Research Article

Effect of Zinc Oxide Nanoparticles (ZnO-NPs) on Seed Germination Characteristics in Two Brassicaceae Family Species: Camelina sativa and Brassica napus L

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Received 27 February 2022; Revised 9 April 2022; Accepted 17 May 2022; Published 7 June 2022

Academic Editor: Ram Prasad

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Introduction. Zinc is one of the essential micronutrients for living organisms; so, the right performance of several enzymes depends on this element. This micronutrient is a regulator of phytohormones and chlorophyll synthesis, and also, it is an essential element for the carbohydrates’ metabolisms in plants. Considering the relatively high solubility of ZnO-NPs and also the ability of plants to uptake and accumulate these nanoparticles in their biomass, ZnO-NPs can be used as an effective nanofertilizer for plants’ growth. Methods. In the present study, zinc oxide nanoparticles synthesized using chemical method and the effect of ZnO-NPs (as a nanofertilizer) on seeds’ germination, seedlings’ rootlet, seedlings’ plumule, and seedling’s vigour index in two oilseed crops from the Brassicaceae family, including Brassica napus L. and Camelina sativa, were investigated. After treating the seeds with different concentrations of ZnO-NPs (from 0.1 to 1000 ppm) for 6 days, the germination percentage (GP) of each treatment was measured. Results. The results indicated an increase in GP for both plants treated with 10 ppm ZnO-NPs. For B. napus, the maximum GP occurred in treated seeds with 5 ppm ZnO-NPs which showed a 30% increase of GP compared with the control condition. For Camelina, this maximum GP was observed in 0.1 ppm concentration of ZnO-NPs which showed a 15% increase compared with the control condition. After the germination test, germinated seedlings were planted in Hoagland hydroponic solution and treated with ZnO-NPs again for a week. For both species, treatment with ZnO-NPs showed a great effect on rootlet growth, while the effects of these treatments on plumule were negligible. The maximum rootlet length was observed in treated B. napus seedlings with 5 ppm ZnO-NPs which showed a 32% increase in this parameter compared with the control condition. In contrast, the high concentrations of ZnO-NPs showed toxic effects on B. napus seedlings’ rootlets. Results showed a 41% decrease in B. napus seedlings treated with 50 ppm ZnO-NPs compared with control seedlings. Similar results were observed in the treated seedlings of Camelina. For Camelina seedlings treated with 1 ppm ZnO-NPs, 15% increase in rootlets’ length was observed, while treated Camelina seedlings with 50 ppm ZnO-NPs showed a 68% decrease in rootlet length compared with the control condition. The results of this study indicated the potential of using ZnO-NPs as nanofertilizer for B. napus and Camelina in low concentrations (lower than 10 ppm). In addition, these results suggest the toxicity effects of these nanoparticles on both species in concentrations higher than 50 ppm.
1. Introduction

