SIZE-DEPENDENT EFFECTS OF ZNO AND FE_2O_3 NANOPRIMING ON GERMINATION DYNAMICS AND SYNCHRONY IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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EFECTELE DEPENDENTE DE DIMENSIUNE ALE TRATAMENTULUI CU NANOPARTICULE DE ZNO ȘI FE_2O_3 ASUPRA DINAMICII ȘI SINCRONIZĂRII GERMINĂRII LA FLOAREA-SOARELUI (HELIANTHUS ANNUUS L.)

Waleed Khaled Kaddem AL-SUDANI [1,2], Rawaa Shakir Shnain AL-SHAMMARI [3,4], Jasim Hafedh AL SAEDI [3], Giorgiana Diana Carmen ANGHELESCU [1], Maria MERNEA *[3], Mihai Gabriel MATACHE*[5], Dan Florin MIHAILESCU [1,3]

[1] Doctoral School of Biology, Faculty of Biology, University of Bucharest, Bucharest / Romania;
[2] Ministry of Trade in Iraq, The General Company for Foodstuff Trade, Baghdad / Iraq;
[3] Department of Anatomy, Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, Bucharest / Romania;
[4] Ministry of Agriculture in Iraq, Baghdad/ Iraq;
[5] National Institute of Research – Development for Machines and Installations Designed for Agriculture and Food Industry – INMA Bucharest, Bucharest/ Romania.
Tel: +40213181573; E-mail: maria.mernea@bio.unibuc.ro
Tel: +40 727 957 693; E-mail: matache@inma.ro
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ABSTRACT

Nanopriming enhances seed germination and early vigor by delivering micronutrients at the nanoscale level. This study evaluated the size-dependent effects of ZnO (7 nm, 100 nm) and Fe_2O_3 (4.5 nm, 16.7 nm) nanoparticles (NPs) on sunflower (Helianthus annuus L.) germination dynamics. Seeds were primed with 10–100 µg/mL NPs, and germination was assessed through mean germination time (MGT), germination speed index (GSI), and synchrony (CV). NP type and size, but not concentration, significantly affected MGT and GSI. ZnO 7 nm enhanced speed and uniformity, while Fe_2O_3 4.5 nm delayed and desynchronized germination. These results show that nanoparticle physicochemical properties critically modulate germination, highlighting ZnO nanopriming for improved seedling emergence.

REZUMAT

Tratamentul semințelor cu nanoparticule (NP) îmbunătățește germinarea și vigurozitatea timpurie prin livrarea micronutrienților la scară nanometrică. Acest studiu a investigat efectele dependente de dimensiune ale NP de ZnO (7 nm, 100 nm) și Fe_2O_3 (4.5 nm, 16.7 nm) asupra dinamicii germinării semințelor de floarea-soarelui (Helianthus annuus L.). Semințele au fost tratate cu 10–100 µg/mL NP, iar germinarea a fost evaluată prin timpul mediu de germinare (MGT), indicele de viteză a germinării (GSI) și sincronizarea (CV). Tipul de NP și dimensiunea, dar nu și concentrația, au afectat semnificativ MGT și GSI. ZnO 7 nm a accelerat și uniformizat germinarea, în timp ce Fe_2O_3 4,5 nm a întârziat și desincronizat germinarea. Rezultatele descriu rolul proprietăților fizico-chimice ale NP în modularea germinării și subliniază efectul pozitiv al ZnO pentru apariția răsadurilor.

INTRODUCTION

Modern agriculture faces increasing challenges due to security and sustainability issues, including growing global food demand, climate variability, soil degradation, and the emergence of pathogens that resist conventional control strategies *(Paulikienė et al., 2025)*. These constraints impose a need of developing environmentally sustainable practices that promote efficient early plant growth and long-term crop resilience. Stress factors related to climate change, such as elevated temperatures, increased atmospheric CO_2 concentrations, and soil salinization, have a negative impact on seed vigor and germination, resulting in inhibited, delayed or heterogeneous emergence *(Chaudhry et al., 2022)*. Germination represents a sensitive and decisive phase of the plant life cycle. Environmental stress acting during this stage can impair stand formation and reduce yield potential. Therefore, a major objective of climate-resilient agriculture is represented by the enhancement of efficiency and uniformity of seed germination. This can be achieved by supporting rapid seedling development, improving tolerance to abiotic stress, and reducing the need for agrochemical inputs *(Gohari et al., 2025)*.

In this context, seed priming strategies were effective in reducing residual dormancy and thermoinhibition, contributing to more stable crop establishment within sustainable agricultural systems *(Reed et al., 2022)*.

Seed priming is a widely used technique to overcome dormancy and improve germination performance. Beyond the early stages, priming can also influence the development of mature plants, leading to greater stress tolerance and higher micronutrient content *(Acharya et al., 2020; Faizan et al., 2024; Granata et al., 2024; MacDonald et al., 2025)*. Among the different priming approaches, nanopriming has emerged as an effective method that not only enhances germination, but also reduces the need for fertilizers and pesticides in agriculture *(do Espirito Santo Pereira et al., 2021)*. This strategy makes use of nanoparticles (NPs), whose small size, large surface-to-volume ratio, and strong adsorption capacity provide unique advantages for plant protection and nutrient delivery *(Faizan et al., 2024)*.

