In titanium dioxide treated seeds, only control seeds and seeds treated with low dose of titanium dioxide developed infection, whereas, none of the nano-TiO_2 treated seeds were infected.

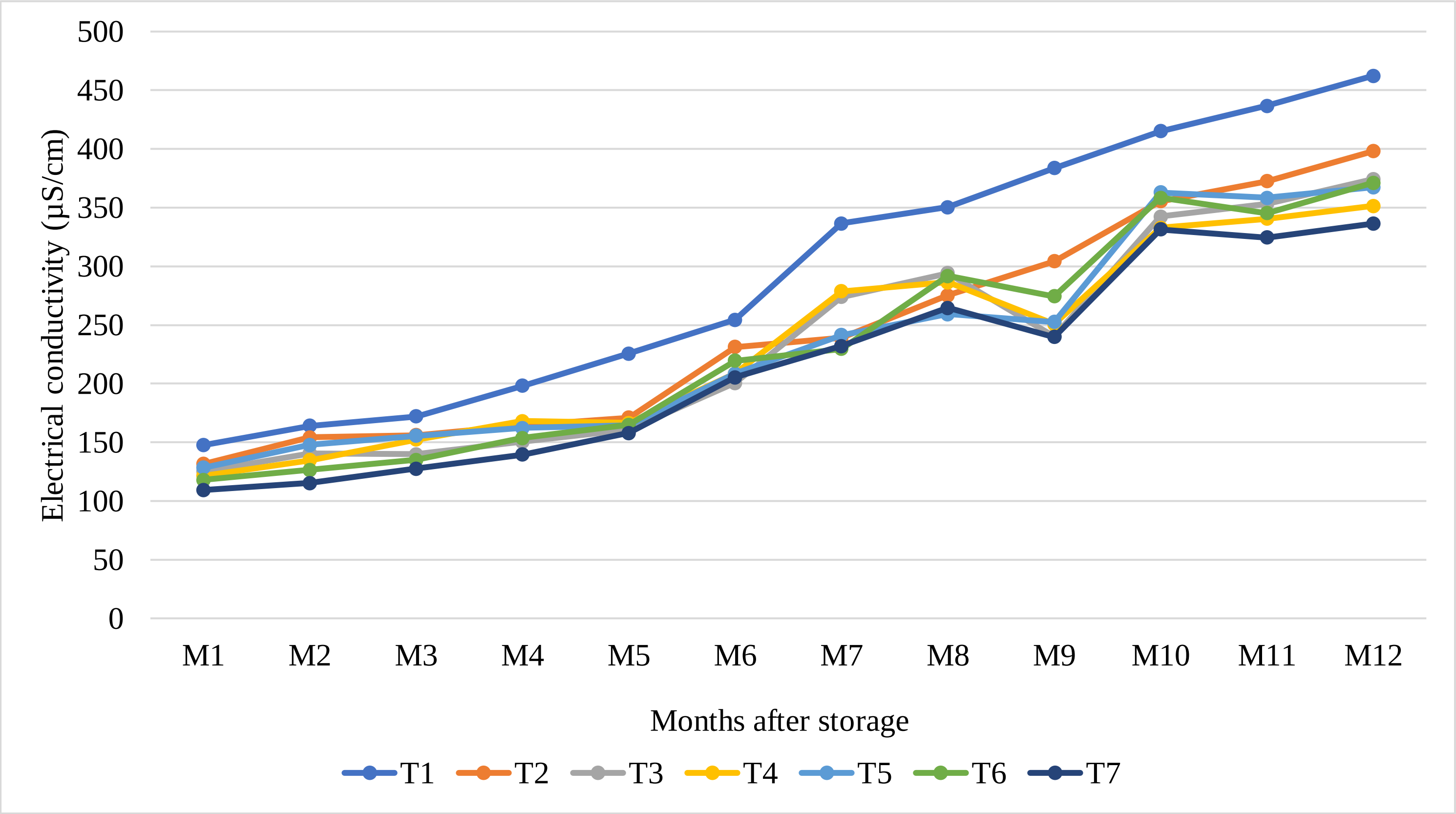


Fig. 6: Effect of titanium dioxide on electrical conductivity of seed leachate during storage

Another advantage of titanium dioxide is that it degenerates very slowly and are therefore able to provide protection against pathogens for long time. Titanium dioxide has also been found to be a good disinfectant, more effective than chlorine and even ozone.

Maness *et al.* (1999) discovered that cell deaths of *E. coli* occurred when growth media containing titanium dioxide nanoparticles were irradiated with near ultra violet light. It was assumed to be due to lipid peroxidation damage to the bacterial cell caused by titanium dioxide particles and it was estimated by using the amount of malondialdehyde (MDA) synthesised as an indicator of cell membrane damage.

5.2.9. SEM analysis of root

The scanning electron microscope analysis of radicles of chilli seeds treated with titanium dioxide along with untreated control revealed significant differences.

The root tips of the seedlings grown from nano-titanium dioxide (500 mg/kg) treated seeds had higher cell proliferation compared to the untreated seeds. In the zone of elongation, the treated seeds presented larger cells than control. Ahmed *et al.* (2016) found that the variation in cell diameter of aleurone layer of wheat did not have any significant effect on rate of germination, while length did.

5.3. Effect of zinc oxide (ZnO) seed treatment on seed quality after accelerated ageing

5.3.1. Germination

Zinc oxide treated seeds of chilli were subjected to accelerated ageing for one day (Table 24). The loss in germination was nearly fifty per cent. The germination per cent of the aged seeds were determined by roll paper towel method. Treatments with zinc oxide such as T7 - nano-ZnO @ 500 mg/kg (49 %), T4 - ZnO @ 1300 mg/kg (43 %), T6 - nano-ZnO @ 250 mg/kg (41.33 %) and T3 - ZnO @ 900 mg/kg (41.00 %) retained maximum germination after accelerated ageing.