Providing food and raw material for a growing population is some of the major human challenges in the 21st century. To meet this growing need for agricultural products, new strategies have been employed in recent years such as chemical and organic fertilizers as well as soil microbial inoculation [1]. However, the production of agricultural products still faces many challenges such as lack of sufficient cultivation area, climate change, and various pests [2]. During the last few decades, especially after the Green Revolution which was accompanied by the emergence of new and high-yielding varieties, chemical fertilizers, pesticides, and chemical herbicides, many of these challenges were partially overcome [3]. Nevertheless, using the chemicals to cultivate barren lands and increase farm production efficiency has had significant effects on the environment such as accumulation of nitrate in groundwater and phosphate in surface waters which led to eutrophication and algal bloom [4, 5]. To address these problems and create a sustainable agricultural industry, alternative technologies with lower toxicity alternative methods are developing [6, 7]. It seems that in the near future, nanotechnology by collaboration with transgenic crops gives new meaning to precision agriculture techniques, field monitoring, and use of herbicides, pesticides, and fertilizers. [8]. Nanoparticles can act as magic bullets that contain herbicides, pesticides, fertilizers, or even genes, moving to a specific plant cell organ and releasing their contents [9]. In recent years, the use of functionalized nanoparticles has received much attention due to their positive effects [10]. However, before these such engineered nanomaterials (ENMs) can be used on an industrial scale, more studies must be done on their effects on plants, humans, and the environment to ensure their health and nontoxicity [11]. Indeed, a study on application of metal-oxide nanoparticles for Cucumis sativus L. showed high accumulation of ZnO and CuO nanoparticles in biomass compared to Zn^{2+} and Cu^{2+} [12], while another study on Zea mays showed higher toxicity of these ions compared with corresponding metal-oxide nanoparticles [13]. Also, there are serious concerns on emission of synthesized nanoparticles, they can improve the quantity and quality of human health [14] and plant products by increasing grain GP, increasing vigor index and growth rate, increasing plant photosynthetic activity and nitrogen metabolism, and increasing carbohydrate and protein synthesis [8]. Also, there are some studies which show the great potential of nanoparticles as Fullerol as nano-antioxidant which reduce the H_{2}O_{2} and as a result increase the plant’s tolerance toward the salinity stress [15]. Metal oxide nanoparticles have the greatest potential for use as nanofertilizers. Among these, zinc oxide nanoparticles have received much attention due to their unique and desirable properties in agriculture. Due to its properties, such as suitable photoluminescence, large specific surface area, low toxicity, and long life, this nanoparticle can have many applications as an antibacterial agent, chemical absorbent, and additive to polymers and as a catalyst [16]. Biologically, zinc is a vital micronutrient for living organisms, so the proper functioning of many enzymes depends on the presence of this element. [17]. It generally acts as an activating metal for enzymes such as aldose, lectinase, cysteine desulphhydrase, histidine deaminase, carbonic anhydrase, dehydropeptidase, and glycyl-glycine dipeptidase and also involved in synthesis of tryptophan and auxins, and alternative methods are developing [18, 19]. The available forms of zinc in the soil are water-soluble, exchangeable, and complex, which are readily available to the plant. Around the world, the average concentration of this metal in the soil is between 10 ppm and 300 ppm; however, the available amount of this metal is much less than its total concentration [20]. Plant access to zinc depends on several factors such as pH, phosphorus level, organic matter content, clay adsorption, interaction with other nutrients, and climatic conditions. Other cations such as Cu^{2+}, Fe^{3+}, and Mn^{2+} prevent the plant from absorbing Zn^{2+} due to competition with zinc ions at the same carrier sites [21]. Zinc deficiency in plants slows down photosynthesis and reduces nitrogen metabolism, which results in reduced flowering, fruit development, and ultimately reduced crop production [22]. Zinc oxide is used as a source of zinc in agricultural fertilizers and can be used to coat plant seeds, immerse roots, and inject into trees to alleviate zinc deficiency [23]. However, zinc oxide (ZnO) is almost insoluble in water so it cannot be used as an efficient source of this micronutrient. In some fertilizers, zinc oxide along with zinc sulfate is used as fertilizer to solve this problem [24]. Conventional zinc fertilizers, when added to the soil, gradually form a sediment (e.g., ZnCO_{3}) and are removed from the plant availability or are adsorbed superficially to metal (such as iron and aluminum) oxides, which in turn prevents the plant from accessing this element [25]. On the other hand, zinc oxide nanoparticles are significantly soluble in water and also plants are able to absorb and accumulate zinc oxide nanoparticles in their biomass. Indeed, it has been proven that nanoparticles can be effective in providing zinc nanofertilizers with high solubility and distribution properties, and this means a turning point in the agricultural industry. [26]. Nanoparticles have the ability to be absorbed through roots as well as leaves and can affect growth, crop production, biological membrane integrity, and also plant organ and seed development [27]. One of the applications of nanoparticles in agriculture is to improve the germination of plant seeds. The penetration of nanoparticles into the plant through the roots is possible from two paths: the apoplastic pathway and the simplastic pathway [28]. The response of plants to the presence and infiltration of nanoparticles depends on many factors such as nanoparticle type, size, concentration in the environment, duration of exposure, plant type, and whether the nanoparticle is a micronutrient required by the plant (as copper, zinc, or iron) [29]. Syu et al. in a study evaluated the effect of triangular, spherical, and decahedral silver nanoparticles on Arabidopsis [30] which observed highest root growth promotion (RGP) associate with decahedral morphology, while spherical nanoparticles did not show any notable RGP effect. Also, studies showed that in acidic soils, direct adding of zinc to the soil will cause severe Fe deficiency in dicots which shows preference of foliar applications of Zn nanofertilizers in such cases [31]. Different size of ZnO-NPs shows noticeable different effect on Brassica pekinensis L germination rate and root/
shoot elongation. Studies show that spherical nanoparticles with 30 nm diameter significantly inhibit the growth and germination parameters compared to larger nanoparticles (150 nm diameter) [32]. This study tried to investigate the effect of zinc oxide nanoparticles as a nanofertilizer on the GP of *Camellia* and *B. napus*. According to previous surveys, no report of the effect of these nanoparticles on *Camelina sativa* has been published until this study.

