Mid storage seed invigoration with nanoparticles in chilli (*Capsicum annuum* L.)

by

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2023

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THESIS

Submitted in partial fulfilment of the
requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

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

2023

DECLARATION

I hereby declare that the thesis entitled **Mid storage seed invigoration with nanoparticles 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: 17-11-2023

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CERTIFICATE

Certified that this thesis entitled **Mid storage seed invigoration with nanoparticles in chilli (*Capsicum annuum* L.)** is a record of research work done independently by **Ms Gagana G (2021-11-129)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the advisory committee of **Gagana G (2021-11-129),** 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 **Mid storage seed invigoration with nanoparticles in chilli (*Capsicum annuum* L.)** may be submitted by **Gagana G (2021-11-129),** in partial fulfilment of the requirement for the degree.

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ACKNOWLEDGEMENT

As a prelude to my thanksgiving, I wish to bow my head before the Almighty God for showering me the strength, knowledge, ability and making me confident and optimistic throughout my journey and enabled me to complete the thesis work successfully on time.

At this juncture, I would like to thank with lots of love, my godparents, ***Appa (Gangarevaiah), & Amma (Mahadevamma)*** *for their profound love, boundless affection and incessant inspiration which supported me to stay on track and I thankfully remember* ***Anna(Renu), Akka (Rashmi), Attige (Bhoomika) and Niece (Lashi)****, who has been showering blessings and care upon me from the heavens.*

With immense pleasure I avail this opportunity to express my deep sense of whole hearted gratitude and indebtedness to my major advisor ***Dr. Dijee Bastian****, Professor (Plant Breeding and Genetics), Department of Seed Science & Technology and Chairperson of my Advisory Committee for her precious suggestions, constant care and support, extreme patience and untiring assistance and also constructive criticism throughout the course of study starting from its preparatory stage to submission of thesis. This work would not have been possible without her valuable guidance and support. I consider myself extremely fortunate in having the privilege of being able to be guided by her.*

I would like to express my extreme indebtedness and obligation to ***Dr. Namboodiri Raji Vasudevan,*** *Assistant Professor (Plant Breeding and Genetics), Head i/c Centre for IPR, Technology Management and Trade****,*** *College of Agriculture, Vellanikkara and member of my advisory committee for her meticulous help, unwavering encouragement, forbearance, timely support and critical examination of the manuscript that has helped a lot for the improvement and preparation of the thesis.*

I sincerely thank ***Dr. C R Rashmi*** *Professor and Head, Department of Plant Pathology for her support, ever willing help, scholarly suggestions and expert advice and help in taking the observations on seed microflora and interpretation of results.*

I express my heartiest gratitude to ***Dr. Smitha Revi*** *Assistant Professor*

Department of Agricultural Entomology, College of Agriculture, Vellanikkara and member of my advisory committee for his encouragement, timely support, valuable guidance and creative suggestions throughout the period of my study.

It gives me great pleasure in acknowledging the support and help of all the non-teaching staff members of Department of seed Science and Technology, **vinod chettan, Smitha chechi**, **Nithu chechi, Hidha chechi, Jeana chechi, Ashwadhi chechy, Bilha, Athira, Ambadi and Dixon** for their sincere co-operation, timely help and assistance during the research work. It would be an omission, should I forget to mention and acknowledge the help extended by the farm laboures of Department of seed Science and Technology, **Abhilash chettan, Uthaman chettan, Dhamu chettan, Omana chechy,Shashi chetton, Shantha chechy, Ajitha chechy, Gijitha chechi, Latha chehi and Sindhu chechi** without whose help it would have been difficult to conduct the study.

I thankfully reminisce the services rendered by all the staff members of **Students Computer club, College library, Academic cell, College office** and **Central library, KAU.** I also thank **Mr.Pauly Manjaly (tcr educare).**

I am particularly thankful for the support and co-operation of my classmates **Adheena, Monica** and **Sai** throughout the duration of my MSc. work. I also extend my heartfelt thanks to my seniors **Adarsh chettan**, **Tabhsheer chettan**, **Vaishnavi chechy**, **Arya chechi and Olivia chechi** I wish to express my sincere gratitude to my dear juniors **Harshitha, Chethan, Prashanth, Priyadarshan, Poonam for** their whole hearted support. It gives me great pleasure in acknowledging the love and gratitude of my beloved friends **Deepu, Pandya, Vinu**, **Apoorva, Anjali, Malappa, Ajmeer, Basu, Chethan,** and all others for their cooperation, time and presence in my research. A word of wholehearted apology to those whom I have not mentioned in person and a note of thanks to everyone who helped me for the successful completion of this endeavor.

Gagana G

Dedicated to Lord Krishna,

my parents and my guide

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LIST OF ABBREVIATIONS

ISTA	: International Seed Testing Association
mL	: milli litre
h	: hours
EC	: electrical conductivity
mg	: milli gram
kg	: kilo gram
$\mu S\ cm^{-1}$	: micro Siemens per centimetre
ppm	: Parts per million
mm	: milli metre
nm	: nano metre
m	: metre
µm	: micro metre
°C	: degree Celsius
Pa	: Pascal
L	: Litre
ha	: Hectare
cc	: cubic centimeters
ANOVA	: Analysis of Variance
ZnO	: zinc oxide
nZnO	: nano-zinc oxide
SE_m	: standard error of error mean sum of squares
CD	: critical difference
var.	: variety
cv.	: cultivar
NP	: nano particle
IMSCS	: Indian Minimum Seed Certification Standard
mg/kg	: milligram per kilogram
°N	: degrees north
°E	: degrees east
G	: gauge
DAT	: Days after transplanting
MAS	: months after storage
%	: per cent
et al.	: *et alia* (Latin: 'and others')
V.I.	: vigour index
PDA	: Potato Dextrose Agar

Introduction

1. INTRODUCTION

Chilli (*Capsicum annuum* L.) is an important spice and vegetable crop belonging to the family Solanaceae. It is native to tropical South America and was introduced to India in the 16th century probably by Portuguese and Arab traders (Antonio *et al*., 2018). India is the world's second largest producer, exporter and consumer of chilli after China, and is considered the secondary centre of diversification.

The area, production and productivity of green chillies in India is 4.17 lakh ha, 4.50 lakh MT, and 10.8 MT. respectively. Andhra Pradesh is the leading chilli growing state in India followed by Telangana and Madhya Pradesh, (APEDA, 2021). Kerala dedicates 1,720 hectares of land to chilli cultivation, achieving an annual yield of 7,650 MT. The average chilli productivity in Kerala is 4.45 MT per hectare (India stat, 2022).

Vegetable seed production requires a significant financial investment and preserving surplus stock for more than one season presents a serious problem due to the gradual deterioration of seeds during storage. Seed deterioration is a natural process that affects all types of seeds during storage. This process involves various physiological and biochemical changes that occur as seeds naturally age. Wastage of seeds is highly regrettable, because of their high economic value. Extending the storage life of seeds is consistently a financially advantageous choice, provided that the method is both cost effective and easily implementable. The mid storage hydration-dehydration treatment method, relatively a low cost technology has been successfully followed for the preservation of seeds for a number of vegetables (Basu and Rudrapal, 1982).

A significant challenge in the chilli seed industry is the difficulty in storing and maintaing the seed quality, especially when exposed to fluctuating environmental conditions. Chilli seeds possess an exceptionally thin seed coat, which facilitates the smooth emergence of both the radicle and shoot. However, this thin seed coat renders the seeds extremely vulnerable to external environmental factors, significantly increasing the seed mortality.

Mid storage seed treatment is a technique employed to enhance the viability of seeds during extended storage periods for various seed types (Pan and Basu, 1985). It is a seed management technique that focuses on enhancing the physiological condition of seeds, resulting in improved germination rate, extended shelf life, and superior performance compared to untreated seeds (Basu, 1994). These treatments are not only effective in maintaining seed viability but also in boosting the productivity of the subsequent crop (Mandal and Basu, 1986). Mid storage invigoration treatment, with or without the use of chemicals, has proven to be successful in enhancing the vigour, viability, and overall productivity in several crops, including rice, wheat, tomato, and sunflower. When seed germination falls slightly below the minimum seed certification standards before sowing, it becomes very important to a consider measures that can enhance seed viability. Application of mid storage invigoration treatments is highly recommended to not only improve the germination of unused seed stocks but also extend their storage life (Renugadevi *et al*., 2006). These treatments are essential to revitalize low-vigour seeds, enabling them to achieve improved emergence and establishment, while also extending their storability. Mid storage seed treatment is particularly effective for seeds with low to medium vigour.

The mid storage seed treatment process encompasses the removal of inhibitors and harmful metabolic byproducts from the seeds, promotion of seed germination, reduction in moisture uptake by treated seeds and prevention of lipid peroxidation reactions (Dey and Mukherjee, 1984).

Nanotechnology has the potential to revolutionize seed enhancement by offering novel methods and materials for improving seed quality. This potential arises from the unique characteristics of nanotechnology, such as its expansive surface area, reactivity, and adaptable pore sizes, which make it a powerful force in advancing agriculture and biotechnology.

Complete prevention of seed senescence may not be achievable, but the utilization of metal oxide nanoparticles in seed treatments can effectively slow down the deteriorative processes associated with seed aging. One of the protective mechanisms of nanoparticles involves the suppression of free radicals within seeds through oxidation-reduction reactions (Zheng *et al.*, 2005). This process releases oxygen, further stimulating the respiration of germinating seeds. Among the most commonly utilized nanoparticles for this purpose are metal oxides, including zinc (Zn), titanium (Ti), copper (Cu), silver (Ag), silicon (Si) and iron (Fe).

In agriculture, zinc is an essential mineral for plant growth and development. Zinc oxide nanoparticles (NPs) find application as nano-fertilizers due to their capacity to address zinc deficiency and enhance seed germination and plant growth. Zinc oxide, being a vital micronutrient, plays a pivotal role in augmenting dry matter production (Mendez-Arguello *et al.*, 2016). This micronutrient contributes significantly to the synthesis of carbohydrates, proteins, lipids and nucleic acids in plants, thereby boosting total leaf area and fostering plant growth (Tarafdar *et al.,* 2013).

Zn NPs supplementation promote seed germination, plant growth and improve crop yield and quality (Kolencik *et al*., 2019). It is a fundamental component of many enzymes that power diverse metabolic processes in many kinds of crops. Additionally, it has been shown that Zn NPs increase the reactivity of phytohormones, notably Indole Acetic Acid (IAA), which promotes the effects of phytostimulants. In order to promote faster seedling development, zinc-enriched Zn NPs may increase IAA levels in roots (sprouts). As nanoparticles can infiltrate through cracks in the seed coat it can effectively suppress free radicals. This interaction results in the enhancement of seed vigour, contributing to improved germination and early seedling growth.

In this context, the present study entitled "Mid storage seed invigoration with nanoparticles in chilli (*Capsicum annuum* L.)" was carried out to enhance the seed quality of aged chilli seeds through the application of nanoparticles as mid storage seed treatments with the following goals.

1. To study the effect of metal zinc oxide nanoparticles when administered as midterm seed treatment on subsequent crop performance and seed yield
2. To assess the impact of midterm seed treatment with metal zinc oxide nanoparticles on seed quality and longevity.

Review of literature

2. REVIEW OF LITERATURE

Seed is the carrier of technology from one generation to another. Seeds are often stored for extended periods, and their viability diminishes quickly as seeds, being inherently hygroscopic, readily absorb moisture from the surrounding humid atmosphere. When combined with elevated temperatures, this absorption process speeds up the natural aging of seeds, resulting in a significant decline in vigour, viability, and potential crop yield. Therefore, it is essential to implement protective measures that enhance germination and field emergence while extending the quality and longevity of seeds.

Seed invigoration can improve germination, seedling emergence, field establishment and plant vigour. Seed treatments maybe applied as presowing, prestorage and mid storage treatments. When applied as mid storage treatments, they have the potential to mitigate the damage caused by aging, by partially rejuvenating the seed vigour. Additionally, these treatments enhance both the viability and productivity of seeds kept in storage. Many researchers observed that mid-term hydration-dehydration treatments can be effective in improving germination and seedling vigour in stored seeds (Mandal *et al*., 2000).

"Nanotechnology is the art and science of manipulating matter at nano". It deals with manipulation of matter with at least one dimension size ranging from 1 to 100 nano meter. Seed treatment using nano scale particles is a promising approach, as the materials can be better absorbed due to the small particle size. Seed priming and coating with nanoparticles, are considered eco-safe and economically prudent (Sharma *et al*., 2018).

Zinc (Zn) deficiency is the second most common deficiency after Fe-deficiency in plants. Treatments with nano ZnO particles at low concentrations, lead to adsorption on the seed coat and penetration through tissue layers, improving germination and crop biomass.

Numerous studies have documented the advantageous impact of specific nanoparticles on enhancing the seed quality of several crops such as tomato (Sridhar, 2012), chilli (Gayathri, 2017; Mathew *et al.,* 2021; Adarsh, 2023), wheat (Rizwan *et* *al.,* 2019), onion (Laware and Raskar, 2014), maize (Mahesh *et al*., 2022) and sunflower (Meenakshi *et al.*, 2020).

In this chapter, the influence of mid storage seed treatment with nanoparticles on crop development and yield, as well as on seed quality and longevity are presented below;

2.1 Effect of mid storage correction with inorganic nanoparticles in chilli on crop growth and yield

2.1.1 Days to first flowering

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Seed treatment with nano ZnO @ 500 mg kg^{-1} of seed led to earlier flowering (73.33 days), when compared to control, which exhibited flowering at 83.67 days.	Adarsh, 2023
Other crops		
Gerbera	The treatment with 20 mg L^{-1} nanoparticle-SiO_2 exhibited the shortest time taken for flower emergence at 62.25 days, which was earlier when compared with control (81.08 days.)	Alikhani *et al.*, 2021
Lily	The plants treated with 50 ppm AgNPs clearly exhibited an earlier onset of flowering, commencing at 106 days, which was two or three days ahead of the control plants, which initiated flowering at 109 days.	Byczynska *et al.*, 2023
Peanut	Seeds treated with nano ZnO at 1000 ppm reported early flowering, at 27.24 days, while the untreated control flowered later at 29.95 days	Prasad *et al.*, 2012

2.1.2 Days to 50 per cent flowering

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Seed treatments with nano ZnO @ 400 mg/kg of seed (82.67 days) resulted in an early onset of 50 per cent flowering compared to control (88.33 days).	Adarsh, 2023
	Treatment with 0.5 g kg^{-1} nano powder of custard leaf resulted in lesser number of days to flowering. Treated plants flowered by 79^{th} day and control flowered at 87^{th} days.	Sandhya, 2016
Tomato	Nano Zinc treatment at 800ppm resulted in a reduced number of days taken to reach 50 per cent flowering (38.87 days) as compared to control (46.84 days).	Sharma *et al*., 2022
Other crops		
Maize	Seed treatment with ZnO NPs (20-30 nm) at 15 mg L^{-1} significantly decreased the time needed for 50 per cent tasseling, with a minimum of 51.73 days, whereas control required 56.87 days. Similarly, the treatment resulted in a minimum of 54.47 days to reach 50 per cent silking, while the control took of 59.60 days to achieve 50 per cent silking.	Mahesh *et al*., 2022
Strawberry	Seed dressing with a combination of 150 ppm ZnO NPs and 150 ppm FeO NPs resulted in early flowering, occurring at 59.40 days, while the control recorded 66.44 days.	Kumar *et al*., 2017

2.1.3 Plant height

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Seed treatment with nano ZnO @400 mg kg^{-1} of seed exhibited the tallest plant height, measuring 42.94 cm. The control recorded a plant height of 35.62 cm.	Adarsh, 2023
	The use of nano ZnO @ 1300 mg kg^{-1} of seed led to the tallest plant, with a height of 67.3 cm, as compared to control (60.33 cm).	Mathew *et al.,* 2021
Chilli	The application of ZnO NPs at 75 mg L^{-1} had a notable impact on plant height, resulting in a significant increase to 61.0 cm compared to the control treatment, (55.0 cm).	AL-Zuhairi *et al.*, 2020
Tomato	Nano $FeSO_4$ at 5 per cent increased plant height to 136.8 cm, which was higher compared to the control (121 cm).	Sakya and Sulandjari, 2019
	Treating seeds with Zn NP suspension at 800 ppm resulted in the tallest plants, reaching a height of 144.22 cm, whereas the control with a height of 68.73 cm.	Raliya *et al.,* 2015
Other crops		
Maize	Seed treatment with ZnO nanoparticles (20-30 nm) at a concentration of 15 mg L^{-1} resulted in a significantly higher plant height of 103.67 cm, compared to control (96.0 cm).,	Mahesh *et al.*, 2022

Okra	ZnO nanoparticles at a concentration of 25 mg L^{-1} were found to significantly enhance total plant height (34.0 cm), outperforming hydropriming, which resulted in a height of 23.0 cm, across various ZnO NPs concentrations ranging from 5 to 200 mg L^{-1}.	Keerthana *et al*., 2021
Fodder maize	Plant height significantly increased when exposed to ZnO nanoparticles at a concentration of 20 mg L^{-1} in comparison to treatments involving $ZnSO_4$ nanoparticles, bulk materials, and control.	Tondey *et al*., 2021
Wheat	The application of 100 mg L^{-1} ZnO NPs resulted in an increase in plant height to 91.16 cm, surpassing the control (75.05 cm).	Popovic *et al*., 2020
Pearl millet	Seeds treated with AgNPs at 20 mM level resulted in a 29 % increase in plant height when compared to control.	Khan *et al*., 2020
Wheat	ZnO NPs @ 100 mg L^{-1} treatment led to a 37.0 per cent increase in plant height compared to control, treatment with 20 mg L^{-1} Fe NPs resulted in a 35% increase.	Rizwan *et al.,* 2019
Rice	Among the four different concentrations of ZnO nanoparticles (0, 0.5, 1.0, and 5.0 g L^{-1}), ZnO NPs at a concentration of 5.0 g L^{-1} significantly enhanced plant height to 74.2 cm, while the control recorded a plant height of 61.0 cm.	Bala *et al*., 2019
Sorghum	Nano ZnO @ 500 ppm resulted in a significantly higher plant height of 186.7cm compared to bulk $ZnSO_4$ (185cm).	Poornima and Koti, 2019

Finger millet	Treatment with nano ZnO @ 500 ppm resulted in a plant height of 120.50 cm, while the control recorded 85.0 cm.	Saraswathi *et al*., 2018
Fodder cowpea	Seed treatment with nano zinc oxide (nZnO) at concentrations of 250, 500, and 750 ppm revealed that the 500 ppm concentration resulted in a significantly higher plant height of 133.9 cm compared to the control (92.7 cm).	Srinivasan *et al*., 2017
Peanut	Seeds treated with nano ZnO and bulk $ZnSO_4$ at a concentration of 1000 ppm recorded plant height of 15.40 cm and 12.42 cm, respectively. In contrast, the control exhibited a plant height of 8.22 cm.	Prasad *et al*., 2012
Okra	Plant height was significantly improved in crops grown from 5-month-old seeds subjected to mid storage dry and wet treatments. Notably, the soaking and drying treatment resulted in a remarkable increase in plant height (67.0 cm), whereas untreated seeds exhibited a comparatively lower plant height of 55.0 cm.	Guha *et al*., 2011

2.1.4 Branches per plant

Crop	**Details of the experiment**	**Reference**
Solanaceae		
Chilli	Seed treatment with nano ZnO @ 400 mg kg^{-1} of seed recorded the highest number of branches (12.67), while control recorded 4.27 branches.	Adarsh, 2023
	The application of ZnO @ 75 mg L^{-1} had a notable effect on the number of branches per plant, resulting in an average of 29.4 branches per plant.	AL-Zuhairi *et al*., 2020

	In contrast, the control treatment exhibited an average of 23.5 branches per plant.	
Other crops		
Grain cowpea	Seed priming with $ZnSO_4$ @ 0.05 per cent resulted in a higher number of branches per plant, with an average of 3.67 branches, compared to the control 3.27 (branches per plant).	Raj *et al.,* 2021
Okra	Among the different treatments of ZnO (5, 10, 25, 50, 100, and 200 mg L^{-1}) application of ZnO nanoparticles at a concentration of 25 mg L^{-1} significantly increased the total number of branches to 7, compared to hydropriming, which resulted in 3 branches.	Keerthana *et al.*,2021
Mung bean	The highest branches per plant (15) was observed when a combination of silver nanoparticles (10 ppm) and zinc nanoparticles (6 ppm) was used as treatment. The control recorded 10.1 branches.	Wasaya *et al.*, 2020

2.1.5 Fruits per plant

Crop	Details of the experiment	Reference
Solanaceae		
	Seed treatment with nZnO @ 500 mg kg^{-1} of seed exhibited the highest number of fruits per plant, with an average of 108.67, surpassing control, which had 73.86 fruits per plant.	Adarsh, 2023

Chilli	Pre storage seed treatment utilizing nano ZnO @ 800 mg L^{-1} per seed resulted in the highest number of fruits per plant, averaging 109.67 fruits, surpassing control, which had an average of 72.23 fruits per plant.	Adarsh, 2023
	Seeds treated with nano ZnO @ 1300 mg kg^{-1} of seed exhibited the highest fruits per plant, producing 122 fruits, in contrast to the control (85.33).	Mathew *et al*., 2021
	ZnO NPs treatment at a concentration of 75 mg L^{-1} produced an average of 16.8 fruits per plant, whereas the control treatment yielded an average of 9.0 fruits per plant.	AL-Zuhairi *et al*., 2020
Tomato	The application of 5.0 per cent $FeSO_4$ resulted in an increased number of fruits (5) compared to the control, which had 3.3 fruits.	Sakya and Sulandjari, 2019
	The treatment with 100 mg kg^{-1} ZnO nanoparticles promoted the highest fruit yield compared to other treatments.	Raliya *et al*., 2015
Other crops		
Maize	Seed treatment with ZnO nanoparticles (20-30 nm) at a concentration of 15 mg L^{-1} resulted in significantly higher values for both the number of kernels per cob and the number of kernel rows per cob, with values of 480.27 and 17.13 respectively, compared to all other treatments.	Mahesh *et al*., 2022

Grain cowpea	Seed priming with $ZnSO_4$ @ 0.05 per cent resulted in a higher number of pods per plant (24.13), as compared to the control (11.06).	Raj *et al.,* 2021
Okra	Among the different ZnO concentrations tested (5, 10, 25, 50, 100, and 200 mg/L), the application of ZnO nanoparticles at a concentration of 25 mg/L significantly improved the number of pods, resulting in 13 pods, compared to hydropriming, which had 4 pods.	Keerthana *et al.,* 2021
Mung bean	The highest number of pods per plant (24) was observed in the treatment with Ag nanoparticles at 10 ppm and Zinc nanoparticles at 6 ppm used in combination. In contrast, the control had 14.30 pods per plant.	Wasaya *et al*., 2020
Fodder cowpea	Seed treatment with different levels of nano zinc oxide (nZnO) at concentrations of 250, 500, and 750 ppm showed that the 500 ppm concentration resulted in a significantly higher number of pods (20) compared to the control, which had 11 pods.	Srinivasan *et al.,* 2017
Wheat	The application of Cu-NPs at concentrations ranging from 10 to 50 ppm revealed that the treatment with 30 ppm of Cu-NPs resulted in a significantly higher number of spikes per plant, measuring 19.33. In contrast, the control yielded 13 spikes per plant.	Hafeez *et al.,* 2015
Onion	Seeds treated with a concentration of 30 µg ml^{-1} of ZnO NPs yielded the highest number of seeded fruits per umbel, with a total of 228.68, while the control produced 203.64 seeded fruits.	Laware and Raskar, 2014

Okra	The number of fruits per plant significantly increased in crops grown from 5-month-old seeds subjected to mid storage dry and wet treatments. In particular, the soaking and drying treatment resulted in a significant increase, yielding 10 fruits per plant, while untreated seeds produced a comparatively lower number, at five fruits per plant.	Guha *et al*., 2011
Cotton	Crops resulting from the mid storage seed treatment, which was administered to 10-month-old seeds using a di-sodium phosphate (10^3 m) solution, displayed the highest count of sympodia per plant at 15.3 just slightly exceeding the control (fresh seeds) with 15.1	Ponnuswamy and Karivathararaju,1996

2.1.6 Fruit length

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Seed treatment using nano ZnO @ 700 mg kg^{-1} of seed resulted in the longest fruits, averaging 4.87 cm, while control recorded 4.47 cm.	Adarsh, 2023
	The longest fruit length, measuring 7.40 cm, was achieved when nano ZnO @1300 mg kg^{-1} seed, surpassing the fruit length of 6.05 cm in the control.	Mathew *et al*., 2021
Other crops		
Maize	Seed treatment with nano ZnO (20-30 nm) at a concentration of 15 mg L^{-1} resulted in a	Mahesh *et al*.,2022

	significantly higher cob length of 19.55 cm, while the control recorded 16.87 cm.	
Okra	Among the different ZnO concentrations tested (5, 10, 25, 50, 100 and 200 mg L^{-1}), the use of ZnO nanoparticles at a concentration of 25 mg L^{-1} significantly improved pod length, reaching 17.2 cm, compared to hydropriming, which resulted in a pod length of 7.3 cm.	Keerthana *et al.*,2021
Grain cowpea	Seed priming with $ZnSO_4$ at a concentration of 0.05% resulted in a higher pod length of 17.47 cm, as compared to the control, which had a pod length of 14.99 cm.	Raj *et al.,* 2021
Wheat	The application of 100 mg L^{-1} ZnO NPs resulted in an increase in the spike length of wheat genotypes to 10.7 cm, surpassing the control (10.0 cm).	Popovic *et al.*, 2020
Barley	Plants were individually treated with chelated nano ZnO and Fe_2O_3 suspensions at different growth stages. The results showed a significant increase in spike length, measuring 10.12 cm and 11.06 cm, respectively, compared to control (68.0cm)	Janmohammadi *et al.*, 2016
Maize	The seeds treated with nano ZnO @ 400 ppm resulted in the longest cob length, measuring 16.40 cm, which was 18 per cent longer than the control.	Subbaiah, 2014
Okra	The length of the fruit exhibited a significant increase in crops cultivated from 5-month-old seeds, that had undergone mid storage dry and wet	Guha *et al.*, 2011

	treatments. Notably, the soaking and drying treatment resulted in a fruit length of 10.68 cm, whereas untreated seeds yielded comparatively shorter fruits length (8.73 cm)	

