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Effect of Nanopriming with Zinc Oxide and Silver Nanoparticles on Storage of Chickpea Seeds and Management of Wilt Disease

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ABSTRACT

Chickpea is an important pulse crop of India, but its productivity is quite low due to several biotic and abiotic stresses. Low seed vigor is one of the issues that occur due to changes in different biochemical properties during improper storage condition. To overcome the biochemical activity during storage, seed priming is a promising strategy. In the present study, two nanopriming agents *viz*., Zinc Oxide Nanoparticles (ZnONPs) and silver Nanoparticles (AgNPs) were evaluated for biochemical activity (Peroxidase activity, alpha amylase activity, total soluble sugar, total protein) of chickpea at 2, 4, 6 and 9 months storage period after priming for 1, 2, and 6 hours. Result showed increased activity of Peroxidase (POX) with increase of storage time, but the rate of increase was comparatively low when seeds were nanoprimed with ZnONPs. Similarly, alpha amylase activity and protein content were recorded highest and Total Soluble Sugar (TSS) was found lowest in ZnONPs primed seed. Out of the different priming times, 6 hrs was found to be the best at 9 months of storage with positive effect on biochemical parameters. Among the biotic stresses, disease caused by *Fusarium oxysporum* f.sp. *ciceri* has been considered as a destructive one, which causes yield loss up to 10% every year. To overcome the biotic stress and enhance the storage life, chickpea seeds were primed with ZnONPs and AgNPS at 100 ppm alone and in combination with each other. We found positive effect on seed germination (%), plant growth, and yield attributing parameter and negative effect on per disease incidence of *F. oxysporum* f.sp. *ciceri*.

Keywords: Biochemical parameters, *Cicer arientinum* L., *F. oxysporum*, Storage life.

INTRODUCTION

Seeds are basic and key input for agriculture. Of all crops, pulses have a specific importance for the vegetarian population of our country as it act as major source of protein. Per capita availability of pulses per day is only 47 g, as against the minimum requirement of 104 g (WHO) (Vishwas *et al*., 2017). Among the pulses, chickpea, *Cicer arientinum* L. (Family: Fabaceae, Sub-family: Faboidae) occupies a predominant position and is considered as a king of pulses. Its different types are variously known as Gram, Bengal gram, Garbanzo bean as well as Egyptian pea.

India produces about 150 million tons of food grains per year, but losses have remained static at 10% (Ali *et al*., 2015). More than 60% of the net area is sown in under rainfed system. It is quite appropriate to develop technologies for rainfed agriculture. Major problems in chickpea cultivation in rainfed system are poor seed germination, seed dormancy, less plant vigor and disease and pest attack. Besides, improper storage is also another reason for lower production and an average of 6% out

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of total 10% loss takes place during storage of food grains. In storage condition, seeds lose their vigor due to different biochemical changes (Maiti and Moreno- Limon, 2001).

Among the different diseases, wilt caused by *Fusarium oxysporum* f. sp. *ciceri* has been considered as a destructive one, which causes yield loss up to 10% every year (Kumar *et al.,* 2014). To overcome this disease, seed priming is the most promising strategy for management of modern crop and production.

Seed priming is an effective tool for increasing seed germination and plant growth that will event-ually increase productivity under different environmental conditions and stresses (Khalaki et al., 2020). Efficient seed germination promotes successful establishment and deep root system of plants. Seed priming methods, particularly nanopriming is more effective mainly because of its small size and unique physicochemical properties. Since plant species are physiologically different, they differ in their uptake of nanoparticles in nanopriming, and, hence, in their rate and manner of growth. Most previous studies have separately investigated the effect of nanomaterial on seed germination, growth, and development of one plant at a time (Khalaki et al., 2016). However, very few studies have reported nanopriming effects using different particles on seed germination and seedling growth of forage and medicinal plants together. The need to produce high seed quality efforts have been directed towards the seed priming, which in tern shorten germination time and helps to overcome the seed dormancy. Seed priming advances seed germination process without emergence of radical and enough to activate the biochemical events. The product of these changes persists following desiccation and are available on re-imbibition of water during seed planting, enabling completion of germination rapidly, leading to a uniform crop stand and synchronized flowering and fruiting. Additionally, these physiological treatments induce tolerance to certain environmental stress (Vanangamudi *et al*., 2008). Various priming methods like osmopriming, hydropriming, solid matrix priming, biopriming, nanopriming etc. are potentially used in seed technology.

"Nanopriming" is a new and fascinating method that can be used for increasing seedling vigor index, improvement of germination percentage, and seedling growth. Nowadays, engineered nanomaterials are the most important index of the nanotechnology area, have entered all aspects of human life and their various applications are quickly expanded due to their new characteristics compared to the corresponding bulk materials (Roozbeh *et al*., 2017). Seed nanopriming is mostly done on the effect of germination process in the field of research. Silver (Ag), Zinc Oxide (ZnO), gold (Au) Nanoparticles (NPs) etc. are some of the different nanoparticles used as a nanopriming agents. We hypothesized that the nanoparticle as priming agent will have positive effect on enhancement of storage life of chick pea with more plant stand against *Fusarium* wilt disease.

The aim of the present study was to investigate the effect of nanopriming agents (Ag and ZnO nanoparticles) on the biochemical parameters of chickpea seeds at different storage periods and to study the performance of the nanoprimed seeds in pot conditions on per cent disease incidence of *Fusarium wilt* and plant growth and yield attributing parameters of chickpea.

MATERIALS AND METHODS

This research was carried out in the Nanotechnology Laboratory of Department of Plant Pathology, Assam Agricultural University (AAU), Jorhat, during 2017-2018 and 2018-2019. In the present study, chickpea seeds (var. JG-16) were used to prime with two nanoparticles *viz*., Zinc Oxide (ZnO) and silver (Ag) Nanoparticles (NPs).