Previous studies have reported several benefits of nanopriming, including improved seed germination, growth, and yield *(Acharya et al., 2020; Nile et al., 2022; Tamindžić et al., 2023)*, enhanced seed vigor *(Ochoa-Chaparro et al., 2025, 2025; Yang et al., 2024)*, stimulated metabolism *(Hasanaklou et al., 2023; Mahakham et al., 2017; Yang et al., 2024)*, increased levels of polyphenolic compounds *(Al-Sudani et al., 2024; Geremew et al., 2025)*, and better stress tolerance in plants *(Alhammad et al., 2023; Faizan et al., 2024; Ghosh et al., 2024; Lee et al., 2024; Yavari et al., 2023)*. However, the field is still developing, and further research is needed, as some studies have also highlighted potential toxic effects of NPs on plants *(Abbasi Khalaki et al., 2021; Hatami et al., 2016)*.

The effects of nanopriming are highly dependent on several interrelated parameters – NP type, size, concentration and plant species addressed. Together, these factors create a complex interplay that shapes the overall effectiveness of NP-based seed treatments. First, the type of NP plays a critical role, as different materials, such as ZnO, Fe, Ti, Ag, Au, etc., exhibit distinct solubility, reactivity, and interactions with plant tissues, leading to variable effects on germination, growth, and stress responses *(Yang et al., 2024)*. Second, NP size influences bioavailability and cellular uptake, with smaller particles generally penetrating seeds more effectively, but also potentially causing higher oxidative stress due to increased surface reactivity *(Djanaguiraman et al., 2024)*. Third, the concentration of NPs determines whether their impact is beneficial or inhibitory; low to intermediate doses often stimulate germination and seedling vigor, while higher concentrations can induce phytotoxicity *(Freire et al., 2024)*. Finally, the plant species itself affects the outcome, as differences in seed coat structure, metabolism, and inherent stress tolerance can modulate the plant's response to nanopriming. For instance, silver NPs improved germination and growth in *Satureja hortensis (Nejatzadeh, 2021)*, but inhibit growth and reduce cellular viability in *Lemna gibba (Oukarroum et al., 2013)* and *Oryza sativa* L. *(Thuesombat et al., 2014)*.

The effect of priming on seed germination can be assessed using various metrics derived from daily counts of germinated seeds. Germination percentage and germination rate provide an overall measure of viability and speed *(Kader et al., 2005)*, while additional indices such as mean germination time (MGT), germination speed index (GSI), and germination synchrony (CV) offer deeper insights into the timing, vigor, and uniformity of germination *(Kader et al., 2005)*. Lower MGT and higher GSI values are associated with faster and more vigorous germination *(Ranal et al., 2009; Talská et al., 2020)*, while lower CV (or higher synchrony) indicates a more uniform germination event, which is desirable for uniform stand establishment and mechanized harvesting *(Tomaz et al., 2018)*.

In a previous study, the influence of NP type, size, and concentration on the germination kinetics of *Helianthus annuus* L. (sunflower) seeds was investigated *(Al-Sudani et al., 2024)*. It was found that 100 nm ZnO NPs significantly enhanced germination, whereas 4.5 nm Fe_2O_3 NPs delayed it. In addition, ZnO NP treatment increased total phenolic content, with 7 nm ZnO NPs producing the strongest effect. The extracted polyphenols effectively inhibited protein glycation. Regarding the germination metrics considered in that study, the analysis focused on overall germination behavior, including the final germination percentage, the temporal progression of germination, and germination rates *(Al-Sudani et al., 2024)*.

The aim of the present study was to extend the previous analysis by systematically evaluating how NP type (ZnO vs. Fe_2O_3), size (4.5 nm, 7 nm, 16.7 nm, 100 nm), and concentration (0 – 100 µg/mL) affect the kinetics and synchrony of sunflower seed germination. Specifically, the effects of ZnO and Fe_2O_3 NPs of different sizes on MGT, GSI, and CV were compared, thereby assessing not only whether seeds germinate, but also how rapidly and uniformly the germination process unfolds. By focusing on these indices, the present study provides an in depth understanding of nanopriming effects that is directly relevant to sowing management, prediction of crop establishment, and optimization of agricultural output.

MATERIALS AND METHODS

Seed material and nanoparticles

The nanopriming experiments were carried out using seeds of *Helianthus annuus* L. (sunflower), Suria hybrid, obtained from Ciproma Sem (Bucharest, Romania). The seeds were sterilized by sequential washing under running tap water for 5 minutes, followed by immersion in 1% (v/v) sodium hypochlorite solution for 3 minutes with continuous agitation. The seeds were then rinsed thoroughly with sterile distilled water (3–4 times) to remove residual chlorine.

Two commercial ZnO NP formulations were used in this study. The first was a nanopowder with a manufacturer-reported average particle size of 7–13 nm (cat. no. 44299, 10 g, CAS 1314-13-2, Alfa Aesar, Ward Hill, MA, USA), referred as "7 nm ZnO NPs" throughout the manuscript for simplicity. The second was an aqueous 20 wt.% dispersion with a reported particle size below 100 nm (cat. no. 721077-100G, CAS 1314-13-2, Sigma-Aldrich, St. Louis, MO, USA), referred to as "100 nm ZnO NPs". These nominal sizes are used as representative descriptors, consistent with standard practice for commercial nanopowders and dispersions.

