Seed invigoration with nanoscale particles in chilli (*Capsicum annuum* L.)

By

Adarsh P V

(2020-11-023)

DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR- 680 656

KERALA, INDIA

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DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

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2023

DECLARATION

I hereby declare that the thesis entitled **Seed invigoration with nanoscale particles in chilli (*Capsicum annuum* L.)** is a bonafide record of research done by me during the course of study and the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara
Date:

Adarsh P V
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CERTIFICATE

Certified that this thesis entitled **Seed invigoration with nanoscale particles in chilli (*Capsicum annuum* L.)** is a record of research work done independently by **Mr Adarsh P V (2020-11-023)** 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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CERTIFICATE

We, the undersigned members of the advisory committee of **Adarsh P V (2020-11-023)**, a candidate for the degree of **Master of Science in Agriculture**, with a major field in **Seed Science and Technology**, agree that this thesis entitled **Seed invigoration with nanoscale particles in chilli *(Capsicum annuum* L.)** may be submitted by **Adarsh P V (2020-11-023)**, in partial fulfilment of the requirement for the degree.

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Adarsh P V

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

Introduction

1. INTRODUCTION

Chilli (*Capsicum annuum* L.), belongs to the nightshade (Solanaceae) family. Its centre of origin is Mexico (South America), and was introduced by Portuguese traders to India by the end of the 15th century.

Both dried and green chilli fruits are inevitable in the Indian diet. In India, green chilli is cultivated in 417.8 thousand hectares with a production of 450.4 thousand MT. The average productivity of green chilli in India is 10.8 MT/ha with Andhra Pradesh leading the production 495270 MT. In Kerala, 1720 ha of land is cultivated with an annual production of 7650 MT. The average productivity of chilli in Kerala is 4.45 MT/ha (Indiastat, 2022).

Indian vegetable seed industry is developing at a rapid phase. Since independence, Government of India policies has liberalized and encouraged the seed trade in India. In India, a several private seed companies with global bases are actively involved in the manufacturing of vegetable seeds, leaving the public sector well behind. Constrains like high cost of seed production has increased price of the chilli seeds. Vegetable seeds especially hybrid seeds are expensive due to the involvement of more labour and other inputs. (Sudha *et al.*, 2006). The market's demand for vegetable seeds is uncertain. Compared to cereal seeds, the surplus cannot be consumed by humans. As a result, excessive vegetable seed production will result in significant economic loss (Sharma, 2011). Modern agriculture, which places a major emphasis on technology and precision, expects that each and every seed should germinate and produce a vigorous seedling.

The most important part of seed production is the maintenance of seed vigour and viability from harvesting to planting. Hence seed storage environment is a key component of seed production. Seeds germinate slowly both in normal and stress condition (Wein, 1999; Demir and Okcu, 2004). The non-starchy endosperm in the seeds serves as a mechanical barrier to the developing embryo, inhibiting the germination process (Andreoli and Khan, 1999). Even if they had reached the essential physiological growth to promote germination, the seeds exhibit varying degrees of dormancy that prevent germination. Slow germination can increase the susceptibility of seeds and young seedlings to infections (Samarah *et al.,* 2016). Due to its slow germination seed producers may face the problem of infection of the seed which results in low seed yield and quality. The external agents that effect seed quality during storage are seed moisture, relative humidity and seed microflora. High temperature and relative humidity, which are typical to Kerala, encourage quick absorption of moisture,and hence,the storage life of chilli seeds is ultimately shortened under normal storage condition. Although seed deterioration is an irreversible and inevitable process, it can be slowed down as far as possible either by storing the seeds in a controlled environment or by seed treatement. Seed treatment continues to be the greatest alternative strategy to retain the seed quality because controlled conditions are extremely expensive. A variety of chemicals are used to prime the seeds in order to stop or slow down seed deterioration during storage and increase seed storage life (Basu, 1993). Similarly mid-storage seed invigoration treatments which leads to an improvement in physiological status of seed, improved germinability and greater storability of seeds (Basu, 1994). Mid- storage treatments are highly warranted since they improve the germination of the unutilized seed stock and also prolong its storability (Renugadevi *et al*. 2006). These treatments invigorate low vigour seeds to achieve better emergence.

Nanotechnology, is an emerging field of science, with a great potential to improve agriculture. Nano particles have unique traits such as a high surface area to weight ratio, the capacity to penetrate cells, and high adsorption .They have proved to enhance the germination, seedling vigour, and physiological parameters of seeds in many crop plants. Nanoparticles, acting as antioxidants, are an effective scavenger of free radicals and suppress the propagation of lipid peroxidation.

Zinc is a micro nutrient involved in many aspects of plants metabolic and structural process such as photosynthesis, respiration, the production of chlorophyll and chloroplast function (Zulfiqar *et al.,* 2020). Recent studies have demonstrated ZnO efficacy as a fungicide due to their ability to prevent the growth of disease-causing fungi in plants. The seed invigoration treatments, such as seed priming and coating with ZnONPs, are the application modes that are regarded as being relatively environmentally friendly and economically viable. These seed treatments involve adding ZnONPs in low concentrations, which adsorbs on the surface of the seed coat, and it penetrates and move through the seed tissue layers, greatly enhancing the germination and crop biomass. These reports show a clear trend toward the creation of nano-based seed invigoration treatments, possibly as a result of bio- and eco-safety concerns associated with the handling and foliar application of the nano-Zn particles, which could be better addressed by seed invigoration methods. The use of nanoparticles depends greatly on their particle size, so it is important to determine the appropriate concentration and size before using them because high doses could stunt the growth of seedlings (Neto *et al.,* 2020).

The present study entilted 'Seed invigoration with nanoscale particles in chilli (*Capsicum annuum* L.)' was to access the following objectives:

1. To elucidate the effect of zinc oxide nanoparticles as a presowing seed treatment on the growth and yield of chilli.
2. To elucidate the effect of zinc oxide nanoparticles as a prestorage seed treatment on the growth and yield of chili.
3. To assess the effect of nanoparticles as mid storage treatment in chilli

Review of literature

2. REVIEW OF LITERATURE

Zinc is an essential micronutrient that is part of six different classes of enzymes. It is involved in many biochemical and physiological processes (Broadley *et al*., 2007). Zinc deficiency in plants leads to stunted growth, chlorosis of leaves, small leaves and spikelet sterility. In India, zinc has been recognized as the fourth most important yield-limiting nutrient after nitrogen, phosphorus and potassium. Zinc sulphate is the most commonly used zinc fertilizer and is available in both crystalline monohydrate ($ZnSO_4.H_2O$) and heptahydrate ($ZnSO_4.7H_2O$). Zinc sulphate is insoluble in water and is costly when compared with Zinc Oxide which is water soluble.

Zinc can be applied to plants through different methods such as soil application, foliar spray, and seed treatment (Tondey *et a*l., 2021). In the soil application method, the nutrient applied may get fixed or precipitated, thus making them unavailable to plants (Rengel 2015).

Foliar application is effective but only low quantities can be applied as high doses cause foliage damage. Therefore, multiple application is required at critical growth stages of the crop, making it time and labour-intensive. Thus, the best option is seed treatment. Seed priming with Zinc positively affects seed vigour, early seedling growth, germination, growth and biomass production (Mondal and Bose 2019; Nciizah *et al.,* 2020).

Nanotechnology has the potential to improve agriculture. Nanoparticles (NPs) with a size of 100 nm (or less) have unique physicochemical properties with high surface area, high reactivity and tunable pore size and particle morphology (Dileepkumar,2015). Seed nano invigoration involves seed surface treatment with nano-formulation of active ingredients which is expected to exhibit a positive effect on seed metabolism and signaling pathways (Pereira *et al.,* 2021)

Zinc oxide is regarded as one of the most important materials among nanoparticles because of its stability, non- toxicity, low cost of manufacture, and environmental friendliness. The physiochemical properties of ZnO nanoparticles allow them to penetrate the plant tissue and interfere with several metabolic processes. Treated seeds show greater seed metabolic efficiency. According to Mirales *et al.,* (2012), ZnO can pierce the seed coat and induce embryonic differentiation by activating enzymes involved in seed dormancy breakdown. Zinc oxide nanoparticles not only enhance seed germination but they also improve seed longevity (Gayathri,2019)

In this chapter, the impact of seed invigoration with nanoparticles has been briefly examined for its role in crop growth and yield and for enhancing seed longevity under the following heads

2.1 Effect of nanoparticle seed treatment on crop growth and yield

2.2 Effect of nanoparticle seed treatment on seed quality as midstorage correction

2.3 Seed infection in chilli

2.1 Effect of nanoparticle seed treatment on crop growth and yield

2.1.1 Days to 50 percent flowering

Crop	Details of the experiment	Reference
Solanaceae		
Tomato	Lesser days for 50 percent flowering (38.87days) was observed when seeds were treated with Nano Zn at 800 ppm suspension.	Sharma *et al.*, 2022
Chilli	Treatment with 0.5 g kg^{-1} nanopowder of custard leaf r resulted in lesser number of days to flowering by eight days while treated plants floered by 79th day control flowered only after 87 days.	Sandhya, 2016
Other crops		
Onion	30 µg $ml^{-1}$of ZnO NP treatment initiated flowering by 51.44 when compared with control (66.28days)	Laware and Raskar, 2014

Strawberry	Treatment (150 ppm ZnO NPs + 150 ppm FeO NPs) resulted in early flowering (59.40days) whereas, maximum days to 50 percent flowering was observed in control (66.44 days).	Kumar *et al*., 2017
Gerbera	The shortest time taken for flowering was 62.25 days in Nano SiO_{22} treatment and while it took 81.08 days to flower in control.	Alikhani *et al*., 2021

2.1.2 Plant height

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Nano ZnO @ 1300 mg kg^{-1} treatment resulted in the highest plant height of 67.3 cm while the least was recorded in control(60.33cm)	Mathew *et al*., 2021
Tomato	Plants treated with 250mg kg^{-1} of Titanium Oxide and Zinc Oxide recorded 24.5 percent increase in plant height as compared to control.	Raliya *et al*., 2015
Other crops		
Wheat	There were significant differences in plant height when seeds were treated with 0.01%, 0.02%, 0.03 % titanium oxide nanoparticle .	Jaberzadeh *et al*., 2013
Sorghum	Nano ZnO 500 ppm spray recorded significantly higher plant height (186.7cm) compared to control (112.33cm)	Poornima and koti, 2019
Wheat	Plant height increased by 37 percent and 35 percent over control at 100 mg L^{-1} ZnO NPs and 20 mg L^{-1} Fe NPs, respectively	Rizwan *et al*., 2019
Wheat	Compared to the control, plant height increased when ZnO NPs concentration in the seed priming solution increased to 100 mg L^{-1}.	Popovic *et al*., 2020
Watermelon	Maxima and Riverside cultivars showed a considerable increase in the length of the main vine in the Ag NP-treated plants compared to the other treatments.	Acharya, 2020
Pearl millet	Plants treated with 20mM Ag NP recorded an increase in growth and biomass	Khan *et al*., 2020
Fodder maize	ZnO NPs at 20 mgL^{-1} significantly increased plant height when compared to $ZnSO_4$ NPs, bulk and control treatments	Tondey *et al*., 2021

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Compared with control(85.33), the nano particle seed invigoration treatment performed well. Among treatments, nano ZnO @ 1300 mg kg-1 recorded the highest fruits per plant (122)	Mathew *et al*., 2021
Tomato	Compared to control, the $FeSO_4$ 5 percent treatment enhanced the number of fruits.	Sakya and Sulandjari, 2019
Solanum lycopersicum	Fruit yield and lycopene content were maximum at 100 $mgkg^{-1}$ when compared to control.	Raliya *et al*., 2015
Other crops		
Peanut	ZnO dose of 1000ppm seed dressing increased the number of pods per plant in peanuts.	Manjumdar and Venkatesh, 2001
Cowpea	Double-dose treatments with TiO_2 at 125 cc/ha produced more pods per plant (33.33) than single-dose treatments (27.33).	Owalde and Ogunleti, 2008
Peanut	When compared to chelated $ZnSO_4$, nanoscale ZnO particles reported 29.5 percent and 26.3 percent higher pod yields at a 15 times lower dose.	Prasad *et al*., 2012
Bitter melon	Carbon nanoparticle treatments led to an increase in fruit number by up to 59 per cent when compared to control.	Kole *et al*., 2013
Fennel	By applying 6 ppm of TiO_2 NPs, a rise in fruit number per plant, was observed.	Khater and Osman, 2015
Chickpea	Nano-iron chelate fertilizer treatment increased pod number per plant by 48 compared to control.	Valadkhan *et al*., 2015
Mung bean	Pods per plant increased to (24) pod per plant in combined Ag and Zn nanoparticle treatment at 20 and 6 ppm concentrations respectively compared to controlb(14.30).	Wasaya *et al*., 2020
Grain cowpea	Seeds primed in $ZnSO_4$ 0.05% for 4 hours recorded higher number of pods per plant (24.13) compared to control (11.06)	Raj *et al.,* 2021

2.1.3 Fruits per plant

2.1.4 Fruit length

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	In comparison to the control, the nano particle seed invigoration treatment performed well. Among the treatments, nano ZnO @ 1300 mg kg-1 recorded the highest fruit length (7.40 cm) compared to control (6.05cm)	Mathew *et al*., 2021
Other crops		
Maize	The longest cob length, 16.40 cm, was obtained from seeds treated with nano ZnO @ 400 ppm, 18 percent longer than control.	Subbaiah, 2014
Barley	At a concentration of 2000 ppm, foliar spraying of nano-fertilizer (ZnO and Fe_2O_3) and nano-titanium dioxide ($nTiO_2$) solutions in barley were found to be effective in grain mass spike length.	Janmohammadi *et al*., 2016
Wheat	Spike length increased in treatment with nano particles at higher concentrations	Rizwan *et al*., 2019
Wheat	Compared to the control, spike length increased when ZnO NPs concentration in the seed priming solution was increased (up to 100 mg L^{-1}).	Popovic *et al*., 2020
Grain cowpea	Seeds primed in $ZnSO_4$ 0.05% for 4 hours produced higher pod length (17.47 cm) compared to the control (14.99)	Raj *et al.,* 2021

2.1.5 Fruit weight at maturity

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Compared to the control, the nano particle seed invigoration treatment performed well. Among treatments nano ZnO @ 1300 mg kg^{-1} recorded the highest fruit weight (3.46g) control (1.04g)	Mathew *et al*., 2021
Tomato	Compared to the control, the $FeSO_4$ 5 percent application enhanced the weight of fruits.	Sakya and Sulandjari, 2019

Other crops		
Borage (*Borago officinalis*)	Treatment with AgNPs from 20 to 60 ppm resulted in higher yield when compared with control.	Seif *et al.* 2011
Carrot	ZnO and FeO nanoparticles were administered as foliar sprays in various levels of 50 ppm, 100 ppm, and 150 ppm in carrot variety Pusa Rudhira. Treatments with nano ZnO @ 1000 ppm and nano FeO @ 50 ppm resulted in a maximum fruit weight of 72.33 g.	Elizabeth *et al*., 2017
Maize	Application of nano ZnO (0.16 mg/seed) particles in maize recorded the highest cob weight and enhanced number of rows per cob over the control	Estrada-Urbina *et al.*, 2018

2.1.6 Fruit yield

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Nano ZnO @ 1300 mg kg^{-1} of seed recorded highest fruit yield per plant (422.70g) compared to control 195.50g	Mathew *et al*., 2021
Other crops		
Carrot	Nanoparticles were applied in variety Pusa Rudhira as foliar spray at different doses.Maximum fruit yield was obtained when treated with nano ZnO @1000 ppm along with nano FeO @ 50 ppm	Elizabath *et al*,. 2017
Rice	Seeds of variety Tarom Hashemi were treated bulk Zinc and nano Zinc. Nano Zn treatment resulted in the highest grain yield than control by 12.6 per cent	Kheyri *et al*., 2019

2.1.7 Seed yield per plant

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	When compared to control(17.96g), the nano particle seed invigoration treatments performed	Mathew *et al*., 2021

	well. Among treatments nano ZnO @ 1300 mg kg^{-1} recorded highest seed yield (41.14 g).	
Other crops		
Borage (*Borago officinalis*)	Treatment with AgNPs from 20 to 60 ppm resulted in higher yield when compared to control	Seif *et al.* 2011
Green gram	Seeds primed in $ZnSO_4$@ 0.05 percent for 4 hours recorded the highest seed yield (1446 kg ha^{-1})	Usman *et al*., 2014
Pearl millet	When treated with nanoparticles, grain yield in variety HHB67 was increased by 37.7 percent compared to the control (1065 $kgha^{-1}$)	Tarafdar *et al*., 2014
Cowpea	Seeds primed in ZnSO4 10^{-3} M solution recorded higher seed yield (1100.5 kg ha^{-1}).	Arun *et al*., 2017
Sorghum	Higher grain yield was recorded in 500 ppm Nano treatment foliar spray (63unit) as compared to 1000 ppm bulk treatment (57.5unit).	Poornima and Koti, 2019
Mung bean	Combined Ag and Zn nanoparticle treatment at 20 ppm and 6 ppm concentrations recorded higher yields when compared to the control	Wasaya *et al*., 2020
Soybean	Compared to other treatments, nano ZnO @160mg/kg registered the highest seed yield.	Yusefi -Tanha *et al*., 2020

2.1.8 Seeds per fruit

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	In comparison to the control, the nano particle seed invigoration treatment performed well. Among treatments nano ZnO @ 1300 mg kg-1 recorded the highest seeds per fruit (62)	Mathew *et al*., 2021
Arabdopsis thaliana	The average number of seeds per pod increased to 57$\pm$ in plants treated with 10g/ml Gold NP, compared to 43$\pm$ in control plants	Kumar *et al*., 2013
Other crops		
Maize	The number of grains per row in maize seeds exposed to nano ZnO at 400 ppm was higher (38.5), which was 36% more than in the control treatment.	Subbaiah, 2014
Chickpea	Nano-iron chelate fertilizer, increased seed number per pod by 17 percent when compared to	Valadkhan *et al*., 2015

	the control	
Fodder cowpea	Cu nano particle at 30 ppm greatly enhanced the, the number of grains per pod	Srinivasan *et al*., 2017
Rice	Nano ZnO 5gL $^{-1}$ treatment gave higher number of seeds (294) when compared to other nanoparticle treatments	Bala *et al*., 2019
Grain cowpea	Seeds priming with $ZnSO_4$ 0.05% for 4 hours resulted in higher number of seeds per pod (16.0)	Raj *et al.,* 2021
Soybean	In comparison to untreated plants seeds treated with 200ppm nano Fe_3O_4 solution recorded an increase in the number of seeds per pod by 43.27percent.	Dola *et al*., 2022

2.1.9 100 Seed weight

Crop	Details of the experiment	Reference
Other crops		
Cowpea	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)	Owolade and Ogunleti , 2008
Peanut	Different doses of both bulk and nano ZnO were applied to seeds of the variety K-134. The seed treatments were administered at doses of 2g/ L and 13g/L. Treatments at the nanoscale performed better than bul k treatments. The maximum 100 kernel weight was re ported on seeds treating with nano ZnO @ 2g /15 L $^{-1}$, which was 36.25g.	Prasad *et al*., 2012
Wheat	50 ppm SNP produced maximum 100 grain weight (4.73) followed by 25 ppm (4.66) against control (3.25).	Jhanzab *et al*., 2015
Fodder cowpea	Cu nano particle at 30 ppm greatly enhanced the the weight of 100 grains,.	Srinivasan *et al*., 2017

2.2 Effect of nanoparticle seed treatment on seed quality as midstorage correction

2.2.1 Midstorage correction

Seed is a biological entity deterioration of seed is unavoidable beyond physiological maturity until it dies or next planting. Loss of seed viability is a major problem facing seed industry and any effort to enhance storability is a boon to seed. The deterioration is slowed down by advocating mid- storage treatments.

Mid-storage seed invigoration treatments are physiological procedures that aim to improve the physiological status of seeds, leading to improved germination, increased storability, and improved performance over the corresponding untreated seeds (Basu, 1994). This approach differs from traditional pre-sowing treatment, which involves quick soaking and drying treatments of stored seeds that allow sufficient time gap before sowing. The treatments works well for crop viability maintenance and improving crop productivity (Mandal and Basu,1986).

There are many postulates regarding the mode of action of hydration, dehydration midstorage treatment. Age-induced biochemical lesions that occurred during the previous dry storage are repaired by cellular enzymatic repair system during the period of hydration.This repair would counteract the normal deteriorative process of ageing and explain their prolonged survival in storage compared to dry seeds(Villiers and Edgcumbe, 1975) and minimization of free radical reactions by counteraction of lipid peroxidation (Wilson and McDonald, 1986 and McDonald, 1999).Ward and Powell(1983) opined that such cellular repair mechanism operate favourably during hydration in seeds which has previously undergone some deterioration. According to Villiers and Edgcumbe (1975), repairing essential bio-organelles in fully imbibed seeds would increase the seed's storage life. Damaged molecules would build up in dry stored seeds in the absence of a repair mechanism. According to Villiers (1974), when dry lettuce seeds were fully imbibed, both the senescence and the damage to the chromosomes and membranes were clearly reversed. Villiers and Edgcumbe (1975) noted that in fully imbibed seeds, repair of essential bio-organelles would occur, increasing the seed's storage life. With dry stored seeds, molecular damage would build up in the absence of a repair mechanism. According to Villiers (1974), when dry preserved lettuce seeds were fully imbibed, both the senescence and the damage to the chromosomes and membranes were clearly undone.

A decrease in lipid peroxidation reactions may be related to the effectiveness of hydration dehydration treatments in preventing seed damage induced by ageing and radiation exposure (Rudarpal and Basu, 1982). Using artificial ageing, Jeng and Sung (1994) discovered that peanut (*Arachis hypogaea* L.) seed lipid peroxidation increased and that more peroxides subsequently accumulated. Hence, it is suggested that the beneficial effect of hydration and dehydration leads not only to cellular repair but also to the control of free radical reactions. In the case of lima, Ramamoorthy *et al* (1989) suggested that four month old seed, when soaked in water for 30 minutes, maintained viability. Savino *et al*., (1979) reported that when the low quality seed was subjected to hydration treatment through chemicals or water,it resulted in improved vigour and viability which might be due to the cellular repair mechanism.