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Source of the Nanoparticles

The nanoparticles were collected from the Nano Lab of Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam. AgNPs and ZnONPs were synthesized mediating *Trichoderm asperellum* (Kaman and Dutta, 2019) and wet chemical process (Kaushik and Dutta, 2017), respectively. Synthesized nanoparticles were characterized by UV-VIS spectroscopy, electron micrography (SEM and TEM), FTIR, Zetasizer and DLS. For AgNPs characteristic surface plasmon absorption band was observed at 420 nm with Zeta potential of -1.34 mV, size of 27.64 nm and roughly spherical in morphology (Kaman and Dutta, 2018). On the other hand, the used ZnONPs had average size of 33.4 nm with PDI of 0.697, with negative zeta potential value of -20.7 mV, spherical in shape with zinc and oxygen as elemental composition (Kaushik and Dutta, 2017)

Source of the Pathogen and Preparation of Inoculum for Pot Experiment

The pathogen *Fusarium oxysporum* f. sp *ciceri* was collected from the culture of Department of Plant Pathology, AAU, Jorhat, Assam and maintained the pure culture by periodic transfer to fresh PDA slants and stored in refrigerator at 4^{o}C. Mass culture of *F. oxysporum* was done in sterilized Maize meal Sand Media (MSM). Two mycelial disc (5 mm diam.) of *F. oxysporum* from fresh PDA medium was transferred aseptically in Erlenmeyer flasks (500 mL) containing MSM and was incubated in BOD incubator (Make: REICO) for seven days at 25 ± 1^{0}C. On 8^{th} day, MSM with fungal inoculum containing fungal mycelium, micro and macrop conidia etc. was inoculated to pot mixture at 0.2% (w/w) seven days prior to the seed sowing so that the pathogen settles well in the pot mixture.

Preparation of Pot Mixture for Pot Experiment

For pot experiment, pot mixture was prepared by mixing soil (sandy loam), sand, and compost at 1:1:1 ratio and sterilized in autoclave (Make: Equitron) at 121^{0}C at 15 lb pressures for 15 minutes consecutively for three days. Nutrients like N, P, K were added in the form of Urea, SSP and MOP three days prior to the seed sowing.

Standardization of Priming Technique

For standardization of nanopriming agents, seeds were treated with ZnONPs and AgNPs alone and in combination with each other for 1, 2, and 6 hours and shade dried till moisture reached 9%. The treatment combination used were: T_1= Control, T_2= Recommended pesticide treatment (Malathion 5% dust per 2.5 g kg^{-1}+Carbendazim with 0.1% concentration), T_3= Nanopriming with ZnONPs at 100 ppm (for 1, 2 and 6 hours), T_4= Nanopriming with AgNPs at 100 ppm (for 1, 2 and 6 hours), and T_5= Nanopriming with ZnONPs at 100 ppm+AgNPs at 100 ppm (for 1, 2 and 6 hours). Treated seeds were packed in HDPE semipermeable bag and stored for 2, 4, 6 and 9 months. The experiment was arranged in Completely Randomized Design (CRD) with 6 replications.

Effect of Nanopriming on Biochemical Parameters and Storage Life

Estimation of Total Soluble Sugar (TSS)

The total soluble sugar content was estimated by using the method of Yemm and Willis (1954). For the estimation of TSS of storage chickpea seeds, 20 mg was homogenized in 5 mL of 80 per cent hot ethanol and centrifuged at 4,000 rpm for 20 minutes. The supernatant was collected and the residue was again centrifuged with 5 mL of 80 per cent hot ethanol at 4,000 rpm for 20 minutes. Both supernatants were mixed

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together. The known amount of ethanol extract was evaporated to dryness in a test tube on a water bath and cooled at room temperature. One mL of distilled water was added in a test tube and mixed thoroughly. To each test tube, 4 mL of anthrone reagent (200 mg of anthrone reagents was dissolved in 100 mL of concentrated sulfuric acid) was added and mixed thoroughly and gently heated on water bath at 100^{o}C for 10 minutes, cooled rapidly under running tap water, and absorbance was measured at 630 nm against reagent blank. The amount of total soluble sugar was calculated using standard curve prepared from graded concentration of glucose. Calculation of the concentration of TSS in the test tube were determined from the standard curve and expressed as per cent or mg g^{-1} dry weight.

Estimation of Total Protein (TP)

The total protein content was estimated by using the method of Lowry *et al.* (1951). The reagents used for estimation of total protein were: **(**a) Phosphate buffer (0.1M, pH 7.6), (b) Solution A: 2% Na_2CO_3 in 0.1N NaOH (20 g of sodium carbonate and 4 g sodium hydroxide was dissolved in distilled water of volume 1 liter), (c) Solution B: 1% $CuSO_4.5H_2O$ solution (1 g of copper sulfate was dissolved in 100 of distilled water) and 2% potassium sodium tartrate was dissolved in 100 mL of distilled water. Both 1% $CuSO_4.5H_2O$ solution and 2% potassium sodium tartrate were mixed in equal volume before use, (d) Solution C: Carbonate-Cu++ solution (this solution was prepared by mixing 50 mL of Solution A and 1 mL of Solution B), (e) Solution D: Folin- Ciocalteu Reagent (equal amount of water was added to the reagent before used), (f) Standard protein solution: 25 mg of bovine serum albumin was dissolved in 100 mL of water.

For estimation of total protein, one gram of chickpea seeds were homogenized in 5 mL of phosphate buffer and centrifuged at 4,000 rpm for 20 minutes. The supernatant was collected and the residue was again centrifuged with 5 mL of phosphate buffer at 4,000 rpm for 20 minutes. Both supernatants were mixed together. In 0.2 mL of phosphate extract, 5 mL of Carbonate- Cu++ solution was added and mixed well by vortexing. After 10 minutes, 0.5 mL of Folin- Ciocalteu reagent was added and mixed well vortexing and absorbance was measured at 660 nm against blank reagent. The amount of total protein was calculated by using standard curve prepared from bovine serum albumin stock solution and expressed as mg/g or 100 g sample.

Estimation of Alpha-Amylase Activity (Bernfield, 1955)

The estimation of alpha-amylase activity was measured by the method of Bernfield (1955) and expressed in milligram of maltose equivalent. The reagents used for doing alpha-amylase were: (a) 0.1M Sodium Acetate buffer (pH- 4.7), (b) Acetic acid 0.2M (1.5 mL glacial acetic acid was added in 100 mL distilled water), (c) 1% Starch solution (1 g starch was dissolved in 100 mL of 0.1M Sodium acetate buffer), (d) Dinitrosalicylic (DNS) acid reagent (1 g DNS, 0.2 g crystalline phenol and 0.05g sodium sulfite was dissolved in 100 mL of 1% NaOH),(e) 40% Potassium Sodium Tartrate (PST), (e) Standard maltose solution (50 mg maltose was dissolved in 50 mL of distilled water).