2.1.7 Fruit weight at maturity

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Pre sowing seed invigoration with nano ZnO @ 500 mg kg^{-1} seed led to the highest fruit weight recorded at 2.27 grams, a significant improvement compared to the control, which had a fruit weight of 1.83 grams.	Adarsh, 2023
	Treating the seeds with nano ZnO @ 1300 mg kg^{-1} of seed led to the highest fruit weight, measuring 3.46 grams and 1.04 grams observed in the control.	Mathew *et al.*, 2021
Tomato	The application of 5.0 per cent $FeSO_4$ resulted in an increased fruit weight of 136.56g compared to the control, which had a fruit weight of 90.95g.	Sakya and Sulandjari, 2019
Other crops		
Grain Cowpea	Seed priming with $ZnSO_4$ at a concentration of 0.05 per cent resulted in a higher pod weight of 17.05g per plant compared to the control, which had a pod weight of 16.9g per plant.	Raj *et al.,* 2021
Mungbean	The highest pod weight of 78.00g was observed in treatment with silver nanoparticles (AgNPs) at 10 ppm and zinc nanoparticles (ZnNPs) at 6ppm in	Wasaya *et al.*, 2020

	combination. This was in contrast to the control, which had a pod weight of 20g.	
Okra	The fruit weight showed a substantial increase in crops grown from 5-month-old seeds that had been subjected to mid storage dry and wet treatments. Specifically, the soaking and drying treatment yielded the highest fruit weight at 10.74g, while untreated seeds produced comparatively lighter fruits, weighing in at 8.01g.	Guha *et al.*, 2011

2.1.8 Fruit yield

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Seed invigoration with nano ZnO @ 500 mg/kg of seed produced the highest yield, measuring 224.56 grams. This yield was notably greater than that of the control, which yielded only 130.53 grams.	Adarsh, 2023
	Seed treated with custard leaf powder @ 500 mg kg^{-1} seed recorded fruit yield of 160.21g Lowest fruit yield was obtained in control (130.53g).	Adarsh, 2023
	The application of nano ZnO @ 1300 mg kg^{-1} of seed resulted in the highest fruit yield per plant, with a remarkable 422.70g, which significantly surpassed the control yield of 195.50g.	Mathew *et al.*, 2021
	The plants treated with ZnO NPs at a concentration of 75 mg L^{-1} had a yield of 119.2g	AL-Zuhairi *et al.*, 2020

	per plant, compared to the control treatment which had a yield of 64.0g $plant^{-1}$.	
Other crops		
Okra	The fruit yield per plant exhibited a significant increase in crops cultivated from 5-month-old seeds that underwent mid storage dry and wet treatments. The soaking and drying treatment resulted in the highest fruit yield, producing 104.2 grams of fruit, while untreated seeds yielded a 43.9g.	Guha *et al.*, 2011

2.1.9 Seed yield per plant

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Seed invigoration with nano ZnO @ 500 mg kg^{-1} of seed produced the highest yield, measuring 24.02 grams. This yield was notably greater than that of the control, which yielded only 11.35g.	Adarsh, 2023
	Compared to the control (17.96g), seeds treated with nano ZnO @ 1300 mg kg^{-1} of seed achieved the highest seed yield of 41.14g.	Mathew *et al.*, 2021
Other crops		
Grain Cowpea	Seed priming with $ZnSO_4$ at a concentration of 0.05% resulted in a higher seed yield of 1446 kg ha^{-1}, compared to the control with a yield of 1246 kg ha^{-1}.	Raj *et al.,* 2021

Cowpea	Seeds subjected to priming in a 10^{-3} M $ZnSO_4$ solution exhibited a significantly higher seed yield (1100.5 kg ha^{-1}) compared to control.	Arun *et al.,* 2017
Fodder cowpea	Seed treatment with different levels of nano zinc oxide (nZnO) at concentrations of 250, 500, and 750 ppm revealed that the treatment with 500 ppm concentration resulted in significantly higher seed yield (23.9g pot^{-1}) compared to the control (15.1g pot^{-1}).	Srinivasan *et al*., 2017
Okra	Crops grown from 5-month-old seeds, which were subjected to mid storage dry and wet treatments, showed a significant increase in the number of seed yield per plant. Specifically, the treatment with red chilli powder resulted in a noteworthy increase in the number of seed yield per plant (5g), while the untreated seeds produced a relatively lower yield of 2.18g.	Guha *et al*., 2011
Cotton	Crops resulting from the mid storage seed treatment, involving the application of a 0.5% Dithane M-45 solution to 10-month-old seeds, demonstrated the highest cotton yield per plant at 91.8g, narrowly surpassing the yield of water soaked seeds at 86.9g.	Ponnuswamy and karivathararaju,1996

2.1.10 Number of Seeds per fruit

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Pre storage seed invigoration with nano ZnO @ 500 mg kg^{-1} of seed led to a higher number of seeds per fruit, with 73.80 seeds, compared to the control (55.91).	Adarsh, 2023
Chilli	Pre sowing seed invigoration with nano ZnO @ 800 mg/kg of seed resulted in a greater number of seeds per fruit, averaging 67.59 seeds, in contrast to the control, which had 53.80 seeds per fruit.	Adarsh, 2023
	The treatments utilizing nano ZnO @ 1300 mg kg^{-1} achieved the highest number of seeds per fruit (62).	Mathew *et al*., 2021
Other crops		
Soybean	Seeds treated with a 200-ppm nano $FeSO_4$ solution demonstrated a 43.27 per cent increase in the number of seeds per pod compared to untreated plants.	Dola *et al*., 2022
Mungbean	The highest number of seeds per pod, at 14.00 seeds per pod, was observed in combined treatment of Ag nanoparticles at 10 ppm and Zinc nanoparticles at 6 ppm. Control, recorded 8.6 seeds per pod.	Wasaya *et al*., 2020

Maize	Maize seeds exposed to nano ZnO @ 400 ppm exhibited a higher number of grains per row (38.5), representing a 36 per cent increase compared to the control treatment.	Subbaiah, 2014
Okra	Crops grown from 5-month-old seeds, which were subjected to mid storage dry and wet treatments, showed a significant increase in the number of seeds per capsule. In particular, the soaking and drying treatment resulted in a noteworthy boost, with 52 seeds per capsule, while untreated seeds had a lower count of 44 seeds per capsule.	Guha *et al.,* 2011

2.1.11 100 Seed weight

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Seed invigoration with nano ZnO @ 600 mg kg^{-1} of seed resulted in the highest 100-seed weight (0.43 grams), compared to 400mg nano ZnO kg^{-1} of seed (0.36g).	Adarsh, 2023
Other crops		
Okra	Crops grown from 5-month-old seeds by mid storage dry and wet treatments showed a significant increase in 1000-seed weight. In particular, the use of bleaching powder led to a substantial enhancement in 1000-seed weight, reaching 63.5 grams, while untreated seeds had lower weight of 53.5 grams.	Guha *et al.,* 2011

2.2 Storage studies

2.2.1 Germination

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	Seeds treated with nano ZnO @ 400 mg kg^{-1} of seed retained the highest germination per cent of 64.33 per cent after three months of storage, which is approximately five per cent higher than the control (59.67%).	Adarsh *et al.*, 2023
	After 3 months of storage, seeds subjected to by mid storage invigoration with nano ZnO @ 400 mg per kilogram of seed exhibited the highest germination per cent of 64.4 per cent, surpassing the control (52.67%).	Adarsh *et al.*, 2023
	After seven months of storage, seeds treated with nano ZnO @ 250 mg kg^{-1} of seed exhibited the highest germination rate at 65.66%, exceeding the germination rate of the control (58%).	Mathew *et al.*, 2021
	Seeds that had undergone treatment with nano-TiO_2 at concentrations of 500, 900, 1300, and 1500 mg kg^{-1} maintained a germination rate of 60 per cent after ten months of storage.	Gayathri, 2019
	Dry dressing seeds with nano and bulk ZnO and TiO_2 at rates of 750, 1000, and 1250 mg kg^{-1}revealed varying effects. The highest germination, at 75.0 per cent, was observed in seeds treated with nano ZnO @ 1000 mg kg^{-1},	Kumar *et al.*, 2019

	while the lowest germination (66%) was recorded in TiO2 at 750 mg kg^{-1}.	
Chilli	Seeds treated with 0.75g of ZnO NPs exhibited a notably higher germination per cent of 65.7 per cent, while untreated seeds exhibited a germination rate of 62.0 per cent.	Afrayeem and Chaurasia, 2017
Other crops		
Tomato	Eight-month-old tomato seeds (*cv*. Co3) were subjected to by mid storage hydration-dehydration treatments with water and various antioxidants. Among these treatments, the use of disodium phosphate (10 mM) resulted in a higher germination rate for tomatoes at 52%, surpassing the control achieved (34%).	Rani *et al.,* 1996
Brinjal	Eight-month-old seeds of brinjal (*cv*. Annamalai) underwent by mid storage hydration-dehydration treatment with water and various antioxidants. Notably, the treatment involving disodium phosphate (10 M) exhibited superior germination in brinjal, reaching 58 per cent, as compared to the control, which showed a lower germination rate of 48%.	Rani *et al.,* 1996
Maize	The pre-sowing seed treatment with ZnO nanoparticles (20-30 nm) at a concentration of 15 mg L^{-1} resulted in a significantly higher germination per cent of 96.0 per cent compared to other treatments.	Mahesh *et al*., 2022
Onion	Seeds treated with TiO_2 nanoparticles at a concentration of 40 ppm exhibited a higher	Khan *et al*., 2022

	germination per cent (58.5%) compared to control (50.75%) after three months of storage.	
Cotton	The control treatment of delinted seeds of *cv.* H 1300 showed the maximum reduction in germination per cent at 20.09 per cent, while the minimum reduction of 14.29 per cent was observed in delinted seeds of H 1098-I when seeds were nano primed with ZnO NPs at a concentration of 400 ppm.	Singh *et al.*, 2022
Flax	Among the different concentrations of ZnO NPs used for seed priming (50, 100, 150 ppm), the highest germination per cent of 87.0 per cent was observed with 500 ppm ZnO NPs, while the germination per cent 80% with slightly lower at 150 ppm ZnO NPs.	Bayat *et al.*, 2022
Okra	ZnO nanoparticles at a concentration of 25 mg L^{-1} were observed to significantly improve germination (89%), surpassing the performance of hydropriming, which achieved a germination rate of 63.0 per cent, across a range of ZnO NPs concentrations from 5 to 200 mg L^{-1}.	Keerthana *et al.*, 2021
Sunflower	Seeds treated with ZnO NPs at a concentration of 1000 mg kg^{-1} recorded a higher germination per cent of 85 per cent at the end of six months of storage, which was 5 per cent higher than the untreated seeds 80 per cent	Meenakshi *et al.*, 2020
Rice	Variety CN^{-1} 794-2 was subjected to six different nano TiO_2 dosages, namely 0, 10, 20, 50, 80, and 100 ppm. Among these treatments,	Debnath *et al.*, 2020

	the 20 ppm and 50 ppm doses exhibited notably higher germination rates of 98 per cent.	
Soyabean	The seed treatment with FeNPs at a concentration of 500 ppm resulted in significantly higher seed germination of 92.5 per cent compared to the control, which had a germination rate of 59.50 per cent after 10 months of storage.	Sandeep *et al*., 2019
Maize	Aged seeds were treated with TiO_2 nanoparticles at concentrations of 200, 400, 600, and 800 mg kg^{-1}. Notably, the lowest concentration of Nano-TiO_2 (200 mg kg^{-1}) significantly higher germination per cent (88%), compared to the control (70%).	Vijayalakshmi *et al*., 2018
Wheat	Treating seeds with 50 ppm ZnO nanoparticles resulted in a higher germination per cent of 98.6 per cent, whereas soaking seeds in a higher concentration of 300 ppm ZnO resulted in a slightly lower germination per cent of 97.3 per cent.	Rawat *et al*., 2018
Onion	Six-month-old seeds were subjected to treatments with nano ZnO, Ag, CuO, and TiO_2 at various concentrations ranging from 750 to 1500 mg kg^{-1}. Highest germination per cent of 72 per cent when recorded in seeds treated with 1000 mg kg^{-1} of ZnO NPs. This was notably higher compared to the control (60%).	Anandaraj, and Natarajan 2017
Maize	Among various nano zinc oxide concentrations (800 ppm, 1000 ppm, 1200 ppm, 1400 ppm, and	Meena *et al.,* 2017

	1600 ppm), 100% germination was observed with 1000 ppm and 1200 ppm, while the lowest germination rate was observed in the 1600 ppm treatment (40%).	
Soyabean	The by mid storage seed invigoration with five months old seeds, using various salts and botanicals, including control, water, NaCl (10-3 M), KI (10-3 M), Na_2HPO4 (10-3 M), iodine (0.1%), $CaCl_2$·2H2O (1%), pongamia leaf extract (1%), and custard apple leaf extract (1%). However, it was observed that among these treatments, the use of iodine (0.1%) for by mid storage seed invigoration yielded significantly better results, with a germination rate of 86.75 per cent, compared to the control (82.13%) after 4 months of storage	Patil *et al.*, 2017
Mungbean	Seeds treated with nano ZnO @ concentrations of 20, 40, 60 and 100mg exhibited enhanced germination. The highest germination rate was observed in seeds treated with 20 mg (100.0%), followed by 40mg (95.0%).	Jayarambabu *et al.*, 2014
Onion	Seeds treated with a concentration of 10µgml^{-1} of ZnO NPs reported the highest seed germination (96.52%), while the untreated seeds exhibited a slightly lower seed germination rate of 94.28%.	Raskar and Laware, 2014
Ground nut	Seeds were treated with ZnO nanoparticles (1000 mg kg^{-1}), recorded a germination of 75 per cent which is 20 per cent higher than control.	Shyla and Natarajan 2014

	Seeds treated with nano ZnO and bulk $ZnSO_4$ at a concentration of 1000 ppm exhibited notable increases in germination rate, measuring 99% and 90.01%, respectively, compared to the control (85.03%).	Prasad *et al.*, 2012
Okra	The by mid storage seed invigoration treatment of medium-vigour (5-month-old) okra by soaking-drying, recorded 52.5% germination, while control recorded 42.7 %.	Guha *et al.*, 2012
Cluster bean Cluster bean	After a 4-month storage period, the by mid storage seed invigoration involving various chemicals and botanicals demonstrated their superior performance compared to untreated seeds. In particular, the treatment using KH_2PO4 (10^{-3}M) exhibited a significantly higher germination rate of 82 per cent, while the control recorded lesser value of 65 per cent.	Renugadevi *et al.*, 2006 Renugadevi *et al.*, 2006
Cluster bean	The by mid storage seed invigoration treatment applied to aged seeds involved various methods, among which the soaking and drying method exhibited a notably significant impact on seed quality. This method increased the germination per cent to 80 per cent, which was 10 per cent higher than the control.	Renugadevi *et al.*, 2006
Mustard	Seed treatment in by mid storage involved osmo conditioning with a PEG solution of 0.75 MPa and hydropriming. The results indicate that germination per cent significantly increased through osmo priming. When seeds were tested immediately after priming, the germination per	Kalyani *et al.*, 1998

	cent was 91 per cent, and after10 months of storage, it remained relatively high at 85 per cent.	
Safflower	Various by mid storage hydration-dehydration treatments, including soaking, moisture equilibration, and moist-sand conditioning, were applied to 9-month-old seeds. After 8 months of storage, the control group recorded a germination rate of 50 per cent, while soaking exhibited the highest germination per cent at 71 per cent per cent, followed by moisture equilibration at 58 per cent, and moist sand conditioning at 52 per cent, each followed by the drying process.	Ramamoorthy and Natarajan, 1996
False flax (*Camelina sativa*)	The highest germination per cent (GP) was observed when seeds were treated with varying concentrations of ZnO NPs, ranging from 0.1 to 1000 ppm. Notably, the maximum GP was achieved at 0.1 ppm ZnO NPs, resulting in a significant 15% increase in germination compared to control.	Ramamoorthy and Natarajan, 1996
Cotton	10-month-old seeds were subjected to a midterm storage treatment with a 10^{-3} M solution of disodium phosphate for a duration of 2 hours. This treatment resulted in the highest germination per cent of 62 per cent as compared to control (40%) after 8 months of storage.	Ponnuswamy and Karivaratharaju, 1996
	After 15 months of storage under ambient conditions, by mid storage and pre-sowing treatments were performed on lettuce seeds.	

Lettuce	Among these by mid storage treatments, moisture equilibration-drying (81.1%) and dipping-drying (74.7%) exhibited significantly higher germination per cent compared to the control, which had a germination rate of 27.5 per cent.	Pan and Basu, 1984
Carrot	After 19 months of storage under ambient conditions, by mid storage and pre-sowing treatments were applied to carrot seeds. Among these by mid storage treatments, both moisture equilibration-drying and dipping-drying (77.8% and 77.9%) respectively demonstrated significantly higher germination per cent (77.8% and 77.9%) compared to the control, (23.9%).	Pan and Basu, 1984

2.2.2 Shoot length

Crop	**Details of the experiment**	**Reference**
Solanaceae		
Chilli	Seeds treated with nano ZnO @ 400 mg kg^{-1} of seed and stored for three months retained the highest shoot length, measuring 4.54 cm. In contrast, the control recorded a shoot length of 3.19 cm.	Adarsh, 2023
	The by mid storage seed invigoration using nano ZnO @ 400 mg kg^{-1} resulted in the highest recorded shoot length of 4.54 cm, surpassing the control (3.19 cm) after a 3-month storage period.	Adarsh, 2023

	At the end of the seven-month storage period, seeds treated with nano ZnO @ 1000 mg kg^{-1} exhibited a higher shoot length of 5.57 cm, surpassing the control recorded shoot length of 4.44 cm.	Mathew *et al*., 2021
	The treatment with a dosage of 1000 mg kg^{-1} resulted in the maximum shoot length observed at 4.3 cm, surpassing the control value of 3.9 cm.	Kumar *et al*., 2020
	Lower concentrations of ZnO NPs (0.25 and 0.50g) showed a significant decrease in shoot length, however, higher concentrations (0.75g) of ZnO NPs showed increased shoot length.	Afrayeem and Chaurasia, 2017
Other crops		
Onion	Seeds treated with TiO_2 nanoparticles at a concentration of 40 ppm exhibited greater shoot length (4.58 cm) compared to the control (4.06 cm) after three months of storage.	Khan *et al*., 2022
Flax	Seed priming at different concentrations of ZnO NPs for seed priming (50, 100, 150 ppm), it was observed that the maximum shoot length of 5.9 cm occurred with 50 ppm ZnO NPs.	Bayat *et al*., 2022
Maize	Presowing seed treatment with ZnO nanoparticles (20-30 nm) at a concentration of 15 mg L^{-1} resulted in a significantly higher shoot length of 22.45cm compared to all other treatments	Mahesh *et al*., 2022
Okra	Seeds treated with ZnO nanoparticles at concentrations ranging from 5 to 200 mg L^{-1} exhibited varying effects on shoot length. Notably, the treatment with 25 mg L^{-1} of ZnO NPs resulted	Keerthana *et al*., 2021

	in a significant increase in shoot length, measuring 18 cm, compared to hydropriming, which yielded a shoot length of 11 cm.	
Sunflower	Seeds treated with Zinc oxide nanoparticles (ZnO NPs) at a concentration of 1000 mg kg^{-1} recorded a shoot length of 14 cm at the end of six months of storage, which was higher than the untreated seeds (13.3 cm).	Meenakshi *et al.*, 2020
Soybean	Ten-month-old seeds treated with 500ppm Fe NPs recorded a shoot length of 14.64 cm. Control restored at 11.06cm..	Sandeep *et al.*, 2019
Maize	Dry dressing aged seeds with TiO_2 nanoparticles at concentrations of 200, 400, 600, and 800 mg kg^{-1} resulted in a significant increase in shoot length. The most substantial improvement was observed at the concentration of 200 mg kg^{-1} Nano TiO_2, where the shoot length reached 11.91 cm, compared to the control (10.78 cm).	Vijayalakshmi *et al.*, 2018
Rice	Seed priming with 1000 ppm nano ZnO resulted in a 34.88% increase in shoot length when compared with control.	Rameshreddy *et al.*, 2017
Maize	Among the different nano zinc oxide concentrations (800 ppm to 1600 ppm), the treatment with 1200 ppm recorded the highest shoot length of 3.9 cm, whereas the lowest shoot length of only 2.7 cm was observed in the 1600 ppm treatment.	Meena *et al.*, 2017
	Seeds were wet dressed with normal and nano ZnO @ 500 ppm, 1000 ppm and 2000 ppm for 2 and 4	

	hours each. Seed treatment with nano grades of ZnO @ 1000 ppm recorded a higher shoot length of 1.94 cm.	Tiwari, 2017
Onion	The application of nanoparticles, specifically ZnO, Ag, CuO, and TiO_2, at varying concentrations between 750 and 1500 mg kg^{-1}, resulted in a significant enhancement of shoot length in six-month-old seeds. Among these, seeds treated with ZnO and Ag NPs at 1000 mg kg^{-1} displayed the longest shoot length (7.5 cm), compared to the control (6.0 cm).	Anandaraj and Natarajan, 2017
Onion	Seeds treated with 10µgml^{-1} of ZnO NPs exhibited the highest shoot length at 7.76 cm, whereas seeds treated with 40µgml^{-1} of ZnO NPs displayed a shorter shoot length of 5.52cm.	Raskar and Laware, 2014
Groundnut	Seeds were treated with ZnO nanoparticles at a dosage of 1000 mg kg^{-1}, recorded a shoot length of 20.97 cm, which was higher than the control (16.92 cm).	Shyla and Natarajan, 2014
Groundnut	Seeds treated with nano ZnO and bulk $ZnSO_4$ at a concentration of 1000 ppm exhibited notable increase in shoot length of 6.71 cm and 4.32 cm, respectively, compared to the control (3.11 cm).	Prasad *et al.*, 2012
Okra	The by mid storage seed invigoration treatment of medium-vigour (5-month-old) okra seeds using powdered pharmaceutical formulations resulted in greater seedling length following by mid storage soaking and drying.	Guha *et al.*, 2012

Cluster bean	The by mid storage seed invigoration treatments, which involved various chemicals and botanicals, consistently demonstrated their superiority over untreated seeds both initially and after four months of storage. Among these treatments, the application of KH_2PO_4 (10^{-3}M) resulted in a significantly increased shoot length at four months after storage measuring 11.8cm, while the control recorded a value of 8.2cm.	Renugadevi *et al*., 2006
	In the by mid storage seed invigoration treatment for aged seeds, various methods were employed. Among these, the soaking and drying method demonstrated a more pronounced effect on seed quality with increased shoot length of 14.3 cm, compared to control which had a shoot length of 11.9 cm.	Renugadevi *et al*., 2006
Mustard	Mid storage seed treatment included osmo conditioning with a 0.75 MPa PEG solution and hydropriming. Specifically, equilibration in moist muslin for 36 hours during hydropriming showed positive effects on shoot length. Immediately after priming, the shoot length measured 6.91 cm, and even after natural aging, it remained relatively high at 6.05 cm, when compared to the control.	Kalyani *et al.***,** 1998

2.2.3 Root length

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	After three months of storage, seeds treated with nano ZnO @ 400 mg kg^{-1} of seed retained the highest root length of 9.24 cm, in contrast to the control (6.94 cm).	Adarsh, 2023
	When seeds were subjected to by mid storage seed invigoration using nano ZnO @ 400 mg kg^{-1}, while exhibited the longest root length, measuring 8.79 cm. In contrast, the control group had a root length of 6.94 cm after 3 months of storage.	
	Seeds treated with nano ZnO @ 50 mg kg^{-1} exhibited longer roots (9.37 cm) after 7 months of storage compared to control, (6.82 cm).	Mathew *et al.*, 2021
	The seeds treated with zinc oxide nanoparticles at a concentration of 1000 mg kg^{-1} exhibited the highest recorded root length of 11.90 cm, surpassing the control with value of 9.19 cm.	Kumar *et al.*, 2019
Other crops		
Maize	Presowing seed treatment with ZnO nanoparticles (20-30 nm) at a concentration of 15 mg L^{-1} resulted in a significantly higher root length of 19.02 cm compared to all other treatments	Mahesh *et al.*, 2022
Rapeseed	Seeds were subjected to varying concentrations of ZnO-NPs ranging from 0.1 to 1000 ppm. The highest root length was recorded at a concentration	Sarkhosh *et al.*, 2022

	of 5 mg L^{-1} of ZnO-NPs, exhibiting a notable 32 per cent increase compared to control.	
False flax	Seeds were subjected to treatment with varying concentrations of ZnO-NPs, ranging from 0.1 to 1000 ppm. The treatment 1 mg/L ZnO-NPs resulted in a 15 per cent increase in root length compared to the control.	Sarkhosh *et al*., 2022
Flax	Seed priming with different concentrations of ZnO NPs (50, 100, 150 ppm), it was observed that the maximum root length of 7.1 cm was recorded in seeds treated with 50 ppm ZnO NPs.	Bayat *et al*., 2022
Okra	Seeds treated with ZnO nanoparticles at concentrations ranging from 5 to 200 mg L^{-1} exhibited varying effects on root length. Notably, the treatment with 25 mg/L of ZnO NPs resulted in a significant increase in root length, measuring 7.8 cm, compared to hydropriming (4.26 cm).	Keerthana *et al*., 2021
Sunflower	Seeds treated with zinc oxide nanoparticles (ZnO NPs) at a concentration of 1000 mg kg^{-1} recorded a root length of 25.9 cm at the end of six months of storage, which was higher than the untreated seeds (23.8 cm).	Meenakshi *et al*., 2020
Soyabean	Seed treatment with FeNPs at a concentration of 500 ppm significantly increased root length to 17.85 cm, surpassing the control with a root length of 15.39 cm after 10 months of storage.	Sandeep *et al*., 2019
	Dry dressing aged seeds with TiO_2 nanoparticles at concentrations of 200, 400, 600, and 800 mg kg^{-1} led to a significant increase in root length. The most	Vijayalakshmi *et al*., 2018