Dollypan and Basu (1985) opined that mid storage hydration treatment in carrot for two hours effectively reduced physiological deterioration in storage and showed better field emergence. Srinivasan and Saxena (2001) reported that priming on partially aged seeds of radish cv. Chinese pink improved seed longevity for up to 10 months when stored at ambient condition

2.2.2 Seed quality assessment

2.2.2.1 Germination

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	After ten months of storage, seeds treated with nano-TiO_2 at concentrations of 500 mg/kg, 900 mg/kg, 1300 mg/kg, and 500 mg/kg maintained 60 percent germination.	Gayathri, 2019
	Seeds when treated with bulk ZnO at 130mgkg^{-1} and 500 mgkg^{-1} ZnO nanoparticles exhibited high performance in maintaining germination above 60 percent at the end of 10 months of storage.	Gayathri, 2019
	Seeds were dry dressed with 750, 1000,and 1250mg Kg^{-1} nano and bulk ZnO and TiO_2 respectively and highest germination was recorded by nano ZnO at 1000mgKg^{-1}	Kumar *et al* ., 2019
Nicotiana tabacum	265 - 530 µmol L-^{1}of Se NPs stimulated plant germination and growth.	Domokos-Szabolcsy *et al*., 2012
Other crops		
Ground nut	It was reported that seeds treated with ZnO NPs at a dose of 1000 mg kg^{-1} increased germination (77%)	Shyla and Natarajan 2014
Fenugreek	20µg mL^{-1}of Ag NPs showed the highest germination (78per cent) over the control (64.44 per cent)	Hojjat and Hojjat 2015
Onion	Zinc oxide (ZnO), silver (Ag), copper oxide (CuO), and titanium oxide (TiO_2) at a dosage of 1000 mg kg-1 each increased onion germination (72%), as compared to control (60%).	Anandaraj, and Natarajan 2017
Rice	Rice variety CN-1794-2 was exposed to six different nano TiO_2 dosages at 0, 10, 20, 50, 80, and 100 ppm for 48 hours. Compared to the control, treatment with a 20 ppm nanoparticle solution increased seed germination by 85%. In treatments 20 ppm and 50 ppm, recorded higher germination of 98% and 98% .	Debnath *et al*., 2020
Wheat	Seeds of wheat variety Lok -1 were treated with different concentrations (2000,4000,6000,8000 and 10000)ppm of nano ZnO. The germination rate of seeds treated with nano ZnO at 2000ppm was 100percent. At greater doses, a gradual decline in germination was also	Bagawade and Jagtap, 2018

	observed	
Onion	Seed treated with TiO_2 nanoparticles @40ppm for 2 hours re corded superior germination percentage.	Khan *et al.*, 2022
Cotton	When seeds were nanoprimed with 400 ppm of ZnO NPs and 200 ppm of TiO_2 NPs, the seeds exhibited the highest levels of germination.	Singh *et al.*, 2022

2.2.2.2 Shoot length

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Lower concentration reduced seedling growth, but higher concentration resulted increased shoot length	Afrayeem and Chaurasia, 2017
	Maximum shoot length (4.3cm) was observed with the treatment 1000mg/kg compared to control (3.9cm)	Kumar *et al*., 2020
Other crops		
Groundnut	Treatment with a concentration of 1000 ppm caused a significant increase in shoot length	Prasad *et al*., 2012
Radish	The longest shoot length of 2.21 cm, was observed in seeds treated with nano TiO_2 at 100 mg L^{-1}.	Haghighi *et al.,* 2014
Wheat	Treatment upto 60mgkg^{-1} recorded an increase in shoot length .Further increase was not affected shoot length.	Rafique *et al*., 2014
Groundnut	ZnO treatment of 1000 mgkg^{-1} and Ag treatment at 1250mgkg^{-1} promoted maximum shoot length	Shyla and Natarajan, 2014
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 shoot length of 1.94 cm.	Tiwari, 2017
Wheat	In comparison to seed soaking at 300 ppm concentration, seed treatment with nanoparticles at 50 ppm concentration recorded higher shoot length	Rawat *et al*., 2018

2.2.2.3 Root length

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Seeds treated with zinc oxide nanoparticles at 1000 mg kg^{-1} recorded highest value maximum root length (11.90 cm) when compared to control 9.19cm.	Kumar *et al*., 2019
Other crops		
Groundnut	Seeds treated with nanoscale ZnO particles at a concentration of 1000 ppm caused a significant increase in root length	Prasad *et al*., 2012
Groundnut	Nanoscale ZnO showed higher root growth of seedlings compared to bulk $ZnSO_4$ and control	Prasad *et al*., 2012
Mustard	Root length increased in treatments upto 200 ppm of silver nanoparticle concentration. Sharp increases of 167 and 277 % in root length were observed at 25 and 50 ppm respectively.	Sharma *et al*., 2012
Wheat	Treatment upto 60mg/kg recorded an increase in root length further increase did not affect root length	Rafique *et al*., 2014
	The study revealed that, in comparison to seed soaking at 300 ppm concentration, seed treatment with nanoparticles at 50 ppm concentration enhanced root length	Rawat *et al*., 2018

2.2.2.4 Dry weight

Crop	Details of the experiment	Reference
Solanaceous crop		
Chilli	Seeds were wet dressed with nano ZnO at 0, 100, 200, and 500 ppm. Higher seedling dry weight was observed at lower doses.	Garcia-Lopez *et al*., 2018
Other crops		

Chickpea	Chickpea seeds were treated with 100, 500, and 1000 ppm nano ZnO. 1000 ppm resulted in greater seedling dry weight among the treated seeds.	Hajra and Mondal, 2017
Wheat	The study found that, in comparison to seed soaking at 300 ppm concentration, seed treatment with nanoparticles at 50 ppm concentration increases seedling dry weight,	Rafique *et al.*, 2014
Wheat	When compared to untreated control seedlings, seedlings exposed to 2500 ppm of ZnONPs showed a significant increase in dry weight (17.0 %).	Alsuwayyid *et al.*, 2022

2.2.2.5 Vigour index

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Vigorous seedlings were seen in seed treated with nano ZnO at 1000 mg kg^{-1} (1285), while control recorded the least value (861).	Kumar *et al.*, 2020
Chilli	The performance of seeds treated with bulk ZnO at 1300 mg kg^{-1} and ZnO nanoparticles at 500 mg/kg in maintaining seed vigour for the full ten months of storage.	Gayathri, 2019
Other crops		
Rice	Significant difference in seed vigour above control were observed in rice seeds treated with nano TiO_2 at 0, 10, 20, 50, 80, and 100 ppm. The maximum vigour was seen in seeds treated with nano TiO_2 @ 20 ppm.	Debnath *et al.*, 2020
Onion	Seed treated with TiO_2 nanoparticles @ 40 ppm for 2 hours generated seedlings with high vigour indices.	Khan *et al.*, 2023
Maize	Positive effect on seed vigour index was seen after treatment with ZnO nanoparticles of size 20–30 nm at a dosage of 15 mgL^{-1} in both the Vigor indices.	Mahesh *et al.*, 2022

Cotton	When seeds were nanoprimed with 400 ppm of ZnO NPs and 200 ppm of TiO_2NPs, the seeds exhibited the highest levels of seed vigour indices seedling establishment and speed of emergence under field conditions.	Singh *et al*., 2022

2.2.2.6 Electrical conductivity of seed leachate

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Using nano ZnO at 500 $mgkg^{-1}$ and 250 mg/kg decreased electrolyte leakage from seeds during storage	Gayathri, 2019
	Electrical conductivity was considerably impacted by the nanoparticle seed treatments. When compared to the control, nano ZnO at 1000 mg kg^{-1} had the lowest EC (0.118 dSm-1) (0.149dSm^{-1}) .	Kumar *et al*., 2020
Other crops		
Groundnut	Electrical conductivity, was least in seeds treated with 1000 mg kg^{-1} TiO_2	Shyla and Natarajan, (2014)
Maize	When treated with various doses, nano TiO_2 @ 200 mg kg-1 showed lower EC (0.278 dSm-1) than the control.	Vijayalakshmi *et al*., 2018
Soybean	Seed treated with TiO_2 nanoparticles @ 40ppm for 2 hours registered the least electrical conductivity (0.951 dSm^{-1}) as compared to control after 3 months of storage.	Sandeep *et al*., 2019
Sunflower	ZnO NPs treated seeds showed low electrical conductivity of seed leachate (1.003units) at six months of ageing while it was higher in untreated seeds (1.175units)	Meenakshi *et al*., 2020

2.3. Seed infection in chilli

The ability of crops to establish themselves and reach their maximum yield and value potential is significantly influenced by the quality of the seeds that are used in the planting process. Storage fungus (*Aspergillus* and *Penicillium* spp.) can significantly reduce seed viability, as they penetrate seeds stored at moisture contents that are in equilibrium with ambient relative humidity between 65 and 90 per cent (Christensen and Meronuck, 1986). When the moisture content is high respiratory activity increases and spontaneously heat is produced which reduces seed quality. Storage fungi are saprophytes or weak pathogens which are widely distributed Infection by this fungi cause discoloration of embryos, decreases in the germination and the production of mycotoxins.

The elements contributing to the growth of microorganisms during storage include moisture, aeration and temperature. Seed vigour and germinability decrease as infections become more prevalent with increase in storage period. The seeds may be discoloured and emit unpleasant odours. Numerous toxins made by fungi have an adverse effect on seedling growth. Nutrients are lost due to biochemical changes that occur inside the seed . The pathogens affecting seed health in storage are enlisted below:

Pathogen observed	Reference
Aspergillus niger, *A. flavus* *Alternaria alternata* *Bipolaris spicifera* *B. australiensis* *Cephalosporium* sp. *Cladosporium* spp.. *Colletotrichum capsici* *Curvularia lunata* *Drechslera* spp *Fusarium moniliforme,* *F. solani* *Penicillium* spp *Macrophomina phaseolina* *Rhizoctonia solani* *Verticillium albo-atrum* *Trichoderma harzianum*	Sharfun-Nahar *et al*., 2004
Aspergillus flavus *Aspergillus niger* *Penicillium digitatum*	Balogun *et al.,* 2005
Aspergillus niger *Colletotrichum capsica* *Curvularia lunata* *Fusarium oxysporum* *Macrophomina phaseolina*	Jogi *et al.,* 2010

Penicillium citrinum *Rhizopus nigricans*	
Aspergillus. flavus *A. niger* *Bispora betulina* *Botryotrichum piluferum* *Colletothrichum capsici* *Humicola dimorphospora* *Humicola fuscoatra* *Periconia byssoides* *Phoma* spp. *Phomopsis* spp	Chigoziri and Ekefan, 2013
Aspergillus flavus *Colletotrichum capsici* *Curvularia lunata* *Fusarium moniliforme* *Rhizopus stolonifera*	Alam *et al*., 2014
Alternaria alternate *C.gloeosporioides* *C. acutatum* *Colletotrichum capsici* *Fusarium oxysporum* *F.sporotrichioides*	Machenahalli *et al*., 2014
Alternaria spp *Aspergillus flavus* *Aspergillus niger* *Pencillium* spp	Navya, 2016
Alternaria spp *Aspergillus* spp *Pencillium* spp	Sandhya, 2016
Aspergillus flavus *Aspergillus niger capsici* *Colletotrichum* sp. *Fusarium solani* *Penicillium* spp. *Rhizopus* spp.	Chauhan *et al*., 2018
Aspergillus flavus *Aspergillus niger* *Rhizopus* sp.	Herbert, 2020
Aspergillus flavus *Aspergillus niger*	Mathew, 2020
Alternaria spp *Aspergillus flavus*	Gayathri, 2019

Aspergillus niger *Mucor* spp.	
Aspergillus astus *A. flavus* *A. niger* *A. tereus* *Absidia corymbifera* *Aspergillus acaleatus* *Curvularia lunata* *Fusarium oxysporum* *Humicola* sp *Penicillium funiculosm* *Phytophthora undulate* *Rhizoctonia bataticola*	Chaudhari *et al*., 2022
Rhizopus sp. *Aspergillus fumigates* *Penicillium* sp.	Mabel *et al*., 2022

Materials and methods

3. MATERIALS AND METHODS

The study entitled 'Seed invigoration with nano scale particles in chilli '(*Capsicum annuum* L.)' was conducted at Department of Seed Science and Technology, College of Agriculture, Kerala Agricultural University, Vellanikkara from December 2021 to December 2022.

3.1. Experimental site

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

3.2. Climatic conditions

Vellanikkara, Thrissur district, is located at 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. The temperature ranged between 22.6^{0}C to 36.1^{0}C and RH ranged between 58 and 88% during the study period (Table 1)

3.3. Experimental material

Chilli seeds of variety Ujwala, harvested during November 2021, procured from the Instructional Farm, College of Agriculture, Vellanikkara were used in the present investigation.

3.4. Experimental details

The study was divided into three as listed below:

Experiment 1 : Effect of pre-sowing treatment with inorganic nanoparticles in chilli.

Experiment 2 : Effect of pre-storage treatment with inorganic nanoparticles in chilli.

Experiment 3 : Effect of mid- storage correction with inorganic nanoparticles in chilli

Table 1: Monthly meteorological data during the study period December 2021 to December 2022

Months	Temperature		Relative humidity(%)	Rainfall (mm)	Rainy days
	Mean maximum(°C)	Mean minimum(°C)			
December2021	32.5	23.3	67.0	19.2	1.0
January2022	33.3	22.6	64.0	0.0	0.0
February2022	34.8	23.3	58.0	0.0	0.0
March2022	36.1	24.7	74.0	1.7	0.0
April20222	34.2	25.1	77.0	84.3	7.0
May2022	31.1	24.0	85.0	422.0	23.0
June2022	31.3	23.6	84.0	391.8	19.0
July2022	29.3	23.5	88.0	628.8	21.0
August2022	29.9	23.6	84.0	563.3	15.0
September2022	31.1	23.7	81.0	167.5	12.0
October2022	32.0	23.6	77.0	69.6	9.0
November2022	32.4	23.0	73.0	75.4	7.0
December2022	32.2	22.6	67.0	91.7	3.0

3.4.1 Experiment 1: Effect of pre-sowing treatment with nanoparticles in chilli

Design: Randomized Block Design (RBD)

Experiment is conducted in ujwala variety with eight treatments with three replications with a plot size 2 m× 2 m with plant to plant spacing 45cm×45cm.

Seeds were subjected to wet and dry seed treatment with nanosized zinc oxide (ZnO), and nanosized custard leaf powder respectively.

3.4.1.1 Wet seed treatment

ZnO nanopowder of SIGMA –ALORICH having <100nm particle size was used. The ZnO nanoparticles were dispersed in distilled water by sonicating for 15 minutes at room temperature (Kumar, 2020). The seeds were soaked in the solution for three hours. Seed soaked in distilled water act as a control. The solution was drained and the soaked seeds were dried to a moisture of less than eight per cent.

3.4.1.2 Dry seed treatment

The seeds were shade dried to reduce the moisture content to below eight percent before administering seed invigoration treatment. The seeds were dry dressed with the required quantity of nano custard leaf powder as detailed in the table. The glass bottles containing seeds and nanoparticles were manually shaken gently for three minutes, five times in a span of three hours.

3.4.1.3 Synthesis of custard leaf nano sized powder

The custard leaf nanopowder was synthesized through ball milling from macro sized particles using high energy ball mill. Fresh custard leaves were collected, dried in the shade, and then dried at 60^0 C in a hot air oven. The dry sample was processed in a mixer grinder and passed through a 0.1mm wire mesh sieve. These 'normal grade powders' were subjected to additional ball milling using a FRITSH, PULVERISETTE 7 HIGH ENERGY BALL MILL at 600 rpm with a 15-minute on-off cycle for three hours to bring down the particle size to the nano scale. These substances were known as 'nano powders.' They were further evaluated in the particle size analyzer to ensure they were of the nano size required and utilised for seed treatment

3.4.1.4. Ball milling

Principle

The ball mill is a crucial piece of equipment for grinding crushed materials; it is a cylindrical device that rotates around a horizontal axis partially filled with the components to be ground and the grinding medium. Based on the critical speed theory, grinding operates. The critical speed can be considered as the point at which the steel balls, which are in charge of grinding the particles, begin to rotate in the direction of the cylindrical device, preventing further grinding. At a specific velocity, the centrifugal force causes the ground sample material and grinding balls to bounce off the inner wall of the grinding bowl, cross the bowl diagonally at an extremely high speed, and impact the material to be ground on the opposite wall of the bowl. The grinding jar's steel grinding balls are lined with high carbon steel plates, porcelain, or silica pebbles and subjected to superimposed rotational forces known as coriolis forces. Ball mills are used for medium- and fine-scale reduction of abrasive materials. Size reduction is achieved in a ball mill by the impact of the balls. High dynamic energies are released due to the interaction between the frictional and impact forces created by the speed differential between the balls and grinding jars. The interaction of these forces results in a high and very effective degree of sample size reduction .

3.4.1.5 Particle size analyzer

Particle size and the distribution pattern of synthesized sample suspensions were performed by dynamic light scattering (DLS). The sample's physical characteristics will determine the dynamic range, which is 0.3 nm to 8 m. Concentration, the strength of light scattering from the sample and the presence of large, undesirable particles all impact the lower limit. The upper limit is affected by the sample density. The DLS relies entirely on Brownian motion and not on gravitational settling; The SZ-100 measures the zeta potential of a suspension to calculate the charge on the surface of particles. The sample is injected into a disposable cell, and the zeta potential is derived from a measurement of the particle electrophoretic mobility.

The sample's zeta potential is commonly used as dispersion stability indicator. Large magnitude zeta potential values predict the stability of an electrostatically stabilised suspension. To aid formulators in creating novel goods with a long shelf life, the zeta potential is frequently tested as a function of pH or other changes in the chemistry. On the other hand, knowing the conditions at which the zeta potential is zero (i.e., the sample is at the isoelectric point) enables one to select the ideal flocculation and separation conditions. Japanese company Horiba Scientific Nanoparticle SZ-100 was used to make the determination.

A sample size of 0.05mg was dispersed in 20 ml distilled water, sonicated for 15 min, and the suspension was analyzed under dynamic light scattering method using 90^0 or 173^0 at 25^0C

3.4.1.6 Scanning Electron Microscope (SEM)

SEM FEI QUANTA 250 was used to characterize the size and morphology of the nanoparticles. In a Scanning Electron Microscope surface of the specimen is scanned by a small electron beam in time with the cathode ray tube's spot (CRT). Electrons scatter elastically and inelastically as a result of this incident beam, and different electromagnetic radiation is also produced. After that, various secondary signals can be found, such as X-rays, Auger electrons, backscattered electrons, secondary electrons, or cathode luminescence. The intensity of the secondary signal will generally change based on the surface shape, chemistry, physical state, etc. while the main electron beam is rastered across a substrate. The contrast can be noticed by altering the CRT spot's brightness using the discovered signal's amplified form.

The double-sided sticky carbon conducting tape was dusted with a sample of test nanoparticles (0.5 to 1 mg) and attached to a 12 mm aluminium stub. The sample surface was examined at various magnifications, and images were captured.

Details of seed treatment used for chilli seeds

Treatment	Dose
T_1	Control
T_2	300mg nano ZnO/Kg of seed
T_3	400mg nano ZnO/Kg of seed
T_4	500mg nano ZnO /Kg of seed
T_5	600mg nanoZnO/Kg of seed
T_6	700mg nanoZnO/Kg of seed
T_7	800mg nanoZnO/Kg of seed
T_8	500mg nano Custard leaf powder/Kg of seed

The four week old seedlings of treated seeds were transplanted to the main field after four weeks from the nursery. The experimental crop was raised as per Package of practices recommendations of Kerala Agricultural University (KAU,2016).

3.4.2 Experiment 2: Effect of pre-storage treatment with inorganic nanoparticles in chilli

3.4.2.1 Method of seed packing and storage

Seeds treated as detailed in experiment I were dried to a moisture content of less than 8 per cent. The treated and untreated seeds of each treatment were packed separately in 700 gauge polythene bags and stored under ambient conditions for a period of six months after assessing the initial seed quality parameters and they were subsequently sown to raise the crop raised as per POP recommendations (KAU, 2016) .

Plate No 1. Transplanting of seedling

3.4.3 Experiment 3: Effect of mid-storage correction with inorganic nanoparticles in chilli

Three month old seeds were treated with nanoparticles as enumerated under Experiment I and stored for three months. Observations on seed germination (%), shoot length (cm), root length (cm), dry weight of seedling (g), and electrical conductivity (ECunit) of seed leachate were recorded initially after seed treatment and subsequently at monthly intervals. Seed microflora (%) and seed moisture (%) were recorded during the start and end of the storage.

3.5 Observations

3.5.1 Biometrical observations

In each replication, five plants were selected randomly from each replication and tagged for taking observation. All biometric observations detailed below were recorded at the appropriate growth stages in the tagged plants and averaged to compute the data of a given replication for each treatment

3.5.1.1 Days to first flowering

Day from sowing to the day on which the first flower appeared was considered as days to first flowering.

3.5.1.2 Days to 50%flowering

The plants were observed daily for flowering . When 50 per cent for flowering of the plants in the plot had flowered it was considered as 50 per cent flowering. The number of days required for 50 per cemt flowering was computed as the number of days from the date of sowing to 50 per cent flowering.

3.5.1.3 Plant height (cm)

height was measured on the 120thday after transplanting and it was measured from ground level to the tip of the main stem, and the average was expressed in centimetre.

3.5.1.4 Number of branches per plant

The number of branches per plant was counted at physiological maturity and the average worked out.

3.5.1.5 Fruits per plant

The number of fruits in each of the five tagged plants was collected, counted and expressed in numbers.

3.5.1.6 Fruit length (cm)

The average length of ten fruits from each plant was measured using a meter scale from the distal end to proximal length and expressed in centimetre.

3.5.1.7 Fruit weight at maturity (g)

The ten dried fruits taken for measuring the fruit length were weighed individually and averaged.

3.5.1.8 Seeds per fruit

In each replication tagged plants fruits were harvested and dried, seeds from the tagged plants fruits were carefully removed. Seeds in each fruit were counted and calculated the average number of seeds per fruit.

Plate No 2. Harvesting of chilli

3.5.1.9 100 seed weight(g)

Eight samples of 100 well filled seeds drawn randomly from the tagged plants of replication were weighed, and the average was expressed in grams

3.5.1.10. Seed yield per plant (g)

The seeds taken from the fruit tagged plants dried and cleaned using sieve size of 1.5mm were weighed and expressed in grams..

3.5.2 Seed quality parameters

3.5.2.1 Germination

The germination test was conducted using roll paper towel or sand medium, 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. For each set, two brown corrugated paper towel sheets were moistened (not dripping wet, just wet). One wet paper towel was placed in a shallow plastic container, and seeds (100 for each set) were evenly distributed on it before being covered with another wet paper towel sheets were then rolled together, wrapped in butter paper, and held together loosely with rubber bands. After properly labeling, rolled-in towels with seeds were stacked vertically. The rolled towels were placed in buckets of water in a slanting position inside a seed germinator which was kept at 25$\pm$ 2 °C temperature and 92% relative humidity. The germination percentage was calculated by counting the number of normal seedlings on the 14th day and averaging the observation and percent.

3.5.2.2 Seedling shoot length (cm)

Ten normal seedlings were selected randomly from each replication of the treatment on 14thday of germination and the shoot length was measured from the base to collar region of the seedling, and the average was taken and expressed in centimeters.

3.5.2.3 Seedling root length (cm)

The root length of the seedlings chosen for shoot length measurement was measured from the collar region to the tip of the root of seedling's root on the 14^{th} day using a scale , and the mean root length was expressed in centimeters.

3.5.2.4 Seedling dry weight (mg)

Using same 10 seedlings used for measurement of both root length and shoot length were used for estimate the dry weight of seedling. The seedlings were enclosed in butter paper and dried in an oven at 80^0C for 24 hours. The seedlings were then weighed using an automatic digital balance, and the average dry weight was calculated 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 and expressed as whole number Abdul Baki and Anderson (1973)

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

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

3.5.2.6 Electrical conductivity of seed leachate (dSm^{-1})

Three replicates of five grams of seeds were drawn from each treatment to estimate the electrical conductivity of seed leachate. For surface sterilization, randomly selected seeds were immersed in 0.1% mercuric chloride ($HgCl_2$) for one minute. These seeds were thoroughly washed several times in distilled water to remove treated chemical residues then it was soaked in 50 ml of distilled water for 24 hours. The electrical conductivity (EC) of seed leachate collected in a beaker after decanting was measured with a digital conductivity meter (EUTECH CON-510), and the mean value was recorded as micro Siemens per centimeter (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. The weight of the container and lid before and after filling with the seeds was measured. The low constant temperature method recommended by ISTA was used to measure the moisture content of the seeds (ISTA1985). Five grams of seed were taken from each replication and distributed equally in a glass container. Before and after adding the seeds, the container's weight with the lid was measured. The samples in the container were dried for 17$\pm$ hours in a hot air oven set at 103$\pm$2 °C. When the containers were finished drying, they were kept in a desiccator to cool for 30-45 minutes. After cooling, the weight of the container and its lid was again measured, and was calculated as per the standard.

$$\text{Moisture content(\%)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

Where,

M_1: Weight of the container with lid

M_2: Weight of the container with lid + seeds before drying

M_3: Weight of the container with lid + seeds after drying

3.5.2.8 Seed infection (%)

Seed infection was detected using the blotter paper method and agar plate method as recommended by ISTA (1999).

3.5.2.8.1 Blotter paper method

To identify seed infection blotter paper method of Neergard (1979) was followed. This technique employed sterilized petriplates with three layers of blotting sheets and sterilized water to soak the blotting papers. Under aseptic conditions of laminar airflow, 25 seeds were distributed evenly such that the outermost layer contained 16 seeds, the middle layer eight seeds, and the centre one seed. For seven days, the petriplates were incubated at 20°C in an alternate cycle of 12 hours of darkness and 12 hours of light. On the eighth day, a stereo binocular microscope was used to examine the plates for the presence of seed microflora, and the number of infected seeds was tallied and recorded in percent.

3.5.3.8.2 Agar plate method

In the agar plate method, seeds were surface sterilized with 0.1 percent mercuric chloride, followed by thorough rinsing in sterile water three times to eliminate mercuric chloride residues. Excessive water in the seed was removed by keeping it on sterile filter paper. Under aseptic condition, potato dextrose agar (20ml) was poured into the sterilized plate and the lid kept slightly open till the media was set, and seeds were placed at equidistance. After plating, they were packed in a polythene cover and incubated for six days under a bell jar. Under a stereo-binocular microscope, the plates were examined, and the percentage of infected seeds was counted and recorded.

3.6 Statistical analysis

Statistical analysis was done using General R-shiny based Analysis Platform Empowered by Statistics (GRAPES) developed by Kerala Agricultural University. The

Plate No 3. Estimation of seed microflora

ranking of treatments was done by using Duncan's Multiple Range Test (DMRT).

Each observation's recorded data was examined using an ANOVA table for a completely randomised design to test for differences, as shown below:

3.6.1 ANOVA for completely randomized design

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

Where,

t: Treatments

n: Number of observations

MST: Treatment mean sum of squares

MSE: Error mean sum of squares

3.6.2 DMRT test for ranking

Duncan's Multiple Range Test (DMRT) is applied for evaluating and ranking all possible Pairs of treatment means, particularly for experiments with large 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 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

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

MSE: error mean sum of squares

R: number of replications.

The (t-1) values of shortest significant ranges were calculated as follows:

$$R_p = \frac{(rp)(SEm)}{\sqrt{2}}$$

Where,

t: number of treatments

SEm: standard error obtained in step

rp: tabulated value of ranges which are significant

p: The difference in rank between the treatment pairs means to be compared

(p = t for the highest and lowest means).

(iii) After identifying all the treatment means which do not differ significantly from each other was grouped.

(iv) The obtained results were presented using alphabetical notation.

Results

4. RESULTS

The present study 'Seed invigoration with nanoscale particles in chilli (*Capsicum annuum* L.)' was undertaken in the Department of Seed Science and Technology, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur. The research was conducted to assess the impact of seed treatments using nano scale particles when administered as presowing and prestorage treatments and to study the effect as mid storage correction in chilli variety Ujwala.

Results obtained from field and storage studies are presented in this chapter.

4.1 Characterisation of nano size custard apple leaf powder

The leaves of custard apple tree were collected, shade dried and powdered. This normal grade powder was synthesized into nano size powders using top down approach by employing high energy ball milling for six hours at the rate of 600 rpm. Custard leaf powder particle size was reduced from bulk to nanoparticle.

4.1.1 Particle size analyser

The particle size analyzer was used to estimate the average particle size and distribution pattern of nano powders using the dynamic light scattering method. The particle sizes of custard leaf powder were analysed as 259nm, 341nm and 379nm respectively (Fig1)

4.1.2 Scanning Electron Microscope (SEM)

The SEM picture revealed confirmed the size reduction from normal to nano size (Plate 1).

4.2 Effect of pre-sowing treatment with inorganic nanoparticles in chilli.

4.2.1 Analysis of variance

The analysis of variance on yield attributes such as days to first flowering, days to 50 percentflowering, plant height (cm), branches per plant, fruits per plant, fruit length (cm), fruit weight at maturity (g), seed yield per plant (g), 100 seed weight (g) and seeds per fruit revealed highly significant differences among the treatments in Ujwala (Table 2).