2. Experimental

2.1. Materials. All chemical materials used in this study (including sodium hydroxide, zinc acetate, and Hoagland components: KNO₃, Ca(NO₃)₂·4H₂O, FeSO₄·7H₂O, EDTA, MgSO₄·7H₂O, H₂BO₃, MnCl₂·4H₂O, ZnSO₄·7H₂O, CuSO₄·5H₂O, Na₂MoO₄·2H₂O, and KH₂PO₄) were purchased from Sigma-Aldrich (Iran). Varieties used in this project were a double haploid (*DH*) line of spring cultivar of *B. napus* and DH1052 line of *Camelina sativa* which were purchased from Bistoon Shafa Co., Iran.

2.2. Zinc Oxide Nanoparticle (ZnO-NP) Synthesis. Zinc oxide nanoparticles were synthesized using chemical method. A 10 mM solution of zinc acetate dehydrates was prepared, and a 2 M solution of sodium hydroxide (NaOH) was added dropwise to the solution until its pH reached 12. The solution was then stirred at room temperature for 2 hours to obtain a white solution which indicated the synthesis of zinc hydroxide. Zinc hydroxide colloids were separated from the solution using a centrifuge at 6000 rpm for 10 minutes, and three wash steps were performed using distilled water to remove unreacted ions and excess NaOH. The resulting white precipitate was dried in an oven at 60°C for 24 hours [33].

2.3. Characterization of Nanoparticles. In order to confirm the synthesis of ZnO-NPs, UV-Vis spectroscopy analysis (Shimadzu-CPS-240A) was performed. The morphology, surface properties, and size of the synthesized ZnO-NPs were investigated using scanning electron microscopy (SEM, Hitachi-SU3500). X-ray diffraction (XRD, Siemens-Diffraclometer D8) was used to determine the crystal structure of ZnO nanofertilizer. Fourier transform infrared (FTIR, Shimadzu-Irprestige-21) spectrometry was also used to investigate the functional groups present on the surface of ZnO-NPs. In order to analyze the elements in the synthesized nanoparticles, energy-dispersive X-ray spectroscopy (EDX, Hitachi-SU3500) was used.

2.4. Seed Germination Assay. For surface sterilization, seeds were washed with 70% ethanol for 2 minutes and immediately washed again three times with distilled water. The seeds were then washed well for 5 minutes using a 1.5% sodium hypochlorite solution. Finally, the surface sterilization process was completed by rinsing four times with distilled water. In order to investigate the effect of ZnO-NPs, liquid solutions containing different concentrations of these nanoparticles (0, 0.1, 0.5, 1, 5, 10, 50, 100, 500, and 1000 mg/L) were used [34]. In the first stage of the experiment, 20 surface sterilized seeds were placed in each petri plate (containing 10 mL of nanoparticle solutions). Seeds were allowed to germinate *in vitro* in darkness at 25°C for six days. During this stage, the number of germinated seeds was counted and the germination percentage (GP%) was calculated (daily and cumulative) according to following equation [35]:

\[
\text{GP\%} = \frac{\text{germinated seeds}}{\text{total seeds}} \times 100, \tag{1}
\]

In the second stage, in order to evaluate the effect of
ZnO-NPs on root/shoot length, seedlings were transferred from petri plates to hydroponic Hoagland’s nutrient media (without Zn micronutrient), treated with different concentration of ZnO-NPs and allowed to grow for 7 days in vitro (at 25°C and photoperiodic: 16-hours light and 8-hours dark). At the end of the second stage, plantlets’ rootlet length (from the bottom of the hypocotyl to the tip of the root) and plumule length (from the lowest point of the hypocotyl to the point of growth of the cotyledon) were measured and vigor index was calculated using following equation [36]:

\[ \text{Vigour index} = \frac{\text{Average root length (mm)}}{\text{Average shoot length (mm)}} \times \text{Germination percentage} \]  