Maize	notable enhancement was observed at the 200 mg kg^{-1} Nano-TiO_2 concentration, where the root length reached 19.45 cm, surpassing the control (18.78 cm).	
Sunflower	Treating the seeds with 50 ppm ZnO nanoparticles resulted in increased root length of 8.4 cm, whereas soaking seeds at a higher concentration of 300 ppm ZnO led to a slightly lower root length of 7.7 cm.	Rawat *et al.*, 2018
Onion	six-month-old seeds were subjected to treatments with nano ZnO, Ag, CuO and TiO_2 at various concentrations ranging from 750 to 1500 mg kg^{-1}. Seeds treated with ZnO and Ag NPs @ 1000 mg kg^{-1} produced the lengthiest root length (6.3 cm) than control (5.4cm)	Anandaraj, and Natarajan 2017
Maize	Among the different nano zinc oxide concentrations (ranging from 800 ppm to 1600 ppm), the treatment with 1200 ppm nano zinc oxide resulted in the highest root length of 6.5 cm, while the lowest root length was observed in the 1600 ppm treatment, measuring only 3.55 cm.	Meena *et al.*, 2017
Rice	Seed priming with 1000 ppm nano ZnO resulted in a 22.34 per cent increase in root length compared to control.	Rameshreddy *et al.*, 2017
Onion	Seeds treated with a concentration of 10$\mu g ml^{-1}$ of ZnO NPs reported a root length of 4.38cm, while the seeds treated with 40 $\mu g ml^{-1}$ of ZnO NPs showed a reduced root length of 3.25 cm.	Raskar and Laware, 2014

Groundnut	Seeds treated with ZnO nanoparticles at a dosage of 1000 mg kg^{-1} exhibited a root length of 17.98 cm, surpassing the root length of the control (15.21 cm).	Shyla and Natarajan, 2014
Groundnut	Seeds treated with nano ZnO and bulk ZnSO4 at a concentration of 1000 ppm exhibited notable increase in root length, measuring 11.81 cm and 6.72 cm, respectively, compared to control (5.02 cm).	Prasad *et al.*, 2012
Cluster bean	In the by mid storage seed invigoration treatment of aged seeds, the soaking and drying method demonstrated a more substantial impact on seed quality by increasing root length to 10.8 cm, compared to the control (11.3 cm).	Renugadevi *et al.*, 2006
Mustard	Osmo conditioning with a 0.75 MPa PEG solution and hydropriming were given as by mid storage seed treatments. The root length immediately after osmopriming was 6.24 cm, and after 10 months of storage, it remained relatively high at 4.69 cm,	Kalyani *et al.*, 1998

2.2.4 Seedling Dry weight

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	After three months of storage, the highest dry weight was observed in seeds treated with nano ZnO @ 400 mg kg^{-1} of seed (24.96 mg), while the control recorded a value of21.94 mg.	Adarsh, 2023
	Mid storage seed invigoration with nano ZnO @ 400 mg kg^{-1} resulted in the highest dry weight	Adarsh, 2023

	(24.96 mg), when compared with control (21.94 mg) after a 3-month storage period.	
Chilli	Maximum dry matter production was observed in seeds treated with nano ZnO 1000 mg kg^{-1} (22.63 mg) after seven months of storage, this significantly exceeded the dry matter production of the control, which was 20.35 mg.	Mathew *et al*., 2021
	Seeds dressed with nano ZnO @ concentrations of 100 ppm exhibited a higher seedling dry weight of 44.13 g compared to 500 ppm (41.84 g).	Lopez *et al*., 2018
Brinjal	Eight-month-old seeds of brinjal (*cv*. Annamalai) underwent by mid storage hydration-dehydration treatment with water and various antioxidants. Among these treatments, the use of disodium phosphate (10 M) resulted in a higher seedling dry weight in brinjal, measuring 1.4 mg, compared to control which exhibited a seedling dry weight of 1.0 mg.	Rani *et al.,* 1996
Tomato	Eight-month-old seeds of tomato (*cv*. Co 3) were subjected to by mid storage hydration-dehydration treatments using water and various antioxidants. Among these treatments, disodium phosphate (10 M) resulted in a higher seedling dry weight in tomatoes, measuring 1.6 mg, as compared to the control (1.2 mg).	
Other crops		
Wheat	Seedlings exposed to 2500 ppm of ZnO NPs exhibited a significant increase in dry weight, with	Alsuwayyid *et al*., 2022

	a 17.0 per cent higher dry weight compared to untreated control.	
Onion	Seeds treated with TiO_2 nanoparticles at a concentration of 40 ppm exhibited a significantly higher dry weight of 0.259g compared to the control after three months of storage.	Khan *et al.*, 2022
Cotton	Nano prime treatment with ZnO NPs at 400 ppm followed by TiO_2NPs at 100 ppm. The control treatment of fuzzy seeds (H 1300) exhibited the highest reduction in seedling dry weight at 28.34per cent, whereas the minimum reduction of 19.20 per cent was observed in delinted seeds of H 1098-i.	Singh *et al.*, 2022
Maize	The presowing seed treatment with ZnO nanoparticles (20-30 nm) at a concentration of 15 mg/L resulted in a significantly higher seedling dry weight of 2.75 g compared to other treatments.	Mahesh *et al.*, 2022
Okra	Seeds treated with ZnO nanoparticles at concentrations ranging from 5 to 200 mg/L exhibited varying effects on root length. Notably, the treatment with 25 mg/L of ZnO NPs resulted in a significant increase in seedling dry weight, measuring 2.8g, compared to hydropriming (7.8g).	Keerthana *et al.,* 2021
Sunflower	Seeds treated with Zinc oxide nanoparticles (ZnO NPs) at a concentration of 1000 mg kg^{-1} recorded a dry matter production of 0.300g at the end of six months of storage, which was higher than the untreated seeds (0.261g).	Meenakshi *et al.*, 2020

Soyabean	Seed treatment with Fe NPs at a concentration of 500 ppm resulted in a significantly higher seedling dry weight at 1.127 g, in contrast to the control, which exhibited a dry weight of 11.06 g after 10 months of storage.	Sandeep *et al.*, 2019
Maize	Dry dressing aged seeds with TiO_2 nanoparticles at concentrations of 200, 400, 600, and 800 mg kg^{-1} resulted in a significant increase in dry weight. The most notable improvement was observed at the concentration of 200 mg/kg Nano-TiO_2, where the dry weight reached 1.340g, in contrast to the control, which had a dry weight of 1.009 g.	Vijayalakshmi *et al.*, 2018
Wheat	Treating seeds with 50 ppm ZnO nanoparticles resulted in an increased seedling dry weight of 11.5g, whereas soaking seeds at a higher concentration of 300 ppm ZnO led to a slightly lower seedling dry weight 10.6 g.	Rawat *et al.*, 2018
Soyabean	The by mid storage seed invigoration with five months old seeds, using various salts and botanicals, including control, water, NaCl (10-3 M), KI (10-3 M), Na_2HPO4 (10-3 M), iodine (0.1%), $CaCl_2 \cdot 2H2O$ (1%), pongamia leaf extract (1%), and custard apple leaf extract (1%). However, it was observed that among these treatments, the use of iodine (0.1%) for by mid storage seed invigoration yielded significantly better results, with a germination rate of 86.75 per cent, compared to the control, which had a germination rate of 82.13per cent. after 4 months of storage	Patil *et al.*, 2017

Chickpea	Chickpea seeds were treated with 100, 500, and 1000 ppm nano ZnO. Concentration 1000 ppm resulted in greater seedling dry weight among the treated seeds	Hajra and Mondal, 2017
Onion	Seeds treated with a concentration of 10µgml^{-1} of ZnO NPs exhibited the highest dry weight, measuring 70.44mg. In contrast, seeds treated with 40µgml^{-1} of ZnO NPs displayed a lower dry weight of 52.42mg.	Raskar and Laware, 2014
Wheat	The study found that, in comparison to seed soaking at 300 ppm concentration, seed treatment with nanoparticles at 50 ppm concentration increase the seedling dry weight.	Rafique *et al.*, 2014
Cluster bean	Mid storage seed invigoration with KH_2PO4 (10^{-3} M) exhibited the highest dry weight (158 mg), in contrast to the control which showed a dry weight of 118 mg after the four-month storage duration.	Renugadevi *et al.*, 2006
Cluster bean	During the by mid storage seed invigoration treatment of aged seeds, various methods were employed. Among these methods, the soaking and drying approach exhibited a notably more significant effect on seed quality, resulting in an increase in dry weight of162mg, as compared to the control (137 mg).	Renugadevi *et al.*, 2006
Cotton	A by mid storage treatment involving soaking 10-month-old seeds in a 10^{-3} M disodium phosphate solution for 2 hours resulted in the highest seedling dry weight (52 mg). In comparison, the control	Ponnuswamy and Karivaratharaju, 1996

	exhibited a lower seedling dry weight of 45 mg after 8 months of storage.	

2.2.5 Vigour index

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	After three months of storage, seeds treated with nano ZnO @ 400 mg kg^{-1} exhibited the highest vigour index I (836) and vigour index II (1606), surpassing the control with values of vigour index I (533) and vigour index II (1156), respectively.	Adarsh, 2023
	by mid storage seed invigoration with the application of nano ZnO @ 400 mg kg^{-1} per seed exhibited the highest seed vigour indices I (836) and II (1606), as compared to the control, which recorded values of 533 and 1156, respectively, after a 3-month storage period.	Adarsh, 2023
	Seeds treated with nano ZnO @ 1000 mg kg^{-1} retained superiority in maintaining, vigour index I (925.88) and a vigour index II (1472), at seven months after storage both of these treatments of 652 and 1181 for vigour index I and II respectively.	Mathew *et al.*, 2021
	The seed treated with nano ZnO @ 1000 mg kg^{-1} exhibited the highest seed vigour index, with a value of 1285, whereas the control recorded the least value (861).	Kumar *et al.*, 2020

	The performance of seeds treated with bulk ZnO at 1300 mg kg^{-1} and ZnO nanoparticles at 500 mg kg^{-1} in maintaining seed vigour for the full ten months of storage.	Gayathri, 2019
Other crops		
Maize	Pre-sowing seed treatment with ZnO nanoparticles (20-30 nm) at a concentration of 15 mg L^{-1} resulted in the significantly highest values for Vigour Index I and II, with values of 3981 and 264.10, respectively, surpassing all other treatments.	Mahesh *et al*., 2022
Rape seed	Comparing the control condition to various concentrations of ZnO-NPs ranging from 0.1 to 1000 ppm, the higher seedling vigour index was observed at concentrations within the range of 0.1 to 10 mg L^{-1} ZnO-NPs.	Sarkhosh *et al.,* 2022
Cotton	When seeds were nano treated 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
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
Sunflower	Seeds treated with zinc oxide nanoparticles (ZnO NPs) at a concentration of 1000 mg kg^{-1} recorded a higher vigour index of 2544 at the end of six	Meenakshi *et al*., 2020

	months of storage, which was higher than the untreated seeds (2362).	
Soyabean	The seed treatment with Fe NPs at a concentration of 500 ppm resulted in a significantly higher seed vigour index 2890, in contrast to the control (1433.03) after 10 months of storage.	Sandeep *et al.*, 2019
Wheat	Treating seeds with 50 ppm ZnO nanoparticles resulted in an increased seed vigour index I and vigour index II (1159 and 1141). Water soaking seeds at a higher concentration of 300 ppm ZnO led to a slightly lower seed vigour index I and vigour index II (1090 and 1031).	Rawat *et al.*, 2018
Soyabean	Mid storage seed invigoration of five months old seeds was carried out using various salts and botanicals, including control, water, NaCl (10-3M), KI (10-3 M), Na_2HPO_4 (10-3 M), Iodine (0.1%), $CaCl_2.2H_20$ (1%), Pongamia leaf extract (1%), and custard apple leaf extract (1%). Notably, among the treatments, the by mid storage invigoration with iodine (0.1%) produced a significantly higher seed vigour index of 2768 compared to the control, which yielded a seed vigour index of 1924 after 4months of storage.	Patil *et al.*, 2017
Onion	Seeds that were treated with nano ZnO, Ag, CuO, and TiO2, at different concentrations ranging from 750 to 1500 mg kg^{-1}, significantly improved the seed vigour index in 6 months old seeds, which revealed that the highest vigour Index was	Anandaraj, and Natarajan 2017

	observed in seeds treated with ZnO NPs @ 1000 mg kg^{-1} (998) than control (692).	
Maize	Seed treatment with different nano zinc oxide concentrations (ranging from 800 ppm to 1600 ppm) showed that 1200 ppm nano zinc oxide recorded the highest seed vigour index of 1040, while the lowest seed vigour index was observed in the 1600 ppm treatment, which was only 250.	Meena *et al.,* 2017
Rice	Seed priming with 1000 ppm nano ZnO resulted in a 45.54 per cent increase in Seed Vigour Index I compared to the control.	Rameshreddy *et al*., 2017
Groundnut	Seeds treated with ZnO nanoparticles at a dosage of 1000 mg kg^{-1}, recorded significantly high vigour index (2949), which was higher than control (1759).	Shyla and Natarajan, 2014
	Seeds treated with nano ZnO and bulk $ZnSO_4$ at a concentration of 1000 ppm exhibited notable increase in seed vigour index, measuring 1701 and 910 respectively, compared to the control (693).	Prasad *et al*., 2012
Cluster bean	Mid storage seed invigoration with the application of KH_2PO_4 (10^{-3}M) exhibited the highest seed vigour index (1786), as compared to the control, which recorded value of 978, after a 4-month storage period.	Renugadevi *et al*., 2006
	The by mid storage seed invigoration treatment for aged seeds, various methods were utilized. Notably, the soaking and drying method demonstrated a significantly more profound impact	Renugadevi *et al*., 2006

	on seed quality by increasing the seed vigour index of 2168, while control had a vigour index of 1624.	
Cotton	A by mid storage treatment involving a 2-hour soaking of 10-month-old seeds in a 10-3 M disodium phosphate solution maintained the highest seed vigour index of 3224, in contrast to the control, which exhibited a vigour index of 1800, after 8 months of storage.	Ponnuswamy and Karivaratharaju, 1996

2.2.6 Electrical conductivity of seed leachate

Crop	Details of the experiment	Reference
Solanaceae		
Chilli	After three months of storage, seeds treated with nano ZnO @ 500 mg kg^{-1} exhibited the lowest electrical conductivity, measuring 68.2 µS cm^{-1}, in contrast to the control which recorded a higher electrical conductivity of 159.20 µS cm^{-1}.	Adarsh, 2023
	After a 3-month storage period, seeds subjected to by mid storage invigoration with nano ZnO @ 500 mg kg^{-1} exhibited the lowest electrical conductivity of 68.2 μScm^{-1}. In comparison, the control showed a higher electrical conductivity of 159.2 µS cm^{-1}.	Adarsh, 2023
	Seeds treated with nano ZnO @ 250 mg kg^{-1} showed the lowest electrical conductivity of seed leachates, measuring 295 µS cm^{-1} seven months	Mathew *et al.*, 2021

	after storage which was significantly lower than control (404.66 µS cm^{-1}).	
	Electrical conductivity was considerably impacted by the nanoparticle seed treatments. When compared to the control (0.149 dS m^{-1}), nano ZnO @ 1000 mg kg^{-1} had the lowest EC (0.118 dS m^{-1}).	Kumar *et al*., 2020
Tomato	The by mid storage hydration-dehydration treatment was applied to eight-month-old tomato seeds (*cv*. Co 3) using water and various antioxidants. Among these treatments, disodium phosphate (10 mM) showed a notable reduction in electrical conductivity in tomatoes, measuring 0.060 dS/m, compared to the control, which registered a higher electrical conductivity of 0.080 dS/m.	Rani *et al.,* 1996
Brinjal	The by mid storage hydration-dehydration treatment with water and various antioxidants was applied to eight-month-old brinjal seeds (*cv*. Annamalai). Among the treatments, the use of disodium phosphate (10 mM) resulted in lower electrical conductivity in the brinjal seeds, measuring 0.055 dS m^{-1}. In comparison, the control exhibited higher electrical conductivity at 0.0680 dS m^{-1}.	Rani *et al.,* 1996
Other crops		
Onion	Seeds treated with TiO_2 nanoparticles at a concentration of 40 ppm exhibited the lowest electrical conductivity (0.951 dS m^{-1}) compared to the control after 3 months of storage	Khan *et al*., 2023

Cotton	The maximum increase in electrical conductivity (56.92%) was observed in the control treatment of fuzzy seeds of H 1300, while the minimum increase (34.43%) was found in delinted seeds of H 1300, when the seeds were nano primed with ZnO NPs at a concentration of 400 ppm.	Singh *et al.*, 2022
Maize	The pre-sowing treatment of seeds with ZnO nanoparticles (20-30 nm) at a concentration of 15 mg L^{-1} resulted in significantly lower electrical conductivity (0.165 dS m^{-1}), whereas the maximum conductivity was recorded in control (0.246 dS m^{-1}).	Mahesh *et al.*, 2022
Sunflower	Seeds treated with Zinc oxide nanoparticles (ZnO NPs) at a concentration of 1000 mg kg^{-1} recorded a low electrical conductivity of seed leachate (1.003 dS m^{-1}) at the end of six months of storage, which was lower than the untreated seeds that recorded an electrical conductivity of 1.175 dS m^{-1}.	Meenakshi *et al.*, 2020
Soybean	The seed treatment with Fe NPs at a concentration of 500 ppm control resulted in significantly lower electrical conductivity (0.43 dS m^{-1}), whereas the maximum conductivity was recorded in the control (0.59 dSm^{-1}), after 10 months of storage.	Sandeep *et al.*, 2019
Maize	When aged seeds were dry dressed with TiO_2 nanoparticles at concentrations of 200, 400, 600, and 800 mg kg^{-1}, the lowest electrical conductivity was observed at the concentration of 200 mg kg^{-1}, (0.361 dS m^{-1}), while the highest conductivity was recorded in the control (0.278 dS m^{-1}).	Vijayalakshmi *et al.*, 2018

Soybean	The mid storage seed invigoration of five months old seeds with various salts and botanicals, including control, water, NaCl (10-3M), KI (10-3 M), Na_2HPO_4 (10-3 M), Iodine (0.1%), $CaCl_2.2H_20$ (1%), pongamia leaf extract (1%), and custard apple leaf extract (1%), it was observed that the treatment involving mid storage invigoration with iodine (0.1%) resulted in a significantly lower electrical conductivity (0.473 dS m^{-1}) compared to the control (0.542 dS m^{-1}) after 4 months of storage.	Patil *et al.*, 2017
Cluster bean	Mid storage seed invigoration were applied to aged seeds. Among the treatments soaking and drying method had a significantly more positive impact on seed quality by reducing electrical conductivity to 0.240 dS m^{-1}, when compared to control (0.280 dS m^{-1}).	Renugadevi *et al.*, 2006
	Mid storage seed invigoration with chemicals and botanicals significantly outperformed over the untreated seeds. After a four-months storage period, the electrical conductivity of the seed leachate was notably lower in seeds treated with KH_2PO_4 (10^{-3} M), measuring 0.252 dS m^{-1}, in contrast to control, registered a higher electrical conductivity (0.280 dS m^{-1}).	Renugadevi *et al.*, 2006
Mustard	A comparison of the by mid storage seed treatments between osmo conditioning (0.75 MPa PEG) and hydropriming revealed that the electrical conductivity of the leachate was substantially reduced in osmo priming treatment, immediately after treatment (42.0 dS m^{-1}) and continued with	Srinivasan *et al.,* 1998

	lower values even after 10 months of storage (63.6 dS m^{-1}), when compared to control.	
Safflower	Mid storage hydration-dehydration treatments were applied to 9-month-old seeds, and the results after 8 months of storage revealed that the lowest electrical conductivity of 0.195 dS m^{-1} was achieved through the soaking-drying method, moisture equilibration-drying (12.5 dS m^{-1}), and moist sand conditioning-drying (12.2 dS m^{-1}). This was in contrast to the control, which had an electrical conductivity of 0.328 dS m^{-1}.	Ramamoorthy and Natarajan, 1996

2 . 2 . 7 Seed infection in chilli

Seed is a small embryonic plant which is an efficient means of introducing plant pathogens into a new area as well as providing a means of their survival from one cropping season to another. Seed-borne pathogens can contribute to seed decay during germination and the mortality of seedlings, leading to poor crop establishment and reduced crop growth and productivity (Amza,2018). Microorganism infection may also cause an increase in electrolyte leakage, which is apparently due to the damage of the cell membrane and seed integuments (Neergaard, 1977 and Herman, 1983). These pathogens, whether present externally or internally in the seeds, can cause seed abortion, rot, necrosis, reduced germination capacity, and seedling damage.

The principal storage fungus belongs to the Genera *Aspergillus, Penicillium, Rhizopus, Sclerotium and Fusarium* (Mashilla, 2004).

Zinc oxide nanoparticles (ZnO NPs), due to their antimicrobial properties, have found utility in improving crop nutrition, increasing biomass, and enhancing overall productivity by restraining the proliferation of pests and pathogens (Anderson *et al*., 2017). Another notable benefit of utilizing ZnO NPs is their relatively lower toxicity to plants and the associated soil microorganisms when compared to other types of metal oxide nanoparticles.

Literature pertaining to the seed microflora recorded in various crops is listed below:

Crop	Micro-organisms observed	Reference
Chilli	*Aspergillus*	Adarsh,2023
	Aspergillus flavus, *Aspergillus niger* *Rhizopus* sp.	Herbert, 2020
	Aspergillus flavus *Aspergillus niger*	Mathew, 2020
	Alternaria spp *Aspergillus flavus* *Aspergillus niger* *Mucor spp.*	Gayathri, 2019
Chilli	**Blotter paper method**: *A. niger* (16.18%), *flavus* (15.75%), *Fusarium solani* (10.50%), *Rhizopus* spp. (7.43%), *Colletotrichum capsici* (6.81%) *Penicillium* spp. (5.75%) **Agar plate method**: *niger* (27.54%) *A. flavus* (20.62%) *Fusarium solani* (7.26%) *Penicillium spp.* (3.50%) *Rhizopus spp.* (3.20%) *Colletotrichum capsica* (2.59%)	Chauhan *et al.,* 2018
Tomato And brinjal	*Aspergillus* *Rhizopus* *Fusarium* *Cladosporium*	Patekar, 2017

Materials and methods

3. MATERIALS AND METHODS

The research project titled "Mid storage seed invigoration with nanoparticles in chilli (*Capsicum annuum* L.)" was carried out in the Department of Seed Science and Technology, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur during the year 2022-2023. The study aimed to assess the effect of nanoparticles on crop performance, seed yield, quality and longevity when administered as midterm storage treatment. The details of the materials used and the methods adopted during the course of the investigation are furnished in this chapter.

3.1 Experimental site

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

3.2 Climatic conditions

Vellanikkara, a suburban area in Thrissur, is approximately 22.25 meters above mean sea level, situated at coordinates, 10.5452°N latitude and 76.2740°E longitude. This region typically experiences a hot and humid climate The monthly mean meteorological data recorded during the course of study (July 2019 to August 2020) ranged from 29.6 to 36.8 °C temperature and 49 to 89 % relative humidity are presented in Appendix I.

3.3 Experimental material

The present investigation was conducted using the chilli seeds of variety Ujwala, harvested in March 2022, procured from the Agricultural Research Station, Mannuthy.

3.3.1 Salient features of chilli variety Ujwala

Ujwala is a high-yielding multiple-resistant (mosaic, bacterial wilt and leaf roller attacks) chilli variety released in 1996 through pureline selection from CA 219-1-19-6, by Kerala Agricultural University. Ujwala is characterized by its deep green fruits that transition to a dark brown shade upon ripening. These fruits, usually clustered closely together, measure approximately 6 cm in length. Ideally versatile, chilli variety Ujwala is well-suited for both fresh green chilli consumption and drying purpose. This variety's notable feature is its high pungency, which makes it well-suited for extracting oleoresins and colouring pigments.

3.4 Experimental details

The current study was structured with two experiments, outlined as follows:

Experiment 1: Effect of mid- storage correction with inorganic nanoparticles in chilli

Experiment 2: Seed storage studies

3.4.1 Experiment 1: Effect of mid- storage correction with inorganic nanoparticles in chilli

3.4.1.1 Design and plan of layout

The experiment was laid out in a Randomized Block Design with seven treatments and three replications. The details of the experiment area as follows,

Design	: Randomized block design
Replication	: Three
Treatments	: Seven
Plot size	: 2m × 2m
Spacing	: 45 cm × 45 cm

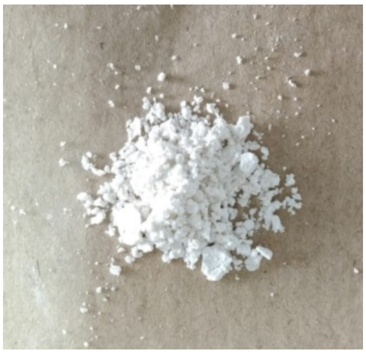
3.4.1.2 Treatment details:

Six-month-old seeds were exposed to wet and dry seed treatments using nanosized zinc oxide (ZnO) and nanosized custard leaf powder, respectively as outlined in Table 1. The control consisted of untreated seeds were kept as control.

3.4.1.3 Wet seed treatment

ZnO nano powder with particle sizes of less than 100nm, obtained from SIGMA-ALORICH, was employed in this study. The nanoparticles were evenly distributed in distilled water through a 15-minute sonication process (Kumar, 2019). Chilli seeds were subsequently soaked in different solutions, as detailed in Table 1, for a duration of three hours. While the control seeds were soaked in distilled water.

