SEED INVIGORATION WITH NANOPARTICLES FOR SEED YIELD AND QUALITY IN CHILLI (*Capsicum annuum* L.)

by

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(2018-11-154)

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2020

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THESIS

Submitted in partial fulfilment of the requirements for the degree of

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Faculty of Agriculture
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DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA
2020

DECLARATION

I hereby declare that this thesis entitled **"Seed invigoration with nanoparticles for seed yield and quality in chilli (*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 **Riya Mary Mathew**

Date: (2018-11-154)

CERTIFICATE

Certified that this thesis entitled "**Seed invigoration with nanoparticles for seed yield and quality in chilli (*Capsicum annuum* L.)**" is a record of research work done independently by **Ms. Riya Mary Mathew (2018-11-154)** 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.

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We, the undersigned members of the advisory committee of **Ms. Riya Mary Mathew (2018-11-154)**, 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 nanoparticles for seed yield and quality in chilli (*Capsicum annuum* L.)"** may be submitted by **Ms. Riya Mary Mathew**, in partial fulfilment of the requirement for the degree.

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Riya Mary Mathew

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LIST OF ABBREVIATIONS

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
ppm	: Parts per million
mm	: milli metre
nm	: nano metre
m	: metre
µm	: micro metre
°C	: degree Celsius
Pa	: Pascal
L	: Litre
ha	: Hectare
cc	: cubic centimeters
ANOVA	: Analysis of Variance

ZnO : zinc oxide

nZnO : nano-zinc oxide

TiO_2 : titanium dioxide

$nTiO_2$: nano-titanium dioxide

SE_m : standard error of error mean sum of squares

CD : critical difference

var. : variety

cv. : cultivar

NP : nano particle

IMSCS : Indian Minimum Seed Certification Standard

mg/kg : milligram per kilogram

°N : degrees north

°E : degrees east

G : gauge

DAT : Days after transplanting

MAS : months after storage

% : per cent

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

V.I. : vigour index

PDA : Potato Dextrose Agar

VFPCK : Vegetable and fruit promotion council Kerala

Introduction

1. INTRODUCTION

Chilli (*Capsicum annuum* L.) is the largest produced spice crop in Asia. India is the largest producer followed by China. In India, Andhra Pradesh (49%), Karnataka (15%), Maharashtra (6%) and Tamil Nadu (3%) constitute nearly 75 per cent of the total area under chilli production. During 2018-19, in India, chilli was grown over an area of 364 thousand ha with a production of 3720 thousand MT (NHB, 2019).

In Kerala the net cropped area was 1.42 thousand ha with a production of 10.95 thousand MT and Palakkad, Thiruvananthapuram, Kollam were the major chilli producing districts during 2018. Chilli seed like all other agricultural commodities, invariably contains a high moisture content (60-85%) at the time of harvest, which must be brought down to 8-12 per cent. Poor seed storage is one of the major constraints in chilli production. The high temperature (25-37°C) and high humidity (70-90 %) prevailing in Kerala, makes storage, a still more difficult task.

Conservation of seed vigour and viability during storage is crucial. Seed qualities like germination and vigour can be considerably affected at any stage of storage. Storage and preservation of quality seed stocks till the next season is as important as producing quality seeds (Singh and Gill, 1996). Quality seed production begins from the time of harvest to planting. The importance of seed storage has been recognized ever since humans began to domesticate plants. Seeds are stored to facilitate planting in the following season and this practice is advantageous to seed growers and farmers to carry over seeds for more years.This results in accumulating supplies of desired seed stocks for use in years of low production (USDA, 1978). Seeds confirming to the prescribed standards in terms of high genetic purity, physical purity, germination per cent, low moisture content and good health are required to raise productivity.

Storage potential of seeds varies with the crop, variety and environment. Seeds with greater storage potential possess higher vigour and can be stored for longer time. The loss of viability impairs the biological value or function of seeds, which is to protect and nourish the living cells of the embryo, until seedling is established. Seed deterioration is inevitable and the best that can be done is to control its rate. Seed treatments can be defined as operations which aim to mitigate seed deterioration, to increase productivity through improving seed quality, good seedling establishment and minimizing crop loss by managing pest and diseases.

Seed deterioration can be alleviated with seed invigoration. Seed invigoration with nanoparticles is an emerging area and is successful in many crops. "Nanotech", is the study of manipulating matter on an atomic and molecular scale. The term ‘nano’ can be defined as the molecular aggregates with at least one dimension between 1 and 100 nm .The positive effects of nanoparticles include enhanced germination per cent, length of root and shoot and vegetative biomass of seedlings in many crop plants (Agarwal and Rathore, 2014). Nanotechnology has the potential to protect plants, monitor plant growth, detect plant diseases, weed management, stress tolerance, increase global food production, enhance food quality and reduce waste for sustainable intensification. Nano particles are being developed for slow release of fertilizers for plants. This makes nutrients more available to nanoscale plant pores and therefore result in efficient nutrient use (Suppan 2013). The mechanism by which nano particles alter biological activities is dependent on their shape, size, surface properties and composition. Growth of microorganism and attack of insects on seeds causes detrimental effects on germination due to loss of protein, total oil content, increased fatty acid and carbohydrates. These effects causes biochemical changes in seeds. The knowledge about the use of nanoparticles as antimicrobial agent will help in commercializing nanoparticle as a safer method for improving seed health. (Jo and Kim, 2009)

Treatment with zinc oxide nanoparticles (ZnO) and titanium dioxide (TiO_2) nanoparticles have been reported to have a positive impact on seedling vigour and storability. The most commonly used and widely applied types of nano particles are Zn NPs and they are known to have antibiotic properties. Zinc (Zn) is considered as an essential micronutrient for both animals and plants and this metal is extremely essential for enzyme system of plants as it acts as metal components, cofactors and other regulatory factors of many enzymes (Prasad *et al.* 2012). Zinc is essential for pollen function, chlorophyll production, germination and fertilization. Zinc NPs are toxic to several cell lines hence it is utilized for treating cancer cells and many other bacterial cells. Moreover, Zn NPs are utilized in sensors, solar cells, photocatalytic purposes etc. (Varseem *et al.,* 2010)

TiO_2 NPs are having high stability, photo-catalytic activity and low costs. They are eco-friendly and safe for human. At lower concentrations, TiO_2 NPs promotes early seedling growth and seed germination. Nano-TiO_2 treatments could markedly promote aged seeds vigour and chlorophyll biosynthesis particularly, the ribulose-1,5-bisphosphate carboxylase/ oxygenase (Rubisco) activity and the photosynthesis efficiency (Gao *et al.,* 2006).The reason for the increased germination per cent could be the generation of hydroxide and superoxide anions by nano - TiO_2 that increased intake of oxygen and water needed for quick germination.

In consideration of the above, the present study entitled 'Seed invigoration with nanoparticles for seed yield and quality in chilli (*Capsicum annuum* L.)', was conducted with the following objectives.

i. To standardize the optimum dose of nano ZnO and nano TiO_2 for seed treatment to increase yield in chilli.

ii. To standardize the optimum dose of nano ZnO and nano TiO_2 for seed treatment to improve viability and prolong seed longevity during storage

iii. To compare the efficacy of titanium dioxide and zinc oxide based on their particle size (normal-size and nano-sized powder) on field performances.

Review of Literature

2. REVIEW OF LITERATURE

Seed invigoration is any treatment which is physical, chemical or biological, applied to seed for improving the physiological status of the seed and thereby results in improved seed quality, better storage life and field performance. In any crop, seeds have to be invariably stored. During storage, seeds being hygroscopic in nature, absorbs moisture from the atmosphere and accelerates the ageing process with consequent loss of quality of seeds (Teckrony and Egli, 1997). It is seen that substandard seed quality generally delays seed germination and produces unhealthy seedlings which subsequently impairs the crop yield. Hence, seed invigoration is inevitable for maintaining qualities like germination, viability, vigour and yield potential of the seed. Seed treatments using nanoparticles is one such seed treatment practice which is currently gaining importance.

Nanotechnology is an emerging discipline with novel applications in agriculture. The nanoparticles whose size is 100 nm (or less than 100nm) in one or more dimensions have unique properties and they have the potential to improve plant metabolism. Zinc oxide nanoparticles increases biomass accumulation, maintains membrane integrity and helps in the functioning of several enzymes (Burman. *et al*., 2013). Nano TiO_2 plays a key role in absorption of inorganic nutrients and breakdown of organic substances. It also helps to remove oxygen free radicles and thus increases photosynthetic rate (Khot *et al.,* 2012)

In this context, effect of seed invigoration with nanoparticles has been briefly reviewed in this chapter as,

2.1 Impact of nanoparticle seed treatment on crop growth and yield
2.2 Impact of nanoparticle seed treatment on seed quality
2.3 Seed infection in chilli

2.1 Impact of nanoparticle seed treatment on crop growth and yield

2.1.1 Plant height

Treatment used	**Crop**	**Details of the experiment**	**Reference**
Bulk and nano grade ZnO	Peanut	Seeds of variety K-134 was treated with doses of bulk and normal ZnO. Chelated bulk $ZnSO_4$ was used as a primary source of Zn. The seed treatments were given at the concentrations of 2g / 15 Land 13g /15 L along with NPK (30-40-50). Nano scale treatments showed superior results than bulk treatments. Seeds treated with nano ZnO @ 2g / 15 L recorded taller plants than other treatments (43.80 cm).	Prasad *et al*., 2012
Normal and nano TiO_2	Wheat	Seeds treated with 0.01%, 0.02%, 0.03 % titanium nanoparticle and bulk titanium showed significant differences in plant height.	Jaberzadeh *et al*., 2013
Nano ZnO and TiO_2	Tomato	ZnO @ 250 mg /kg recorded an increase in plant height by 24.5 % while the same dose of TiO_2(250 mg /kg,) did not have any significant effect	Raliya *et al*., 2015
Nano TiO_2	Barley	Application of nano TiO_2 at 500 and 1000 mg kg^{-1} resulted in a gradual increase in plant height with an increase in dosage.	Mattiello and Marchio, 2017

ZnO nanoparticles	Wheat	Wheat seeds of variety Lassani-2008 were treated with different concentrations of ZnO @ 0, 25, 50, 75, and 100 mg L^{-1}. The crop was raised in greenhouse conditions and height of each plant recorded.The plant height increased by 37 per cent when the seeds were treated with ZnO @ 100 mg L^{-1}.	Rizwan *et al*., 2018
ZnO nanoparticles	Red radish	Application of ZnO and FeO at 50 ppm and 60ppm respectively on red radish variety 'Champion' recorded in an increase in plant height.	Mahmoud *et al.,* 2019

2.1.2 Fruits per plant

Treatment used	**Crop**	**Details of the experiment**	**Reference**
Bulk TiO_2	Cowpea	Treatments with TiO_2 at 125 cc/ha (double dose) exhibited more number of pods per plant (33.33) which was followed by single dose of TiO2 at 125 cc/ha (27.33), double dose (25) and single dose of TiO2 at 62 cc/ha (24).	Owalde *et al*., 2008
Nano Zn	Pomegranate	Nanoparticles of Zn at 120mg L^{-1} was found to be efficient for obtaining increased number of fruits per branch	Davarpanah *et al*., 2017
Nano ZnO	Mango	Treatments with Nano zinc @ 0.5 g/l and 1 g/l recorded highest fruit yield and it caused indirect effect on number of fruits per plant.	Zagzog *et al*., 2017

2.1.3 Fruit length

Treatment used	**Crop**	**Details of the experiment**	**Reference**
Bulk TiO_2	Cowpea	Treatments with TiO_2 at 125 cc/ha exhibited the highest pod length of 18.33 cm.	Owalde *et al*., 2008
Nano ZnO	Maize	Seeds treated with nano ZnO @ 400 ppm recorded the highest cob length of 16.40 cm, which was 18% more than control.	Subbaiah, 2014
Nano Zn and TiO_2	Barley	Higher spike length was seen when treated with ZnO nanoparticle and TiO_2 nanoparticle.	Janmohammadi *et al*., 2016
ZnO NP and FeO NP	Carrot	Plant material used for the treatment was Pusa Rudhira. Nanoparticles were applied in different doses (50ppm, 100 ppm and 150 ppm)of ZnO and FeO separately and in combined form. The results revealed that the highest root length of 19.75 cm were observed in treatment with nano ZnO @1000 ppm along with nano FeO @ 50 ppm.	Elizabath *et al*, 2017
Nano TiO_2	Rice	Rice seeds were treated with nano TiO_2 @ 0, 10, 20, 50, 80 and 100 ppm in three replications. An increase in panicle length of 3.2 per cent was observed when treated with nano TiO_2 @ 50 ppm than the control. But at higher (100 ppm) doses the treatments showed negative effects on panicle length.	Debnath *et al.,* 2020

2.1.4 Fruit weight at maturity

Treatment used	**Crop**	**Details of the experiment**	**Reference**
Nano TiO_2	Barley	Nano particles were given at the rate of 0.01 %, 0.02 %, and 0.03 %. The results revealed that weight of spikelets increased when treated with 0.03 % nano TiO_2 (6.7t/ha).	Moaveni *et al*., 2011
Nano and bulk TiO_2	Wheat	Wheat variety 'Pishtaz' were treated with 0.01%, 0.02%, 0.03% bulk and nano grade TiO_2. The highest ear weight was obtained when treated with nano TiO_2 @ 0.02 %and least ear weight when treated with TiO_2 @ 0.01%.	Jabersadeh *et al*., 2013
ZnO NP and FeO NP	Carrot	Plant material used for the treatment was Pusa Rudhira. Nanoparticles were given as foliar spray in different doses of 50ppm, 100 ppm and 150 ppm of ZnO and FeO separately and in combined form. The results revealed that the highest fruit weight of 72.33 kg was observed in treatment with nano ZnO @1000 ppm along with nano FeO @ 50 ppm.	Elizabath *et al*, 2017

2.1.5 Fruit yield

Treatment used	**Crop**	**Details of the experiment**	**Reference**
Bulk TiO_2	Cowpea	Treatments with TiO_2 at 125 cc/ha (double dose) exhibited the highest yield of 948.90 kg.	Owalde *et al*., 2008

Nano TiO_2	Barley	Treatments were given with different doses of nano TiO_2 and bulk TiO_2. The results revealed that treatments at 0.03% recorded the highest yield of 5.7 t/ha.	Moaveni *et al*., 2011
Bulk and normal ZnO	Peanut	Seeds of variety K-134 was treated with doses of bulk and normal ZnO. The seed treatments were effeced at the concentrations of 2g / 15l and 13g /15l along with NPK (30-40-50). Nano scale treatments showed superior results than bulk treatments. Seeds treated with nano ZnO @ 2g / 15 l recorded more yield (3121.54 kg/ ha) than bulk treatments.	Prasad *et al*., 2012
Nano titanium dioxide	Coriander	Coriander plants were treated twice with different doses of nano titanium dioxide at 2, 4 and 6 ppm at 30 DAP and 60 DAP. The treatment exhibited significant increase in growth parameters. The highest yield of 106.5 g was obtained when seeds were treated with nanoTiO_2 at 6 ppm.	Khater, 2015
Nano ZnO	Carrot	Nanoparticles were applied in Pusa Rudhira as foliar spray at different doses of 50ppm, 100 ppm and 150 ppm of ZnO and FeO separately and in combined form. The results revealed that maximum fruit yield was	Elizabath *et al*, 2017

		obtained when treated with nano ZnO @1000 ppm along with nano FeO @ 50 ppm. Along with individual effect, interaction effect was also found to be significant.	
Nano Zn	Mango	Number of fruits per plant was recorded after 48 days of full bloom in mango trees. Treatments with Nano zinc @ 1 g/l showed increased fruit yield by 37.28 per cent than control.	Zagzog *et al*., 2017
Bulk and normal ZnO	Rice	Seeds of variety Tarom Hashemi were treated without Zn, bulk Zn and nano Zn. The yield increased in both bulk and nano Zn seed treatments, but it was not significantly different. Nano Zn recorded the highest grain yield than control by 12.6 per cent and bulk Zn recorded 9.2 per cent.	Kheyri *et al*., 2019

2.1.6 Seeds per fruit

Treatment used	**Crop**	**Details of the experiment**	**Reference**
Bulk TiO_2	Cowpea	Treatments with TiO_2 at 125 cc/ha (double dose) exhibited more number of seeds per pod of 18.67.	Owalde *et al*., 2008
Nano ZnO	Maize	Nano ZnO @ 400 ppm showed more number of grains per row (38.50) which was 36% more than to control treatment.	Subbaiah, 2014
Nano ZnO	Rice	Higher numbers of seeds (294) were recorded in 5 g L^{-1} of nano ZnO treatment when compared to other nanoparticle treatments.	Bala *et al*., 2019

Bulk and nano ZnO	Rice	The results showed that nano Zn recorded more number of filled grains per panicle (175.1), while the control showed the least value (88.3 filled grains).	Kheyri *et al.*, 2019

2.1.7 Seed yield per plant

Treatment used	**Crop**	**Details of the experiment**	**Reference**
Bulk and nano ZnO	Pearl millet	The variety HHB 67 was utilized for the experiment. Normal grade zinc oxide and nano grade zinc oxide was sprayed over germinated seedlings @ 10 mg L^{-1}. Grain yield was found to be increased by 37.7 per cent over control when treated with nanoparticles.	Tarafdar *et al.*, 2014
Nano TiO_2 and Zn	Barley	Seeds were treated with nano iron -chelate, nano zinc and foliar application was given with nano- TiO_2 at 2000 ppm along with control. Nano treatments were applied at the time of initiation of the tillering stage, booting, and milky stage. The results revealed that nano particles showed greater potential in obtaining higher yield. Nano Zn along with nano TiO_2 @ 2000 ppm recorded the highest yield.	Janmohammadi *et al.*, 2016
Nano TiO_2	Rice	Rice seeds were treated with nanoTiO_2 @ 0, 10, 20, 50, 80 and 100 ppm. The treated seeds exhibited higher values for several growth parameters.	Debnath *et al.*, 2020

		The results revealed that seed treatment with 20 ppm of nano TiO2 recorded increased seed yield by 19.22 per cent than control.	
Nano ZnO	Soybean	Nano ZnO @ 160 mg/kg showed the highest seed yield when compared to all other treatments.	Yusefi -Tanha *et al.*,2020

2.1.8 100 seed weight

Treatment used	**Crop**	**Details of the experiment**	**Reference**
Bulk TiO_2	Cowpea	The treatments irrespective of number of doses, and concentration showed significant differences. Treatments with TiO_2 at 125 cc/ha exhibited the highest 100 seed weight (18.47 g)	Owalde *et al.*, 2008
Bulk and nano ZnO	Peanut	Seeds of variety K-134 was treated with various doses of bulk and nano ZnO. The seed treatments were given at the concentrations of 2g / 15 L and 13g /15 L along with NPK (30-40-50). Nano scale treatments showed superior results than bulk treatments. Seeds treated with nano ZnO @ 2g / 15 L recorded highest 100 kernal weight of 36.25g.	Prasad *et al.*,2012

2.2 Impact of nanoparticle seed treatment on seed quality

2.2.1 Germination

Treatment used	Crop	Details of the experiment	Reference
Nano ZnO	Soybean	Seeds treated with nano ZnO at 1000 mg L^{-1} increased seed germination.	Lopez-Moreno *et al.*, 2010