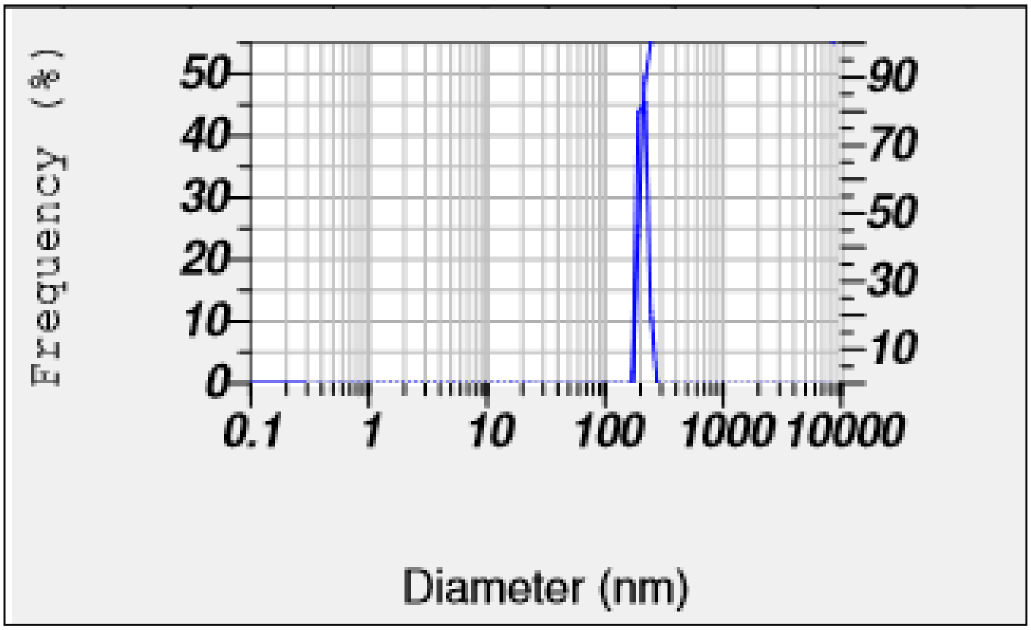

Fig 1. Particle size distribution of custard leaf powder

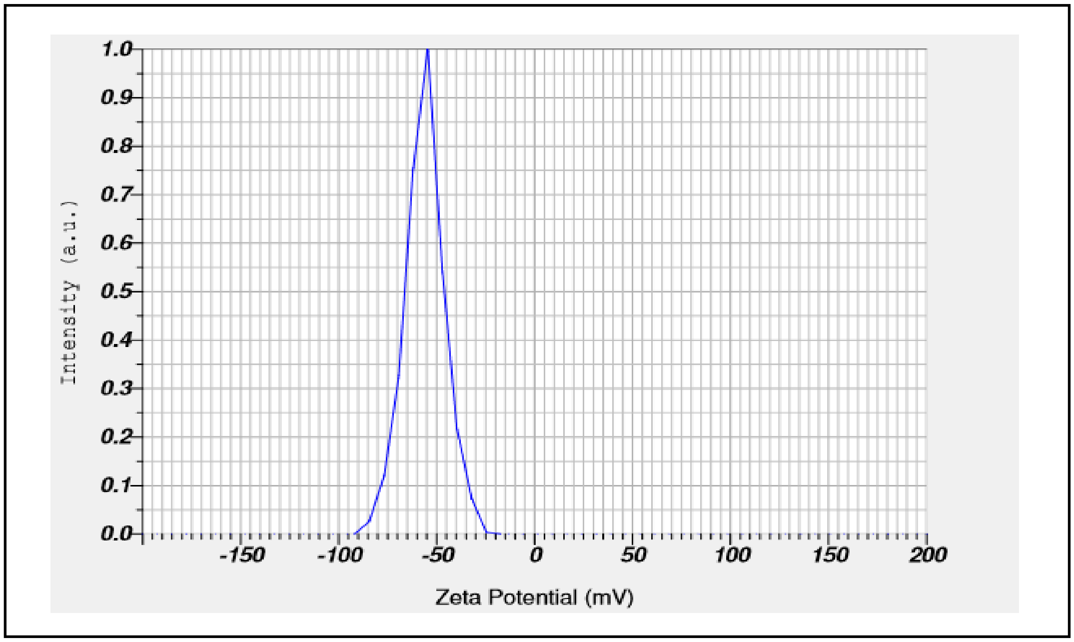

Fig 2. Zeta Potential of custard leaf powder

Zeta Potential (Mean) : -40.6 mV

Z-Average : 15185.8 nm **PI : 6.247**

Table 2. Analysis of variance of the effect of pre-sowing seed treatment with nano scale particle on yield and yield contributing characters in chilli

Sources of variation	Degrees of freedom	Mean sum of squares										
		Plant height (cm)	Branches/ plant (nos.)	Days to first flowering (nos.)	Days to 50 % flowering (nos.)	Fruits/ plant (nos.)	Fruit length (cm)	Fruit weight at maturity (g)	Fruit yield/plant (g)	No. of seeds/ fruit (nos.)	100 seed weight (g)	Seed yield per plant (g)
Replications	2	2.19	0.60	2.79	3.17	44.35	0.02	0.00	35.26	0.04	296.06	0.86
Treatments	12	38.26**	35.96**	5.69**	17.21**	618.35**	0.44**	0.08**	3654.50**	69.95**	1481.71**	56.75**
Error	24	1.49	1.51	1.17	4.64	41.30	0.02	0.00	166.37	8.86	170.78	0.95
SE		0.70	0.71	0.62	1.24	3.71	0.08	0.02	7.44	1.71	7.54	0.56
CV (%)		3.24	17.50	1.44	2.57	7.35	3.05	2.08	7.45	4.93	3.23	5.82

Significant at 5 % level *

Significant at 1 % level **

Table 3. Effect of presowing seed treatments with nanoparticles on growth parameters in chilli

Treatment	Plant height (cm)	Branches/ plant (nos.)	Days to first flowering (nos.)	Days to 50 % flowering (nos.)
T_1: Control	35.62^{cd}	4.27^{c}	77.67^{a}	88.33^{a}
T_2: 300mg/kg ZnO	34.13^{d}	5.80^{c}	75.00^{bcd}	82.67^{bc}
T_3: 400mg/kg ZnO	42.94^{a}	12.67^{a}	74.67^{bcd}	79.67^{c}
T_4: 500mg/kg ZnO	40.22^{b}	11.70^{a}	73.33^{d}	84.67^{ab}
T_5: 600mg/kg ZnO	35.75^{cd}	5.40^{c}	74.00^{cd}	84.33^{b}
T_6: 700mg/kg ZnO	33.84^{d}	8.20^{b}	74.33^{cd}	83.67^{b}
T_7: 800mg/kg ZnO	42.02^{ab}	4.07^{c}	75.33^{bc}	84.33^{ab}
T_8: 500mg/kg Custard leaf powder	36.67^{c}	4.13^{c}	76.33^{ab}	83.67^{b}
SEm	0.70	0.710	0.62	1.240
CD (0.05)	2.14	2.15	1.90	3.77

Table 4. Effect of presowing seed treatments with nanoparticles on fruit and seed traits

Treatment	Fruits/ plant (nos.)	Fruit length (cm)	Fruit weight at maturity (gm)	Fruit yield/plant (g)	No. of seeds/ fruit (nos.)	100 seed weight (g)	Seed yield per plant (g)
T_1: Control	72.23^{c}	4.47^{b}	1.83^{d}	130.53^{d}	53.80^{d}	0.42^{a}	11.35^{f}
T_2: 300mg/kg ZnO	79.93^{bc}	4.37^{bc}	1.95^{c}	154.14^{c}	55.53^{cd}	0.41^{ab}	14.72^{de}
T_3: 400mg/kg ZnO	77.60^{bc}	4.50^{b}	2.23^{a}	161.23^{c}	60.48^{bc}	0.36^{d}	16.04^{d}
T_4: 500mg/kg ZnO	98.87^{a}	4.19^{c}	2.27^{a}	224.56^{a}	57.37^{cd}	0.42^{a}	24.02^{a}
T_5: 600mg/kg ZnO	72.33^{c}	4.54^{b}	2.05^{b}	143.09^{cd}	62.73^{ab}	0.43^{a}	14.11^{e}
T_6: 700mg/kg ZnO	101.80^{a}	4.87^{a}	1.93^{c}	191.81^{b}	65.80^{a}	0.39^{c}	19.02^{c}
T_7: 800mg/kg ZnO	109.67^{a}	3.68^{d}	2.07^{b}	219.47^{a}	67.59^{a}	0.39^{c}	21.56^{b}
T_8: 500mg/kg Custard leaf powder	86.80^{b}	4.87^{a}	1.88^{cd}	160.21^{c}	59.70^{bc}	0.41^{ab}	13.51^{e}
SE_m	3.71	0.078	0.024	0	1.72	7.54	0.564
CD (0.05)	11.25	0.24	0.07	22.58	5.21	22.89	1.71

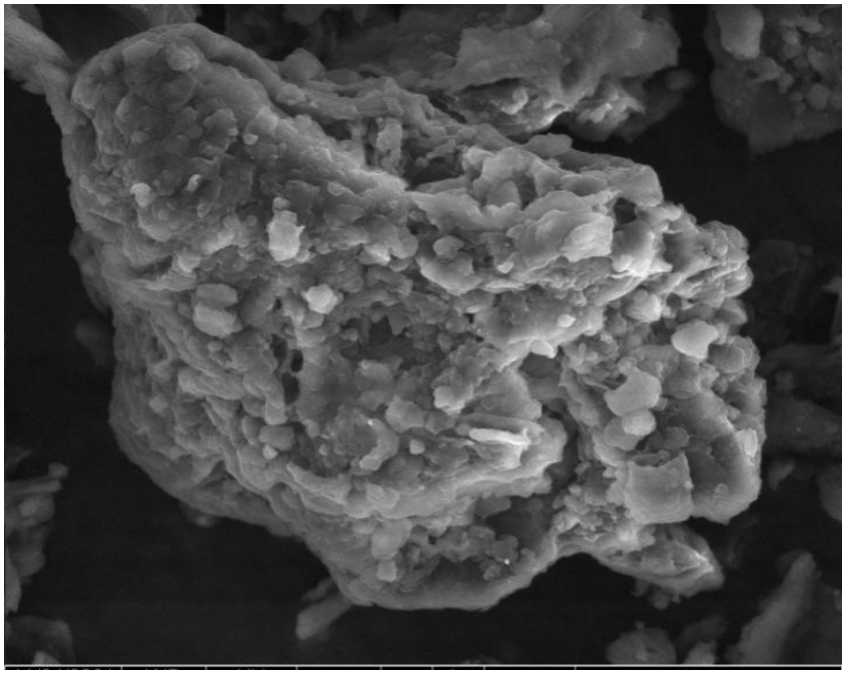

Plate 4. Custard leaf powder after 3 hours of ball milling mag 5000 ×

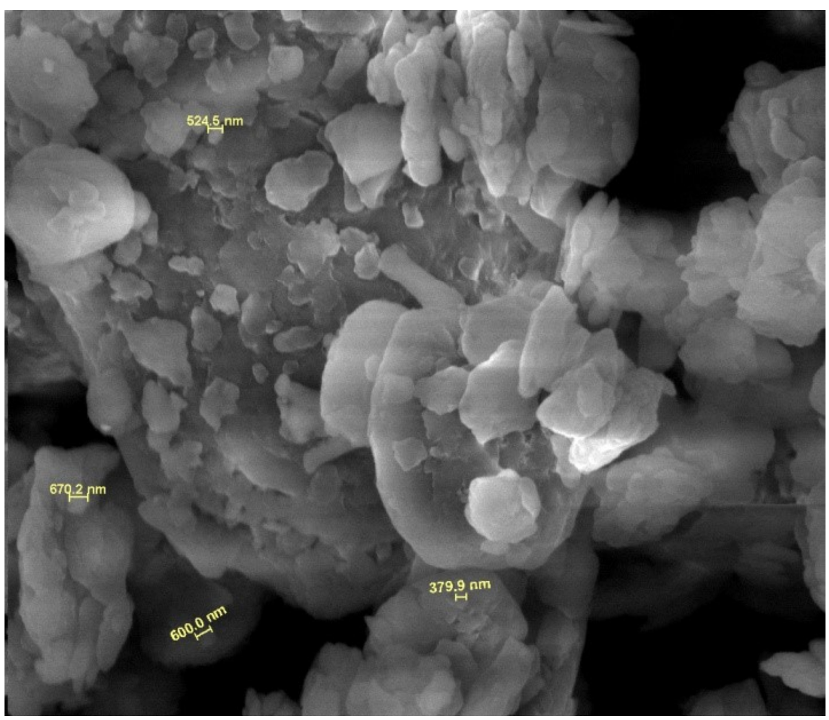

Plate 5. Custard leaf powder after 6 hours of ball milling mag 1000×

4.2.2 Days to first flowering

Significant differences were observed among the seed treatments (Table 3). Seed treatment with nanoparticles were found to have favourable effect on days to first flowering.

Among the treatments seed treated with nano ZnO @ 500 mg kg^{-1} of seed(T_4) recorded 73.33 days for first flowering which is the lowest among the different treatments. Treatments T_5-nano ZnO @ 600 mg kg^{-1} of seed is on par with T_6-nano ZnO @ 700 mg kg^{-1} of seed, (74days, 74.33days) while the control recorded 77.67 days to first flowering.

4.2.3 Days to 50 % flowering

The treatments recorded significant differences for days to fifty per cent flowering (Table 3). It ranged from 79.67 days in T_3- nano ZnO @ 400 mg $kg^{-1}$of seed to 88.33days in control Treatment T_2-300 mg kg^{-1} of seed (82.67 days) was on par with T_5-nano ZnO 600 mg $kg^{-1}$of seed (84.33 days).

4.2.4 Plant height (cm)

Treatments recorded significant differences for plant height (Table 3). Treated seeds produced taller plants than control. The plant height ranged between 33.84 cm in T_6- nano ZnO @ 700mg $kg^{-1}$of seed and 42.94 cm in T_3- nano ZnO @ 400 mg $kg^{-1}$of seed. Treatment T_7-nano ZnO @ 800 mg $kg^{-1}$of seed (42.02cm) was on par with Treatment T_3 - nano ZnO @ 400 mg kg^{-1} of seed (42.94cm) and T_4- nano ZnO @ 500 mg $kg^{-1}$of seed (40.22cm). Treatment T_2- nano ZnO @ 300 mg $kg^{-1}$of seed (34.13cm), T_6-nano ZnO @ 700 mg Kg^{-1} of seed (33.84cm), T_5- nano ZnO @ 600 mg kg^{-1} of seeds (35.75cm) were on par with the control (35.62cm).

4.2.5 Number of branches per plant

Significant differences were observed in the case of number of branches per plant when the seeds were treated with nanoparticle (Table 3). Among the treatments T_3- nano ZnO @ 400 mg kg^{-1} of seed recorded the highest number of branches (12.67) followed by T_4 – nano ZnO @ 500 mg kg^{-1} of seed (11.70) and both being on par followed by the T_8 and control (4.13 and 4.27)(Table3). T_7- nano ZnO @ 800mg kg^{-1} of seed recorded the least number of branches per plant (4.07).

4.2.6 Fruits per plant

There existed significant differences among the treatments in case of fruits per plant (Table 4). T_7- nano ZnO @ 800 mg $kg^{-1}$of seed (109.67), T_6 - nano ZnO @ 700 mg kg^{-1} of seed (101.6), T_4- nano ZnO @ 500mg kg^{-1} of seed (98.87) produced more number of fruits compared to control (72.23). T_5- nano ZnO @ 600 mg $kg^{-1}$of seed recorded least number of fruits (72.33) and was on par with control (72.23).

4.2.7 Fruit length (cm)

Treatments recorded significant differences in case of fruit length (Table 4).

Nanoparticle seed treatments T_6- nano ZnO @ 700 mg kg^{-1} of seed (4.87cm), T_8- Custard leaf powder (4.87cm) T_5- nano ZnO @ 600 mg $kg^{-1}$of seed (4.54cm), T_3- nano ZnO @ 400 mg kg^{-1} of seed (4.50 cm) seed produced longer fruits compared to control (4.47cm). The lowest fruit length was observed in T_7- nano ZnO @ 800 mg kg^{-1} seeds (3.68cm).

4.2.8 Fruit weight at maturity (g)

Among the treatments T_4- nano ZnO @ 500 mg kg^{-1} of seed and T_3- nano ZnO @ 400 mg kg^{-1} of seed were on par and having highest fruit weight at maturity (2.27g, 2.23g) this was followed by T_7- nano ZnO @ 800 mg kg^{-1} of seed, T_5- nano ZnO @ 600 mg kg⁻

[1]of seed (2.07g, 2.05g) compared to control (1.83g). The treatment T_2- nano ZnO @ 300 mg kg^{-1} seed (1.95g), T_6- nano ZnO @ 700mg kg^{-1} of seed (1.93g), T_8- custard leaf powder@ 500mg kg^{-1} of seed (1.88g) were on par with control(1.83) (Table 4).

4.2.9 Fruit yield/ plant

Significant differences were found among the treatments (Table 4). T_4- nano ZnO @ 500 mg kg^{-1} of seed recorded highest yield (224.56g) which is on par with T_7- nano ZnO @ 800 mg kg^{-1} of seed (219.47g). TreatmentsT_2- nano ZnO @ 300 mg Kg^{-1} of seed, T_3- nano ZnO @ 400 mg kg^{-1} of seed and T_8- custard leaf powder @ 500 mg kg^{-1} seed recorded fruit yield of 154.14g, 161.23g, and 160.21g respectively. Lowest fruit yield was obtained in control (130.53g).

4.2.10 Seeds per fruit

Compared to untreated seeds treated seeds recorded higher number of seeds per fruit (Table 4). Seeds treated with T_5-nano ZnO @ 600 mg kg^{-1} of seed recorded highest number of seeds (56.67) which was on par with T_6 - nano ZnO @ 700 mg kg^{-1} of seed (65.8) and T_7 - nano ZnO @ 800 mg kg^{-1} of seed (67.59). Among the treatments T_2- nano ZnO @ 300 mg kg^{-1} of seed recorded lowest number of seeds per fruit (55.3).

4.2.11 100 seed weight(g)

Significant difference was found among the treatments.100 seed weight was varied from 0.36g T_3-nano ZnO @ 400mg kg-1 of seed to 0.43g in T_5- nano ZnO @ 600 mg kg^{-1} of seed (Table 4)

4.2.12. Seed yield/ plant

The treatments differed among themselves for the trait seed yield per plant (Table 4). Seeds treated with T_4- nano ZnO @ 500 mg kg^{-1} of seed (24.02g) recorded the highest seed yield compared to control (11.35g). Treatments T_5- nano

ZnO @ 600 mg kg^{-1} of seed (14.11g) and T_8- custard leaf powder @ 500 mg kg^{-1} of seed (13.51g) were on par with each other and treatments T_2 - nano ZnO @ 300 mg kg^{-1} of seed (14.72g) and T_3- nano ZnO @ 400 mg kg^{-1} of seed (16.04g) were on par with each other. T_1 control recorded the least seed yield per plant (11.35g).

Incidence of mites and thrips was also observed.

Table 5. Analysis of variance of pre storage seed treatment seed yield and fruit yield attributes in chilli

Sources of variation	Degrees of freedom	Mean sum of squares										
		Plant height (cm)	Branches/ plant (nos.)	Days to first flowering (nos.)	Days to 50 % flowering (nos.)	Fruits/ plant (nos.)	Fruit length (cm)	Fruit weight at maturity (gm)	Fruit yield/plant (g)	No. of seeds/ fruit (nos.)	100 seed weight (g)	Seed yield per plant (g)
Replications	2	0.62	2.39	1.54	5.54	2.00	0.24	3.20	23340.07	23.88	0.00	3.81
Treatments	12	28.86**	25.31**	17.79**	30.28**	413.91**	0.42	1.99**	43535.45**	186.42**	0.00**	242.21**
Error	24	0.88	2.40	5.45	6.35	15.01	0.25	0.48	2940.05	38.79	0.00	1.98
SE		0.54	0.89	1.35	1.46	2.24	0.29	0.40	31.31	3.60	0.01	0.81
CV (%)		2.60	13.39	2.94	2.88	4.35	10.36	12.57	11.03	10.07	3.65	0.96

Significant at 5% level *

Significant at 1%level**

Table 6. Effect of prestorage seed treatments with nanoparticles on biometric observations in chilli

Treatment	Plant height (cm)	Branches/ plant	Days to first flowering	Days to 50 % flowering
T_1: Control	36.11^{c}	6.60^{d}	83.67^{a}	93.33^{a}
T_2: 300mg/kg ZnO	37.84^{b}	10.53^{bc}	80.33^{abc}	86.33^{bc}
T_3: 400mg/kg ZnO	36.77^{bc}	13.06^{ab}	77.33^{c}	82.00^{c}
T_4: 500mg/kg ZnO	33.91^{d}	14.86^{a}	76.33^{c}	86.67^{b}
T_5: 600mg/kg ZnO	33.35^{de}	11.13^{bc}	78.67^{bc}	88.67^{b}
T_6: 700mg/kg ZnO	36.92^{bc}	14.70^{a}	79.67^{abc}	87.67^{b}
T_7: 800mg/kg ZnO	41.98^{a}	12.96^{ab}	81.67^{ab}	89.00^{ab}
T_8: 500mg/kg Custard leaf powder	32.07^{e}	8.70^{cd}	77.67^{bc}	86.67^{b}
SE_m	0.54	0.89	1.35	1.46
CD (0.05)	1.64	2.71	4.09	4.41

Table 7. Effect of prestorage seed treatments with nanoparticles on fruit and seed traits

Treatment	Fruits/ plant (nos.)	Fruit length (cm)	Fruit weight at maturity (gm)	Fruit yield/plant (g)	No of seeds/ fruit(nos.)	100 seed weight (g)	Seed yield per plant (g)
T_1: Control	73.86[f]	4.40	5.51[bc]	406.74[d]	55.91[cd]	0.42[a]	37.17[e]
T_2: 300mg/kg ZnO	91.83[c]	4.63	4.61[c]	423.68[bcd]	64.36[abc]	0.41[ab]	41.88[d]
T_3: 400mg/kg ZnO	84.57[d]	5.60	4.88[c]	412.71[cd]	57.48[cd]	0.36[c]	42.65[c]
T_4: 500mg/kg ZnO	108.67[a]	4.69	7.10[a]	770.68[a]	73.80[a]	0.42[a]	60.56[a]
T_5: 600mg/kg ZnO	83.00[de]	4.55	6.13[ab]	507.27[bc]	58.60[cd]	0.43[a]	55.77[b]
T_6: 700mg/kg ZnO	101.10[b]	4.85	4.80[c]	482.54[bcd]	50.93[d]	0.39[b]	44.64[c]
T_7: 800mg/kg ZnO	91.77[c]	4.89	5.56[bc]	510.02[b]	62.00[bc]	0.39[b]	57.74[b]
T_8: 500mg/kg Custard leaf powder	77.50[ef]	5.06	5.41[bc]	420.91[bcd]	71.94[ab]	0.41[ab]	40.33[d]
SEm	2.24	0.29	0.40	31.31	3.60	0	0.81
CD (0.05)	6.79	NS	1.21	94.96	10.91	0.03	2.47

4.3 Effect of pre storage treatment with inorganic nanoparticles in chilli

4.3 Field performance of seeds treated with nano particle on yield attributes

4.3.1 Analysis of Variance

The analysis of variance on yield attributes such as days to first flowering, days to 50 % flowering, plant height (cm), branches per plant, fruits per plant, fruit length (cm), fruit weight at maturity (g), seed yield per plant(g), 100 seed weight (g) and seeds per fruit revealed highly significant differences among the treatments in Ujwala (Table5)

4.3.2 Days to first flowering

Significant differences were observed among the seed treatments (Table 6). Seed treatment with nanoparticles were found to have early flowering in plants compared to control (83.67). Among the treatments, seeds treated with T_4- nano ZnO @ 500 mg kg^{-1} of seed (73.33) flowered early and was on par with T_3 - nano ZnO @ 400 mg kg^{-1} of seed(77.33),(T_8) custard leaf powder 500mg kg^{-1},T_5 nano ZnO @600mg kg^{-1} of seed and (T_6) nanoZnO @700mg kg^{-1} of seed T_7- nano ZnO @ 800 mg kg^{-1} of seed (81.67) and T_2- nano ZnO 300 mg kg^{-1} (80.33) took more days for first flowering. Control recorded 83.67 days for first flowering.

4.3.3 Days to 50 % flowering

The treatments recorded significant differences in case of days to 50 percent flowering. Among the treatments (Table 6) seeds treated with T_3- nano ZnO@ 400 mg $kg^{-1}$of seed (82 days) showed early 50% flowering compared to all other treatments. There were no significant differences between treatments T_4- nano ZnO @ 500 mg kg^{-1} of seed (86.67 days), T_5- nano ZnO @ 600 mg kg^{-1} of seed (88.67days), T_6- nano ZnO@ 700 mg kg^{-1} of seed (87.67 days) , T_8- custard leaf powder 500 mg Kg^{-1} of seed (86.67days). T_7- nano ZnO @ 800 mg kg^{-1} of seed (89 days) was on par with T_1 (control) (93.33days).

4.3.4 Plant height (cm)

Treatments recorded significant differences in case of plant height (Table 6). Treated seeds produced taller plants when compared to control (36.11cm). Among the treated seeds, least plant height was recorded in T_8- Custard leaf powder 500 mg kg^{-1} of seed(32.07cm) while the tallest plants were observed by T_7- nano ZnO 800 mg kg^{-1} of seed (41.98 cm) followed by T_2- nano ZnO @ 300 mg kg^{-1} of seed (37.84 cm).T_2 is on par with T_3-nano ZnO 400 mg $kg^{-1}$of seed (36.77 cm) There were no significant differences between T_3- nano ZnO @ 400 mg kg^{-1} of seed (36.77 cm) and T_6- nano ZnO @ 700 mg kg^{-1} of seed (36.92 cm).