For estimation of alpha-amylase activity, the storage chickpea seeds were homogenized in 5 mL of ice cold 10 mM Calcium chloride solutions over night at 4^{0}C or 3 hours at room temperature and centrifuged the extract at 6,000 rpm at 4^{0}C for 10 minutes, and the supernatant was made up to 10 mL with 10 mM calcium chloride. The supernatant was used as enzyme source. In a test tube, 1 mL of supernatant was taken and added with 1 mL starch solution, 2 mL DNS solution and 1 mL PST solution and mixed it properly by using vortex mixture and heat in a boiling water bath for 5 minutes and dark orange red

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color was developed and absorbance was measured at 560 nm against blank reagent. The alpha amylase activity was calculated by using standard curve prepared form maltose solution.

Estimation of Peroxidase Activity

The peroxidase activity was estimated by using the methods of Putter (1974). The reagents used for doing peroxide activity were: (a) Phosphate buffer 0.1M (pH 7.0), (b) Guaiacol solution 20 mM (0.24 mL guaiacol was dissolved in 100 mL of water), (c) Hydrogen peroxide solution (0.14 mL of 30% H_2O_2 was diluted in 100 mL of water).

For estimation of peroxidase activity, one gram of storage chickpea seeds were homogenized in 3 mL of phosphate buffer by grinding in a pre-cooled mortar and pestle and centrifuged the extract at 18,000 rpm at 5^0C for 15 minutes. The supernatant was used as an enzyme source by making 10 mL with phosphate buffer. In a test tube, 0.1 mL of enzyme extract was taken and added with 3 mL of phosphate buffer, 0.05 mL guaiacol solution and 0.03 mL hydrogen peroxide solution. The mixture was well shaken and placed in a spectrophotometer by putting the mixture in a cuvette. The time required for the mixture optical density to be increased by 0.1Δt at 436 nm was recorded and used in calculations.

Calculation of Peroxidise Activity

Since the extinction coefficient of guaiacol dehydrogenation product at 436 nm under the condition specified is 6.39 per micromole, the enzyme activity per liter of extract was calculated as, Enzyme activity ($U\ L^{-1}$)= $(3.18\times0.1\times1000/6.39\times1\times\Delta t\times0.1)= 500/\Delta t$.

Δt=time change in minute

Based on the above biochemicals parameters, best priming time and highest storage months were selected and compared.

Effect of Nanopriming against Wilt of Chickpea and Plant Growth and Yield Parameters

The best treatment combinations found in the above experiments were selected for the pot experiment conducted in net house of Department of Plant Pathology, Assam Agricultural University (AAU), Jorhat, Assam.

The treatment combination used were: $\mathbf{T_1}$= Uninoculated control (No seed treatment+No pathogen), $\mathbf{T_2}$= Inoculated control (*F. oxysporum* f.sp. *cicero* at 0.02%), $\mathbf{T_3}$= Recommended pesticide treatment (Malathion 5% dust per 2.5g kg^{-1}+Carbendazim with 0.1%)+*F. oxysporum* f. sp. *ciceri*, $\mathbf{T_4}$= Nanopriming with ZnONPs at 100 ppm (for 6 hours)+*F. oxysporum* f.sp. *ciceri*, $\mathbf{T_5}$ = Nanopriming with AgNPs at 100 ppm (for 6 hours)+*F. oxysporum* f. sp. *ciceri* and $\mathbf{T_6}$= Nanopriming with ZnONPs at 100 ppm+AgNPs at 100 ppm (for 6 hours)+*F. oxysporum* f. sp. *ciceri*. Observation on seed germination percentage (%), shoot length and root length (cm), number of branches per plant, fresh and dry weight of plant at final harvest (g), seedling vigor index, number of pods per plant, seeds per pod, seed yield per plant (g $plant^{-1}$), 1,000 seed weight (g) and disease incidence (%) etc. were recorded as per following procedure.

Physiological Parameters of Priming Storage Chickpea Seeds

Seed germination percentage and moisture content

Seed germination was studied following the between paper methods (ISTA, 1985). Fifty seeds were placed equidistantly between two seeds of germination paper soaked in water, rolled and tagged and

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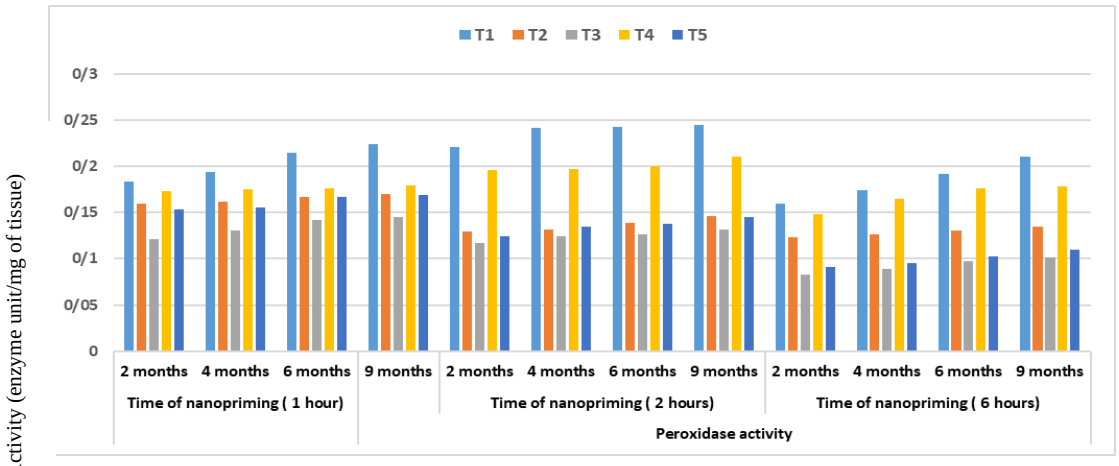

Figure 1. Effect of time of nanopriming (1, 2, and 6 hourrs) on peroxidase activity of chickpea seeds at different storage periods (2, 4, 6, and 9 months).

incubated inside germinator at 30^0C. Final count was taken on 8^{th} day. Observation was recorded on seed germination percentage.

