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Research Article

Effect of ZnO nano priming on germination and root length of soybean seeds (*Glycine max* L.)

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Abstract: Nano-priming is a pioneering method of treating seeds that improves seed germination, growth, and yield by imparting resilience to several plant stressors. Zinc oxide (ZnO) is a nanomaterial with a specific surface area, high pore volume, low toxicity, and an extended lifetime, and used in nano-priming. This study aimed to determine the effect of ZnO nanoparticles (NPs) on seed germination and root length in determining the optimum concentration of ZnO-NPs for soya plants. The transmission of electron microscopy (TEM) and zeta potential measurements were used to characterize ZnO-NPs. Soya seeds were treated with different concentrations of ZnO-NPs (0, 250, 500, 1000 and 2000 mgL^{-1}) for 24 h. to determine the optimum concentration of ZnO-NPs for selected variants. After priming, the germination percentage and root length of each treatment were measured. The effect of ZnO nanoparticles (in soya plants was investigated by comparing them with seeds germinated in a control (hydro-priming) medium. The investigation demonstrated that the high concentration of ZnO NPs had an adverse impact on both seed germination and root length. Based on this, it was suggested that studies should be conducted including different concentrations of ZnO nanoparticles, which are thought to have a complex structure, to understand the mechanism of action, to find the appropriate concentration for soybean plants, and to increase seed germination.

1. INTRODUCTION

Seed priming, which often occurs during the first stage of germination, triggers metabolic processes that result in a higher percentage of germination and a faster emergence rate (Nile *et al*., 2022). At the same time, this process increases the ability of seeds to survive abiotic challenges and provides for their survival (Khan *et al*., 2023). Seed priming techniques often used include hydropriming (using water), Osmo priming (using polyethylene glycol and inorganic salts), hormonal priming, and nutritional priming. (Paparella *et al*., 2015; Sytar *et al*., 2019). Classic hydro priming involves soaking the seeds in water before drying them. So, seeds absorb water rapidly, increasing germination rates, and seedlings emerge uniformly.

Over the last decade, the use of nanoparticles for nutrient priming has significantly increased, surpassing other seed preparation methods (Zain *et al*., 2023). The process of nano priming forms nanopores in the embryo, aiding in water absorption (Gupta *et al*., 2024). In addition, nanoparticle (NP) stimulates amylase, which in turn promotes seed germination, causing starch

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degradation. Nano-priming mechanisms in seeds may speed up the growth of roots by changing the transcription of genes that control metabolic processes, such as making phytohormones (Imtiaz *et al*., 2023). Nanoparticles' most important roles in seed preparation are their ability to create electron exchange and their higher surface interaction capacity with different parts of plant cells and tissues. Different metal-based nanoparticles, along with biogenic and polymeric nanoparticles, have been used as seed pretreatment agents in nutrient priming. These include Ag, Au, Cu, Fe, FeS2, TiO2, Zn, and ZnO NPs. Among them, researchers are interested in Zn NPs because they are different from other metal oxide nanoparticles in that they can photocatalyze and photo-oxidize chemical and biological species (Chikkanna *et al*., 2019). Zinc doesn't dissolve well in soil, so plants don't have any of it. Zn NPs applications can solve this problem by providing plants with a form of zinc that dissolves better and is easier to use because it is very reactive. Rameshraddy *et al*. (2017) have demonstrated that priming seeds with Zn NPs enhances their Zn content, thereby promoting improved seedling growth and yield.

Nanoparticles' size, zeta potential, type, and concentration (Santás-Miguel *et al*., 2023) can either help seeds germinate or stop them from doing so (Alobaiddy & Zorer Çelebi, 2022; Das *et al.*, 2020; Hassanisaadi *et al.*, 2022; Li *et al*., 2021). In addition to NP characteristics, the effect of ZnO nanoparticles on plants also varies with plant age and species (Burman *et al*., 2013). ZnO nanoparticles have been shown to aid in the growth of plants such as mung bean (*Vigna radiata*), chickpea *(Cicer arientinum*), cucumber (*Cucumis sativus*), alfalfa *(Medicago sativa* L.), and tomato (*Solanum lycopersicum*) (Mahajan *et al*., 2011; de la Rosa *et al.*, 2013). Research on the exposure of ZnO NPs should include many plant species and variants since the impacts of ZnO NPs are unique to plants (Wang *et al.*, 2023). Further investigation is required to fully comprehend the consequences of NPs and reveal their concentration and variant dependence. The possibility that the ZnO NPs may have beneficial effects on the germination process makes the studies valuable in this area.

Given their economic significance, practical convenience, and various variants, soybeans are ideal model plants to test the impacts of these nanoparticles. Before the widespread application of ZnO NPs in agricultural environments, it is necessary to develop empirical models based on controlled experiments and field research. There is a limited amount of research that particularly examines the biological impacts of ZnO NPs on the process of seed germination and the development of various plant varieties (Faizan *et al.*, 2020; Thounaojam *et al*., 2021). The studies indicate that high concentrations such as 500 mg/kg (Yoon *et al.*, 2014), 500 mgL (Lopez-Moreno *et al.*, 2010) and 400 mg/kg (Yusefi-Tanha *et al*., 2020) of ZnO NP negatively affects the growth and development of soybeans. On the other hand, low concentrations 50 mgL (Gaafar *et al.*, 2020), <160 mg/kg (Yusefi-Tanha *et al*., 2020) effect positively root development and seed production, as well as 200 mg/kg the optimum root development in soybeans (Yusefi – Tanha *et al*., 2022). There are still unresolved scientific uncertainties surrounding the influence of nanoparticles on plants, namely different soybean variants with higher concentrations. Therefore, this research used a highly economic variant of *Glycine max* L. as a model to describe the effects of ZnO NPs on seed germination and root length.