4.3.5 Number of branches per plant

Significant difference was observed in case of number of branches per plant (Table 6), when the seeds were treated with nanoparticle. Among the treatments seed plants T_4- nano ZnO @ 500 mg kg^{-1} of seed had highest number of branches (14.86) which is comparable with T_6- nano ZnO @ 500 mg kg^{-1} of seed (14.70) , T_3- nano ZnO @ 400 mg $kg^{-1}$of seed (13.06) and T_7-nano ZnO @ 800 mg kg^{-1} of seed (12.96). Least number of branches per plant was observed in case of control (6.60) and among the treatments T_2 – 300 mg kg^{-1} of seed showed least number of branches per plant (10.53).

4.3.6 Fruits per plant

Significant difference was observed in case of fruits per plant (Table 7).When treated seed is compared with control, treated seed recorded higher number of fruits per plant. Highest number of fruits per plant was observed in case of T_4 – nano ZnO @ 500 mg $kg^{-1}$of seed (108.67) followed by T_6- nano ZnO @ 700mg $kg^{-1}$of seed (101.1), T_2- nano ZnO @ 300 mg kg $^{-1}$ of seed (91.83) Among the nanotreatments least number of fruits per plant was T_8- nano ZnO @ 500 mg kg^{-1} of seed custard leaf powder gives lowest number of fruits per plant (77.50).

4.3.7 Fruit length (cm)

There was no differences among the treatments for fruit length. (Table 7)

4.3.8 Fruit weight at maturity (g)

Significant difference were observed among the treatments (Table 7). Highest fruit weight was observed in case of T_4- 500 mg kg^{-1} of seed (7.10g) which is comparable with T_5- 600 mg kg^{-1} of seed (6.13g).

4.3.9 Fruit yield/ plant

Seeds treated with nanoparticles. T_4- nano ZnO @ 500 mg kg^{-1} of seed recorded highest yield (770.68g) followed by T_7- nano ZnO @ 800 mg kg^{-1} of seed (510.02g)(Table 7). Least fruit yield was obtained in control (406.74g).

4.3.10 Seeds per fruit

Treated seed resulted in more number of seeds per fruit when compared with control (55.91)except T_6 700mg kg^{-1} of seed (50.93) (Table 7). T_4 –nano ZnO @ 500 mg kg^{-1} of seed recorded more number of seeds per fruit (73.80) which is comparable with T_8- custard leaf powder @ 500 mg kg^{-1} of seed (71.94) and T_2 nano ZnO @ 300mg kg^{-1} of seed (64.36) .

4.3.11 100 seed weight(g)

Significant differences was found among the treatments in case of 100 seed weight (Table 7). 100 seed weight varied from 0.43g in T_5- nano ZnO @ 600 mg kg^{-1} of seed, to 0.36g in T_3- nano ZnO @ 400 mg kg^{-1} of seed.100 seed weight of control was 0.42 g.

4.3.12 Seed yield/plant (g)

There was significant variations among the treatments in seed yield per plant compared to control (Table 7). Seeds treated with T_4- nano ZnO @ 500 mg kg^{-1} of seed (60.56 g) recorded highest seed yield. Followed by T_5- nano ZnO @ 600 mg kg^{-1} of seed (55.77g), T_7-nano ZnO @ 800 mg $kg^{-1}$of seed (57.74g), T_3- nano ZnO @ 400 mg kg^{-1} of seed (42.65g), T_6-nano ZnO @ 700 mg kg^{-1} of seed (44.64g), T_2 - nano 300 mg kg^{-1} of seed (41.88g), T_8- Custard leaf powder @ 500 mg $kg^{-1}$of seed (40.33g). Least seed yield observed in control (37.17g).

Incidence of mites and thrips were also observed.

Table 8. Initial seed quality parameters before mid- term storage treatment

Seed quality parameters		Pre storage	After three months of storage
Germination percent (%)		74.00	62.00
Shoot length(cm)		5.40	4.88
Root length (cm)		9.37	9.29
Seedling dry weight (mg)		25.35	25.09
Vigour Index 1		1092.00	879.00
Vigour Index 2		1875.00	1556.00
Electrical conductivity (μScm^{-1})		60.60	70.20
Moisture content (%)		7.10	7.24
Seed microflora (%)	**Blotter paper method**	0.00	24.00
	Agar plate method	5.00	37.50

Table 9. Effect of midstorage treatments with nanoparticles on germination and electrical conductivity of chilli

	Germination (%)				Electrical conductivity((μScm^{-1})			
	After initial treatment	**1MAS**	**2MAS**	**3MAS**	**After initial treatment**	**1MAS**	**2MAS**	**3MAS**
T_1: Control	61.00^{cd}	59.67^{cd}	56.00^{d}	52.67^{de}	94.70^{a}	104.33^{a}	141.77^{a}	159.20^{a}
T_2: 300mg/kg ZnO	62.33^{bc}	60.67^{bcd}	57.00^{cd}	54.33^{cd}	79.43^{b}	86.20^{c}	116.40^{b}	140.60^{b}
T_3: 400mg/kg ZnO	70.33^{a}	68.00^{a}	65.33^{a}	64.33^{a}	69.57^{fg}	74.33^{e}	101.33^{e}	110.60^{fg}
T_4: 500mg/kg ZnO	64.67^{b}	63.67^{b}	60.33^{bc}	59.67^{b}	68.20^{g}	74.36^{e}	97.80^{e}	108.43^{g}
T_5: 600mg/kg ZnO	58.33^{d}	55.67^{e}	53.67^{de}	51.33^{de}	71.90^{de}	80.40^{d}	101.40^{e}	113.20^{f}
T_6: 700mg/kg ZnO	60.67^{cd}	58.00^{de}	55.33^{d}	50.00^{de}	76.97^{c}	84.30^{c}	112.40^{bc}	126.70^{d}
T_7 : 800mg/kg ZnO	58.33^{d}	55.00^{e}	50.67^{e}	49.67^{e}	71.00^{ef}	84.10^{c}	110.40^{cd}	130.90^{c}
T_8 : 500mg/kg Custard leaf powder	64.33^{b}	62.67^{bc}	61.67^{ab}	58.33^{bc}	73.53^{d}	95.20^{b}	107.10^{d}	122.30^{e}
SEm	0.75	1.13	1.34	1.49	0.87	0.99	1.56	0.88
CD (0.05)	2.25	3.39	4.01	4.47	2.62	2.97	4.68	2.65

***MAS- Month after storage**

4.4 Effect of mid- storage correction with inorganic nanoparticles in chilli

4.4.1 Analysis of variance

The analysis of variance indicated that highly significant differences existed among the treatments for germination per cent, seedling shoot length and root length, seedling dry weight, seedling vigour indices, electrical conductivity of seed leachate and seed infection percent in Ujwala.

4.4.2 Germination (%)

Seed before storage recorded a germination per cent of 72.After three months of storage germination per cent reduced to 62 per cent (Table 8). The seeds were treated with different doses of nano scale ZnO and nanoscale custard apple leaf powder. After treatment T_3- nano ZnO @ 400 mg kg^{-1} of seed recorded highest germination per cent (70.33) (Table 9).

There was significant difference among treatments throughout the storage period. After one month of storage, T_3 – nano ZnO @ 400 mg kg^{-1} of seed recorded the highest germination per cent (68) followed by T_4 – nano ZnO @ 500 mg kg^{-1} of seed (63.67) .The latter being on par with T_8- custard leaf powder 500 mg $kg^{-1}$of seed (62.67) and T_2- nano ZnO @ 300 mg $kg^{-1}$of seed (62.33). Least germination per cent was seen in T_7– nano ZnO @ 800 mg kg^{-1} of seed (60.67). At the end two months of storage two treatments namely T_3 – nano ZnO @ 400 mg kg^{-1} of seed and T_4 – nano ZnO @ 500 mg kg^{-1} of seed retained a germination per cent of above 60 per cent. However, at the end of the storage period only treatment T_3 – nano ZnO @ 400 mg kg^{-1} of seed could retain a germination per cent of 64.33 per cent.

4.4.3 Electrical conductivity (μScm^{-1})

Electrical conductivity of fresh seeds before storage was (60.6 μScm^{-1}) which later increased to (70.2μScm^{-1}) after three months of storage (Table 8). After treatment T_4- nano ZnO @ 500 mg kg^{-1} of seed recorded the lowest electrical conductivity (68.2μScm^{-1}). It was observed that electrical conductivity increased with the increase in storage period. At the end of storage the electrical conductivity of all treatments were less than control. (Table 9)

There was significant difference between treatments. In the first month after treatment all the treatments recorded an electrical conductivity less than control (104.33 μScm^{-1}).However T_3- nano ZnO @ 400 mg kg^{-1} of seed (74.33 μScm^{-1}) and T_4- nano ZnO @ 500 mg kg^{-1} of seed (74.36 μScm^{-1}) retained the lowest electrical conductivity.

The same trend was observed at the end of the storage period with T_4 – nano ZnO @ 500 mg $kg^{-1}$of seed (108.43μScm^{-1}) and T_3- nano ZnO @ 400 mg kg-^{1} of seed (110.6 μScm^{-1}) recording the least values while the electrical conductivity of control was (159.2 μScm^{-1}).

Table 10. Effect of midstorage treatments with nanoparticles on shoot length and root length length of chilli

	Shoot length(cm)				Root length(cm)			
	After initial treatment	**1MAS**	**2MAS**	**3MAS**	**Initial**	**After initial treatment**	**2MAS**	**3MAS**
T_1: Control	3.48^{e}	3.33^{cd}	3.24^{cd}	3.19^{ef}	7.52^{c}	7.36^{c}	7.20^{bc}	6.94^{c}
T_2: 300mg/kg ZnO	3.60^{e}	3.58^{bcd}	3.45_{c}	3.39^{de}	7.91^{b}	7.86^{b}	7.51^{b}	7.21^{b}
T_3: 400mg/kg ZnO	4.54^{a}	4.43^{a}	4.35^{a}	4.21^{a}	9.24^{a}	9.10^{a}	8.98^{a}	8.79^{a}
T_4: 500mg/kg ZnO	4.05^{c}	3.99^{ab}	3.90^{b}	3.86^{bc}	9.09^{a}	9.00^{a}	8.87^{a}	8.70^{a}
T_5: 600mg/kg ZnO	3.85^{d}	3.76^{bc}	3.70^{b}	3.59^{cd}	7.15^{d}	7.13^{c}	7.00^{c}	6.82^{c}
T_6: 700mg/kg ZnO	3.25^{f}	3.23^{d}	3.09^{d}	3.01^{f}	6.50^{e}	6.46^{d}	6.38^{d}	6.20^{d}
T_7 : 800mg/kg ZnO	3.63^{e}	3.43^{cd}	3.31^{cd}	3.23^{ef}	6.20^{e}	6.13^{d}	5.96^{e}	5.85^{e}
T_8 : 500mg/kg Custard leaf powder	4.30^{b}	4.39^{a}	4.18^{a}	4.10^{ab}	8.95^{a}	8.86^{a}	8.68^{a}	8.54^{a}
SEm	0.06	0.16	0.08	0.10	0.11	0.13	0.12	0.08
CD (0.05)	0.17	0.47	0.25	0.31	0.34	0.39	0.36	0.25

***MAS- Month after storage**

4.4.4 Shoot length (cm)

Shoot length reduced from (5.40cm) to (4.88cm) within three months of storage (Table 8). Seedling shoot length reduced with the time period of storage. Significant differences were noticed among the treatments throughout the storage period .Treatment T_3- nano ZnO @ 400 mg kg^{-1} of seed recorded the highest shoot length (4.54cm)(Table 10)

After one month of storage, T_3-nano ZnO @ 400 mg kg^{-1} of seed (4.43cm) and both being on par T_8- custard leaf powder @ 500 mg kg^{-1} (4.39cm) recorded higher values for the trait treatment T_6-nano ZnO @ 700 mg $kg^{-1}$of seed recorded the least value.

At the end of storage control recorded a shoot length (3.19 cm).

4.4.5 Root length (cm)

Root length showed a similar trend as in shoot length. There was significant difference was observed among different treatments throughout the storage period. Root length of seeds when stored for three months reduced from (9.24cm) in treatment T_3-nano ZnO @ 400 mg kg^{-1} of seed to T_7 nano ZnO @ 800mg kg^{-1} of seed (5.85cm). Treatment T_3-nano ZnO @ 400 mg kg^{-1} of seed recorded the highest root length throughout the storage period followed by T_4- 500 mg kg^{-1} of seedand T_8- custard leaf powder @ 500 mg kg^{-1} of seed. Mean while the treatment, T_7- nano ZnO @ 800 mg $kg^{-1}$of seed (Table 10) recorded the least values. At the end of the storage period control recorded a root length of 6.94cm.

4.4.6 Seedling dry weight (mg)

Seedling dry weight reduced gradually with an increase in the period of storage (Table 11). Fresh seeds having seedling dry weight of (25.35 mg) reduced to (25.09 mg) by three months of storage (Table 8). There was no significant differences among the treatment immediately after treatment. Significant difference was found among different treatments throughout the storage period. At 3MAS highest dry weight was observed in case of T_3- nano ZnO @ 400 mg kg^{-1} of seed (24.96mg) which was on par with T_2 nano ZnO @ 300mg kg^{-1} of seed,T_4 nano ZnO @ 500mg kg^{-1} of seed and T_7 nano ZnO @ 800mg kg^{-1} of seed . Treatments T_2- nano ZnO @ 300 mg kg^{-1} of seed, T_4 – 500 mg kg^{-1} ZnO T_5 – 600 mg kg^{-1} ZnO and T_7 - 800mg/kg ZnO were on par with each other throughout the storage period.

Table 11. Effect of midstorage treatments with nanoparticles on seedling dry weight of chilli (mg)

Seedling dry weight(mg)				
	After initials treatment	**1MAS**	**2MAS**	**3MAS**
T_1: Control	23.06	22.86^{d}	22.39^{d}	21.94^{c}
T_2: 300mg/kg ZnO	24.56	24.36^{abc}	24.24^{abc}	24.39^{ab}
T_3: 400mg/kg ZnO	25.22	25.12^{a}	25.03^{a}	24.96^{a}
T_4: 500mg/kg ZnO	25.15	24.92^{ab}	24.74^{ab}	24.35^{ab}
T_5: 600mg/kg ZnO	24.36	24.01^{abcd}	23.92^{abc}	23.76^{ab}
T_6: 700mg/kg ZnO	23.73	23.57^{cd}	23.36^{cd}	23.27^{b}
T_7: 800mg/kg ZnO	24.76	24.55^{abc}	24.43^{abc}	24.31^{ab}
T_8: 500mg/kg Custard leaf powder	24.30	23.87^{bcd}	23.65^{bc}	23.57^{b}
SEm	0.45	0.39	0.39	0.40
CD (0.05)	NS	1.16	1.18	1.20

***MAT- Month after storage**

Table 12. Effect of midstorage treatments with nanoparticles on Vigour index I and Vigour index II of chilli

	Vigour index I				Vigour index II			
	Initial	**1MAS**	**2MAS**	**3MAS**	**Initial**	**1MAS**	**2MAS**	**3MAS**
T_1: Control	550^{bcd}	637^{d}	585^{c}	533^{c}	1406^{d}	1365^{de}	1254^{d}	1156^{d}
T_2: 300mg/kg ZnO	447^{d}	694^{c}	625^{c}	576^{c}	1531^{bcd}	1479^{bcd}	1382^{bc}	1325^{bc}
T_3: 400mg/kg ZnO	465^{cd}	920^{a}	871^{a}	836^{a}	1774^{a}	1708^{a}	1636^{a}	1606^{a}
T_4: 500mg/kg ZnO	721^{a}	827^{b}	771^{b}	748^{b}	1626^{b}	1587^{ab}	1492^{b}	1454^{b}
T_5: 600mg/kg ZnO	611^{ab}	607^{de}	575^{cd}	535^{c}	1421^{d}	1337^{e}	1285^{cd}	1218^{cd}
T_6: 700mg/kg ZnO	492^{cd}	562^{ef}	524^{d}	460^{d}	1440^{cd}	1367^{cde}	1293^{cd}	1163^{d}
T_7 : 800mg/kg ZnO	566^{bc}	526^{e}	470^{e}	451^{d}	1445^{cd}	1350^{de}	1238^{d}	1208^{cd}
T_8 : 500mg/kg Custard leaf powder	516^{bcd}	831^{b}	793^{b}	737^{b}	1565^{bc}	1497^{bc}	1458^{b}	1376^{b}
SE_m	36.88	18.16	17.49	15.31	44.96	43.79	41.12	43.21
CD (0.05)	111.85	54.45	52.44	45.92	136.35	131.28	123.28	129.54

***MAS- Month after storage**

4.4.7 Vigour index 1

Vigour index I of fresh seeds reduced from 1092 in first month of storage to 879 in third month of storage (Table 8). The mean vigour index I declined gradually during storage .Initial after treatment shows that vigour index is high in case of T_4- nano ZnO @ 500 mg $kg^{-1}$of seed (721). It is clearly evident from the Table that treatments after one month of storage showed a significant reduction.(Table 12)

After one month of storage highest vigour index 920 was recorded in T_3-nano ZnO @ 400 mg kg^{-1} of seed followed by T_4- nano ZnO @ 500 mg kg^{-1} of seed (827). At two months after storage T_3- nano ZnO @ 400 mg kg^{-1} of seed show the highest vigour (871) followed by T_4- nano ZnO @ 500 mg kg^{-1} of seed (771). which is on par with T_8-custard leaf powder @ 500 mg kg^{-1} (793).

At the end of storage vigour index I was highest in case of T_3 – nano ZnO @ 400 mg kg^{-1} (836) followed by T_4- 500 mg $kg^{-1}$of seed (748).

4.4.8 Vigour index II

Vigour index II reduced from 1875 to 1556 by the third month of storage (Table 8). The vigour index II was reduced gradually to the end of storage. It was observed that immediately after treatment vigour index II is high in case of T_3- nano ZnO @ 400 mg kg^{-1} of seed (1708). There was significant differences among the treatments throughout the period of storage. One month after treatment T_3 - nano ZnO @ 400 mg kg^{-1} of seed shows highest seed vigour index II (1708) which is in statistically on par with T_4-nano ZnO @ 500 mg kg^{-1} of seed (1587). Lowest seed vigour index II was observed in T_5- nano ZnO @ 600 mg $kg^{-1}$of seed (1337). At the end of storage, T_3- nano ZnO @ 400 mg $kg^{-1}$of seed has highest seed vigour index II (1606) followed by T_4 nano ZnO @ 500 mg $kg^{-1}$of seed (1454) which is comparable with T_8- custard leaf powder @ 500 mg kg^{-1} of seed (1208)(Table 12).

Table 13. Effect of midstorage treatments with nanoparticles on moisture content of chilli

	Moisture content(%)	
	Prestorage	**After 3 month storage**
T_1: Control	7.12	7.23
T_2: 300mg/kg ZnO	7.02	7.15
T_3: 400mg/kg ZnO	6.92	7.03
T_4: 500mg/kg ZnO	6.60	6.97
T_5: 600mg/kg ZnO	6.99	7.16
T_6: 700mg/kg ZnO	7.30	7.43
T_7: 800mg/kg ZnO	7.16	7.32
T_8: 500mg/kg Custard leaf powder	6.88	7.19
CD	NS	NS

*MAS : Months after storage

4.4.9 Seed moisture content

Seed moisture content increased from 7.10 to 7.24 at the end of the third month of storage (Table 8). There was no significant differences among the treatments. There was a marginal increase in seed moisture content at the end of storage. There is no significant variation in moisture content from the start till the end of storage (Table 13).

4.4.10 Microflora per cent (%)

Fresh seeds when stored for three months, the seed microflora increased from 5 to 37.5 per cent in agar plate method (Table 14). Results of seed microflora (%) after treatment shows that seed infection was low in case of T_8 –custard leaf powder 500 mg

kg^{-1} of seed and T_2- nano ZnO @ 300 mg kg^{-1} of seed in both agar method (3.33, 23.33) and blotter method (1.33,18.67). In case of blotter method, by three months of storage, microflora per cent increased from 0 to 24 per cent. Blotter method comparatively showed less infection compared to agar method. Microflora per cent in seed increased with time.At end of storage there existed significant differences among the treatments. In case of agar plate method low infection was seen in case of T_8- custard leaf powder 500 mg kg^{-1} of seed (33.33) which was on par with T_2 –nano ZnO @ 300 mg kg^{-1} of seed (43.33). Control showed highest microflora per cent (96.67). Similar observation was observed in case of blotter method. Lower seed infection was observed in case of T_8 – custard leaf powder 500mg kg^{-1} of seed(26.67%) followed by T_2 – nano ZnO @ 300 mg kg^{-1} of seed (41.33) where as high infection is observed in case of control (89.33). *Aspergillus* sp and *Rhizopus* sp were observed in both methods of microflora testing.

Table 14. Effect of midstorage treatments with nanoparticles on seed microflora (%) in chilli

	Seed Infection (%)			
	Blotter paper method		Agar plate method	
	After initial treatment	After 3 month of storage	After initial treatment	After 3 month of storage
T_1: Control	62.67 (52.36)[a]	89.33 (71.01)[a]	66.6 (54.78)[a]	96.67 (83.85)[a]
T_2: 300mg/kg ZnO	18.67 (25.39)[e]	41.33 (39.99)[c]	23.33 (28.78)[d]	43.33 (41.07)[b]
T_3: 400mg/kg ZnO	61.33 (51.61)[ab]	85.33 (67.80)[ab]	63.33 (52.78)[ab]	83.33 (66.14)[a]
T_4: 500mg/kg ZnO	46.67 (43.05)[bcd]	78.66 (62.82)[b]	53.33 (46.92)[bc]	83.33 (70.07)[a]
T_5: 600mg/kg ZnO	40.67 (39.06)[cd]	86.66 (68.63)[ab]	46.67 (43.08)[c]	93.33 (77.71)[a]
T_6: 700mg/kg ZnO	49.33 (44.62)[abc]	83.33 (65.43)[ab]	56.67 (48.93)[abc]	83.33 (70.78)[a]
T_7: 800mg/kg ZnO	34.00 (35.30)[d]	84 (66.89)[ab]	60.00 (50.85)[ab]	86.67 (72.71)[a]
T_8: 500mg/kg Custard leaf powder	1.33 (3.85)[f]	26.67 (31.03)[d]	3.33 (35.21)[d]	33.33 (35.22)[b]

*Figures in parentheses are arc-sine transformed values

Discussion

5. DISCUSSION

The quality of seed is crucial in crop performance. Good quality seed alone increase plant yield upto 15per cent. Yield of the crop is determined by its planting value which depends on germination, seed vigour, and seed health. High germination per cent is essential for establishing an optimum plant population with the recommended seed rate of crop. Seed vigour ensures performance of a seed in field under normal and adverse conditions, and in storage . Seed enhancement technique like pre sowing, prestorage, and mid storage seed enhancement techniques are widely used in agriculture (Sharma *et al*., 2015). Nanotechnology is recently being used in agriculture as a potential seedling growth regulator /enhancer. Seed treatment with nanoparticle result in better absorption. /coating of seed and thus improve the seed quality/vigour. The efficacy of nano treatments in enhancing plant characters was reported by and Salama (2012) in maize bean.

The present study was conducted to assess the impact of nanoparticle treatments of zinc oxide in chilli seeds as pre sowing treatments as well as prestorage treatments and as midstorage correction treatment to prolong seed longevity. The findings of the study are discussed in this chapter.

5.1 Effect of presowing treatments with nanoparticles on growth and yield in chilli

5.1.1 Vegetative characters

Treating seeds with nano ZnO @ 500 mg kg^{-1} of seed resulted in earlier flowering (73.33 days) when compared with control (77.67 days) Seeds treated with nano ZnO @ 400 mg kg^{-1} of seed recorded the minimum in days to 50 per cent flowering (79.67 days) nearly nine days ahead of control. Similar observations were reported by Laware and Raskar (2014) in onion and Sharma *et al*., (2022) in tomato . Laware and Raskar (2014) opined that Zinc induced early flowering in plants by supplying vital plant growth-promoting compounds that while supported the reproductive phase.

Application of nano ZnO @ 400 mg kg^{-1} of seed (42.94cm) and nano ZnO @ 800 mg kg^{-1} of seed (42.02cm) resulted in taller plants when compared with control (35.62) which is in accordance with the findings of Mathew(2020) in Chilli

Rengeland and Graham (1995) reported that the increasing zinc content from 0.25 µg to 0.70 µg seed significantly improved root and shoot growth. High zinc content in seed act as starter fertilizer and improve germination and seedling growth. Kobayashi and Mizutani, (1970) opined that since zinc is a precursor in auxin production, application of zinc in nanoscale may enhance the IAA activity in the roots which in turn increase plant growth rate and results in extended inter-nodal length. According to Elizabeth *et al*., (2017) nano Zn treatments may help plants to expand their roots and shoots, enhancing cell permeability and supplying nutrients.

The number of branches per plant increased in nano ZnO treatments when compared with control. Among the treatments nano ZnO @ 400 mg kg^{-1} of seed recorded the highest number of branches (12.67) when compared to control (4.27). Similar observation was reported by Jabeen *et al*., (2018) in tomato. The fundamental function of Zn in protecting and maintaining the structural stability of cell membranes, protein synthesis, membrane function, cell elongation, and tolerance to environmental stress, may account for the increase in vegetative growth (Welch *et al*., 1982).