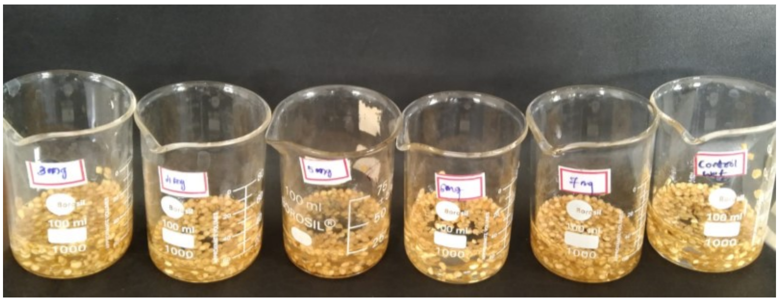
A)



ZnO Nanoparticles

Magnetic stirrer

Solution kept for sonicating for dispersing nanoparticles



Seeds soaked in treatment solution

B)

Nano custard leaf powder

Seeds treated with nanoparticles

Plate 1: Seed treatments with nanoparticles in chilli
A) Wet seed treatment B) Dry seed treatment

After soaking, excess solution was removed, and the treated seeds were dried until their moisture content reached below 8 per cent.

Table 1: Details of the treatments

Treatment	Details
T_1	Control
T_2	300 mg nano ZnO/kg of seed
T_3	400 mg nano ZnO/kg of seed
T_4	500 mg nano ZnO/kg of seed
T_5	600 mg nano ZnO/kg of seed
T_6	700 mg nano ZnO/kg of seed
T_7	500 mg nano Custard leaf powder /kg of seed

3.4.1.4 Dry treatment

The seeds were subjected to shade drying to reduce their moisture content to below eight per cent before administering the seed invigoration treatments. The seeds were dry dressed with the required quantity of nano custard leaf powder as detailed in Table 1. To ensure proper mixing, glass bottles containing both the seeds and nanoparticles were manually shaken gently for 3 minutes, five times over a span of three hours.

The treated seeds were initially raised in a nursery, and subsequently, seedlings aged between 6 and 7 weeks were transplanted to the main field. The experimental crop was raised as per Package of Practices Recommendations of Kerala Agricultural University (KAU,2016).

3.4.2 Experiment 2: **Seed storage studies**

Seeds from the seven treatments in Experiment I were separately dried to reduce their moisture content to less than eight per cent. After drying, they were packed into 700-gauge polyethylene bags, with each bag containing 75 grams of

Field preparation

Transplanting

Irrigation

Vegetative stage

Flowering stage

Fruiting stage

Harvesting

Seed extraction

Collected seeds

Plate 2: Field performance of midstorage corrected chilli

seeds. These bags were then stored under normal ambient conditions for a duration of six months. At monthly intervals, samples were collected to assess the seed quality parameters. The experiment was carried out using a Completely Randomized Design (CRD) with seven treatments and three replications.

3.5 Collection of experimental observations

In each replication of every treatment, five plants were chosen randomly. Various biometric measurements viz; Plant height (cm), 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), seeds per fruit, fruit yield per plant(g), seed yield per plant(g), 100 seed weight (g), incidence of pests and disease were recorded at the appropriate growth stages. These observations were averaged to calculate the data specific to a particular replication of a treatment.

3.5.1. Days to first flowering

The average number of days to first flowering was calculated based on the number of days from the date of sowing and the appearance of the first flower in the tagged plants.

3.5.2 Days to 50 per cent flowering (DFF)

The plants were monitored on a daily basis to track their flowering progress. Total number of days from date of sowing to the date on which 50 per cent of the plants initiated flowers in the tagged plants was recorded and average was calculated.

3.5.3 Plant height

Plant height was measured from the base of the plant to the top of fully opened leaf of the main shoot at 120th day after transplanting. Measurements were taken from the main shoots of five plants in each treatment and the average height of the single plant is calculated and expressed in centimetres.

3.5.4 Number of branches

The number of branches per plant was taken by counting the primary branches at the time of last harvest and the average was recorded.

3.5.5 Fruits per plant

The total number of fruits harvested from labelled plants was counted, and the average number of fruits per plant was calculated

3.5.6 Fruit length (cm)

Ten fruits were selected from each tagged plant in each replication, and their lengths were measured on a meter scale between proximal and distal ends. The average fruit length was noted in centimeters (cm).

3.5.7 Fruit weight at maturity (g)

Ten individual fruits were weighed separately to determine their fresh weight using a weighing balance. The average fruit weight, was expressed in grams (g).

3.5.8 Seeds per fruit

In each replication, seeds from five randomly chosen fruits from the tagged plants were carefully extracted and manually counted. Subsequently, the average number of seeds per fruit was calculated.

3.5.9 100 seed weight (g)

Three random samples of 100 well-filled seeds were selected from the tagged plant in each replication. These samples were individually weighed, and the resulting average seed weight was expressed in grams.

3.5.10 Seed yield per plant (g)

The weight of seeds extracted from the fruits of the tagged plants was promptly measured using a digital weighing balance, and the average weight expressed in grams (g).

3.5.3 Seed quality parameters

Observations

Seed samples from each replication of every treatment were randomly selected for the analysis of seed quality parameters such as seed germination per cent (%), seedling root length (cm), seedling shoot length (cm), seedling dry weight (mg), and electrical conductivity (EC) of seed leachate (dSm^{-1}). These observations were recorded at monthly intervals. Additionally, observations related to seed microflora and seed moisture content (%) were documented both at the beginning and end of the storage period. The specific procedures for determining these seed quality parameters are outlined below.

3.5.3.1 Seed germination per cent (%):

The germination test was conducted using the "between paper" method following the guidelines outlined by ISTA (International Seed Testing Association) in 2001. For this test, a roll paper towel served as the substrate. From each replication, four sets of one hundred seeds were randomly selected. These seeds were then arranged on a moistened paper towel and covered with another layer of wet paper towel. The two layers of paper towels, along with the seeds, were rolled together. These rolled paper towels were placed upright in a container filled with water. The experimental setup was maintained under consistent conditions of 25 ± 2°C temperature and 95 ± 2 per cent relative humidity. On the 14^{th} day, the number of healthy seedlings was counted, and the results were expressed in a per cent.

$$\text{Seed germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds sown}} \times 100$$

3.5.3.2 Seedling shoot length (cm)

On the 14^{th} day of the germination test, ten healthy seedlings were chosen randomly from each replication of the treatments. The length between the collar portion and the tip of the shoot was measured, and the average value was calculated and expressed in centimetres.

Day 1: Moistened roll towel paper containing seeds

Rolling of paper towel

Rolled towels kept for germination

Untreated (Control)

Treated (600 mg ZnO/kg)

Day 14: Germinated seedlings on opening the roll towel

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

3.5.3.3 Seedling root length (cm)

On the 14^{th} day, the root length of the seedlings, which were selected for shoot length measurement, was determined by measuring from the collar region to the tip of the root using a scale. The average root length was then calculated and expressed in centimeters.

3.5.3.4 Seedling dry weight (mg)

The estimation of seedling dry weight was conducted using the same ten seedlings that were utilized for measuring seedling shoot and root length. These seedlings were enclosed in a butter paper cover and subjected to drying in a hot air oven, which was maintained at a constant temperature of 85 ± 1°C, for a duration of 24 hours. Following the drying process, the seedlings were allowed to cool within a desiccator for a period of 45 minutes. Subsequently, their weight was measured and expressed in milligrams, (Copeland and McDonald in 2001).

3.5.3.5 Seed vigour indices

Seed vigour indices was calculated using the germination per cent obtained in the germination test. The vigor index was calculated as described by Abdul-Baki and Anderson (1973).

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

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

3.5.3.6 Electrical conductivity of seed leachate (μScm^{-1})

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

3.5.3.7 Seed moisture content (%)

The moisture content of the seeds was determined at the beginning and end of the storage period using the low constant temperature method outlined in the ISTA (International Seed Testing Association) guidelines (ISTA 1985). In this procedure, five grams of seeds from each replication of the treatments were carefully selected and evenly spread across the surface of a container. The container chosen was made of non-corrosive material, such as metal or glass, with a thickness of about 0.5mm. Both the container and its lid were weighed before and after filling with seeds.

Next, the container was placed inside a hot air oven that was maintained at a temperature of 103±2 °C, and the seeds were dried for a duration of 17±1 hours. The drying period was considered to have started once the oven reached a temperature of 103 °C. Upon completion of the prescribed drying time, the container was taken out from the hot air oven and transferred to a desiccator to cool for a period of 30-45 minutes. After cooling, the container, along with its lid, was reweighed. The seed moisture content was then determined using the following formula:

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

Where,

M_1 - Weight of empty moisture bottle with lid

M_2 - Weight of moisture bottle with seed sample before drying

M_3 - Weight of moisture bottle with sample after drying

This process allowed for the precise measurement of seed moisture content in accordance with the ISTA (1985) guidelines, employing controlled drying at a constant temperature.

3.5.3.8 Seed infection

A seed health test was performed by both blotter papper method and agar plate method of following the guidelines ISTA (1999). These methods are commonly used for assessing the health and viability of seeds.

3.5.3.8.1 Blotter paper method

The standard ISTA blotter test first proposed by Neergaard (1979) was used to identify seed microflora on blotter paper. In sterile Petri plates, three layers of sterilized blotting paper were retained. The plates were filled with sterile water, and the extra water was drained after the filter paper had soaked. In order to preserve aseptic conditions laminar air flow was used to space out 25 chilli seeds evenly over the blotter paper. Eight seeds were in the middle, one was in the centre, and there were 16 seeds on the outermost layer. Following plating, the seeds were incubated at 20 °C for seven days using an alternating cycle of 12 hours of darkness and 12 hours of light. The plates were examined for the presence of seed microflora on the eighth day using a stereo binocular microscope. The per cent contamination was arrived at by counting the number of contaminated seeds and expressing the same in per cent. Slides were prepared to analyse the physical characteristics of the conidia, conidiophores, and fruiting bodies, in order to identify the casual organism.

3.5.3.8.2 Agar plate method

Seeds were surface sterilized using mercuric chloride solution (0.1%), followed by three thorough rinses in sterile water to eliminate any residual mercuric chloride. The excess water on the seeds was carefully removed by placing them on sterile filter paper. In aseptic conditions, 20 mL of potato dextrose agar (PDA) was poured into a sterilized Petri plate, and the lid was left slightly open until the medium solidified. The sterilized seeds were then evenly placed at equidistance on the PDA surface. After plating, the Petri plates were closed and kept in a polythene cover and subjected to a six-day incubation period under a bell jar. Subsequently, the plates were examined under a stereo-binocular microscope, and the number of infected seeds was meticulously counted and expressed in per cent.

3.6 Statistical analysis

The statistical analysis of the laboratory experiment data was conducted utilizing the agricultural analysis software as WASP (Web Agri Stat Package 2.0). In order to assess significance, critical difference was calculated at a 5 % and 1 % probability level for all F-tests that demonstrated significance. The ranking of the treatments that exhibited statistical significance was performed using Duncan's

Multiple Range Test (DMRT). The treatment efficacy parameters consisting of per cent age data (germination per cent) were subjected to arcsine transformation. The zero values present in the data were converted to 1/n value, where 'n' is the number of observations

3.6.1 ANOVA for Randomized Block Design

The data collected for each observation in Experiment I were analysed using ANOVA to assess difference among two or more independent groups.

Source of variation	Degrees of freedom (df)	Sum of squares (SS)	Mean square MS = SS/df	Computed Fvalue
Replication	r-1	RSS	RMS	RMS/EMS
Treatment	t-1	TrSS	TrMS	TrMS/EMS
Error	(r-1) (t-1)	ESS	EMS	
Total	n-1	TSS		

3.6.2 ANOVA for Completely Randomized Design

The data collected for each observation in Experiment II were analyzed usingANOVA so as to test the difference between two or more independent groups.

Source of variation	Degree of freedom (df)	Sum of squares(SS)	Mean squareMS = SS/df	Computed F
Treatment	t – 1	TrSS	TrSS	TrSS/EMS
Error	n– t	ESS	EMS	
Total	n – 1	TSS		

Where,

r= No. of replications

t = No. of treatments

n =Total number of observations

TrSS = Sum of squares of treatment

ESS= Sum of squares of error

TSS= Sum of squares of total

RMS = Mean square of replication

TrMS = mean square of treatments

EMS = Mean square of error

3.6.3 DMRT test for ranking

Duncan's Multiple Range Test (DMRT) is utilized to assess and rank all possible pairs of treatment means, especially in experiments with a large number of treatments. DMRT establishes numerical boundaries that aid in categorizing the diference between any two treatments as either significant or non-significant. The calculation of DMRT values primarily relies on the specific standard error (SEm) associated with the pair of treatments being compared. For ranking data, Gomez and Gomez (1976) proposed the following procedure:

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

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

$$SEm = \frac{\sqrt{2MSE}}{r} \times 100$$

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

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

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

- 't' represents the number of treatments.
- 'SEm' signifies the standard error acquired in step ii.
- 'rp' corresponds to the tabulated values of ranges that are deemed significant.

'p' denotes the difference in rank between the pairs of treatment means being compared, with 'p' being equal to 't' for the highest and lowest means

iv. the treatment means that do not exhibit significant difference from each other were identified and grouped together.

v. the obtained results were presented using alphabetical notation, where the same alphabet was assigned to all treatments connected by the same vertical line. This notation helps visually represent and group treatments with similar effects or outcomes.

Results

4. RESULTS

The study titled "Mid-term storage seed invigoration with nanoparticles in chilli (*Capsicum annuum* L.)" was conducted in the Department of Seed Science and Technology, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur. The primary objective of this research was to evaluate the impact of seed treatments using nanoparticles on crop performance, seed yield, quality, and longevity when applied as a mid-term storage treatment. The results obtained for seed quality, plant growth, and yield parameters are presented below in this chapter.

4.1 Initial seed quality assessment

The initial quality assessment of the six-month-old seeds was carried out at the beginning of the experiment (Table 2). Seeds exhibited an initial germination of 67.37 per cent, with seedling shoot, root length, seedling dry weight with values of 4.68 cm, 6.81 cm, and 24.1mg respectively. The vigour index-I and vigour index-II were calculated as 774 and 1595 respectively. The electrical conductivity of the seed leachate was recorded at 192.33 μScm^{-1}. The initial moisture content of the seeds was 7.53 per cent, and the seed infection per cent was observed to be 47.15 per cent (blotter method) and 53.30 per cent (agar plate method).

4.2 Effect of ZnO nanoparticles on seed quality parameters immediately after treatment

4.2.1 Germination per cent

Significant variation in germination was observed in chilli seeds which were subjected to different doses of nano zinc oxide (Table 3). The germination rate of untreated seeds (Control) was consistently lower compared to the treated seeds, regardless of the specific treatment applied.

The highest germination rate of 73.31 per cent was noted in T_6 (ZnO @ 700 mg kg^{-1} seed) which was statistically on par with T_5 (nano ZnO @ 600 mg kg^{-1} of seed) at 72.58%, the untreated seeds exhibited significantly lower germination rate of 67.04 percent.

4.2.2 Seedling shoot length (cm)

Shoot length varied between 5.55 cm T_6 (nano ZnO 700 mg kg^{-1} of seed) and 4.53 cm in control (Table 3). Treatments T_5 (nano ZnO 600 mg kg^{-1} of seed) at 5.47 cm, and T_4 (nano ZnO 500 mg kg^{-1} of seed) at 5.12 cm were statistically on par with each other. Treated Seeds exhibited greater shoot length when compared to control.

4.2.3 Seedling root length (cm)

Seed treatment with nano ZnO had a noticeable impact on seedling root length, as indicated in Table 3.

The highest root length was observed in T_6 (nano ZnO 700 mg kg^{-1} of seed) at 7.36 cm, followed by T_5 (nano ZnO 600 mg kg^{-1} of seed) at 7.29 cm. It was noted that T_2 (nano ZnO 300 mg kg^{-1} of seed) had a comparatively lower root length among the seed treatments, measuring 7.01 cm, while the control exhibited a root length of 6.88 cm.

4.2.4 Seedling dry weight (mg)

Significant variation in seedling dry weight was observed among chilli seeds treated with different doses of nano zinc oxide with six-month-old seeds, as illustrated in Table 3. However, it was consistently noted that the treated seeds exhibited higher dry weight compared to the control seeds.

Among the different treatments, T_6 (nano ZnO @ 700 mg kg^{-1} of seed) displayed the highest seedling dry weight at 24.16 mg, which was statistically comparable to T_5 (nano ZnO @ 600 mg kg^{-1} of seed) at 24.06 mg, T_4 (nano ZnO @ 500 mg kg^{-1} of seed) at 23.87 mg, while the control recorded a dry weight of 23.16 mg.

4.2.5 Vigour index I

The highest vigour index-I value (945) was observed in seeds treated with nano ZnO @ 700 mg kg^{-1}, followed by nano ZnO @ 600 mg kg^{-1} (929). T_2 (nano ZnO @ 300 mg kg^{-1}) exhibited a lower seed vigour index-I (852). Control Showed significantly lower seed vigour index-I of 765 (Table 3).

4.2.6 Vigour index II

Chilli seeds subjected to various doses of nano zinc oxide treatments displayed significant variation in vigour index-II (Table 3). The highest vigour index-II value (1771) was observed in seeds treated with nano ZnO @ 700 mg kg^{-1}, closely followed by those treated with nano ZnO @ 600 mg kg^{-1} (1752). In contrast, T_2 (nano ZnO @ 300 mg kg^{-1}) exhibited a lower seed vigour index-II at 1685 among the different treatments, while the control had significantly lower seed vigour index-II (1553).

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

The lowest mean electrical conductivity was observed in seeds treated with treatment (T_6) nano ZnO @ 700 mg kg^{-1} and nano ZnO @ 600 mg kg^{-1} (192 µS cm^{-1}), while the highest mean value was recorded in the control (193.33 µS cm^{-1}) .

4.2.8 Seed microflora

Seed treatments with nano ZnO revealed non-significant difference in seed infection, as observed in Table 3.

There was no seed infection observed in seed treated with nano ZnO. Infection was only observed in the control seeds, with rates of 53.3 per cent in the agar plate method and 47.15 per cent in the blotter paper method. The presence of *Aspergillus* species was detected in both of these methods.

4.3 Effect of mid- storage correction with nanoparticles on growth and yield parameter in chilli

4.3.1 Analysis of variance

The analysis of variance revealed highly significant variation for all the traits under study namely, days to first flowering, days to 50 per cent 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 (Table 4).

4.3.2 Days to first flowering

The number of days taken for first flowering varied significantly among the treatments (Table 5). Seeds treated with 500 mg kg^{-1} of nano custard leaf powder (T_7) were the earliest to flower, which was four days earlier than the untreated control

(78days). Treatments T_5 (nano ZnO @ 600 mg kg^{-1} of seed) and T_6 (nano ZnO @ 700 mg kg^{-1} of seed) with value of 74.78 days.

4.3.3 Days to 50 % flowering

Significant variation was observed among the treatments with regard to the days to fifty per cent flowering, (Table 5).

Table 2: Initial seed quality parameters of chilli var. Ujwala

Seed quality parameter		Value
Germination (per cent)		67.37
Seedling shoot length (cm)		4.68
Seedling root length (cm)		6.81
Seedling dry weight (mg 10 $seedlings^{-1}$)		23.69
Vigour index-I		774
Vigour index-II		1595
Electrical conductivity (µS cm^{-1})		192.33
Seed infection (per cent)	Blotter paper method	47.15
	Agar plate method	53.30
Seed moisture content (per cent)		7.53

Table 3. Effect of midterm storage correction with nanoparticles on seed quality parameters immediately after treatment in six-month-old chilli seeds

Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Dry weight (mg)	Vigour Index I	Vigour Index II	Electrical conductivity ($\mu S\ cm^{-1}$)	Seed infection (%)	
								Agar plate method	Blotter paper method
T_1: Control	67.04^{e}	4.53^{c}	6.88^{d}	23.16^{c}	765^{f}	1553^{g}	193.33^{a}	53.30	47.15
T_2: 300 mg nano ZnO/kg of seed	71.79^{d}	4.87^{bc}	7.01^{c}	23.47^{abc}	852^{e}	1685^{f}	192.67^{a}	0	0
T_3: 400 mg nano ZnO/kg of seed	71.99^{cd}	4.95^{bc}	7.17^{b}	23.77^{abc}	872^{d}	1711^{d}	192.60^{a}	0	0
T_4: 500 mg nano ZnO/kg of seed	72.05^{cd}	5.12^{ab}	7.22^{ab}	23.87^{ab}	889^{c}	1720^{c}	192.07^{a}	0	0
T_5: 600 mg nano ZnO/kg of seed	72.84^{ab}	5.47^{a}	7.29^{a}	24.06^{ab}	929^{b}	1752^{b}	192.00^{a}	0	0
T_6: 700 mg nano ZnO/kg of seed	73.31^{a}	5.55^{a}	7.36^{a}	24.16^{a}	945^{a}	1771^{a}	192.00^{a}	0	0
T_7: 500 mg nano custard leaf powder/ kg of seed	72.59^{bc}	4.95^{bc}	7.27^{a}	23.45^{ab}	886^{c}	1702^{e}	192.00^{a}	0	0
SEm	0.13	0.06	0.08	0.13	7.51	9.06	0.93	0	0
CD (0.05)	0.41	0.17	0.25	0.39	22.77	27.49	NS	NS	NS

Table 4. Analysis of variance for the impact of midterm storage correction with nanoparticles on yield attributing 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 (gm)	Fruit yield/plant (g)	No. of seeds/ fruit (nos.)	100 seed weight (g)	Seed yield/ plant (g)
Treatments	6	83.5220**	0.2750**	5.3109**	9.5801**	252.9370**	0.4871**	0.1104**	6219.9430**	64.1593**	0.0024**	16.9752**
Replications	2	11.4100	0.0060	2.0164	0.6759	8.4347	0.0350	0.0008	66.3401	5.5804	0.0012	0.1860
Error	12	2.9944	0.0210	0.5839	0.7098	11.6714	0.0120	0.0017	17.5557	1.5493	0.0004	0.0819
SEm		0.9941	0.0840	0.4412	0.4864	1.9724	0.0635	0.0238	2.4191	0.7163	0.0125	0.1652
CD (5%)		3.0078	0.2590	1.3594	1.5499	6.0777	0.1957	0.0734	7.4538	2.2072	0.0385	0.5091
CD (1%)		4.3157	0.3630	1.9058	2.1013	8.5224	0.2744	0.1029	10.4498	3.0943	0.0539	0.7138

Table 5. Effect of mid storage correction with nanoparticles on growth and yield parameters in chilli

Treatment	Days to first flowering (no.)	Days to 50 % flowering (no.)	Plant height (cm)	Branches/ plant (no.)	Fruits/ plant (no.)	Fruit length (cm)	Fruit weight at maturity (g)	Fruit yield/plant (g)	Number of seeds/ fruits	100 seed weight (g)	Seed yield / plant (g)
T_1: Control	78.00[a]	87.17[a]	65.27[d]	4.93[d]	84.30[d]	4.34[c]	1.73[d]	360.96[g]	48.17[f]	0.350[c]	19.55[e]
T_2: 300 mg nano ZnO/kg of seed	76.67[b]	85.67[b]	71.47[c]	5.10[cd]	95.80[c]	4.97[b]	1.96[c]	374.71[f]	51.33[e]	0.395[b]	20.81[d]
T_3: 400 mg nano ZnO/kg of seed	76.11[bc]	85.89[b]	72.53[c]	5.30[bc]	96.70[c]	5.10[ab]	2.08[b]	393.63[d]	53.03[d]	0.403[ab]	21.07[d]
T_4: 500 mg nano ZnO/kg of seed	75.11[cd]	83.33[c]	75.87[b]	5.53[ab]	103.67[b]	5.23[ab]	2.25[a]	436.27[c]	56.53[c]	0.416[ab]	23.45[b]
T_5: 600 mg nano ZnO/kg of seed	74.78[d]	82.89[c]	76.87[b]	5.80[a]	112.57[a]	5.36[ab]	2.31[a]	478.73[a]	61.50[a]	0.431[a]	25.76[a]
T_6: 700 mg nano ZnO/kg of seed	74.78[d]	82.67[c]	80.40[a]	5.63[a]	105.33[b]	5.55[a]	2.13[b]	462.09[b]	59.27[b]	0.433[a]	25.50[a]
T_7: 500 mg nano custard leaf powder/ kg of seed	74.22[d]	83.33[c]	79.60[a]	5.30[bc]	93.67[c]	5.41[ab]	2.07[b]	388.09[e]	56.30[c]	0.413[ab]	22.44[c]
SEm	0.44	0.49	0.99	0.08	1.97	0.06	0.03	2.42	0.72	0.012	0.17
CD (0.05)	1.36	1.50	3.08	0.26	6.08	0.20	0.11	7.45	2.21	0.038	0.51

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

The treatment involving seeds treated with nano ZnO @ 700 mg kg^{-1} (T_6) demonstrated the shortest duration to achieve fifty per cent flowering (82.67 days), followed closely by treatment T_5 (nano ZnO @ 600 mg kg^{-1} of seed) with 82.89 days and T_7 (500 mg nano custard leaf powder/ kg of seed) with 83.33 days. The control took 87.17 days to attain fifty per cent flowering.

4.3.4 Plant height (cm)

Significant difference in plant height was observed among the treatments (Table 5) with the treated seeds resulting in taller plants, when compared to control (87.17cm).

Among the treatments, the tallest plants were observed with 80.40 cm in T_6 (nano ZnO @ 700 mg kg^{-1} of seed), and T_7 (nano custard leaf powder at 500 mg kg^{-1} of seed) with 79.60 cm. Treatment T_4 (nano ZnO @ 500 mg kg^{-1} of seed) and treatment T_5 (nano ZnO @ 400 mg kg^{-1} of seed) were on par with each other recording plant heights of 75.87 cm and 76.87 cm respectively.