The study's goal was to determine whether we could use different amounts of ZnO NPs as nano priming agents instead of hydropriming to aid in soya seed germination and improve their metabolic activity. The research further aimed to evaluate the influence of ZnO NPs on soybean plants and ascertain the optimal dosage for maximum effectiveness. We can use ZnO NPs to increase the Zn concentration in soybean seeds, which enhances their nutritional quality and speeds up germination. This approach may serve as an alternative to traditional hydropriming methods.

2. MATERIAL and METHODS

2.1. Plant Material

In our research, we used ANP 2018 seeds from a widely cultivated and easily grown soybean variety. The variety's thousand-grain weight is 121–131 g, and the growing period is near the middle (I. product: 135–140 days, II. product: 108–112 days) (ANP 2018, 2023). We obtained the variety of seeds from the Eastern Mediterranean Agricultural Research Institute Directorate.

2.2. Characterization of ZnO Nanoparticles

The ZnO NP used in the research was purchased from Nanography Turkey (https://shop.nanografi.com.tr). The nanoparticles that are provided have a purity level of at least 99 percent and an average size of less than 100 nanometers. The characterization research of ZnO nanoparticles included assessing the dimensions and distribution of the particles. The study was performed at Canakkale Onsekiz Mart University Science and Technology Application and Research Centre (COBİLTUM) utilizing a JEOL JEM-1400 PLUS Transmission Electron Microscope (TEM) model. The Zetasizer apparatus at the Turkish Energy, Nuclear, and Mining Research Institute (TENMAK) was used to estimate the zeta potential, which measures the level of repulsion or attraction between particles.

2.3. Seed Priming Method

This study used two distinct methodologies to investigate the effects of water and nanoparticles on seed preparation: Hydropriming, which simply employed water, and nano-priming, which included the dispersion of ZnO nanoparticles in water. The seeds were mixed in a magnetic stirrer in a 0.1% sodium hypochlorite solution for surface sterilization for ten minutes. Following sterilization, the seeds were thoroughly rinsed three times with deionized water to eliminate any traces of chlorine. Subsequently, the seeds were left to dry naturally (Mohamed *et al*., 2019). To manufacture the seeds, ZnO nanosuspensions with varying concentrations (0, 250, 500, 1000, and 2000 mgL$^{-1)}$ were produced by dispersing the particles in deionized water using ultrasonic vibration (200 W, 37 kHz) for 30 minutes (Hòe *et al.*, 2018). The seeds that had been prepared were thereafter rinsed 3–4 times (with each rinse lasting 3 minutes) using distilled water and then dried until they regained their initial moisture content. Subsequently, the seeds were packed in polythene bags and kept at ambient temperature until their next use.

2.4. Seed Germination Parameters

The seeds were placed inside a petri dish with a diameter of 10 cm, resting on two filter paper discs. The germination experiment was carried out in three replicates, and each treatment group consisted of a total of 100 seedlings. The seeds were incubated in an oven at a temperature of 24 ± 2 °C to initiate germination.

2.4.1. *Screening of different concentrations of ZnO NPs and priming time for seed priming*

To determine the ideal concentrations of nanoparticles for experimental purposes, four solutions were created with ZnO NPs at concentrations of 250 mgL^{-1}, 500 mgL^{-1}, 1000 mgL^{-1}, and 2000 mgL^{-1}. Soybean seeds were soaked in ZnO NP solutions at varying concentrations for 24 h at room temperature with continuous aeration and shaking. Hydroprimed seeds were soaked in deionized water for the same period (Mohamed *et al*., 2019). Each petri dish was filled with ten seeds and coated with filter paper. The dishes were then sealed with parafilm tape. Ultimately, all petri dishes were incubated in an oven at a temperature of 24 ± 2 °C. The germinating seeds were quantified using a binocular stereo microscope based on the appearance of a 2 mm root. The germination percentages and root lengths in mm were measured at time intervals of 15, 21, 24, 48, 72, and 96 hours (Hòe *et al*., 2018).

2.5. Statistical Analysis

We presented the data as the mean and standard deviation of three replicates for each treatment. They were then put through a two-way analysis of variance (ANOVA) using the R and R Studio (R Studio Team, 2020; R Core Team, 2021). We used a two-way ANOVA test to evaluate the effect of two grouping variables (treatment and duration) on a response variable, germination percentage or root length. Four ZnO NP solutions and the untreated control group comprised the five levels of the first factor, which related to the concentrations the seeds encountered (treatment). The second factor is related to temporal measurement, specifically the six-time intervals during which we conducted the measurements. The ANOVA assumptions were checked using the Shapiro-Wilk test for normal distribution and Levene's test for homogeneity of variance. Next, we performed a square root transformation on the root length data to meet the required assumptions. We used Duncan's multiple-range test to validate the statistical significance of the average difference between certain pairs of groups. (Açıkgöz *et al*., 2004).