3. Results and Discussion

3.1. Characterization of ZnO-NPs

3.1.1. Chemical Synthesis of ZnO-NPs: Coprecipitation. The coprecipitation method includes three phases: nucleation, growth, and termination. During the nucleation phase, Zn$^{2+}$ was reduced and colloidal solution of Zn(OH)$_2$ was produced. This step could be determined through the virtual change of the solution’s color, from transparent to white. In the growth phase, effective parameters on size, shape, and final ZnO-NPs morphology include ions’ concentration, solution temperature, stirring speed, and time. More time in the aging process causes more colloids to aggregate and bigger particle production. According to the following equation, during the termination phase, further reduction occurs by dehydration of zinc hydroxide using heat treatment, and

Figure 2: SEM image of ZnO-NPs (a) at 4 μm scale, (b) at 2 μm scale, (c) at 1 μm scale with clear spindle shape, and (d) at 500 nm scale.
the final result is ZnO-NP production [37].

Nucleation phase: $\text{Zn} (\text{CH}_3\text{COO})_2 + 2\text{NaOH} \rightarrow \text{Zn(OH)}_2(S) + 2\text{CH}_3\text{COONa}_{\text{aq}}$, Termination phase: $\text{Zn(OH)}_2 \xrightarrow{\text{Heat Treatment}} \text{ZnO}$.

3.1.2. Initial Confirmation of ZnO-NP Synthesis: UV-Vis Spectroscopy Analysis. UV-Vis spectroscopy analysis was used for initial confirmation of ZnO-NP synthesis. As shown in Figure 1, the produced nanoparticles have a maximum absorption at a wavelength of 358 nm. The resulting curve can also be used to determine the size of the synthesized nanoparticles [38]. Since ZnO-NPs have a maximum absorption at a wavelength between 350 and 380 nm, the result of this test was considered as a preliminary confirmation of ZnO-NP synthesis [39]. Indeed, shorter maximum absorption’s wavelength indicates nanoparticles with smaller size [40]; therefore, primary investigation of synthesized ZnO-NPs showed the relatively small size of these particles.

3.1.3. Size and Morphology of Synthesized Nanoparticles: SEM Analysis. In order to more accurate investigation of the size and morphology of nanoparticles, SEM analysis was used. According to Figure 2, synthesized nanoparticles had almost spindle shape with size $45 \pm 5$ nm. Such small size and spindle shape is the result of high concentration of Zn$^{2+}$ (supersaturated solution), sequential core production during the nucleation phase, and optimized aggregation during the growth phase. Forces that form the final shape of nanoparticles include electrostatic attraction, collision

![Figure 3: EDX analyses show the elemental composition of synthesized nanoparticles (mostly zinc and oxygen).](image1)

![Figure 4: XRD patterns of synthesized ZnO-NPs.](image2)
Figure 5: FTIR spectra of synthesized nanoparticle.

Figure 6: Effect of different levels of ZnO-NPs on *B. napus* seedlings’ (a) root length, (b) shoot length, and (c) vigour index.
(caused by stirring), polarity, and high surface energy of ZnO-NPs [41].

3.1.4. Elemental Analysis of Synthesized Nanoparticles: EDX Analysis. Also, in order to investigate the elemental composition of synthesized nanoparticles, EDX analyses were hired. This technique is a unique method for elemental analysis or chemical characterization of the samples. This method is based on the interaction of X-ray excitation sources and the sample, and the reason for this unique feature is that each element has a unique atomic structure that allows it to create a unique series of peaks in its electromagnetic emission spectrum. According to Figure 3, major elements existed in the samples were zinc and oxygen which shows negligible impurity in the synthesized nanoparticle.

3.1.5. Crystal Structure of Synthesized Nanoparticles: XRD Pattern. Since the resulting pattern for each crystal structure is unique, by examining the XRD pattern shown in Figure 4 and comparing the peaks in this diagram with the standard mode, the synthesis of ZnO-NPs was confirmed with certainty. The resulting diagram has peaks in points with length (2θ) equal to 002, 023, 100, 101, 102, 103, 112, and 202, which confirm the crystallinity of synthesized ZnO-NPs. These results are quite similar to the results of previous studies on the synthesis of ZnO-NPs by chemical and green methods [42, 43].