In addition, two γ-Fe_2O_3 NP types synthesized in-house by laser pyrolysis were employed. These were previously described and extensively characterized *(Baciu et al., 2019; Lungu et al., 2023)*. The sample referred to as "4.5 nm Fe_2O_3 NPs" corresponds to the SFnew2 sample reported by *Lungu et al., (2023)*. Transmission electron microscopy (TEM) analysis indicated a mean particle diameter of approximately 4.2 nm, with predominantly spherical morphology and a tendency to form chain-like aggregates. X-ray diffraction (XRD) analysis revealed a mean crystallite size of 4.4 nm and a cubic spinel structure characteristic of magnetite/maghemite (Fe_3O_4/γ-Fe_2O_3). Selected-area electron diffraction (SAED) patterns revealed diffraction planes consistent with γ-Fe_2O_3 *(Lungu et al., 2023)*.

The sample referred to as "16.7 nm Fe_2O_3 NPs" corresponds to the SFnew11 sample described by *Baciu et al., (2019)*. XRD analysis indicated a magnetite/maghemite (Fe_3O_4/γ-Fe_2O_3) composition, Fe/O ratios supporting γ-Fe_2O_3 as the dominant phase. The crystallite size estimated from XRD patterns was ~ 17 nm, depending on the diffraction plane used *(Baciu et al., 2019)*. The value of 16.7 nm is used here for consistency with earlier reports *(Al-Sudani et al., 2024)*.

Stock NP suspensions were freshly prepared in deionized water and diluted to final concentrations of 10, 20, 40, 60, and 100 µg/mL. Prior to use, NP suspensions were dispersed by sonication for 30 min. The selected NP concentration range (10–100 µg/mL) was based on the previous findings indicating that these concentrations are sufficient to modulate germination responses *(Al-Sudani et al., 2024)* and is consistent with ranges reported in the literature *(Esmaeili et al., 2025; Kumar et al., 2025; Sarkhosh et al., 2022; Yang et al., 2023)*.

Nanopriming and germination protocol

The detailed germination protocol has been previously described in *(Al-Sudani et al., 2024)*. Briefly, the sunflower seeds, 15 per replicate (n = 3), were soaked for 4 h at 25 °C in NP suspensions at the concentrations listed above (Figure 1a). For each condition, a control was included in which seeds were soaked in deionized water alone.

Fig. 1 – Experimental setup of the nanopriming assay for sunflower seeds.

(a) Tubes with sunflower seeds soaked in different NP solutions. (b) Seeds transferred to Petri dishes immediately after soaking in the NP solution. (c) The same seeds after 5 days of germination. The figure is reproduced from *(Al-Sudani et al., 2024)*, published by MDPI, Basel, Switzerland, under the terms of the Creative Commons Attribution (CC BY) license (*https://creativecommons.org/licenses/by/4.0/*).

After the soaking period, seeds were carefully blotted on sterile filter paper to remove excess liquid and then transferred to 100 mm Petri dishes lined with moist filter paper (Figure 1b). The dishes were sealed with Parafilm to maintain humidity and placed in a dark germination chamber at 25 ± 1 °C. Moisture was maintained by adding sterile water as needed to prevent desiccation. The seeds were left to germinate for a total of 10 days (Figure 1c). The number of germinated seeds was recorded daily by counting the number of seeds with visible radicle protrusion of at least 2 mm.

Calculation of germination kinetics indices

The kinetics of seed germination was evaluated using the indices described below, calculated based on the daily counts of germinated seeds from each replicate.

Mean germination time (MGT) was calculated according to the equation provided in *(Orchard, 1977)*:

$$MGT = \sum(n_i \cdot t_i)/\sum n_i \quad (1)$$

The germination speed index (GSI) was determined using the equation described in *(Domingues Neto et al., 2024)*:

$$GSI = \sum n_i/t_i \quad (2)$$

The germination synchrony was estimated based on the coefficient of variation of germination times (CV), calculated as in *(Silva et al., 2019)*:

$$CV = \text{SD of germination times}/\text{MGT} \quad (3)$$

where n_i is the number of seeds germinated at time t_i (the time - here measured in days - since sowing) and SD is the standard deviation of germination times.

Statistical analysis

All analyses were conducted in Python (v. 3.10) using the pandas, NumPy, SciPy, statsmodels, matplotlib, and seaborn libraries. Germination indices (MGT, GSI, and CV) were calculated for each replicate.

Effect of NP concentration

For each NP type and size, the influence of NP concentration on germination indices was evaluated using Kruskal–Wallis tests. When appropriate, pairwise Mann–Whitney U tests with Holm correction were applied for post-hoc comparisons.

Effect of NP type and concentration

A two-way ANOVA including the interaction term (NP type × concentration) was used to assess the combined effect of NP type and concentration. Given the small sample size (n = 3 per group), formal normality testing was not performed. Therefore, ANOVA results were interpreted as complementary to the non-parametric analyses.