Nano ZnO	Rice	Irrespective of dose of treatments, application of nano ZnO (10-1000 mgL^{-1}) recorded 100 per cent germination. The results revealed that seed treatments did not adversely affect seedling growth.	Boonyanitipong *et al.* 2011
Nano and bulk titanium dioxide	Wheat	Seed were treated with both nano and bulk TiO_2 at 0, 5, 20, 40, 60 and 80 mgL^{-1}. It was observed that treatment with nano TiO_2 @ 60ppm (76 per cent) showed greater potential in improving germination and least values (41%) were recorded in bulk TiO_2 @ 60 ppm. It was seen that, mean germination time was improved by 31.8 per cent when treated with nano TiO_2 at 40 ppm when compared to control.	Feizi *et al*., 2013
Nano TiO_2	Tomato Onion	Seeds were incorporated with nano TiO_2 at 0, 100, 200, and 400 mg L^{-1}. Treatments with 100 and 200 mg L^{-1} showed superior effects on germination. Nano TiO_2 @ 100 mg L^{-1} recorded the highest values in tomato (100%) and onion (30%).	Haghighi *et al.,* 2014
Nano TiO_2	Radish	Seeds were treated with different doses of nano TiO_2 (0, 100, 200, and 400 mg L^{-1}). At 400 mg L^{-1}, the highest germination (100%) was observed.	Haghighi *et al.,* 2014
Nano ZnO	Mungbean	ZnO nanoparticles for seed treatment was prepared by dissolving nanoparticles in distilled water by placing on sonicator for 15 minutes. Seeds were treated with 20, 40, 60 and 100mg nano ZnO and reported an increase in germination. Treated seeds showed the highest germination at	Jayarambabu *et al*., 2014

		20 mg (100%) followed by 40mg (95 %)	
Nano ZnO	Maize, soyabean, pigeon pea, okra	Seeds were treated with nano-scale ZnO at 25 mg and 50 mg. The results of germination test revealed that treated seeds showed better germination of 98 – 100 per cent compared to control.	Adhikari *et al.*,2016
Nano ZnO and TiO2	Cowpea	Nano ZnO (0, 2, 4, 8, 10, 15 ppm) and nano TiO2 (0, 10, 20, 30, 40, 50 ppm) was incorporated into cowpea seeds. Highest germination per cent was recorded when treated with 20 ppm nano TiO_2 (98) and 2 ppm nano ZnO (98). An increase of germination by 22.91 per cent was observed when treated with both the nanoparticles.	Priya, 2016
Nano ZnO	Chilly	Seeds were treated with nano ZnO at 0.0, 0.25, 0.50 and 0.75g. Highest seed germination (65.7 %) was observed when treated with 0.75 g nano particle.	Afrayeem and Chaurasia, 2017
Nano TiO_2	Chickpea	Seeds of variety PBG-7 was wet dressed with nano ZnO and TiO_2 at 100, 500 and 1000 ppm. Nano TiO_2 at 500 ppm showed highest germination.	Hajra and Mondal, 2017
Nano ZnO	Maize	Nano zinc oxide treatments (800 ppm, 1000 ppm, 1200 ppm 1400 ppm and 1600 ppm) showed superior performances. Treatments with 1000 and 1200 ppm recorded 100 per cent germination.	Meena *et al.*, 2017
Bulk and nano ZnO and TiO_2	Chilly	Seeds were dry dressesd with nano and bulk ZnO and TiO_2 each at 750, 1000 and 1250 mg kg^{-1}. Nano ZnO at 1000 mg kg^{-1} showed highest	Kumar, 2019

		germination of 75 per cent and least values (66%) was observed in TiO_2 at 750 mg kg^{-1}.	
Bulk and nano ZnO	Chilly	Seeds treated with nanoparticles of ZnO at 500 mg/kg and bulk ZnO at 1300 mg/kg showed superior performances in maintaining germination above 60 per cent till the end of ten months of storage.	Gayathri, 2019
Bulk and nano TiO_2	Chilly	Seed treatments with nano-TiO2 @ 500 mg/kg, 900 mg/kg, 1300 mg/kg and 500 mg/kg retained 60 per cent germination at the end of ten months of storage.	Gayathri, 2019
Nano ZnO	Wheat	Wheat variety Lok-1 was treated with various doses of nano ZnO (10000, 8000, 6000, 4000 and 2000 ppm). Seeds treated with nano ZnO @ 2000 ppm exhibited 100 per cent germination. A gradual reduction in germination was observed at higher concentrations. The shoot length and root lengths are decreased with increase in concentrations of nanoparticles	Bagawade and Jagtap, 2020

Nano TiO_2	Rice	Rice variety CN-1794-2 were treated with six different doses of nano TiO_2 at 0, 10, 20, 50, 80 and 100 ppm for 48 hours. Significantly higher differences were found in all the treatments irrespective of concentration of nanoparticles. Seed germination was improved by 85 per cent when treated with 20ppm nanoparticle solution when compared to control. Higher germination of 98 per cent and 98 per cent was recorded in treatments with 20 ppm and 50 ppm.	Debnath *et al.*, 2020

2.2.2 Seedling length

Treatment used	**Crop**	**Details of the experiment**	**Reference**
Nano ZnO	Rice	At low concentration of 10 mg L^{-1}, nano ZnO showed positive effect on root length.	Boonyanitipong *et al.*, 2011
Nano TiO_2	Tomato	Seeds treated with nano TiO_2 (100, 200 and 400 mg L^{-1}) were grown under greenhouse conditions. At 400 mg L^{-1} seedling recorded the highest root length of 4.5 cm.	Haghighi *et al.,* 2014
Nano TiO_2	Raddish	Seeds treated with nano TiO_2 @ 100 mg L^{-1} recorded the highest shoot length of 2.21 cm.	Haghighi *et al.,* 2014

Nano ZnO and nano TiO2	Cowpea	ZnO and TiO_2 nanoparticles at various doses showed significant effects on seedling length. Nano ZnO at 2 ppm and nano TiO_2 at 20 ppm showed superior results. At low concentration, ZnO and TiO2 nanoparticles showed good effect on root and shoot length. An increase of root length by 65.96% and shoot length by 61.82% was observed when treated nano TiO_2.	Priya, 2016
Nano ZnO	Chilly	Seeds were treated with nano ZnO at 0.0, 0.25, 0.50 and 0.75g. The results showed that at lower concentrations (0.25 and 0.50g) a decrease in shoot and root lengths was seen and at 0.75 g an increase in length was observed.	Afrayeem and Chaurasia, 2017
Nano ZnO and TiO_2	Chickpea	Longer roots of 12.36 cm was produced when treated with nano TiO_2 at 1000 ppm when compared to nano ZnO (0.93 cm) treatments. In case of shoot length, nano ZnO @ 500 ppm produced shoots of 7.6 cm and 9.2 cm by nano TiO_2 at 1000ppm.	Hajra and Mandal, 2017
Nano ZnO	Maize	Nano ZnO treatments were given at the rate of 800, 1000, 1200, 1400, 1600 ppm. Seed treatments at 1200 ppm of nano ZnO exhibited shoot length of 3.9 cm and root length of 6.5 cm.	Meena *et al*., 2017

Normal and nano ZnO	Maize	Seeds were wet dressed with normal and nano ZnO at 500 ppm, 1000 ppm and 2000 ppm for 2 and 4 hours each. Seed treatment with nano grades of ZnO @ 1000 ppm recorded higher root length of 6.82 cm and shoot length of 1.94 cm. It was observed that at higher doses of treatment like 2000 ppm root length was reduced.	Tiwari, 2017
Bulk and nano ZnO and TiO_2	Chilly	Seed treatment with nano ZnO at 1000 mg kg^{-1} recorded maximum shoot length of 4.3 cm and root length of 11.90 cm.	Kumar *et al.,* 2019
Nano ZnO	Wheat	Wheat variety Lok-1 treated with nano ZnO @ 2000 ppm exhibited the highest shoot and root length.	Bagawade and Jagtap, 2020
Nano TiO_2	Rice	Significant differences in seedling length was observed when treated with nano TiO_2 at 0, 10, 20, 50, 80 and 100 ppm. Nano seed treatment @ 50ppm and 80 ppm recorded the highest root length and shoot respectively when compared to other treatments.	Debnath *et al*., 2020

2.2.3 Dry weight

Treatment used	**Crop**	**Details of the experiment**	**Reference**
Nano ZnO	Chickpea	Nano ZnO treated seeds at lower doses (1.5 ppm) improved dry matter accumulation in seedlings	Burman *et al*., 2013
Nano and bulk titanium dioxide	Fennel	At 5 ppm and 80 ppm highest shoot biomass was recorded in bulk TiO_2 (1.18 mg) and nano TiO_2 (1.16 mg) respectively. Bulk TiO_2 at 20 ppm and	Feizi *et al.,* 2013

		nano TiO_2 at 5ppm and 20 ppm showed the highest root biomass.	
Nano TiO_2	Tomato	Seeds were treated with various doses of nano TiO_2 (100, 200 and 400 mg L^{-1}). Highest seedling dry weight of 0.74 g was observed in seeds treated with nano titanium dioxide at 400 mg L^{-1}.	Haghighi *et al.*, 2014
Nano TiO_2	Raddish	Treatments using nano TiO_2 at 100 mg L^{-1} recorded the highest seedling dry weight of 0.21g.	Haghighi *et al.*, 2014
Nano ZnO and nano TiO2	Cowpea	Seedling dry weight showed significant differences when treated with various doses of nano ZnO (0, 2, 4, 8, 10, 15 ppm) and nano TiO2 (0, 10, 20, 30, 40, 50 ppm). Nano ZnO at 8 ppm and nano TiO_2 at 20 ppm showed superior results. An increase of dry weight by 118.6% in nano ZnO and 148.8 per cent in nano TiO_2 was observed.	Priya, 2016
Nano ZnO	Chickpea	Nano ZnO treatments at 100, 500 and 1000 ppm was incorporated into chickpea seeds. Among the treated seeds, treatments at 1000 ppm recorded higher seedling dry weight.	Hajra and Mondal, 2017
Zinc oxide NPs	Chilli	Seeds were wet dressed with doses of nano ZnO at 0, 100, 200 and 500 ppm. Lower doses recorded higher seedling dry weight.	García-López *et al.*, 2018

2.2.4 Vigour index

Treatment used	**Crop**	**Details of the experiment**	**Reference**
Nano and bulk titanium dioxide	Fennel	Application of nano TiO_2 exhibited significant improvement in vigour index while bulk TiO_2 reduced seedling vigour index II. Nano TiO_2 @ 5 ppm recorded the higher values for vigour index II compared to other treatments.	Feizi *et al*., 2013
Nano ZnO and nano TiO2	Cowpea	Seed treatment with nano ZnO at 2 ppm and nano TiO_2 at 20 ppm showed superior results than control. An observed increase of seed vigour by 100 per cent and 116.45 per cent respectively was seen in nano ZnO and nano TiO_2.	Priya, 2016
Nano ZnO and TiO_2	Onion	Treatments at 750, 1000, 1250 and 1500 mg kg^{-1} was given using nano grades of ZnO and TiO_2. Seeds treated with nano grades of ZnO and TiO_2 at 1000 mg kg^{-1} recorded the highest seedling vigour of 998 and 795 respectively.	Anandaraj and Nataraja, 2017
Nano ZnO	Maize	Nano ZnO treatments were given at the rate of 800, 1000, 1200, 1400, 1600 ppm. Seed treatments at 1200 ppm of nano ZnO recorded the highest vigour index I of 1040.	Meena *et al*., 2017

Normal and nano ZnO	Maize	Seeds were wet dressed with normal and nano ZnO at 500 ppm, 1000 ppm and 2000 ppm for two and four hours each. Seed treatment with nano grades of ZnO @ 1000 ppm recorded the high vigour index I of 861.4 and 845.4 when soaked at two and four hours respectively.	Tiwari, 2017
Nano ZnO	Peanut	Nano scale ZnO of 25 mm particle size at a concentration of 1000 ppm exhibited the higher seed vigour index I of 1701.	Prasad *et al.*, 2012
Bulk and nano ZnO and TiO_2	Chilly	Vigorous seedlings were observed in treatments with nano ZnO at 1000 mg kg^{-1} (1285) and least values in control (861).	Kumar *et al.*, 2019
Bulk and nano ZnO	Chilly	Seeds treated with nanoparticles of ZnO at 500 mg/kg and bulk ZnO at 1300 mg/kg showed superior performances in maintaining seed vigour till the end of ten months of storage.	Gayathri, 2019
Bulk and nano TiO_2	Chilly	Seed treatments with nano-TiO_2 @ 500 mg/kg and 1300 mg/kg recorded high seed vigour.	Gayathri, 2019
Nano TiO_2	Rice	Rice seeds were treated with nano TiO_2 at 0, 10, 20, 50, 80 and 100 ppm showed significant differences in seed vigour over control. Seeds treated with nano TiO_2 @ 20ppm recorded the highest vigour.	Debnath *et al.*, 2020

2.2.5 Electrical conductivity of seed leachate

Treatment used	**Crop**	**Details of the experiment**	**Reference**
Nano zinc oxide	Groundnut	Seeds of variety VRI-2 was treated with 750, 1000, 1250 mg/kg of seeds of nano ZnO. Seed treatments @ 1000 mg kg^{-1} recorded lower electrical conductivity of 0.347dSm^{-1} at the end of 12 months of period of storage.	Shyla and Natarajan, 2014
Nano titanium dioxide	Maize	Nano TiO_2 @ 200 mg kg $^{-1}$ exibited lower EC (0.278 dSm^{-1}) compared to control when treated with various doses.	Vijayalakshmi *et al*., 2018
Bulk and nano grade ZnO	Chilly	The nanoparticle seed treatments significantly influenced electrical conductivity. Nano ZnO at 1000 mg kg^{-1} recorded the lowest EC (0.118 dSm^{-1}) compared to control (0.149 dSm^{-1}).	Kumar *et al*., 2019
Nano and bulk Zno	Chilly	Nano ZnO at 500 mg/kg and 250 mg/kg reduced leakage of electrolyte from seeds during the period of storage	Gayathri, 2019

2.3 Seed infection in chilli

Seed infections are caused by fungi, bacteria and viruses which results in inferior seed quality and reduction in yield. Loss of seeds at the time of storage is the greatest challenge in quality seed production. Fungus causes 70% of diseases in several crops. They invade host plants and establish infection by colonizing from inside.

Temperature, moisture, aeration etc. are the factors contributing growth of microbes during storage. Germinability and vigour of seeds get reduced as pathogens are present with the advancement of storage. The seeds may show discolouration and produce abnormal odours. Several toxins produced by fungus causes negative impact on growth of seedling. Thus, biochemical changes occurs inside the seed due to which nutrients are lost (Aher, 2013). The pathogens causing diseases in chilly are enlisted below.

Pathogen observed	**Reference**
Alternaria alternata, , A. niger, A. flavus, Cephalosporium sp., Bipolaris australiensis, B. spicifera, Cladosporium spp., Drechslera spp., Colletotrichum capsici, Rhizoctonia solani, Curvularia lunata, Macrophomina phaseolina, Fusarium moniliforme, F. solani, Penicillium spp., Verticillium albo-atrum and Trichoderma harzianum,.	Sharfun-Nahar *et al.*, 2004
Penicillium digitatum, Aspergillus flavus and Aspergillus niger	Balogun *et al.*, 2005
Aspergillus niger, Rhizopus nigricans, Alternaria alternate, Colletotrichum capsica, Macrophomina phaseolina, Penicillium citrinum, Fusarium oxysporum and Curvularia lunata,	Jogi *et al.*, 2010
Agar plate method:, *Aspergillus niger and A. flavus, Colletothrichum capsici, Bispora betulina, Humicola*	Chigoziri and Ekefan, 2013

fuscoatra, Phoma spp, Humicola dimorphospora, , Periconia byssoides, Botryotrichum piluferum, Phomopsis spp,	
Colletotrichum capsici, Rhizopus stolonifer, Aspergillus flavus. Fusarium moniliforme and Curvularia lunata,	Alam *et al.*, 2014
Colletotrichum capsici, Alternaria alternate, C. gloeosporioides, Fusarium sporotrichioides, C. acutatum and F. oxysporum.	Machenahalli *et al*., 2014
Aspergillus niger, Pencillium spp., Aspergillus flavus, and Alternaria spp.	Navya, 2016
Aspergillus spp. Alternariaspp, and Pencilliumspp	Sandhya 2016
Aspergillus flavus, Aspergillus niger, Rhizopus spp., Penicillium spp., Colletotrichum capsici, Fusarium solani	Chauhan *et a*l., 2018
Aspergillus flavus, Alternaria spp Aspergillus niger, and Mucor spp.	Gayathri, 2019
Curvularia pallescens, Myrothecium sp., Fusarium verticillioides, Cladosporium sphaerospermum, ,Fusarium solani, Aspergillus flavus, Aspergillus niger, Penicillium sp., Colletotrichum gloesporioides, Colletotrichum capsici, Rhizopus sp. And Macrophomina phaseolina	Ghyasi *et al.*, 2020

Materials and Methods

3. Materials and methods

The 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 May 2019 to March 2020.

3.1 Experimental site

The field and laboratory experiments were conducted in the Department of Seed Science and Technology, College of Horticulture, Vellanikkara, Thrissur.

3.2 Climatic conditions

Vellanikkara, of Thrissur district, is located 22.25 m above mean sea level and its co-ordinates lies between 10.5452 °N and 76.2740 °E. A hot and humid climate prevails in this region.

3.3 Experimental material

Chilli seeds of variety Anugraha, harvested in May 2019 were procured from Vegetable And Fruit Promotion Council Keralam at Alathur, Palakkad.

3.4 Experimental details

The study was divided into two experiments as listed below:

Experiment 1: Field performance of dry dressed inorganic nanoparticles on seed yield in chilli

Experiment 2 - Seed storage studies with wet dressed inorganic nanoparticles

3.4.1 Experiment 1: Field performance of dry dressed inorganic nano particles on seed yield in Chilli

3.4.1.1 Layout of the experimental field

Design: Randomized Block Design (RBD)

Variety: Anugraha

Treatments: 13

Replication: 3

Total number of plots: 39

Plot size: 5 m X 1.5 m

Spacing: 45 cm × 45 cm

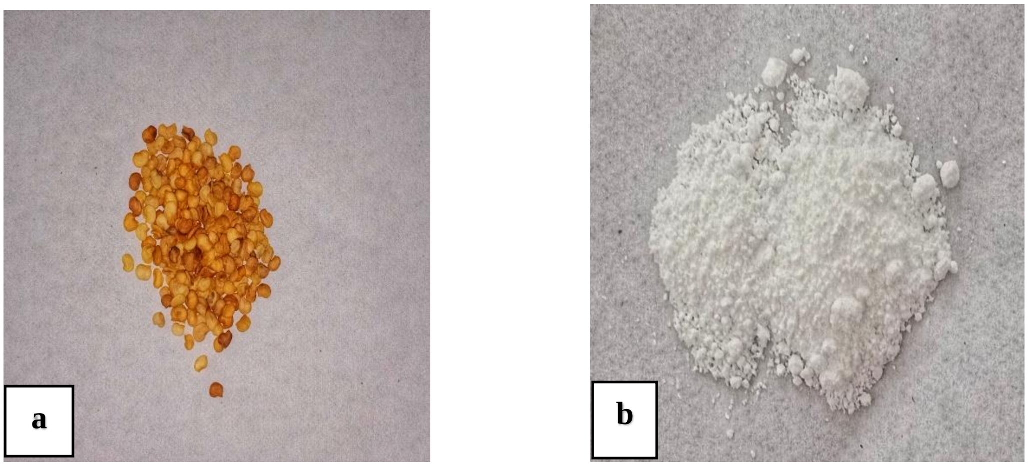
Number of plants per plot: 20

3.4.1.2 Treatment details

Seeds of chilli variety Anugraha were dry dressed with bulk and nano sized zinc oxide (ZnO) and titanium dioxide (TiO_2) particles in a Randomized block Design with thirteen treatments in 3 replications.