3.1.6. Chemical Bonds in Synthesized Nanoparticles: FTIR Spectra Analysis. FTIR analysis was performed, using frequencies of 400 to 4000 cm⁻¹ infrared waves and the resulting spectrum shown in Figure 5. As shown in Figure 5, the two main absorbance bands are related to the zinc oxide nanoparticles (478 cm⁻¹) and the hydroxide bond (3421 cm⁻¹). The presence of a strong hydroxide bond indicates that despite the completion of the first reaction (formation of zinc hydroxide colloids), the second reaction step (dehydration of zinc hydroxide) is not completed and part of the zinc hydroxide remains, without dehydration. Also, the presence of weak bands in three regions: 999-1084 cm⁻¹, 1500-1620 cm⁻¹, and 2850-2925 cm⁻¹, indicates the presence of C-O bond, C=O bond, and CH3 bond, respectively. The presence of these bonds indicates the presence of small amounts of zinc acetate in the resulting precipitate, indicating that only a small fraction of the raw material remains without any reaction. The results are in complete agreement with the results of previous studies using similar methods in ZnO-NP synthesis [33].

3.2. Seed Germination and Seedling Assay. ZnO-NPs show different effects on different plant species. Studies show that even the application methods of these nanoparticles (foliar or root feed solution) can have completely different effects on germination and plant growth parameters [44]. Also, sensitivity to ZnO-NPs varies greatly from species to species. For example, a study on Black gram showed that treatment of seeds with a concentration of 600 mg/L ZnO-NPs significantly affects the germination parameters as far as, the highest GP, maximum root length, maximum germination length, and also the highest seedling vigor were observed at this concentration [45]. Another study on the effect of ZnO-nanorods on symbiotic relationship of P. indica and B. oleracea shows synergistic effect of this nanorod and P. indica on B. oleracea growth and also P. indica biomass too [46]. Studies on Arachis hypogaea have shown that treatment of seeds with high concentration of ZnO-NPs (1000 ppm) increases the GP, increases the seedling vigor, causes earlier flowering, and increases the leaves’ chlorophyll. The role of these particles in the roots and stems’ growth of this plant was also proven. ZnO-NPs increased the pod production by 34% in the treated samples compared to the control. In a study on Capsicum annum L., it was found that the treatment of seeds with a concentration of 750 ppm ZnO-NPs increases GP, root length, and stem length [47]. In some species even a low concentration of ZnO-NPs shows significant effect. In a study on pearl millet, a significant increase in root growth, buds, protein content, and pigment was observed in concentrations less than 10 ppm of ZnO-NPs (foliar application) [48].

However, in most of the studied species, ZnO-NPs in relatively low concentrations improved germination and seedling growth, while high concentrations of these nanoparticles showed toxic effects on seedlings. Studies on Triticum aestivum showed that the treatment of seeds with a concentration of 50 ppm ZnO-NPs has a positive effect on germination, the number of roots, and overall plant growth [26]. In contrast, the treatment of Triticum aestivum seeds with 400 ppm has shown completely inhibitory effects on root growth and germination of seedlings [49]. Many studies have been done on the effects of ZnO-NPs on Cicer arietinum. In the foliar treatment of 10-day-old seedlings of Cicer arietinum with 1.5 and 10 ppm ZnO-NPs, maximum growth was observed at a concentration of 1.5 ppm, while a concentration of 10 ppm showed an inverse effect on root growth [50]. In another study on the Cicer arietinum, Pandey et al. showed that ZnO-NPs increases the phytohormones such as indole acetic acid (IAA) in the roots, which in turn improved the root growth [51]. In a study on corn, it was found that a concentration of about 10 ppm increases the

**Figure 7:** Effect of ZnO-NPs on the B. napus seedlings’ growth.
GP, while showing different effects on root growth [52]. In another study on maize, it was observed that concentrations higher than 800 ppm ZnO-NPs cause inhibitory and toxic effects on germination and seedlings’ growth [53]. Further research on the effect of ZnO-NPs on maize shows a toxic effect at concentrations above 400 ppm [54]. But, studies report different observations in some cases. In a study on the toxicity of ZnO-NPs on Lactuca sativa and some other species, Lin and Jing observed that even high concentrations of this nanoparticle (2000 mg/L) did not have a negative effect on seed germination. On the other hand, they found that the germination rate of ryegrass and corn was decreased up to 50% even at low concentrations of ZnO-NPs (20 to 50 mg/L) [55].