Effect of NP size

The effect of NP size was examined using Kruskal–Wallis tests on data pooled across all concentrations and within each NP type. Significant effects were further explored using Holm-corrected pairwise Mann–Whitney U tests.

Correlation analysis

Spearman rank correlation coefficients were computed to evaluate relationships among MGT, GSI, and CV, both across all NP treatments pooled together and separately for each combination of NP type and size.

Data are presented as mean ± standard deviation. Statistical significance was set at $p < 0.05$. All plots were generated in matplotlib and seaborn, with error bars indicating standard deviations and boxplots used to visualize pooled distributions.

RESULTS

The calculated MGT, GSI and CV values for seeds treated with 4.5 nm Fe_2O_3 NPs, 16.7 nm Fe_2O_3 NPs, 7 nm ZnO NPs and 100 nm ZnO NPs are presented in Figure 2. Across all treatments, MGT ranged from 3.31 ± 0.04 days (0 µg/mL ZnO 7 nm) to 4.38 ± 1.07 days (20 µg/mL ZnO 100 nm), with Fe_2O_3 NPs generally producing slightly longer germination times than ZnO (Figure 2a). For Fe_2O_3, MGT values tended to increase moderately with NP concentration, particularly for the 4.5 nm particles, whereas the 16.7 nm Fe_2O_3 NPs exhibited more stable and homogeneous values. The treatment with 4.5 nm Fe2O3 NPs showed the greatest variability among all conditions. ZnO treatments showed overall lower and more homogeneous MGT, especially for the 7 nm NPs.

GSI values ranged between 3.82 ± 0.38 (40 µg/mL Fe_2O_3 16.7 nm) and 4.68 ± 0.02 (0 µg/mL ZnO 7 nm) (Figure 2b). With a few exceptions, the replicate values were homogeneous across the treatment conditions. Fe_2O_3 treatments generally produced moderately lower GSI values compared with ZnO, with the 16.7 nm Fe_2O_3 NPs showing more variable outcomes across concentrations. In contrast, ZnO NPs, particularly the 7 nm particles, consistently led to higher and more homogeneous GSI values, suggesting faster germination rates under these conditions.

CV ranged from 0.22 ± 0.03 (0 µg/mL ZnO 7 nm) to 0.46 ± 0.07 (60 µg/mL Fe_2O_3 16.7 nm). The variability of CV values obtained for most treatments was larger among replicates than in the case of other indices, substantial heterogeneity in the germination response among individual seeds. The CV values in Figure 2c reflect the differences in germination uniformity among treatments. Fe_2O_3 NPs tended to induce higher variability, particularly for the 16.7 nm particles at intermediate concentrations (40–60 µg/mL), whereas ZnO treatments, especially with 7 nm particles, maintained relatively low CV values, indicating more uniform germination.

These descriptive results establish a baseline for evaluating the effects of NP type, size, and concentration on germination dynamics in the subsequent statistical analyses.

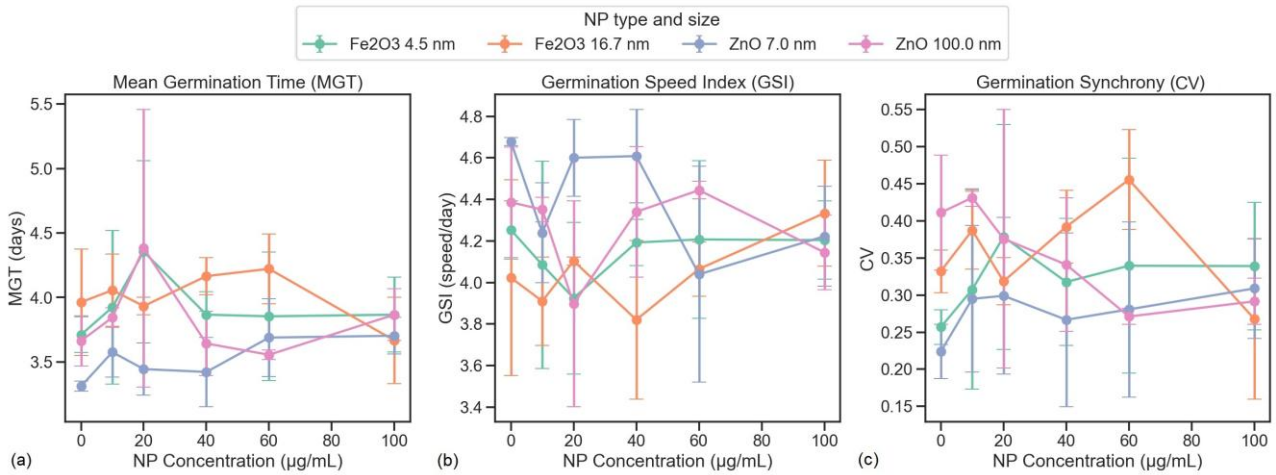

Fig. 2 – Gemination indices calculated for the seeds treated with different NPs as a function of NP concentration.
(a) mean germination times - MGT, (b) germination speed indices – GSI, (c) germination synchrony – CV. *Data are shown for all NP types and sizes (Fe_2O_3 4.5 nm and 16.7 nm; ZnO 7 nm and 100 nm). The symbols represent mean values ± SD of three replicates.*

Effect of NPs concentration on the germination of seeds

To address the impact of NP concentration on the germination indices Kruskal–Wallis tests were applied. The results indicated no statistically significant effects of NP concentration on MGT, GSI, or CV for any specific combination of NP type and size, with statistical p values exceeding 0.05. Two cases were identified in which p values were closer to the cutoff, namely GSI values for seeds treated with increasing concentrations of 7 nm ZnO NPs (p = 0.061) and CV values for seeds treated with 16.7 nm Fe_2O_3 NPs (p = 0.056). In these cases, a trend was observed, suggesting that higher NP concentrations might slightly influence GSI or CV; however, these effects did not reach statistical significance.