3.4.1.3 Dry seed treatment

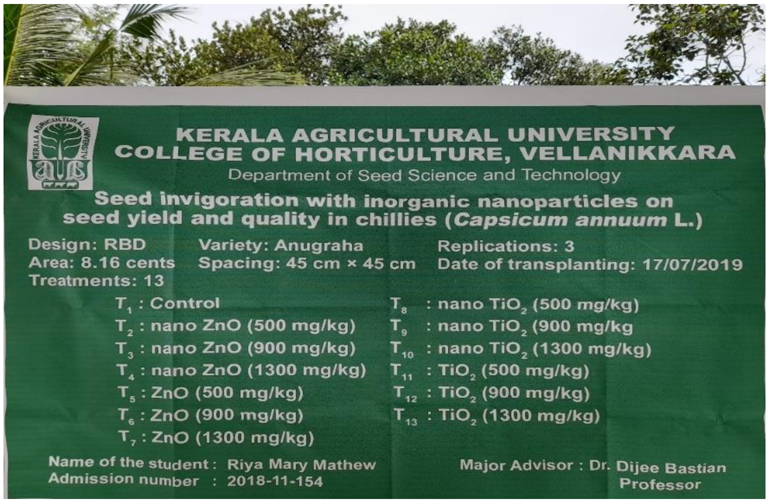
1g seeds of chilli were dry dressed with normal grade ZnO, nano grade ZnO , normal grade TiO_2 and nano grade TiO_2 in screw capped glass bottles at room temperature using the specified quantity of chemicals detailed in Table 1. The glass bottles along with seed and nanoparticles was shaken gently 5 times for 3 min, at an interval of 3hrs. Untreated seeds served as control.



Untreated seeds **Chemical for seed treatment**

Seeds treated with nanoparticle

PLATE 1: Dry seed treatments in chilli



KERALA AGRICULTURAL UNIVERSITY
COLLEGE OF HORTICULTURE, VELLANIKKARA
Department of Seed Science and Technology

Seed invigoration with inorganic nanoparticles on seed yield and quality in chillies (*Capsicum annuum* L.)

Design: RBD
Variety: Anugraha
Replications: 3
Area: 8.16 cents
Spacing: 45 cm × 45 cm
Date of transplanting: 17/07/2019
Treatments: 13

T_1 : Control
T_2 : nano ZnO (500 mg/kg)
T_3 : nano ZnO (900 mg/kg)
T_4 : nano ZnO (1300 mg/kg)
T_5 : ZnO (500 mg/kg)
T_6 : ZnO (900 mg/kg)
T_7 : ZnO (1300 mg/kg)
T_8 : nano TiO_2 (500 mg/kg)
T_9 : nano TiO_2 (900 mg/kg
T_{10} : nano TiO_2 (1300 mg/kg)
T_{11} : TiO_2 (500 mg/kg)
T_{12} : TiO_2 (900 mg/kg)
T_{13} : TiO_2 (1300 mg/kg)

Name of the student : Riya Mary Mathew
Admission number : 2018-11-154

Major Advisor : Dr. Dijee Bastian
Professor

PLATE 2: View of experimental plot

Table 1: Details of seed treatment used for dry-dressing of chilli seeds

Treatment	**Details**
T_1	Control (Untreated seeds)
T_2	500 mg nano ZnO/ kg of seed
T_3	900 mg nano ZnO/ kg of seed
T_4	1300 mg nano ZnO/ kg of seed
T_5	500 mg ZnO/ kg of seed
T_6	900 mg ZnO/kg of seed
T_7	1300 mg ZnO/kg of seed
T_8	500 mg nano TiO_2/kg of seed
T_9	900 mg nano TiO_2/kg of seed
T_{10}	1300 mg nano TiO_2/kg of seed
T_{11}	500 mg TiO_2/kg of seed
T_{12}	900 mg TiO_2/kg of seed
T_{13}	1300 mg TiO_2/kg of seed

The treated seeds were raised in nursery and transplanted to main field after four weeks. The experimental crop was raised as per Package of practices recommendations of Kerala Agricultural University, to study the field performance of dry dressed inorganic nanoparticles on seed yield.

3.4.2. Experiment 2 - Seed storage studies with wet dressed inorganic nanoparticles

3.4.2.1 Treatment details

Chilli seeds were wet dressed with different doses of nano sized zinc oxide (ZnO) and titanium dioxide (TiO_2) particles.

Design: Completely Randomized Design (CRD)

Variety: Anugraha

Treatments: 13

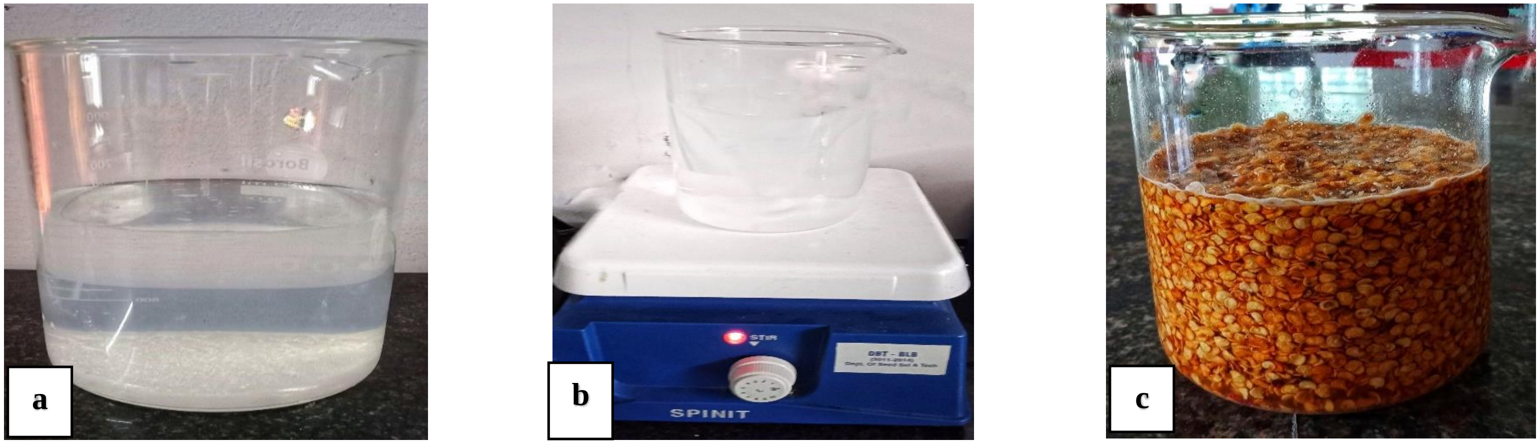
Replication: 3

3.4.2.2 Wet seed treatment

The nanoparticles were dispersed in distilled water by sonicating for 15 min (Kumar, 2019). The chilli seeds were soaked in different solutions as mentioned in Table 2, for three hours. Seeds soaked in water served as control. Soaked seeds were then removed and dried back to a moisture content of less than 8 per cent.

Table 2: Details of seed treatment used for wet-dressing of chilli seeds

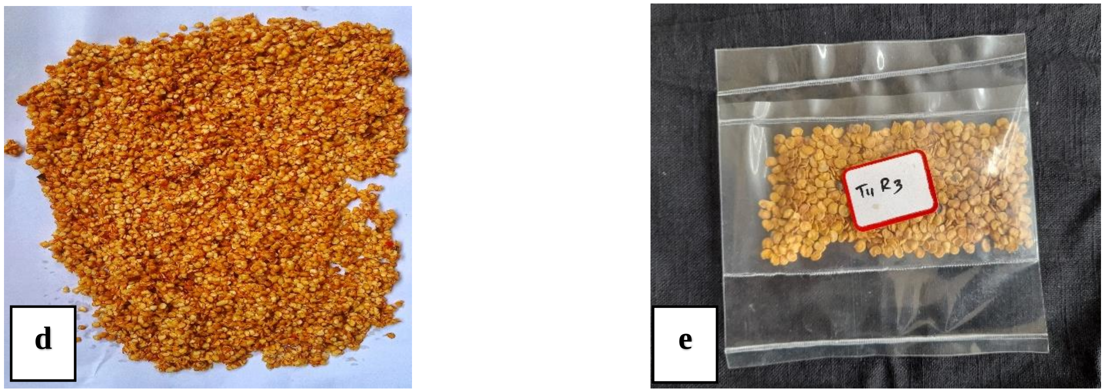
Treatment	Details
T_1	Control (Untreated seeds)
T_2	100 mg nano ZnO/ kg of seed
T_3	250 mg nano ZnO/ kg of seed
T_4	500 mg nano ZnO/ kg of seed



Solution containing nanoparticles

Solution kept for sonicating for dispersing nanoparticles

Seeds soaked in treatment solution



Drying of seeds after wet dressing

Seeds packed after wet dressing for storage

PLATE 3: Wet seed treatment using nanoparticles

T_5	750 mg nano ZnO/ kg of seed
T_6	1000 mg nano ZnO/ kg of seed
T_7	1250 mg nano ZnO/ kg of seed
T_8	100 mg nano TiO_2/ kg of seed
T_9	250 mg nano TiO_2/ kg of seed
T_{10}	500 mg nano TiO_2/ kg of seed
T_{11}	750 mg nano TiO_2/ kg of seed
T_{12}	1000 mg nano TiO_2/ kg of seed
T_{13}	1250 mg nano TiO_2/ kg of seed

3.4.2.3 Method of seed packing and storage

The treated and untreated seeds in each treatment were packed separately in 700 gauge polythene bags and stored under ambient conditions for a period of seven months. Separate seed lots were maintained in each replication of a treatment for ease of drawing seed samples to record seed quality parameters at monthly intervals as well as reduce the inadvertent imbibition of moisture by the stored seeds during seed sampling.

The packed seeds were stored at ambient conditions and observations like seed germination (%), shoot length (cm), root length (cm), dry weight of seedling (g) and electrical conductivity (EC) of seed leachate were recorded at monthly intervals and seed micro flora (%) and seed moisture (%) were recorded during the start and end of the storage.

3.5 Observations

3.5.1 Experiment 1: Field performance of dry dressed inorganic nano particles on seed yield in Chilli

Five plants in each replication of each treatment were randomly selected. All biometric observations *viz.,* plant spread (cm), plant height (cm), days to harvest, fruits per plant, fruit length (cm), fruit weight (g) at maturity, fruit yield per plant (g), seeds per fruit, seed yield per plant (g) and 100 seed weight (g) were recorded at appropriate growth stages in the tagged plants and averaged to compute the data pertaining to a given replication of a treatment.

3.5.1.1 Plant height (cm)

On the 120th day after transplanting, plant height was measured from ground level to the tip of the main stem and the average was expressed in centimetre.

3.5.1.1 Plant spread (cm)

Plant spread is the maximum width of the plant as measured at its widest part from leaf tip to leaf tip at maturity. The plant spread was measured using a metre scale and the average expressed in centimeters.

3.5.1.2 Days to harvest

The number of days taken from transplanting to first harvest was counted and the average expressed as whole numbers.

3.5.1.3 Fruits per plant

Total number of fruits harvested per plant was counted and the average expressed in numbers.

3.5.1.4 Fruit length (cm)

Ten fruits from each plant were measured from distal end to proximal length using meter scale and the average length was expressed in centimetres.

3.5.1.5 Fruit weight at maturity (g)

Fruits collected from each plant were weighed using weighing balance and average was expressed in grams.

3.5.1.6 Fruit yield per plant (g)

The fruits collected from each plant were weighed, and the yield of fruits per plant was computed and expressed in grams.

3.5.1.7 Seeds per fruit

Seeds from the fruits of the tagged plants were extracted carefully in each replication. The number of seeds per fruit was counted and average number of fruits were worked out.

3.5.1.8 Seed yield per plant (g)

The seeds extracted from the fruits of the tagged plants were weighed using weighing balance and expressed in grams

3.5.1.10 100 seed weight

Three samples of 100 well filled seeds drawn at random from the tagged of a replication were weighed and the average expressd in gram.

3.5.2 Experiment 2: Seed storage studies with wet dressed inorganic nanoparticles

Observations on germination per cent, shoot and root length of the seedling (cm), seedling dry weight, seed moisture content (%), electrical conductivity (E.C.) and Seed microflora infections were assessed as detailed below.

3.5.2.1 Germination (%)

Germination test was conducted using roll paper towel as the substratum following the standard procedure advocated by ISTA (1995). Four replications of hundred seeds each were drawn from each replication of a treatment, for conducting germination test. The seeds were placed between two layers of germination paper and rolled. The rolled towels were placed in the buckets containing water in a slanting position inside a seed germinator maintained at 25 ± 2 °C temperature and 95 ± 2 per cent relative humidity. The germination per cent was worked out by counting the number of normal seedlings on the 14th day and the average expressed in per cent.

3.5.2.2 Seedling shoot length (cm)

On the 14th day of germination test, ten normal seedlings from each replication of the treatment were selected randomly and the shoot length was measured from the base to the collar region of the seedling and the average was expressed in centimeters.

3.5.2.3 Seedling root length (cm)

On the 14th day, the root length of the seedlings selected for measurement of the shoot length, was measured from the collar region to the tip of the root of the seedling and the mean root length was expressed in centimeters.

3.5.2.4 Seedling dry weight (mg)

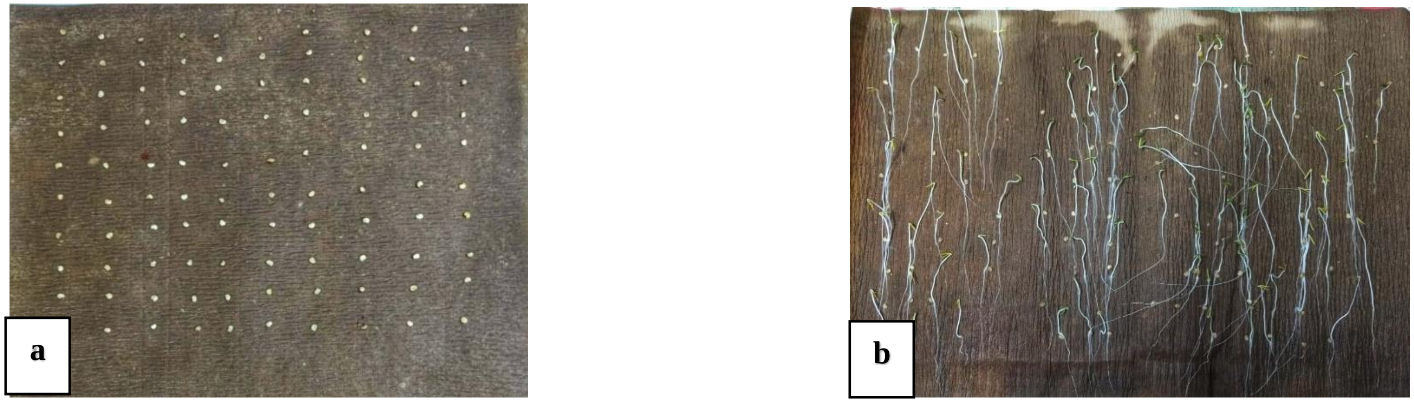
Seedling dry weight estimation was done using the same ten seedlings used for measurement of seedling shoot and root length. The seedlings were placed in butter paper covers and transferred to an oven and dried at 80^0 C temperature for 24 h. These were then weighed using an automatic digital balance and the average seedling dry weight computed and expressed in milligrams (mg).

3.5.2.5 Seed Vigour Indices

Seed vigour indices was calculated using the germination percentage obtained in the germination test. The vigor index was calculated adopting the method of Abdul Baki and Anderson (1973).

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

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



100 seeds placed on a moistened roll towel paper

Seeds germinated on roll towel at 14th day

10 seedlings selected for measuring shoot and root length

PLATE 4: Germination test using between paper method

3.5.2.6 Electrical conductivity of seed leachate (µS cm-1)

Three replicates of five gram of seeds were drawn from each treatment for estimating electrical conductivity of seed leachate. For surface sterilization, randomly selected seeds were treated in 0.1% mercuric chloride ($HgCl2$) for one minute. These seeds were thoroughly washed with distilled water several times for removing the residues of treated chemicals and was soaked in 50 ml of distilled water for 24 hours. Electrical conductivity (EC) of the seed leachate collected in a beaker after decanting was measured using digital conductivity meter (EUTECH CON-510) and the mean value was recorded as micro Siemens per centimetre (µS cm^{-1}).

3.5.2.7 Seed moisture content (%)

Seed moisture content was measured using the low constant temperature procedure advocated by ISTA (1985). Seed samples (5 g each) were drawn from each replication and were evenly placed in a container made up of glass. Weight of the container along with lid before and after filling with the seeds was measured. The samples in the container were placed in hot air oven maintained at 103±2 °C and dried for 17±1 h. After drying, the containers was placed in a dessicator for cooling for 30-45 minutes. The weight of the container along with its lid was taken again after cooling and the seed moisture content in per cent was estimated using the formulae.

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

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

3.5.2.8 Seed infection (%)

Detection of seed infection was conducted using blotter paper method and agar plate method as recommended by ISTA (1999).

3.5.2.8.1 Blotter paper method

The procedure described by Neergard (1979) was followed for detecting seed infection using blotter paper method. Sterilized petriplates with three layers of blotter papers were used in this method and sterilized water was used to soak the blotter papers. Twenty five seeds were placed equidistantly in such a way that, the outer most layer consisted of 16 seeds, eight in the middle and one seed at the centre under aseptic conditions of laminar air flow. Incubation of the petriplates was done under alternate cycle of 12 h darkness and 12 h light at 20±2 ^{0}C, for seven days. The plates were observed for the presence of seed microflora on the eighth day under stereo binocular microscope and the number of infected seeds were counted and recorded in per cent.

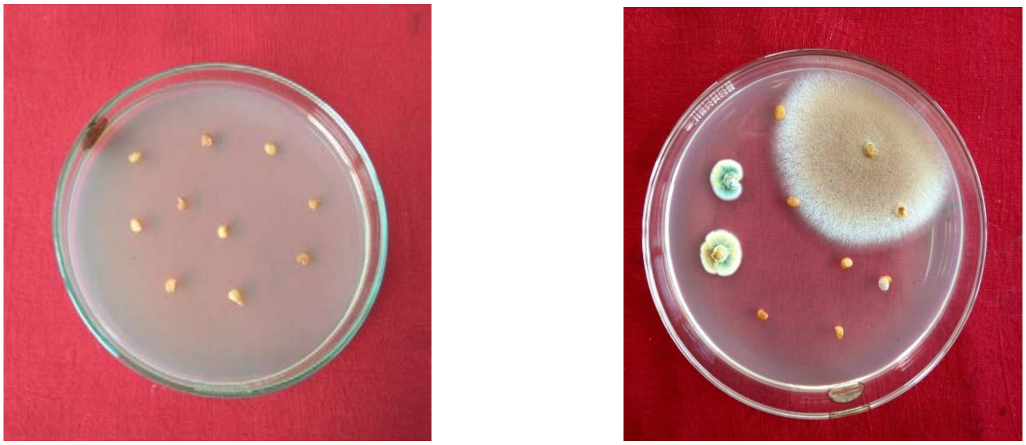
3.5.2.8.2 Agar plate method

In agar plate method, seeds were surface sterilized using 0.1 per cent mercuric chloride and then washed thoroughly three times in sterile water to remove residues of mercuric chloride. The seeds were kept in sterile filter paper to remove excessive water and were placed on petriplates having potato dextrose agar media, under aseptic conditions of laminar air flow. After plating, they were incubated for six days after packing in polyethylene cover, under bell jar. The plates were observed under stereo-binocular microscope and the number of infected seeds were counted and recorded in per cent.

3.6 Statistical analysis

The statistical analysis of the data was performed using Web Agri Stat Package (WASP) developed by Indian Council of Agricultural Research and ranking of significant treatments was done using Duncan's Multiple Range Test (DMRT). For all F- test critical difference was calculated at 5 per cent probability. The zero values present in the data were converted to 1 n value, where 'n' is the number of observations.

Agar plate method



Initial Final

Blotter paper method

Initial Final

PLATE 5: Testing seeds for identification of seed microflora

The recorded data in each observation were analyzed using ANOVA table for completely randomized design to test the differences 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	$\text{SST}_{\circ}$		

Where,

t: Treatments

n: Number of observations

MST: Treatment mean sum of squares

MSE: Error mean sum of squares

3.6.1 DMRT test for ranking

For the evaluation and ranking of all possible Pairs of treatment means, Duncan's Multiple Range Test (DMRT) is applied, particularly for experiments having big numbers of treatments. To classify the difference between any two treatments as significant or non-significant, numerical boundaries are created by DMRT and its value calculation depends primarily on the specific standard error (SEm) of the pair of treatments being compared. The procedure for ranking of data suggested by Gomez and Gomez (1976) is given below:

i. The treatment means are ranked in the increasing or decreasing order according to the order of preference

ii. Calculate standard error of error mean sum of squares (SEm) by using the formula

$$\text{SEm} = \frac{\sqrt{2MSE}}{r}$$

Where,

MSE: error mean sum of squares

R : number of replications.