Speed of Germination

Seeds of different treatments were also evaluated in terms of Speed of Germination Index (SGI). Fifty seeds were placed equidistantly between two seeds of germination paper soaked in water, rolled and tagged and incubated inside germinator at 30 ^{0}C (ISTA, 1985). Number of seeds germinated daily was counted till completion of germination. From the observation data, speed of germination index was calculated as per the following formula:

Speed of germination= $X_1/n_1+(X_2–X_1)/n_2+(X_3–X_2)/n_3....(X_n–X_{n-1})/n_n$

Where, X_1, X_2, X_3.....= Number if seedling germinated on n_1, n_2, n_3.....days.

Shoot Length, Root Length, Seedling Fresh Weight and Seedling Dry Weight and Seedling Vigor Index

To study the effect of priming seedling vigor index, normal seedling showing normal root and shoot growth were randomly selected. The root and shoot portion were separated, their length was measured, and mean seedling length was calculated. The Seedling Vigor Index (SVI) was calculated using the following formula:

SVI= Seedling length×Germination (%)

RESULTS

Results obtained on different biochemical parameters like peroxidase activity; alpha amylase activity, total soluble sugar, and total protein on different months of storage are presented as follows:

Effect of Nanopriming on Peroxidase Activity (POX) in Chickpea Seeds

In this study, it was found that the POX enzyme activity increases with increase of storage periods. POX activity was highest in the control (non-primed seeds), but the rate of increase was comparatively lower when seeds were primed with ZnONPs at 100 ppm in all periods of storage for 1, 2 and 6 hours. This was followed by seeds treated with both ZnONPs at 100 ppm and AgNPs at 100 (Figure 1).

Effect of Nanopriming on Alpha Amylase Activity in Chickpea Seeds

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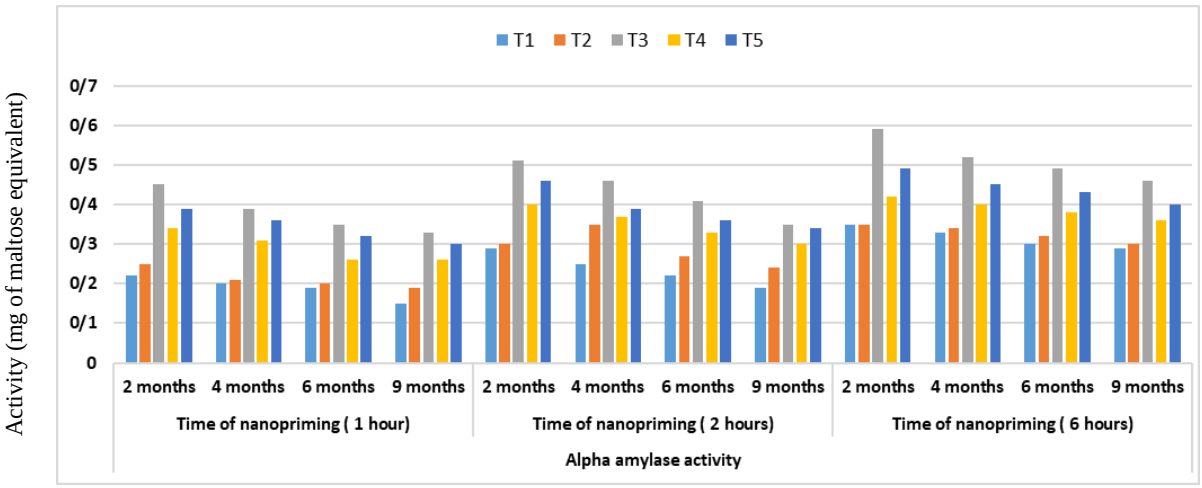

Figure 2. Effect of time of nanopriming (1, 2, and 6 hours) on alpha amylase activity of chickpea seeds at different storage periods (2, 4, 6, and 9 months).

Alpha amylase is an indicator of seed germination. The data recorded showed that the alpha amylase activity decreases with increase of storage period. The alpha amylase activity was recorded highest in seeds treated with ZnONPs at 100 ppm in all the periods of storage for 1, 2 and 6 hours. This was followed by seeds treated with both ZnONPs at 100 ppm and AgNPs at 100 ppm (Figure 2). The lowest concentration of alpha amylase activity was recorded in the control, where seeds were non-primed.

Effect of Nanopriming on Total Soluble Sugar and Total Protein in Chickpea Seeds

Highest protein content was recorded when ZnONPs was used as nanopriming agent in all periods of storage in all priming times (1, 2 and 6 hours.) (Figure 3). This was followed by nanopriming with ZnONPs at 100 ppm+AgNPs at 100 ppm. The lowest protein content was recorded in the control with highest Total Soluble Sugar (TSS). TSS content was at the lowest concentration in seeds primed with ZnONPs at 100 ppm in all the period of storage in all priming time (1, 2 and 6 hours.). This was followed by seeds primed with both ZnONPs at 100 ppm and AgNPs at 100 ppm (Figure 4).

Effect of Nanopriming on Physiological Parameters of Chickpea Seeds in Suppression of Wilt Disease

The study on the effect of nanopriming showed that all the treatments had positive effect on plant growth and yield parameters. Seeds primed with nanoparticles for 6 hours caused significant reduction in seedling infection by the pathogen (Table 1). Highest seed germination percentage were recorded for seeds nanoprimed with ZnONPs at 100 ppm (85.46%), followed by seeds treated with both ZnONPs and AgNPs at 100 ppm. Amongst the nanoprimed seeds, the minimum seed germination (65.00 %) was observed in seeds treated with AgNPs at 100 ppm with lowest in the inoculated control (50.76%). Seeds treated with the recommended chemical showed a germination percentage of 71.56% (Table 1). Lowest percent disease index (PDI) (6.16%) was recorded in seeds priming with ZnONPs at 100 ppm. This was followed by seed primed with both the combination of ZnONPs at 100 ppm and AgNPs at 100 ppm