3. FINDINGS

3.1. Characterisation of ZnO Nanoparticles

Transmission electron microscopy (TEM) analysis of ZnO NPs at various concentrations revealed that the ZnO NPs had an average size of 30-50 nm and exhibited a spherical morphology. Furthermore, the process of combining or gathering together in the cluster of sponge-like particles was documented and shown in Figure 1. The analysis revealed that a solution containing 250 mgL^{-1} of ZnO NPs had an average zeta potential of 11 ± 3.82 mV (Figure 2a). The solutions containing 500 mg/L^{-1}, 1000 mg/L^{-1}, and 2000 mg/L^{-1} of ZnO nanoparticles exhibited average zeta potentials of 11.1 ± 3.36 mV (Figure 2b), 9.25 ± 3.23 mV (Figure 2c), and 7.79 ± 3.10 mV (Figure 2d), respectively

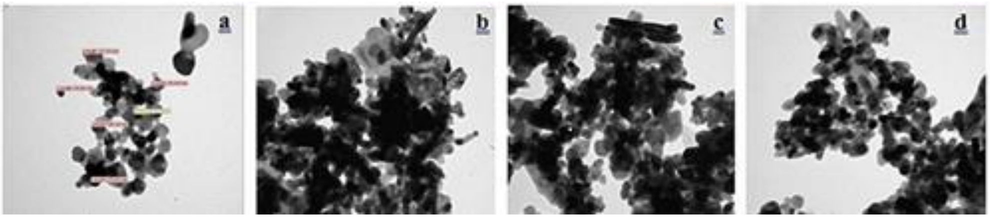

Figure 1. TEM micrograph of dispersion and dispersion of ZnO suspension at four different concentrations (250, 500, 1000, 2000 mgL^{-1}). (x 300000) **(a)** 250 mgL^{-1}, **(b)** 500 mgL^{-1} particle aggregation, **(c)** 1000 mgL^{-1} particle aggregation, **(d)** 2000 mgL^{-1} particle aggregation.

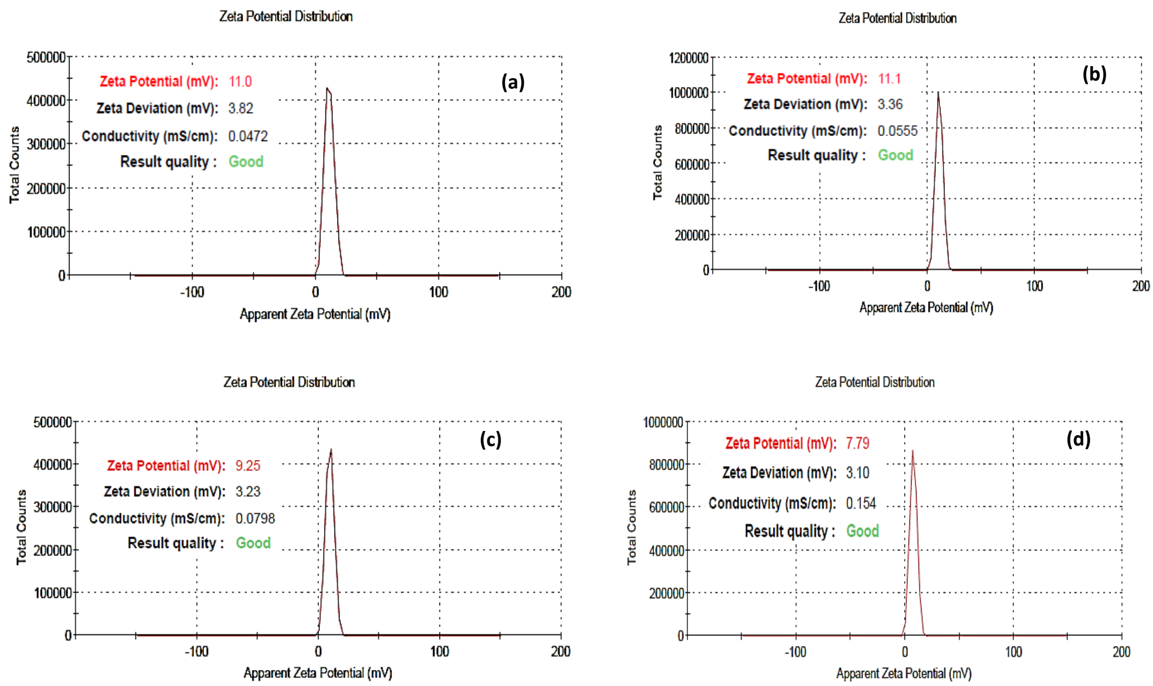

Figure 2. Zeta potential values of ZnO suspension at four different concentrations (250, 500, 1000, 2000 mgL^{-1}). (a) 250 mgL^{-1}, (b) 500 mgL^{-1}, (c) 1000 mgL^{-1}, (d) 2000 mgL^{-1}.

3.2. Accumulation of ZnO Nanoparticles in Seed Coat

The images of soya seeds treated with different concentrations of ZnO nanoparticles at the end of 24 hours were examined by Scanning Electron Microscopy (SEM). It was observed that as the number and concentration of nanoparticles penetrating the seed increased, the accumulation in the seed coat increased significantly in parallel (see Figure 3).

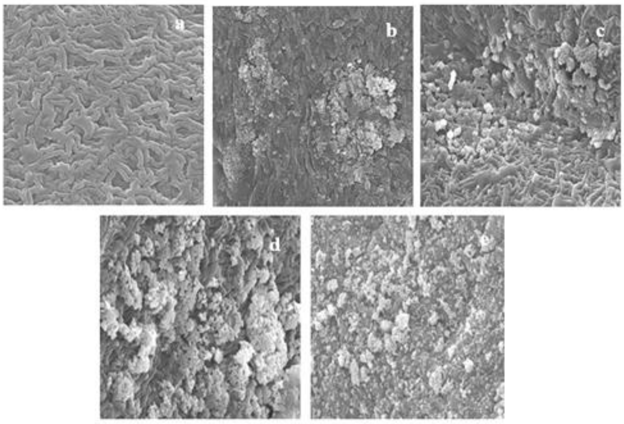

Figure 3. SEM micrograph of nanoparticle accumulation in seed coat. (x 5000) (a) Untreated, (b) 250 mgL^{-1}, (c) 500 mgL^{-1}, (d) 1000 mgL^{-1}, (e) 2000 mgL^{-1}.