3.2.1. Effect of ZnO-NPs on Rootlet and Plumule Growth. There are several previous reports which show the positive effect of ZnO-NPs on overall growth of seedlings. However, in some species, inhibitory effects on growth parameters as rootlet and plumule were observed at high concentrations of these nanoparticles. In the current study, a similar trend was observed in the case of Camelina sativa and Brassica napus; so, low concentration of ZnO-NPs has positive effect on rootlet and plumule growth, while high concentration of ZnO-NPs decreases the length of plumule and rootlet.
Effect of ZnO-NPs on Growth of Brassica napus L. Seedlings. As shown in Figure 6, up to a concentration of 10 mg/L of ZnO-NPs, a positive effect on the rootlet length of B. napus seedlings was observed, so that the maximum of this positive effect can be seen in seedlings treated with 5 mg/L (Figure 6(a)). These results showed that canola seedlings treated with 5 mg/L ZnO-NPs increased the rootlet length by 32% compared to the control. In contrast, further increase in the concentration of ZnO-NPs has an inhibitory effect on seedlings’ rootlet growth, so that the concentration of 50 mg/L reduced the rootlet growth by 41% compared to the control plate (Figure 6(a)). Figure 6(b) shows the effect of different concentrations of ZnO-NPs on the length of B. napus plumule. Based on these results, no significant relationship was observed between the length of the plumule and the concentration of ZnO-NPs. Figure 6(c) shows the effect of ZnO-NPs on seedlings’ vigor index. Seedling vigor index as a combination of parameters such as GP, rootlet, and plumule length is a very illustrative index to express the overall effect of ZnO-NPs on the quality and quantity of germination. According to Figure 6(c), the optimum concentration of ZnO-NPs for maximum seedling vigor index

Figure 10: Germination percentage (GP) data of B. napus seeds recorded for 6 days: (a) 3D bar graph and (b) radar graph.
of *B. napus* is between 0.1 and 10 mg/L. Also, this index clearly shows the negative effect of concentrations higher than 50 mg/L on the *B. napus* seedlings.

Figure 7 has shown the effect of ZnO-NPs on the *B. napus* seedlings. This image shows the positive effect of low concentrations (up to 10 mg/L) on seedlings' rootlet and inhibitory effect of higher concentrations (above 50 mg/L) on *B. napus* rootlet growth.

Other studies have been performed on the effect of ZnO-NPs on plants of the *Brassicaceae* family. In a study on *Brassica juncea*, it was observed that 25 ppm shows positive effects on seedling growth, while 100 ppm of these nanoparticles has a toxic effect. In this study, the effect of ZnO-NPs on *Brassica napus* as one of the most important plants of the *Brassicaceae* family has been investigated, which showed similar results to *Brassica juncea* [56]. Other research on the long-term effects of ZnO-NPs on *B. napus* also confirms these results [57].

The effect of ZnO-NPs on the overall growth of *Camelina sativa* seedlings is shown in Figure 8. This figure shows the effect of ZnO-NPs on rootlet and plumule length and also *Camelina* seedling vigour index. As shown in Figure 8(a), ZnO-NPs up to 10 mg/L show a positive effect on the rootlet growth of *Camelina* seedlings and the maximum of this positive effect is observed in seedlings treated with 1 mg/L ZnO-NPs. According to this diagram, rootlets' length of *Camelina* seedling treated with 1 mg/L ZnO-NPs showed 15% increase compared to the control. However, further increase in the concentration of ZnO-NPs caused a drastic inhibitory effect on the rootlet growth; thus, 50 mg/L ZnO-NPs reduced the rootlets' length by 68% compared to the control (Figure 8(a)). Figure 8(b) shows the effect of ZnO-NPs on the *Camelina* seedlings' plumule. As can be seen in this diagram, no significant relationship was observed between plumule length and ZnO-NP concentrations. Figure 8(c) shows the effect of ZnO-NPs on the seedling vigor index of *Camelina* seedlings. According to this diagram, the optimum concentration of ZnO-NPs on the *Camelina* seedling vigor index is also between 0.1 to 10 mg/L that is similar to *B. napus*. Also, the diagram in Figure 8(c) clearly shows the negative effect of ZnO-NPs on *Camelina* seedlings at concentrations above 50 mg/L.