The analysis suggests that the presence and characteristics of the NPs were more influential than the specific concentration within the tested range (10-100 µg/mL). Therefore, the combined effects of NP concentration and NP type (Fe_2O_3 or ZnO) on seed germination were further examined.

Effect of NPs type and concentration on the germination of seeds

The effects of NP type (Fe_2O_3 vs ZnO) and NP concentration on seed germination (Figure 3 a-c) were analyzed using two-way ANOVA. For MGT, NP type had a significant effect ($F_{1,60}$ = 10.03, p = 0.002), whereas NP concentration ($F_{5,60}$ = 1.17, p = 0.336) and the interaction between NP type and concentration ($F_{5,60}$ = 0.62, p = 0.687) were not significant, indicating that the two NP types consistently affected MGT regardless of concentration.

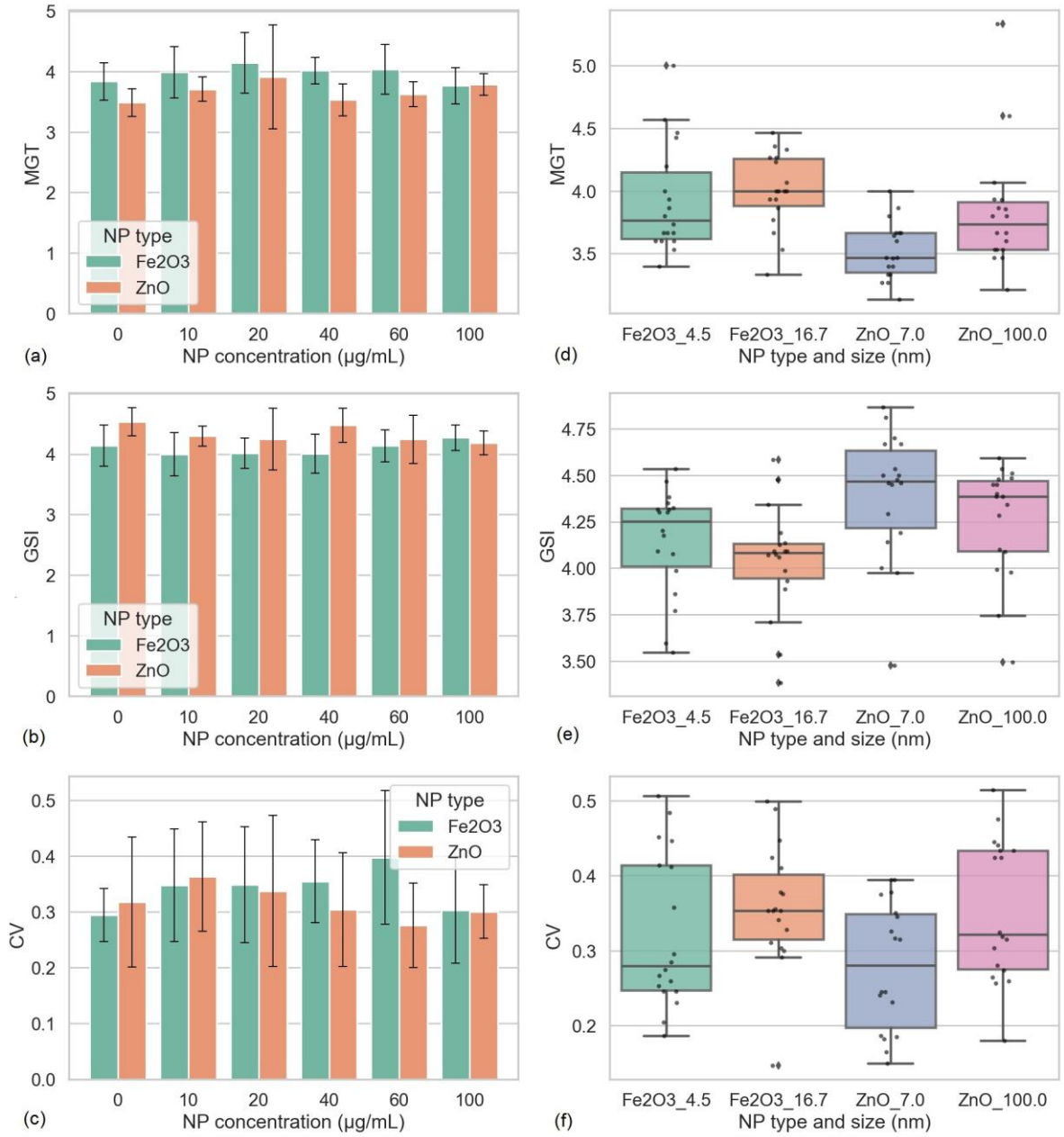

Fig. 3 - Effect of NP type and size on germination parameters.