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

$$R_p = \frac{(r_p)\,(SE_m)}{\sqrt{2}}$$

Where,

t : number of treatments

SEm: standard error obtained in step ii

rp: tabulated value of ranges which are significant

p: difference in rank between the pairs of treatment means to be compared (p = t for the highest and lowest means).

iv. After identifying all the treatment means which do not differ significantly

from each other was grouped.

v. The obtained results were presented using alphabetical notation.

Results

4. RESULTS

The results obtained from field and storage studies on seed invigoration with nanoparticle on seed yield and quality in chilli conducted in the Department of Seed Science and Technology, College of Horticulture, Vellanikkara are presented in this chapter. The data obtained with respect to seed quality, plant growth and yield parameters were analysed statistically and presented below.

4.1: Field performance of dry dressed inorganic nanoparticles on seed yield in chilli

4.1.1 Analysis of variance

The analysis of variance of the observations recorded is presented in Table 3. The treatments were highly significant for yield attributes such as plant spread (cm), plant height (cm),days to harvest (days), number of fruits per plant, fruit length (cm), fruit weight (g) at maturity, fruit yield, number of seeds per fruit, seed yield per plant (g) and 100 seed weight (g).

4.1.2 Plant spread (cm)

The treatments with normal and nano grades of ZnO and TiO_2 exhibited significant differences in plant spread (Table 4). Treated seeds produced more plant spread than control (42.66 cm). Among them, nano grade ZnO and TiO_2 performed better than normal grade seed treatments. Seeds treated with T_4: nano ZnO @ 1300 mg kg^{-1} of seed (60.1 cm) found to have highest plant spread followed by T_{10}- nano TiO_2 @ 1300 mg kg^{-1} of seed (56cm) and T_9- nano TiO_2 @ 900 mg kg^{-1} of seed (54 cm). These treatments were found to be on par with each other. The least plant spread was seen in control (42.66 cm) while comparing to other treatments.

4.1.3 Plant height (cm)

The chilli seeds treated with normal and nano grades of ZnO and TiO_2 exhibited significant differences in plant height (Table 4). The treated seeds produced taller plants than control (60.33 cm). Among normal and nano grade seed treatments, seeds treated with nano grade ZnO and TiO_2 were found to have favourable effect on plant height.

Table 3: Analysis of variance of seed yield and fruit yield attributes in chilli

Sources of variation	Degrees of freedom	Mean sum of squares									
		Plant spread (cm)	Plant height (cm)	Days to harvest	Fruits per plant	Fruit length (cm)	Fruit weight (g) at maturity	Fruit yield	Seeds per fruit	Seed yield per plant(g)	100 seed weight (g)
Replications	2	12.29	2.57	0.231	8.94	0.373	0.028	656.48	2.33	1.27	0.000
Treatments	12	62.19**	11.79**	74.63**	490.38**	0.227**	0.385**	15953.27**	39.70**	180.32**	0.007**
Error	24	12.57	1.12	0.203	3.56	0.023	0.018	221.38	4.86	0.87	0.000
SE		2.895	0.866	0.135	1.540	0.123	0.109	12.148	1.800	0.764	0.000
CV (%)		7.043	1.632	0.486	1.892	2.134	4.412	4.898	3.856	3.201	2.207

Significant at 5 % level *
Significant at 1 % level **

Table 4: Influence of normal grade and nano grade ZnO and TiO_2 on yield attributes in chilli

Treatment	Plant spread (cm)	Plant height (cm)	Days to harvest (nos)	Fruits/ plant (nos.)	Fruit length (cm)	Fruit weight at maturity(g)	Fruit yield (g)
T_1- Control	42.66^{f}	60.33^{e}	90^{f}	85.33^{i}	6.43^{g}	2.30^{f}	195.5^{f}
T_{2}- n ZnO@500	48.20^{cdef}	62.00^{e}	90^{ef}	90.00^{gh}	6.90^{ef}	2.86^{cd}	255.00^{e}
T_{3}- n ZnO@900	46.66^{ef}	66.00^{bc}	92^{c}	88.00^{hi}	6.80^{f}	2.80^{d}	246.33^{e}
T_{4} -n ZnO@1300	60.10^{a}	67.30^{a}	92^{cd}	122.00^{a}	7.40^{a}	3.46^{ab}	422.70^{a}
T_{5}- ZnO@500	50.10^{bcde}	65.33^{bcd}	92^{c}	98.33^{e}	7.06^{cde}	2.93^{cd}	288.50^{d}
T_{6}- ZnO@900	50.90^{bcde}	66.46^{ab}	91^{de}	103.66^{d}	7.33^{ab}	3.26^{b}	338.73^{c}
T_{7}- ZnO@1300	46.80^{def}	65.00^{bcd}	90^{f}	87.66^{hi}	6.90^{ef}	2.83^{cd}	251.30^{e}
T_{8}- nTiO_2@500	49.30^{cde}	65.80^{bcd}	91^{ef}	93.00^{fg}	7.23^{abcd}	2.56^{e}	238.70^{e}
T_{9}- n TiO_2@900	54.00^{bc}	66.33^{ab}	90^{f}	117.66^{b}	7.30^{abc}	3.30^{b}	388.30^{b}
T_{10}- nTiO_2@1300	56.00^{ab}	64.10^{d}	90^{f}	114.66^{bc}	7.00^{cde}	3.33^{ab}	382.13^{b}
T_{11}-TiO_2 @500	52.66^{bcd}	64.50^{cd}	91^{ef}	111.66^{c}	7.40^{a}	3.53^{a}	394.56^{b}
T_{12}-TiO_2@900	49.70^{cde}	65.60^{bcd}	105^{a}	95.00^{f}	7.10^{bcde}	3.03^{c}	288.30^{d}
T_{13}- TiO_2@1300	47.10^{def}	66.23^{abc}	103^{b}	89.33^{h}	7.00^{def}	2.90^{cd}	259.10^{e}
SE_m	2.895	0.866	0.135	1.540	0.123	0.109	12.148
CD (0.05)	5.976	1.789	0.759	3.180	0.254	0.224	25.075

Tallest plants were produced by T_4: nano ZnO @ 1300 mg kg^{-1} of seed (67.3 cm) followed by T_6 : ZnO @ 900 mg kg^{-1} of seed (66.46 cm) ,T_9 : nano TiO_2 @ 900 mg kg^{-1} of seed (66.33 cm) and T_{13} : TiO_2 @ 1300 mg kg^{-1} of seed (66.23 cm) which were on par with each other. Least plant height was recorded in control (60.33 cm) and among the treated seeds least plant height was recorded in T_2: nano ZnO @ 500 mg kg^{-1} of seed (62 cm).

4.1.4 Days to harvest (days)

Significant differences were found among the seeds treated with normal and nano grades of ZnO and TiO_2 (Table 4). Days to harvest varied from 90 to 105 days. Irrespective of the doses of various treatments, T_2 -nano ZnO @ 500 mg kg^{-1} of seed, T_9 -nano TiO_2 @900 mg kg^{-1} of seed, T_{10} -nanoTiO_2 @1300 mg kg^{-1} of seed , T_7 - ZnO @ 1300 mg kg^{-1} of seed and control were harvested at 90 DAT. Among the treatments, delayed harvest (105 days) was recorded in seeds treated with T_{13} -TiO_2 @ 1300 mg kg^{-1} of seed.

4.1.5 Fruits per plant (Nos.)

Chilli seeds treated with ZnO and TiO_2 recorded more number of fruits than untreated seeds (Table 4). Among them, nano grade ZnO and TiO_2 showed good results than normal grade seed treatments. Seed treatments with T_4 -nano ZnO @ 1300 mg kg^{-1} of seed (122) produced more number of fruits followed by T_{9-} nano TiO_2 @ 900 mg kg^{-1} of seed (118) which were on par with T_{10-} nano TiO_2 @ 1300 mg kg^{-1} of seed (115). Least number of fruits were recorded in T_3- nano ZnO @ 900 mg kg^{-1} of seed (88), T_{7-} ZnO @ 1300 mg kg^{-1} of seed (88) and control (88).

4.1.6 Fruit length (cm)

There existed a significant variation among the treatments for fruit length (Table 4). In general it is observed that TiO_2 was more effective in improving the trait. Treatments T_4 -nano ZnO @ 1300 mg kg^{-1} of seed and T_{11} -TiO_2 @ 500 mg $kg^{-1}$of seed recorded the highest value and were on par (7.40cm). Among the treatments, control recorded lowest fruit length (6.4 cm).

4.1.7 Fruit weight at maturity (g)

All treatments recorded higher values than control for fruit weight at maturity with significant differences among themselves (Table 4). Seeds treated with T_{11} -TiO_2 @ 500 mg kg^{-1}of seed recorded highest fruit weight (3.53 g) at maturity followed by T_4 -nano ZnO @ 1300 mg kg^{-1} (3.46g) and T_{10} - nano TiO_2 @1300 mg kg^{-1} of seed (3.33 g).These treatments were on par with each other. In general, TiO_2 seed treatments exhibited superiority for this trait.

4.1.8 Fruit yield per plant (g)

Significant differences were found among the seeds treated with normal and nano grades of ZnO and TiO_2 (Table 4). Nano ZnO @ 1300 mg kg^{-1} of seed (T_4) recorded highest fruit yield per plant (422.70 g). T_{11} -TiO_2 @ 500 mg kg^{-1} of seed, T_9 -nanoTiO_2 @900 mg kg^{-1} of seed and T_{10} -nanoTiO_2 @1300 mg kg^{-1} of seed which were on par with each other recorded fruit yield of 394.56 g, 388.30 g, 382.13 g respectively. Least fruit yield per plant (195.50g) was recorded by control.

4.1.9 Number of seeds per fruit

Compared to untreated seeds, number of seeds per fruit was higher in treatments with normal and nano grades of ZnO and TiO_2 (Table 5). Seeds treated with T_{6-} ZnO 900 mg kg^{-1} of seed (62) recorded more number of seeds per fruit followed by T_4 -nano ZnO @ 1300 mg kg^{-1} of seed (61), T_{12}-TiO_2 @ 900 mg kg^{-1} of seed (61), T_{11}-TiO_2 @ 500 mg kg^{-1} of seed (60), T_{9-} nano TiO_2 @ 900 mg kg^{-1} of seed (59) and T_{10-} nano TiO_2 @ 1300 mg kg^{-1} of seed (59) which were on par with each other compared to control (50). The number of seeds were less in T_{8-} nano TiO_2@ 500 mg kg^{-1} of seed (53) among treated seeds.

In general, normal grades of ZnO and TiO_2 exibited superiority in recording more seed yield per fruit than nano grade seed treatments.

Table 5: Influence of normal grade and nano grade ZnO and TiO_2 on seed yield and seed weight in chilli

Treatment	Seeds/fruit (nos)	Seed yield/plant (g)	100 seed weight
T_1- Control	50.33^{f}	17.18^{h}	0.40^{e}
T_{2-} n ZnO@500	54.66^{de}	24.62^{e}	0.50^{bc}
T_{3-} n ZnO@900	58.66^{abc}	23.22^{ef}	0.45^{c}
T_{4} -n ZnO@1300	61.33^{a}	41.14^{a}	0.55^{a}
T_{5-} ZnO@500	56.33^{bcd}	28.80^{d}	0.52^{b}
T_{6-} ZnO@900	62.33^{a}	35.49^{c}	0.55^{a}
T_{7-} ZnO@1300	54.33^{de}	21.43^{g}	0.45^{d}
T_{8-} $nTiO_2$@500	52.33^{ef}	24.32^{e}	0.50^{bc}
T_{9-} n TiO_2@900	59.00^{ab}	38.17^{b}	0.55^{a}
T_{10-} $nTiO_2$@1300	59.00^{ab}	37.20^{b}	0.55^{a}
T_{11}-TiO_2 @500	60.00^{ab}	36.84^{bc}	0.55^{a}
T_{12}-TiO_2@900	61.00^{ab}	29.62^{d}	0.52^{b}
T_{13-} TiO_2@1300	55.00^{cde}	22.10^{fg}	0.45^{c}
SE_m	1.800	0.764	0.000
CD (0.05)	3.716	1.578	0.019

1.1.10 Seed yield per plant (g)

Significant variations were observed for seed yield per plant among the treatments with treatments performing better than control. (Table 5). Seeds treated with T_4 -nano ZnO @ 1300 mg kg^{-1} of seed (41.14 g) recorded highest seed yield compared to control (17.18 g). Treatments with T_9- nano TiO_2 @ 900 mg kg^{-1} of seed (38.17 g), T_{10}- nano TiO_2 @ 1300 mg kg^{-1} of seed (37.20) and T_{11}-TiO_2 @ 500 mg kg^{-1} of seed (36.84) were found to be on par with other. Lowest seed yield was recorded in seeds treated with T_7- ZnO @ 1300 mg kg^{-1} of seed (21.43g) among treated seeds.

4.1.11 100 seed weight (g)

Significant differences was found among the seeds treated with normal and nano grades of ZnO and TiO_2 (Table 5). 100 seed weight varied from 0.55g in T_9 - nano TiO_2 @ 900 mg kg^{-1} of seed, T_{11} -TiO_2 @ 500 mg kg^{-1} of seed, T_4 -nano ZnO @ 1300 mg kg^{-1} of seed, T_{10} - nano TiO_2 @ 1300 mg kg^{-1} of seed and T_6 -ZnO @ 900 mg kg^{-1} of seed to 0.40 g in control. Least seed weight was recorded in seeds treated with T_7- ZnO @ 1300 mg kg^{-} of seed (0.45g) among the treated seeds. In general, nano grades of ZnO and TiO_2 exibited greater potential in recording more seed weight than normal grade seed treatments. Irrespective of various doses of treatments, both ZnO and TiO_2 performed well.

4.2 Seed storage studies with wet dressed inorganic nanoparticles

4.2.1 Initial seed quality

Initial seed quality prior to storage was determined and are presented in the Table 6. Seed quality parameters like germination, shoot length, root length, seedling dry weight, vigour index – I and II, electrical conductivity of seed leachates and seed microflora infestation were recorded significant differences existed among the treatments except for shoot length.

Table 6: Seed quality of chilly before storage

Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Dry weight (mg)	EC (µS cm^{-1})	VI I	VI II	Moisture content (%)	Microflora (agar plate method) (%)
T_1- Control	70.00	5.10	7.63	25.00	268.33	885	1777	7.10	16.66
T_2 - ZnO@100	71.60	5.96	9.53	26.66	221.00	1105	1894	7.00	0
T_3 - ZnO@250	72.00	6.06	9.33	26.33	204.33	1105	1901	7.00	0
T_4 - ZnO@500	69.00	5.43	9.80	26.00	237.66	1056	1800	6.30	0
T_5 - ZnO@750	70.00	5.86	10.16	26.66	249.33	1128	1861	6.30	0
T_6- ZnO@1000	71.66	6.10	9.83	27.33	210.33	1132	1945	7.00	0
T_7 -ZnO@1250	69.00	5.70	9.50	26.33	249.67	1048	1807	7.40	0
T_8 -TiO2@100	69.33	5.46	8.13	27.00	261.66	941	1869	7.30	0
T_9 -TiO_2 @250	70.66	5.20	8.33	26.33	254.33	954	1848	7.60	0
T_{10} -TiO_2@500	70.33	5.90	9.50	28.33	223.00	1085	1975	6.40	0
T_{11} -TiO_2@750	72.00	6.03	8.93	27.66	201.66	1074	2012	6.90	0
T_{12}- TiO_2@1000	72.00	6.10	9.23	27.33	191.66	1105	1965	7.60	0
T_{13}-TiO2@1250	68.66	5.20	8.43	26.33	279.67	924	1788	6.30	0
CD (0.05)	NS	0.182	NS	NS	NS	NS	NS	NS	NS

Table 7: Influence of nano particles of ZnO and TiO$_2$ on germination of chilli

Treatment	Months of storage						
	M1	M2	M3	M4	M5	M6	M7
T$_1$- Control	71.66^{cd} (57.843)	69.66^{c} (56.584)	67.33^{d} (55.152)	65.33^{d} (53.930)	62.33^{f} (52.14)	60.66^{f} (51.16)	58.00^{f} (49.60)
T$_2$ - ZnO@100	73.00^{abc} (58.696)	72.33^{ab} (58.271)	71.33^{abc} (57.629)	70.33^{ab} (56.998)	68.33^{c} (55.75)	66.33^{bc} (54.54)	62.33^{cd} (52.14)
T$_3$ - ZnO@250	74.60^{a} (59.781)	73.33^{a} (58.910)	73.00^{a} (58.696)	72.33^{a} (58.266)	70.33^{ab} (56.99)	69.66^{a} (56.58)	65.66^{a} (54.13)
T$_4$ - ZnO@500	72.33^{bcd} (58.275)	70.66^{bc} (57.216)	69.66^{bcd} (56.584)	68.33^{bc} (55.757)	66.33^{d} (54.54)	65.66^{cd} (54.13)	60.66^{de} (51.16)
T$_5$ - ZnO@750	72.00^{bcd} (58.061)	71.6^{abc} (57.843)	69.66^{bcd} (56.584)	67.33^{cd} (55.146)	65.66^{d} (54.13)	63.66^{de} (52.93)	59.66^{ef} (50.57)
T$_6$- ZnO@1000	73.33^{abc} (58.910)	72.00^{ab} (58.061)	72.66^{ab} (57.845)	72.00^{a} (58.061)	70.66^{ab} (57.20)	68.33^{ab} (55.75)	64.00^{bc} (53.13)
T$_7$ -ZnO@1250	71.33^{cd} (57.210)	70.33^{bc} (57.003)	69.66^{bcd} (56.584)	67.33^{cd} (55.146)	64.66^{de} (53.52)	62.33^{ef} (52.14)	59.33^{ef} (50.37)
T$_8$ -TiO2@100	71.66^{cd} (57.845)	70.66^{bc} (57.216)	68.33^{d} (55.757)	66.66^{cd} (54.742)	63.66^{ef} (52.93)	60.66^{f} (51.16)	58.66^{ef} (49.99)
T$_9$ -TiO$_2$ @250	72.00^{bcd} (58.061)	71.33^{abc} (57.629)	69.33^{cd} (56.374)	68.00^{c} (55.552)	65.66^{d} (54.13)	63.33^{de} (52.73)	59.66^{ef} (50.57)
T$_{10}$ -TiO$_2$@500	72.66^{bcd} (58.266)	72.33^{ab} (58.266)	71.00^{abc} (57.424)	70.30^{ab} (56.998)	68.33^{c} (55.75)	65.66^{cd} (54.13)	63.66^{bc} (52.93)
T$_{11}$ -TiO$_2$@750	74.00^{ab} (59.345)	73.00^{a} (58.696)	72.66^{ab} (57.845)	71.66^{a} (57.843)	69.33^{bc} (56.37)	66.00^{cd} (54.13)	62.33^{cd} (52.14)
T$_{12-}$ TiO$_2$@1000	74.66^{a} (59.781)	73.33^{a} (58.910)	73.00^{a} (58.482)	72.33^{a} (58.266)	71.33^{a} (57.633)	69.33^{a} (56.37)	65.33^{a} (54.54)
T$_{13}$-TiO2@1250	70.60^{d} (57.210)	70.33^{bc} (58.910)	69.00^{cd} (56.172)	66.66^{cd} (54.742)	62.33^{f} (52.14)	60.66^{f} (51.16)	58.66 (49.99)
SE$_m$	0.66	0.69	0.71	0.66	0.55	0.72	0.65
CD (0.05)	1.364	1.438	1.467	1.372	1.151	1.483	1.342

Highest shoot length of 6.10 cm was recorded in treatments with nano ZnO @ 1000 mg kg^{-1} of seed (T_6) and nanoTiO$_2$ @ 1000 mg kg^{-1} of seed (T_{12}).