Enhanced lipid peroxidation mediated by free radical and peroxide might explain the loss of seed viability during ageing (Sung, 1996). Gidrol *et al.* (1998)

speculated that the decrease in germination per cent may be due to degradation of the mitochondrial membrane by free radicals. Damage to the energy currency of the cell cause decreased energy supply for the germinating seeds.

A major role is played by zinc in protection and maintenance of structural integrity of cell membranes (Welch *et al*., 1982), protein synthesis, membrane functioning, cell elongation and tolerance to environmental stresses. Plants emerging from seeds with low zinc had poor seedling vigour and poor field establishment, while the presence of high zinc content in seed could stimulate seed germination and vigour.

The difference in the ability of various seed lots to withstand accelerated ageing and produce normal seedlings is dependent on various factors such as seed coat characteristics, chemical composition and anti-oxidative ability of seeds (Vijayakumar and Vijayakumar, 2015).

The capability of accelerated ageing to increase lipid peroxidation and decrease the functioning of antioxidant enzymes have been documented by Hsu and Sung (1997) and Bailly *et al.* (1998). Accumulation of reactive oxygen species (H_2O_2 and malondialdehyde) as a result of lipid peroxidation of the seed's cell membrane and mitochondrial deterioration were mainly responsible for the decline in seed longevity during storage (Li *et al.*, 2019).

5.3.2. Seedling length

Root length and shoot length of chilli seedling invigorated with zinc oxide were found to be highly reduced after accelerated ageing (Table 25).

McDonald (1999) opined that seed deterioration from ageing might have led to diminished seedling growth as a ramification of reduced respiration and lesser mitochondria in cells.

Zinc oxide treatments like nano-ZnO @500 mg/kg (T7) and bulk-ZnO @ 1300 mg/kg (T4) were able to produce longest shoots (4.36 cm and 4.80 cm) and roots (5.78 cm and 5.48 cm), while control seeds recorded 2.64 cm (shoot length) and 3.56 cm (root length). Nanoparticles of zinc oxide were better at improving the root growth compared to even highest doses of bulk particles. Nano-ZnO @ 500 mg/kg (T7) produced 4.36 cm long shoot and 5.78 cm long roots, whereas bulk-ZnO @ 500 mg/kg (T2) produced 2.94 cm long shoot and 5.04 cm long root, respectively.

Pandey *et al.* (2010) discovered a rise in IAA level in the seeds soaked in nano-ZnO suspension for five days compared to the immersion in de-ionized water. This might be due to the ability of nano-zinc oxide to catalyse IAA production and subsequently enhance germination.

5.3.3. Seedling dry weight

The seedling dry weight of seeds invigorated with T4 - ZnO @ 1300 mg/kg (26.15 mg) and T7 - nano-ZnO @ 500 mg/kg (21.65 mg) after artificial ageing were found to highest (Table 26), which was lesser than the dry matter generation of T2 - bulk-ZnO @ 500 mg/kg (17.05 mg).

Raju and Rai (2017) ascribed the rise in dry matter production by titanium oxide treatment to the enhanced production of hydrolytic enzyme in the early germination phases. This aided effective mobilization of available food reserves of the seeds which resulted in the early emergence and improved growth of the seedlings.

5.3.4. Vigour indices

Vigour index-I and vigour index-II of seeds treated with zinc oxide were higher than control seeds (Table 27).

Vigour index-I of nano-ZnO @ 500 mg/kg treated seeds (497) and ZnO @ 1300 mg/kg (441) were the highest. While T7 (nano-ZnO @ 500 mg/kg) recorded 497, treatment with T2 (ZnO @ 500 mg/kg) recorded only 139 as its vigour-index-I. Vigour index-II estimation also revealed T4 (1125) and T7 (1064) as the best treatments, while control seeds produced less vigorous seedlings (72). On comparing the particle-size efficiency, T7 (nano-ZnO @ 500 mg/kg) produced more vigorous seedlings (1064) than T2 - 500 mg/kg of bulk-ZnO (297).

The decreased seed vigour is because of seeds’ reduced capacity to germinate and produce vigorous seedlings which might be due to seed deterioration resulting

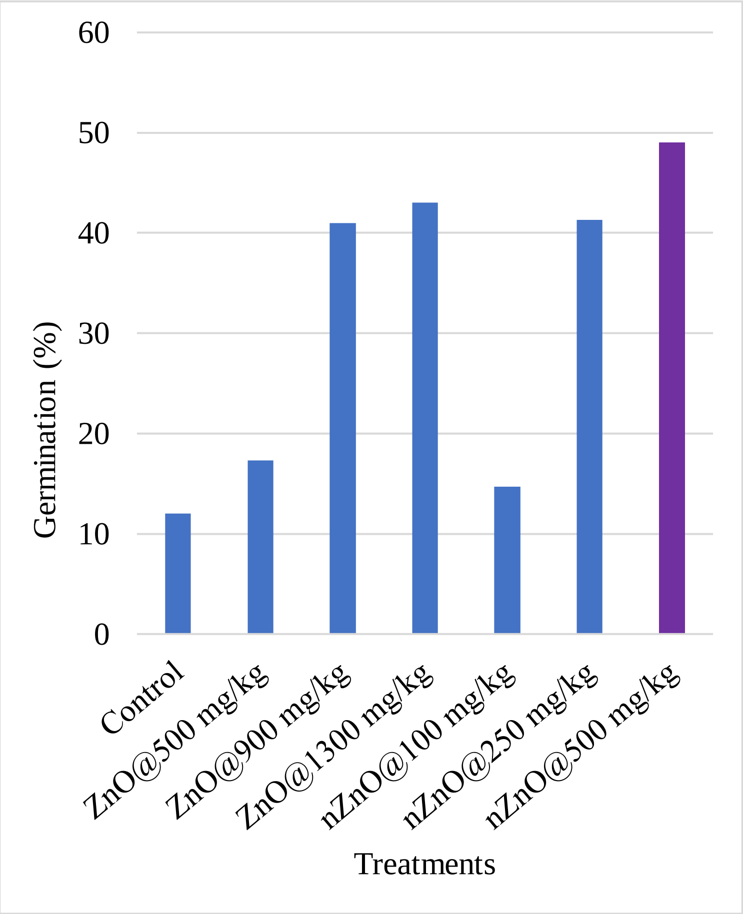


Fig. 7: Viability of zinc oxide treated chilli seeds after one day of accelerated ageing (1 MAS)