(a–c) Effect of NP type on mean germination time (MGT, a), germination speed index (GSI, b), and germination synchrony (CV, c), irrespective of NP size. Data were pooled across all NP concentrations. Bars represent mean ± SD of three replicates per NP type. (d–f) Effect of NP size on MGT (d), GSI (e), and CV (f), irrespective of NP concentration. Data were pooled across all concentrations for each NP type. Boxplots show median, interquartile range, and individual data points for each NP size.

Post-hoc comparisons confirmed that the differences were attributable to NP type rather than concentration. Similarly, for GSI, NP type was significant ($F_{1,60}$ = 10.51, p = 0.002), while concentration ($F_{5,60}$ = 0.69, p = 0.631) and the interaction ($F_{5,60}$ = 1.28, p = 0.286) were not. In contrast, none of the factors significantly affected the CV (p > 0.28 for all), indicating that variability in germination was not influenced by NP treatment. Overall, these results suggest that NP type, but not concentration, drives consistent differences in germination performance across the tested conditions.

Effect of NPs size on the germination of seeds

To address the effect of NP size on the germination indices with values pooled over all concentrations (Figure 3 d-f) a Kruskal-Wallis test was applied followed by post-hoc pairwise Mann–Whitney U test. Results showed that NP size significantly influenced MGT (H = 19.79, p < 0.001), the post-hoc analysis revealing that the effect of 7 nm NPs was significantly different than the effect of 4.5 nm NPs (p = 0.017) and 16.7 nm NPs (p = 0.003). The impact of NP size also resulted to be significant on GSI (H = 13.87, p = 0.003), the post-hoc analysis showing a significant difference between the effects of 7 nm NPs and 16.7 nm NPs (p = 0.01) and on CV (H = 8.06, p = 0.045).

Overall, these analyses show that smaller NPs (4.5 nm Fe_2O_3 and 7 nm ZnO) often differed from larger NPs (16.7 nm Fe_2O_3 and 100 nm ZnO) for MGT and GSI. Within-type comparisons confirmed a significant difference for MGT between 7 nm and 100 nm ZnO ($p = 0.017$), and for CV between 7 nm and 100 nm ZnO ($p = 0.030$).

Correlations among gemination indices

Spearman correlation analysis was performed to evaluate the relationships between MGT, GSI, and CV. When all NP treatments were pooled, a strong negative correlation was observed between MGT and GSI ($\rho = -0.85$), indicating that faster-germinating seeds (higher GSI) tended to have shorter germination times. MGT also showed a positive correlation with CV ($\rho = 0.79$), suggesting that prolonged germination was associated with reduced uniformity. These relationships were largely preserved when data were analyzed separately by NP type. For Fe_2O_3 NPs, the MGT – GSI correlation was particularly strong ($\rho = -0.90$), accompanied by an even tighter association between MGT and CV ($\rho = 0.91$). ZnO NPs displayed slightly weaker but still consistent correlations ($\rho = -0.79$ and $\rho = 0.76$, respectively).

When NP size was further considered (Figure 4 a-d), the strongest relationships were found for Fe_2O_3 NPs of 4.5 nm (MGT–GSI: $\rho = -0.95$; MGT–CV: $\rho = 0.92$), suggesting that under these conditions, seed germination dynamics were tightly coordinated. For Fe_2O_3 NPs of 16.7 nm, correlations remained positive but were weaker. ZnO NPs followed a similar trend: 7 nm ZnO induced moderately strong correlations ($\rho \approx -0.81$ and 0.70), whereas 100 nm ZnO showed an intensified negative correlation between MGT and GSI ($\rho = -0.92$) but a weaker link with CV ($\rho = 0.64$).

Overall, these results indicate that MGT, GSI, and CV are interdependent germination descriptors, where shorter mean germination times and higher speed indices correspond to more synchronous germination. The strength of these relationships appears to depend on both NP type and particle size, with Fe_2O_3, particularly at smaller sizes, producing the most consistent coupling among germination parameters.

DISCUSSIONS

These findings indicate that nanopriming with ZnO and Fe_2O_3 NPs can finely modulate the germination dynamics of sunflower seeds, not by altering the final germination percentage *(Al-Sudani et al., 2024)*, but by influencing the timing, synchrony, and uniformity of germination. The previous study on the same experimental system showed that, while germination success remained high across all NP treatments, the germination rate and kinetics were strongly affected by NP type, size, and concentration. Specifically, extreme NP sizes (4.5 nm Fe_2O_3 and 100 nm ZnO) produced the most pronounced effects, with 4.5 nm Fe_2O_3 reducing and 100 nm ZnO enhancing germination speed. The intermediate NP sizes (16.7 nm Fe_2O_3 and 7 nm ZnO) showed little to no deviation from control conditions *(Al-Sudani et al., 2024)*.