Figure 9 shows the treated *Camelina* seedlings with three ZnO-NP concentrations: control, 10 mg/L, and 50 mg/L. As shown in this image, low concentration (10 mg/L) of ZnO-NPs has a positive effect on the overall growth of *Camelina* seedlings. On the other hand, high concentration of these nanoparticles (higher than 50 mg/L) severely decreases the overall growth (specially rootlet growth).

Similar results have been observed in other species in the *Brassicaceae* family. Xiang et al. in a study on *Brassica rapa* observed that concentrations above 80 mg/L have a severely negative effect on rootlet and plumule length [32]. According to these researchers, the reason for such an inhibitory effect was the production of the free hydroxide (OH•) group as well as the bioaccumulation of Zn in the roots and buds of treated seedlings. Nevertheless, in some other species of the *Brassicaceae* family, a completely different result is observed. In a study on *Arabidopsis*, concentrations above 400 ppm prevented germination, root growth, and reduced number of seedlings [44]. In another study on *Arabidopsis*, these inhibitory effects on plant growth were reported even at concentrations below 5 ppm [58].

3.2.2. Effect of ZnO-NPs on Germination and Vigour Index. As discussed above, previous studies showed different effect on germination and vigour index of a different plant. In some cases, the effect of nanoparticles on plant growth was not just as a result of direct effect of ZnO-NPs on plant but it could be a consequence of these nanoparticles on rhizosphere bacterium [12]. In this study, positive effect was observed on seeds’ GP and also VI at low concentration of ZnO-NPs. However, increasing the concentration upper than 100 ppm caused an adverse effect on both of *B. napus* and *Camelina*, so that the *Camelina* showed higher sensitivity to ZnO-NPs.

(1) Effect of ZnO-NPs on *Brassica napus* L. Seed Germination. In order to investigate the nutritive and/or toxic effects of ZnO-NPs on *B. napus* and *Camelina*, a wide range of nanoparticle concentrations (0.1 to 1000 mg/L) in hydroponic solution were used. As shown in the bar and radar diagrams in Figure 10, increasing the nanoparticles to a concentration of 50 mg/L showed a positive effect on the GP of *B. napus* seeds. Best results were observed at 5 mg/L.
in which a 30% increase in GP was observed compared to the control. Nevertheless, higher concentrations of ZnO-NPs (greater than 100 mg/L) caused a relative decrease in GP; so, the GP of *B. napus* showed a decrease of 5% at 100 mg/L and 10% at 1000 mg/L compared with the control plate.

Figure 11 shows the effect of ZnO-NPs on *B. napus* germination in three concentrations: control (zero), 5 mg/L, and 1000 mg/L. This image shows the significant effect of ZnO-NPs in increasing the GP of *B. napus* seeds at 5 mg/L and also the inhibitory effect of these nanoparticles at 1000 mg/L.

In a similar study on some *Brassicaceae* species, Singh et al. by examining the effect of bulk ZnO particles found that these particles had a toxic effect on *Brassica oleracea* var. *capitata* and *Brassica oleracea* var. *botrytis* and reduced GP and vigour index in treated seedlings. On the other hand, the use of ZnO-NPs increased GP, bud growth, pigments, sugars, protein percentage, and even the amount of antioxidants in these two species [24]. In contrast, in the study of the toxicity effect of zinc nanoparticles on germination of *Sinapis alba* (another species of the *Brassicaceae* family), ZnO nanoparticles showed a significant inhibitory effect on GP [59]. In fact, a study on comparing the effect of different metal-oxide nanoparticles on four different vegetable plants
11-8 mg/L of Zn\(^{2+}\) in the environment, which has reduced results of the current study, 1000 mg/L of ZnO-NPs released Allium cepa roots NPs on root growth by 40%. A study on inhibitory effects on mitochondrial membrane and chromosomal anomalies on mitochondrial membrane and chromosomal inclusion of radish, cucumber, tomato, and alfalfa and showed that Zn-NPs have highest inhibitory effect compare to TiO\(_2\), Al\(_2\)O\(_3\), and CuO nanoparticles [60]. According to the results of the current study, 1000 mg/L of ZnO-NPs released 11-8 mg/L of Zn\(^{2+}\) in the environment, which has reduced root growth by 40%. A study on inhibitory effect of ZnO-NPs on Allium cepa roots suggests that this nanoparticles in high concentrations increase ROS production, causing anomalies on mitochondrial membrane and chromosomal deflection or fraction [61].