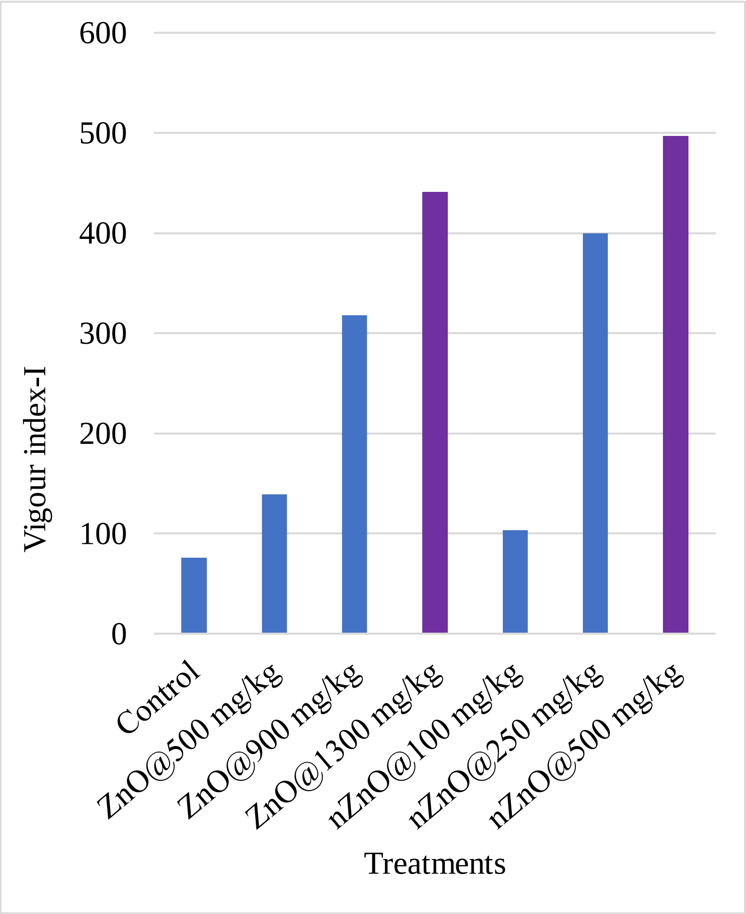


Fig. 8: Effect of zinc oxide on vigour index-I after accelerated ageing (1 MAS)

from accelerated ageing of seed (Singh, 1989). Ageing might increase the rate of respiration of seed and consequently affect the protein and DNA synthesis of the developing embryo. The speed of transport of storage nutrients slows down, causing a decline in seedling vigour (Murthy *et al.*, 2003). Seeds have a defensive system to overcome oxidative stress by using anti-oxidant enzyme network. But high concentration of H^+ ions in mitochondria during ageing disrupts this mechanism and increases the activity of protein degrading enzymes (protease) causing cell death (Kibinza *et al*., 2011). This results in loss of seed vigour.

5.3.5. Electrical conductivity

The electrical conductivity is an indirect measurement of the seed vigour (AOSA, 1983). A basic cause of differences in vigour could be dependent on the state of integrity of seed coat (cell membrane) which is based on deteriorative biochemical as well as physical changes occurring within the seed.

Among various doses of treatment with zinc oxide, the electrical conductivity of the seed leachate (Table 28) varied. Control seeds measured the highest value of electrical conductivity (299.67 µS/cm), while all the treated seeds were found to be on par with each other. So, irrespective of the dosage and particle size, zinc oxide acts as a membrane protector in chilli seeds.

Usually, the electrical conductivity increase after seeds are subjected to accelerated ageing due to membrane deterioration and metabolic changes occurring in the seed (Gupta *et al.*, 2005). The presence of nano particles of titanium dioxide was able to slow down the rate of membrane degradation and it might have resulted in the reduced amount of seed nutrient exudates indicated by the lower values of EC.

On the contrary, Abdul-Baki and Anderson (1970) found no difference in electrical conductivity measurement of seed leachate of barley seeds accelerated aged for 12 days, even though a drastic reduction in the germination was observed.

5.3.6. Seed moisture content

The seed moisture content of the chilli seeds treated with zinc oxide was found to vary from the initial value after being subjected to one day of accelerated ageing (Table 29).

An increase in seed moisture content could escalate the respiration rate and subsequent utilization of stored food material leading to the reduction in seed viability. The high moisture also invites the growth of micro organisms which are detrimental to seed quality. Similar findings were also reported by Sugandhi and Selvaraju (2017) in groundnut seeds subjected to accelerated ageing.

5.3.7. Seed microflora

Many reports have been made regarding the antibacterial and antifungal properties of nanoparticles.

The seed infection percent of zinc oxide treated seeds after accelerated ageing (Table 30) were found to be higher than the seed infection during normal storage.

Among the zinc oxide treatments, the minimum seed infection per cent was seen in seeds dry dressed with T7 - nano-ZnO @ 500 mg/kg (blotter method-5.33 percent; agar method-10.00 per cent), whereas, normal ZnO @ 500 mg/kg (T2) developed 53.33 per cent (blotter paper method) and 43.33 per cent (agar plate method) infection. Other doses of nano zinc oxide treatment (T5 and T6) also produced lower infection than seed treatment with normal-grade zinc oxide at all doses.