4.2.2 Quality of seed during storage

4.2.2.1 Analysis of variance

The analysis of variance of the observations recorded at monthly intervals for seven months of storage revealed that there existed high significant differences for seed quality parameters among the various doses of nano grade ZnO and TiO$_2$ treatments used to wet dress the seeds.

4.2.2.2 Germination (%)

Seed treatment with various doses of nano grades of ZnO and TiO$_2$ exibited significant differences in germination per cent throughout the storage period (Table 7). The germinability of seeds were found to decline in both treated and untreated seeds (control) over the period of storage. However, it was seen that, treated seeds recorded higher germination compared to control for most part of the storage period.

At 1MAS germination ranged from 74.66 per cent in nanoTiO$_2$@1000 mg kg^{-1} of seed (T_{12}) to 70 per cent in nanoTiO$_2$ @ 1250 mg kg^{-1} of seed (T_{13}). All the treatments including control retained germination above the prescribed 60 per cent by IMSCS (Indian Minimum Seed Certification Standards) till sixth month of storage.

Among the treatments T_{3-} nano ZnO 250 mg kg^{-1} of seed (65.66) recorded maximum germination followed by T_{12-} nano TiO$_{2-}$ 1000 mg kg^{-1} of seed (65.33) which were on par with each other compared to control (58) at the end of storage *i.e.,* seven MAS. Seeds treated with T_{3-} nano ZnO 250 mg kg^{-1} of seed (65.66) which was on par with T_{12-} nano TiO$_{2-}$ 1000 mg kg^{-1} of seed (65.33) and T_{6-} nano ZnO 1000 mg kg^{-1} of seed (64.00) which was on par with T_{10-} nano TiO$_2$ 500 mg kg^{-1} of seed (63.66), T_2 -nano ZnO-100 mg kg^{-1} of seed (62.33) and T_{11-} nano TiO$_2$ 750 mg kg^{-1} of seed (62.33) retained more than 60 per cent germination after seven months of storage period.

In general, the seeds treated with nano ZnO @ 250 mg kg^{-1} of seed(T_3)and nano TiO_2@1000 mg kg^{-1} of seed (T_{12}) showed superiority in recording highest germination per cent.

4.2.2.3 Shoot length (cm)

Significant influence of storage period and seed treatments were seen in seedling shoot length (Table 8). The shoot length were found to decline in both treated and untreated seeds (control) over the period of storage. However, it was seen that, treated seeds recorded higher shoot length compared to control.

Among the treatments, T_{6-} nano ZnO 1000 mg kg^{-1} of seed (5.57 cm), T_{3-} nano ZnO 250 mg kg^{-1} of seed (5.26 cm), T_2 - nano ZnO-100 mg kg^{-1} of seed (5.21 cm), T_{11-} nano TiO_2 750 mg kg^{-1} of seed (5.19 cm) and T_{10-} nano TiO_2 500 mg kg^{-1} of seed (5.13 cm) exhibited highest shoot length at seven MAS. The treatments were found to be on par with each other. It was seen that T_{13-} nano TiO_{2} -1250 mg kg^{-1} of seed (4.07 cm) recorded lower shoot length among the seed treatments at the seven MAS.

4.2.5 Root length (cm)

Seed treatment using nano grades of ZnO and TiO_2 had a noticeable effect on seedling root length (Table 9). The treatments were found to be significantly different from each other. Over the period of storage, a gradual decrease was observed in seedling root length.

After 7 MAS, T_{5-} nano ZnO 50 mg kg^{-1} of seed (9.37 cm) produced longer roots followed by T_6- nano ZnO@1000 (8.89). Among the treated seeds, nano TiO_2@100 mg kg^{-1} of seed (T_8) recorded shorter root length (7.38 cm).

Table 8: Influence of nano particles of ZnO and TiO_2 on shoot length (cm) of chilli

Treatment	Months of storage						
	M1	M2	M3	M4	M5	M6	M7
T_1- Control	5.03^{g}	4.92^{h}	4.81^{i}	4.76^{i}	4.68^{i}	4.59^{i}	4.44^{de}
T_2 - ZnO@100	5.80^{b}	5.69^{d}	5.53^{d}	5.40^{d}	5.38^{c}	5.32^{d}	5.21^{abc}
T_3 - ZnO@250	5.83^{b}	5.71^{d}	5.61^{c}	5.53^{c}	5.48^{b}	5.37^{c}	5.26^{ab}
T_4 - ZnO@500	5.38^{de}	5.31^{f}	5.25^{fg}	5.10^{f}	5.07^{f}	4.96^{f}	4.82^{bcd}
T_5 - ZnO@750	5.51^{cd}	5.33^{f}	5.18^{g}	5.08^{g}	4.92^{g}	4.83^{g}	4.72^{cd}
T_6- ZnO@1000	6.03^{a}	6.00^{b}	5.98^{a}	5.81^{a}	5.71^{a}	5.61^{a}	5.57^{a}
T_7 -ZnO@1250	5.63^{c}	5.53^{e}	5.36^{e}	5.28^{e}	5.15^{e}	4.94^{f}	4.81^{bcd}
T_8 -TiO2@100	5.32^{e}	5.31^{f}	5.28^{f}	5.16^{f}	5.06^{f}	4.86^{g}	4.64^{d}
T_9 -TiO_2 @250	5.18^{f}	5.03^{g}	4.96^{h}	4.87^{h}	4.75^{h}	4.69^{h}	4.52^{de}
T_{10} -TiO_2@500	5.84^{b}	5.73^{d}	5.50^{d}	5.40^{d}	5.31^{d}	5.27^{e}	5.13^{abc}
T_{11} -TiO_2@750	6.00^{a}	5.87^{c}	5.72^{b}	5.63^{b}	5.47^{b}	5.31^{d}	5.19^{abc}
T_{12}- TiO_2@1000	6.10^{a}	6.13^{a}	6.00^{a}	5.83^{a}	5.71^{a}	5.51^{b}	4.71^{cd}
T_{13}-TiO2@1250	5.10^{fg}	4.93^{h}	4.79^{i}	4.68^{j}	4.40^{j}	4.30^{j}	4.07^{e}
SE_m	0.063	0.025	0.036	0.001	0.001	0.001	0.238
CD (0.05)	0.134	0.050	0.077	0.032	0.031	0.037	0.490

Table 9: Influence of nano particles of ZnO and TiO$_2$ on root length (cm) of chilli

Treatment	Months of storage						
	M1	M2	M3	M4	M5	M6	M7
T$_1$- Control	7.51^{j}	7.32^{l}	7.21^{l}	7.11^{k}	7.03^{l}	6.91^{m}	6.82^{l}
T$_2$ - ZnO@100	9.39^{c}	9.21^{e}	9.18^{d}	9.03^{d}	8.97^{c}	8.76^{d}	8.63^{d}
T$_3$ - ZnO@250	9.20^{de}	9.13^{f}	9.00^{e}	8.91^{e}	8.74^{e}	8.62^{f}	8.51^{e}
T$_4$ - ZnO@500	9.10^{e}	9.02^{g}	8.91^{f}	8.72^{f}	8.62^{f}	8.55^{g}	8.35^{g}
T$_5$ - ZnO@750	10.00^{a}	10.23^{a}	10.03^{a}	9.83^{a}	9.77^{a}	9.54^{a}	9.37^{a}
T$_6$- ZnO@1000	9.78^{b}	9.61^{b}	9.49^{b}	9.32^{b}	9.22^{b}	9.03^{b}	8.89^{b}
T$_7$ -ZnO@1250	9.22^{d}	9.00^{g}	8.81^{g}	8.72^{f}	8.51^{g}	8.43^{h}	8.33^{g}
T$_8$ -TiO2@100	8.02^{i}	8.00^{k}	7.89^{k}	7.71^{j}	7.69^{k}	7.53^{l}	7.38^{k}
T$_9$ -TiO$_2$ @250	8.29^{h}	8.13^{j}	8.10^{j}	7.94^{i}	7.79^{j}	7.64^{k}	7.52^{j}
T$_{10}$ -TiO$_2$@500	9.35^{c}	9.24^{d}	9.18^{d}	8.94^{e}	8.81^{d}	8.68^{e}	8.48^{f}
T$_{11}$ -TiO$_2$@750	8.87^{f}	8.54^{h}	8.35^{i}	8.25^{h}	8.03^{i}	7.92^{j}	7.87^{i}
T$_{12-}$ TiO$_2$@1000	9.78^{b}	9.53^{c}	9.31^{c}	9.25^{c}	8.96^{c}	8.85^{c}	8.71^{c}
T$_{13}$-TiO2@1250	8.38^{g}	8.51^{i}	8.44^{h}	8.32^{g}	8.22^{h}	8.07^{i}	7.91^{h}
SE$_m$	0.025	0.001	0.036	0.025	0.001	0.025	0.001
CD (0.05)	0.048	0.029	0.077	0.047	0.031	0.053	0.021

Table 10: Influence of nano particles of ZnO and TiO$_2$ on dry weight (mg) of chilli

Treatment	Months of storage						
	M1	M2	M3	M4	M5	M6	M7
T_1- Control	26.13^{e}	25.83^{d}	25.00^{i}	23.72^{i}	22.12^{f}	21.84^{e}	20.35^{i}
T_2 - ZnO@100	26.86^{c}	26.01^{cd}	25.36^{f}	25.03^{d}	24.26^{bc}	23.54^{ab}	22.04^{c}
T_3 - ZnO@250	26.82^{c}	26.32^{c}	25.00^{i}	24.46^{h}	24.03^{cd}	23.42^{b}	21.97^{e}
T_4 - ZnO@500	26.02^{e}	25.77^{d}	25.51^{e}	24.91^{e}	23.13^{e}	22.64^{d}	21.92^{d}
T_5 - ZnO@750	25.51^{f}	25.25^{e}	25.13^{g}	24.82^{f}	23.12^{e}	21.95^{e}	19.13^{j}
T_6- ZnO@1000	26.93^{c}	26.83^{b}	26.51^{a}	25.63^{b}	25.01^{a}	23.73^{a}	22.63^{a}
T_7 -ZnO@1250	25.20^{g}	25.54^{e}	25.03^{h}	24.62^{g}	23.55^{de}	22.05^{e}	20.74^{g}
T_8 -TiO2@100	26.16^{e}	26.22^{c}	25.92^{d}	23.75^{k}	22.42^{f}	22.18^{e}	21.23^{f}
T_9 -TiO$_2$ @250	25.31^{fg}	25.07^{e}	24.89^{j}	23.91^{j}	22.00^{f}	21.20^{f}	20.71^{h}
T_{10} -TiO$_2$@500	27.97^{a}	27.43^{a}	26.28^{b}	25.76^{a}	24.40^{cd}	22.96^{c}	21.22^{f}
T_{11} -TiO$_2$@750	26.98^{b}	26.82^{b}	25.12^{g}	24.39^{i}	23.91^{cd}	22.45^{d}	20.72^{h}
T_{12}- TiO$_2$@1000	26.51^{d}	26.22^{c}	26.12^{c}	25.51^{c}	24.76^{ab}	23.32^{b}	22.41^{b}
T_{13}-TiO2@1250	26.00^{e}	25.73^{d}	24.25^{k}	22.92^{m}	21.03^{g}	19.62^{g}	18.05^{k}
SE$_m$	0.025	0.001	0.036	0.025	0.001	0.025	0.001
CD (0.05)	0.245	0.317	0.014	0.013	0.539	0.268	0.019

4.2.6 Dry weight (mg)

Seedling dry weight differed significantly throughout the storage period irrespective of seed treatments in a declining trend with the advancement of the period of storage (Table 10). At 7 MAS, Maximum dry matter production was recorded in T_{6-} nano ZnO 1000 mg kg^{-1} of seed (22.63 mg),T_{12-} nano TiO_{2-} 1000 mg kg^{-1} of seed (22.41 mg),and T_2 - nano ZnO-100 mg kg^{-1} of seed (22.04 mg) compared to control (20.35 mg) at seven MAS.

4.2.7 Vigour index I

Significant differences due to seed treatments in vigour index I was observed during the period of storage of seven months (Table 11). It was seen that seed vigour declined during the advancement of storage. At the initial month of storage, vigour index I varied between 1133 in nano ZnO @ 1000 mg kg^{-1} of seed (T_6) and 845 in control.

At seven MAS T_{6-} nano ZnO @ 1000 mg kg^{-1} of seed (925.88) retained superiority in maintaining vigour index I followed by T_{10-} nano TiO_2 @ 500 mg kg^{-1} of seed (867) which was on par with T_{12} -nano TiO_2 @ 1000 mg kg^{-1} of seed (857.62), T_2 -nano ZnO @ 100 mg kg^{-1} of seed (844) and T_{5-} nano ZnO @ 50 mg kg^{-1} of seed (841) compared to control (652).

4.2.8 Vigour index II

Significant differences due to seed treatments in vigour index II was observed during the period of storage and it declined during the advancement of storage (Table 12). Seeds treated with T_{12} -nano TiO_2 @ 1000 mg kg^{-1} of seed (1472) which was on par with T_{3-} nano ZnO 250 mg kg^{-1} of seed (1457) and T_{6-} nano ZnO @ 1000 mg kg^{-1} of seed (1448) retained superiority in maintaining vigour index II. Among the seed treatments T_{13-} nano $TiO_{2\,-}$1250 mg kg^{-1} of seed (1059) exhibited lowest vigour index II.

Table 11: Influence of nano particles of ZnO and TiO_2 on Vigour Index I of chilli

Treatment	Months of storage						
	M1	M2	M3	M4	M5	M6	M7
T_1- Control	845^{g}	825^{g}	809^{g}	799^{f}	789^{b}	775^{h}	652^{g}
T_2 - ZnO@100	10701^{cde}	1048^{de}	1023^{de}	1007^{cd}	991^{a}	981^{cde}	844^{bcd}
T_3 - ZnO@250	1095^{bc}	1083^{bc}	1067^{bc}	1054^{ab}	1038^{a}	1022^{ab}	781^{e}
T_4 - ZnO@500	1056^{de}	1034^{de}	1015^{de}	1004^{d}	857^{b}	962^{def}	821^{cde}
T_5 - ZnO@750	10823^{bcd}	1084^{bc}	1055^{bc}	1039^{bc}	1023^{a}	1001^{bc}	841.30^{bcd}
T_6- ZnO@1000	1133^{a}	1119^{a}	1503^{a}	1085^{a}	1057^{a}	1049^{a}	926^{a}
T_7 -ZnO@1250	1047^{e}	1027^{e}	1013^{de}	997^{d}	982^{a}	955^{ef}	798^{e}
T_8 -TiO2@100	912^{f}	909^{f}	899^{f}	879^{e}	864^{b}	847^{g}	705^{f}
T_9 -TiO_2 @250	934^{f}	913^{f}	906^{f}	888^{e}	869^{b}	855^{g}	718^{f}
T_{10} -TiO_2@500	1079^{bcde}	1064^{cd}	1042^{cd}	1016^{cd}	1006^{a}	990^{cd}	867^{b}
T_{11} -TiO_2@750	1066^{cde}	1033^{de}	1009^{e}	995^{d}	973^{a}	948^{f}	814^{de}
T_{12}- TiO_2@1000	1107^{ab}	1101^{ab}	1084^{b}	1058^{ab}	1041^{a}	1022^{ab}	858^{bc}
T_{13}-TiO2@1250	930^{f}	928^{f}	913^{f}	897^{e}	873^{b}	856^{g}	703^{f}
SE_m	16.98	16.56	15.53	15.83	46.11	14.86	20.47
CD (0.05)	34.92	34.06	31.93	32.56	94.80	30.56	42.09

Table 12: Influence of nano particles of ZnO and TiO$_2$ on Vigour Index II of chilli

Treatment	Months of storage						
	M1	M2	M3	M4	M5	M6	M7
T$_1$- Control	1893^{c}	1799de	1683^{g}	1549^{hi}	1379^{f}	1324^{f}	1181^{f}
T$_2$ - ZnO@100	1961^{b}	1881^{bc}	1809^{bcd}	1761^{bc}	1658^{bc}	1561^{bc}	1374^{b}
T$_3$ - ZnO@250	2002^{ab}	1929^{ab}	1825^{bc}	1769^{bc}	1690^{b}	1631^{a}	1457^{a}
T$_4$ - ZnO@500	1882^{cd}	1821^{cde}	1777^{cdef}	1702de	1534^{d}	1487^{d}	1330^{bc}
T$_5$ - ZnO@750	1837^{def}	1809de	1751^{def}	1671^{ef}	1518^{d}	1398^{e}	1142^{f}
T$_6$- ZnO@1000	1975^{ab}	1932^{ab}	1900^{a}	1845^{a}	1767^{a}	1622^{ab}	1449^{a}
T$_7$ -ZnO@1250	1797^{f}	1773^{e}	1744^{ef}	1657^{ef}	1523^{d}	1375^{ef}	1230^{e}
T$_8$ -TiO2@100	1875^{cde}	1853^{cd}	1771^{cdef}	1583^{gh}	1427^{ef}	1326^{f}	1246de
T$_9$ -TiO$_2$ @250	1822^{ef}	1788de	1726^{fg}	1626^{fg}	1451^{e}	1343^{ef}	1236^{e}
T$_{10}$ -TiO$_2$@500	2024^{a}	1984^{a}	1866^{ab}	1812^{ab}	1623^{c}	1507^{cd}	1351^{b}
T$_{11}$ -TiO$_2$@750	2029^{a}	1957^{a}	1800^{cde}	1748^{cd}	1657^{bc}	1474^{d}	1291^{cd}
T$_{12-}$ TiO$_2$@1000	1979^{ab}	1923^{ab}	1898^{a}	1845^{a}	1766^{a}	1616^{ab}	1472^{a}
T$_{13}$-TiO2@1250	1838^{def}	1809de	1673^{g}	1528^{i}	1311^{g}	1190^{g}	1059^{g}
SE$_m$	26.45	32.63	28.96	26.37	31.61	29.78	23.28
CD (0.05)	54.315	67.089	59.558	54.231	64.992	61.248	47.882

Table 13: Influence of nano particles of ZnO and TiO$_2$ on electrical conductivity of seed leachate (µScm^{-1}) of chilli

Treatment	Months of storage						
	M1	M2	M3	M4	M5	M6	M7
T$_1$- Control	295.66^{a}	316.33^{a}	334.67^{a}	362.33^{a}	381.00^{a}	401.33^{a}	404.66^{a}
T$_2$ - ZnO@100	234.66^{h}	247.00^{h}	261.33^{f}	286.67^{g}	297.33^{f}	315.33^{h}	319.67^{f}
T$_3$ - ZnO@250	215.33^{j}	222.00^{k}	234.33^{i}	262.67^{k}	281.66^{hi}	283.66^{l}	295.00^{i}
T$_4$ - ZnO@500	254.67^{f}	270.66^{f}	295.66^{d}	306.33^{f}	320.66^{e}	333.67^{f}	347.00^{d}
T$_5$ - ZnO@750	283.67^{c}	296.66^{c}	314.66^{c}	320.33^{d}	343.00^{c}	350.67^{e}	372.00^{c}
T$_6$- ZnO@1000	217.00^{j}	226.33^{j}	246.66^{h}	267.33^{j}	286.33^{gh}	293.66^{j}	301.00^{gh}
T$_7$ -ZnO@1250	261.33^{e}	227.00^{j}	255.00^{g}	272.00^{i}	294.33^{fg}	319.33^{g}	331.00^{e}
T$_8$ -TiO2@100	285.33^{c}	290.00^{d}	323.33^{b}	342.00^{c}	363.33^{b}	371.00^{c}	386.67^{b}
T$_9$ -TiO$_2$ @250	272.33^{d}	287.00^{e}	294.00^{d}	316.33^{e}	330.67^{d}	353.33^{d}	372.66^{c}
T$_{10}$ -TiO$_2$@500	246.66^{g}	261.33^{g}	272.33^{e}	283.67^{h}	296.33^{i}	305.66^{i}	308.66^{g}
T$_{11}$ -TiO$_2$@750	221.00^{i}	234.67^{i}	254.66^{g}	266.00^{j}	281.66^{hi}	285.33^{k}	290.33^{i}
T$_{12-}$ TiO$_2$@1000	203.00^{k}	215.33^{l}	229.66^{j}	243.67^{l}	274.33^{i}	292.66^{j}	299.33^{h}
T$_{13}$-TiO2@1250	290.00^{b}	311.00^{b}	322.66^{b}	349.66^{b}	368.33^{b}	383.66^{b}	397.67^{a}
SE$_m$	0.987	1.169	1.029	1.339	3.960	0.750	3.814
CD (0.05)	2.029	2.404	2.117	2.754	8.153	1.544	7.842

Table 14: Influence of nano particles of ZnO and TiO_2 on seed moisture and seed infection at the end of storage in chilli

Treatment	Moisture content (%)	Seed infection	
		Blotter paper method	Agar plate method
T_1- Control	7.13	3.33	13.33
T_2 - ZnO@100	7.16	0	10.00
T_3 - ZnO@250	7.03	0	0
T_4 - ZnO@500	6.70	0	0
T_5 - ZnO@750	6.93	0	0
T_6- ZnO@1000	7.23	0	0
T_7 -ZnO@1250	7.50	0	0
T_8 -TiO2@100	7.43	0	0
T_9 -TiO_2 @250	7.76	0	10.00
T_{10} -TiO_2@500	7.06	0	0
T_{11} -TiO_2@750	7.06	0	0
T_{12}- TiO_2@1000	7.70	0	0
T_{13}-TiO2@1250	6.43	0	0
SE_m	0.48	1.30	4.71
CD (0.05)	NS	NS	9.69

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

Electrical conductivity of seed leachates of seeds treated with various doses of nano grades of ZnO and TiO_2 was significantly increasing throughout the storage period irrespective of the treatments (Table 13). It was seen that treated seeds recorded lower values while comparing with control.