(2) Effect of ZnO-NPs on Camelina sativa Seed Germination. As shown in the bar and radar diagram in Figure 12, increasing the ZnO-NPs to 10 mg/L showed a positive effect on the GP of Camelina. The maximum of this positive effect was observed at a concentration of 0.1 mg/L, in which a 15% increase in GP compared to the control plate was observed. The results of this study also showed the inhibitory effect of ZnO-NPs on the germination of Camelina seeds at concentrations above the 50 mg/L, so that the GP of Camelina seeds decreased by 10% at 50 mg/L and 35% at 1000 mg/L of ZnO-NPs. These diagrams indicate that Camelina is more sensitive to high concentrations of ZnO-NPs compared to B. napus.

Figure 13 shows the effect of ZnO-NPs on the germination of Camelina seeds in three concentrations: control, 10 mg/L, and 1000 mg/L. This image obviously shows the positive effect of ZnO-NPs in increasing the GP of Camelina seeds at the concentration of 10 mg/L and inhibitory effect of these particles at the concentration of 1000 mg/L.

A similar trend has been observed in some other species. For example, a study on Cicer arietinum showed that 1.5 ppm of ZnO-NPs improves vigour index, while a concentration of 10 ppm has a negative effect on growth parameters [50]. Research on Vigna radiata has also shown that low concentrations of ZnO-NPs (20 ppm) significantly improve growth parameters [62]. However, some species, such as Oryza sativa, have reacted quite differently to high concentrations of ZnO-NPs. Both foliar (5000 ppm) and hydroponic (1000 ppm) treatment of Oryza sativa with ZnO-NPs improved the GP and vigour index of these seeds which is quite different to the observed results for B. napus and Camelina. Similar results have been observed in the study on Arachis hypogaea, so that 1000 ppm of ZnO-NPs improved GP and seedling vigor index [63].

3.3. Conclusion. ZnO-NPs increase the synthesis of tryptophan, one of the precursors of the IAA which leads to increasing the growth, germination rate, and biomass production. ZnO-NPs also increase the expression and activity of the nitrate reductase and carbonic anhydrase, two enzymes which play key roles in nitrogen uptake and chlorophyll synthesis, respectively. These all indicate reasons for positive effect on B. napus and Camelina seeds and seedlings treated by ZnO-NPs up to 10 ppm which increase the GP and rootlet growth and result in a significant increase in seedling vigor index.

On the other hand, in the treatment of seedlings and seeds with high concentrations of ZnO-NPs, entered nanoparticle to the cells may interact with cytoplasmic and endomembranes’ proteins or even organelle, which leads to changes in metabolism and cellular signaling which stimulates a cellular response similar to that occurring during oxidative signaling. In order to overcome the toxicity of oxidative stress, the plant activates the enzymatic antioxidants (CAT, POX, and SOD) and produces nonenzymatic antioxidants (proline, ascorbate, and glutathione). This oxidative stress can also lead to a decrease in gas exchange capacity, thus a reduction in photosynthesis. The results of the current study also confirm the fact that high concentrations of ZnO-NPs cause toxic effects in Camelina and B. napus. As mentioned, concentrations higher than 50 ppm reduced GP and rootlet growth, resulting a sharp decrease in seedling vigor index of both species. These results indicate that ZnO-NPs can be used as nanofertilizers on optimal concentrations of about 1 to 10 ppm for these species.

Data Availability

All data used to support the findings of this study are included in the article.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.
Acknowledgments

The authors are thankful to Razi University and Maragheh University of Medical Sciences, for providing all necessary research facilities and moral supports to carry out this research.

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