Arciniegas-Grijalba *et al.* (2017) suggested that the antifungal effect of zinc oxide nanoparticles could be due to the production of ROS (reactive oxygen species) and/or Zn^{2+} ions. The target organ of nano-ZnO is the wall of the fungus, which is made of β-1,3-D-glucan, chitin, glucan, mannan and galactomannan among others. Zinc oxide nanoparticle affect the functioning of N-acetylglucosamine (precursor to chitin synthesis) and β-1,3-D glucan synthase (involved in β-1,3-D-glucan synthesis) resulting in inhibition of mycelial growth.

5.4. Effect of titanium dioxide (TiO_2) seed treatment on seed quality after accelerated ageing

5.4.1. Germination

Titanium dioxide treated seeds also showed a marked decrease in germination after being subjected to artificial ageing (Table 31). Seeds dry dressed with T7 - nano-TiO_2 @ 500 mg/kg (51.67 %), T4 - TiO_2 @ 1300 mg/kg (47.33 %) and T3 - TiO_2 @ 900 mg/kg (44.67 %) had a higher germination per cent. This is in agreement with other studies which reported the ability of titanium dioxide to improve germination of aged seeds (Zheng *et al.*, 2005). The seeds which are able to perform well after accelerated ageing are considered to be good storers, whereas, those with reduced germination are considered to be poor storers (Delouche and Baskin, 1973).

Nanoparticles can penetrate the seed coat and impart a beneficial response on seed germination (Feizi *et al.*, 2013). Smaller size of nanoparticles enables it to easily enter through the cracks present on the outer seed surface, react with free radicals resulting in enhanced seed vigour and viability. NP treatment could have enhanced the physiological performance of seeds by quenching of free radicals.

Ahmed *et al.* (2016) isolated the scutellum and aleurone layer of naturally aged and accelerated aged wheat seeds. They discovered a positive correlation between nuclei staining intensity of scutellum cells and seed germination, and also that a wider length:width ratio of nuclei was detrimental to germination during natural ageing. In accelerated aged seeds, scutellum was found to be disorganized, the cell layers crumbled and honey-comb structure of cells was absent.

5.4.2. Seedling length

Titanium dioxide treated seeds produced variations in seedling shoot length and root length after being subjected to accelerated ageing (Table 32). Higher doses of nano and bulk titanium dioxide were able to develop longer roots and shoots. Shoot length (4.34 cm) and root length (5.72 cm) were highest in seeds treated nano-

TiO_2 @ 500 mg/kg (T7). Bulk-sized TiO_2 @ 500 mg/kg (T2) produced much shorter shoot (2.87 cm) and root (2.02 cm).

The ability of titanium dioxide NPs to improve root growth in cucumber were reported by Andersen *et al.* (2016).

The root length increase was higher in titanium dioxide NP treated seeds rather than zin oxide NP treated seeds. This enhancement is possibly due to an increased level of some nutrient (Fe and Mg) and increased chlorophyll (a and b) biosynthesis aided by titanium dioxide (Kuzel *et al*., 2003). Lei *et al.*, (2008) advocated the potential of titanium dioxide NPs to reduce hydrogen peroxide (H_2O_2), superoxide radicals and malonyl dialdehyde content of aged chloroplasts by activating superoxide dismutase (SOD), catalase (CAT), ascorbateperoxidase (APX), and guaiacolperoxidase (GPX).

5.4.3. Seedling dry weight

Titanium dioxide treated seeds also differed significantly in their dry biomass (Table 33). Higher doses of both nano and bulk titanium dioxide performed well for this parameter. The seedling dry weight of TiO_2 @ 900 mg/kg (19.31 mg), nano-TiO_2 @ 500 mg/kg (18.45 mg), TiO_2 @ 1300 mg/kg (17.15 mg) and nano-TiO_2 @ 250 mg/kg (17.00 mg) were found to be on par with each other. Normal grade titanium dioxide @ 500 mg/kg (T2) was able to produce 15.43 mg dry biomass, whereas nano titanium dioxide @ 500 mg/kg (T7) produced 18.45 mg, resulting in a 16 per cent increase due to nanoparticle treatment.

Seedling dry weight reduction could be assigned to the increased rate of catabolic changes occurring in the seed which is beyond the threshold at which cell repair mechanisms can function effectively (Vasudevan *et al.*, 2012).

5.4.4. Vigour indices

In titanium dioxide treatment also both vigour index-I and II of control seeds were much lower than treated seeds (Table 34). Highest vigour index-I was measured in T7 - nanoTiO_2 @ 500 mg/kg (520), whereas an 88 % reduction was observed in T2 - bulk-TiO_2 @ 500 mg/kg (60). Vigour index-II also showed a similar trend. Bulk-TiO_2 @ 500 mg/kg (T2) developed 81 % less vigorous seedlings than nano-TiO_2 @ 500 mg/kg (T7). While vigour index-I of titanium dioxide treated seeds were better than zinc oxide treated seeds, vigour index-II values were higher in zinc oxide treated seeds.

Accelerated ageing of seed leads to the rapid loss of vigour and eventually viability in less vigorous seed lots, and can be used as an effective method to determine the vigour changes during seed storage (Tian *et al*. 2008).

According to Adesanya *et al.* (2018), the performance of seeds after accelerated ageing is well correlated with their activity in field too. Accelerated ageing percent can hence be used to predict the field emergence.

During accelerated ageing, seeds undergo protein deactivation, denaturation and increase in free radicles of oxygen resulting in oxidative damage to cells (Parmoon *et al.*, 2019).

5.4.5. Electrical conductivity

The electrical conductivity of the seed leachates obtained from seeds dry dressed with nano-TiO_2 @ 500 mg/kg (245.67 µS/cm) was lower than other treatments as well as control (308.00 µS/cm) (Table 35).

The leakage of Na^+, Ca^{2+}, K^+ and Mg^{2+} from accelerated aged seeds of sweet pepper increased with the duration of ageing. This might have been because of the interruption of electrolyte transportation caused by the damaged transporting protein in cell membrane (Kaewnare *et al.*, 2011). During ageing, the protein might have become non-functional during its transformation from fluid state to solid-gel state. It could have been followed by the additional deterioration of lipids in the phospholipid bilayer of cell membrane by the action of lipid peroxygenase (LOX) enzyme (Torres and Andrews, 2006) causing higher EC value.