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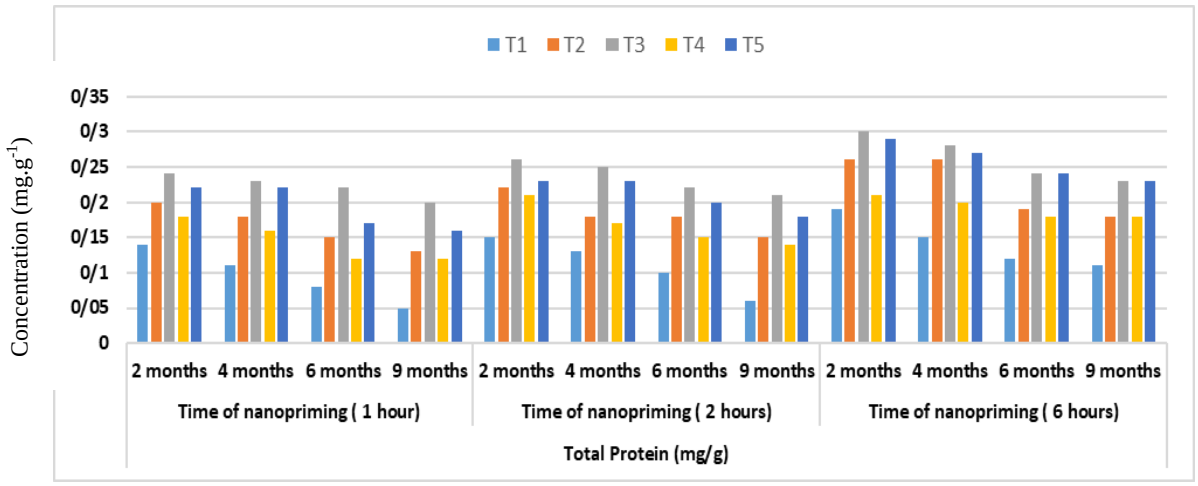

Figure 3. Effect of time of nanopriming (1, 2, and 6 hours) on total protein (mg g^{-1}) of chickpea seeds at different storage periods (2, 4, 6, and 9 months).

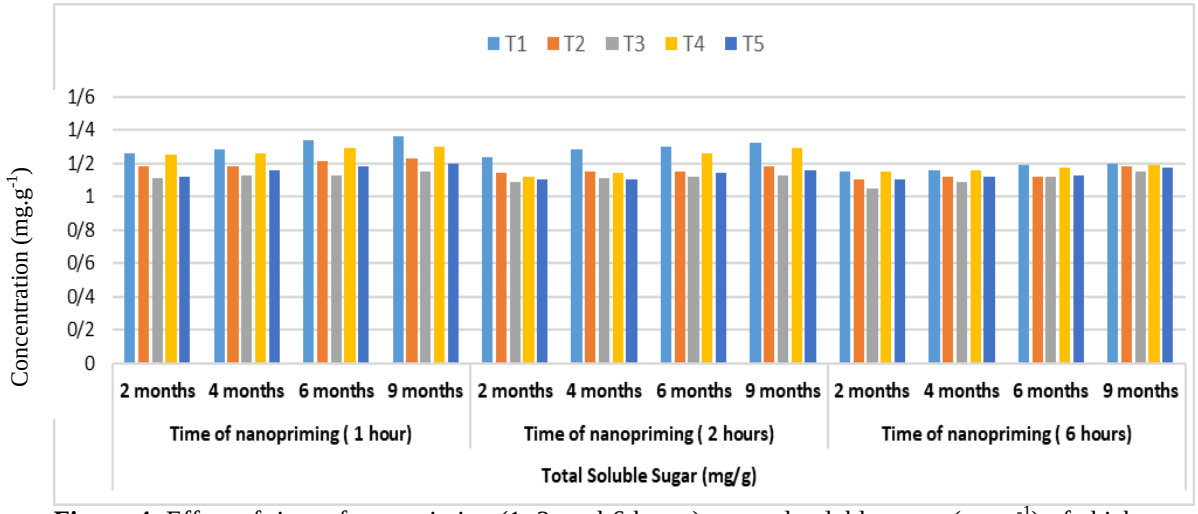

Figure 4. Effect of time of nanopriming (1, 2, and 6 hours) on total soluble sugar (mg g^{-1}) of chickpea seeds at different storage periods (2, 4, 6, and 9 months).

(6.16%). Seeds primed with recommended chemical check showed PDI of 7.03%.

Study on effect of nanopriming of plant growth parameter showed that when ZnONP at 100 ppm was used as priming agents, it showed the highest plant growth parameters like root length (15.21 cm), shoot length (56.00 cm), number of branches (5), fresh weight of roots (0.77 g) and shoots (31.62 g) and dry weight of roots (0.64 g) and shoot (7.19 g). This was followed by seeds treated with both the combination of ZnONPs at 100 ppm and AgNPs at 100 ppm and seeds primed with the recommended chemicals.

Effect on Seedling Vigor Index

The highest seedling vigor index (6085.60) was recorded when the seeds were primed with ZnONPs at 100 ppm (for 6 hours)+*F. oxysporum* f. sp. *ciceri* (Table 1). This was followed by seeds treated with both ZnONPs at 100 ppm and AgNPs at 100 ppm with seedling index of 4224.27. Lowest seedling vigor index (2535.71) was recorded in inoculated control and seeds primed with

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the recommended chemical check showed the seedling vigor index of 3446.17.

Effect of Nanopriming on Seed Production of Chickpea Seeds

In nanoprimed seeds, the highest pods per plant was recorded when seeds were primed with ZnONPs at 100 ppm (23.00) (Table 2). This was followed by seeds treated with both ZnONPs @ 100 ppm and AgNPs at 100 ppm. The lowest pods/plant (16 nos.) was recorded in inoculated control. Data presented in Table 2 shows the highest seeds per pod in T_4 and T_6 where seeds were treated with ZnONPs at 100 ppm alone and in combination with AgNPs at 100 ppm.

Similarly, seed yield per plant (2.19 g) and 1000 seed weight (319.66 g) were recorded highest in seeds treated with ZnONPs at 100 ppm. This was followed by seeds treated with both ZnONPs at 100 ppm and AgNPs at 100 ppm. Lowest seed yield per plant (1.68 g) and 1,000 seed weight (117.16 g) were recorded in inoculated control. Seeds primed with the recommended chemical check had a seed yield per plant and 1,000 seed weight of 1.79 and 219.83 g, respectively.