Seeds treated with T_{3}- nano ZnO @ 250 mg kg^{-1} of seed (295 µScm^{-1}) which was on par with T_{11}- nano TiO_2 @ 750 mg kg^{-1} of seed (290.33 µScm^{-}1) had least electrical conductivity of seed leachates compared to control (404.66 µScm^{-1}) at seven MAS. Among the treated seeds T_{13}- nano TiO_2 @ 1250 mg kg^{-1} of seed (397.67 µScm^{-}1) recorded highest value at seven MAS.

4.2.10 Seed moisture content (per cent)

A marginal increase in moisture content was noticed during the advancement of storage period (Table 14). Insignificant differences was found when seeds treated with both nano ZnO and TiO_2.

4.2.11 Seed microflora (per cent)

Seed treatments with both nano ZnO and TiO_2 showed significant difference in seed infection (Table 14). The control seeds showed higher infection in both agar plate method and blotter paper method irrespective of the treatments.

In case of agar plate method, control showed an infection of 13.3% and in nano ZnO @ 100 mg kg^{-1} of seeds (T_2) and nano TiO_2 @ 250 mg kg^{-1} of seeds (T_9), 10 % seed infection was observed. Only control seeds showed an infection of 3.33% in blotter paper method. The presence of *Aspergillus flavus* and *Aspergillus niger* was observed in both methods.

Discussion

5. Discussion

Seed is the vital input for sustainable agriculture since most of the food crops are produced from seeds. To feed the ever growing population, new management practices that provides high productivity, healthy ecosystem and cost effectiveness along with good quality seeds are required (Kumar, 2012). Seed treatment with nanoparticle is one such practice. Rapid seed germination and uniform crop stand are crucial for economic sustainability in agriculture. Nanoparticles promotes plant growth and development thereby enhancing crop yield.

Hence the present study was conducted to assess the impact of nanoparticle treatments of zinc oxide and titanium dioxide on yield and seed quality in chilli. The findings of the study are discussed in this chapter.

5.1 Impact of seed invigoration using inorganic nanoparticles of zinc oxide and titanium dioxide on yield in chilli

5.1.1 Effect of Nano grade zinc oxide on yield attributes

Regardless of the treatments all the yield parameters recorded superior results in treatments with nano grades of ZnO. The efficacy of nano treatments in enhancing plant characters was reported by Salama (2012) in maize and bean.

Application of nano ZnO@ 1300 mg kg^{-1} of seed (T_4) obtained highest plant spread (60.1 cm), number of fruits per plant (122), fruit length (7.40cm), fruit weight (3.53 g), fruit yield (422.7 g), 100 seed weight (0.55 g) and seed yield (41.14 g). While nanoparticles of ZnO @ 900 mg kg^{-1} of seed (T_3) recorded highest plant height. In case of number of days to harvest, T_2 (500 mg kg^{-1} of seed) recorded least days (90 DAT).

Subbaiah (2014) by using nano ZnO at 400 ppm obtained highly significant results for yield, plant height, cob length and number of grains per cob when compared to control in maize. Raliya *et al.* (2015) in tomato, and García-López *et al.* (2018) in pepper reported that plant growth increased significantly throughout the growth stages when using nanoparticles for seed treatments. The impact of seed treatment with nanoparticles on fruit yield attributes was noticed by Khanm *et al.* (2017) in tomato

Mahdieh *et al.* (2018) in bean and Sadak and Bakry (2020) in flax. Similar results on seed yield attributes was noticed by Rezaei *et al.* (2015) in soyabean, and Poornima and Koti (2019) in sorghum and Sadak and Bakry (2020) in flax.

5.1.2 Effect of Normal grade zinc oxide on yield attributes

Normal grade ZnO treatments was effective for improving traits like number of seeds per fruit (ZnO @ 1300 mg kg^{-1} of seed) and 100 seed weight (ZnO @ 900 mg kg^{-1} of seed). .

Potarzycki and Grzebisz (2009) inferred that when sufficient Zn was applied, seed yield in maize could be significantly increased. This increment was due to the increase in yield attributes like cob length, thousand kernel weight, number of kernels per cob and number of rows per cob and the enhancement in nitrogen use efficiency by the application of Zn. Ziaeyan and Rajaie (2009) and Vazin (2012) in maize reported similar effects of normal grade Zn

Zinc is an important factor in synthesis of indole-acetic acid (IAA), proteins, chlorophyll, carbohydrates etc. It also helps in biosynthesis of cytochromes, detoxification of reactive oxygen species (ROS) and reduces cadmium (heavy metal) uptake by plants (Buchanan *et al*., 2000). When Chickpea seeds were treated with nano ZnO, an increment in IAA levels was observed in roots which in turn increased plant growth (Avinash *et al*., 2010).

Agronomic efficiency of Zn treatments are influenced by particle size. When particle size is reduced, number of particles per unit weight is increased and thus surface area is also increased (Mortvedt, 1992). This enhances uptake of Zn. These nanoparticles have good catalytic surface, high activity, adsorb more water and are rapidly dispersible (Khanm *et al*., 2017).As the Zn content in seeds are higher, it will hinder the pathogen invasion during germination and growth of seedling thus ensures good crop stand and better yield (Marschner,1995). When Zn shortage occurs, plants make physiological adjustments to maintain homeostasis (Grusak, 2002). These processes may adversely affect yield and quality.

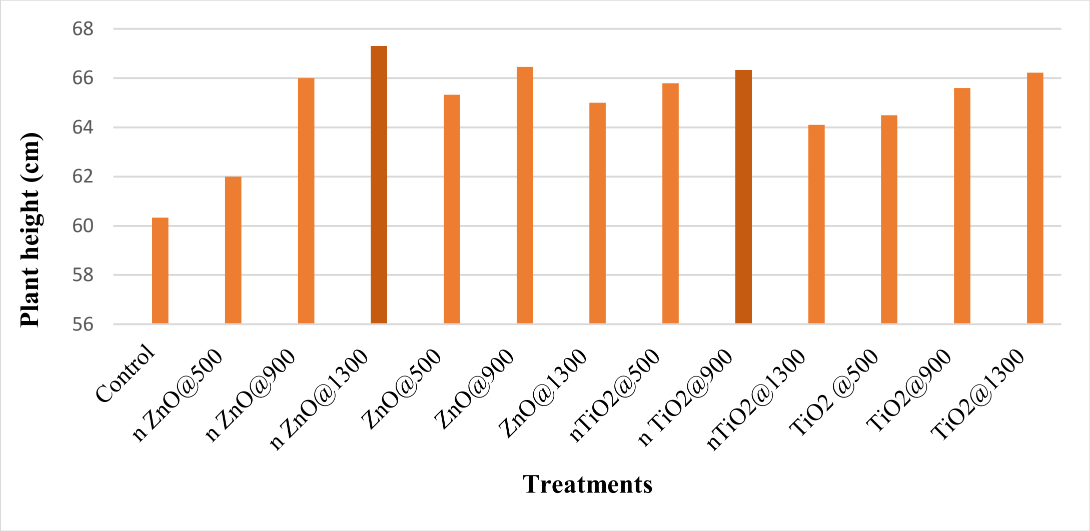


Fig. 1. Influence of normal grade and nano grade and TiO_2 on plant height in chilli

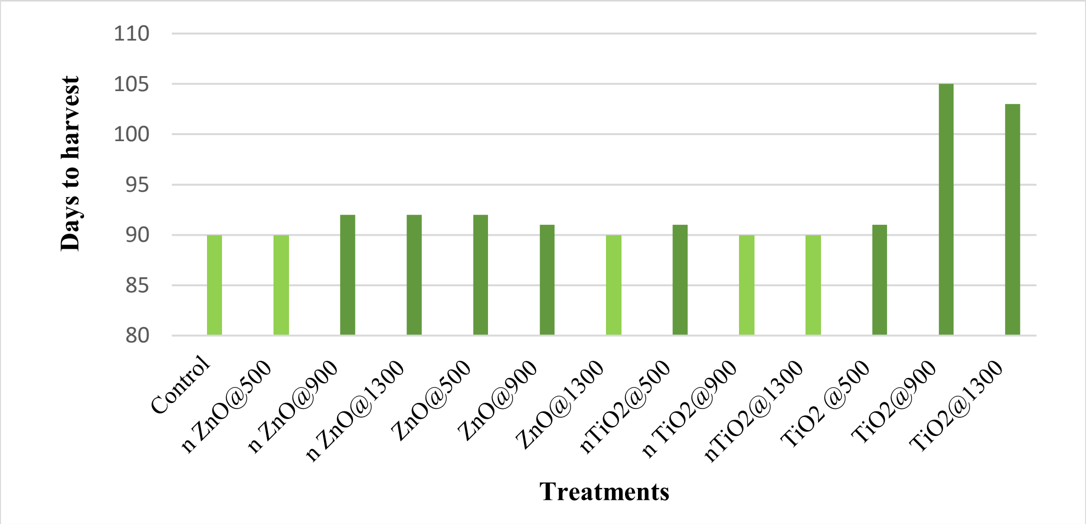


Fig. 2. Influence of normal grade and nano grade ZnO and TiO_2 on days to harvest in chilli

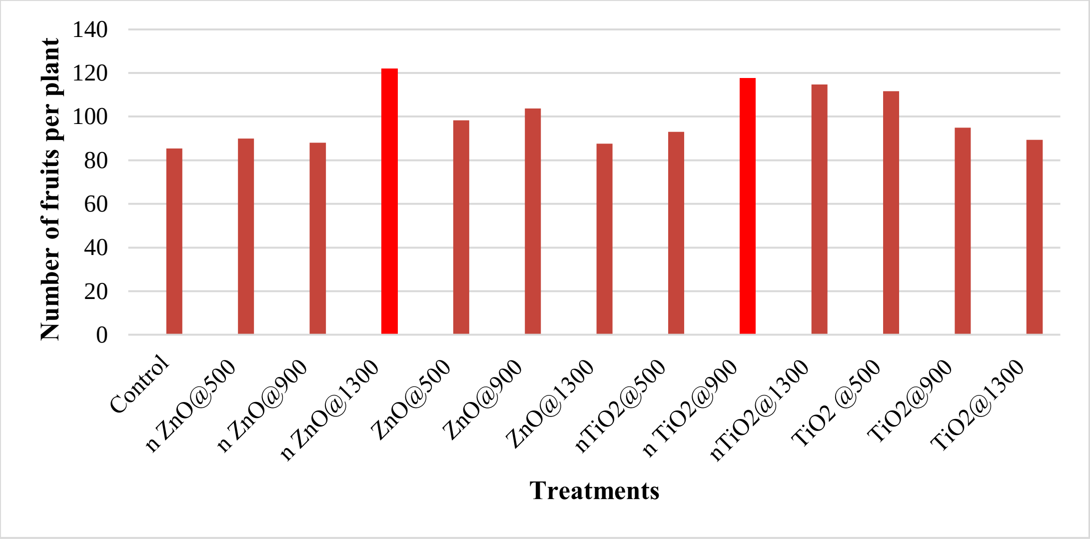


Fig. 3. Influence of normal grade and nano grade ZnO and TiO_2 on number of fruits per plant in chilli

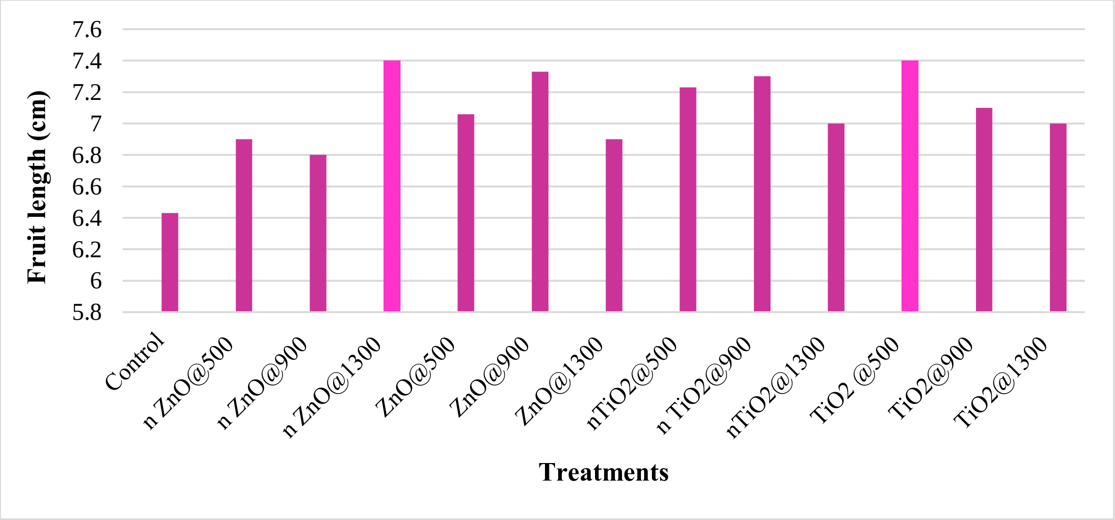


Fig. 4. Influence of normal grade and nano grade ZnO and TiO_2 on fruit length in chilli

5.1.4 Effect of Nano grade titanium dioxide on yield attributes

Significant variations was seen in seeds treated with nano grades of TiO_2. Among the TiO_2 treatments, nanoTiO_2 @ 900 mg kg^{-1} of seed (T_9) recorded highest plant height (66.33 cm), number of fruits per plant (118), seed yield (38.17), 100 seed weight (0.55g) and the least days to harvest (90 DAT). Nano TiO_2 @ 1300 mg kg^{-1} of seed (T_{10}) recorded highest plant spread (56 cm) and 100 seed weight (0.55 g).

An increase in yield and yield promoting traits was observed by Debnath *et al.,* (2020) in rice when treated with nano TiO_2 at 500, 1000 and 2000 mg kg$^{−1}$. Impact of nano TiO_2 on plant characters was observed by Jaberzadeh *et al.* (2013) in wheat and Mattiello and Marchio (2017) in barley. Similar findings on fruit and seed yield attributes was reported by Owolade *et al*., (2008) in cowpea, Moaveni *et al.* (2011) in barley and Khater (2015) in coriander.

5.1.4 Effect of normal grade titanium dioxide on yield attributes

Normal grades of TiO_2 also reported significant effects on yield characters. TiO_2 @ 500 mg kg^{-1} of seed (T_{11}) recorded longest fruits (7.40 cm), fruit yield (394.56), fruit weight (3.53 g) and TiO_2 @ 900 mg kg^{-1} of seed (T_{12}) reported more number of seeds (61) among TiO_2 treatments. This was in confirmation with the observations of Grajkowski and Ochmian, (2007) in raspberry and Haghighi *et al*., (2014) in tomato.

Titanium is involved in plant metabolism by improving absorption of other nutrients like Iron and Magnesium. The major impact of titanium are enhancement of chlorophyll content, improvement of essential elements in tissues and yield in chilli (Hruby *et al*., 2002). Enhancement in plant growth and yield might be due to the action of titanium in several cellular mechanisms. For instance, increase in photosynthetic rate is one of the major reason. (Jabersadeh *et al.*, 2013). The photosynthetic efficiency is increased when light energy is transformed to active electrons in chloroplast by Ti nano particles and thus stimulates Rubisco activase complex. This amplification of photosynthesis promotes yield (Moaveni *et al*., 2011). By the application of nano TiO_2, Rubisco carboxylase activity increased by 2.67 times over control (Gao *et al.,* 2006).

Titanium is not an essential element for growth of plants as per criteria for essentiality by Arnon and Stout (1939) but several studies agrees that Ti have positive impact on plant growth and development. Hong *et al.* (2005) stated that, redox reactions that occur when nano TiO_2 enters the chloroplast, might trigger oxygen evolution and electron transport.

5.1.6 Influence of nanoparticles over bulk treatments

Nanoparticles interact with the living cells at molecular level. They act on plant metabolism by regulating genes, interferes with oxidative process or by providing micronutrients. Nanoparticles have the ability to penetrate the seed coat and results in increased water absorption. Thus seed germination is improved (Hatami *et al*., 2014)

In the above experiment, nanoparticle treatments were found to perform better than normal grade treatments for many of the traits. According to Poornima and Koti (2019), the reason may be due to the increased uptake and translocation efficiency of nano particles over bulk forms.The bulk particles have high solubility and low retention time inside the plant system. Hence, the bioavailability of particles for the uptake are reduced.

As the retention time of nanoparticles are increased inside the plant system, they are translocated to all plant parts. Hence, the contents of the nanoparticles will be available for an extended period to the plant. This stimulates physiological and biochemical processes of plant system (Subbaiah, 2014). A gradual increase of nutrient uptake can be seen as granular size is reduced. Liscano *et al.* (2000) inferred that weight of granules of 1.5 mm is less than 2 mm or 2.5 mm. The use of smaller sized granules for same weight results in better distribution of nutrients and thus better nutrient uptake.

5.2. Influence of nanoparticles on storage life of chilli

Nanoparticles possess smaller particle sizes, higher surface area and increased proportion of reactive surface atoms when compared to bulk particles (Wigginton *et al*., 2007). Such unique properties led to wide range of application in the different fields. Seed treatment with various nanoparticles have the potential to meet crop requirements.