5.4.6. Seed moisture content

The seed moisture content of the chilli seeds treated with titanium dioxide was found to vary from the initial moisture content after an accelerated ageing of

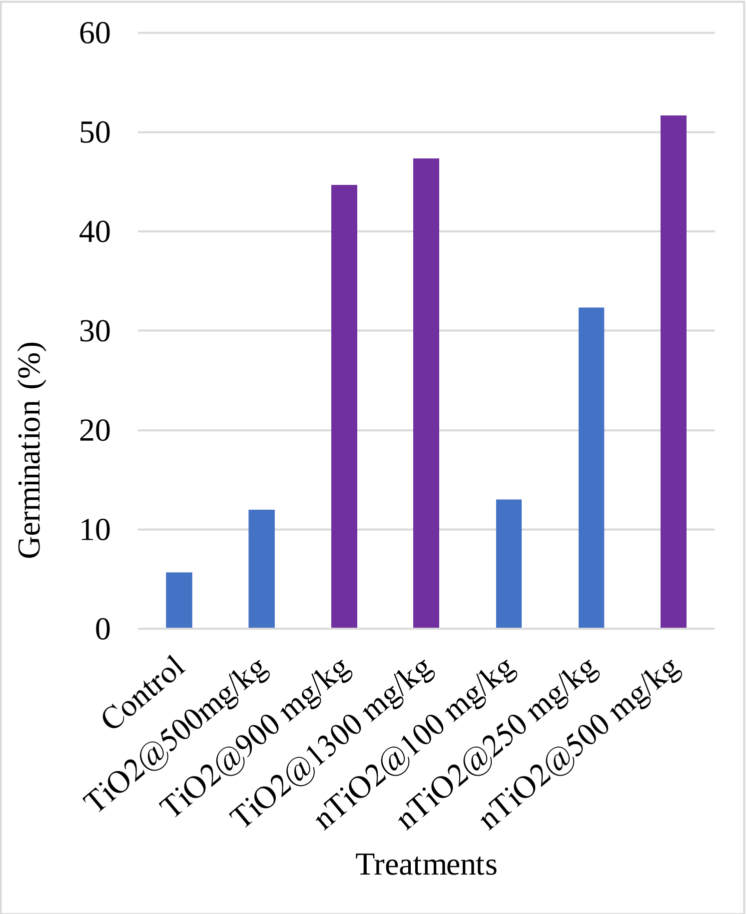


Fig. 9: Viability of titanium dioxide treated chilli seeds after one day of accelerated ageing (1 MAS)

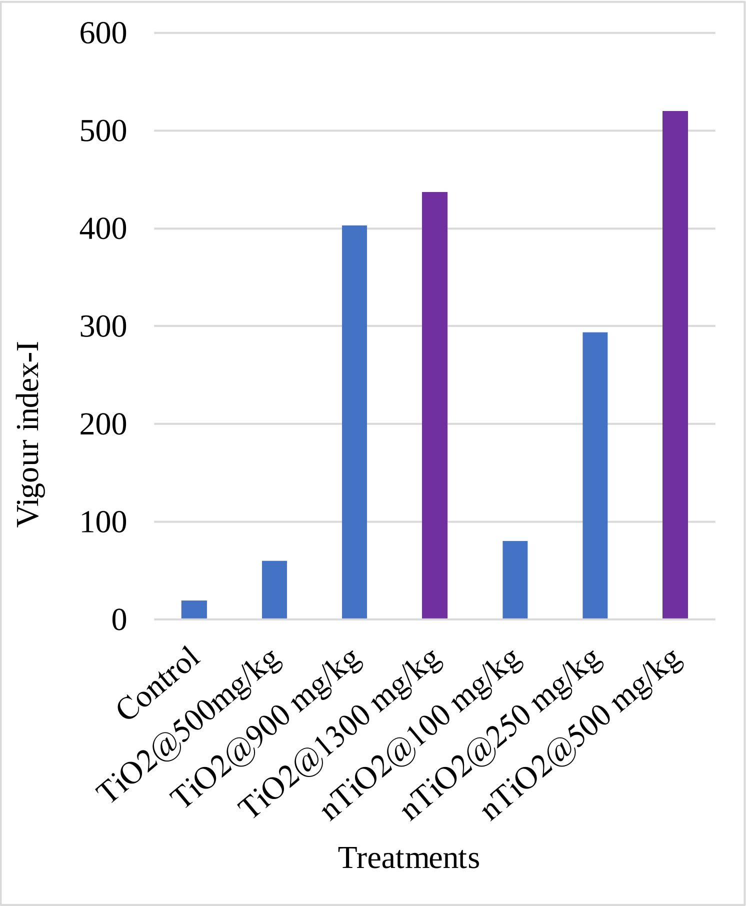


Fig. 10: Effect of titanium dioxide on vigour index-I after accelerated ageing (1 MAS)

one day (Table 29). Control seeds maintained a higher moisture content than treated seeds.

The increase in seed moisture content during ageing might be due to the disorganization of cell membranes (Ellis *et al.*, 1992). It was opined by Kapoor *et al.* (2011) that rate of cell membrane deterioration accelerated and seed moisture increased as the period of accelerated ageing prolonged.