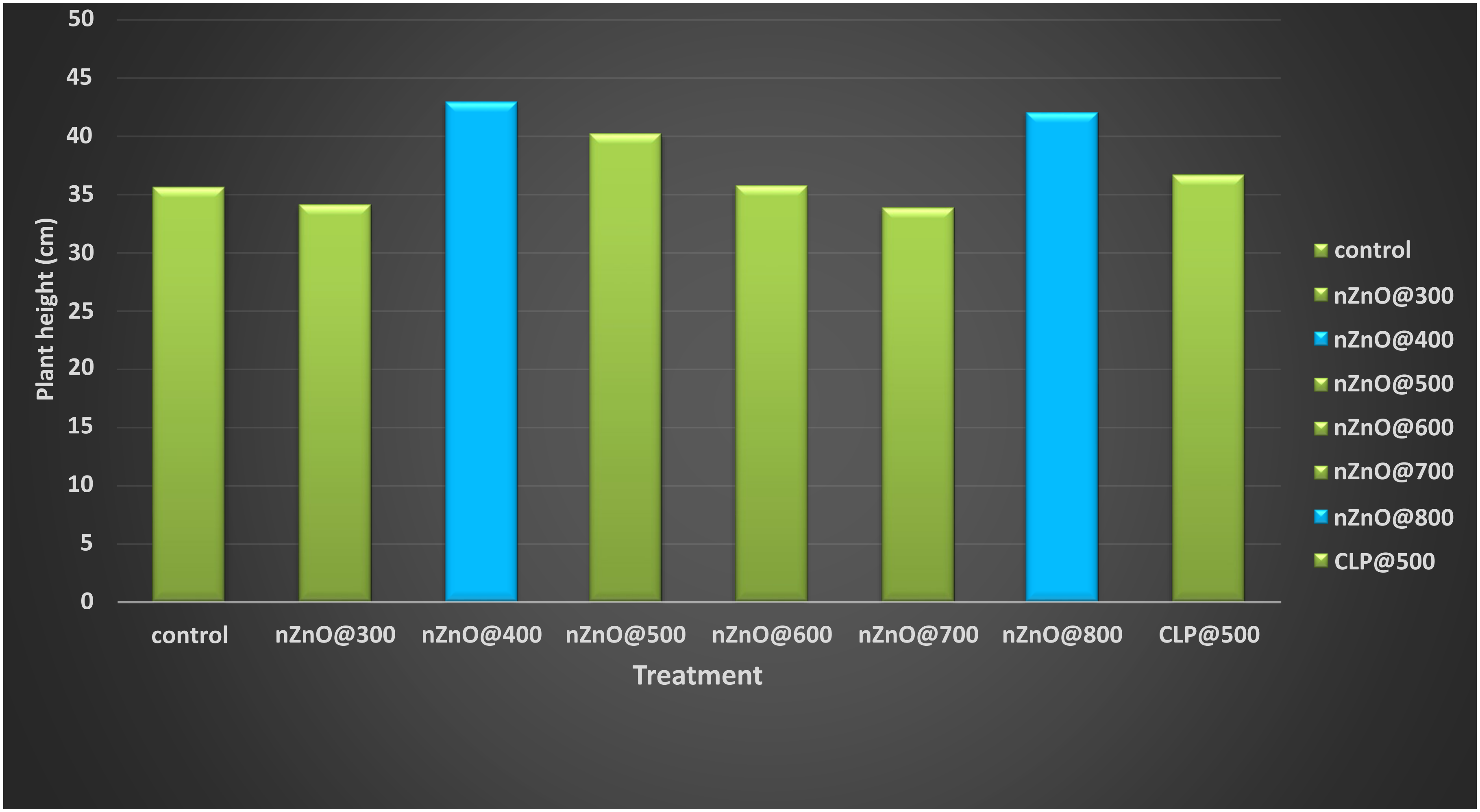

Fig 3. Effect of presowing treatments with nanoparticles on plant height

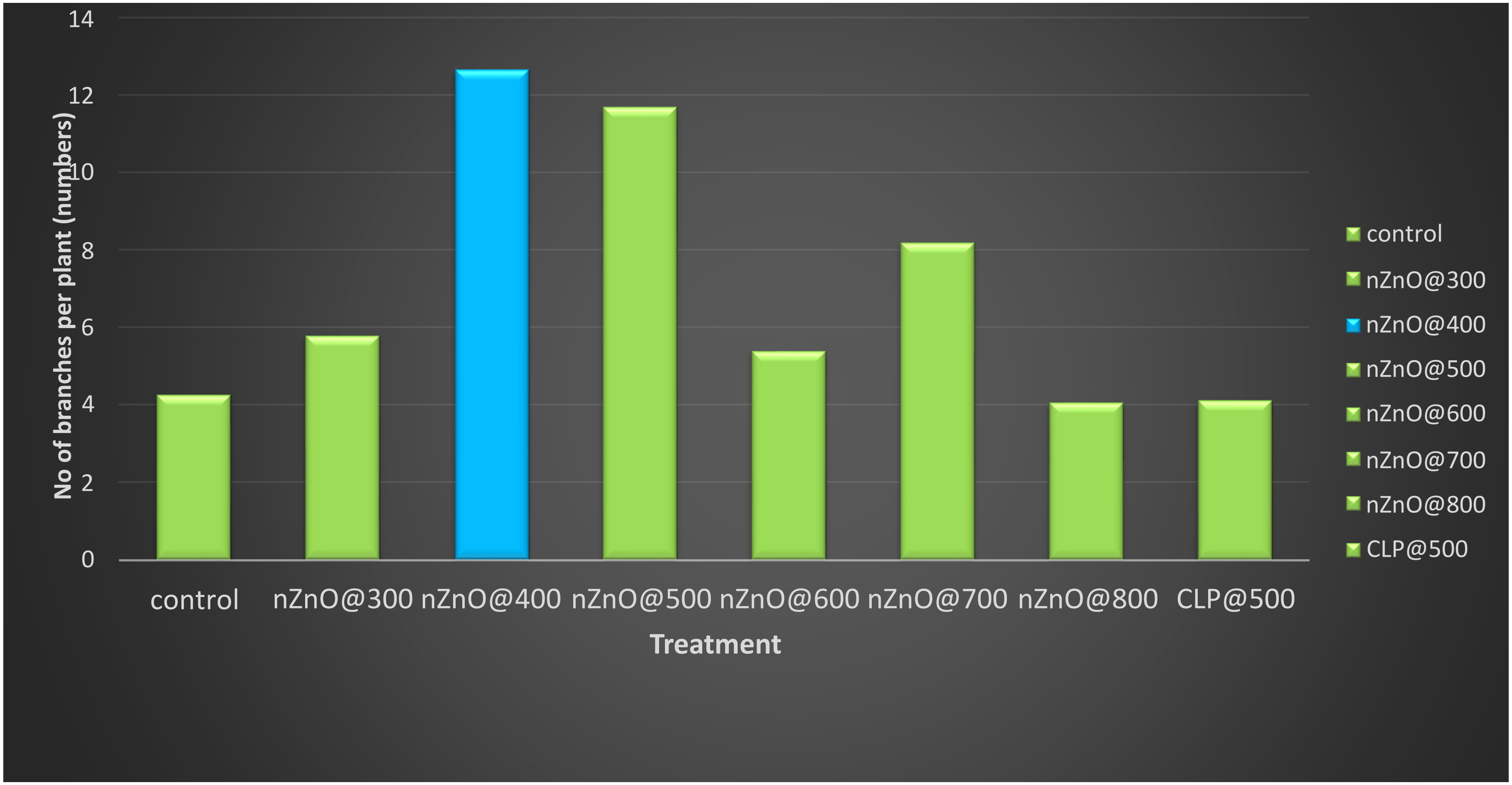

Fig 4. Effect of presowing treatments with nanoparticles on number of branches per plant

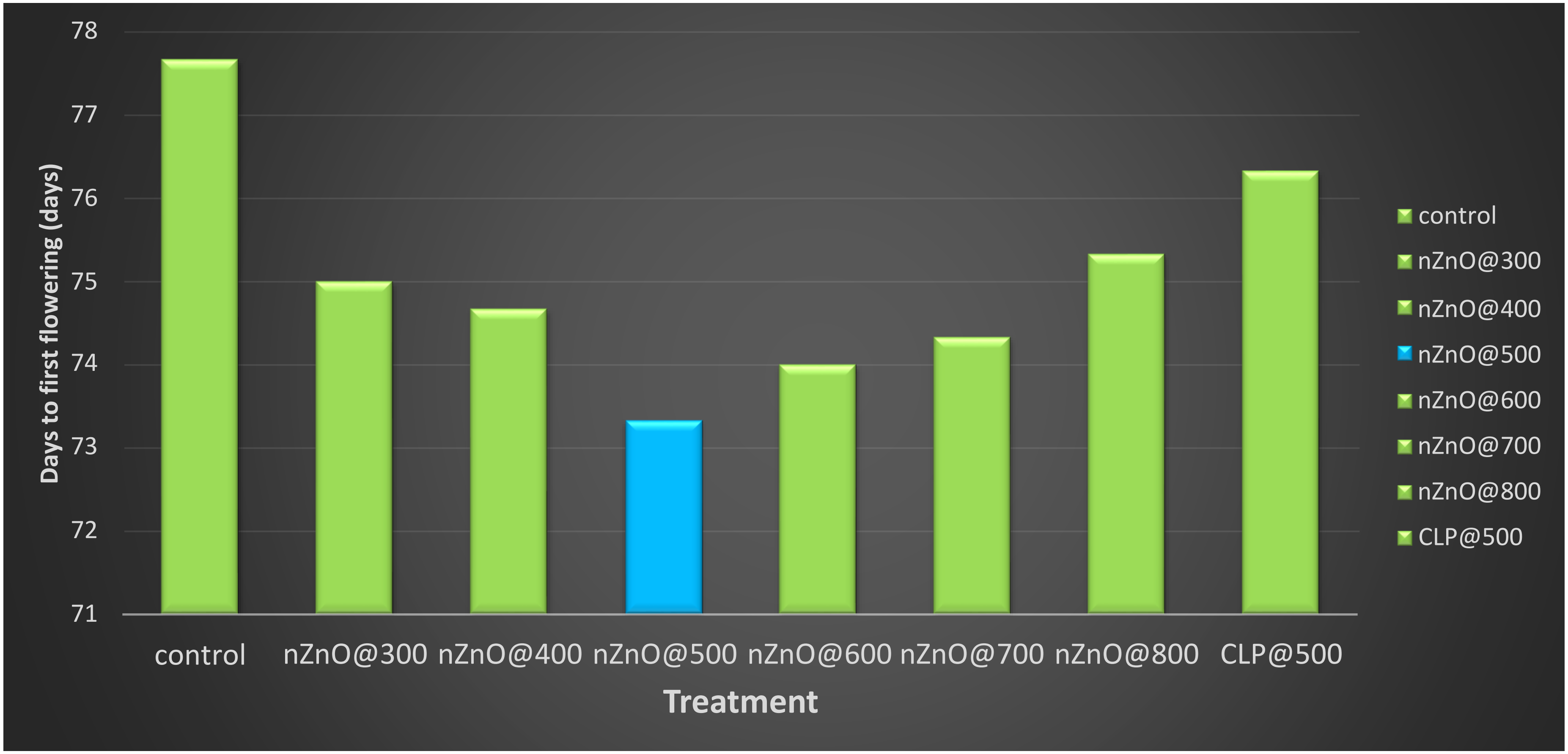

Fig 5. Effect of presowing treatments with nanoparticles on days to first flowering

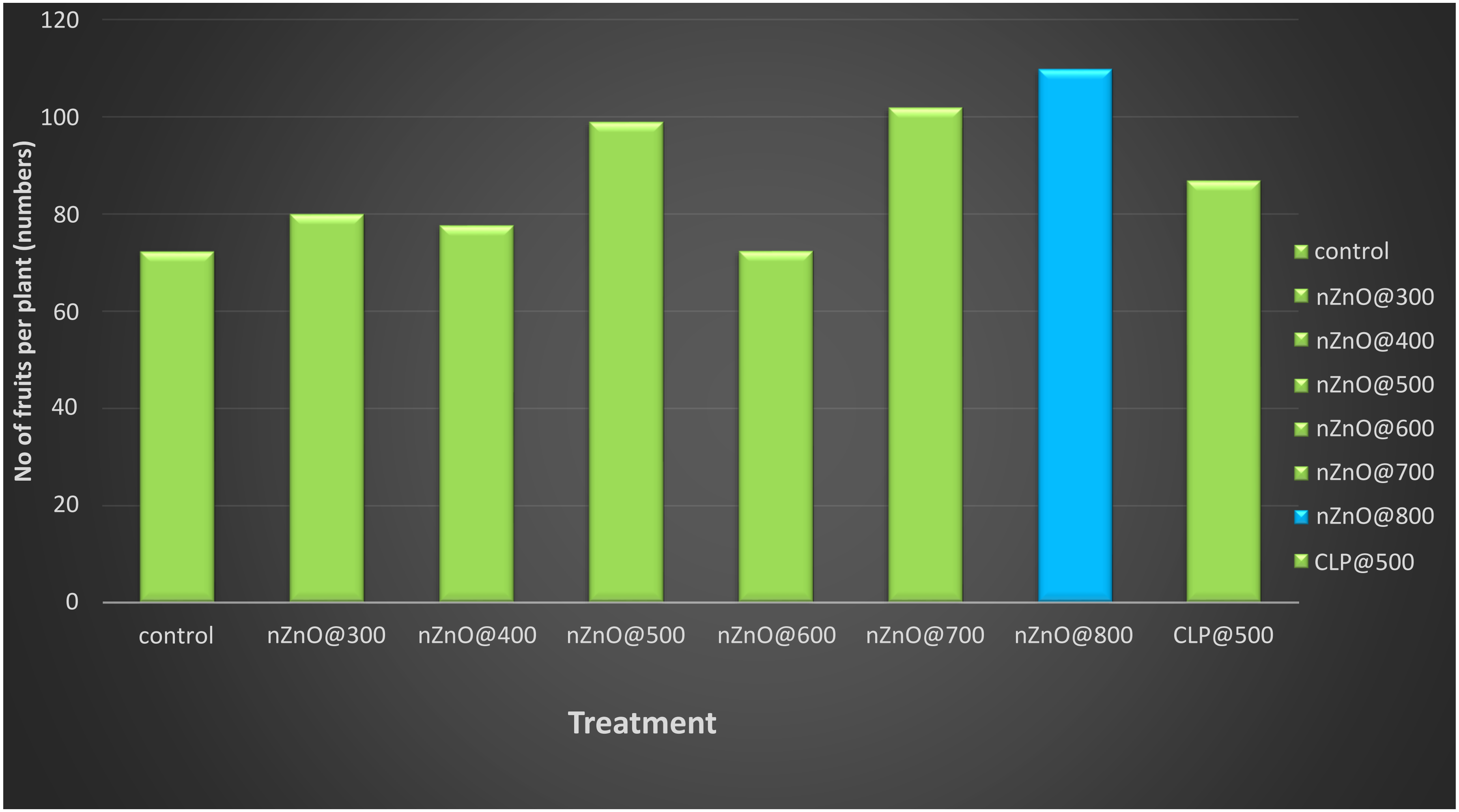

Fig 6. Effect of presowing treatments with nanoparticles on number of fruits per plant

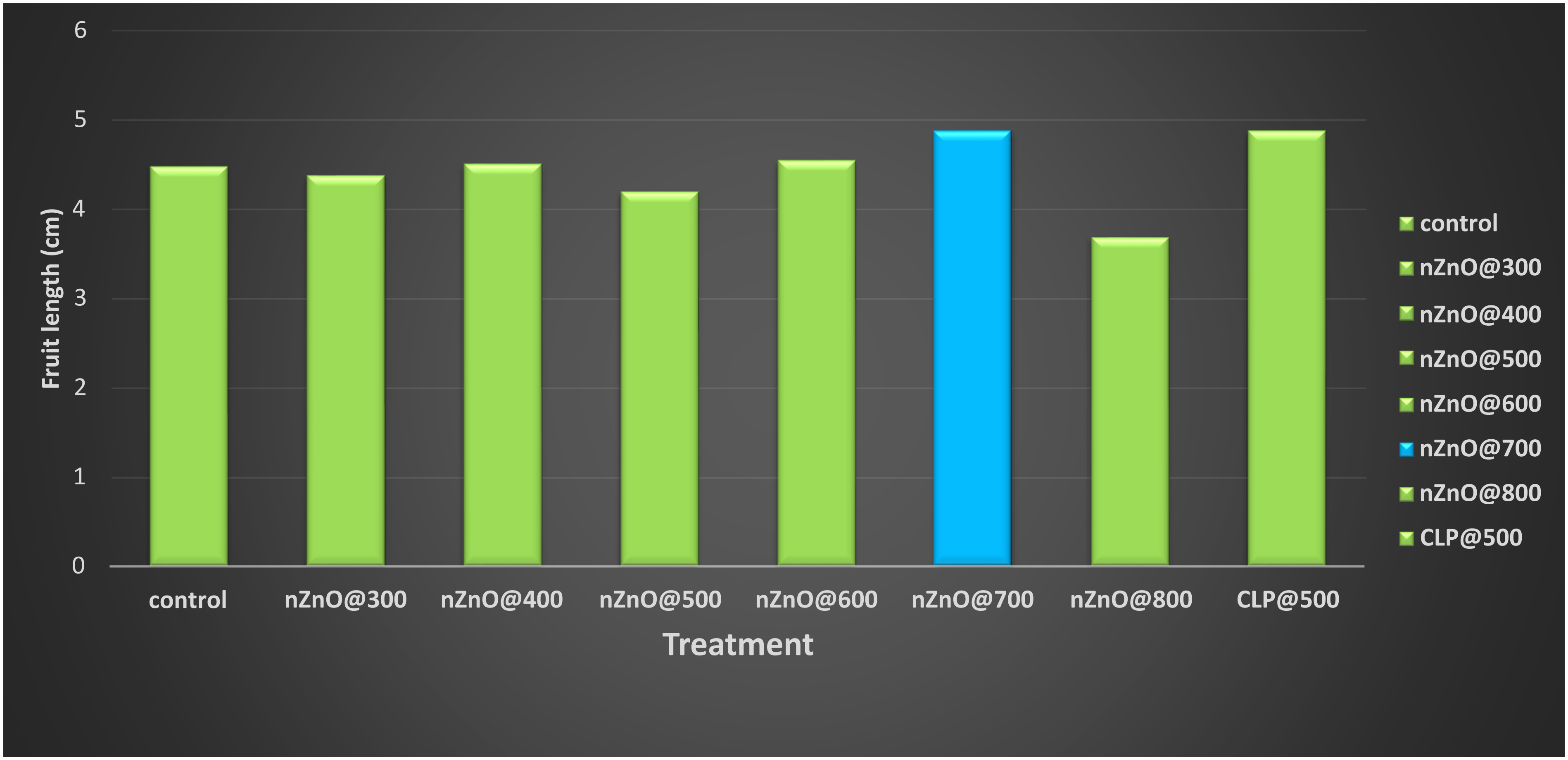

Fig 7. Effect of presowing treatments with nanoparticles on fruit length

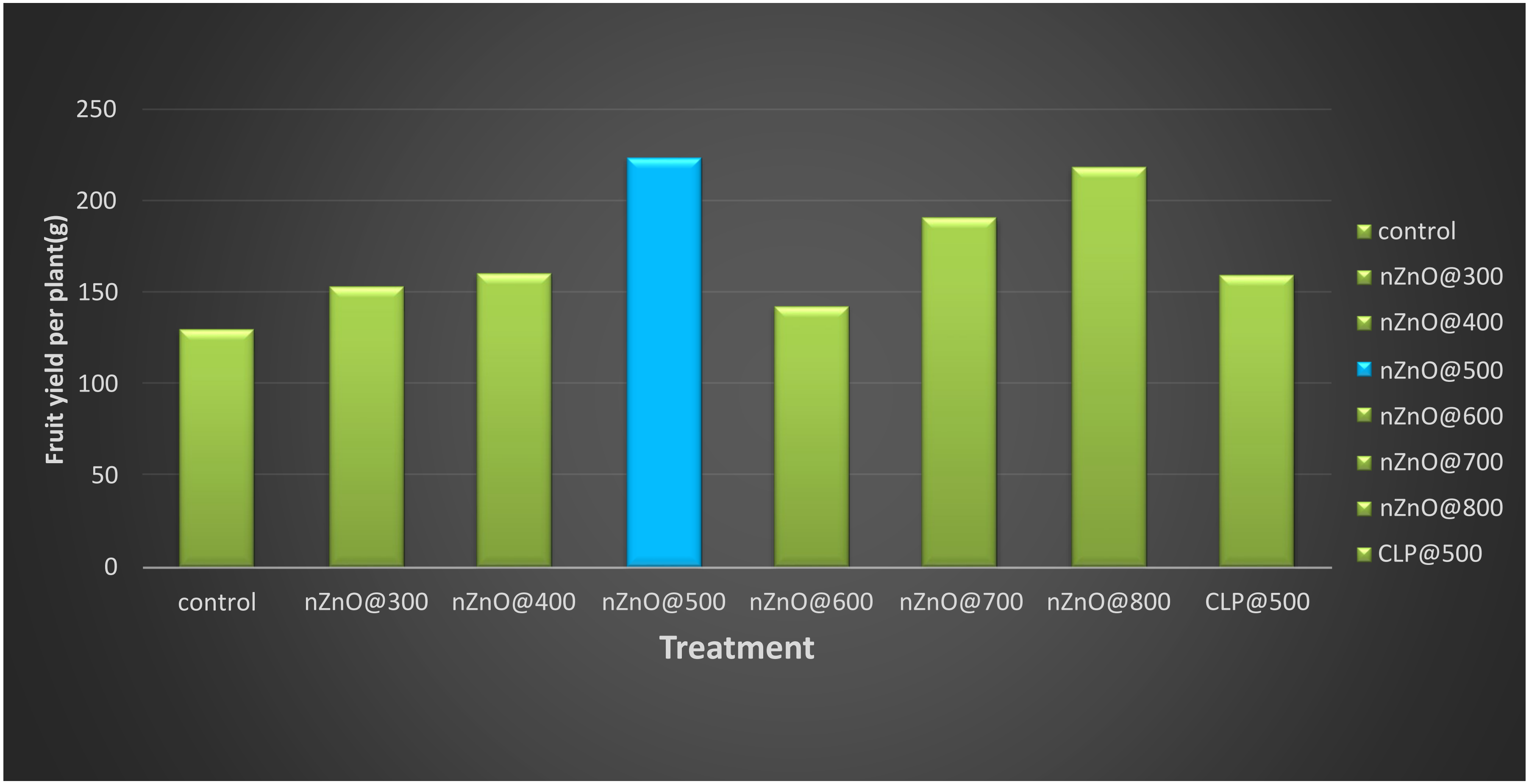

Fig 8. Effect of presowing treatments with nanoparticles on fruit yield per plant (g)

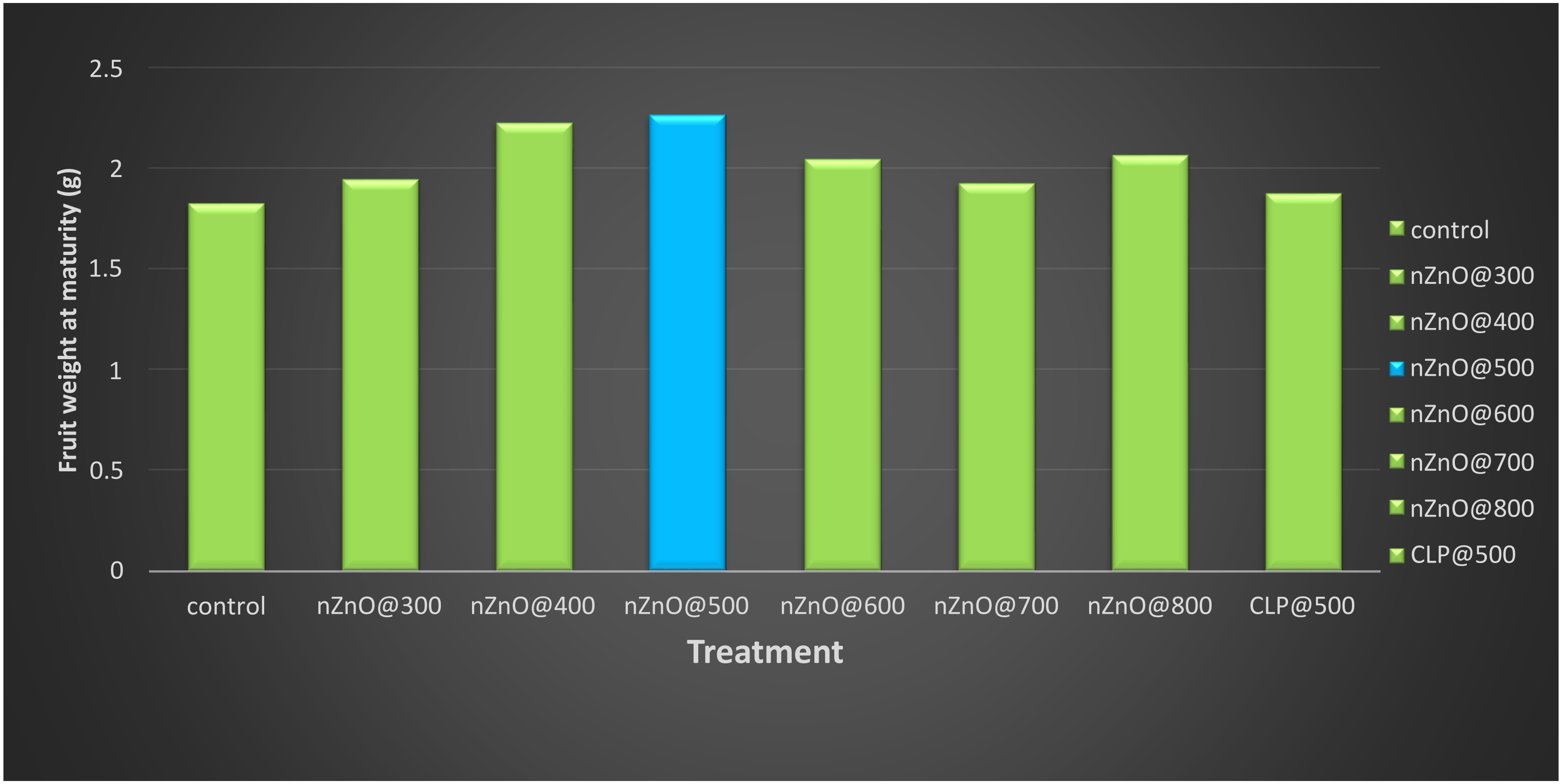

Fig 9. Effect of presowing treatments with nanoparticles on fruit weight at maturity