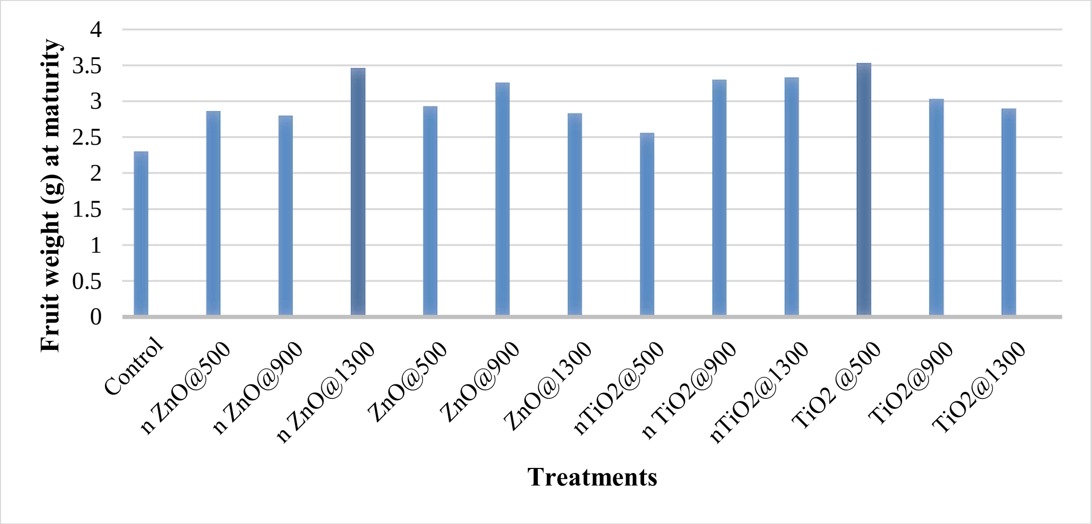


Fig. 5. Influence of normal grade and nano grade ZnO and TiO_2 on fruit weight at maturity in chilli

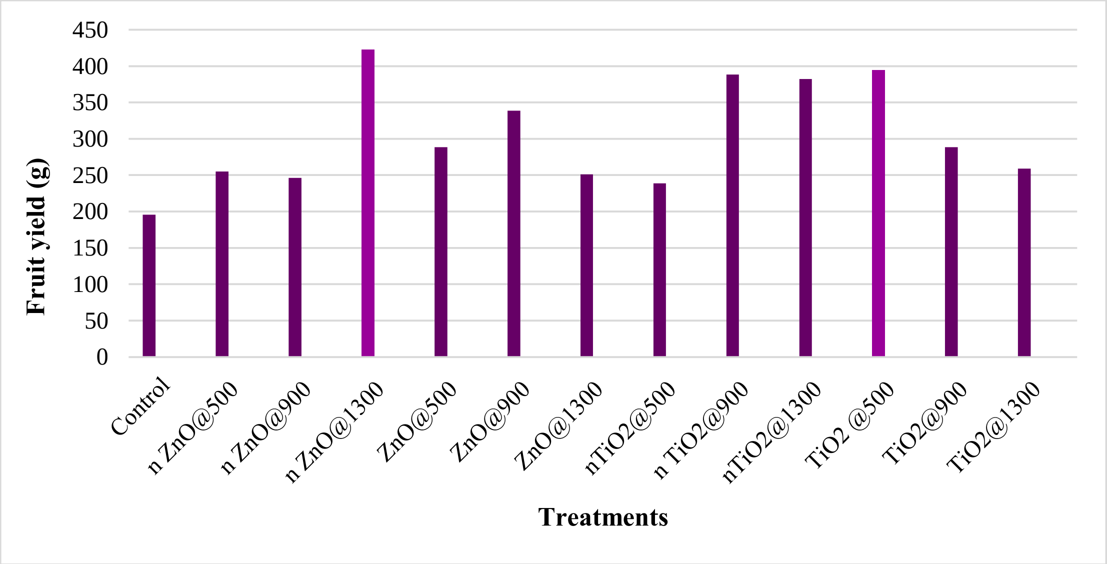


Fig. 6. Influence of normal grade and nano grade ZnO and TiO_2 on fruit yield in chilli

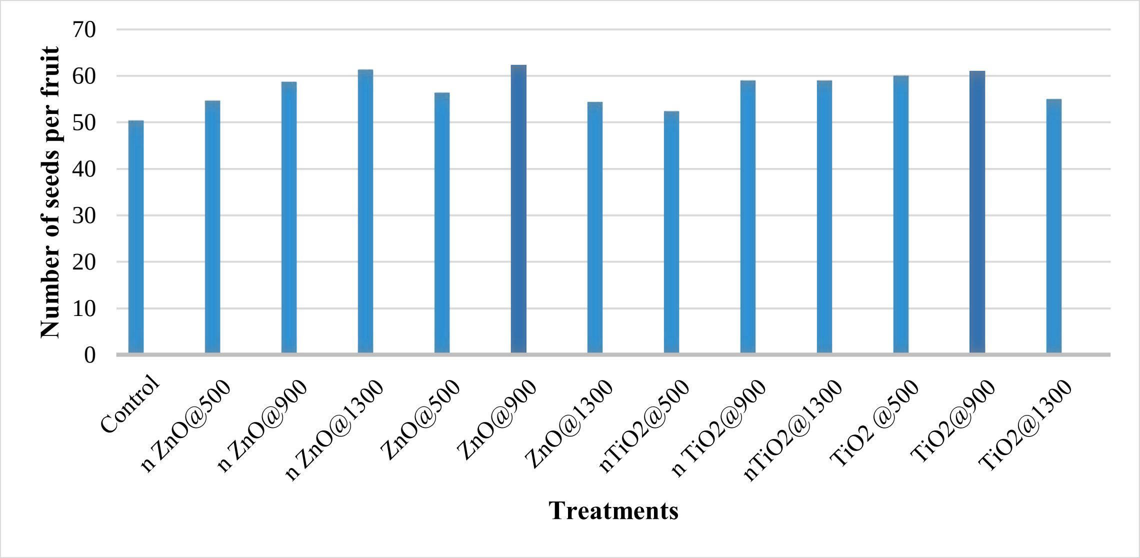


Fig. 7. Influence of normal grade and nano grade ZnO and TiO_2 on number of seeds per fruit in chilli

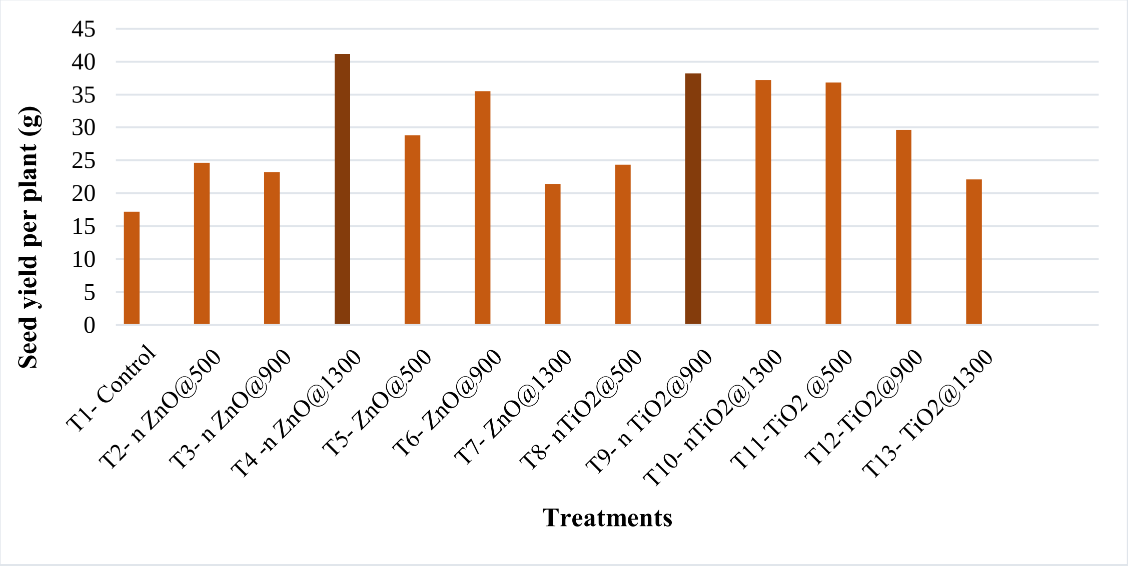


Fig. 8. Influence of normal grade and nano grade ZnO and TiO_2 on seed yield per plant in chilli

It can be applied either by soaking in solution containing specific concentration for specific time period (seed priming) or as seed coating. Seed deterioration is the loss of viability, quality and vigor of seeds either due to adverse environmental conditions or aging of seeds.

The exact reason for loss of viability is unknown, but according to Chiub *et al.* (1995) the primary reason is that, the non-enzymatic peroxidation of free radicles damages the membrane of seeds during storage. Even though seed quality is mainly governed by genetic makeup, seeds may get deteriorated during storage. This occurs due to mechanical damages, changes in temperature and humidity, presence of storage insects and pathogen, seed moisture content etc. during storage. Relative humidity is an important factor that directly influences the moisture content of seeds during storage. Thus physiological and biochemical changes occurs inside the seed and hence seed quality is reduced.

Fluctuating relative humidity and temperature are the major constraints for storage in chilli seeds. High relative humidity increase moisture content in seeds. High temperature along with increased moisture will enhance respiration which triggers deterioration. This results in reduced germination per cent and loss of vigour in seeds at the end of storage period (Barua *et al.,* 2009). Increased moisture may harbour storage pests and pathogens which in turn makes seeds unusable. In case of chilly seeds, it loses viability faster due to free fatty acid accumulation. So it is important to invigorate seeds to maintain viability and vigour throughout the period of storage.

5.3 Impact of seed invigoration using inorganic nanoparticles of zinc oxide and titanium dioxide on seed quality in chilly

5.3.1 Germination per cent

Various doses of nano grades of ZnO and TiO_2 exhibited significant differences in germination per cent throughout the storage period (Table 7). Irrespective of the treatments a decline in germination was seen over the period of storage. These results was in consonance with findings of Nagendra *et al*. (2017) in oriental pickling melon, Athmaja *et al*. (2019) in ash gourd, Navya (2016), Sandhya (2016) and Gayathri (2019) in chilli. The deterioration of seed quality was less in treated seeds compared to untreated seeds. According to Farooq *et al.* (2009) primed seeds show synchronized germination than control.

An increase in germination was seen in the initial month than the readings taken before storage. Gayathri (2019) recorded similar results and explained that the increment is due to the dormancy present in chilli during initial stages of growth.

In the seven months of storage period, all the treatments including control maintained the prescribed 60 percent germination by IMSCS (Indian Minimum Seed Certification Standards) till sixth month. At the end of storage ZnO@100 mg kg^{-1} of seed(T_2), ZnO@250 mg kg^{-1} of seed (T_3), ZnO@500 mg kg^{-1} of seed (T_4), ZnO@1000 mg kg^{-1} of seed (T_6**)**, TiO_2@500 mg kg^{-1} of seed (T_{10}), TiO_2@750 mg kg^{-1} of seed (T_{11}) and TiO_2@1000 mg kg^{-1} of seed (T_{12}) retained germination above 60 % (Figure 9) .

The highest germination of 65.66 % was recorded by nano ZnO @ 250 mg kg^{-1} of seed (T_3). Khanm *et al.* (2017) noticed significant germination (93.33 %) in tomato seeds when treated with nano ZnO at 400 ppm. Nano ZnO may affect the antioxidant system of plants which is important in mitigating the negative impact of reactive oxygen species (ROS) on photorespiration and photosynthesis (Burman *et al.,* 2013). It also increases indole acetic acid levels in roots thus growth rate is increased (Lu *et al*., 2002).

Nano TiO_2 @ 1000 mg kg^{-1} of seed (T_{12}) recorded highest germination (65.33) at the end of storage period. When nano-TiO_2 enter into cells, redox reactions occurs through superoxide ion radical and free radicles will be removed from the germinating seeds. The oxygen thus formed will be used for respiration and germination of seed is promoted (Zheng *et al.,* 2005).

Several studies reported the impact of ZnO and TiO_2 seed treatments in different crops. Meena *et al.* (2017) in maize and Baddar and Unrine (2018) in wheat reported impact of ZnO and Mahmoodzadeh *et al.* (2013) in canola, Haghighi *et al.* (2014) in Tomato, onion and radish and Debnath *et al.* (2020) in rice reported impact of TiO_2.

5.3.2 Seedling length (cm)

Seedling length was significantly influenced by storage period and seed treatments (Table 8). Treated seeds recorded highest seedling length than untreated seeds. Both shoot and root lengths were found to decline in all the treatments throughout the period of storage. Similar findings were reported by Sandhya (2016) and Gayathri (2019) in chilli variety Anugraha.

In case of shoot length, T_{6}- nano ZnO 1000 mg kg^{-1} of seed (5.57 cm) which wqas on par with T_{3}- nano ZnO 250 mg kg^{-1} of seed (5.26 cm), T_{2} - nano ZnO-100 mg kg^{-1} of seed (5.21 cm), T_{11}- nano TiO_2 750 mg kg^{-1} of seed (5.19 cm) and T_{10}- nano TiO_2 500 mg kg^{-1} of seed (5.13 cm) recorded higher shoot length at the end of storage (7 months). Similar observations on effect of ZnO nanoparticles was reported by Narendhran *et al.* (2016) in sesame, Afrayeem and Chaurasia, (2017) in chilli, and Raju and Rai, (2017) in pigeon pea. The impact of titanium dioxide nanoparticles was observed in crops like canola (Mahmoodzadeh *et al.,* 2013), radish (Haghighi *et al.,* 2014) and maize (Vijayalakshmi *et al*., 2018).

Radicle growth was higher in nano ZnO @ 750 mg kg^{-1} of seed (T_{5}) (9.37 cm) followed by T_{6}- nano ZnO@1000 (8.89) at the end of storage period. This was in confirmation with the observations of Pokhrel and Dubey, (2013) in maize, Segatto *et al.* (2018) in maize and sesame and Maity *et al.* (2018) in sorghum on impact of ZnO treatments. Laware and Raskar (2014) in onion and Hajra and Mondal (2017) in chickpea noticed the action of TiO_2 on radicle development.

Mahajan *et al.* (2011) observed that, seedling growth declined at higher concentrations of ZnO. At 20 ppm both root and shoot growth was at its maximum in mung bean and in gram, at 1ppm significant increase in root and shoot growth by 53.13% and 6.38% was recorded respectively. Similarly, Maity *et al.* (2018) also revealed that ZnO at lower doses of 750 mg kg^{-1} was beneficial for growth in sorghum over higher dose of 1000 and 1250 mg kg^{-1}.

Zheng *et al.* (2005) noticed the impact of nano TiO_2 on seedling growth and reported that due to smaller particle size of nano TiO_2 it can be easily penetrated into the seed and enhances the growth in spinach and Clément *et al.* (2013) noted that increment in root growth of flax seeds was due to antimicrobial activity of nano TiO_2.

5.3.3 Dry weight (mg)

Significant differences was seen in dry matter production when treated with nanoparticles of ZnO and TiO_2 in a declining trend. In chilli, Gayathri (2019) reported similar trend when treated with nanoparticles of ZnO and TiO_2.

At the end of storage period, higher dry weight of 22.63 mg 10^{-1} seedlings was recorded in T_6 -nano ZnO 1000 mg kg^{-1} of seed. Similar observations was reported in pigeon pea (Raju and Rai, 2017), chickpea (Burman *et al.*, 2013) and wheat (Rizwan *et al.,* 2018).

In general, irrespective of treatments, as concentration of nanoparticle increased a decline in dry matter production was reported (Burman *et al.*, 2013). According to Zheng *et al.* (2005) as the chlorophyll content, rubisco activity and photosynthetic rate increased dry matter accumulation increased. He concluded that, the particle size and physiology of plants are related.

5.3.4 Vigour indices

Vigour indices of stored seeds declined during the advancement of storage (Table 11, 12). According to Kavitha (2002), when enzyme and amino acid synthesis decreases, loss of seed vigour might occur.

Higher seed vigour index I was recorded by nano ZnO @ 1000 mg kg^{-1} of seed (925.88) followed by T_{10}- nano TiO_2 @ 500 mg kg^{-1} of seed (866.93) at the end of storage (Figure 10). Nano TiO_2 @ 1000 mg kg^{-1} of seed (T_{12}) (1471.81) which was on par with ZnO treatments like nano ZnO@ 250 mg kg^{-1} of seed (T_3) and T_6- nano ZnO @ 1000 mg kg^{-1} of seed (1448.75) maintained higher seed vigour index II at the end of period of storage (Figure 11). Several authors reported similar effects in seeds treated with ZnO and TiO_2 nanoparticles.

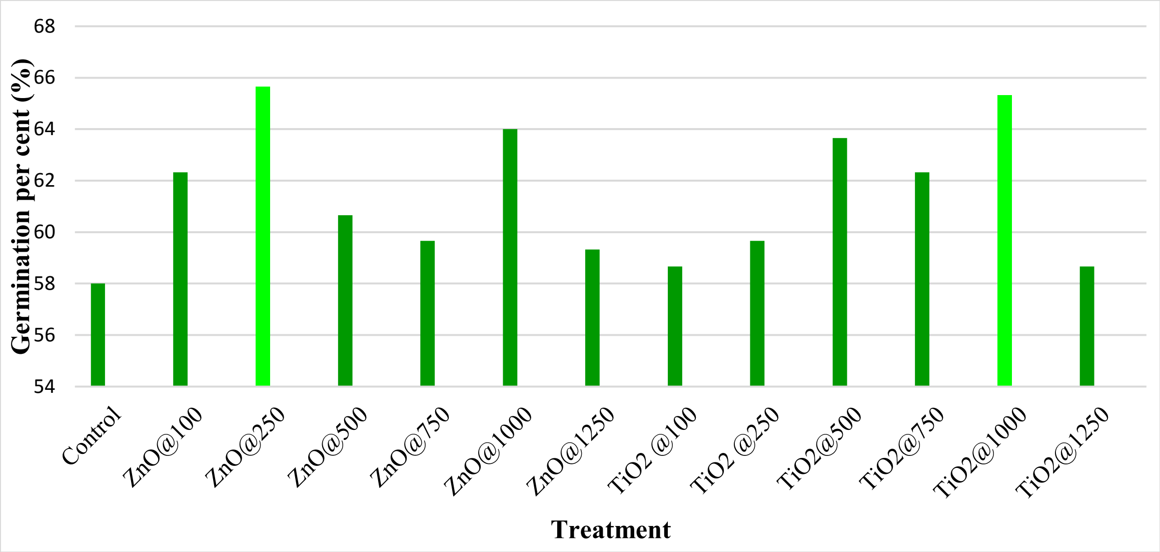


Fig. 9. Influence of nano grade ZnO and TiO_2 in maintaining seed germination after seven months of storage

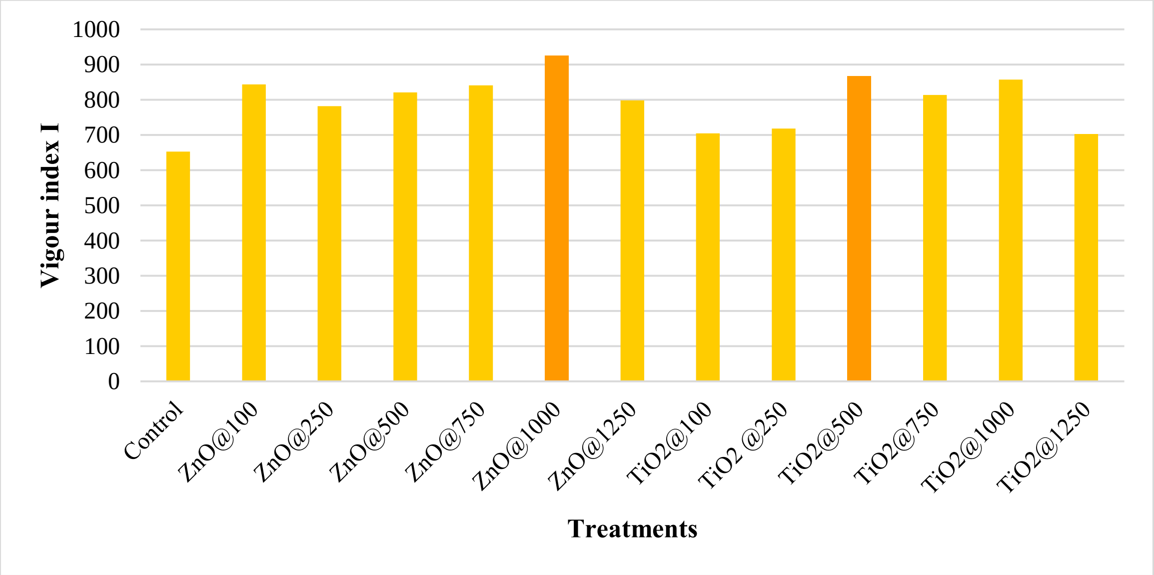


Fig. 10. Influence of nano grade ZnO and TiO_2on vigour index I after seven months of storage

Shyla and Natarajan (2014) in groundnut, Jalill and Yousef (2015) in rice and Maity *et al*. (2018) in cowpea reported the effects of nanoTiO_2 and Korishettar *et al.* (2016) in pigeon pea, Subbaiah *et al*. (2016) in maize and García-López *et al*. (2018) in chilli published the effects of nano ZnO treatments.