DISCUSSION

Seed priming is considered to be the important approach in emergence of crops as well as in storage condition (Pill *et al*., 2009; Rakshit *et al*., 2014). Maintenance of good quality seeds is the clear understanding of the biochemical events during storage. In the present study we found that, with the increase in storage periods, the biochemical parameters like alpha amylase activity and total protein decreased and peroxidase activity and Total Soluble Sugar (TSS) increased. This may be due to the reduction of concentration of protein that leads to increase in TSS concentration as observed in the present study. Out of different nanopriming time, 6 hrs was found to be the best which results in biochemical parameters of chickpea at 9 months of storage. Peroxidase enzyme activity was the lowest in seeds that were nanoprimed with ZnONPs at 100 ppm. This indicated that, due to priming, firmness of seed increases resulting the more storage life. On the other hand, alpha amylase activity was lower in ZnONPs treated seeds. This indicates that the low rate of germination percentage of chickpea seeds in ZnONPs primed seeds reduces compared to other treatments. Ramegowda (1992) reported that a decrease in the activity of enzymes *viz*., alpha amylase, and peroxidase coupled with progressive ageing of rice seeds. He further authenticated that alpha amylase and peroxidase enzymes were more directly involved in the maintenance of better germination of differentially aged seeds. Zafar *et al*. (2016) reported that treatment with ZnONPs increases the biochemical parameters in *Brassica nigra* seedlings.

Biochemical studies have been done to amylase the effect of nanoparicles on biochemical changes of storage chickpea seeds, which were treated for three different hours (2, 4, and 6 hours) and stored for 9 months. Result on POX activity showed that with increase of storage time, POX activity increased. However, the rate of increase was comparatively low when seeds were nano-primed with ZnONPs. In case of alpha amylase activity, lowest concentration was recorded in the control, but seeds nano-primed with ZnONPs showed the highest activity of alpha amylase. Highest protein content was recorded when ZnONPs was used as nano-priming agents. On the other hand, the highest TSS was recorded in the control with lowest in nano-primed with ZnONPs.

During our experiment, we found significantly the lowest Percent Disease Incidence (PDI) when seeds were primed for 6 hrs with ZnONPs at 100 ppm followed by seeds primed with ZnONPs at 100 ppm+AgNPs at 100 ppm. The former treatment also showed significantly positive effect on germination percentage, plant

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Table 1. Effect of ZnONPs and AGNPs priming (6 hours) on physiological parameters of chickpea seeds in suppression of wilt disease.[a]

Treatments*	Physiological parameters									
	Time of nanopriming (6 hours)									
	PDI	Seed germination (%)	Shoot length (cm)	Root length (cm)	Branches (nos)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)	Seedling vigour index
T_1	0.00	52.73	44.00	8.71	4.00	10.72	4.39	0.64	0.20	4090.27
T_2	90.00	50.76	42.50	7.46	4.00	5.81	1.97	0.33	0.19	2535.71
T_3	7.03	71.56	45.66	7.85	4.00	8.14	3.20	0.51	0.20	3446.17
T_4	6.16	85.46	56.00	15.21	5.00	31.62	7.19	0.77	0.64	6085.60
T_5	7.18	65.00	43.16	10.01	3.00	13.75	3.21	0.40	0.19	3756.04
T_6	6.19	78.57	47.50	10.65	4.00	14.81	5.47	0.69	0.27	4224.27
S.Ed (±)	0.21	2.36	1.71	0.38	0.53	0.44	0.47	0.03	0.03	14.14
C.D (p=0.05)	0.42	2.17	3.52	0.78	1.07	0.90	0.97	0.06	0.06	29.14

[a] Data are mean of 6 replications [**T_1**: Uninoculated control (No seed treatment+No pathogen), **T_2**: Inoculated control (*F. oxysporum* f. sp. *ciceri* at 0.02%), **T_3**: Recommended pesticide treatment (Malathion 5% dust per 2.5 g kg^{-1} + Carbendazim with 0.1%)+*F. oxysporum* f. sp. *ciceri*, **T_4**: Nanopriming with ZnO NP at 100 ppm+*F. oxysporum* f. sp. *ciceri*, **T_5**: Nanopriming with AgNP at 100 ppm+*F. oxysporum* f.sp. *ciceri*, **T_6**: Nanopriming with ZnO NP at 100 ppm+Ag NP at 100 ppm+*F. oxysporum* f. sp. *ciceri*]

Table 2. Effect of nanopriming with ZnONPs and AgNPs (6 hours) on seed production in chickpea plant.[a]

Treatments	Seed production			
	Time of biopriming (6 hours)			
	Pods/Plant (Number)	Seeds/Pod (Number)	Seed yield/Plant (g)	1000 Seed weight (g)
T1: Uninoculated control (No seed treatment+No pathogen)	17.00	1.00	1.71	170.16
T2: Inoculated control (*F. oxysporum* f. sp. *ciceri* at 0.02%),	16.00	1.00	1.68	117.16
T3: Recommended pesticide treatment (Malathion 5% dust per 2.5 g kg^{-1}+Carbendazim with 0.1%)+*F. oxysporum* f.sp. *ciceri*	20.00	2.00	1.79	219.83
T4: Nanopriming with ZnO NP at 100 ppm (for 6 hrs)+*F. oxysporum* f. sp. *ciceri*	23.00	2.00	2.19	319.66
T5: Nanopriming with AgNP at 100 ppm (for 6 hours)+*F. oxysporum* f. sp. *ciceri*	18.00	1.00	1.76	197.66
T6: Nanopriming with ZnONP at 100 ppm+AgNP at 100 ppm (for 6 hours)+*F. oxysporum* f.sp. *ciceri*	21.00	2.00	2.05	295.83
SEd (±)	0.47	0.26	0.20	0.44
CD (P= 0.05)	0.96	0.52	0.40	0.90

[a] Data are mean of 6 replications.

growth parameters, and chickpea seed production in pot condition as compared to unprimed seeds by reducing seedling infection. The lowest PDI recorded in ZnONPs primed and AgNPs primed seeds may be due to the antifungal activity of the nanoparticles against *F. oxysporum* as reported earlier by Yehia *et al.* (2013), Karimiyan *et al.* (2015), and Ouda (2014). The reduction in disease indirectly helped in proper health management and ultimately showed increased plant growth parameters and yield attributing characteristics. Antifungal activity of ZnONPs against *F. graminearum* was also reported by Dimkpa *et al*. (2013). They reported that ZnONPs was significantly more inhibitory to fungal growth than micro-sized particles of ZnO, although both types of particles released similar levels of soluble Zn, indicating size-dependent toxicity of the particles. AgNPs was reported to have antifungal activity against a multiple fungal pathogen including *Fusarium* spp. (Alananbeh *et al.,* 2017). A study conducted to find out the effect of *T. asperellum* mediated AgNPs on physiology of tea plant showed that AgNPs at 100 ppm can induce the plants in increasing physiological parameters like chlorophyll content, moisture content, relative water content, TSS, total protein and biochemical parameters like lipid peroxidation, (MDA content) and secondary metabolites like phenol, alkaloid, and flavonoid content of treated plants without any harmful effect on the plant (Ahmed and Dutta, 2019). In an another study, we found that AgNPs (size 8.26 nm with polydispersity index of 0.857 and zeta potential value of -1.34 m) as soil treatment has positive impact on soil pH, microbial count, organic carbon, and microbial biomass carbon, except total microbial count (Ahmed and Dutta, 2020); and nuclear magnetic resonance spectroscopy study showed the presence of the AgNPs within the cell of the treated plants and had positive impact on cellular metabolites (Ahmed and Dutta, 2020).