4.3.5 Branches per plant

Treatments varied significantly among themselves for branches per plant (Table 5). Among the treatments, T_5 (nano ZnO @ 600 mg kg^{-1} of seed) exhibited the highest number of branches (5.8), followed by T_6 (nano ZnO @ 700 mg kg^{-1} of seed) with 5.63 and T_4 (nano ZnO @ 500 mg kg^{-1} of seed) with 5.53 branches which were on par with each other. The control recorded the least number of branches per plant (4.93).

4.3.6 Fruits per plant

Application of nano ZnO treatment to chilli seeds resulted in higher number of fruits, when compared to untreated seeds (Table 5). Among these, seed treatment using T_5 (nano ZnO @ 600 mg per kg of seed) resulted in the highest number of fruits (112.57), followed by T_6 (nano ZnO @ 700 mg per kg of seed) with 105.33 fruits, which was statistically on par with T_4 (nano ZnO @ 500 mg per kg of seed) with 103.67 fruits. Among the treated seeds, the lowest number of fruits was observed in T_2 (nano ZnO @ 300 mg per kg of seed) with 95.80 fruits followed by control (84.30 fruits).

4.3.7 Fruit length(cm)

Chilli seeds treated with nano ZnO treatments exhibited significant variation in fruit length, as indicated in Table 5. Treatment T_6 (nano ZnO @ 700 mg kg^{-1} of seed) recorded lengthier fruits (5.55 cm), followed by T_7 (nano custard leaf powder 500 mg kg^{-1} of seed) with a fruit length of 5.41 cm, T_4 (nano ZnO @ 500 mg kg^{-1} of seed) at 5.23 cm, and T_3 (nano ZnO @ 400 mg kg^{-1} of seed) at 5.10 cm. The control recorded the lowest fruit length of 4.34 cm.

4.3.8 Fruit weight at maturity (g)

All the treatments recorded significantly higher fruit weight at maturity when compared to control (Table 5).

Among the treatments, seeds treated with nano ZnO @ 600 mg kg^{-1} (T_5) yielded the highest fruit weight at maturity (2.31 grams). This was followed by T_4 (nano ZnO @ 500 mg kg^{-1} of seed), with a fruit weight of 2.25 grams, and T_6 (Nano ZnO @ 700 mg kg^{-1} of seed), producing a fruit weight of 2.13 grams. The control had the lowest fruit weight at maturity (1.73 grams).

4.3.9 Fruit yield/ plant

Significant variation was observed among the seeds subjected to treatments with nano particles, as shown in Table 5. The seeds treated with nano ZnO @ 600 mg kg^{-1} exhibited the highest fruit yield per plant (478.73 grams). Treatments nano ZnO treatments at 700 mg kg^{-1} of seed (T_6) and 500 mg kg^{-1} of seed (T_4) resulted in fruit yields of 462.09g and 436.27g respectively. The control recorded the lowest fruit yield per plant (360.96 grams).

4.3.10 Number of seeds per fruit

Chilli seeds subjected to nano ZnO treatments reported significant variation in the number of seeds per fruit (Table 5). The treatment T_5 (600 mg nano ZnO kg^{-1} of seed) recorded the most significant increase, with 61.5 seeds per fruit. Followed closely by T_6 (nano ZnO applied at 700 mg kg^{-1} of seed) with 59.27 seeds per fruit, T_4 (500 mg nano ZnO/ kg of seed) and T_7 (nano clustered leaf powder at 500 mg kg^{-1} of seed) with 56.53 and 56.30 seeds per fruit, respectively. These treatments were statistically outperformed the untreated control (48.17 seeds per fruit).

4.3.11 100 seed weight (g)

Significant variation was noted among the seed treatments, as depicted in Table 5. Among the treatments, seeds treated with 700 mg nano ZnO kg^{-1} of seed (T_6) recorded the highest 100-seed weight (0.443 grams), closely followed by seeds treated with nano ZnO @ 600 mg kg^{-1} of seed (0.430 grams), while control recorded 0.357 grams.

4.3.12 Seed yield/plant (g)

The study revealed significant variation in seed yield per plant among the treatments (Table 5). The highest seed yield was achieved in seeds treated with nano ZnO @ 600 mg/ kg of seed (T_5), recording 25.76g, which significantly exceeded the control (19.55g).

Treatments T_6 (nano ZnO @ 700 mg/ kg of seed) resulted in a seed yield of 25.5g, while T_4 (nano ZnO @ 500 mg/ kg of seed) had a yield of 23.45g.

4.4 Seed storage studies

The seeds obtained from the previous experiment were dried to a moisture content of less than eight per cent. Subsequently, they were stored in polythene covers (700G) under normal ambient conditions for a duration of six months. The seed storage investigation was organized in a completely randomized design, comprising three replications for each of the seven treatments. The evaluation of seed quality parameters was carried out at monthly intervals throughout the storage period.

4.4.1 Initial seed quality assessment before storage

The initial seed quality parameters were evaluated before the initiation of the storage study, and the results are documented in Table 6. Significant difference was observed in seed quality parameters, such as germination, shoot length, root length, vigour indices (I and II), and seed microflora infestation among the treatments. However, there were no significant difference was observed for seedling dry weight, electrical conductivity of seed leachates and moisture content.

4.4.1.1 Germination (%)

Percentage of germination ranged from 59.80 per cent in T_1 to 68.07 per cent in T_5. The highest germination per cent age was observed in T_5 (600 mg nano ZnO/kg of seed) with 68.07 per cent, and this value was statistically comparable to the germination of 67.10 per cent in T_6 (700 mg nano ZnO/kg of seed). The lowest germination percentage was recorded in control (59.80%).

4.4.1.2 Seedling shoot length (cm)

Significant variation was observed in the seedling shoot length among the treatments. The range of shoot length varied from 4.58 cm (T_1) to 5.20 cm (T_5). Treatment T_5 (600 mg nano ZnO/kg of seed) recorded a shoot length of 5.20 cm and was on par with T_6 (700 mg nano ZnO/kg of seed) and T_4 (500 mg nano ZnO/kg of seed). The lowest shoot length was recorded in the control (T_1) with a length of 4.58 cm.

4.4.1.3 Seedling root length (cm)

Seedling root length exhibited significant variation, ranging from 7.53 cm in T_1 to 8.76 cm in T_5. The highest value of 8.95 cm, was observed in T_5 (600 mg nano ZnO/kg of seed which is on par T_6 (700 mg nano ZnO/kg of seed), with a root length of 8.85 cm and T_4 (500 mg nano ZnO/kg of seed) with a root length of 8.76 cm. The lowest root length, 7.53 cm, was recorded in control (T_1).

4.4.1.4 Seedling dry weight (mg)

There were no differences among the treatments for Seedling dry weight

4.4.1.5 Vigour index I

Among different treatments the initial mean vigour index varied from 725 (T_1) to 963 (T_5). The highest vigour index (963) was observed in T_5 (600 mg of nano ZnO /kg of seed), which was statistically on par with T_6 (700 mg of nano ZnO /kg of seed).

4.4.1.6 Vigour index II

The treatments demonstrated notable variation in vigour index II, ranging from 1363 (T_1-Control) to 1640 (T_5-600 mg of nano ZnO /kg of seed).

4.4.1.7 Electrical conductivity (μScm^{-1})

The impact of the treatments on the initial electrical conductivity of seed leachates was found to be statistically non-significant, with values ranging from 87.427 $\mu S\ cm^{-1}$ (T_5) to 92.98 $\mu S\ cm^{-1}$ (T_1).

4.4.1.8 Moisture content (%)

The initial seed moisture content did not show significant difference among the treatments, ranging from 6.26 per cent in T_5 (600 mg of nano ZnO/kg of seed) to 6.58 per cent in control (T_1).

Table 6. Effect of mid storage seed treatment with nanoparticles on seed quality parameters before seed storage in chilli

Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Dry weight (mg)	Vigour Index I	Vigour Index II	Electrical conductivity (μScm^{-1})	Moisture content (%)	Seed infection (%)	
									Agarplate method	Blotterpaper method
T_1: Control	59.80^{e}	4.58^{d}	7.53^{c}	23.80^{a}	725^{e}	1363^{d}	92.98^{a}	6.58	3.17^{a} (10.15)	1.83^{a} (7.65)
T_2: 300 mg nano ZnO/kg of seed	63.78^{d}	4.71^{cd}	7.73^{c}	24.05^{a}	794^{d}	1491^{c}	90.67^{a}	6.45	2.00^{ab} (7.90)	1.17^{abc} (6.19)
T_3: 400 mg nano ZnO/kg of seed	64.50^{cd}	4.90^{bc}	8.21^{b}	24.10^{a}	847^{c}	1527^{bc}	90.70^{a}	6.26	1.67^{bc} (7.33)	1.23^{ab} (6.35)
T_4: 500 mg nano ZnO/kg of seed	65.93^{cd}	5.03^{ab}	8.76^{a}	24.40^{a}	910^{b}	1571^{abc}	89.19^{a}	6.43	1.43^{bc} (6.87)	1.10^{abc} (6.00)
T_5: 600 mg nano ZnO/kg of seed	68.07^{a}	5.20^{a}	8.95^{a}	24.52^{a}	963^{a}	1640^{a}	87.42^{a}	6.31	1.43^{bc} (6.83)	0.60^{c} (4.31)
T_6: 700 mg nano ZnO/kg of seed	67.10^{ab}	5.07^{ab}	8.85^{a}	24.53^{a}	934^{ab}	1601^{ab}	87.84^{a}	6.38	0.90^{c} (5.43)	1.00^{bc} (4.96)
T_7: 500 mg nano custard leaf powder/ kg of seed	65.96^{bc}	4.93^{bc}	8.66^{a}	24.45^{a}	897^{b}	1569^{abc}	89.14^{a}	6.39	1.30^{bc} (6.46)	1.30^{ab} (6.46)
SEm	0.71	0.08	0.13	0.29	16.71	24.85	2.35	0.20	0.37	0.20
CD (0.05)	2.15	0.26	039	NS	50.70	75.38	NS	NS	1.12	0.61

* Figures in parentheses are arc-sine transformed values

Table 7. Effect of seed invigoration with nanoparticles on germination per cent (%) in chilli after storage at monthly intervals

Treatment	Months of storage (M)					
	M1	M2	M3	M4	M5	M6
T_1: Control	65.32^{d} (53.93)	68.46^{d} (55.88)	69.33^{d} (56.38)	67.33^{c} (55.14)	66.34^{c} (54.55)	63.11^{d} (52.59)
T_2: 300 mg nano ZnO/kg of seed	69.56^{bc} (56.54)	70.35^{cd} (57.02)	71.83^{bc} (57.94)	71.47^{ab} (57.71)	69.10^{bc} (56.23)	67.21^{c} (55.06)
T_3: 400 mg nano ZnO/kg of seed	1.02^{ab} (57.44)	71.97^{bc} (58.03)	72.40^{bc} (58.31)	71.94^{ab} (58.01)	70.02^{b} (56.80)	68.33^{abc} (55.75)
T_4: 500 mg nano ZnO/kg of seed	71.36^{ab} (57.65)	72.45^{abc} (58.34)	73.17^{ab} (58.82)	73.03^{ab} (58.72)	70.39^{ab} (57.03)	68.56^{abc} (55.90)
T_5: 600 mg nano ZnO/kg of seed	72.51^{a} (58.37)	74.88^{a} (59.92)	75.07^{a} (60.05)	73.70^{a} (59.14)	72.87^{a} (58.60)	70.10^{a} (56.85)
T_6: 700 mg nano ZnO/kg of seed	71.03^{ab} (57.44)	73.48^{ab} (59.02)	73.87^{ab} (59.26)	73.23^{a} (58.84)	71.43^{ab} (57.70)	69.67^{ab} (56.58)
T_7: 500 mg nano custard leaf powder/ kg of seed	68.50^{c} (55.86)	69.94^{cd} (56.75)	71.62^{bc} (57.81)	70.66^{b} (57.21)	69.30^{b} (56.35)	67.47^{bc} (55.25)
SEm	0.69	0.82	0.84	0.74	0.83	0.70
CD (0.05)	2.10	2.50	2.55	2.26	2.54	2.13

* Figures in parentheses are arc-sine transformed values

Table 8. Effect of seed invigoration with nanoparticle on shoot length (cm) in chilli after storage at monthly intervals

Treatment	Months of storage (M)					
	M1	M2	M3	M4	M5	M6
T_1: Control	4.64^{d}	4.73^{c}	4.80^{d}	4.72^{d}	4.63^{c}	4.43^{e}
T_2: 300 mg nano ZnO/kg of seed	4.83^{cd}	4.93^{bc}	5.03^{c}	4.90^{cd}	4.78^{bc}	4.69^{d}
T_3: 400 mg nano ZnO/kg of seed	5.01^{bc}	5.04^{b}	5.16^{bc}	5.13^{abc}	4.96^{ab}	4.80^{cd}
T_4: 500 mg nano ZnO/kg of seed	5.09^{ab}	5.12^{ab}	5.23^{abc}	5.19^{ab}	5.15^{a}	4.93^{abc}
T_5: 600 mg nano ZnO/kg of seed	5.29^{a}	5.33^{a}	5.37^{a}	5.28^{a}	5.21^{a}	5.10^{a}
T_6: 700 mg nano ZnO/kg of seed	5.10^{ab}	5.14^{bc}	5.29^{ab}	5.17^{ab}	5.18^{ab}	5.04^{ab}
T_7: 500 mg nano custard leaf powder/ kg of seed	5.00^{bc}	4.97^{ab}	5.03^{c}	4.97^{bcd}	4.93^{ab}	4.86^{bcd}
SEm	0.07	0.08	0.06	0.07	0.08	0.07
CD (0.05)	0.23	0.25	0.19	0.22	0.25	0.21

Table 9. Effect of seed invigoration with nanoparticles on root length (cm) in chilli after storage at monthly intervals

Treatment	Months of storage (M)					
	M1	M2	M3	M4	M5	M6
T_1: Control	7.73[d]	8.23[c]	8.45[e]	8.33[d]	8.27[e]	7.93[d]
T_2: 300 mg nano ZnO/kg of seed	8.13[c]	8.32[c]	8.79[d]	8.69[c]	8.52[d]	8.32[c]
T_3: 400 mg nano ZnO/kg of seed	8.63[b]	8.73[b]	8.92[cd]	8.83[bc]	8.67[cd]	8.43[c]
T_4: 500 mg nano ZnO/kg of seed	8.80[ab]	8.96[ab]	9.05[bc]	8.96[ab]	8.97[ab]	8.77[ab]
T_5: 600 mg nano ZnO/kg of seed	9.10[a]	9.20[a]	9.27[a]	9.13[a]	9.17[a]	8.97[a]
T_6: 700 mg nano ZnO/kg of seed	8.96[ab]	9.10[ab]	9.14[ab]	9.07[ab]	9.04[a]	8.83[ab]
T_7: 500 mg nano custard leaf powder/ kg of seed	8.73[ab]	8.85[ab]	8.95[bcd]	8.83[bc]	8.78[bc]	8.57[bc]
SEm	0.11	0.11	0.06	0.07	0.07	0.09
CD (0.05)	0.34	0.34	0.19	0.22	0.22	0.28

Table 10. Effect of seed invigoration with nanoparticles on dry weight (mg) in chilli after storage at monthly intervals

Treatment	Months of storage (M)					
	M1	M2	M3	M4	M5	M6
T_1: Control	24.03^{b}	24.20^{b}	24.50^{b}	24.21^{b}	24.05^{b}	23.63^{c}
T_2: 300 mg nano ZnO/kg of seed	24.43^{ab}	24.58^{ab}	24.70^{ab}	24.36^{b}	24.11^{b}	23.96^{abc}
T_3: 400 mg nano ZnO/kg of seed	24.60^{ab}	24.75^{ab}	24.82^{ab}	24.53^{ab}	24.32^{b}	24.12^{abc}
T_4: 500 mg nano ZnO/kg of seed	24.67^{ab}	24.79^{ab}	24.86^{ab}	24.65^{ab}	24.48^{b}	24.18^{ab}
T_5: 600 mg nano ZnO/kg of seed	24.86^{a}	25.19^{a}	25.53^{ab}	25.03^{a}	24.98^{a}	24.41^{a}
T_6: 700 mg nano ZnO/kg of seed	24.73^{ab}	24.93^{a}	25.26^{a}	24.93^{a}	24.52^{b}	24.15^{abc}
T_7: 500 mg nano custard leaf powder/ kg of seed	24.59^{ab}	24.97^{a}	25.19^{ab}	24.69^{ab}	24.20^{b}	23.77^{bc}
SEm	0.15	0.19	0.21	0.16	0.15	0.16
CD (0.05)	0.46	0.57	0.64	0.48	0.45	0.48

Table 11. Effect of seed invigoration with nanoparticles on vigour index I in chilli after storage at monthly intervals

Treatment	Months of storage (M)					
	M1	M2	M3	M4	M5	M6
T_1: Control	808^{f}	887^{e}	918^{e}	880^{d}	856^{e}	780^{e}
T_2: 300 mg nano ZnO/kg of seed	901^{e}	933^{de}	993^{d}	971^{c}	919^{d}	874^{d}
T_3: 400 mg nano ZnO/kg of seed	969^{c}	992^{bc}	1019^{cd}	1004^{bc}	954^{cd}	904^{c}
T_4 : 500 mg nano ZnO/kg of seed	991^{bc}	1021^{bc}	1045^{bc}	1034^{ab}	991^{bc}	939^{b}
T_5: 600 mg nano ZnO/kg of seed	1043^{a}	1088^{a}	1099^{a}	1062^{a}	1044^{a}	986^{a}
T_6: 700 mg nano ZnO/kg of seed	999^{b}	1046^{ab}	1066^{ab}	1045^{a}	1015^{ab}	967^{ab}
T_7: 500 mg nano custard leaf powder/ kg of seed	941^{d}	967^{cd}	1001^{d}	975^{c}	950^{cd}	908^{c}
SEm	8.42	17.63	12.86	11.07	13.51	9.89
CD (0.05)	25.56	53.49	39.01	33.59	41.00	30.00

Table 12. Effect of seed invigoration with nanoparticles on vigour index II in chilli after storage at monthly intervals

Treatment	Months of storage (M)					
	M1	M2	M3	M4	M5	M6
T_1: Control	1498^{c}	1657^{d}	1698^{d}	1629^{d}	1595^{fc}	1490^{e}
T_2: 300 mg nano ZnO/kg of seed	1637^{b}	1729^{cd}	1774^{cd}	1740^{c}	1665^{bc}	1610^{cd}
T_3: 400 mg nano ZnO/kg of seed	1683^{ab}	1781^{bc}	1797^{bcd}	1764^{bc}	1702^{b}	1647^{bc}
T_4: 500 mg nano ZnO/kg of seed	1714^{ab}	1796^{abc}	1819^{abc}	1800^{ab}	1723^{b}	1657^{b}
T_5: 600 mg nano ZnO/kg of seed	1752^{a}	1866^{a}	1896^{a}	1844^{a}	1820^{a}	1710^{a}
T_6: 700 mg nano ZnO/kg of seed	1706^{ab}	1850^{ab}	1886^{ab}	1825^{a}	1752^{ab}	1682^{ab}
T_7: 500 mg nano custard leaf powder/ kg of seed	1626^{b}	1746^{c}	1780^{cd}	1744^{c}	1677^{bc}	1603^{d}
SEm	29.33	25.03	29.97	15.93	27.84	13.59
CD (0.05)	88.96	75.91	90.90	48.32	84.46	41.21

Table 13. Effect of seed invigoration with nanoparticles on electrical conductivity of seed leachate (µS cm^{-1}) in chilli

Treatment	Months of storage (M)					
	M1	M2	M3	M4	M5	M6
T_1: Control	99.14^{a}	116.66^{a}	131.66^{a}	140.23^{a}	153.17^{a}	163.83^{a}
T_2: 300 mg nano ZnO/kg of seed	96.70^{ab}	110.20^{b}	129.43^{ab}	135.50^{b}	149.73^{b}	158.07^{b}
T_3: 400 mg nano ZnO/kg of seed	94.53^{bc}	108.23^{bc}	127.97^{b}	133.80^{b}	147.17^{bc}	156.17^{bc}
T_4: 500 mg nano ZnO/kg of seed	92.87^{cd}	107.03^{c}	125.30^{c}	129.63^{c}	146.43^{c}	153.43^{c}
T_5: 600 mg nano ZnO/kg of seed	90.33^{d}	103.30^{d}	121.66^{d}	124.77^{e}	141.13^{d}	147.80^{d}
T_6: 700 mg nano ZnO/kg of seed	91.16^{d}	105.13^{cd}	122.17^{d}	125.90^{de}	142.37^{d}	148.03^{d}
T_7: 500 mg nano custard leaf powder/ kg of seed	92.33^{cd}	107.06^{c}	123.33^{cd}	128.03^{cd}	146.67^{c}	156.00^{c}
SEm	0.80	0.95	0.83	0.89	0.89	0.96
CD (0.05)	2.45	2.88	2.53	2.70	2.71	2.91

Table 14. Effect of seed invigoration with nanoparticles on moisture content and seed microflora at the end of storage

Treatment	Moisture content (%)	Seed infection(%)	
		Blotter paper method	Agar plate method
T_1: Control	7.05^{a}	58.85^{a}	62.18^{a}
T_2: 300 mg nano ZnO/kg of seed	6.83^{a}	52.57^{b}	55.57^{b}
T_3: 400 mg nano ZnO/kg of seed	6.71^{a}	51.20^{bc}	54.20^{bc}
T_4: 500 mg nano ZnO/kg of seed	6.74^{a}	51.53^{bc}	53.53^{bc}
T_5: 600 mg nano ZnO/kg of seed	6.60^{a}	48.37^{d}	51.7^{cd}
T_6: 700 mg nano ZnO/kg of seed	6.63^{a}	48.01^{d}	49.03^{d}
T_7: 500 mg nano custard leaf powder/ kg of seed	6.77^{a}	50.10^{cd}	52.10^{c}
SEm	0.09	1.28	2.32
CD (0.05)	NS	3.90	7.05

4.4.1.9 Seed Infection (%)

The seed infection was minimal before storage, as shown in Table 6. In the blotter paper method, the highest seed infection was observed in control T_1 with 1.83 per cent infection while the lowest infection was recorded in seeds treated with nano ZnO @ 600 mg kg^{-1} (T_5; 0.6% infection), and these were found to be affected by fungi. Similarly, in the agar plate method, control seeds (T_1) exhibited 3.17 per cent infection, whereas the lowest infection (0.9 %) was found in T_6 (ZnO@700 mg kg^{-1}). The presence of *Aspergillus* was noted in both methods

4.4.2 Assessment of seed quality in storage

4.4.2.1 Analysis of variance

The analysis of variance for the data collected at monthly intervals over a six-month storage period indicated the presence of highly significant difference in seed quality parameters such as germination per cent , seedling shoot length, root length, seedling dry weight, seedling vigour indices, electrical conductivity of seed leachate and seed infection.

4.4.2.2 Germination (%)

Significant variation was noted among the treatments (Table 7). Treated seeds consistently exhibited higher germination rate in comparison to the control throughout the study period. By the end of the 3rd month of storage, the treatments had attained their peak germination followed by a gradual decline, regardless of the treatments, as the period of storage progressed.

The highest germination per cent throughout the storage period was recorded in T_5 (600 mg of nano ZnO per kilogram of seeds) in the third month of storage (75.07%) which was on par with T_6 (700 mg nano ZnO/kg of seed) and T_4 (500 mg ZnO/kg of seed).

At the end of the storage period, T_5 (600 mg of nano ZnO per kilogram of seed) recorded a germination of 70.10 per cent, which was statistically similar to V (700 mg of nano ZnO/kg of seed), The lowest germination per cent was recorded in the control (63.11%).

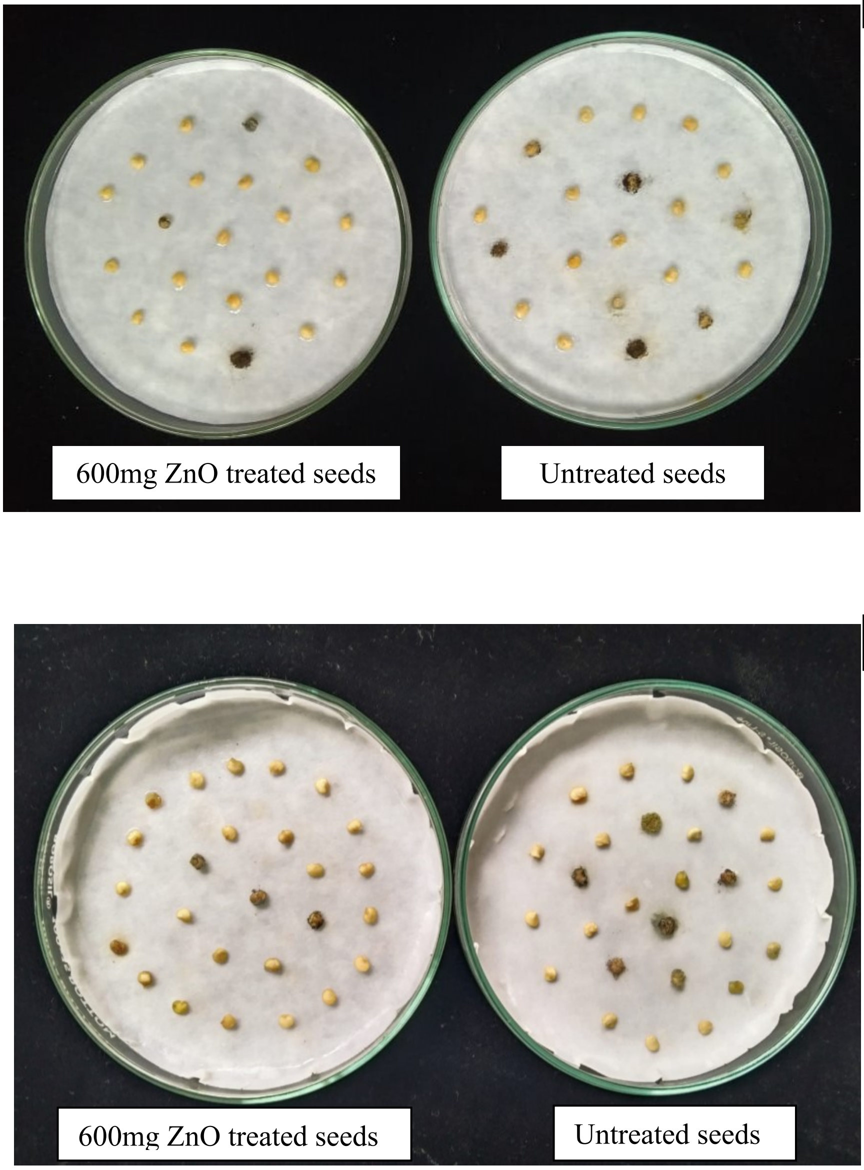


Plate 4: Evaluation of seed microflora in chilli
A) Before storage B) After six months of storage

All treatments, including the control group, maintained germination per cent above the Minimum Seed Certification Standard (MSCS) of 60% throughout the six months of storage.