5.4.7. Seed microflora

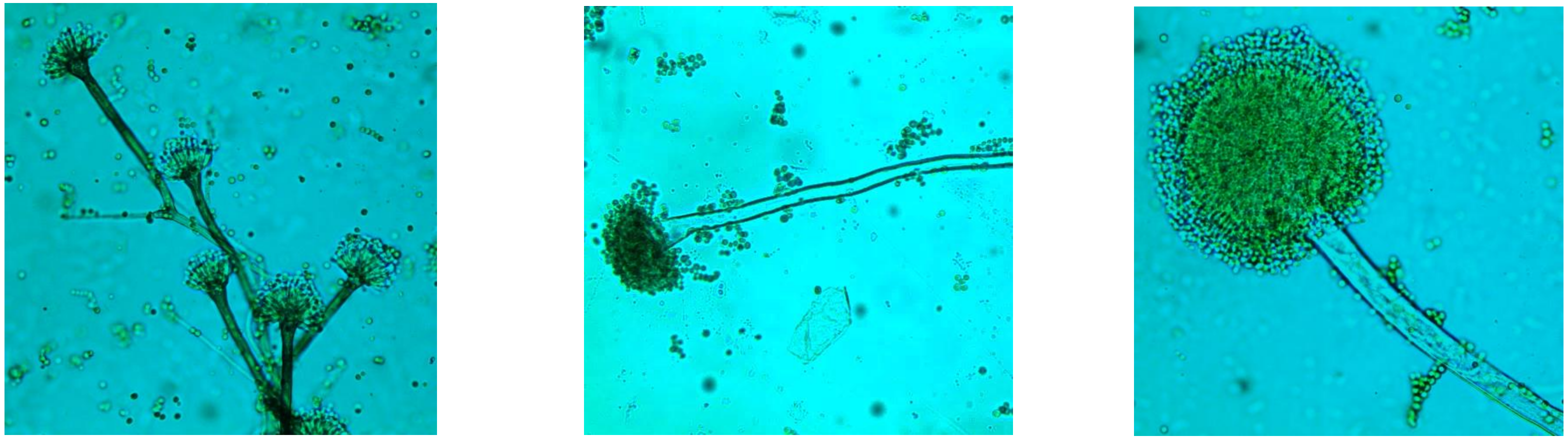
Greater seed infection was observed in titanium dioxide treated seeds after accelerated ageing (Table 37) compared to the seed infection during ambient storage.

Among the titanium dioxide treated seeds, the per cent seed infection was found to be least in T7 - nano-TiO_2 @ 500 mg/kg in both blotter paper (12.00 per cent) and agar plate (6.67 per cent) method. When the same dose of treatment (500 mg/kg) was given as T2 - bulk-TiO_2 @ 500 mg/kg, the per cent of infected seeds was higher. Blotter paper infection was 20.00 per cent and in agar plate method, 10.00 per cent of the seeds were infected.

Antimicrobial effects of titanium dioxide might be due to its oxidative nature and the metabolic processes following its absorption by the plants. In the presence of ultra violet light, the photocatalytic TiO_2 produces singlet oxygen (O=O) and superoxide anion (O_2^-) which could impair cell functioning of microbes due to oxidation (Lyu *et al*., 2017).

Greenhouse (Paret *et al.*, 2013b) and field (Paret *et al*., 2013a) trials showed that using titanium dioxide or zinc could result in significantly reduced bacterial spot severity compared to using untreated controls.

Aspergillus niger *Alternaria* spp. *Mucor* spp.



Aspergillus flavus *Aspergillus flavus* *Aspergillus flavus*

Plate 8: Micro-organisms observed under microscope

Seed invigoration of chilli seeds by dry dressing them with zinc oxide and titanium oxide nanoparticles proved to be an effective way to improve seed quality during storage. During natural ageing, the best treatment was found to be 500 mg/kg of nano-grade zinc oxide and titanium dioxide. At 1300 mg/kg, the bulk-sized zinc oxide and titanium dioxide were able to produce similar results. Amongst all the treatments, electrical conductivity of seed leachates was found to be least in the nano particle (500 mg/kg) treated seeds probably due to the ability of NPs to protect the cell membrane of seeds.

In the aged seeds, significantly higher germination per cent was obtained from zinc oxide and titanium dioxide NP treatments (500 mg/kg) than even the highest dose of normal-grade (1300 mg/kg) particles. The vigour indices, seed infection, and electrical conductivity were also improved with NP invigoration of seeds and it increased with increase in concentration.

In all the characters studied, nanoparticle at the highest dose (500 mg/kg) showed similar or superior results compared to the highest dose of normal grade chemical (1300 mg/kg). When similar doses (500 mg/kg) of both particle size were compared, the nanoparticle application was found to be a more efficient seed invigoration agent. Also, they are capable of entering the plant system through nano-sized pores on seed coat and aid in water and nutrient absorption as the plant grows. The NPs also promote the activity of free radical oxygen scavengers and limit lipid peroxidation damage occurring in the cells.

Chilli seeds accelerated aged at 40 °C and 100 % relative humidity for one day behaved similar to chilli seeds which were over ten months old. Hence, one day of accelerated ageing in chilli could be considered as equivalent to ten months of storage in ambient conditions and this could be used as a time-saving method to estimate how the seed lots behave during long storage periods without having to store them for months.

From all these experiments, it can be inferred that germination per cent is the most efficacious parameter indicating seed quality during both natural ageing and accelerated ageing. Vigour index- I is dependent on the seedling length which may vary according to the light availability, while vigour-index II (determined by seedling dry weight) is more constant. While electrical conductivity was effective in surmising the seed quality under natural ageing condition, no significant difference among the treatments was observed in accelerated aged seeds. As the tests are conducted in controlled environment of laboratory (light availability also quantified), germination per cent, vigour index-I and vigour index-II could be useful tools to indicate the seed quality.

Table 38: Cost of treatment chemicals

Material	**Quantity (g)**	**Cost (Rs)**	**Cost per 1000 mg (Rs)**
ZnO powder	500	768.60	1.5
TiO_2 powder	1000	888.00	0.9
Nano ZnO powder	50	8930.31	178.6
Nano TiO_2 powder	100	15639.82	156.4

As chilli seeds are costly (Rs. 3000/kg) with low and erratic germination (60 per cent), maximum care needs to be taken to improve its germination and field stand. Treatment with nanoparticles of zinc oxide and titanium dioxide up to 500 mg/kg of seeds have been attempted in this study and it was found to be more influential than the same dose of bulk particles zinc oxide and titanium dioxide.