3.3. Effect of ZnO Nanoparticle on Germination of Soybean

The germination percentage progressively increased with time in both the untreated and treatment groups. However, the maximum value was seen at the 72nd and 96th hours (Table 1 and Figure 4). The lowest germination percentage was seen at the 15th hour in both the untreated and treated groups with varying doses of ZnO NPs. Overall, the germination percentages quickly rose after 48 hours in both the untreated and treatment groups. The germination percentage indicated variability during 96 hours of the experiment (F=58.16, df=6, p<0.001).

Table 1. Effect of ZnO nanoparticle on germination of soya bean seeds.

Variety	ZnO NP Concentration (mgL^{-1})	Germination Percentage (%) 15th hour	18th hour	21st hour	24th hour	48th hour	72th hour	96th hour
ANP2018	0	60^{a}	72^{a}	74^{a}	79^{a}	83^{a}	90^{a}	91^{a}
	250	19^{b}	37^{b}	37^{b}	44^{b}	57^{b}	61^{b}	69^{b}
	500	20^{b}	35^{b}	39^{b}	44^{b}	67^{b}	67^{b}	78^{ab}
	1000	18^{b}	26^{b}	33^{b}	40^{b}	55^{b}	63^{b}	79^{ab}
	2000	20^{b}	32^{b}	38^{b}	48^{b}	57^{b}	67^{b}	82^{ab}
MSE (Mean Square Error)		90.2	97.13	110.4	99.3	119.6	42.3	50.4

*There is no difference between the values shown with the same letters.

Similarly, the germination percentage varied within treatments in all temporal measurement groups, and this variation was statistically significant (F=51.25, df = 4, p<0.001). The untreated group significantly contributed to this variation. The germination percentage of the untreated group was higher than that of the treatment groups, and the differences between the germination percentage of the untreated group and that of the treatment groups were statistically significant for all temporal measurement groups (p<0.001). Among treatment groups, the germination percentage of seeds exposed to high concentrations of ZnO NP was high compared to the seeds exposed to low concentrations at the 96th hour of the experiment. Among treatment groups, the germination percentage of seeds exposed to high concentrations of was high compared to the seeds exposed to low concentrations at the 96th hour of the experiment (see Table 1).

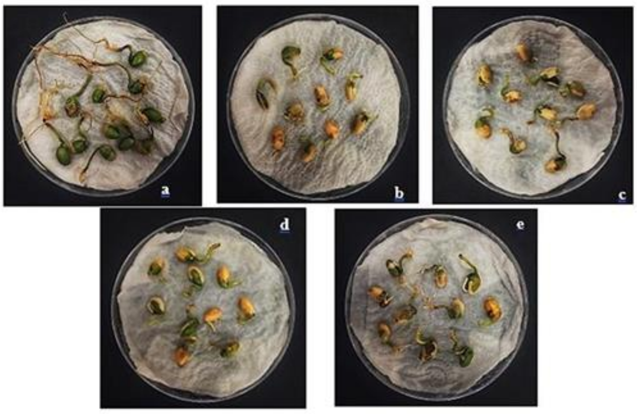

Figure 4. Effect of ZnO nanoparticle on germination percentage of soya bean seeds at the endof 96th hour **a:** control, **b:** 250 mgL^{-1}, **c:** 500 mgL^{-1}, **d:** 1000 mgL^{-1}, **e:** 2000 mgL^{-1}.

3.4. Effect of ZnO Nanoparticle on Root Length of Soybean

Root lengths obtained in the study are presented in Table 2. The root length got longer in the untreated group until they reached their longest point at 96 hours (Figure 5). There was no significant variation in root lengths between the 15th and 24th hours ($p > 0.05$, Table 2). The findings were consistent across all different ZnO NP treatments, indicating that there were no significant changes seen between the 15th and 48th hours ($p > 0.05$). In all treatments, the length of the roots exhibited growth at 72 hours, with the exception of the untreated group. The growth accelerated at the 48th hour in the untreated group (see Table 2). At the 96th hour, all treatment groups, including the untreated group, achieved the highest value. There was a significant difference among the root lengths of all experimental groups ($F = 112.7$, $df=4$, $p<0.001$). Root length was highest at the untreated group ($p<0.001$). The mean root length in the untreated group was longer than that in the all-treatment groups at the all-temporal measurement groups ($p<0.001$).

Table 2. Effect of ZnO nanoparticle on root length of soya bean seeds.

Variety	ZnO NP Conct. (mgL^{-1})	Average Root Length (cm) 15th hour	18th hour	21th hour	24th hour	48th hour	72th hour	96th hour
ANP 2018	0	0.56±0.13^{a}	0.73±0.22^{a}	0.81±0.22^{a}	0.81±0.21^{a}	1.91±0.98^{a}	2.90±1.61^{a}	7.22±5.30^{a}
	250	0.24±0.13^{b}	0.32±0.16^{b}	0.32±0.16^{b}	0.33±0.15^{b}	0.71±0.3^{b}	0.96±0.58^{b}	1.44±1.12^{b}
	500	0.28±0.14^{b}	0.36±0.21^{bc}	0.36±0.21^{bc}	0.37±0.20^{bc}	0.6±0.31^{b}	1.03±0.47^{b}	1.68±1.42^{b}
	1000	0.24±0.11^{b}	0.35±0.19^{bc}	0.43±0.20^{bc}	0.44±0.19^{bc}	0.81±0.4^{b}	0.93±0.53^{b}	1.51±1.29^{b}
	2000	0.29±0.09^{b}	0.43±0.18^{c}	0.45±0.21^{c}	0.45±0.20^{c}	0.78±0.3^{b}	0.95±0.55^{b}	1.52±1.47^{b}
MSE (Mean Square Error)		0.002	0.003	0.003	0.004	0.009	0.02	0.33

*There is no difference between the values shown with the same letters.