4.4.2.3 Seedling shoot length (cm)

Significant variation in seedling shoot length was observed throughout the storage period (Table 8). Seedling shoot length consistently increased up to the third month and later declined as the storage period progressed. Treated seeds recorded higher shoot length when compared to control. The highest seedling shoot length (5.37 cm) was recorded in T_5 (600 mg nano ZnO/kg of seed) in the third month, and the least value was observed in control at the end of storage (4.43 cm).

In the third month, T_5 (600 mg of nano ZnO per kilogram of seed), recorded the highest shoot length of 5.37 cm. This was statistically equivalent to the shoot length in T_6, (700 mg of nano ZnO per kilogram of seed), measuring 5.29 cm among the seed treatments. T_2 (300 mg ZnO/kg of seed) and T_7 (500 mg/kg custard leaf powder) exhibited a lower shoot length of 5.03 cm, while the control recorded the lowest shoot length of 4.80 cm.

At the end of the storage period, the most favourable performance in terms of seedling shoot length (5.10 cm) was observed in T_5 (600 mg nano ZnO/kg of seed). Lower values (4.69 cm) were recorded in T_2 (300 mg ZnO/kg of seed) and control (4.43 cm).

4.4.2.4 Seedling root length (cm)

Significant variation in seedling root length was observed among the treatments throughout the six months of storage, as indicated in Table 9. The average seedling root length exhibited a gradual increase up to the third month of storage, later declined as the storage duration advanced, regardless of the treatments. However, it was observed that treated seeds consistently showed higher root length compared to the control.

During the third month of storage, the highest value was recorded by T_5 (600 mg nano ZnO/kg of seed) in the third month of storage (9.27 cm). It was on par with T_6 (700 mg nano ZnO/kg of seed) followed by T_4 (500 mg nano ZnO/kg of seed), while the control recorded the least value (8.45 cm).

At the end of storage, the mean seedling root length was observed to be higher in T_5 (600 mg nano ZnO/kg of seed) with value of 8.96 cm, which was on par with T_6 (700 mg nano ZnO/kg of seed) and T_4 (500 mg/kg custard leaf powder) with 8.76 cm. While the control recorded the least value (7.93 cm).

4.4.2.5 Seedling dry weight (mg)

Seedling dry weight exhibited significant variation over the months of storage, as detailed in Table 10. The average seedling dry weight showed a gradual increase up to the third month of storage and later declined as the storage period progressed, regardless of the treatments. The highest value of 25.53 mg in treatment T_5 (600 mg nano ZnO/kg of seed) in the third month of storage, while the lowest value of 23.63 mg was observed in the control at the end of storage.

In the sixth month of storage, the seedling dry weight (24.41 mg) was significantly higher in T_5 (600 mg nano ZnO/kg of seed) which was on par with T_4 (500mg nano ZnO/kg of seed) with 24.18 mg, while control recorded the lowest dry weight (23.63 mg).

4.4.2.6 Vigour index I

The data presented in Table 11, clearly indicates that there were significant difference among the treatments for the vigour index I throughout the storage period. The mean vigour index I gradually increased upto third month and later declined until the end of the storage period.

During the third month of storage, the vigour index I (1099) was significantly higher in T_5 (600 mg nano ZnO/kg of seed) which was on par with T_6 (700 mg nano ZnO/kg of seed) at 1066. While control recorded the lowest vigour index I (918).

At the end of the storage period, the vigour index I was significantly higher in T_5 (600 mg of nano ZnO/kg seed) at 985 on par with T_6 (700 mg nano ZnO/kg of seed) at 967. The lowest vigour index I (780) were observed in control.

4.4.2.7 Vigour index II

The data presented in Table 12, clearly represented the significant difference among the treatments for vigour index II throughout the storage period. From third month onwards there was a gradual reduction in vigour index II.

During the third month of storage, the vigour index II (1896) was significantly higher in T_5 (600 mg nano ZnO/kg of seed), which was on par with T_6 (700 mg nano ZnO/kg of seed) with 1886. The control recorded the lowest vigour index II (1698).

In the last month of storage, highest value for vigour index II (1710) was observed in T_5 (600 mg nano ZnO/kg of seed), which was statistically on par with T_6 (700 mg nano ZnO/kg of seed) at 1682. While the least value was recorded in control (1490).

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

Electrical conductivity (Table 13) of seed leachates varied significantly among the treatments during storage. The conductivity gradually increased over the months of storage irrespective of the treatments. The lowest electrical conductivity was recorded in the first month (90.33 µS cm^{-1}) and the highest towards the end of storage (163.83µS cm^{-1}).

In the first month, electrical conductivity of seed leachate was observed highest incontrol (99.14 µS cm^{-1}) which was on par with T_2 (300 mg ZnO/kg of seed) at 96.7 µS cm^{-1}. The least value for EC of seed leachate (90.33 µS cm^{-1}) was observed in T_5 (600 mg nano ZnO/kg of seed) followed by 700 mg nano ZnO/kg of seed (91.16 µS cm^{-1}).

At the end of storage, T_1 (control) had recorded the highest electrical conductivity (163.83 µS cm^{-1}) followed by T_2 (300 mg ZnO/kg of seeds) at 158.06 µS cm^{-1} whereas T_5 (600 mg nano ZnO/kg of seed) recorded the least value (147.8 µS cm^{-1}).

4.4.2.9 Seed moisture content (%)

The moisture content of both treated and untreated seeds showed non-significant variation at the end of storage, as indicated in Table 14. However, there was a marginal increase in seed moisture content in all the treatments in comparison with the value at the start of storage.

4.4.2.10 Seed microflora (%)

The data regarding the average seed infection per cent for each treatment at the end of the storage period is documented in Table 14. Significant variation were observed in this parameter among the various treatments.

In the blotter paper method, a lower seed infection rate was observed in T_6 (700 mg nano ZnO/kg of seed) at 48.01 per cent followed by T_5 (600 mg nano ZnO/kg of seed) with 48.37per cent however, control exhibited significantly higher seed infection of 58.85 per cent, indicating the inferior performance.

Similar observations were recorded in the agar plate method. Lower seed infection rate was observed in T_6, (700 mg of nano ZnO/kg of seed) with value of 49.03 per cent. Closely followed by T_5 (600 mg of nano ZnO per kilogram of seed) at 51.7% and highest seed infection of 62.18% observed in control (T_1), *Aspergillus* spp. were observed in all the treatments.

Discussion

5. DISCUSSION

A seed is an embryonic future plant encased within a protective seed coat. The success of any seed program depends on effectively maintaining the vigour and viability of seeds for the upcoming sowing season. The poor performance of seeds can be attributed to various factors, but one of the most critical factors is the physiological quality of seeds after storage. Ageing is an inherent and irreversible process that causes seed deterioration, resulting in the loss of both vigour and viability. Quality of seed is determined by its genetic and physical purity, health status and physiological quality (Finch-Savage and Bassel 2016). The rapid decline in the vigour and viability of most vegetable seeds during storage is primarily due to the prevailing high temperatures and relative humidity in the storage environment (Bhatia *et al*., 2002; Bellard *et al*., 2006).

The quality and longevity of seeds is significantly influenced by relative humidity and temperature, as it is directly linked to the moisture content of the seeds. (Harrington, 1972 in lettuce; Agarwal, 1993; Dey and Mukerjee, 1984 in soybean; Palanisamy, 1987 in bhendi; Khattra *et al*., 1988 in pigeon pea; Balamurugan *et al.,* 2003 in sunflower). The other factors like oxygen, pressure (Roberts and Abdalla, 1968). microflora (Bhatia *et al*., 2002) and insects (Lande *et al*., 1986 in peanut; Patil *et al*., 2006 in chickpea) also affect the quality of seeds to a greater extent.

The deterioration of seeds during storage is an inevitable process. The decline in seed vigour is typically associated with unavoidable changes that occur, leading to a reduction in germination and vigour. These changes could potentially have an impact on the performance of mature plants, ultimately lowering the final crop yield (Roberts, 1973; Chauhan and Banerji, 1983). It is impossible to completely halt the aging process in seeds, but it can be partially slowed down without compromising the seed quality. Basu *et al*. (1974) employed by mid storage hydration-dehydration treatments to manage the physiological deterioration of stored seeds from different crops. These treatments have led to increased rate and uniformity germination and improved seedling growth (Heydecker 1972; Adarsh, 2023) in chilli, extended storage potential (Basu and Pal, 1980) and enhancements in growth and yield attributes (Guha *et al*., 2012) in okra.

5.1 Effect of Mid storage treatment with nano particle on seed quality of six-month-old chilli seeds

The Mid storage seed treatment of six-month-old of chilli *cv.* Ujwala seeds had a significant impact on various seed quality aspects, including seed germination, seedling shoot length, root length, seedling vigour index, electrical conductivity, and seed microflora.

5.1.1 Initial seed quality assessment

The experiment started with a preliminary quality assessment of the six-month-old seeds. Initial germination (67.37%) was demonstrated by the seeds, resulting in a seedling shoot, root length (4.68 cm and 6.81 cm), and seedling dry weight (24.1 mg). It was determined that the vigour index I was 774 and the vigour index II was 1595. The observed value for the electrical conductivity of the seed leachate was 192.33 μScm^{-1}. Seeds had a moisture level of 7.53% at the beginning, and the per cent age of seed infection was found to be 53.30% on an agar plate method and 47.15% on a blotter method.

5.1.2 Effect of nanoparticles immediately after mid storage treatment

The germination tests conducted immediately after the mid storage treatments with different concentrations of nano zinc oxide and custard leaf powder revealed a positive impact on seed quality when compared to the untreated control. Among the various treatments, Mid storage invigoration using T_6 (700 mg of nano ZnO/kg of seed) recorded remarkably higher seed germination (73.31%), longer seedling shoots length (5.5 cm), greater root length (7.36 cm), enhanced seedling vigour index I (945), and vigour II (1771) and it exhibited lower electrical conductivity (192 $\mu S\ cm^{-1}$) compared to the control 67.04% of germination, shoot (4.53 cm), root length (6.88cm), Dry weight (23.16mg), vigour index I (765) and vigour index II(1553) with highest electrical conductivity (193.33$\mu S\ cm^{-1}$). Seed infection with agar plate method (53.30% and blotter method (47.15%). Similar observations reported by Adarsh (2023) in chilli, Renugadevi *et al*. (2006) in cluster bean and Rani *et al*. (1996) in tomato. Saha *et al.* (1990) obtained that by mid storage soaking-drying treatments of stored seeds greatly reduced the loss of vigour and maintained the viability under accelerated as well as natural ageing conditions. Singh *et al.* (1972) have reported a decline in seed quality over time in storage, which is evidenced by reduced seedling length, diminished dry matter production, and a decrease in vigour indices. Zinc oxide nanoparticles, which can enter seeds through defects in the seed coat and react with free radicals to produce increased seedling vigour, may be responsible for the elevated physiological parameters.

5.2 Effect of mid- storage correction with nanoparticles on growth and yield parameters in chilli

Crops raised from six-month-old seeds subjected to by mid storage treatments with different doses of nano zinc oxide and custard leaf powder observed a significant increase in both growth and yield characteristics when compared to the control.

5.2.1 Vegetative characters

The emergence of floral buds signals a shift from a vegetative to a reproductive phase in plants. The number of days it takes for flowering to occur is determined by a combination of inherent genetic factors and external environmental conditions, including day length, temperature, and stress. Plants respond to various stimuli by either hastening their flowering to produce seeds for the next generation or delaying it through a slowdown in their metabolic processes (Cho *et al*., 2017).

The number of days required for the first flowering and reaching 50 per cent flowering exhibited variation within the treatments, ranging from 74.22 days (T_7- nano custard leaf powder @ 500 mg kg^{-1} of seed) to 78 days (T_1- control) and 87.17 days (T_6- nano ZnO @ 400 mg kg^{-1} of seed) to 87.17 days (T_1- control) respectively. The observed difference among the treatments were statistically significant, occurring approximately five days before the control. Similar observations were reported by Sandhya (2016) in chilli, where the seed treatment with 0.5 g kg^{-1} nano powder of custard leaf resulted in a reduction of eight days in the time to flowering compared to the control. Seed treatments with 400 mg/kg of nano ZnO resulted in an early onset of 50 per cent flowering at 82.67 days, compared to the control in chilli (Adarsh, 2023). Laware and Raskar (2014), suggested that zinc induced early flowering in plants, by providing essential plant growth-promoting compounds that effectively supported the reproductive phase.

The mean plant height showed significant variation among the different treatments, ranging from 65.27 cm in control (T_1) to 80.40 cm in T_5 (700 mg of nano ZnO/kg of seed).

Plant height is a significant morphological quantitative characteristic that has a direct correlation with yield. It is a visible measure of plant growth and is a function of leaf emergence and internodal elongation. As leaves and branches develop on the stem, the development of leaf area and biomass production is closely associated with plant height. The impact of seed treatment was evident in the rapid growth observed in plant height. The results obtained were consistent with the research findings of Adarsh (2023) in chilli, wherein the seed treatment with nano ZnO @ 400 mg/kg of seed exhibited the tallest plant height of 42.94 cm. In okra, by mid storage soaking and drying treatment resulted in a remarkable increase in plant height (67cm) (Guha *et al*., 2011).

The treatments had a significant impact on the branches per plant, with values ranging from 4.93 in the control (T_1) to 5.80 in the treatment with nano ZnO600 mg /kg of seed (T_5). The development of branches is a dynamic process subjected to various genetic and environmental factors (McSteen and Leyser, 2005). The sufficient availability of zinc to the plant may have amplified its catalytic or stimulatory effects on various physiological and metabolic processes within the plant Nagar *et al.* (2022) in tomato. Similar observations were also reported by Adarsh, (2023) and AL-Zuhairi *et al*. (2020) in chilli.

5.2.2 Yield parameters

Every plant progresses through both vegetative and reproductive growth phases to fulfill its life cycle. Yield is considered to be the ultimate manifestation of the physiological and metabolic processes of plant life, influenced by numerous factors. The elements contributing to yield remarkably impact the plant productivity and play a pivotal role in enhancing overall crop production. The utilization of nanoparticles as a by mid storage seed treatment had a significant influence on both seed yield and fruit yield.

The factors contributing to fruit yield, such as the number of fruits per plant, exhibited higher values with seed treatment involving 600 nano ZnO mg/kg (112.57)

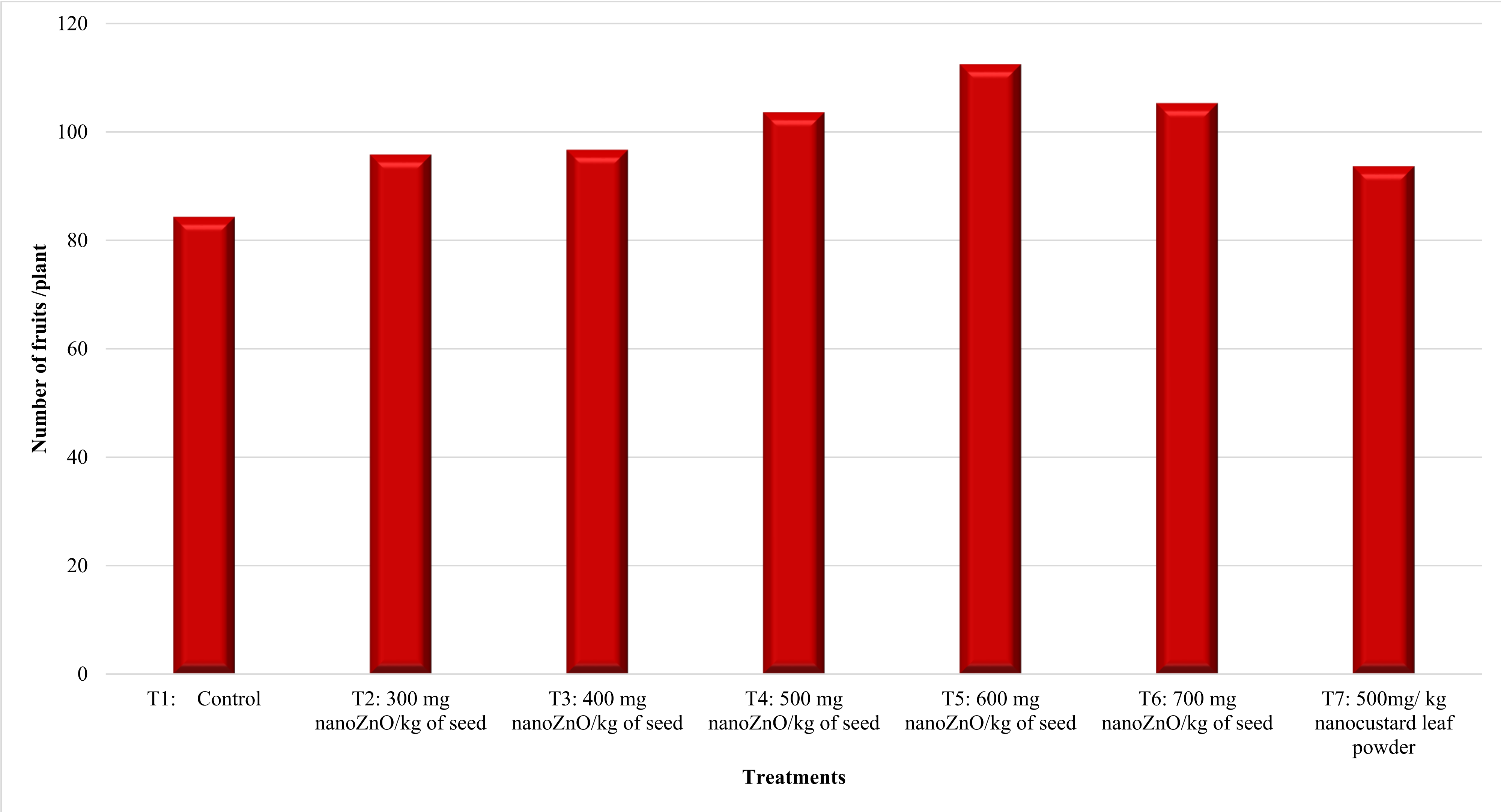


Figure 1. Effect of midstorage correction with nanoparticles on fruits per plant

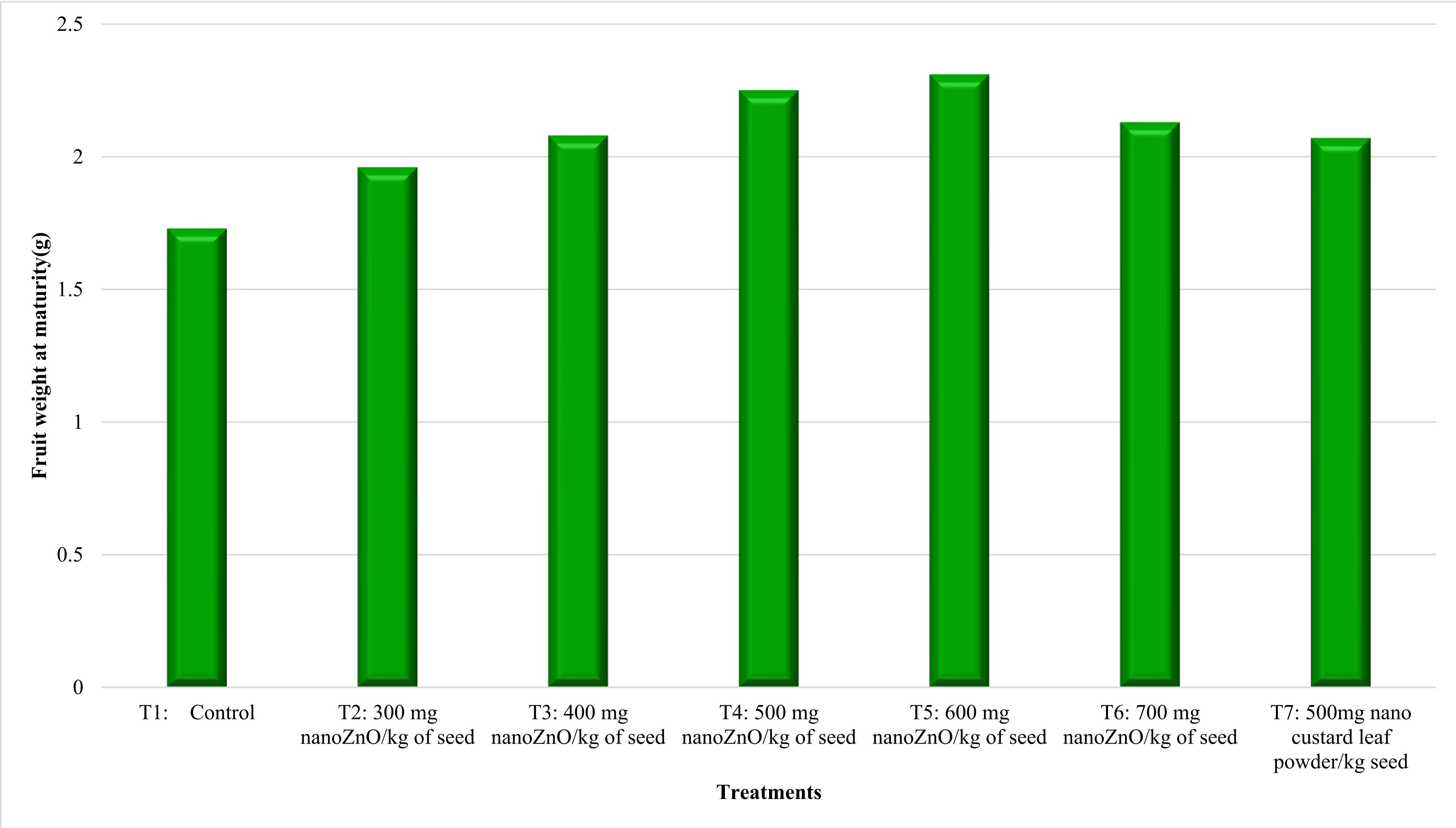


Figure 2. Effect of midstorage correction with nanoparticles on Fruit weight at maturity (gm)

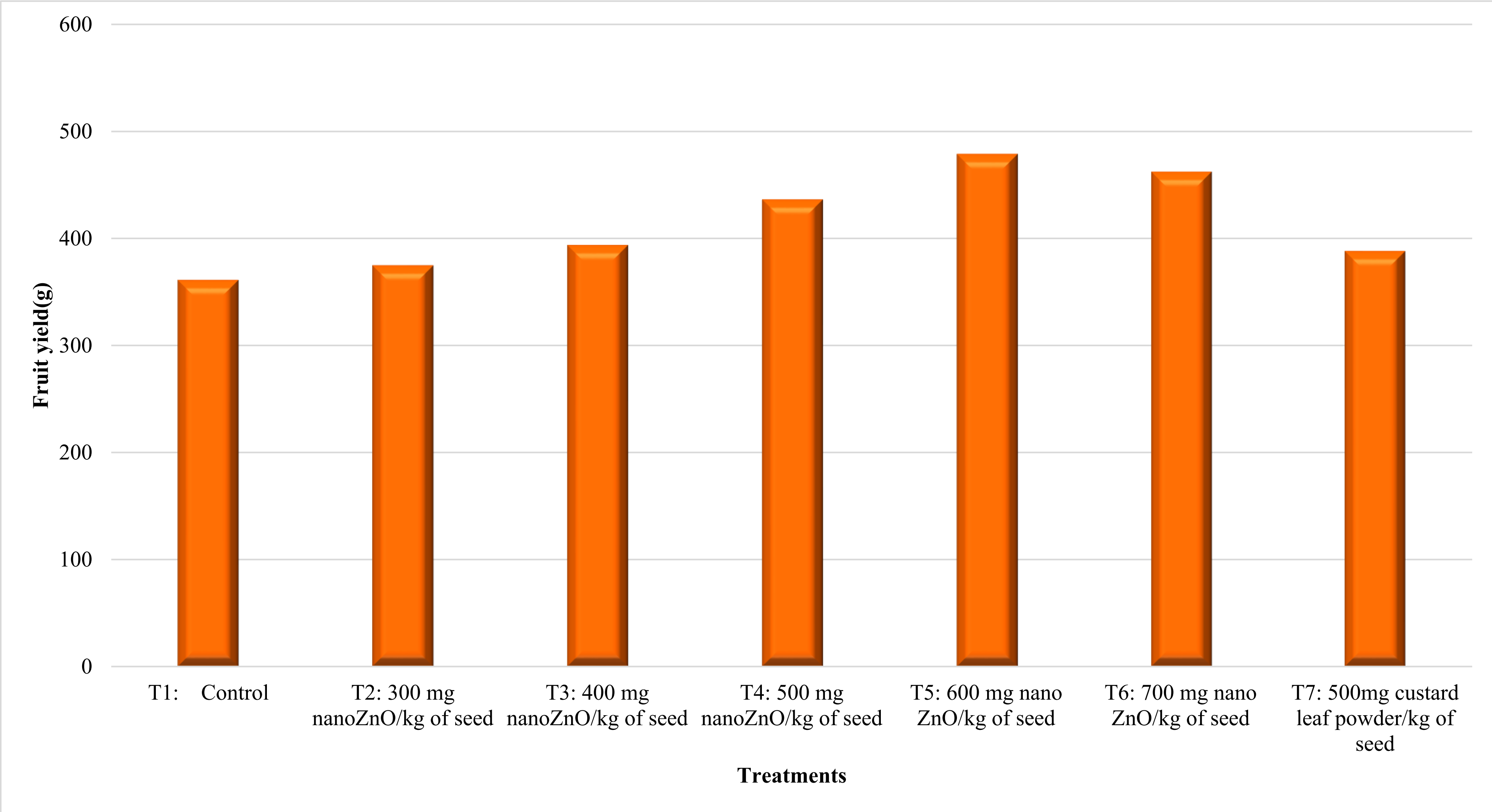


Figure 3. Effect of midstorage correction with nanoparticles on Fruit yield per plant (g)