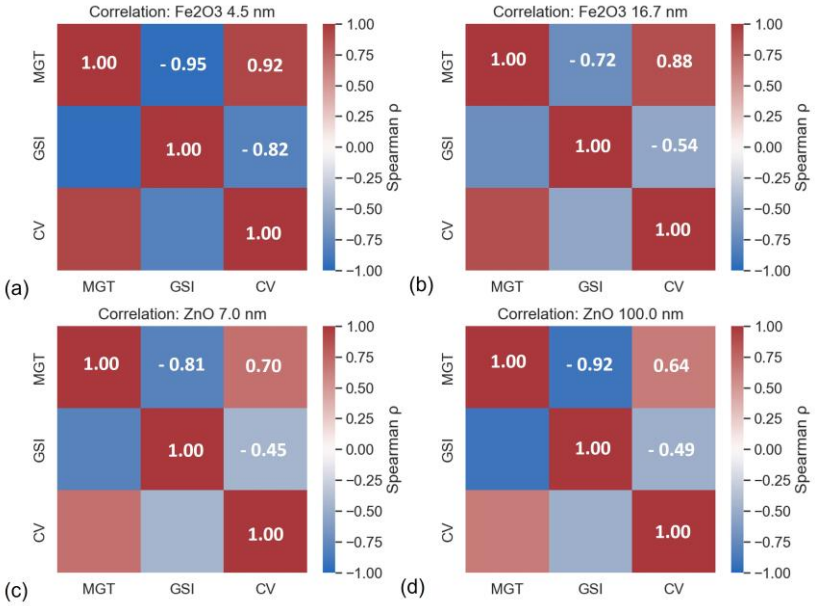

Fig. 4 - Spearman correlation heatmaps of germination indices (MGT, GSI, CV) in sunflower seeds under priming with the the different NPs:

(a) 4.5 nm Fe_2O_3 NPs, (b) 16.7 nm Fe_2O_3 NPs, (c) 7 nm ZnO NPs, (d) 100 nm ZnO NPs.
Positive correlations are shown in red tones, negative correlations in blue tones, and the intensity indicates the strength of correlation.
All squares are annotated with the corresponding Spearman correlation coefficients (ρ) values.

The present study extends those findings *(Al-Sudani et al., 2024)* by introducing germination indices (MGT, GSI, and CV), which provide quantitative insight into the speed (MGT), vigor (GSI), and synchrony (CV) of germination. Consistent with the previous observations, Fe_2O_3 NP treatments produced slightly longer MGTs and more variable CV values, particularly for the 4.5 nm particles, indicating slower and less synchronized germination. In contrast, ZnO NP treatments, especially with 7 nm particles, resulted in shorter and more homogeneous MGT and GSI values, reflecting faster and more uniform germination. Statistical analyses supported these patterns: Kruskal–Wallis tests revealed no significant effect of NP concentration within each NP type and size, while two-way ANOVA indicated a significant effect of NP type on MGT and GSI. NP size had a pronounced influence, particularly at the smallest and largest extremes (4.5 nm Fe_2O_3 and 100 nm ZnO), and Spearman correlations confirmed strong interdependence among the indices, highlighting coordinated germination responses to NP exposure.

The influence of NP size was not a simple function of smaller particles being uniformly beneficial or harmful. Instead, the biological outcome reflected a balance between NP composition, size, and reactivity. The inhibitory effects observed for 4.5 nm Fe_2O_3 NPs likely arise from their higher surface reactivity and enhanced potential to induce oxidative stress, surpassing the antioxidant capacity of the seed. Iron is an essential micronutrient and cofactor in numerous redox enzymes *(Zhang et al., 2025)*; however, excessive Fe or uncontrolled NP dissolution can lead to oxidative imbalance. Previous studies showed that Fe concentration exerts biphasic effects on germination: low Fe levels (≈100 μmol·L^{-1}) stimulated *Vigna radiata* germination, whereas higher concentrations (200–300 μmol·L^{-1}) inhibited it *(Verma et al., 2017)*. Similarly, *Triticum aestivum* exposed to 500 μmol·L^{-1} Fe experienced reduced starch mobilization and oxidative stress *(Zhang et al., 2025)*.

At optimal doses, however, Fe-based nanomaterials can act as effective nanofertilizers, enhancing germination and seedling vigor through increased Fe bioavailability and activation of antioxidant systems. α-Fe_2O_3 NPs at 0.5 mM improved *Abelmoschus esculentus* germination *(Mohan et al., 2025)*, and nα-Fe_2O_3 promoted *Oenothera biennis* germination and biomass accumulation via increased catalase and peroxidase activity, while higher concentrations induced oxidative stress *(Asadi-Kavan et al., 2020)*. Particle size is also critical: smaller Fe_2O_3 NPs (<40 nm) possess higher solubility and improved transport through seed coats *(Asadi-Kavan et al., 2020; Sembada et al., 2024)*, yet their increased reactivity can lead to cellular stress and membrane damage, as observed in *Hordeum vulgare (Tombuloglu et al., 2024)*.