Figure 5. Effect of ZnO nanoparticle on root length of soya bean seeds at the end of 96th hour.

4. DISCUSSION and CONCLUSION

Zeta potential analysis measured the stability of ZnO NPs at different concentrations in this study. The ZnO NPs we studied were moderately stable and dispersed. The ZnO NP parts in the suspension can be used as a seed priming agent (Ateş, 2018). In our study, we measured the zeta potential lower than what Sharma *et al*. (2022) recorded. Furthermore, our study measured the zeta potential positively. There were functional groups on the surface of NP that gave it a positive charge. These groups consisted of hydroxyl (OH) or amine (NH_2) groups. Therefore, the higher pH values (alkaline) of the ZnO NP environment also explain the positive zeta potential (Hunter *et al.*, 1981). This phenomenon contributed to their stability, dispersion, and interactions with biological systems. On the other hand, low stability increases the possibility of particles adhering to one another (Hidayat Chai *et al*., 2018). Our results indicate that ZnO NPs exhibited lower stability at a concentration of 2000 $mgL^{-1,}$ leading to increased particle agglomeration. The SEM visualization supports this conclusion.

Seed germination and early seedling production are crucial phases in plant growth. The positive and detrimental effects of ZnO-NP use on plant development depend on the concentration used (Faizan *et al*., 2020; Rajput *et al*., 2021). In a study with maize and wheat, it was confirmed that germination percentage increased at lower ZnO NP concentrations (100 mgL^{-1}), but decreased variably at higher levels (150–200 - 200 mgL^{-1}) (Srivastav *et al*., 2021). In our study, seeds showed germination in all treatments, suggesting that ZnO NPs had a positive effect. ZnO NPs significantly increase seed water uptake compared to conventional hydropriming (El-Saadony *et al*., 2021; Rai-Kalal &Jajoo, 2021). The studies demonstrate that rapid absorption of water molecules and ZnO nanoparticles during nano-priming allows them to enter the seed through the seed coat's cell wall, leading to the production of ROS, a signal for fast seed germination (Mahakham *et al.,* 2017; Sharma *et al.*, 2021). As a result of ZnO nanocoating applications, increased seed water uptake promotes metabolic activity. Taking in water accelerates the process of breaking down starch by activating germination enzymes such as α-amylase. This makes seed germination much better in the early stages. Nano priming controls the aquaporin and α-amylase genes, making them better at quickly taking in water from the seed, breaking down starch, making more soluble sugar, starting GA signaling pathways, and getting rid of ROS to help seeds germinate faster and healthier (Khan *et al.*, 2023).

Researchers found that priming with ZnO NP increased the germination percentage in soybean (Montanha *et al.,* 2020) and wheat (Munir *et al*., 2018). However, Rosa *et al.* (2013) found that ZnO NPs at a concentration of 1600 ppm slowed the germination of Cucumis sativus (cucumber) by 10% and sped up the germination of *Solanum lycopersicum* (tomato) and *Medicago sativa* (black clover) by 20% and 40%, respectively. In a different study, it was found that high levels of ZnO NPs reduce the growth and germination of pepper seeds and seedlings, showing that ZnO NPs are toxic (García-López *et al.,* 2018). The results of our study are consistent with the literature indicating that high concentrations of ZnO NP reduce the percentage of germination in soybeans. Seeds treated with high concentrations of ZnO nanoparticles may have nanoparticle deposits on the outer surfaces. This situation may naturally block the pores of the seed coat, disrupting the water uptake necessary for germination. As a result, the seed is unable to absorb water sufficiently (Johns & Cahill, 2018). This event disrupts metabolic processes such as enzyme activation, protein synthesis, RNA synthesis, and cell division, among others. Accordingly, cellular oxidative stress begins to increase, and the antioxidant defence system weakens. This may delay the germination of seeds.

As in the germination percentage, previous studies reported that the root length of plants exposed to low ZnO NP concentrations (for example, 10 mgL^{-1}) increased (Itroutwar *et al*. 2020, Nemček *et al*. 2020, Youssef & Elamawi 2020). It is known that zinc is needed to make certain hormones, like auxins and gibberellins, which explains why the length of the radicle and plumule grows when ZnO NPs are present. Consequently, the radicle and plumule length of NP-exposed seeds increase. Zinc is essential for the biosynthesis of endogenous hormones, including auxins and gibberellins, which explains the increase in radicle and plumule length in the presence of ZnO NPs. Consequently, the radicle and plumule length of NP-exposed seeds increase (Cakmak, 2008; Prasad *et al*., 2012). On the other hand, the toxic effect of the high concentration of ZnO NP (1,000 $mgL^{-1)}$ reported as reducing the root length of corn and cucumber (Zhang *et al*., 2015). Also, research has linked high levels of ZnO NP (100–200 mgL^{-1}) to phytotoxicity by creating chromosomal errors, micronuclei, and vacuolated nuclei (Youssef & Elamawi 2020). Our study's result is consistent with this literature: high concentrations of ZnO NP exposure negatively affect root length in soybeans.

We used the nano-priming method to test ZnO nanoparticles on soy bean seeds at 15, 21, 24, 48, 72, and 96 hours to see how they changed the number of seeds that germinated and the length of the roots. The concentrations we tested were 0, 250 mgL^{-1}, 500 mgL^{-1}, 1000 mgL^{-1}, and 2000 $mgL^{-1.}$ ZnO had near-spherical structures with an average particle size of 30–50 nm. In solution, the zeta potential values of four different suspensions of ZnO NPs showed moderate stability and dispersion. At high concentrations, these characterized ZnO NPs had a negative effect on germination percentages and root lengths compared to the control groups. This statement is compatible with the results of previous studies on various plant species and varieties. These findings can help us understand the mechanism of action of high-concentration ZnO nanoparticles. However, given the intricate nature of the impact mechanisms, further research is necessary prior to agricultural application to boost plant growth and mitigate adverse effects.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors. **Ethics Committee Number**: Canakkale Onsekiz Mart University/ Postgraduate Education Institute Ethics Committee Scientific Research Ethics Committee, E-84026528-050.01.04-2200303364.