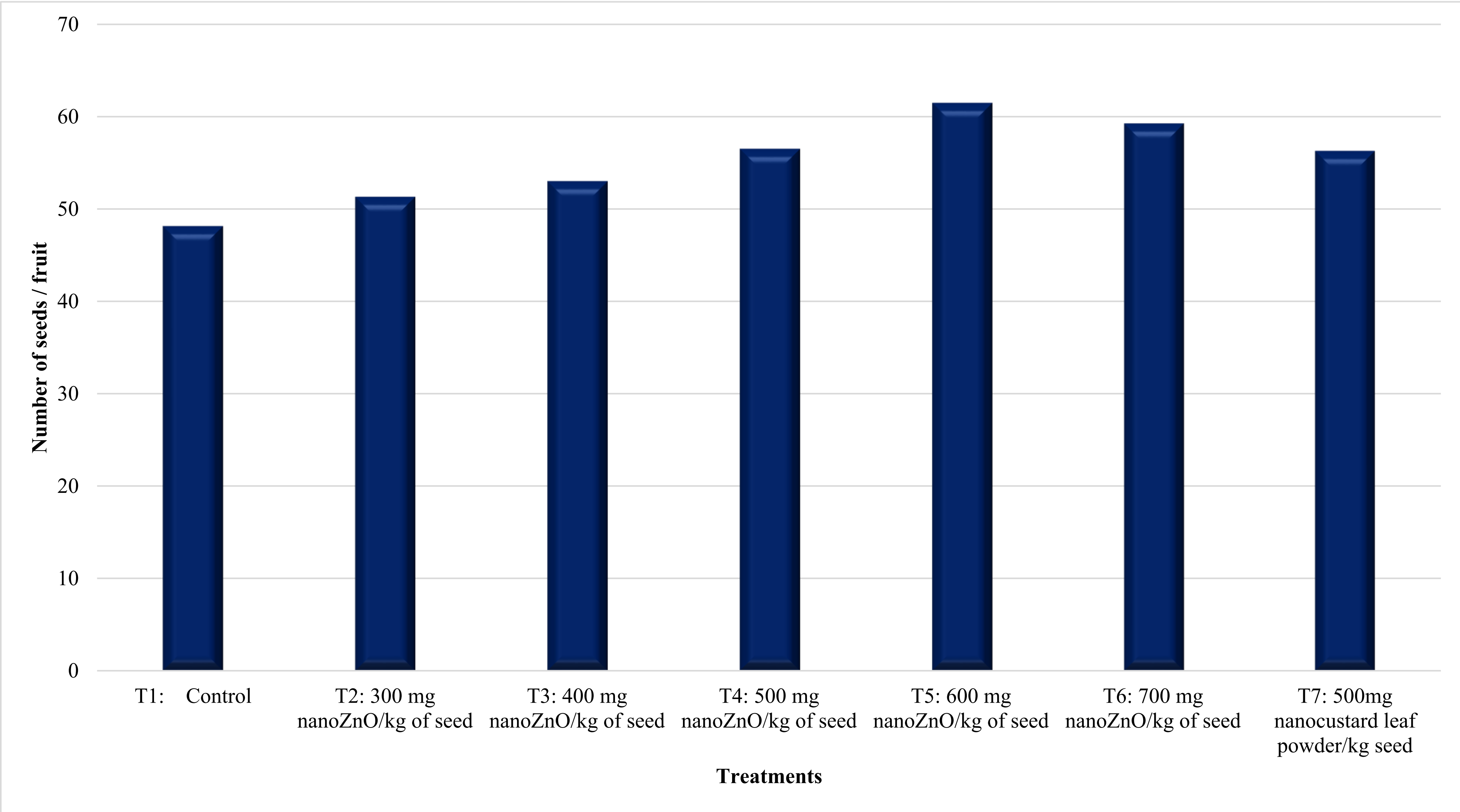


Figure 4. Effect of midstorage correction with nanoparticles on Number of seeds per fruit

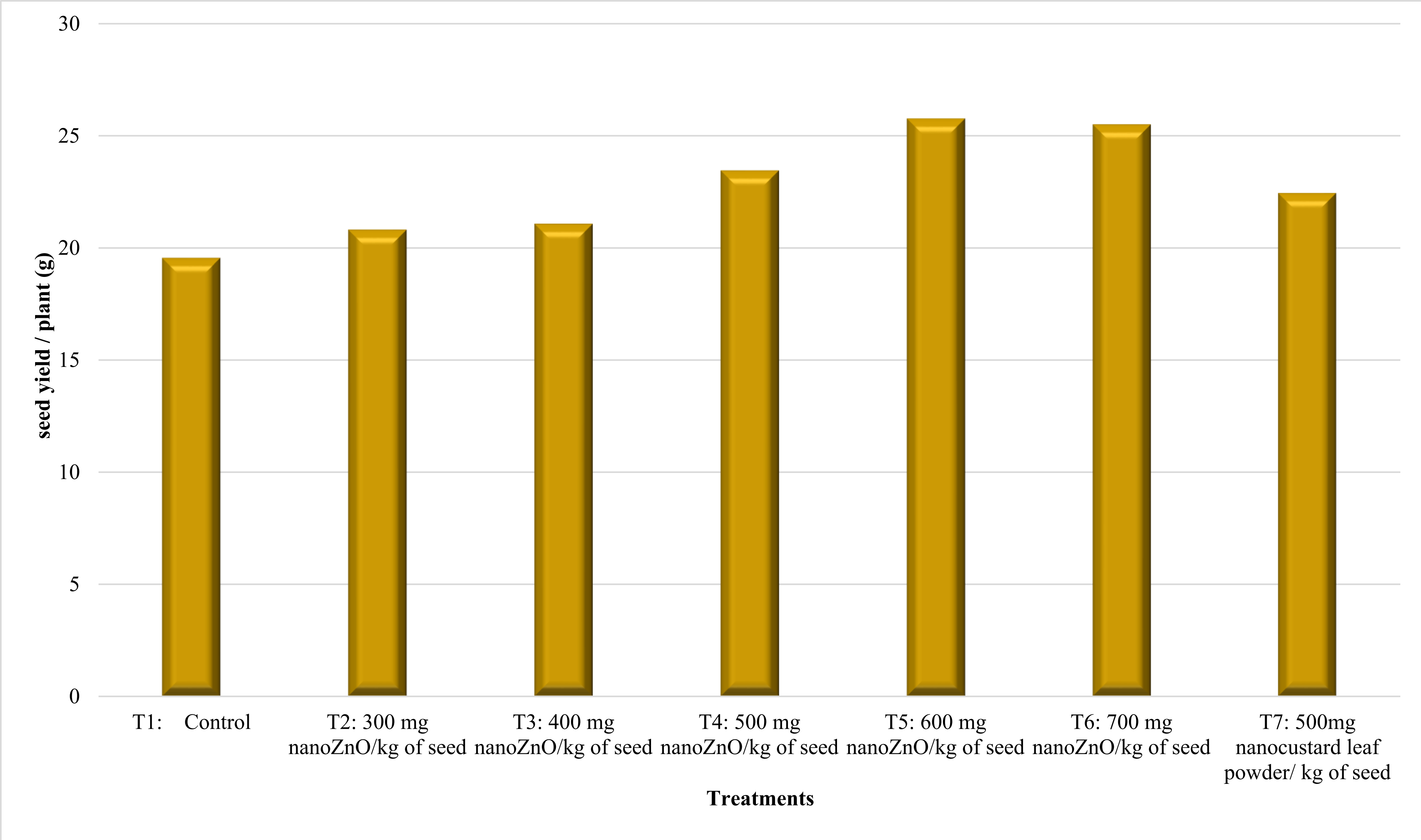


Figure 5. Effect of midstorage correction with nanoparticles on Seed yield per plant

compared to control (84.30) and the fruit length was greater in the seed treated with 700 nano ZnO mg/kg (5.55 cm). Fruit weight at maturity (2.31 g) and fruit yield per plant (478.73 g) were higher in the seed treatment with 600 nano ZnO mg/kg of seed. The applied ZnO NPs ensured an increase in yield due to improvement in the yield attributing characters of the treated plants. The synthesis of proteins, carbohydrates, chlorophyll, and indole-acetic acid (IAA) all depend on zinc. Moreover, it reduces the uptake of cadmium (a heavy metal) by plants which helps in the production of cytochromes and the detoxification of reactive oxygen species (ROS) (Buchanan et al., 2000). A rise in IAA levels was seen in the roots of chickpea seeds treated with nano ZnO, and this, in turn, led to an increase in plant development (Avinash et al., 2010). Particle size affects the agronomic effectiveness of zinc treatments. Particle size reduction increases the number of particles per unit weight, which increases surface area (Mortvedt, 1992). This increases zinc absorption. These nanoparticles are very active, have an excellent catalytic surface, can absorb more water, and disperse quickly (Khanm et al., 2017).

The seed yield per plant was significantly increased when seeds were treated with nano ZnO @ 600 mg/kg of seed, resulted in yield of 25.76g while the yield in control yielded was19.55g. Moreover, the number of seeds per fruit was also positively impacted by the same treatment, with an average of 61.50 seeds per fruit compared to 48.17 seeds in the control. The hundred-seed weight was enhanced when seeds were treated with nano ZnO @ 700 mg /kg of seed, yielding a weight of 0.43 grams per hundred seeds, in contrast to the control, which had a weight of 0.35 grams per hundred seeds. The results obtained are in consistent with the research findings of Guha *et al.* (2011), in by mid storage soaking and drying seed treatment in okra. Adarsh (2023) and Mathew *et al*. (2021) reported comparable results in the context of seed treatment with nano zinc oxide in chili. This may be due to the increased accumulation of nutrients in the seeds over those obtained from other treatments and control. Zinc is recognized as a vital element in protein synthesis and plays a crucial role in multiple enzyme processes, carbohydrate metabolism, and nitrogen fixation, ultimately contributing to the enhancement of seed yield (Yusefi-Tanha et al., 2020).

5.3 Seed storage studies

5.3.1 Evaluation of initial seed quality

The results obtained from the initial quality assessment of seeds obtained in Experiment I indicated the presence of significant variation among the treatment groups for parameters such as germination per cent, shoot length, root length, vigour indices and seed microflora. Among the various concentrations of nano zinc oxide tested, the treatment with nano zinc oxide at 600 mg/kg resulted in the highest germination rate (68.07%). While control exhibited the lowest germination per cent of59.80%.

The root length and shoot length exhibited significant difference with the application of nanoparticles. Seeds treated with zinc oxide nanoparticles at a concentration of 600 mg kg^{-1}, it resulted in the highest shoot length of 5.2 cm and the longest root length of 8.95 cm. In comparison, the control recorded shoot and root lengths of 4.58 cm and 7.53 cm respectively. Seedling dry weight (24.5 mg), seed vigour index I (963) and seed vigour index II (1640), were achieved in the treatment 600 mg of nano ZnO per kg of seed.

Highly significant difference was observed for the presence of pathogens in the seeds, due to nanoparticle seed treatment. Among the nanoparticles treatments, seeds treated with ZnO nanoparticles at a concentration of 600 mg/kg exhibited the lowest seed pathogen infection rate in the agar plate method (0.9%) and in the blotter method (1%). Whereas the control showed the highest seed pathogen infection rate in the agar plate method (3.17%) and blotter method(1.83%).

5.3.2 Seed quality assessment during storage

5.3.2.1 Germination (%)

Significant difference were observed among the treatments for germination per cent from first month to six months of storage. The treatments recorded the highest mean germination per cent at the end of the third month of storage and then reduced gradually irrespective of the treatments with the advancement of storage period. The increased viability after the first month of storage might be due to the primary dormancy found in chilli during the early stages of growth. Fresh chilli (Evans, 1984; Herbert, M. 2020) and tomato (Hilhorst *et al.*, 1998) seeds continue to grow after

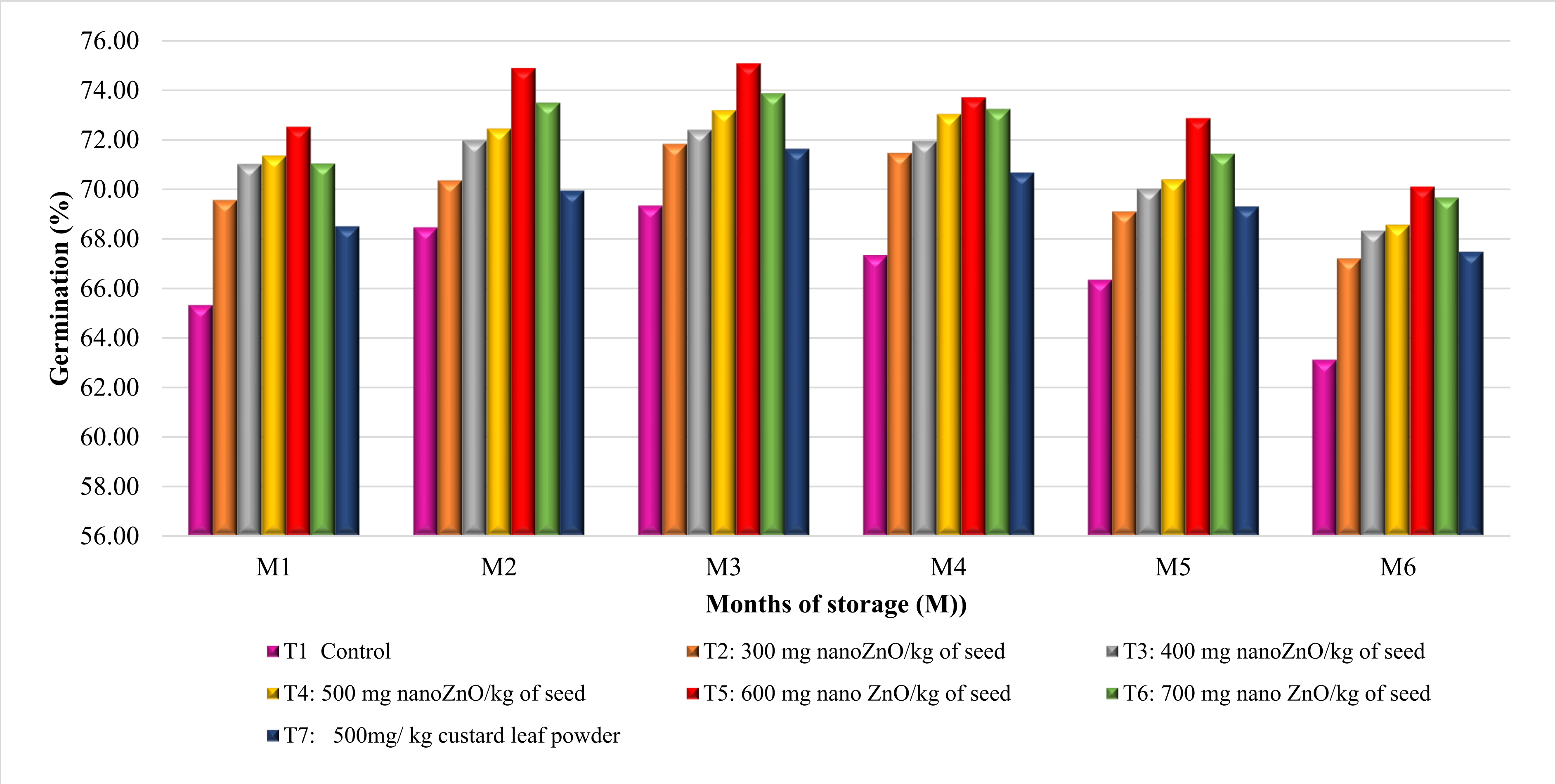


Figure 6. Effect of seed invigoration treatments with nano particles on germination percent (%) in chilli after storage at monthly intervals

harvest, resulting in a decrease in abscisic acid concentration and hence improved germinability. Such gradual reduction in germination per cent with progress of seed ageing was earlier observed by Adarsh (2023), Mathew *et al*. (2013), Navya (2016) and Gayathri (2019) in chilli.

Seed invigoration with different quantities of nano scale particle was able to retain the IMSCS set standards of germination (minimum 60.0 %) up to six-month seed storage. The best dosage was found to be nano ZnO 600 mg/kg of seed (75.07%), which was on par with nano ZnO @ 700 mg/kg seed (73.87%) in the third month. At the end of six-month of storage, seed treatment with 600 mg nano ZnO/kg of seed showed highest germination per cent (70.10%) and whereas lower germination was observed in control (63.11%).

Superior performance of the treatments, nano ZnO 600 mg/kg of seed (T_5), and nano ZnO @ 700 mg/kg seed (T_6) for increased germination per cent observed over the months of storage can be attributed to the improved seed quality resulting from the specific treatments applied in experiment I. The higher seed germination per cent age of ZnO nanoparticles attributed to the higher activity of nano zinc in auxin production (Kobayashi and Mizutani, 1970). This is most likely due to nanoparticles penetrating the cell wall and plasma membrane of root and shoot epidermal cells and accumulating in the vascular tissues, resulting in enhanced cell division and cell elongation.

Zinc oxide nanoparticles were observed to have a positive effect on germination in chilli (Adarsh, 2023, Mathew *et al*., 2013, and Gayathri, 2019) and Maize (Mahesh *et al*., 2022).

5.3.2.2 Seedling length (cm)

Seedling length was greatly affected by storage duration and seed treatments. Seedling shoot and root length significantly varied among the treatments upto sixth months of storage. As the storage period progressed, there was a noticeable decline in shoot and root length, particularly after the third month. When compared to all other treatments, untreated seeds declined more quickly. Adarsh (2023), Navya (2016), and Sandhya (2016) recorded a similar pattern in chili.

After six months of storage of seeds treated with nano ZnO @ 600 mg/kg (T_5) resulted in seedlings with longer shoots (5.10cm). while the least value was recorded in control (4.43 cm). Studies conducted by Maity *et al.* (2018) revealed that lower doses of nano scale zinc oxide (750 mg/kg) were beneficial to shoot length in sorghum seeds.

By the end of the storage period, the average root length of seedlings was notably greater, with 8.97 cm in T_5 (nano ZnO @ 600 mg/kg), which was on par with T_7 (nano ZnO @ 700 mg/kg), which exhibited a length of 8.83 cm, while control recorded 7.93 cm. Similar results were observed by Adarsh (2023), Mathew *et al.* (2021), Gayathri (2019) and Navya (2016) in chilli. Zinc oxide nanoparticles have the potential to elevate the concentration of indole acetic acid in the roots or sprouts, subsequently enhancing the growth rate of seedlings (Pandey *et al*., 2006). Zinc improves the cation exchange capacity of the roots, leading to an increased absorption of crucial nutrients like nitrogen. Availability of Zinc to seed during seed germination have an important physiological role in seedling growth (Kumar *et al*., 2019).

5.3.2.3 Seedling dry weight (mg)

Seedling dry matter content is a measure of physiological vigour and is frequently controlled by metabolites, plant growth hormones and enzymatic activities. (Qualls and Cooper, 1968).

The influence of zinc oxide on seedling dry matter production was prominent during the initial month after storage. However, it was observed that this effect diminished after the third month of storage, regardless of the specific treatments applied. Following a six-month period of storage, the dose of nano ZnO 600 mg/kg showed higher dry matter production in seedlings (24.41 mg/10 seedlings) and the least value was obtained in control (23.36 mg/10 seedlings). Seed treatment with zinc oxide NPs was able to promote the biomass production in chilli as reported by (Adarsh, 2023, Gayathri, 2019, Mathew *et al*., 2021), Maize (Mahesh *et al*., 2022) okra (Keerthana *et al.*, 2021). Zinc is an important micronutrient for plant growth because it supports a number of enzymes that are in the role of controlling different metabolic processes in plants.

5.3.2.4 Vigour index

Seed vigour is the sum of those properties which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence. The vigour index I and II were calculated using an equation developed by Abdul-Baki and Anderson (1973) and is the product of the germination per cent and the seedling length (cm) or seedling dry weight (mg), respectively.

Mean vigour index I and II reached its maximum in the third month (1099 and 1896, respectively), and then gradually decreased until the end of storage. At the end of the six-month storage period, T_5 (600 mg nano ZnO/kg of seed) had significantly higher vigour index I and II values of (986 and 1710, whereas the control recorded the lowest values (780 and 1490). The studies revealed disparate findings by Adarsh, 2023, Gayathri, 2019, Mathew *et al*., 2021) in chilli, Anandaraj and Natarajan (2017) in onion and Meenakshi *et al.* (2020) in Sunflower. The increased per cent age of seed germination and significantly longer seedling length observed in chilli seedlings from seeds obtained from plants treated with zinc oxide nanoparticles it might be due to the translocation of zinc (Zn) from the leaf tissues through the phloem to the seeds during the seed development and maturation process. Nanoparticles donate electrons, which scavenge the free radicals generated in aging seeds, thereby enhancing the seedling vigour index (Kumar *et al*., 2019).

5.3.2.5 Electrical conductivity

The Electrical Conductivity (EC) test serves as an indicator of cell membrane integrity and it has been strongly correlated with plant vigour and field emergence in certain crop species. The fundamental principle behind the EC test is that seeds with lower vigour or greater deterioration exhibit a slower rate of repair of their cell membranes during the water uptake process for germination. As a result, they release higher quantities of solutes into the external environment (Marcos- Filho, 2015).

Both treated and high-quality seeds have the capacity to maintain membrane integrity for extended periods of time, resulting in lower electrical conductivity values recorded from the seed leachates.

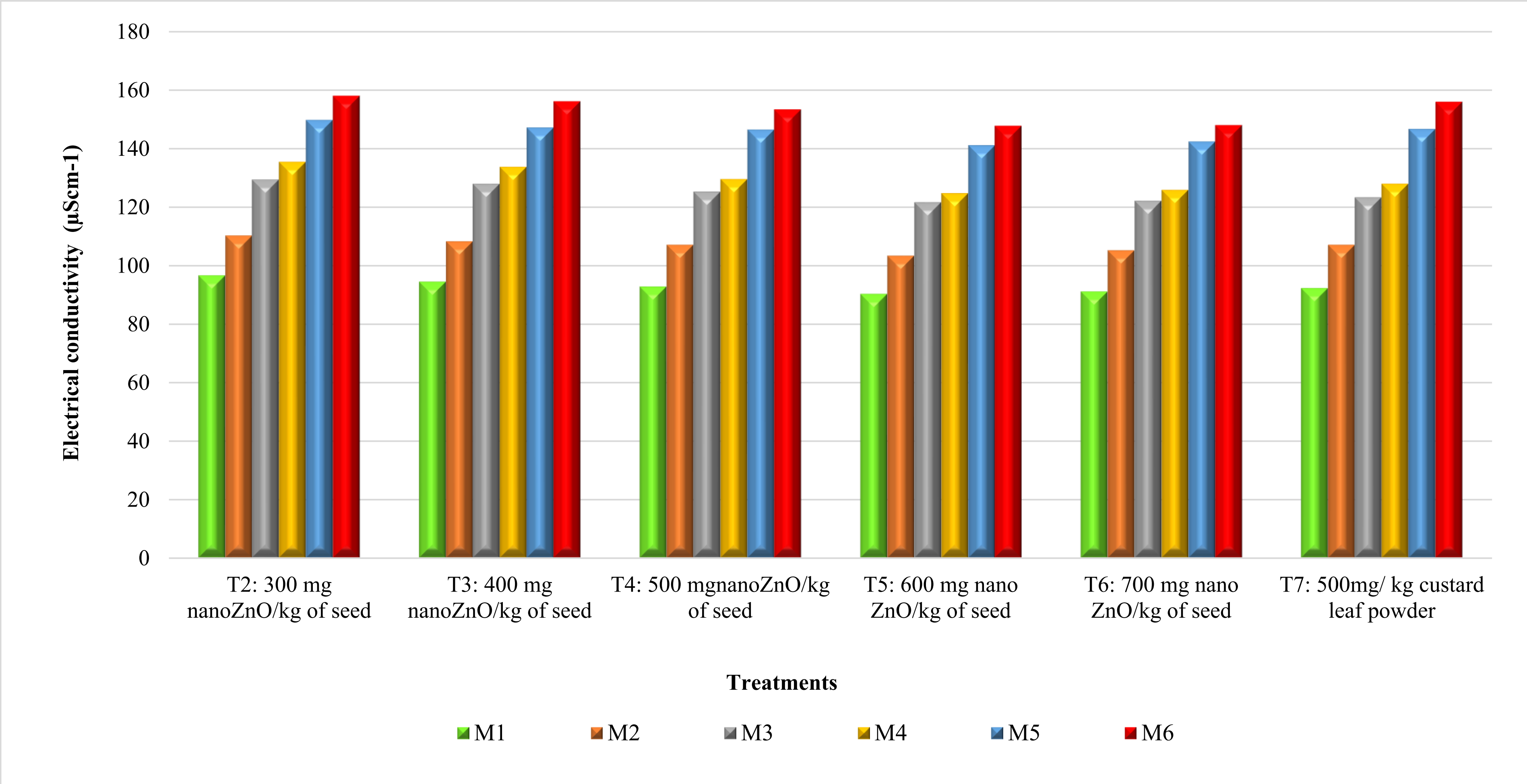


Figure 7. Effect of seed invigoration treatments with nano particles on germination percent (%) in chilli after storage at monthly intervals

The electrical conductivity of seed leachates varied significantly among the treatments throughout the storage period. The conductivity gradually increased over the months of storage irrespective of the treatments. Lowest mean electrical conductivity was recorded in the first month and increased towards the end of storage. When compared to control, the highest mean electrical conductivity (EC) was observed in the first and last months of storage (99.14 µS cm^{-1} and 163.83 µS cm^{-1}, respectively). The results are in line with the findings of Adarsh (2023), Gayathri (2019), Mathew *et al*. (2021) in chilli and Shyla and Natarajan (2016) in peanut.