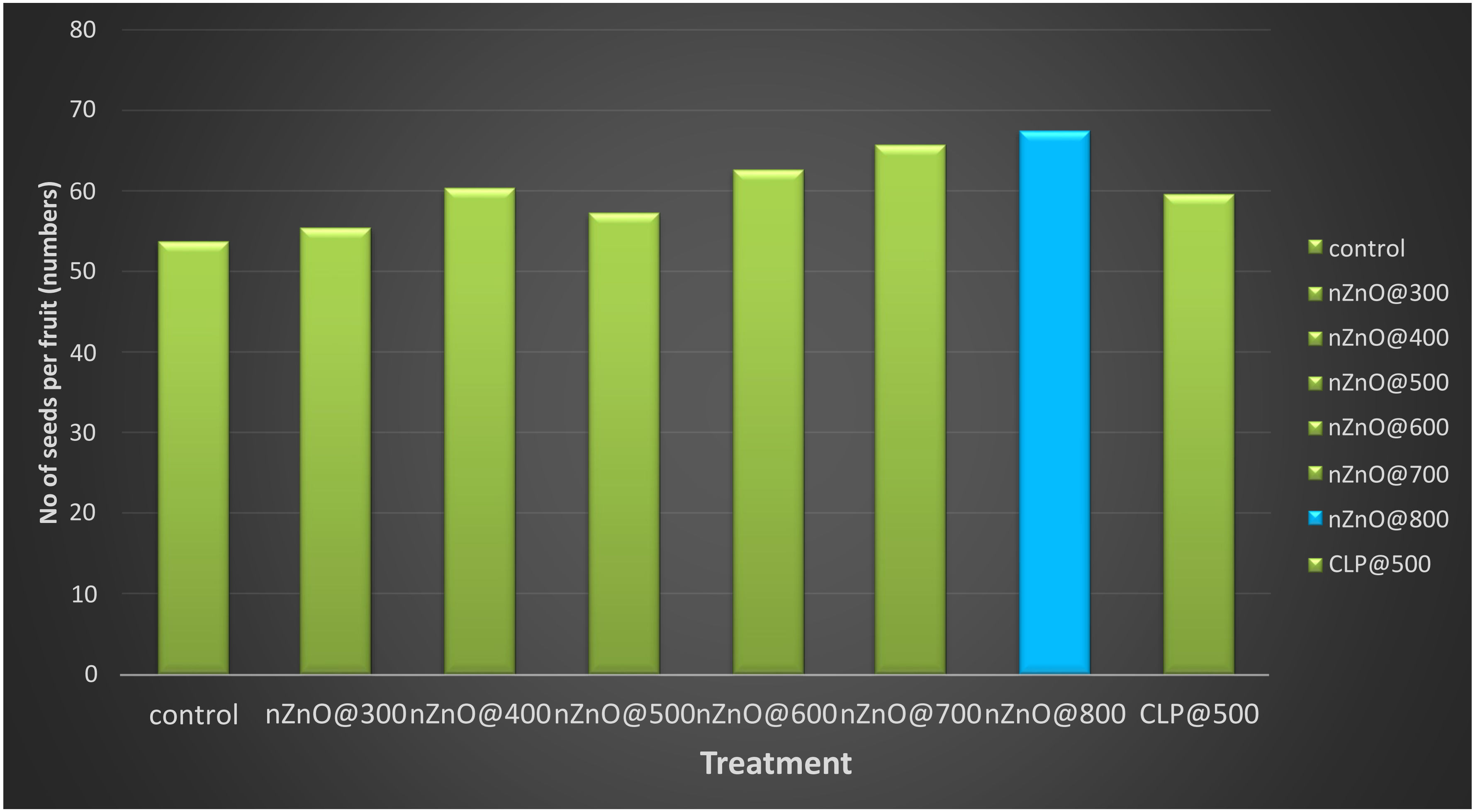

Fig 10. Effect of presowing treatments with nanoparticles on number of seeds per fruit

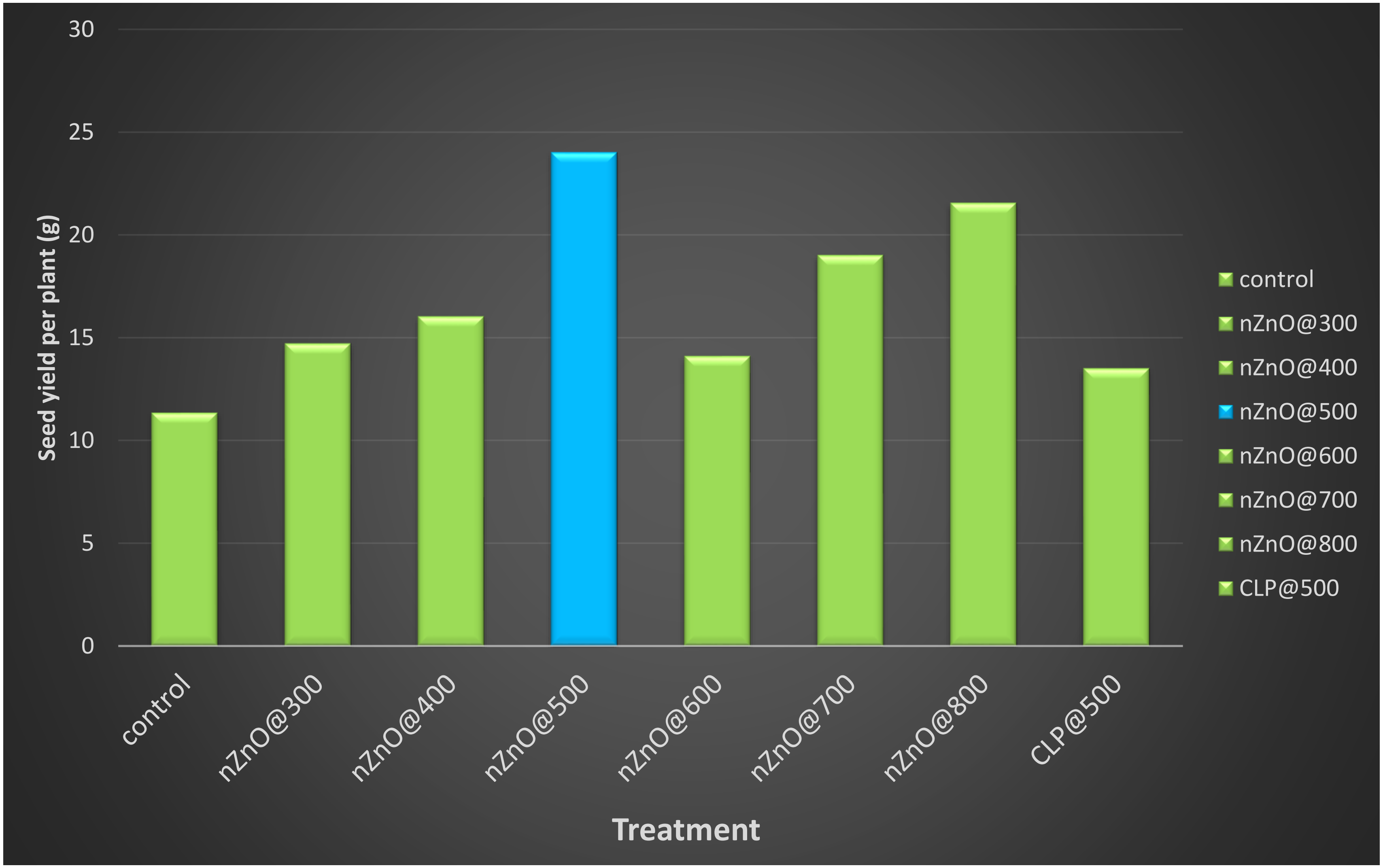

Fig 11. Effect of presowing treatments with nanoparticles on seed yield

5.1.2 Fruit characters

The use of nanoparticles as a seed treatment had a considerable impact on chilli seed yield and yield-contributing factors. Yield contributing factors i.e. fruits per plant was high (109.67) when seeds were treated with nano ZnO @ 800 mg $kg^{-1}$of seed compared to control (72.23), fruit length was high in treatment nano ZnO @ 700 mg kg^{-1} of seed (4.87cm) when compared with control (4.47cm) whereas, fruit weight at maturity (2.27g) and fruit yield/plant (224.56g) was high in treatment nano ZnO @ 500 mg kg^{-1} of seed compared to control. These results are in conformity with the findings of Mathew *et al*., (2021) in chilli, Osman *et al*. (2019), Faizan and Hayat (2019) and Sharma *et al*., (2022) in tomato. Higher absorption of Zn nanoparticle enhances the accumulation of photosynthates in leaves which gets translocated towards the fruit might have result in development of more number of fruits.

5.1.3 Seed attributes

Seed yield per plant was high in case of nano ZnO @ 500 mg kg^{-1} of seed (24.02g) compared to control (11.35g). Number of seeds per fruit was high in case of nano ZnO @ 800 mg $kg^{-1}$of seed (67.59) compared to control (53.8).Hundred seed weight was high when seeds were treated with nano ZnO @ 600 mg kg^{-1} of seed (0.43g) compared to control (0.42g). The findings are in conformity with the works of Mathew *et al*., (2021) in chilli, Sharma *et al*., (2022) in tomato. The role of Zinc as a key element in protein synthesis, nitrogen fixation, carbohydrate metabolism and various types of enzyme activity, might be the reason behind the increased number of seeds per plant (Elham *et al*., 2020).

5.2 Effect of prestorage treatments with nanoparticles on yield attributes in chilli

Treated seeds stored for six months were used to raise a crop to study the effect of pre storage seed treatments in chilli

5.2.1 Vegetative characters

Treatment nano ZnO @ 800 mg kg^{-1} of seed recorded the highest plant height among the treatments. Number of branches were high in the treatment nano ZnO @ 500 mg kg^{-1} of seed. Days to first and fifty per cent flowering was least in case of nano ZnO @ 400 mg kg^{-1} of seed (77.33 days) and (93.33 days). Vegetative growth may be high in nano ZnO treatment because nano particle treated seed maintained high seedling vigour for more than six months compared to control in chilli (Mathew *et al*., 2021) which under field conditions resulted in early flowering and high vegetative growth.

5.2.2 Fruit attributes

Seeds treated with nano ZnO @ 500 mg $kg^{-1}$of seed recorded highest number of fruits per plant (108.67), fruit weight at maturity (7.10g) and fruit yield (770.68g). Effective absorption of Zinc nanoparticle enhanced the accumulation of photosynthates in leaves and which in turn gets translocated to the fruit and and resulted in more number of fruits.

5.2.3. Seed attributes

Seed yield (60.56g) and number of seeds per fruit (73.80) were high in case of nano ZnO @ 500 mg kg^{-1} of seed treatment. Zinc is known to be a crucial component in protein synthesis and is involved in a variety of enzyme processes, carbohydrate metabolism, and nitrogen fixation that enhanced the seed yield attribute (Yusefi-Tanha *et al*., 2020).

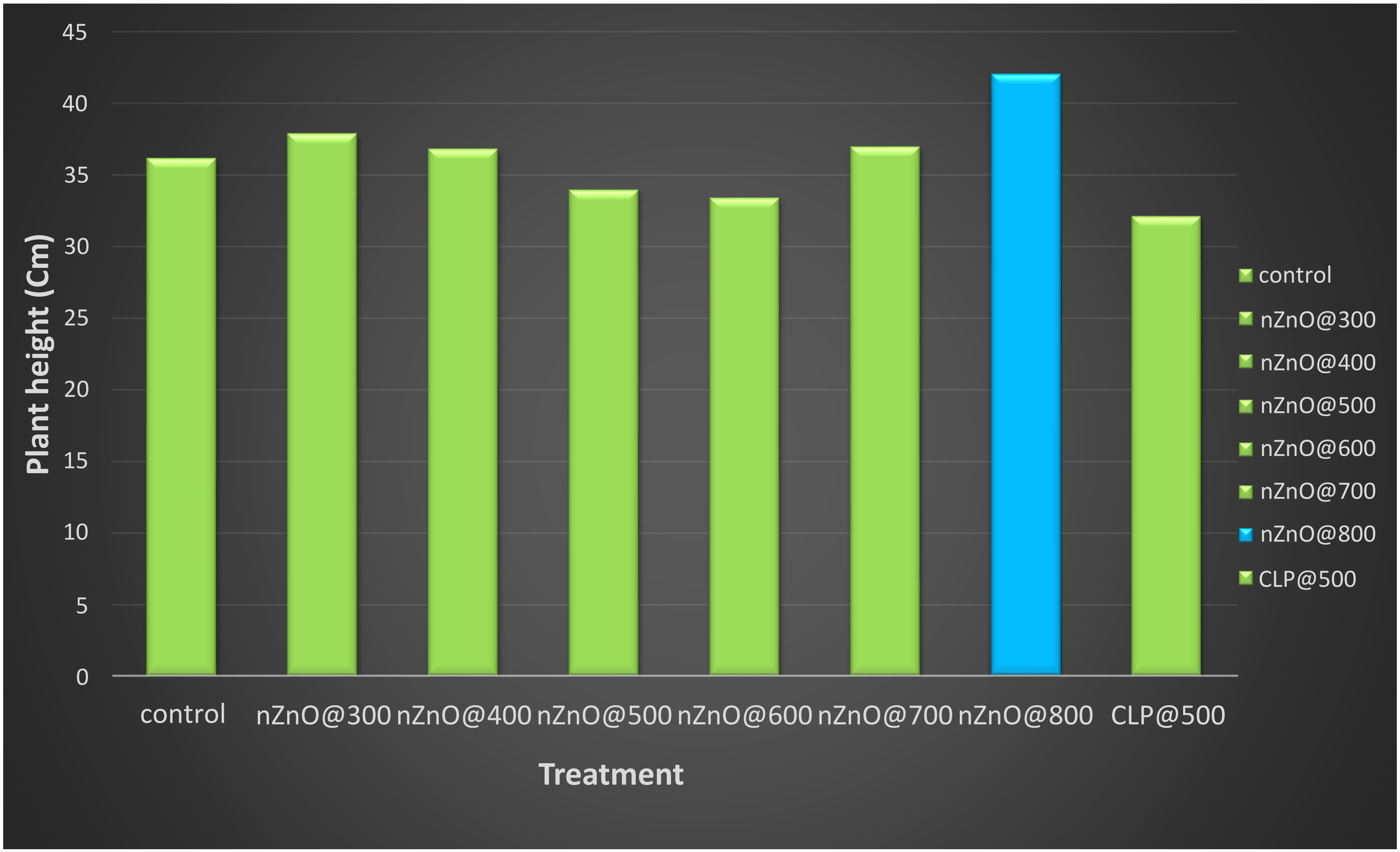

Fig 12. Effect of prestorage treatments with nanoparticles on plant height

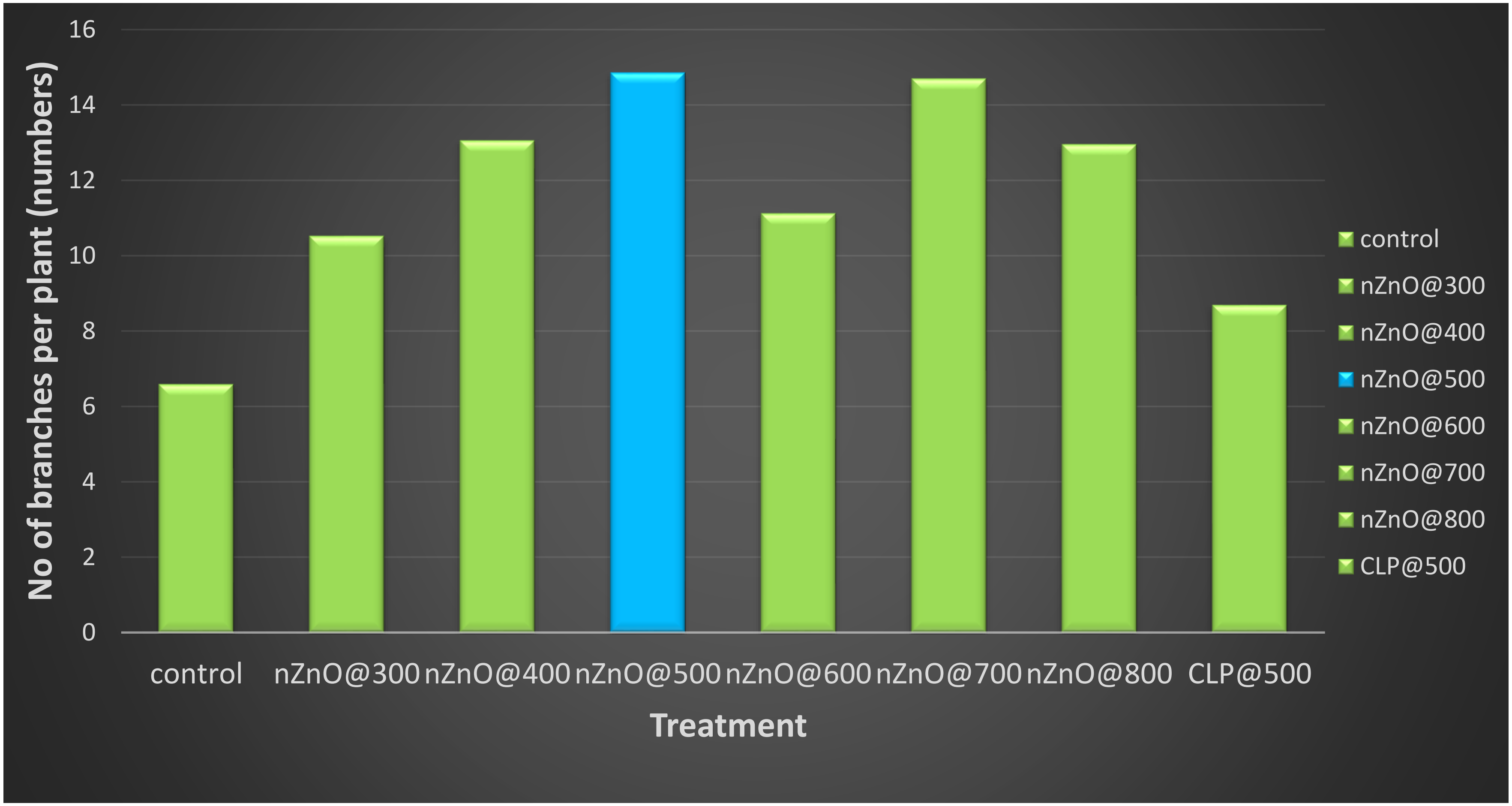

Fig 13. Effect of prestorage treatments with nanoparticles on number of branches per plant

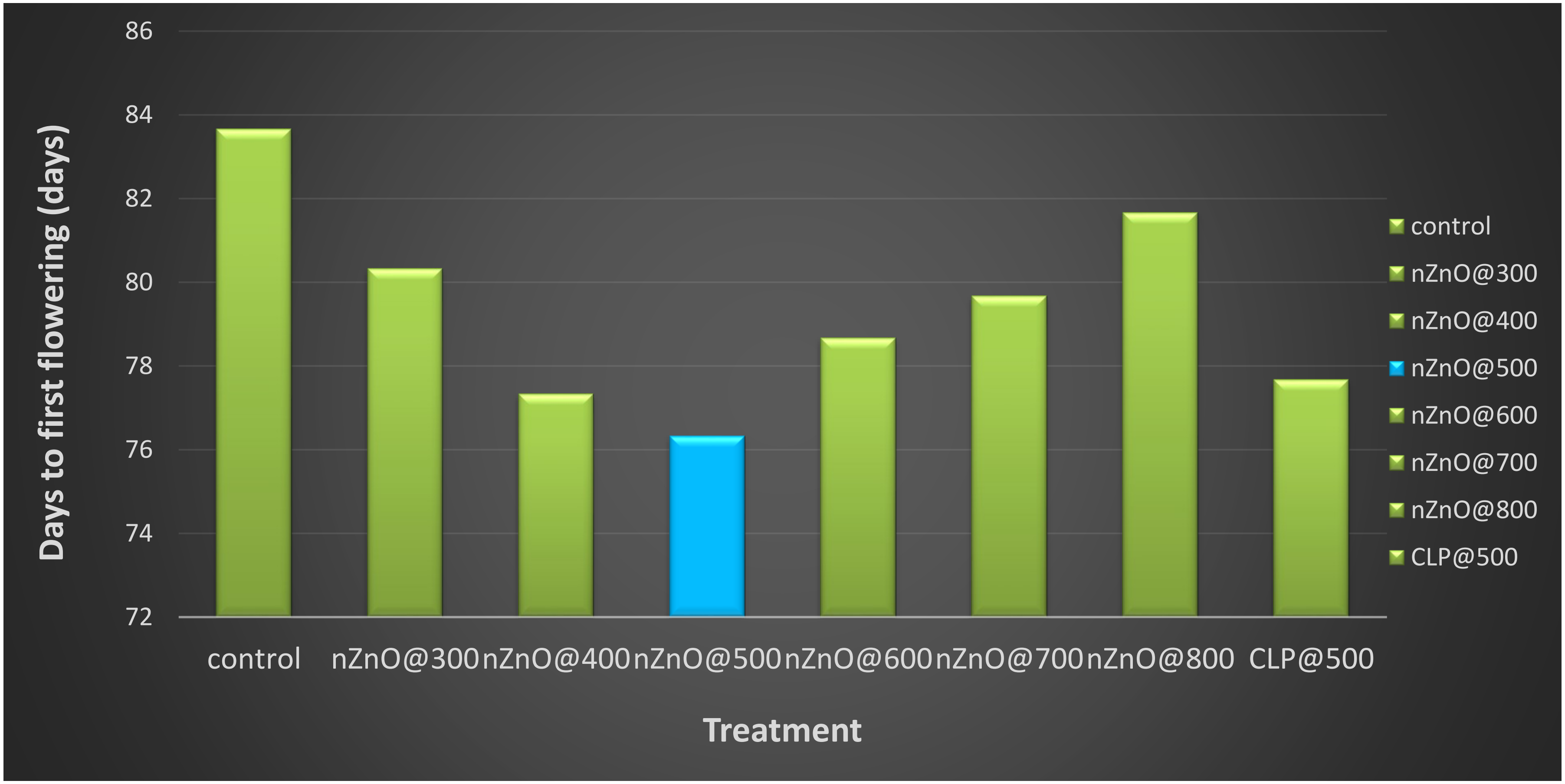

Fig 14. Effect of prestorage treatments with nanoparticles on days to first flowering

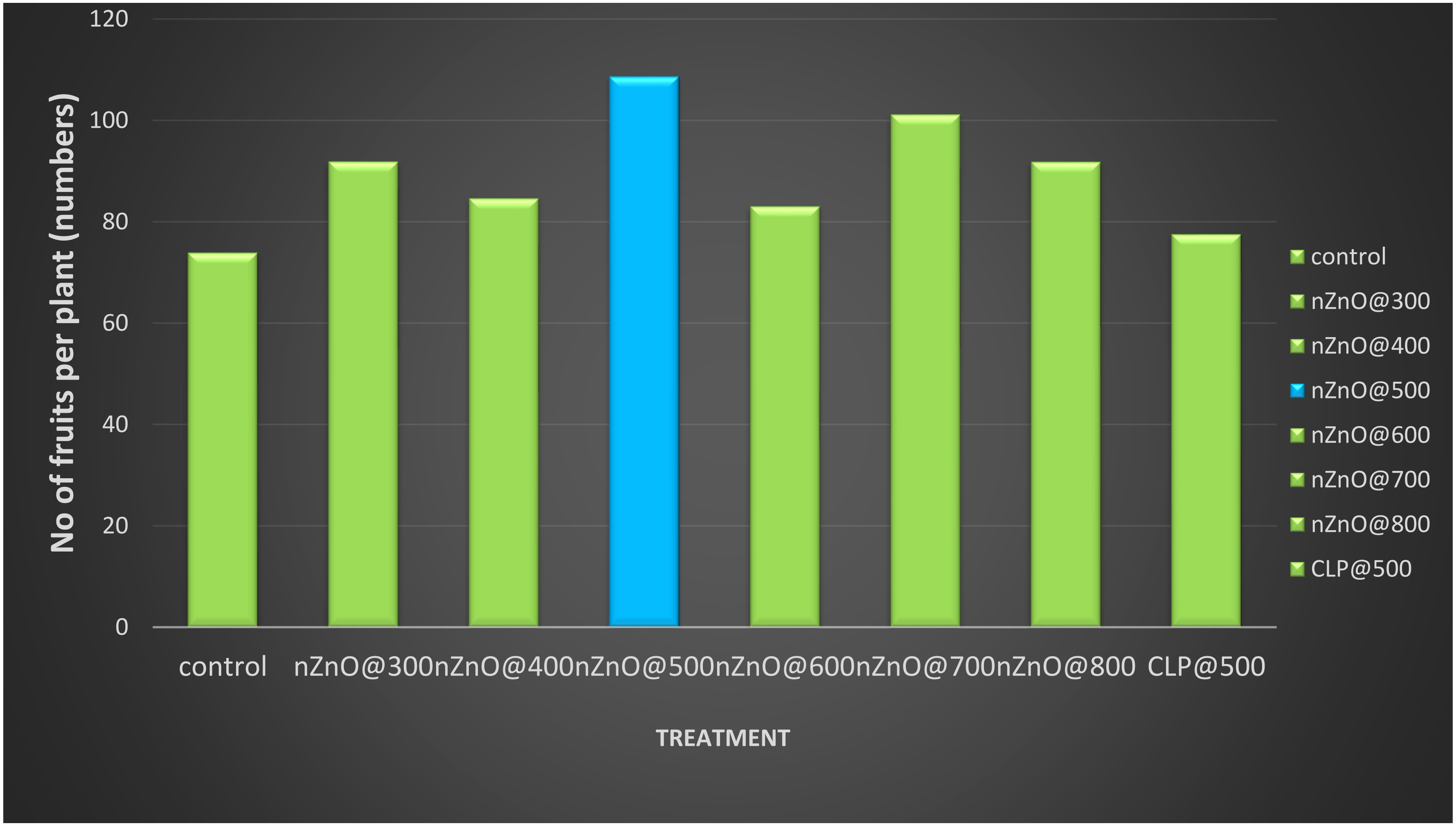

Fig 15. Effect of prestorage treatments with nanoparticles on number of fruits per plant