In contrast, ZnO NP treatments, particularly 7 nm ZnO, appeared to exert beneficial effects, enhancing germination vigor and synchrony. Zinc is an essential element involved in numerous enzymatic and transcriptional processes *(Mossa et al., 2020)*, and its deficiency inhibits RNA and protein synthesis, leading to chlorosis and growth retardation *(Hamzah Saleem et al., 2022)*. In spite of its essential function, unoptimized Zn^{2+} concentrations can be phytotoxic *(Bhattacharjee et al., 2022)*. ZnO NPs can alleviate Zn deficiency due to their small size and enhanced mobility, providing Zn^{2+} ions more efficiently than bulk fertilizers *(Moreno-Lora et al., 2020; Sarkhosh et al., 2022; Zhao et al., 2025)*. Their effects are concentration-dependent and biphasic: lower concentrations (<10 ppm) enhance germination in several species (*Camelina sativa*, *Brassica napus*, *Arachis hypogaea*) *(Prasad et al., 2012; Sarkhosh et al., 2022)*, while higher levels (>500–1000 mg·L^{-1}) become phytotoxic *(Prasad et al., 2012; Rai-Kalal et al., 2021; Zhao et al., 2025)*. This biphasic behavior is consistent with the present findings, where ZnO NPs improved germination quality without compromising final viability, indicating that the concentrations tested were within the stimulatory range.

The observed modulation of germination by Fe_2O_3 and ZnO NPs could be the result of redox-mediated signaling *(Mohan et al., 2025)* and altered seed hydration dynamics *(Mohan et al., 2025; Thunugunta et al., 2018)*. Low levels of reactive oxygen species (ROS) serve as positive signals for seed dormancy release and early metabolic activation *(Mohan et al., 2025)*, while excessive ROS accumulation delays or inhibits germination *(Tao et al., 2023)*. NPs can influence ROS homeostasis *(Fallah et al., 2024; Mohan et al., 2025; Stałanowska et al., 2025)*, antioxidant enzyme activity *(Sarkhosh et al., 2022; Tripathi et al., 2018; Waqas Mazhar et al., 2022; Zhao et al., 2025)*, and hormonal balances *(Kulus et al., 2025; Pandey et al., 2010)*, all of which regulate germination timing. Additionally, NP-induced changes in seed coat permeability and water uptake could affect imbibition rates and, consequently, germination uniformity *(Asadi-Kavan et al., 2020; Yu et al., 2020; Zhao et al., 2025)*.

From an applied perspective, these findings highlight the potential of nanopriming as a promising tool to modulate germination behavior. Properly optimized NP formulations and concentrations could enhance field emergence, promote stress tolerance, and improve synchronization, traits highly valuable in agricultural production. However, the fine threshold between beneficial and inhibitory concentrations underscores the need

for careful dose optimization and environmental risk assessment, as overapplication may disrupt soil microflora or induce phytotoxicity.

In conclusion, this study provides a detailed quantitative analysis of how NP composition and size affect germination dynamics. By integrating germination indices with previously reported germination rate data *(Al-Sudani et al., 2024)*, it is demonstrated that nanopriming with Fe_2O_3 and ZnO NPs influences not only the final outcome but also the pattern of germination, revealing a subtle regulatory layer that is not captured by traditional germination measurements. This represents the first report examining MGT, GSI, and CV in the context of Fe_2O_3 and ZnO nanopriming of sunflower seeds. The results further indicate that while ZnO NPs, particularly 7 nm particles, enhance germination uniformity and vigor, Fe_2O_3 NPs, especially smaller ones, tend to slow and desynchronize germination, likely through the induction of mild oxidative stress.

These findings contribute to understanding the mechanistic and practical dimensions of NP - seed interactions, bridging nanomaterial science and plant developmental physiology. Although the experiments were conducted under controlled Petri dish conditions with a small sample size (n = 3) and without mechanistic biochemical markers, they clearly reveal size- and composition-dependent effects of NPs on sunflower seed germination. These results provide a solid foundation for future studies under soil or field conditions, where additional environmental factors can be explored.

CONCLUSIONS

This study evaluated the influence of NPs with different chemical composition (ZnO and Fe_2O_3), sizes (4.5–100 nm), and concentrations (10–100 µg/mL) on sunflower germination timing indices. While germination percentage and rate are commonly reported and are relevant for crop management, parameters describing germination kinetics and synchrony remain underexplored in many NP priming studies. The results indicate that ZnO NPs, particularly those with a nominal size of 7 nm, significantly accelerate and synchronize sunflower germination, promoting more uniform seedling emergence. In contrast, smaller Fe_2O_3 NPs (4.5 nm) delayed and desynchronized germination. Across the tested concentration range, NP physicochemical properties had a greater impact than concentration, highlighting particle size and chemical composition as the primary drivers of the observed biological responses.

The present work demonstrates that nanopriming with ZnO and Fe_2O_3 NPs modulates sunflower seed germination in a size- and type-dependent manner, with effects extending beyond final germination outcomes to the dynamics and uniformity of the process. The novelty of this study lies in the systematic evaluation of germination timing indices as sensitive descriptors of NP-mediated effects, providing insight into germination features that are directly relevant to agronomic performance. From a practical perspective, enhanced germination speed and synchrony are essential for uniform stand establishment, improved predictability of crop development, and compatibility with mechanized agricultural practices. The results indicate that ZnO NPs, particularly smaller formulations, represent promising candidates for seed nanopriming strategies aimed at improving early crop establishment. Future studies incorporating molecular-level analyses and field trials will be critical for translating these results into agricultural applications.

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