5.3.2.6 Seed moisture content (%)

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

5.3.2.7 Seed microflora

At the end of the storage period, it was observed that the level of seed infection in the ZnO treated seeds was minimal compared to control. Both the blotter paper (58.85%) and the agar plate method (62.18%) showed higher infection in the control and lower infection in seeds treated with 700 mg nano ZnO/kg of seed (blotter paper (48.01%) and the agar plate method (49.03%). This suggests that ZnO is beneficial for providing protection against seed-borne pathogens. Nanoparticles may have an antimicrobial effect on bacteria, viruses, and fungi because of their interaction with their outer membranes, which can inhibit respiration and other metabolic processes and possibly cause death (Kumar *et al.,* 2015). Positive effect of zinc oxide nanoparticles rendering protection against microbial action was reported by (Adarsh ,2023), Gayathri (2019), Mathew *et al*. (2021) in chilli and Chaudhari *et al*. (2022) in groundnut. Antifungal activity of zinc oxide nanoparticles has been reported by Jasim (2015) against *Aspergillus* ssp. zinc oxide nanoparticles cause deformed sporulation, swelling of hyphae, structural malformation and melanisation of hyphae which led to effective antifungal activity.

Table 15: Ranking of nanoparticle treatments based on growth, yield and seedquality parameters

Treatments		T_1	T_2	T_3	T_4	T_5	T_6	T_7
Growth and seed yield parameters		**Ranks**						
Days to first flowering		6	5	4	3	2	2	1
Days to 50 per cent flowering		6	5	4	3	2	1	3
Plant height (cm)		7	6	5	4	3	1	2
Branches per plant		6	5	4	3	1	2	4
Fruits per plant		7	5	4	3	1	2	6
Fruit length (cm)		7	6	5	4	3	1	2
Fruit weight (g)		7	6	4	2	1	3	5
Fruit yield per plant(g)		7	6	4	3	1	2	5
Seeds per fruit		7	6	5	3	1	2	4
Seed yield per plant (g)		7	6	5	3	1	2	4
Hundred seed weight (g)		7	6	5	3	2	1	4
Score		**74**	**62**	**49**	**34**	**18**	**19**	**42**
Rank		**7**	**6**	**5**	**3**	**1**	**2**	**4**
Seed quality parameters								
Germination per cent (%)		7	6	4	3	1	2	5
Shoot length (cm)		7	6	5	3	1	2	4
Root length (cm)		7	6	5	3	1	2	4
Seedling dry weight(mg)		7	5	4	2	1	3	6
Electrical conductivity		7	6	5	3	1	2	4
Vigour index I		7	6	5	3	1	2	4
Vigour index II		7	5	4	3	1	2	6
Seed microflora	Blotter paper method	7	6	5	4	2	1	3
	Agar plate method	7	6	5	4	2	1	3
Score		**63**	**52**	**42**	**28**	**11**	**17**	**39**
Rank		**7**	**6**	**5**	**3**	**1**	**2**	**4**

Ranking and scoring

Treatments were ranked according to their highest to lowest value given scores by assaying 1 to a, 2 to b, 3 to c and 4 to d average the total score obtained from each treatment according to score rank is given in from higher to lower value to determine the best treatments (Herbert, 2020).

The by mid storage seed treatment with nano ZnO @ 600 mg kg^{-1} of seed (T_5) clearly showed the best field performance and seed quality, as verified by the scores obtained from nanoparticle treatments for growth, yield, and seed quality parameters in chilli (Table 15). This treatment was followed by nano ZnO @ 700 mg kg^{-1} of seed (T_6) and nano ZnO @ 500 mg kg^{-1} of seed (T_4).

The present study revealed the positive impact of zinc oxide and custard apple leaf nanoparticles in by mid storage correction with aged seeds. It is one of the physiological seed management techniques, which effect improvement in physiological status of seed, in order to reduce the deterioration process, improve field performance, seed quality and longevity in aged seeds. Mid storage hydration and dehydration lead not only to cellular repair but also to the control of free radical reactions. 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. Mid storage correction techniques, including the use of nanoparticles like zinc oxide, can help counteract the natural decline in seed quality and viability that occurs during storage. This is particularly important for long term seed storage where maintaining the germination potential is critical.

Mid storage correction is crucial for extending the storability of vegetable seeds like chilli, which are expensive and require careful handling to ensure their quality.

The present study is in concurrence with the reports that the mid storage seed treatment is effective in extending the longevity in chilli seeds. Mid storage treatments have to be tried in other ruling varieties of chilli before conclusive recommendations can be given.

Summary

6. SUMMARY

The study "Mid storage seed invigoration with nanoparticles 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 mid- storage correction with nanoparticles on crop performance in chilli

The initial quality assessment of the six-month-old seeds was carried out at the beginning of the experiment. Seeds exhibited an initial germination (67.37%), with seedling shoot and root length (4.68 cm and 6.81 cm), respectively and seedling dry weight (24.1mg). The vigour index-I and vigour index-II were calculated as 774 and 1595 respectively. The electrical conductivity of the seed leachate was recorded at 192.33 µS cm^{-1}. The initial moisture content of the seeds was 7.53 percent, and the seed infection percent was observed to be 47.15% and 53.30 % in blotter method and agar plate method respectively.

Six-month-old seeds were assessed for their quality immediately after Mid storage seed treatments with different doses of nano ZnO and custard leaf powder, Among the various treatments, mid storage invigoration using T_6 (700 mg of nano ZnO/kg of seed) recorded significantly higher seed germination (73.31%), seedling shoot length (5.5 cm), greater root length (7.36 cm), and enhanced seedling vigour index I (945), and vigour index II (1771). It exhibited significantly lower electrical conductivity (192 µS cm-1) compared to the control.

Seeds treated with nano ZnO and nano custard leaf powder were raised to assess their field performance. It was observed that treated seeds exhibited superior performance in comparison to untreated seeds. Significant differences were seen in days to the 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 and 100 seed weight.

- Nano ZnO @ 700 mg kg^{-1} of seed (T_6) performed superior for days to 50.0 per cent flowering (82.67 days), fruit length (5.55 cm), fruit yield per plant (224.56g), plant height (80.40cm) and 100 seed weight (0.433g).
- Nano ZnO @ 600 mg kg^{-1} of seed (T_5) performed superior for branches per plant (5.8), fruits per plant (112.57), fruit weight (2.31g), fruit yield per plant (478.73g), seeds per fruit (61.50), and seed yield per plant (25.76g).
- Nano custard leaf powder @ 500 mg kg^{-1} of seed (T_7) performed superior for days to first per cent flowering (74.22 days).
- After scoring and ranking, it was noted that in the context of mid storage seed invigoration with nanoparticles in chilli, the treatment involving nano ZnO @ 600 mg kg^{-1} of seed (T_5) outperformed all other treatments

6.2 Effect of nanoparticles on seed storage

Seeds treated with nanoparticles during mid storage were sown in the field, and the harvested seeds were packed in 700-gauge polythene bags, and stored under ambient conditions for a duration of six months. Significant variations was observed among the treatments with respect to germination, shoot length, root length, seedling dry weight, vigour indices, and electrical conductivity of seed leachate during the storage period.

- The treatments recorded the higher germination percentage. seedling length, seedling dry weight and seed vigour indices at the end of the third month of storage and then reduced gradually irrespective of the treatments with the advancement of storage period. The electrical conductivity and microflora infection significantly increased throughout the storage period.
- After a three-month storage period, it was observed that nano ZnO applied at @ 600 mg kg^{-1} of seed exhibited superior performance in various seed quality parameters, including germination percentage (75.07%), shoot length (5.37 cm), root length (9.27 cm), seedling dry weight (25.53 mg), vigour index I (1099), and vigour index II (1896).
- At the end of the study period, nano ZnO applied at 600 mg kg^{-1} of seed maintained good seed quality, with a germination percentage of 70.10 per cent, shoot length of 5.10 cm, root length of 8.97 cm, seedling dry weight of 24.41 mg, vigour index I of 986 and vigour index II of 1710.

- Electrical conductivity, was highest in the control (163.83 µS cm^{-1}) and lowest in the seeds treated with Nano ZnO @ 600 mg kg^{-1} of seed (147.80 µS cm^{-1}) after six months of storage.
- Seed infection in agar plate method was noticed in treatment (T_5) nanoZnO @ 600 mg kg^{-1} of seeds (49.03%) and blotter paper method (48.01%) at the end of storage.
- Considering the scoring and ranking results, it can be recommended that Nano ZnO @ 600 mg kg^{-1} of seed (T_5) is an effective choice for improving most of the seed quality parameters during mid storage correction.

Seed treatment with nano ZnO @ 600 mg kg^{-1} was determined to be effective in improving crop performance, preserving seed quality.

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Appendix

Appendix-I

Monthly meteorological data during the study period October 2022 to November 2023

Months	Temperature		Relative humidity(%)	Rainfall (mm)	Rainy days
	Mean maximum (^oC)	Mean minimum (^oC)			
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
January2023	32.7	21.9	56.0	00.0	0.0
February2023	35.4	21.9	49.0	00.0	0.0
March2023	36.1	24.2	57.0	1.7	0.0
April2023	36.8	25.5	67.0	108.3	2.0
May2023	34.7	25.7	74.0	23.4	4.0
June2023	31.8	24.4	83.0	276.3	16.0
July2023	29.6	23.7	88.0	667.3	21.0
August2023	32.5	24.4	77.0	25.3	3.0
September2023	28.3	23.7	87.0	516.7	25.0
October2023	30.4	23.8	79.0	186.8	14.0

Abstract

Mid storage seed invigoration with nanoparticles in chilli (*Capsicum annuum* L.)

By

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(2021-11-129)

ABSTRACT

Submitted in partial fulfilment of the
requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

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2023

ABSTRACT

The entitled "Mid storage seed invigoration with nanoparticles in chilli (*Capsicum annuum* L.)" was carried out in the Department of Seed Science and Technology, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur during the year 2022-2023. The study aimed to assess the effect of nanoparticles on crop performance, seed yield, quality and longevity when administered as midterm storage treatment.

The initial quality assessment of the six-month-old seeds was carried out at the beginning of the experiment. Seeds exhibited an initial germination of 67.37%, with seedling shoot and root length with value of 4.68 cm and 6.81 cm, respectively and seedling dry weight of 24.1mg. The vigour index-I and II were calculated as 774 and 1595 respectively. The electrical conductivity of the seed leachate and initial moisture content were 192.33 µS cm^{-1} and 7.53 % respectively, and the seed microflora was observed to be 47.15per cent (blotter method) and 53.30 % (agar plate method). The seeds were imposed with different treatments of nano zinc oxide (300, 400, 500, 600, and 700mg of nano ZnO) and 500 mg nanosized custard leaf powder per kilogram of seed. The six treatments along with untreated control formed the basis of the study. The quality parameters of treated seeds were studied and significant variation was noticed among treatments. Seeds treated with 700 mg of nano zinc oxide per kilogram of seed recorded significantly higher seed germination (73.31%) and enhanced seedling vigor indices (945, 1771). Mid storage treatment could enhance the germination and vigour of seeds.

The treatments along with control (seven treatments) were raised in a randomized block design with three replications. Observations on growth and yield parameters were recorded at appropriate growth stages in the tagged plants. While all nanoparticles treated seeds performed better than control (untreated seeds) treatments.

Seed treatment with nano ZnO @ 700 mg kg^{-1} of seed (T_6) performed superior for plant height (80.40 cm), days to 50% flowering, (82.67 days), fruit length (5.55 cm), 100 seed weight (g). Treatment with nano ZnO @ 600 mg kg^{-1} of seed (T_5) was effective in improving branches per plant (5.80), fruits per plant (112.57), fruit weight at maturity (2.31g), fruit yield per plant (478.73g), seeds per fruit (61.50), seed yield per plant (25.76g) and 100 seed weight (0.433g). Significant effects were recorded when seeds were treated with nano

custard leaf powder @ 500 mg kg^{-1} of seed (T_7) for days to first flowering (74.22 days).

Seeds obtained from Experiment I were used for seed storage studies (Experiment II). The seeds were dried to a moisture content below eight per cent, bagged in 700 gauge polyethylene bag and stored under ambient conditions for six months. Samples were drawn at monthly interval and seed quality parameters were analyzed. The experiment was laid out in a Completely Randomized Design (CRD) with seven treatments T_1 to T_7 as in experiment I with three replications.

Seed quality parameters such as germination per cent, vigour indices and seedling dry weight declined with the progress of storage, whereas electrical conductivity of seed leachates increased over the storage period. Significant difference were observed among the treatments for germination per cent from first month onwards to the end of storage. All treatments including the control maintained MSCS (Minimum Seed Certification Standard) of 60 per cent germination till the end of six months of storage. At the end of storage, T_5 (Nano ZnO @ 600 mg kg^{-1} of seed) followed recorded higher germination per cent (70.10%), vigour index I and II (986 and1710) respectively.

The influence of nano particle treatments on seed infection was found significant at the start and end of storage. Lowest seed infection rate was observed in seeds treated with nano zinc oxide @ 600 mg kg^{-1} of seeds (T_5) in agar plate method (49.03%) and blotter paper method (48.01%).

Among the treatments, T_5 (nano ZnO @ 600 mg kg^{-1} of seed) was found to have multiple benefits of enhancement of growth and seed yield attributes and maintenance of seed quality during storage. T_6 (nano ZnO @ 700 mg kg^{-1} of seed) and T_4 ((nano ZnO @ 500 mg kg^{-1} of seed), were the next two treatments in term of different parameters.

Hence, it is concluded that mid storage seed treatment with nano ZnO @ 600 mg kg^{1} of seed effectively enhanced seed yield, quality, and longevity in the chilli variety, Ujwala.

സംഗ്രഹം

തൃശ്ശൂർ വെള്ളാനിക്കരയിലെ കേരള അഗ്രികൾച്ചറൽ യൂണിവേഴ്സിറ്റിയിലെ കോളേജ് ഓഫ് അഗ്രികൾച്ചറിലെ സീഡ് സയൻസ് ആൻഡ് ടെക്നോളജി ഡിപ്പാർട്ട്മെന്റിൽ "മുളകിലെ നാനോപാർട്ടിക്കിളുകളുള്ള മിഡ് സ്റ്റോറേജ് സീഡ് ഇൻവിഗറേഷൻ" എന്ന തലക്കെട്ടിൽ നടത്തി (2022-2023 വർഷത്തിൽ). മിഡ്ടേം സ്റ്റോറേജ് ട്രീറ്റ്മെന്റായി നൽകുമ്പോൾ വിളകളുടെ പ്രകടനം, വിത്ത് വിളവ്, ഗുണനിലവാരം, ദീർഘായുസ്സ് എന്നിവയിൽ നാനോകണങ്ങളുടെ സ്വാധീനം വിലയിരുത്താനാണ് പഠനം ലക്ഷ്യമിടുന്നത്.

പരീക്ഷണത്തിന്റെ തുടക്കത്തിൽ ആറുമാസം പ്രായമായ വിത്തുകളുടെ പ്രാഥമിക ഗുണനിലവാര വിലയിരുത്തൽ നടത്തി. വിത്ത് 67.37% പ്രാരംഭ മുളയ്ക്കൽ പ്രകടമാക്കി, തൈകളുടെ തണ്ടിന്റെ നീളവും വേരിന്റെ നീളവും യഥാക്രമം 4.68 സെന്റിമീറ്ററും 6.81 സെന്റിമീറ്ററും മൂല്യവും തൈകളുടെ ഉണങ്ങിയ ഭാരം 24.1 മില്ലിഗ്രാം ആണ്. വീര്യ സൂചിക-I ഉം II ഉം യഥാക്രമം 774 ഉം 1595 ഉം ആയി കണക്കാക്കി. വിത്ത് ലീച്ചേറ്റിന്റെയും പ്രാരംഭ ഈർപ്പത്തിന്റെയും വൈദ്യുതചാലകത യഥാക്രമം 192.33 μScm^{-1} ഉം 7.53 % ഉം ആയിരുന്നു, വിത്ത് മൈക്രോഫ്ലോറ 47.15 ശതമാനവും (ബ്ലോട്ടർ രീതി) 53.30 % ഉം (അഗർ പ്ലേറ്റ് രീതി) ആയി നിരീക്ഷിച്ചു. നാനോ സിങ്ക് ഓക്സൈഡ് (300, 400, 500, 600, 700 മില്ലിഗ്രാം നാനോ ZnO), ഒരു കിലോഗ്രാം വിത്തിന് 500 മില്ലിഗ്രാം നാനോസൈസ്സ് കസ്റ്റാർഡ് ലീഫ് പൗഡർ എന്നിവയുടെ വ്യത്യസ്ത ചികിത്സകൾ ഉപയോഗിച്ചാണ് വിത്തുകൾ ചുമത്തിയത്. ചികിത്സയില്ലാത്ത നിയന്ത്രണത്തോടൊപ്പം ആറ് ചികിത്സകളും പഠനത്തിന്റെ അടിസ്ഥാനമായി. സംസ്ക്കരിച്ച വിത്തുകളുടെ ഗുണനിലവാര പാരാമീറ്ററുകൾ പഠിക്കുകയും ചികിത്സകളിൽ കാര്യമായ വ്യത്യാസം കാണുകയും ചെയ്തു. ഒരു കിലോഗ്രാം വിത്തിന് 700 മില്ലിഗ്രാം നാനോ സിങ്ക് ഓക്സൈഡ് ഉപയോഗിച്ച് സംസ്കരിച്ച വിത്തുകൾ ഗണ്യമായി ഉയർന്ന വിത്ത് മുളയ്ക്കുകയും (73.31%) തൈകളുടെ വീര്യം വർദ്ധിപ്പിക്കുകയും ചെയ്തു (945, 1771). മിഡ് സ്റ്റോറേജ് ട്രീറ്റ്മെന്റ് വിത്തുകളുടെ മുളയ്ക്കലും വീര്യവും വർദ്ധിപ്പിക്കും.

നിയന്ത്രണത്തോടൊപ്പം ചികിത്സകളും (ഏഴ് ചികിത്സകൾ) മൂന്ന് പകർപ്പുകളുള്ള ക്രമരഹിതമായ ബ്ലോക്ക് രൂപകൽപ്പനയിൽ ഉയർത്തി. ടാഗ് ചെയ്ത ചെടികളിൽ ഉചിതമായ വളർച്ചാ ഘട്ടങ്ങളിൽ വളർച്ചയെയും വിളവിനെയും കുറിച്ചുള്ള നിരീക്ഷണങ്ങൾ രേഖപ്പെടുത്തി. എല്ലാ നാനോകണങ്ങളും ചികിത്സിച്ച വിത്തുകൾ നിയന്ത്രണ (ചികിത്സ ചെയ്യാത്ത വിത്തുകൾ) ചികിത്സകളേക്കാൾ മികച്ചതാണ്.

ചെടിയുടെ ഉയരം (80.40 സെ.മീ), ദിവസങ്ങൾ മുതൽ 50% വരെ പൂവിടുമ്പോൾ (82.67 ദിവസം), കായ്കളുടെ നീളം (5.55 സെന്റീമീറ്റർ), 100 വിത്ത് തൂക്കം (ഗ്രാം) എന്നിവയ്ക്ക് നാനോ ZnO @ 700 മില്ലിഗ്രാം കി.ഗ്രാം വിത്ത് (T6) ഉപയോഗിച്ചുള്ള വിത്ത് സംസ്കരണം മികച്ചതാണ്. . നാനോ ZnO @ 600 mg kg^{-1} വിത്ത് (T5) ഉപയോഗിച്ചുള്ള ചികിത്സ ഒരു ചെടിയുടെ ശാഖകൾ (5.80), ഓരോ ചെടിയുടെയും പഴങ്ങൾ (112.57), പഴുത്ത സമയത്ത് (2.31g), പഴങ്ങളുടെ ഭാരം (2.31g), ഒരു ചെടിയിൽ നിന്നുള്ള ഫലം (478.73g) എന്നിവ മെച്ചപ്പെടുത്താൻ ഫലപ്രദമായിരുന്നു. , ഓരോ പഴത്തിനും വിത്തുകൾ (61.50), ഒരു ചെടിയിൽ നിന്നുള്ള വിത്ത് വിളവ് (25.76 ഗ്രാം), 100 വിത്ത് ഭാരം (0.433 ഗ്രാം). നാനോ കസ്റ്റാർഡ് ലീഫ് പൗഡർ @ 500 മില്ലിഗ്രാം കിലോഗ്രാം വിത്ത് (T_7) ഉപയോഗിച്ച് വിത്ത് സംസ്കരിച്ചപ്പോൾ, ആദ്യത്തെ പൂവിടുമ്പോൾ (74.22 ദിവസം) ദിവസങ്ങളിൽ കാര്യമായ ഫലങ്ങൾ രേഖപ്പെടുത്തി.

പരീക്ഷണം I-ൽ നിന്ന് ലഭിച്ച വിത്തുകൾ വിത്ത് സംഭരണ പഠനത്തിനായി ഉപയോഗിച്ചു (പരീക്ഷണം II). വിത്തുകൾ എട്ട് ശതമാനത്തിൽ താഴെ ഈർപ്പം ഉള്ളിടത്തോളം ഉണക്കി, 700 ഗേജ് പോളിയെത്തിലീൻ ബാഗിൽ പൊതിഞ്ഞ് ആറ് മാസത്തേക്ക് ആംബിയന്റ് അവസ്ഥയിൽ സംഭരിച്ചു. പ്രതിമാസ ഇടവേളയിൽ സാമ്പിളുകൾ എടുക്കുകയും വിത്തിന്റെ ഗുണനിലവാര പാരാമീറ്ററുകൾ വിശകലനം ചെയ്യുകയും ചെയ്തു. മൂന്ന് പകർപ്പുകളുള്ള പരീക്ഷണം I-ലെ പോലെ T_1 മുതൽ T_7 വരെയുള്ള ഏഴ് ചികിത്സകളുള്ള ഒരു കംപ്ലീറ്റ്ലി റാൻഡമൈസ്ഡ് ഡിസൈനിലാണ് (CRD) ഈ പരീക്ഷണം തയ്യാറാക്കിയത്.

സംഭരണത്തിന്റെ പുരോഗതിയനുസരിച്ച് വിത്തിന്റെ ഗുണനിലവാര സൂചകങ്ങളായ മുളയ്ക്കൽ ശതമാനം, വീര്യസൂചകങ്ങൾ, തൈകളുടെ ഉണങ്ങിയ ഭാരം എന്നിവ കുറഞ്ഞു. ആദ്യ മാസം മുതൽ സംഭരണത്തിന്റെ അവസാനം വരെ മുളയ്ക്കുന്ന ശതമാനത്തിനുള്ള ചികിത്സകളിൽ കാര്യമായ വ്യത്യാസം കണ്ടു. ആറ് മാസത്തെ സംഭരണം അവസാനിക്കുന്നത് വരെ 60 ശതമാനം മുളപ്പിച്ച MSCS (മിനിമം സീഡ് സർട്ടിഫിക്കേഷൻ സ്റ്റാൻഡേർഡ്) ഉൾപ്പെടെയുള്ള എല്ലാ ചികിത്സകളും. സംഭരണത്തിന്റെ അവസാനം, T5 (Nano ZnO @ 600 mg kg^{-1} വിത്ത്) യഥാക്രമം ഉയർന്ന മുളയ്ക്കൽ ശതമാനം (70.10%), വീര്യ സൂചിക I, II (986, 1710) രേഖപ്പെടുത്തി.

സംഭരണത്തിന്റെ തുടക്കത്തിലും അവസാനത്തിലും വിത്ത് അണുബാധയിൽ നാനോ കണിക ചികിത്സയുടെ സ്വാധീനം ഗണ്യമായി കണ്ടെത്തി. അഗർ പ്ലേറ്റ് രീതിയിലും (49.03%), ബ്ലോട്ടർ പേപ്പർ രീതിയിലും (48.01%) നാനോ സിങ്ക് ഓക്സൈഡ് @ 600 മില്ലിഗ്രാം കിലോഗ്രാം വിത്ത് (T5) ഉപയോഗിച്ച് സംസ്കരിച്ച വിത്തുകളിലാണ് ഏറ്റവും കുറഞ്ഞ അണുബാധ നിരക്ക്.

ചികിത്സകളിൽ, T_5 (നാനോ ZnO @ 600 mg kg^{-1} വിത്തിന്റെ) വളർച്ചയും വിത്ത് വിളവ് ഗുണങ്ങളും വർദ്ധിപ്പിക്കുന്നതിനും സംഭരണ സമയത്ത് വിത്തിന്റെ ഗുണനിലവാരം നിലനിർത്തുന്നതിനും ഒന്നിലധികം ഗുണങ്ങളുണ്ടെന്ന് കണ്ടെത്തി. T6 (വിത്തിന്റെ nano ZnO @ 700 mg kg^{-1}), T_4 ((വിത്തിന്റെ nano ZnO @ 500 mg kg^{-1}), എന്നിവ വ്യത്യസ്ത പാരാമീറ്ററുകളുടെ അടിസ്ഥാനത്തിൽ അടുത്ത രണ്ട് ചികിത്സകളായിരുന്നു.

അതിനാൽ, നാനോ ZnO @ 600 mg kg^{-1} വിത്ത് ഉപയോഗിച്ചുള്ള മിഡ് സ്റ്റോറേജ് വിത്ത് സംസ്കരണം മുളക് ഇനമായ ഉജ്വാലയുടെ വിത്ത് വിളവും ഗുണവും ദീർഘായുസ്സും ഫലപ്രദമായി വർദ്ധിപ്പിക്കുമെന്ന് നിഗമനം.