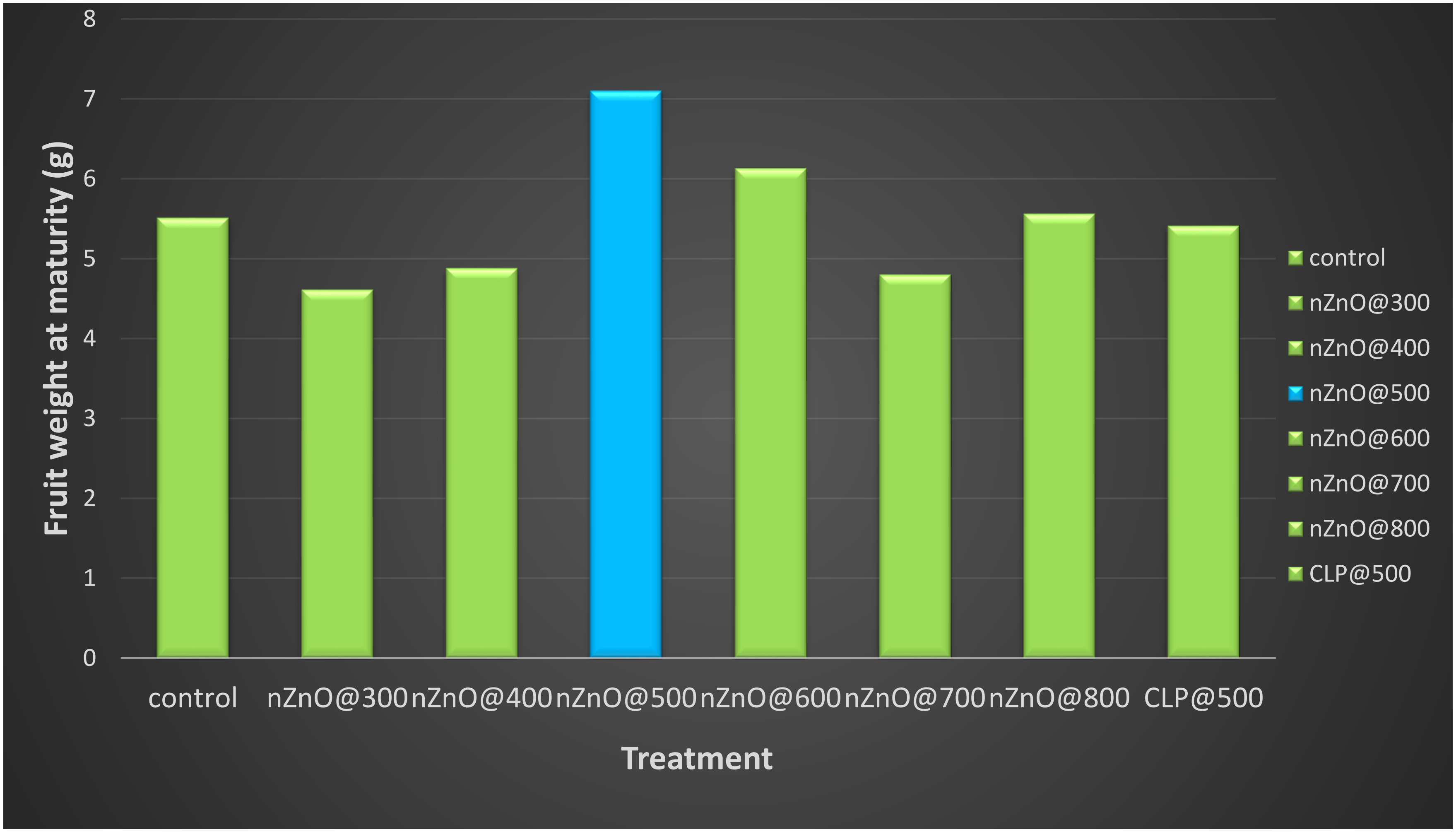

Fig 16. Effect of prestorage treatments with nanoparticles on fruit weight at maturity

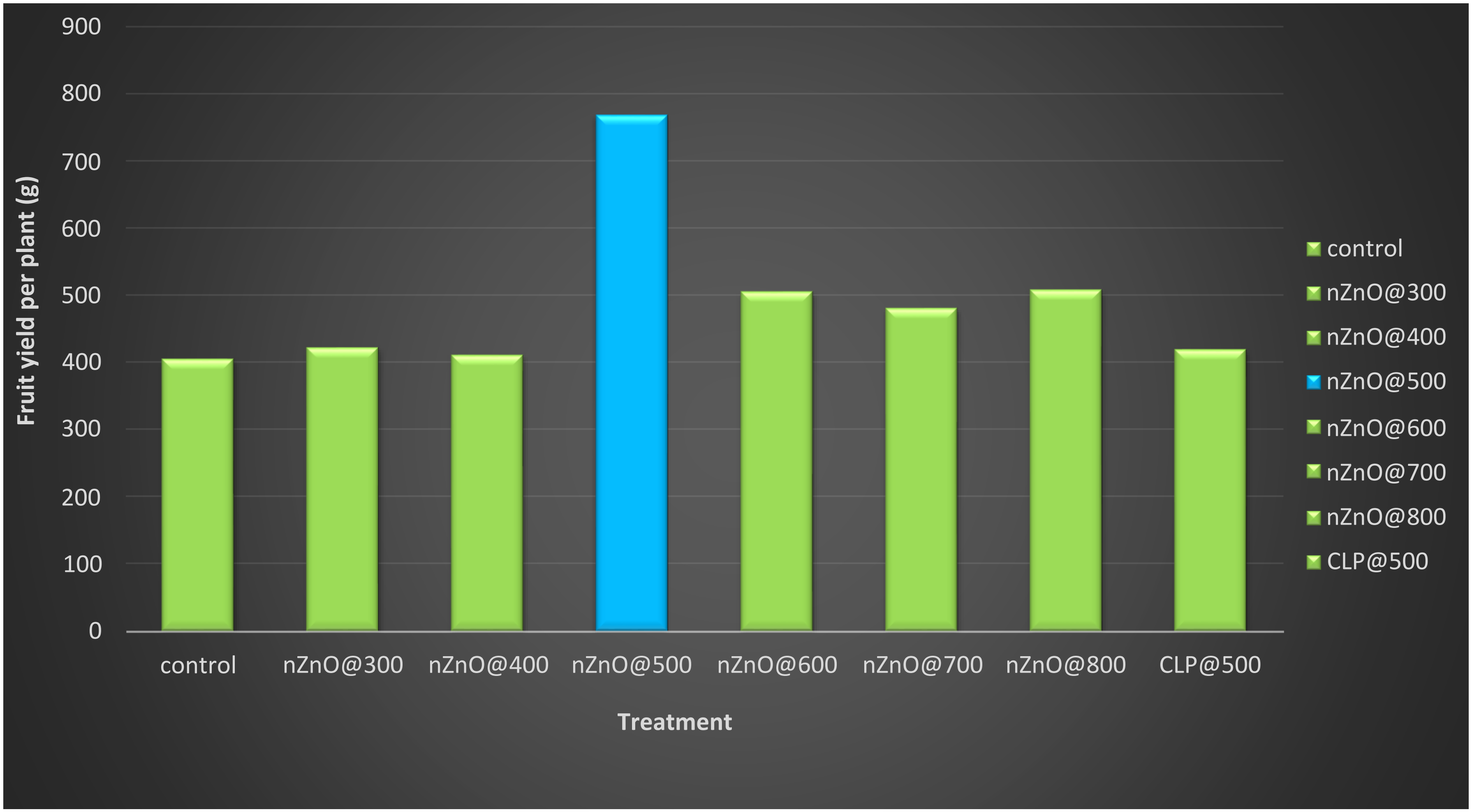

Fig 17. Effect of prestorage treatments with nanoparticles on fruit yield per plant

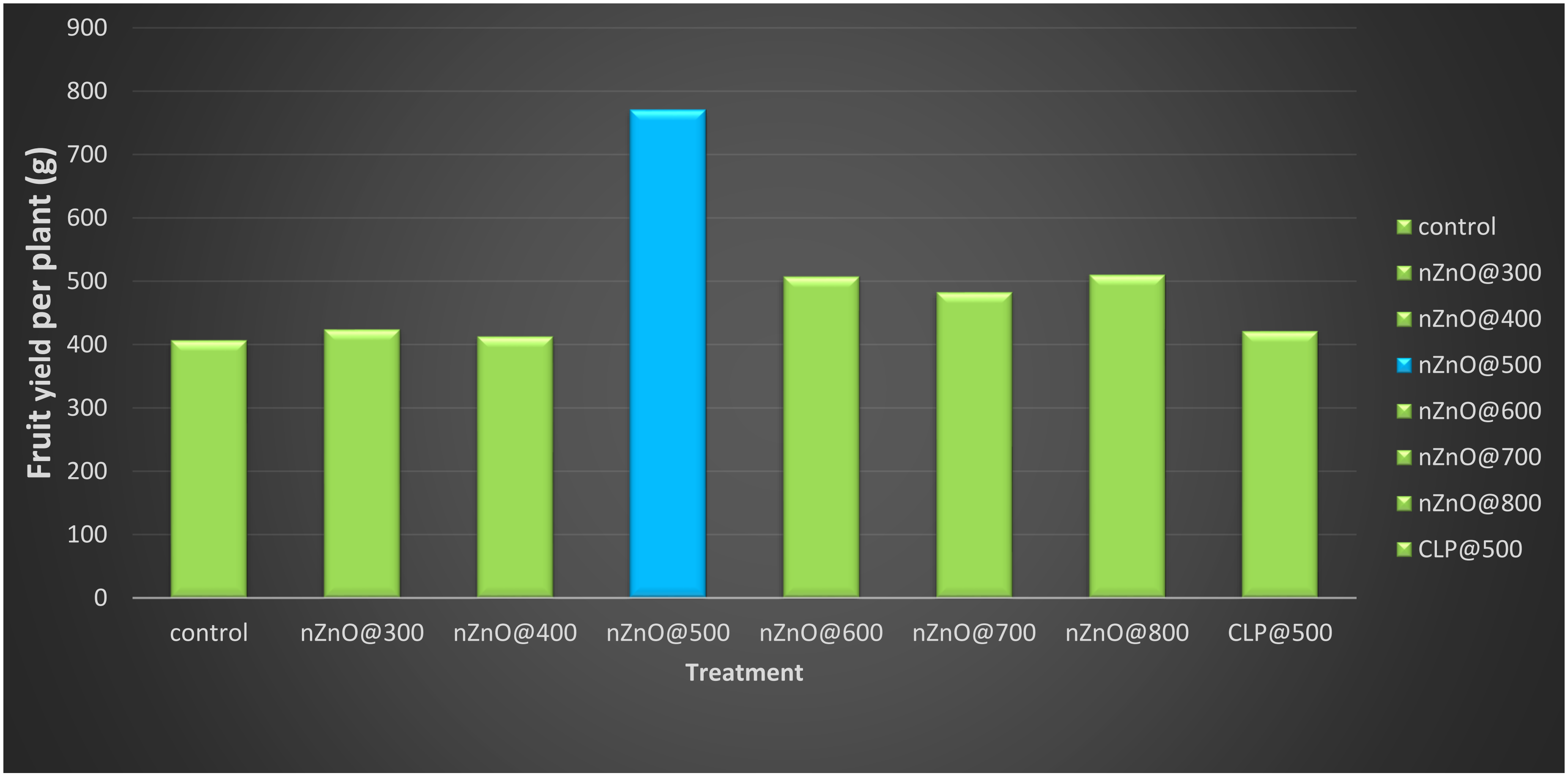

Fig 18. Effect of prestorage treatments with nanoparticles on number of seeds per fruit

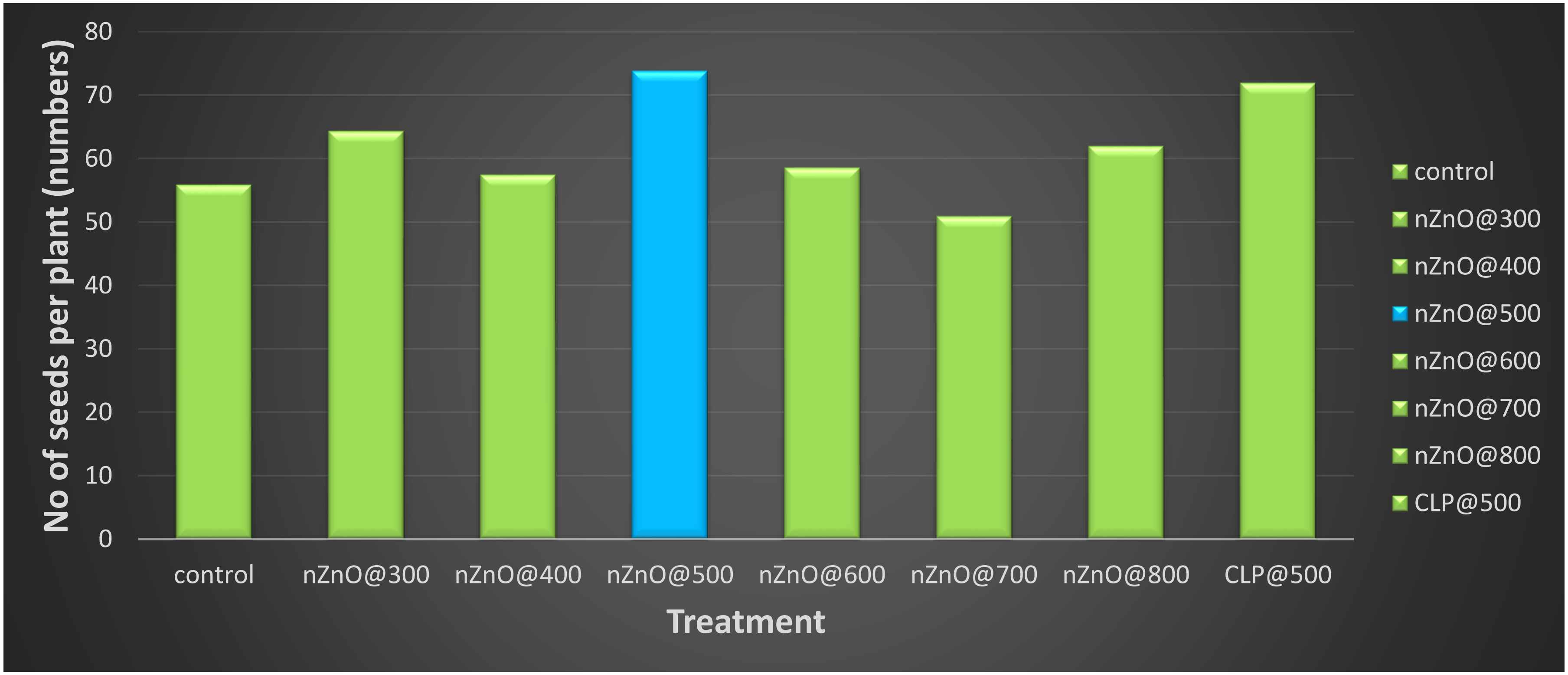

Fig 19. Effect of prestorage treatments with nanoparticles on seed yield

5.3 Effect of mid- storage correction with inorganic nanoparticles in chilli

In general, important parameters including the heritable genetic makeup of the varieties, initial seed quality, storage environment moisture content, relative humidity, and temperature *etc*., have a significant impact on the seed longevity in storage. The most crucial issue for farmers and seed producers is seed viability, which has huge economic ramifications. According to Ellis and Roberts (1977), higher temperature and seed moisture content cause rapid viability loss. Seed deterioration lowers the viability of the seed, which finally results in seed mortality. Moreover, weak seedlings are produced by damaged seeds (Kapoor *et al.,* 2010). Seed deterioration is inexorable, best thing that can be done is to slow down its rate. The rate of seed ageing is determined by the seed's capacity to withstand degradation changes and defence systems, which are unique to each plant species (Mohammadi *et al*., 2011; Sisman and Delibas, 2004).The specific cause of viability loss is unknown, however according to sung and Chiu (1995), the main cause is that during storage, the membrane of seeds is damaged by non-enzymatic peroxidation of free radicals.

Although genetic makeup of the variety mostly determines seed quality, seeds continue to degrade while in storage. This happens as a result of mechanical damage, temperature and humidity fluctuations, pathogens and storage pests.. Midstorage seed invigoration treatments are physiological treatments that aim to improve the physiological status of the seed, enhancing its germination. Mid-storage invigoration treatments are widely recommended in order to increase the germination of unutilized stock and also to extend their storability (Renugadevi *et al*., 2006). Treatment invigorates low vigour seeds to achieve better emergence and establishment. Hydration dehydration treatments counteract physiological deterioration hence ageing damaged caused is prevented by counteraction of lipid peroxidation and free radical reactions. Peroxidation of unsaturated lipid constituents of lipoprotein membranes and production of highly reactive free radical intermediates may destroy the semi permeable nature of membranes. If this chain propagation reaction of free radical is terminated post ageing damage could be prevented. Seed longevity of onion seeds, which are known to be very susceptible to deterioration in storage, were improved by midstorage correction using nanoparticles. (Anandaraj *et al*., 2018).

5.3.1 Effect of mid- storage correction with inorganic nanoparticles on germination (%) in chilli

Treatements with nano grade ZnO showed improvement in germination percent (Table 9). Regardless of the treatments, a decrease in germination was noticed over the storage time. The findings of Navya (2016), Sandhya (2016), Gayathri (2019) and Mathew (2020) in chilli were in concurrence with the results obtained in the present study . After one month of storage nano ZnO @ 400 mg $kg^{-1}$of seed gave highest germination percent (64.33) which is almost five perecent more than control(59.67) also followed minimum germination percent prescribed by IMSCS (Indian Minimum Seed Certification Standards) for chilli seeds. Savithramma *et al*., (2012) opined that nanoparticles may have created new pores on the seed coat during penetration, which activated the process of water uptake, and may have helped to influx the nutrients inside the seed, or may have carried nutrients along with it, which would have resulted in a significant improvement in germination and seedling growth rate.

5.3.2. Seedling length in chilli

Storage time and seed treatments had a big impact on seedling length. Compared to untreated seeds, treated seeds recorded the longest seedling length. Throughout the storage period, it was discovered that the lengths of the shoots and roots decreased in all of the treatments. In the case of the chilli variety Anugraha, The research findings of sandhya (2016) and Gayathri (2019) found outcomes that were comparable. Shoot length was high in case of nano ZnO@ 400 mg $kg^{-1}$of seed (4.43 cm) but control had shoot length of 3.33cm after 1month of storage while root length was high in case of nano ZnO @ 500 mg kg^{-1} of seed (9.24cm) comparable to control (7.36 cm). After three month of storage in case of nano ZnO @ 400 mg $kg^{-1}$of seed shoot length reduced to 4.21cm and root length decreased to (8.79cm) where control shoot length reduced to (3.19cm) and

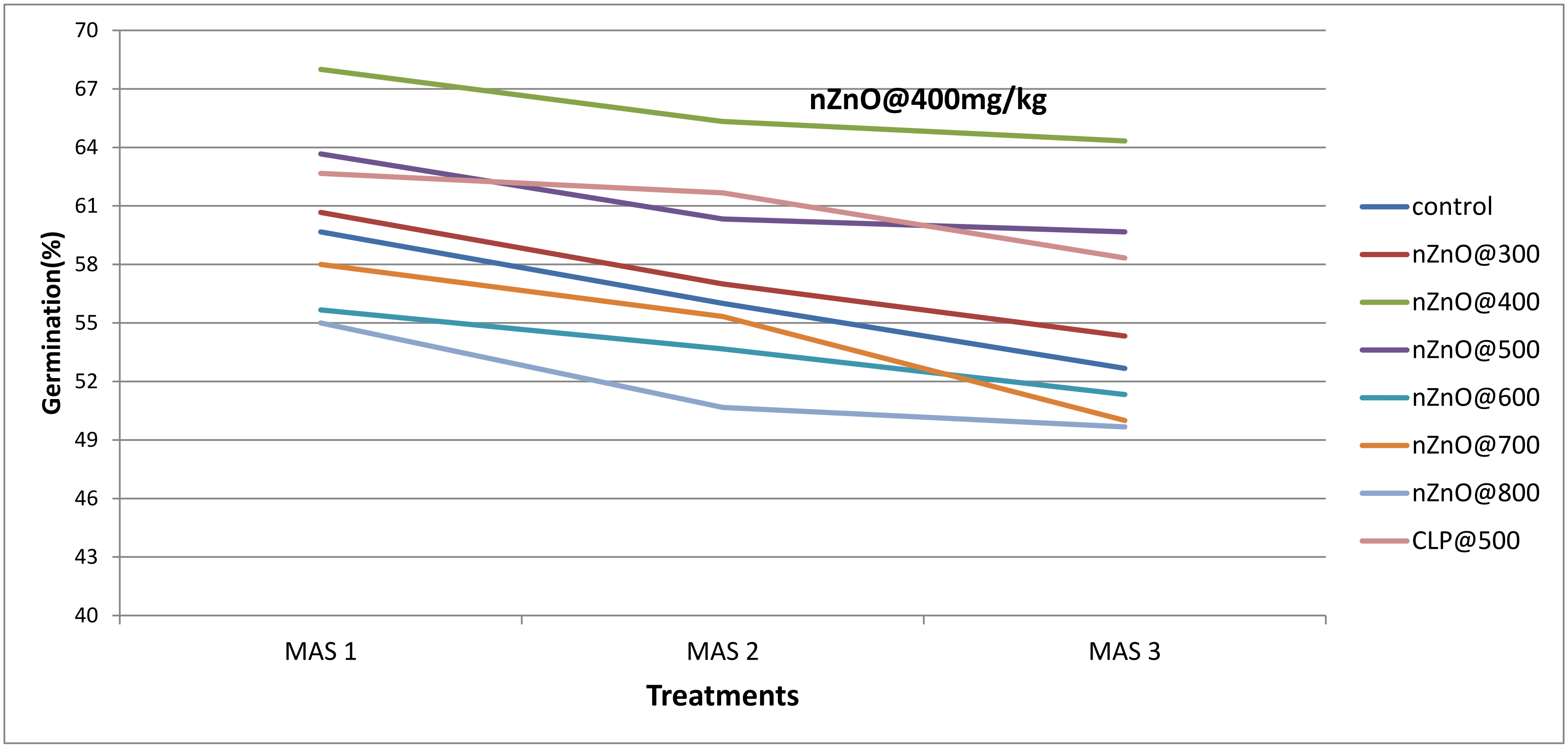

Fig 20. Effect of midstorage treatments with nanoparticles on germination

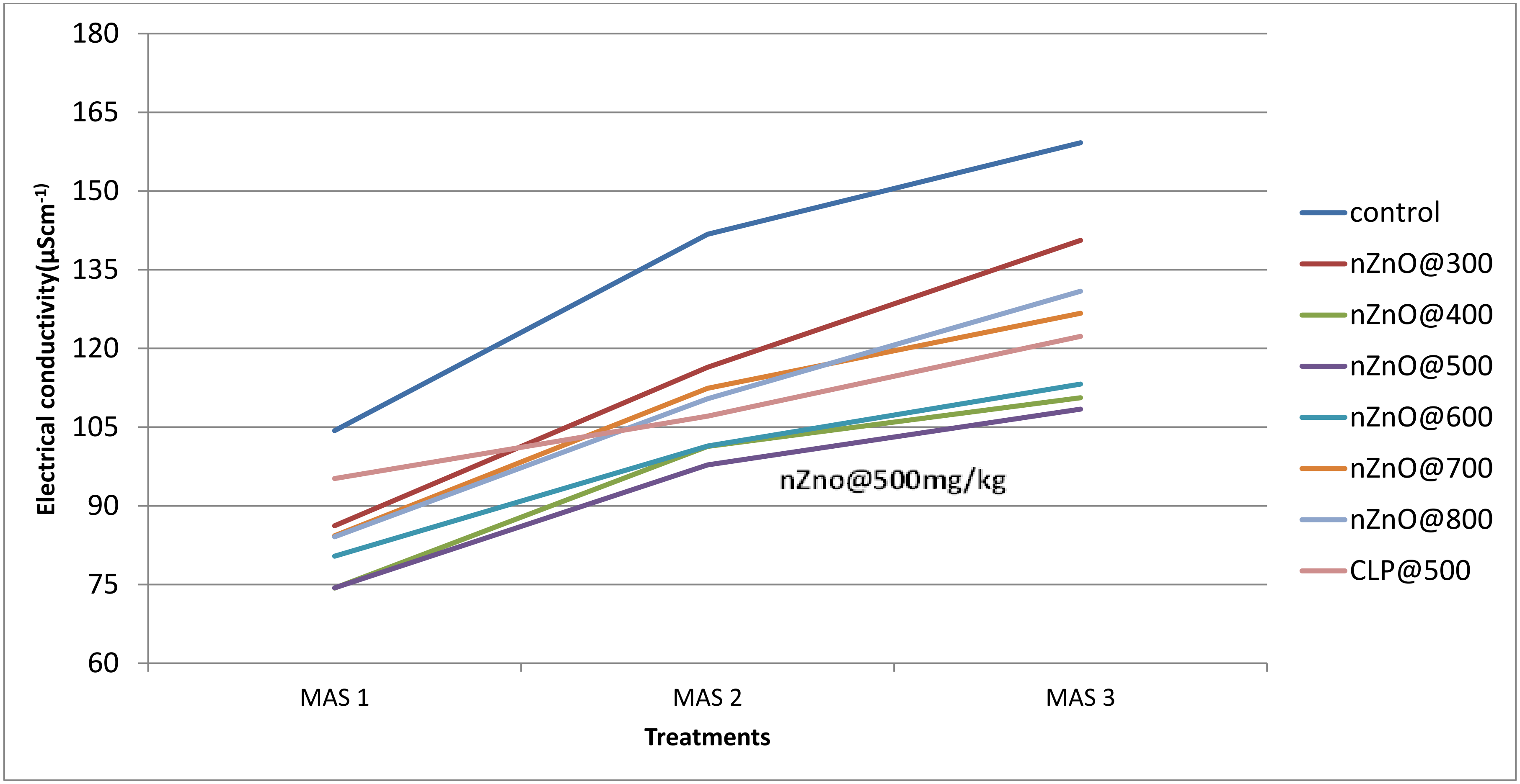

Fig 21. Effect of midstorage treatments with nanoparticles on electrical conductivity (μScm^{-1})

root length to (6.94cm). Similar to seed germination, seedling length significantly increased when Zn was administered in accordance with the authorised amount of ZnO. Zinc is essential for the synthesis of tryptophan, which is a precursor to indole acetic acid (IAA) (Anandraj and Natarajan 2017). The auxin signalling system is crucial for the start of lateral-root development. Zinc can increase the activity of enzymes that convert fat into a source of rich nutrients and energy for improved germination, proteins into amino acids, starch into simple sugars, and proteins into amino acids. Indole acetic acid levels in roots (sprouts) might be raised by zinc oxide nanoparticles, which could then speed up seedling growth.