Authorship Contribution Statement

Burcu Akbay: Investigation, resources, visualization, software, formal analysis, and writing - original draft. **F. Sevil Yalçın**: Methodology, supervision, validation, review and editing.

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REFERENCES

Açıkgöz, N., & Gökçöl, İ.E. (2004). *TOTEMSTAT statistical packet programme: Assessment of biological research on the computer*. Aegean University. ISBN: 975-483-607-8.

Alobaiddy, M., & Zorer Çelebi, Ş. (2022). *Effect of active dry yeast and nano iron fertiliser on some yield and quality characteristics of maize (Zea mays L.) plants grown in alkaline soil* [Unpublished Doctoral dissertation]. Van Yüzüncü Yıl University.

Ateş M. (2018). Measurement and examination techniques of nanoparticles. *Turkish Journal of Scientific Review, 11*(1), 63.

Burman, U., Saini, M., & Kumar, P. (2013). Effect of zinc oxide nanoparticles on growth and antioxidant system of chickpea seedlings. *Toxicological & Environmental Chemistry*, *95*(4), 605-612. https://doi.org/10.1080/02772248.2013.803796

Cakmak, I. (2008). Enrichment of cereal grains with zinc: agronomic or genetic biofortification? *Plant and Soil*, *302*, 1-17. https://doi.org/10.1007/s11104-007-9466-3

Chikkanna, M.M., Neelagund, S.E., & Rajashekarappa, K.K. (2019). Green synthesis of zinc oxide nanoparticles (ZnO NPs) and their biological activity. *SN Applied Sciences*, *1*(1), 117. https://doi.org/10.1007/s42452-018-0095-7

Das, S., Mukherjee, A., Sengupta, G., & Singh, V.K. (2020). *Overview of nanomaterials synthesis methods, characterisation techniques and effect on seed germination: In Nano-Materials as Photocatalysts for Degradation of Environmental Pollutants* (pp. 371-401). Elsevier. https://doi.org/10.1016/B978-0-12-818598-8.00018-3

de la Rosa, G., López-Moreno, M.L., de Haro, D., Botez, C.E., Peralta-Videa, J.R., & Gardea-Torresdey, J.L. (2013). Effects of ZnO nanoparticles in alfalfa, tomato, and cucumber at the germination stage: root development and X-ray absorption spectroscopy studies. *Pure and Applied Chemistry 4th*, *85*(12), 2161-2174. https://doi.org/10.1351/pac-con-12-09-05

El-Saadony, M.T., Saad, A.M., Najjar, A.A., Alzahrani, S.O., Alkhatib, F.M., Salem, E., … Hassan, M.A.A. (2021). The use of biological selenium nanoparticles to suppress *Triticum aestivum* L. crown and root rot diseases induced by Fusarium species and improve yield under drought and heat stress. *Saudi Journal of Biological Sciences*, *28*(8), 4461-4471. https://doi.org/10.1016/j.sjbs.2021.04.043

Faizan, M., Faraz, A., Mir, A.R., Hayat, S. (2020). Role of zinc oxide nanoparticles in countering negative effects generated by cadmium in *Lycopersicon esculentum*. *Journal of Plant Growth Regulation*, *40*, 101-115. https://doi.org/10.1007/s00344-019-10059-2

Gaafar, R., Diab, R., Halawa, M., Elshanshory, A., El-Shaer, A., & Hamouda, M. (2020). Role of zinc oxide nanoparticles in ameliorating salt tolerance in soybean. *Egyptian Journal of Botany*, *60*(3), 733-747. https://doi.org/10.21608/ejbo.2020.26415.1475

García-López, J.I., Zavala-García, F., Olivares-Sáenz, E., Lira-Saldívar, R.H., Díaz Barriga-Castro, E., Ruiz-Torres, NA, ... & Niño-Medina, G. (2018). Zinc oxide nanoparticles boosts phenolic compounds and antioxidant activity of *Capsicum annuum* L. during germination. *Agronomy*, *8*(10), 215. https://doi.org/10.3390/agronomy8100215

Gupta, N., Rai, S.K., Kumar, R., Singh, P.M., Chaubey, T., Singh, V., & Behera, T.K. (2024). *Seed priming with engineered nanomaterials for mitigating abiotic stress in plants*: In Nanotechnology for abiotic stress tolerance and management in crop plants (pp. 229-247). Academic Press. https://doi.org/10.1016/B978-0-443-18500-7.00015-6

Hassanisaadi, M., Barani, M., Rahdar, A., Heidary, M., Thysiadou, A., & Kyzas, G.Z. (2022). Role of agrochemical-based nanomaterials in plants: Biotic and abiotic stress with germination improvement of seeds. *Plant Growth Regulation*, *97*(2), 375-418. https://doi.org/10.1007/s10725-021-00782-w

Hidayat Chai, M.H., Amir, N., Yahya, N., & Saaid, I.M. (2018). *Characterization and colloidal stability of surface modified zinc oxide nanoparticle*. In Journal of Physics: Conference Series, International conference on fundamental & applied sciences, a conference of world engineering, science & technology congress , Kuala Lumpur, Malaysia ,13-15 August, (Vol. 1123, p. 012007). IOP Publishing. https://doi.org/10.1088/1742-6596/1123/1/012007