According to Kumar *et al.* (2019), nanoparticles enter into seeds through the cracks on surfaces of seeds. These particles will react with the free radicles present in the seeds and thus enhances viability and seed vigour. The reason for loss in seed vigour can be lipid peroxidation, accumulation of reactive oxygen species (ROS) (Bailly, 2004) or increase in free radicle concentration (Khan *et al.*, 1996). The ROS interrupts metabolism of seeds by making damage to proteins and lipids causing physiological and biochemical changes (Mittler *et al.*, 2004).

5.3.5 Electrical conductivity

Electrical conductivity is increased when there is electrolytic leakage due to loss in membrane integrity (Ratajczak and Pukacka, 2005). Significant increase in electrical conductivity was seen throughout the storage period in both ZnO and TiO_2 treatments (Table 13). Among the treatments control recorded highest electrical conductivity (404.66 μScm^{-1}) (Figure 12). Similar observations was recorded in seeds of chilli variety Anugraha by Sandhya (2016) and Gayathri (2019). Irrespective of the treatments and doses nanoparticles acts as a protector from seed deterioration.

According to Murali *et al.* (2002) germination and electrical conductivity are related. With the advancement of storage electrical conductivity increases which germination declines. As the permeability of membranes are lost, leaching of organic acids and sugars increases the conductivity of seeds (Sujatha and Srimathi, 2006). This leakage is more in aged seeds.

5.3.6 Seed moisture content (per cent)

Seed moisture content recorded insignificant differences among the treatments at the end of storage (Table 14). Only a marginal increment in moisture was recorded since 700 gauge polyethylene, moisture and vapour impervious packing was used.

5.3.7 Seed microflora (per cent)

Invasion of microorganisms play a major role in seed deterioration. Seed infection was higher at 7th month of storage than in the beginning of storage. Hence, we can say that pathogens have a role in determining period of storage.

Irrespective of the methods, control seeds showed more infection (Table 14). All the treatments except nano ZnO @ 100 mg kg^{-1} of seeds (T_2), nano TiO_2 @ 250 mg kg^{-1} of seeds (T_9) and control didn't exhibit any infection in agar plate method. While in blotter paper method, only control seeds showed seed infection. *Aspergillus flavus* and *Aspergillus niger* was identified from both agar plate and blotter paper method. Presence of *Aspergillus sp.* in stored seeds was observed in chilli by Sandhya (2016), Navya (2016), Chauhan *et al.* (2018) and Gayathri (2019). The pathogens might cause changes in protein, lipids, carbohydrate and vitamins. Reduction in total oil content, increased free fatty acid, discoloration, abnormal odours and caking of seeds also occurs and resulting in loss of germination.

Both ZnO and TiO_2 nanoparticles are effective in controlling pathogen infections. When the nanoparticles come in contact with the fungal membrane, it hinder the metabolic processes which eventually causes fatality of the pathogen. ZnO nanoparticles disrupt cellular functions and it results in deformation of fungal hyphae (He *et al.*, 2011) and also creates structural changes which causes cytoplasm leakage and death in bacteria (Brayner *et al.,* 2010). TiO_2 nanoparticles causes disintegration and deformation of fungal spores and hyphae (Argawy *et al.*, 2017). They also have anti-bacterial properties similar to ZnO particles (Frazer, 2001). According to Perez-de-Luque and Rubiales (2009) these inhibitory actions are due to the release of metabolites and extracellular enzymes.

In general, wet seed dressing with TiO_2 and ZnO nanoparticles were found to be effective in maintaining seed quality throughout the storage period. Higher doses (1250 mg kg^{-1} of seed) were found to be highly effective. Feizi *et al.*, (2012) noticed that TiO_2 nanoparticles showed promontory effects for seedling length at 2ppm and 10 ppm on wheat and at higher doses neutral effects was observed.

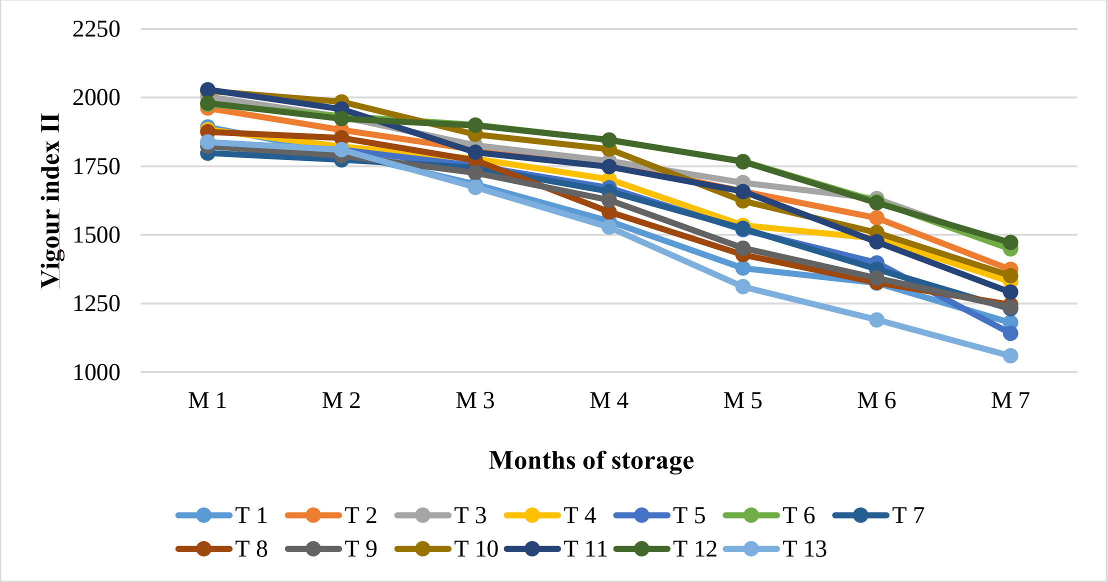


Fig. 11. Influence of nano grade ZnO and TiO_2on vigour index II after seven months of storage

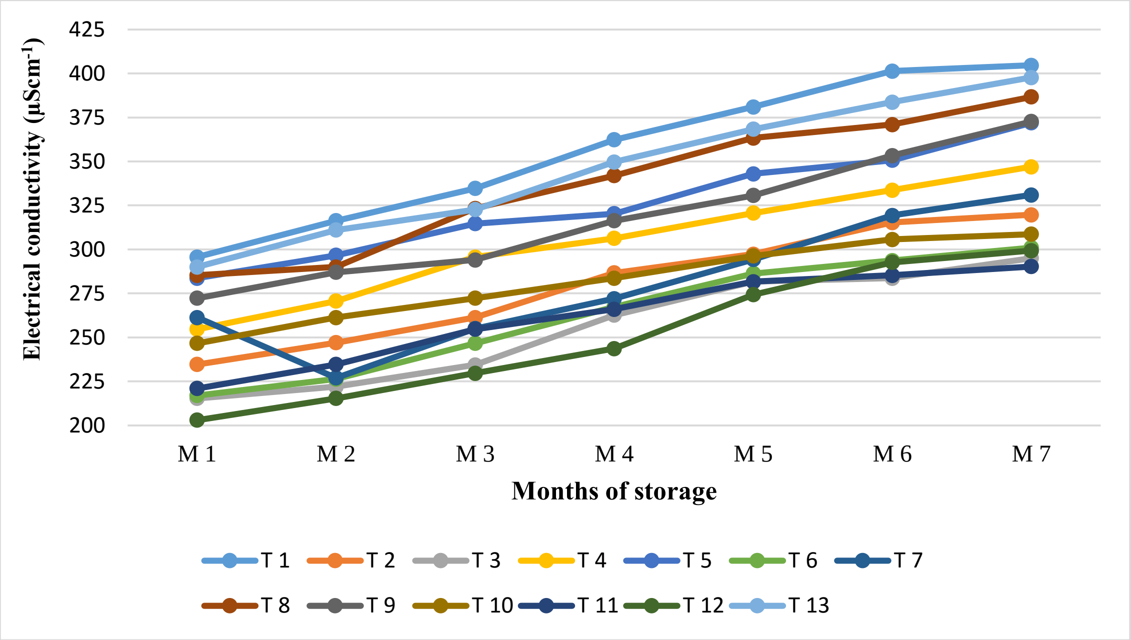
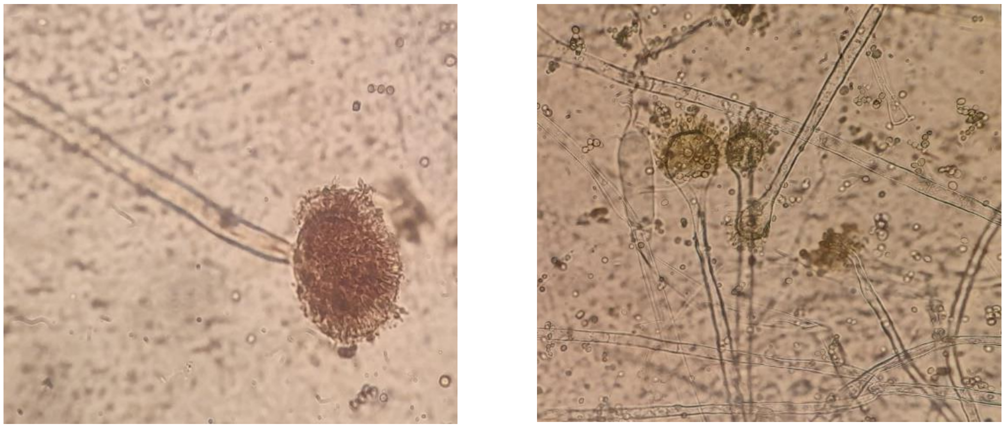


Fig. 12. Influence of nano grade ZnO and TiO_2on electrical conductivity after seven months of storage



Aspergillus flavus

Aspergillus niger

PLATE 6: Microorganisms observed after seven months of storage

FUTURE LINE OF WORK

1. Field performance of seeds wet dressed with nanoparticles is to be ascertained inorder to study its performance on growth parameters.
2. The present study has to be confined to a single variety. Hence it would be advisable to repeat the experiment in other varieties.
3. The study can be extended to ascertain the use of nanoparticle against pest population during storage for effective recommendations.
4. The impact of nanoparticle treatments on biochemical parameters like lipid peroxidation and dehydrogenase activity can be assessed.

Summary

6. SUMMARY

The study "Seed invigoration with nanoparticles for seed yield and quality in chilli (*Capsicum annuum* L.)" was conducted in the Department of Seed Science and Technology, College of Horticulture, Vellanikkara, Kerala Agricultural University, Thrissur. The study was conducted using chilli cv Anugraha. Seed invigoration was done with nano and normal grade ZnO and TiO_2.

6.1 Impact of nano and normal grade ZnO and TiO_2 on seed yield

- The seeds treated with normal and nano grades of ZnO and TiO_2 performed better than untreated seeds. Significant variations was observed for traits such as plant spread, plant height, days to harvest, fruit length, number of fruits per plant, fruit weight at maturity, fruit yield, number of seeds per fruit, seed yield per plant and 100 seed weight.
- Nano ZnO@ 1300 mg kg^{-1} of seed (T_4) performed superior for plant spread, plant height, number of fruits per plant, fruit length, fruit yield and seed yield per plant.
- Normal grade ZnO treatments was effective in improving number of seeds per fruit (ZnO @ 900 mg kg^{-1} of seed).
- Significant effects were recorded when seeds were treated with normal grade TiO_2 @ 500 mg kg^{-1} of seed (T_{11}) for fruit weight at maturity.
- In case of days to harvest, treatments nano ZnO @ 500 mg kg^{-1} of seed (T_2), nano TiO_2 @ 900 mg kg^{-1} of seed (T_9), nano TiO_2@ 1300 mg kg^{-1} of seed (T_{10}), ZnO @ 1300 mg kg^{-1} of seed (T_7) and control recorded the least number of days.
- 100 seed weight of 0.55g was recorded in treatments like TiO_2 @900 mg kg^{-1} of seed (T_9), TiO_2 @ 500 mg kg^{-1} of seed (T_{11}), nano ZnO @ 1300 mg kg^{-1} of seed (T_4), nanoTiO_2 @1300 mg kg^{-1} of seed (T_{10}) and ZnO @ 900 mg kg^{-1} of seed (T_6).

In general, nano grade seed treatments performed better than normal grade treatments in case of ZnO and for TiO_2, both normal and nano grade treatments performed well.

Impact of nano ZnO and TiO_2 on seed storage

- Seeds wet dressed with nano ZnO and TiO_2 were dried and packed in 700 gauge polythene bags and stored for 7 months. The treatments showed significant variations for germination, shoot length, root length, seedling dry weight, vigour indices and electrical conductivity of seed leachate over the period of storage.
- A declining trend was observed for germination, seedling length, dry weight and vigour indices while electrical conductivity and seed infection increased throughout the storage period.
- Nano seed treatments ZnO@100 mg kg^{-1} of seed (T_2), ZnO@250 mg kg^{-1} of seed (T_3), ZnO@500 mg kg^{-1} of seed (T_4), ZnO@1000 mg kg^{-1} of seed (T_6**)**, TiO_2@500 mg kg^{-1} of seed (T_{10}), TiO_2@750 mg kg^{-1} of seed (T_{11}) and TiO_2@1000 mg kg^{-1} of seed (T_{12}) retained germination above 60 % (IMSCS) after seven months of storage and nano ZnO @ 250 mg kg^{-1} of seed (T_3) recorded highest value (65.66).
- Vigour index-I was found to be high in ZnO @ 1000mg kg^{-1} of seeds (T_6**)** (925.88) followed by nano TiO_2 @ 500 mg kg^{-1} of seed (866.93) and vigour index II in TiO_2 @1000 mg kg^{-1} of seed (T_{12}) (1471.81) followed by ZnO @ 250 mg kg^{-1} of seed (1457.58) at the end of storage.
- In case of root length, higher values were recorded by ZnO @750 mg kg^{-1} of seed (9.37 cm) and shoot length by ZnO @ 1000 mg kg^{-1} of seeds (5.57 cm).
- Dry matter production was high in nano ZnO @ 1000 mg kg^{-1} of seed (T_6) (22.41mg).
- Seed treatments at nano ZnO @ 250 mg kg^{-1} of seed (295 µScm$^-$1) followed by nano TiO_2 @ 750 mg kg^{-1} of seed (290.33 µScm^{-1}) were able to maintain lower electrical conductivity of seeds.

- Seed infection in agar plate method was noticed in treatments nanoZnO @ 100 mg kg^{-1} of seeds (T_2) (13.3%) and nano TiO_2 @ 250 mg kg^{-1} of seeds (T_9) (10%) at the end of storage. *Aspergillus niger* and *Aspergillus flavus* were the seed micrflora observed.

Seed treatment with nano ZnO at 1000 mg kg^{-1} of seed was found to be effective in retaining seed longevity.

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SEED INVIGORATION WITH NANOPARTICLES FOR SEED YIELD AND QUALITY IN CHILLI (*Capsicum annuum* L.)

by

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ABSTRACT OF THE THESIS

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ABSTRACT

The study "Seed invigoration with nanoparticles for seed yield and quality in chilli" was conducted in the Department of Seed Science and Technology, College of Horticulture, Vellanikkara during May 2019 with the objective of standardizing the optimum seed treatment dose required for increasing yield, improving quality and prolonging seed longevity. Normal grade and nano grade zinc oxide (ZnO) and titanium dioxide (TiO_2) were used for seed treatment on chilli variety Anugraha.

The study consisted of two experiments. In the first experiment, chilli seeds dry dressed with 500, 900 and 1300 mg kg^{-1} of normal and nano grade ZnO and TiO_2 along with control (thirteen treatments) were raised in a Randomized block design with three replications. While all treated seeds performed better than control (untreated seeds) treatments with nanoparticles performed better than the normal grade particles. Highly significant variations was observed for traits such as plant spread, plant height, days to harvest, fruit length, number of fruits per plant, fruit weight at maturity, fruit yield, number of seeds per fruit, seed yield per plant and 100 seed weight.

Seed treatments with nano ZnO @ 1300 mg kg^{-1} of seed (T_4) performed superior for plant spread (60.1 cm), plant height (67.30 cm), number of fruits per plant (122), fruit length (7.40cm), fruit weight (3·46g), fruit yield (422.70 g) and seed yield per plant (41.14 g). Normal grade ZnO treatments was effective in improving number of seeds per fruit (62) (ZnO @ 900 mg kg^{-1} of seed). Significant effects were recorded when seeds were treated with normal grade TiO_2 @ 500 mg kg^{-1} of seed (T_{11}) for fruit weight at maturity (3.53 g).

In the second experiment the effect of wet seed treatment with nanoparticles on seed storability was assessed. The experiment was laid out in Completely Randomized Design with thirteen treatments in three replications. Treatments include nano ZnO and TiO_2 at 100,250,500 and 100 mg kg^{-1}. Seed quality parameters like germination, seedling length, dry weight and vigour indices showed a declining trend throughout the storage period while electrical conductivity and seed infection increased. Treatments ZnO@100 mg kg^{-1} of seed (T_2), ZnO@250 mg kg^{-1} of seed (T_3), ZnO@500 mg kg^{-1} of seed (T_4), ZnO@1000 mg kg^{-1} of seed (T_6), TiO_2@500 mg kg^{-1} of seed (T_{10}),

TiO_2@750 mg kg^{-1} of seed (T_{11}) and TiO_2@1000 mg kg^{-1} of seed (T_{12}) retained germination above 60 % (IMSCS) at the end of seven months of storage. ZnO @ 250 mg kg^{-1} of seed (T_3) retained the highest germination per cent of 65.66 %.

Vigour index-I was found to be high in ZnO @ 1000mg kg^{-1} of seeds (T_6**)** (925.88) and vigour index II in TiO_2 @1000 mg kg^{-1} of seed (T_{12}) (1471.81) at the end of storage.The electrical conductivity of seed leachate obtained from nano ZnO @ 250 mg kg^{-1} of seed and nano TiO_2 @ 750 mg kg^{-1} of seed recorded the lowest values (295μ$Scm^{-1}$and 290.33 μScm^{-1}).Pathogen infection in seeds were found to be lower in treated seeds than in untreated control. Infection was recorded in nano ZnO @ 100 mg kg^{-1} of seeds (T_2) (13.3%) and nano TiO_2 @ 250 mg kg^{-1} of seeds (T_9) (10%) treatments. The seed storage fungi observed were *Aspergillus niger* and *Aspergillus flavus*.

Seed treatments with inorganic nanoparticles are effective in improving field performance and as well as retaining seed quality in storage. Nano ZnO at 1300 mg kg^{-1} of seeds was the best treatment followed by nano TiO_2 at 900mg kg^{-1} of seeds in improving yield in chilli. Treatments nano ZnO at 250 and 1000 mg kg^{-1} of seed may be used as seed treatments to enhance seed longevity in chilli.

Appendix-I

Monthly meteorological data from June 2019 to March 2020

Months	Temperature (^{0}C)		Relative Humidity (%)	Rainfall (mm)	Rainy days	Mean sunshine hours (hrs/day)	Wind speed (kmph)
	Mean Maximum	Mean minimum					
June 2019	32.2	23.5	83	324.4	15	3.7	1.7
July 2019	30.4	22.8	85	654.4	21	2.6	1.7
August 2019	29.5	21.9	89	977.5	24	1.5	1.5
September 2019	31.2	22	85	419.0	19	3.3	1.4
October 2019	32.4	21.4	80	418.4	16	5.5	1.8
November 2019	32.9	21.7	71	205.0	5	7.5	4
December 2019	32.3	22.1	63	4.4	1	6.7	8.7
January 2020	34.1	22.4	60	0	0	9.4	5.9
February 2020	35.5	23.2	54	0	0	9.5	5.3
March 2020	36.4	24.4	65	33.4	2	8.5	2.8