From the above information, we found that out of the different times of priming, 6 hrs was best, and among the different months of storage, the highest was 9 months. Therefore, all the studied biochemical parameters were considered for the two best treatments (T_3 and T_5) and comparison was made with the control (T_1). Results showed that seeds primed with ZnONPs at 100 ppm for 6 hrs showed highest biochemical activities, which increased the storage life of the chickpea seeds up to 9 months. This was followed by seeds nanoprimed with the combination of both ZnONPs at 100 ppm and AgNPs at 100 ppm (Figure 5).

To conclude, nanopriming with ZnONPs for 6 hrs can help to store the chickpea seeds up to 9 months. We also found positive effect of nanopriming with ZnONPs during study on biochemical parameters like POX and alpha amylase activity, which helped in increasing the storage life of chick pea up to 9 months. Further, 9-months stored ZnONP nanoprimed seeds were also effective in suppressing the infection by *F. oxysporum* and enhanced plant growth parameters and yield attributing parameters.

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REFERENCES

1. Ahmed, A. A. and Dutta, P. 2019. *Trichoderma asperellum* Mediated Synthesis of Silver Nanoparticles: Characterization and Its Physiological Effects on Tea [*Camelia. sinensis* (L.) Kuntze var. assamica (J.

[DOR: 20.1001.1.16807073.2022.24.1.9.9]

Masters) Kitam.] *Int. J. Curr. Microbiol. Appl. Sci.,* **8(4)**: 1215- 1229.
2. Ahmed, A. A. and Dutta, P. 2020. Effect of Green Synthesized Silver Nanoparticles on Soil Properties. *J. Med.Plant. Stud.,* **8(1)**: 34-37.
3. Ahmed, A. A. and Dutta, P. 2020. Confocal Microscopy and Nuclear Magnetic Resonance (NMR) Spectroscopic Studies of Tea Leaves Treated with Green Synthesized Silver Nanoparticles. *Int. J. Chem. Stud.,* **8(2)**: 1162-1165.
4. Alananbeh, K. M., Al-Qudah, Z., El-Adly, A. and Al Refaee, W. J. 2017. Impact of Silver Nanoparticles on Bacteria Isolated from Raw and Treated Wastewater in Madinah, KSA. *Arab. J. Sci. Eng*., **42(1):** 85-93.
5. Ali, J., Sharma, A. and Ran, P. 2015. Overview of Grain Drying and Storage Problems in India. *Int. J. Eng. Res. General Sci.,* **3(5):** 674-78
6. Bernfield, P. 1955. *Methods in Enzymology*. (Eds.): Colowick S. P. and Kaplan N. O. Academic Press, New York, PP. 149-158.
7. Bradford, K. J. 1986. Manipulation of Seed Water Relations via Osmotic Priming to Improve Germination under Stress Conditions. *Hortic. Sci. (USA),* **21(5):** 1105-1112
8. Dimkpa, C. O., McLean, J. E., Britt, D. W. and Anderson, S. 2013. Antifungal Activity of ZnO Nanoparticles and Their Interactive Effect with a Biocontrol Bacterium on Growth Antagonism of the Plant Pathogen *Fusarium graminearum*. *Biometals,* **26(6):** 913-924.
9. Husen, A. and Siddiqi, K. S. 2014. Carbon and Fullerene Nanomaterials in Plant System. *J. Nanobiotechnol.*,**12(1):** 16-24
10. ISTA, 1985, International Rules for Seed Testing.Seed Science and Technology, 13, 307-520.
11. Kaman, P. and Dutta, P. 2019. Synthesis, Characterization and Antifungal Activity of Biosynthesized Nanoparticle. *Indian Phytopathol.*,**72**: 79-88.
12. Karimiyan, A., Najafzadeh, H., Ghorbanpour, M. and Hekmati-Moghaddam, S. H. 2015. Antifungal Effect of Magnesium Oxide, Zinc Oxide, Silicon Oxide and Copper oxide Nanoparticles against *Candida albicans*. *J. Res.Med. Sci.*, **17(10):** 55-60
13. Kaushik, H. and Dutta, P. 2017. Chemical Synthesis of Zinc Oxide Nanoparticle: Its Application for Antimicrobial Activity and Plant Health Management. *Phytopathology*, **107(12):** 38-38.
14. Khalaki, M. A., Moameri, M., Lajayer, B. A. and Astatkie, T. 2020. Influence of Nano-Priming on Seed Germination and Plant Growth of Forage and Medicinal Plants. *Plant Growth Regul.*, VOL?? PP?
15. Khalaki, M. A., Ghorban, A and Moameri, M. 2016. Effect of Silica and Silver Nanoparticles on Seed Germination Traits of *Thymus kotchyanus* in Laboratory Conditions. *J. Rangel. Sci.*, **6(3):** 221-225.
16. Kumar, V., Shahid, M., Singh, A., Srivastava, M., Mishra, A., Srivastava, Y. K., Pandy, S., and Sharma, A. 2014. Effect of Biopriming with Biocontrol Agents *Trichoderma harzianum* (Th. Azad) and *Trichoderma viride* (01pp) on Chickpea Genotype (Radhey). *J. Plant Pathol. Microbiol.*, **5(252):** 2.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.,* **193:** 265-275.
18. Mahakham, W., Theerakulpisut, P., Maensiri, S., Phumying, S. and Sarmah, A. K. 2016. Environmentally benign synthesis of phytochemicals-capped gold nanoparticles as nanopriming agent for promoting maize seed germination. *Science of the Total Environment*, **573:** 1089-1102.
19. Maiti, R. K. and S, Moreno-Limon 2001 Seed and seedling traits in bean (*Phaseolus vulgaris* L.) and its relation to abiotic stress resistence. *Legume Research* **24 (4):** 211-221.
20. Maroufi K, Farahani HA and Aghdam AM (2011) Effect of Nanopriming on germination in sunflower (Helianthus Annus L.). *Advances in Environmental Biology* **6** :3747-3751.
21. Ouda SM (2014) Antifungal activity of silver and copper nanoparticles on two plant pathogens, *Alternaria alternata* and *Botrytis cinerea*. *Research Journal of Microbiology* **9 (1):**34-42.
22. Bernfeld, P. 1955. Amylase α and β. In: "*Methods in Enzymology*", (Eds.) Colowick, S and Kaplan, N. O. Academic Press, New York., (1), 149-158.
23. Pill, W. G., Collins, C. M., Goldberger, B. and Gregory, N. 2009. Responses of Non-Primed or Primed Seeds of 'Marketmore 76' Cucumber (*Cucumis sativus* L.) Clurry Coated with *Trichoderma* Species to Planting in Growth Media Infested with *Pythium*