Hoe, P.T., Linh, T.M., Van, N.T., Buu, N.Q., Mai, N.C., Ban, N.K., & Chau, N.H. (2018). Effects of nanoparticles zinc oxide and nano cobalt on the germination of soybean (*Glycine max* (L.) Merr). *Vietnam Journal of Biotechnology*, *16*(3), 501-508. https://doi.org/10.15625/1811-4989/16/3/13472

Hunter, R.J. (1981). *Zeta Potential in Colloid Science: Principles and applications*. Academic. Press. https://doi.org/10.1016/C2013-0-07389-6

Imtiaz, H., Shiraz, M., Mir, A.R., Siddiqui, H., & Hayat, S. (2023). Nano-priming techniques for plant physio-biochemistry and stress tolerance. *Journal of Plant Growth Regulation*, *42*(11), 6870-6890. https://doi.org/10.1007/s00344-023-10981-6

Itroutwar, P.D., Govindaraju, K., Tamilselvan, S., Kannan, M., Raja, K., & Subramanian, K.S. (2020). Seaweed-based biogenic ZnO nanoparticles for improving agro- morphological characteristics of rice (*Oryza sativa* L.). *Journal of Plant Growth Regulation*, *39*, 717-728. https://doi.org/10.1007/s00344-019-10012-3

Johns, D.A., Cahill D. (2018). *Effect of silicon dioxide nanoparticles on seed germination and growth of four different plant species* [Msc dissertation, Deakin University]. Deakin University. https://hdl.handle.net/10536/DRO/DU:30132925

Khan, M.N., Fu, C., Li, J., Tao, Y., Li, Y., Hu, J., ... & Li, Z. (2022). Seed nanopriming: How do nanomaterials improve seed tolerance to salinity and drought? *Chemosphere*, 136911. https://doi.org/10.1016/j.chemosfer.2022.136911

Li, Y., Liang, L., Li, W., Ashraf, U., Ma, L., Tang, X., ... & Mo, Z. (2021). ZnO nanoparticle-based seed priming modulates early growth and enhances physio-biochemical and metabolic profiles of fragrant rice against cadmium toxicity. *Journal of Nanobiotechnology*, *19*, 1-19. https://doi.org/10.1186/s12951-021-00820-9

López-Moreno, M.L., de la Rosa, G., Hernández-Viezcas, J.Á., Castillo-Michel, H., Botez, C. E., Peralta-Videa, J.R., & Gardea-Torresdey, J.L. (2010). Evidence of the differential biotransformation and genotoxicity of ZnO and CeO_2 nanoparticles on soybean (*Glycine max*) plants. *Environmental Science & Technology*, *44*(19), 7315-7320. https://doi.org/10.1021/es903891gopen_in_new

Mahajan, P., Dhoke, S.K., Khanna, A.S., & Tarafdar, J.C. (2011). Effect of nano-ZnO on growth of mung bean (*Vigna radiata*) and chickpea (*Cicer arietinum*) seedlings using plant agar method. *Applied Biological Research*, *13*(2), 54-61.

Mahakham, W., Sarmah, A.K., Maensiri, S., & Theerakulpisut, P. (2017). Nanopriming technology for enhancing germination and starch metabolism of aged rice seeds using phytosynthesised silver nanoparticles. *Scientific Reports*, *7*(1), 8263. https://doi.org/10.1038/s41598-017-08669-5

Mohamed, E., Harbi, H., & Aref, N. (2019). Radioprotective Efficacy of zinc oxide nanoparticles on γ-ray-induced nuclear DNA damage in *Vicia faba* L. as evaluatedby DNA bioassays. *Journal of Radiation Research and Applied Sciences*, *12*(1), 423-436. https://doi.org/10.1080/16878507.2019.1690798

Montanha, G.S., Rodrigues, E.S., Marques, J.P.R., de Almeida, E., Colzato, M., & Pereira de Carvalho, H.W. (2020). Zinc nanocoated seeds: an alternative to boost soybean seed germination and seedling development. *SN Applied Sciences*, *2*(5), 857. https://doi.org/10.1007/s42452-020-2630-6

Munir, T., Rizwan, M., Kashif, M., Shahzad, A., Ali, S., Amin, N., ... & Imran, M. (2018). Effect of zinc oxide nanoparticles on the growth and zn uptake in whea (*Triticum aestivum* L.) by see priming method. *Digest Journal of Nanomaterials & Biostructures* (djnb), *13*(1).

Nemček, L., Šebesta, M., Urík, M., Bujdoš, M., Dobročka, E., & Vávra, I. (2020). Impact of bulk ZnO, ZnO nanoparticles and dissolved Zn on early growth stages of barley-a pot experiment. *Plants*, *9*(10), 1365. https://doi.org/10.3390/plants9101365

Nile, S.H., Thiruvengadam, M., Wang, Y., Samynathan, R., Shariati, M.A., Rebezov, M., ... Kai, G. (2022). Nano-priming as emerging seed priming technology for sustainable agriculture-recent developments and future perspectives. *Journal of Nanobiotechnology*, *20*(1), 1-31. https://doi.org/10.1186/s12951-022-01423-8

Paparella, S., Araújo, S.S., Rossi, G., Wijayasinghe, M., Carbonera, D., & Balestrazzi, A. (2015). Seed priming: state of the art and new perspectives. *Plant Cell Reports*, *34*, 1281-1293. https://doi.org/10.1007/s00299-015-1784-y

Prasad, T.N.V.K.V., Sudhakar, P., Sreenivasulu, Y., Latha, P., Munaswamy, V., Reddy, K.R., & Pradeep, T. (2012). Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. *Journal of Plant Nutrition*, *35*(6), 905-927. https://doi.org/10.1080/01904167.2012.663443