5.3.3 Effect of mid- storage correction with inorganic nanoparticles on electrical conductivity in chiili

Electrical conductivity increases when there is electrolytic leakage owing to membrane integrity loss (Ratajczak and Pukacka, 2005). Control had the highest electrical conductivity (159.2 μScm^{-1}) among the treatments . Similar obsrevations were recorded in the seeds of the chilli variety Anugraha by Sandhya (2016) and Gayathri (2019) and Mathew (2020). Nanoparticles function as a protector against seed deterioration regardless of treatments and dosages. Electrical conductivity increased from one month after storage (104.33) μScm^{-1} to third month of storage(159.2 μScm^{-1}) in case of control it was least in case of nanoZnO @ 400 mg kg^{-1} of seed (74.33 μScm^{-1}) one month after storage which increased to (110.6 μScm^{-1}) by the third month of storage. Seed treatment with ZnO NP resulted in decreased EC, which can be attributed to improved membrane integrity. ZnO NPs are anticipated to trigger oxidation-reduction events via the superoxide-ion-radical, quenching free radicals and reactive oxygen species in the seeds. The respiration process uses the oxygen released during the quenching of ROS, and this is advantageous for seed germination and seedling growth.

5.3.4 Seedling dry weight in chilli.

Dry weight of seedling improved after treatment with Zinc Oxide Nano particles, although a decline was observed with increase in storage period. Gayathri (2019) and Mathew *et al* ., (2021) reported similar trend when seeds were treated with nanoparticles of ZnO. Among the treatment the improvement in seedling dry weight was high in treatment with nano ZnO @ 400 mg kg^{-1} of seed throughout the storage period (25.12g at 1MAS and 24.96g at 3MAS)

5.3.5. Vigour indices in chilli

Seed vigour indices decreased as storage progressed. Loss of seed vigour may occur when enzyme and amino acid synthesis declines. According to Kavitha (2002). Seed vigour index I and II was highest in case of nano ZnO @ 400 mg kg^{-1} of seed) after one month of storage which declined over the period of storage. Garcia-Lopez *et al*. (2018) Gayathri (2019) and (Mathew *et al* ., 2021) reported similar trends in chilli.

Nanoparticles are said to penetrate seeds through cracks on their surfaces, according to Kumar *et al*. (2019). The interaction of these particles with the free radicles in the seeds will increase viability and seed vigour. Lipid peroxidation, an accumulation of reactive oxygen species (ROS), or an increase in the concentration of free radicals are possible causes of seed vigour loss (Bailly, 2004). By generating damage to proteins and lipids and resulting in physiological and biochemical alterations, ROS disrupt seed metabolism (Mittler *et al*., 2004).

5.3.6 Seed moisture content

At the end of storage period , the seed moisture content showed minimum variations between the treatments (Table 14). Use of proper moisture and vapour resistant packing (700 gauge polyethylene), might be the reason for stability in seed moisture content.

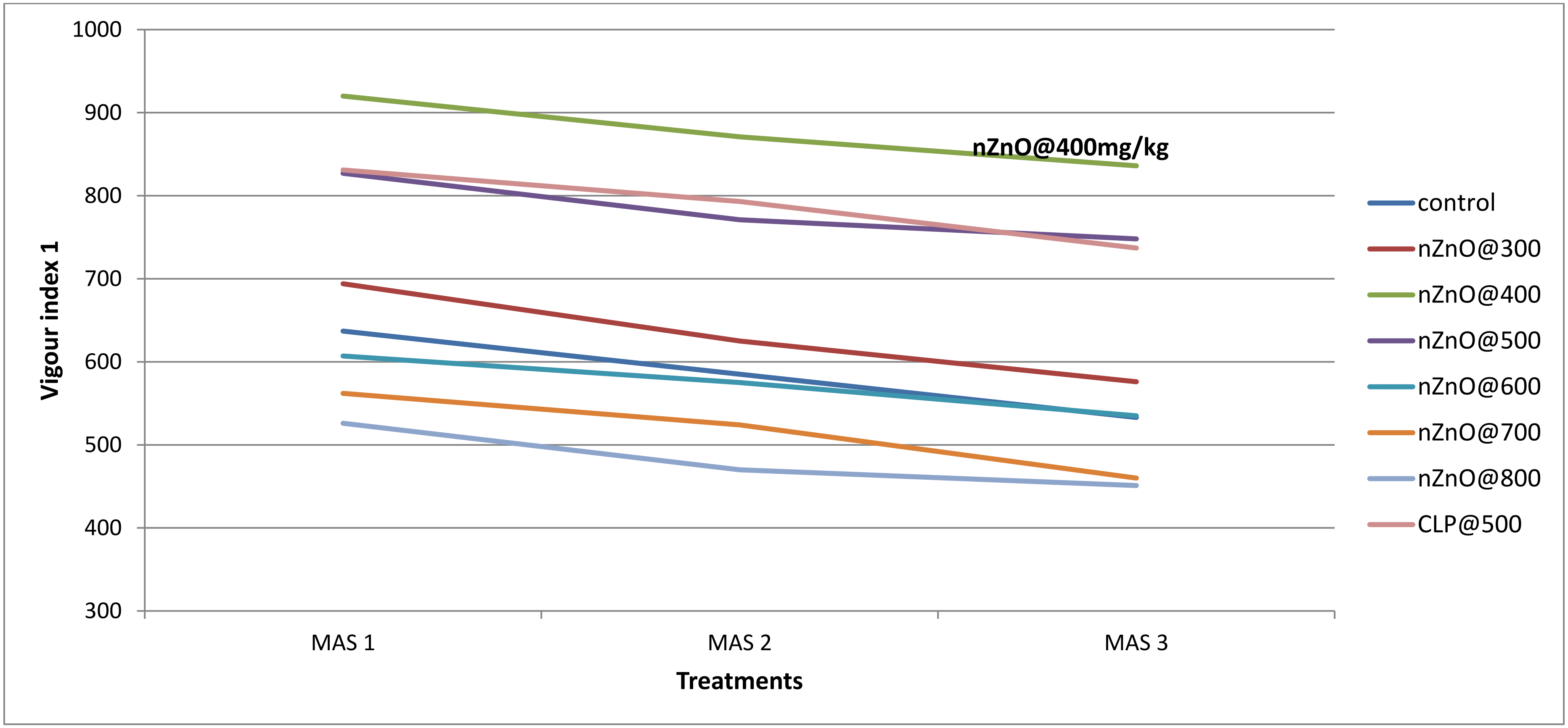

Fig 22. Effect of midstorage treatments with nanoparticles on vigour index I

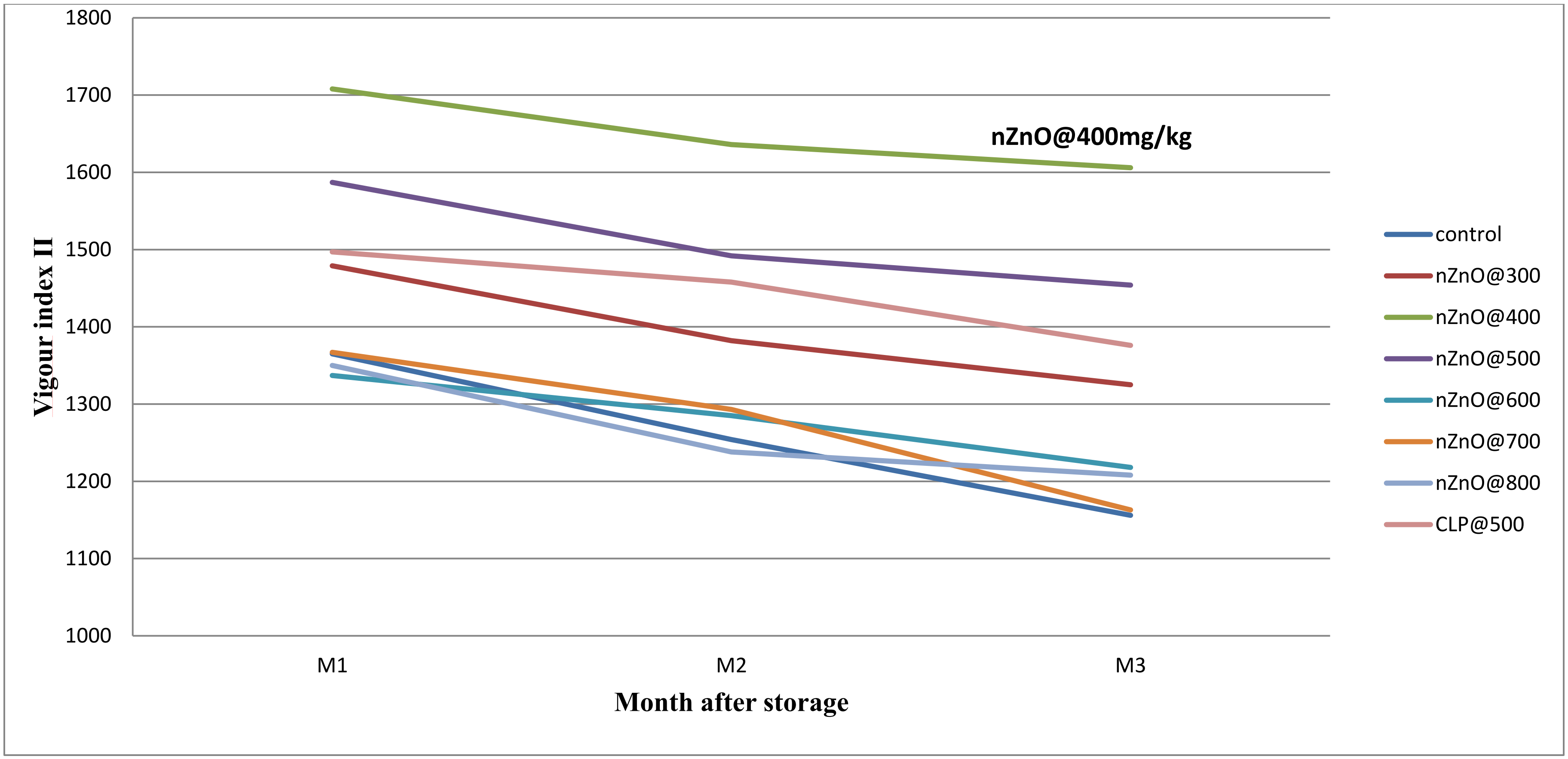

Fig 23. Effect of midstorage treatments with nanoparticles on vigour index II

5.3.7 Seed microflora in chilli

Irrespective of methods control showed highest infection in both blotter paper and agar plate method. Lowest infection observed in case of custard leaf powder treatment. Treatments nano ZnO @ 300 mg kg^{-1} of seed and custard leaf powder @ 500 mg kg^{-1} of seed recorded lowest infection before and at the end of storage in both agar and blotter methods. (23.33, 3.33) agar method (18.67, 1.33) and. Similar observation were recorded by sandhya (2014). In the present mictroflora such as *Aspergillus* sp and and *Rhizopus* sp were observed . *Aspergillus* sp. was found in chilli seeds that had been stored as per reports by Sandhya (2016), Navya (2016), and Gayathri (2019).

Protein, lipids, carbohydrates, and vitamins could all change as a result of the infections. Loss of germination is caused by a reduction in total oil content, and increase in free fatty acids, discoloration, strange odours, and caking of the seeds. The metabolic functions of the pathogen are hampered when the nanoparticles interact with the fungal membrane, which ultimately results in pathogen mortality. ZnO nanoparticles produce structural changes that result in cytoplasm leakage and bacterial mortality, disturb cellular processes, and distort fungal hyphae (Brayner *et al*., 2010; He *et al*., 2011).Custrad leaf has significant fungal activities.Custard leaves has several secondary metabolites such as glycosides, phytosterols, alkaloids, oils, saponins, phenols, and flavonoids. Manju and kumar 2015 have reported acetogenine compound in it which is having high antifungal and antibacterial activity.Hence the improve seed health under storage leading to enhance seed longevity and seed quality.

Table 15. Ranking of pre-sowing seed treatment on yield and yield contributing characters in chilli

Treatment	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Days to first flowering	3	2	1	1	1.5	2	2.5	1.5
Days to 50 % flowering	3	1.5	1	2	2	2	2.5	2
Plant height	3	2	2.5	4	4.5	2.5	1	5
Branches per plant	4	2.5	1.5	1	2.5	1	1.5	4.5
Fruits/ plant (nos)	3	2.5	2.5	1	3	1	1	2
Fruit length	2	2.5	2	3	2	1	4	1
Fruit weight at maturity	4	3	1	1	2	3	2	3.5
Fruit yield/plant	4	3	3	1	3.5	2	1	3
No. of seeds/ fruit	4	3.5	2.5	3.5	1.5	1	1	2.5
100 seed weight	1	1.5	4	1	5	3	2	5
Seed yield per plant	6	4.5	4	1	5	3	2	5
Total	37	28.5	25	19.5	32.5	21.5	20.5	35
Rank	8	5	4	**1**	6	3	2	7

Table 16. Ranking of pre-storage seed treatment on yield and yield contributing characters in chilli

Treatment	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Days to first flowering	3	2	1	1	1.5	2	2.5	1.5
Days to 50 % flowering	3	1.5	1	2	2	2	2.5	2
Plant height	3	2	2.5	4	4.5	2.5	1	5
Branches per plant	4	2.5	1.5	1	2.5	1	1.5	4.5
Fruits/ plant (nos)	3	2.5	2.5	1	3	1	1	2
Fruit length	2	2.5	2	3	2	1	4	1
Fruit weight at maturity	4	3	1	1	2	3	2	3.5
Fruit yield/plant	4	3	3	1	3.5	2	1	3
No. of seeds/ fruit	4	3.5	2.5	3.5	1.5	1	1	2.5
100 seed weight	1	1.5	4	1	5	3	2	5
Seed yield per plant	6	4.5	4	1	5	3	2	5
Total	37	28.5	25	19.5	32.5	21.5	20.5	35
Rank	8	5	4	**1**	6	3	2	7

Table 17. Ranking of mid- storage correction with inorganic nanoparticles in chilli

Treatment		T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Germination (%)		4.5	3.5	1	2	4.5	4.5	5	2.5
Shoot length (cm)		8	7	2.5	2	3	5	6	4
Root length (cm)		5.5	4.5	1	2.5	3.5	6	5.5	1.5
Electrical conductivity		3	2	1	1	3	4	5	1
Vigour index I		3	1.5	1	1.5	1.5	2	1.5	2
Vigour index II		3	3	1	2	3	4	4	2
Seedling dry weight(mg)		4	2.5	1	2	4.5	4	4.5	2
Seed microflora	**Blotter paper method**	2	1	2	2	2	2	2	1
	Agar plate method	4	2	3.5	3	3.5	3.5	3.5	1
Total		37	27	14	18	28.5	35	37	17
Rank		7	4	**1**	3	5	6	7	2

Ranking and scoring

In order to arrive at the best treatments the treatments where given scores based on their DMRT given scores by assaying 1-a, 2-b, 3 to c and 4 to d and treatment score was the average of the DMRT value.Eg if a treatment has a it is assigned 1,ab – es 1.5, abc is 2, bcd 3

Pre sowing treatment

Based on the scores obtained by each treatment in case biometric observation in case effect of pre sowing treatment with inorganic nanoparticle in chilli it was observed that T_4 - nano ZnO @ 500 mg kg^{-1} of seed perfomed well in both fruit yield and seed yield attributes followed by T_7 - nano ZnO @ 800 mg kg^{-1} of seed and T_6 - nano ZnO @ 700 mg kg^{-1} of seed. Least performance was observed in case of control (Table 15).

Pre storage Treatment

Similarly prestorage treatment with nanoparticle in chilli seed T_4 - nano ZnO @ 500 mg kg^{-1} of seed performed well in both fruit and seed yield attributes followed by T_7- nano ZnO @ 800 mg kg^{-1} of seed and T_6 – nano ZnO @ 700 mg kg^{-1} of seed. Least performance was observed in case of Control (Table 16).

Mid storage correction

Based on score seed quality parameters of chilli seed administered with inorganic nanoparticle as mid- storage correction T_3 –nano ZnO @ 400 mg kg^{-1} of seed has the best seed quality attributes followed by T_8 – custard leaf powder @ 500 mg kg-1of seed and T_4- nano ZnO @ 500 mg kg^{-1} of seed (Table 17).

Nanoparticles distinguish themselves from other species through their smaller size, clearly defined outer areas, potential surface energy, and high solubility. Plant absorption is boosted as a result of these distinctive features. As a result, nanotechnology can be applied to agriculture to quickly deliver agrochemicals to the point of use with minimal depletion. Nanoparticles are among the materials with the widest range of uses due to their varied characteristics, functions, and features. ZnO - nanoparticles can be applied to crops to encourage their growth, yield, and quality.

The findings of the present study revealed the positive impact of zinc oxide and custard apple leaf nanoparticles in seed treatments. Zinc being a crucial element in plant growth, its application at nanoscale enhances the efficacy and effectiveness of the treatments. Vegetable seeds like chilli being costly every care must be taken to prolong its storability and therefore pre storage seed treatment and mid storage correction are will be a boon to seed industry.

The present study for want of time has been restricted to one season only hence the results obtained may not be conclusive. More trials with different varieties may help in arriving at proper recommendations.

Summary

6. SUMMARY

The study "Seed invigoration with nanoscale particles in chilli (*Capsicum annuum* L.)" was conducted in the Department of Seed Science and Technology, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur. The study was conducted using chilli *cv* Ujwala. Seed invigoration was done with nano ZnO and nano custard leaf powder.

6.1 Effect of pre-sowing treatment with inorganic nanoparticles in chilli

- The seeds treated with nano ZnO performed better than untreated seeds. Significant variations was observed for traits such as days to first flowering, days to 50 per cent flowering, plant height, number of branches, fruits per plant, fruit length, fruit weight at maturity, fruit yield per plant, seeds per fruit, seed yield per plant and100 seed weight.
- Nano ZnO @ 500 mg kg^{-1} of seed (T_4) performed superior for days to first flowering (73.33 days), fruit weight at maturity (2.27g), fruit yield per plant (224.56g) and Seed yield per plant (24.02g).
- Nano ZnO @ 400 mg kg^{-1} of seed (T_3) performed superior for days to 50 per cent flowering (79.67 days), plant height (42.94cm) and branches per plant (12.67)
- In case of 100 seed weight nano ZnO @ 600 mg kg^{-1} of seed (T_5) recorded the highest value (0.42g).
- Nano ZnO @ 800 mg kg^{-1} of seed (T_7) gave the highest fruits per plant(109.67). Nano ZnO @ 700 mg kg^{-1} of seed and custard leaf powder @ 500 mg kg^{-1} of seed gave highest fruit length(4.87cm).
- Based on scoring and ranking it was observed that nano ZnO @ 500 mg kg^{-1} of seed(T_4) perfomed well compared to all other treatments in case of pre-sowing treatment with inorganic nanoparticles in chilli.

6.2 Effect of pre-storage treatment with inorganic nanoparticles in chilli

- Nano ZnO @ 500 mg kg^{-1} of seed (T_4) treatment have recorded least number of days to first flowering (76.33) followed by nano ZnO @ 400 mg kg^{-1} of seed (T_3) (77.33). Nano ZnO @ 400 mg kg^{-1} of seed (T_3) took least days among the treatments for days to 50 per cent flowering (82).
- Nano ZnO @ 500 mg kg $^{-1}$ of seed gave good result in terms of branches per plant (14.86), fruits per plant(108.67), fruit weight at maturity(7.10g), fruit yield per plant (770.68g), Number of seeds per fruit (73.80)and Seed yield per plant(60.56g).
- There was no significant difference was recorded in fruit length among the treatments.
- Nano ZnO @ 800mg kg^{-1} of seed (T_7) resulted to highest plant height (41.98cm).
- Nano ZnO @ 600 mg kg^{-1} of seed(T_5) recorded highest value for seed weight (0.43g).
- Based on ranking and scoring pre storage treatment with nano ZnO @ 500 mg kg^{-1} (T_4) perfomed well in most of the fruit yield and fruit yield contributing attributes.

6.3 Effect of mid- storage correction with inorganic nanoparticles in chilli

- Seeds treated with nanoparticle showed better performance compared to control in all seed quality parameters during storage.
- After three month germination above s of storage nano ZnO @ 400 mg kg^{-1} of seed (T_3) gave more than 60 percentage germination.
- Declining trend was observed in case of germination percentage , seedling length, seedling dry weight but electrical conductivity and microflora percentage was increased through out the storage .
- Nano ZnO @ 400 mg kg^{-1} of seed gave better result in case of

germination per cent (64.33),shoot length (4.21cm), root length (8.79), seedling dry weight (24.96), vigour index I (836), vigour index II (1606).

- Electrical conductivity was highest in case of control (159.20 μScm^{-1}) lowest in case of nano ZnO @ 500 mg $kg^{-1}$of seed (108.43 μScm^{-1}).
- Seeds treated with custard leaf powder @ 500 mg kg^{-1} of seed treated seed showed lowest microflora infection after three months of storage both in case of blotter method (26.67) and agar method (33.33) followed by nano ZnO @ 300 mg kg^{-1} of seed.
- Based on scoring and ranking nano ZnO @ 400 mg kg^{-1} of seed (T_3) performed well in most of the seed quality parameters which can be recommended for the mid storage correction.

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Seed invigoration with nanoscale particles in chilli (*Capsicum annuum* L.)

By

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(2020-11-023)

ABSTRACT

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Faculty of Agriculture
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ABSTRACT

An experiment was undertaken at the Department of Seed Science and Technology, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur to test the efficacy of nanosized particles of Zinc Oxide and custard apple leaf in seed treatments.. The study consisted of three experiments.

In the first experiment, seeds were treated with the nanoparticles as presowing treatments and the crop raised to ascertain its effect on growth and yield.

Nano ZnO @ 500 mg kg^{-1} of seed (T_4) performed superior for days to first flowering (73.33 days), fruit weight at maturity (2.27g), fruit yield per plant (224.56g), seed yield per plant (24.02g). Nano ZnO@ 400 mg kg^{-1} of seed (T_3) performed superior for days to 50 per cent flowering (79.67days), plant height (42.94cm) and branches per plant (12.67). Nano ZnO @ 500mg kg^{-1} of seed (T_4) perfomed well in case seed yield and seed yield attributes.

In experiment II, seeds were subjected to the same treatments as in the previous experiment and stored for six months. The stored seeds were then used to raise the crop. Nano ZnO @ 500 mg kg^{-1} of seed (T_4) treatment recorded least number of days to first flowering (76.33) hihest number of branches per plant(14.86), fruits per plant(108.67), fruit weight at maturity(7.10g), fruit yield per plant (770.68g), number of seeds per fruit (73.80) and seed yield per plant (60.56g). Nano ZnO @ 500mg kg^{-1}(T_4) of seed perfomed well in case seed yield and seed yield attributes.

In the third experiment, three month old seeds were treated with nanoparticles as enumerated under Experiment I and stored for three months. Seed quality parameters were measured during due course of storage as per ISTA standards at monthly intervals. Highly significant differences were observed among the treatments. At the end of the storage period (three months) nano ZnO @ 400 mg kg^{-1} of seed recorded highest germination value for 60 per cent (64.33),and shoot length (4.21cm), root length

(8.79), seedling dry weight(24.96), vigour index I (836) and vigour index II (1606). Nano Custard leaf powder @ 500 mg kg^{-1} of seed treatment infection after three months of storage both in case of blotter method (26.67) and agar method (33.33) .

Zinc Oxide nano particles @500mg kg^{-1} of seed where found promising as prestorage and presowing treatment and @ 400 mg kg^{-1} of seed was good as a mid storage treatment. The present study indicates that seed longevity, viability and seedling performance can be enhanced by treating the chilli seeds with nano particles both organic and inorganic nano particles