Extending the storage life of chilli seeds for two months will be a big boon to the seed industry. Seed treatments with chilli seeds will cost less than two hundred rupees per kg. Hence seed treatments with nanoparticles offer a viable option for enhancing the storage life of chilli seeds as well as retaining the quality and vigour.

FUTURE LINE OF WORK

Standardisation of temperature, relative humidity and duration of ageing procedure for accelerated ageing in chilli seeds which could give a good correlation to the field establishment of the crop.

The field performance of treated seeds and their correlation to various seed vigour test methods in laboratory such as EC, standard germination, accelerated ageing test, vigour index-I and II could be ascertained.

This study has been restricted to one season and may be repeated for confirmation of results. The study has envisaged seed treatment for one variety. Hence the seed treatments may be repeated for other varieties in chillies and the dosage standardised.

The possibility of using other inorganic nanoparticles for seed treatment such as copper (Cu) NPs and silver (Ag) NPs may be thought of. Moreover, other seed treatments techniques like wet seed treatment may also be attempted to arrive at the most economical and feasible method of seed treatment with nanoparticles in chilli.

Summary

6. SUMMARY

The present study was conducted in the Department of Seed Science and Technology, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur, to elucidate the effect of inorganic nanoparticle treatment on seed quality of naturally-aged and accelerated-aged seeds of chilli.

Seeds of chilli cv Anugraha was exposed to varying doses of nano and bulk particles of zinc oxide and titanium dioxide in four different experiments. In the first two experiments, the treated seeds (zinc oxide - experiment 1 and titanium dioxide - experiment 2) were packed in 700 G polythene bag and stored in ambient conditions for twelve months.

- Seed quality parameters like germination per cent, seedling length, vigour indices and seedling dry weight of all the treated and control seeds gradually decreased with the advance of time.
- Seed solute leakage (measured using electrical conductivity of the seed leachates) and seed infection per cent increased during storage.
- Among the two treatment chemicals, zinc oxide and titanium dioxide were both capable of improving seed longevity during storage.
- During natural ageing, seed invigoration with zinc oxide NPs (500 mg/kg) and zinc oxide (1300 mg/kg) were found to be the best treatments in terms of germination per cent, vigour indices and seed infection per cent. These treatments were able to maintain 60 % germination stipulated by the IMSCS for ten months after storage, while the other treated seeds lost viability after the ninth and control seeds after the eighth month.
- Seed treatment with zinc-oxide nanoparticles (500 mg/kg and 250 mg/kg) were able to reduce electrolyte leakage from the seeds as indicated by lower values of electrical conductivity measured.
- When titanium dioxide was used, four treatments were able to retain 60 % viability up to ten months after storage, *i.e.*, T7 - nano-TiO_2 @ 500 mg/kg,

T3 - TiO_2 @ 900 mg/kg, T4 - TiO_2 @ 1300 mg/kg and T6 - nano-TiO_2 @ 500 mg/kg, while in control seeds only up to eight months.

- With respect to vigour index I and II, the best treatments were found to be T7 - nano-TiO_2 @ 500 mg/kg and T4 - TiO_2 @ 1300 mg/kg.
- In natural ageing, all the treated seeds were effective in reducing electrolyte leakage and reducing seed infection per cent.
- Scanning electron microscope (SEM) analysis of radicles of control, zinc oxide NP (500 mg/kg) and titanium dioxide NP (500 mg/kg) treated seeds showed a faster proliferation cells in the root tip and occurrence of larger cells in the zone of elongation. These observations reinforce the positive effects of nanoparticle seed treatment.

In experiments 3 (zinc oxide) and 4 (titanium dioxide), the treated seeds were subjected to accelerated ageing at 95±2 per cent relative humidity and 40°C for one day and seed quality assessed for a storage period of three months.

- Zinc oxide treated seeds subjected to one day of accelerated ageing revealed marked differences among the treatments. T7 - nano-ZnO @ 500 mg/kg was the best treatment It was able to retain germination by 75.5 % when compared to control and 64.6 % compared to bulk-ZnO @ 500 mg/kg
- The vigour indices of chilli seeds treated with T7 - nano-ZnO @ 500 mg/kg and T4 - ZnO @ 1300 mg/kg were found to higher than that of other treatments. In terms of electrical conductivity, all the treatments were on par with each other. However, the vigour indices of untreated control were higher than those of accelerated aged seeds.
- When the seed dressing was done using titanium dioxide, treatment T7-nano-TiO_2 @ 500 mg/kg and T4 - TiO_2 @ 1300 mg/kg were effective in enhancing the germination and vigour indices. The electrical conductivity and seed infection were least in T7 - nano-TiO_2 @ 500 mg/kg.

From the above experiments, it can be concluded that dry dressing of chilli seeds with zinc oxide and titanium dioxide nanoparticles are effective in enhancing its storability. Seed storability could be prolonged for two months on treatment with ZnO (T3 - 900 mg/kg and T4 - 1300 mg/kg) as well as nano-ZnO (T6 - 250 mg/kg and T7 - 500 mg/kg) compared to untreated seeds. Seed longevity was increased by two months on treatment with TiO_2 (T3 - 900 mg/kg and T4 - 1300 mg/kg) and nano-TiO_2 (T6 - 250 mg/kg and T7 - 500 mg/kg).

Accelerated ageing test also support this result.