R Core Team (2021). *R: A language and environment for statistical computing*. R Foundation

for Statistical Computing, Vienna, Austria. https://www.r-project.org/
Rai-Kalal, P., & Jajoo, A. (2021). Priming with zinc oxide nanoparticles improve germination and photosynthetic performance in wheat. *Plant Physiology and Biochemistry, 160*, 341-351. https://doi.org/10.1016/j.plaphy.2021.01.032
Rajput, V.D., Minkina, T., Kumari, A., Harish Singh, V.K., Verma, K.K., Mandzhieva, S., … Keswani C. (2021). Coping with the challenges of abiotic stress in plants:new dimensions in the field application of nanoparticles. *Plants, 10,* 1221. https://doi.org/10.3390/plants10061221
Rameshraddy, G., Pavithra, J., Mahesh, S., Geetha, K.N., & Shankar, A.G. (2017). Seed priming and foliar spray with nano zinc improves stress adaptability and seed zinc content without compromising seed yield in ragi (*Finger millet*). *International Journal of Pure and Applied Bioscience*, *5*(3), 251-258.http://dx.doi.org/10.18782/2320-7051.2906
RStudio Team (2020). *RStudio: Integrated Development for R*. RStudio, PBC, Boston, MA. http://www.rstudio.com/
Santás-Miguel, V., Arias-Estévez, M., Rodríguez-Seijo, A., & Arenas-Lago, D. (2023). Use of metal nanoparticles in agriculture. A review on the effects on plant germination. *Environmental Pollution*, 122222. https://doi.org/10.1016/j.envpol.2023.122222
Sharma, D., Afzal, S., & Singh, N.K. (2021). Nanopriming with phytosynthesized zinc oxide nanoparticles for promoting germination and starch metabolism in rice seeds. *Journal of Biotechnology*, 336, 64-75. https://doi.org/10.1016/j.jbiotec.2021.06.014
Sharma, P., Urfan, M., Anand, R., Sangral, M., Hakla, H.R., Sharma, S., & Bhagat, M. (2022). Green synthesis of zinc oxide nanoparticles using Eucalyptus lanceolata leaf litter: characterisation, antimicrobial and agricultural efficacy in maize. *Physiology and Molecular Biology of Plants*, *28*(2), 363-381. https://doi.org/10.1007/s12298-022-01136-0
Srivastav, A., Ganjewala, D., Singhal, R.K., Rajput, V.D., Minkina, T., Voloshina, M., & Shrivastava, M. (2021). Effect of ZnO nanoparticles on growth and biochemical responses of wheat and maize. *Plants*, *10*(12), 2556. https://doi.org/10.3390/plants10122556
Sytar, O., Kumari, P., Yadav, S., Brestic, M., & Rastogi, A. (2019). Phytohormone priming: regulator for heavy metal stress in plants. *Journal of Plant Growth Regulation*, *38*, 739-752. https://doi.org/10.1007/s00344-018-9886-8
Thounaojam, T.C., Meetei, T.T., Devi, Y.B., Panda, S.K., & Upadhyaya, H. (2021). Zinc oxide nanoparticles (ZnO-NPs): a promising nanoparticle in renovating plant science. *Acta Physiologiae Plantarum*, 43, 1-21. https://doi.org/10.1007/s11738-021-03307-0
Wang, X., Xie, H., Wang, P., & Yin, H. (2023). Nanoparticles in plants: Uptake, transport and physiological activity in leaf and root. *Materials*, *16*(8), 3097. https://doi.org/10.3390/ma16083097
Yoon, S.J., Kwak, J.I., Lee, W.M., Holden, P.A., & An, Y.J. (2014). Zinc oxide nanoparticles delay soybean development: a standard soil microcosm study. *Ecotoxicology and Environmental Safety*, *100*, 131-137. https://doi.org/10.1016/j.ecoenv.2013.10.014
Youssef, M., & Elamawi, R. (2020). Evaluation of Phytotoxicity, Cytotoxicity, and Genotoxicity of ZnO Nanoparticles in Vicia faba. *Environmental Science and Pollution Research*, *27*(16), 18972-18984. https://doi.org/10.1007/s11356-018-3250-1
Yusefi-Tanha, E., Fallah, S., Rostamnejadi, A., & Pokhrel, L.R. (2020). Zinc oxide nanoparticles (ZnONPs) as a novel nanofertilizer: Influence on seed yield and antioxidant defense system in soil grown soybean (*Glycine max* cv. Kowsar). *Science of the Total Environment*, *738*, 140240. https://doi.org/10.1016/j.scitotenv.2020.140240
Yusefi-Tanha, E., Fallah, S., Rostamnejadi, A., & Pokhrel, L.R. (2022). Responses of soybean (*Glycine max* [L.] Merr.) to zinc oxide nanoparticles: Understanding changes in root system architecture, zinc tissue partitioning and soil characteristics. *Science of the Total Environment*, *835*, 155348. https://doi.org/10.1016/j.scitotenv.2022.155348
Zain, M., Ma, H., Chaudhary, S., Nuruzaman, M., Azeem, I., Mehmood, F., ... & Sun, C. (2023). Nanotechnology in precision agriculture: Advancing towards sustainable crop production.

Plant Physiology and Biochemistry, 108244. https://doi.org/10.1016/j.plaphy.2023.108244

Zhang, R., Zhang, H., Tu, C., Hu, X., Li, L., Luo, Y., & Christie, P. (2015). Phytotoxicity of ZnO nanoparticles and the released Zn (II) ion to corn (*Zea mays* L.) and cucumber (*Cucumis sativus* L.) during germination. *Environmental Science and Pollution Research*, *22*, 11109-11117. https://doi.org/10.1007/s11356-015-4325-x