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aphanidermatum. Scientia Horticulturae, **121:** 54-62.
24. Putter, J. 1974. In: "*Methods of Enzymatic Analysis", 2 (Ed.)* Bergmeyer), Academic Press, New York, 685 PP.
25. Rakshit, A., Pal, S., Meena, S., Manjhee, B., Rai, S., Rai, A. and Singh, H. B. (2014) Seed Bio-Priming: A Potential Tool in Integrated Resource Management. *SATSA Mukhaptra Annual Technical Issue*, **18**: 94-103.
26. Rame, G., 1992. Studies on Seed Senescence and Seed Vigor in Some Genotypes of Rice. PhD. Thesis, Tamil Nadu Agriculture University. Coimbatore.
27. Roozbeh, F., Davoodi, D. and Khayamym, S. 2017. Effects of Nano-Priming, Priming Time and Post-Priming Maintenance on Seed Germination of Sugar Beet. *J. Agri. Res.*, **3(2):** 16-35.
28. Valadkhan, M., Mohammadi, K. and Nezhad, M. T. K. 2015. Effect of Priming and Foliar Application of Nanoparticles on Agronomic Traits of Chickpea. *Biol. Forum*, **7(2):** 599-605
29. Vanangamudi, K., Bhaskaran, M., Bharati, A. and Maregesan, P. 2008. Seed Hardening for Drought Resistance. *Adv. Seed Sci. Technol.*, **1**: 195-200.
30. Vishwas, S., Chaurasia, A. K., Bara, B. M., Debnath, A., Parihar, N. N., Brunda, K. and Saxena, R. 2017. Effect of Priming on Germination and Seedling Establishment of Chickpea (*Cicer arietinum* L.) Seeds. *J. Pharmacogn. Phytochem.*, **6(4):** 72-74.
31. Yehia, R. S. and Ahmed, O. F. 2013. *In Vitro* Study of the Antifungal Efficacy of Zinc Oxide Nanoparticles against *Fusarium oxysporum* and *Penicilium expansum. Afr. J. Microbiol. Res.*,**7(19):** 1917-1923.
32. Yemm, E. W. and Willis, A. J. 1954. The Estimation of Carbohydrates in Plant Extracts by Anthrone. *New Phytologist*, **57:** 509-514.
33. Zafar, H., Ali, A., Ali, J. S., Haq, I. U. and Zia, M. 2016. Effect of ZnO Nanoparticles on *Brassica nigra* Seedlings and Stem Explants: Growth Dynamics and Antioxidative Response. *Front. Plant Sci.*,**7:** 535-547.

اثر نانوپرایمینگ با اکسید روی و ذرات نانو نقره بر انبارداری دانه نخود و مدیریت بیمای پژمردگی

گ. داس، و پ. دوتا

چکیده

نخود از حبوبات مهم هندوستان است ولی بهره‌دهی عملکرد آن به علت چند تنش زیستی وغیرزیستی پایین است. یکی از مسایل در این ارتباط، ضعیف بودن دانه است که به خاطر تغییرات درخواص بیوشیمیایی افتراقی درطی دوران انبارداری نامناسب رخ میدهد. برای غلبه برفعالیتهای بیوشیمیایی، آماده سازی (پرایمینگ) دانه از راه‌کارهای امید بخش است. در پژوهش حاضر، دو عامل نانو پرایمینگ به نام ذرات نانو اکسید روی(ZnONPs) و ذرات نانو نقره (AgNPs) برای فعالیت های بیوشیمیایی نخود (شامل فعالیت پراکسیداز، آلفاامیلاز، کل قند محلول،وپروتئین کل) در طی مدت ۲، ۴، ۶، و ۹ ماه انبارداری و بعد از ۱، ۲، و ۶ ساعت پرایمینگ ارزیابی شد. نتایج حاکی از افزایش فعالیت پراکسیداز (POX) با افزایش دوره انبارداری بود ولی نرخ افزایش در موردی که دانه ها با ZnONPs نانوپرایمینگ شده بود نسبتا کم بود. همچنین، فعالیت آمیلاز و محتوای پروتئین در حد بیشینه ثبت شد

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ولی قند محلول کل (TSS) در دانه‌هایی که با ZnONPs پرایم شده بود در حد کمینه بود. در میان زمانهای مختلف پرایمینگ، تیمار ۶ ساعت پرایمینگ برای ۹ ماه انبارداری بهترین تیمار بود و اثر های مثبتی روی پارامترهای بیوشیمیایی داشت. نیز، در میان تنش های زیستی، بیماری ایجاد شده با *Fusarium oxysporum* f.sp. *ciceri* به عنوان یک بیماری زیانبار تلقی می شود زیرا هر سال باعث ۱۰٪ کاهش عملکرد می شود. برای رفع تنش زیستی و ارتقای شرایط انبارداری، دانه های نخود با ماده ZnONPs و AgNPs به تنهایی یا همراه با هم با ۱۰۰ قسمت در ملیون (ppm)آماده سازی و پرایم شد. در پی آن، اثرات مثبتی روی درصد جوانه زنی دانه، رشد گیاه، و پارامترهای مربوط به عملکرد و اثرات منفی بر ابتلا به بیماری *F.oxysporum* f.sp. *cicero* مشاهده شد.

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