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Appendix

Appendix-I

Monthly meteorological data from February 2018 to May 2019

Month	Temperature (°C)		Mean relative humidity (%)	Rainfall (mm)	Rainy days
	Max.	Min.			
Feb 2018	35.7	22.5	47	5.2	1
Mar 2018	36.7	24.0	59	33.2	2
Apr 2018	36.1	24.8	69	28.9	2
May 2018	33.2	22.6	79	483.6	14
Jun 2018	29.8	23.2	89	730.0	23
Jul 2018	29.6	22.5	88	793.2	22
Aug 208	29.2	22.2	87	928.0	21
Sep 2018	32.2	22.5	75	29.0	1
Oct 2018	32.8	22.9	76	393.0	13
Nov 2018	32.7	23.3	68	66.6	5
Dec 2018	33.0	22.5	63	0.0	0
Jan 2019	32.9	20.4	55	0.0	0
Feb 2019	35.3	23.4	59	0.0	0
Mar 2019	36.7	24.8	65	0.0	0
Apr 2019	36.2	25.5	70	76.4	3
May 2019	34.6	24.9	74	48.8	4

SEED INVIGORATION WITH INORGANIC NANOPARTICLES IN CHILLIES (*Capsicum annuum* L.)

by

GAYATHRI SATHEES

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ABSTRACT OF THE THESIS

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Seed invigoration with inorganic nanoparticles in chillies (*Capsicum annuum* L.)

Abstract

A study entitled ‘Seed invigoration with inorganic nanoparticles in chillies (*Capsicum annuum* L.)’ was conducted in the Department of Seed Science and Technology, College of Horticulture, Vellanikkara between February 2018 and May 2019 to investigate the effect of nano and bulk particles of zinc oxide (ZnO) and titanium dioxide (TiO_2), on longevity of chilli variety Anugraha.

The present study was subdivided into four experiments. In experiment I, fresh seeds of chilli were treated with ZnO (nano and bulk particle) and stored under ambient conditions. Experiment I consisted of dry dressing chilli seeds with TiO_2(nano and bulk). In both the experiments treated seeds performed better than the control. In experiment I, the seeds treated with T_3 - ZnO @ 900 mgkg^{-1}, T_4 - ZnO @ 1300 mgkg^{-1}, T_6 - nano ZnO @ 250 mgkg^{-1} and T_7 - nano ZnO @ 500 mgkg^{-1} were able to maintain the minimum germination standard of 60.00 per cent stipulated by the IMSCS (Indian Minimum Seed Certification Standards) in chilli upto 10 MAS. The vigour index-I and II were found to be high in T_4 (ZnO @ 1300 mgkg^{-1}), T_7 (nZnO @ 500 mgkg$^{-1)}$ and T_3 (ZnO @ 900 mgkg^{-1}). The seed leachate obtained from T_7 gave the lowest measurement (246 µS cm^{-1}) of electrical conductivity. Control seeds were found to contain higher per cent of infection on comparison with the treated seeds.

Chilli seeds treated with T_3 - TiO_2 @ 900 mgkg^{-1}, T_4 - TiO_2 @ 1300 mgkg^{-1}, T_6 - nanoTiO_2 @ 250 mgkg^{-1} and T_7 - nanoTiO_2 @ 500 mgkg^{-1} were able to maintain IMSCS up to ten months of storage, while the control (T_1) lost the germination after eight months, *i.e.*, T_7 (63.00 per cent), T_3 (61.33 per cent), T_4 (61.00 per cent) and T_6 (60.00 per cent). At the end of ten months of storage, all the treated seeds had lower electrical conductivity compared to control seeds. In both experiments, the seed infection per cent was found to be higher in untreated seeds compared to the

treated ones. It was also evident that the treated seeds possessed better seed quality parameters compared to untreated seeds (control).

In experiments III and IV fresh seeds which were treated with various doses of zinc oxide and titanium dioxide respectively, were subjected to accelerated ageing at a temperature of 40±1°C and relative humidity of 100 per cent for one day. In experiment III, the zinc oxide treated seeds after accelerated ageing revealed that seeds treated with T_7 - nano ZnO @ 500 mgkg^{-1} retained a high germination of 49.00 per cent, followed by T_4 (43.00 per cent). In terms of vigour index I and II, T_7 and T_4 were found to be on par with each other. The seed infection was found to be lower in nano ZnO treated seeds compared to control and normal grade ZnO treated seeds. The least infection was detected in T_7 - nano ZnO @ 500 mgkg^{-1}.

Experiment IV consisted of untreated and TiO_2 treated seeds which were subjected to accelerated ageing. Even after artificial ageing, T_7 - nano TiO_2 @ 500 mgkg^{-1} was able to retain 51.67 per cent germination in the first month. It was on par with T_4 - TiO_2 @ 1300 mgkg^{-1} (47.33 per cent) and T_3 - TiO_2 @ 900 mgkg^{-1} (44.67 per cent). The highest vigour index was observed in seeds treated with T_7 - nano TiO_2 @ 500 mgkg^{-1} (520) and it was on par with T_4 - TiO_2 @ 1300 mgkg^{-1} (437). T_7 exhibited the highest vigour index-II (953) and was on par with T_3 - TiO_2 @ 900 mgkg^{-1} (863) and T_4 - TiO_2 @ 1300 mgkg^{-1} (813). The lowest electrical conductivity was obtained from the seed leachate of T_7 (246 µS cm^{-1}). The seed infection per cent was found to be the least in T_7 - nano TiO_2 @ 500 mgkg^{-1} treated seeds.

Scanning electron micrograph of the radicles of seeds treated with nZnO and nTiO_2 @ 500 mgkg^{-1} revealed higher cell proliferation at root tip and larger cells in the zone of elongation.

The results obtained indicate that dry dressing of chilli seeds with zinc oxide and titanium dioxide is effective in improving the seed quality parameters during storage. The results of accelerated ageing test are also in agreement with the results obtained from storage under ambient conditions during natural ageing. Among various doses of zinc oxide T_7 - nano ZnO @ 500 mgkg^{-1} and T_4 - ZnO @ 1300 mgkg^{-1} exhibited the best results, whereas, among titanium dioxide treatments, T_7 -

nano TiO_2 @ 500 $mgkg^{-1}$ and T_4 - TiO_2 @ 1300 $mgkg^{-1}$ seed were found to be the best doses.