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ZnO nanoparticles synthesized by chemical precipitation to increase germination and bioactive compounds in sprouts of *Raphanus sativus* L.

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ABSTRACT

Objective: To examine how priming radish (*Raphanus sativus* L.) sprouts with zinc oxide nanoparticles (NPs-ZnO) affects their germination, photosynthetic pigments, phenolic compounds, and zinc content.

Design/methodology/approach: We evaluated five NPs-ZnO treatments and a control sample with four replications under a completely randomized design.

Results: Sprouts treated with NPs-ZnO showed increased germination variables, photosynthetic pigments, phenolic compounds, and zinc content as compared to untreated radish sprouts.

Study limitations/implications: It is hard to establish a response model for the effects of NPs since their shape, size, surface charge, chemical composition, and concentration may have a differentiated impact on seed germination.

Findings/conclusions: Using NPs-ZnO could be an effective way to enrich crops, since the passage of Zn through plant tissues will cause an accumulation of this micronutrient.

Keywords: *Raphanus sativus*, Nanofertilizer, Zinc oxide.

INTRODUCTION

Due to the growing demand for agricultural products, boosting crop production and productivity is a great challenge worldwide. To increase sustainability in modern agriculture, we must introduce innovative technologies (Rai-Kalal & Jajoo, 2021). Using and applying nanoparticles to stimulate plant growth and modulate plant physiological responses is a relatively recent practice. Nanopriming can improve seed germination and reduce aging (Mahakham *et al.*, 2017) by triggering metabolic processes usually activated during the early phase of germination, which in turn improves germination and emergence

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rates, thus promoting the sustenance of seeds under different abiotic stresses (Singh *et al.*, 2020). Moreover, zinc oxide nanoparticles (NPs-ZnO) have shown positive effects on the growth of mung beans, chickpeas, cucumber, alfalfa, tomato, and other crops (Mahajan *et al.*, 2011; De la Rosa *et al.*, 2013). Radish (*Raphanus sativus*) (Brassicaceae) is an annual horticultural crop consumed worldwide because of its nutritional value (Xie *et al.*, 2018), and has proven to be a healthy food for human diet and health. It is a widely studied species that has been incorporated as a scientific biological model to describe the toxic effects of some chemical substances on germination (Taladrid & Espinosa, 2021). Our objective was to use NPs-ZnO, synthesized through a conventional method of controlled chemical precipitation, to improve germination, growth, bioactive compounds, and zinc concentration in radish sprouts.

MATERIALS AND METHODS

Zinc oxide nanoparticles

We used zinc oxide nanoparticles (NPs-ZnO), measuring between 67 and 71 nm, with a purity of 99%, white color, and structurally hemispherical and polygonal shapes. We synthesized the NPs-ZnO through a controlled chemical precipitation method following Galindo-Guzmán *et al.* (2022), and using zinc acetate $Zn(CH_3COO)_2$ as a precursor.

NPs-ZnO applied treatments

In order to test how different concentrations of NPs-Zno affect seed viability, we applied six treatments, divided into one control sample (deionized water), and 25, 50, 75, 100, and 125 mg L^{-1} NPs-ZnO doses.

Seed germination

We used radish seeds of the Champion variety (Hortaflor®). The seeds were disinfected with 75% ethanol and washed with deionized water. We performed germination tests by placing ten seeds per treatment in 90 mm Petri dishes containing Whatman filter paper. The treatments were applied only once during imbibition, for which we added 5 mL of NPs-ZnO suspension per treatment with a pipette. Four replications per sample were prepared. The Petri dishes were sealed with Parafilm paper and placed in a Novatech CA-550 artificial growth chamber at 22±2 °C for seven days (Don *et al.*, 2013).

Parameters evaluated during the experiment

The trial was completed according to the International Seed Testing Association (ISTA) guidelines, which consider the following parameters: percentage of vigor and germination (%), radicle length (cm), and biomass accumulation in the plumule and radicle in milligrams per sprout.

Determining chlorophyll and carotenoid content

Chlorophyll and carotenoids were determined following the Lichtenthaler & Wellburn (1983) method. We recorded absorbance readings at 665, 649, and 470 nm using a Jenway 7305 UV-visible spectrophotometer.

Total phenol content

The total phenolic compounds were quantified using the Folin-Ciocalteu method (Singleton *et al.*, 1999). Results were reported in gallic acid equivalent mg on a 100 g fresh weight basis (mg equiv AG 100 g^{-1} FW).

Zinc

Zn was quantified in a hydride generation atomic absorption (AA) spectrophotometer (Lab Wrench Varian SpectrAA®, 220Fast model). We presented the results in μg kg^{-1} of dry weight.

Statistical analysis

The experiment used a completely randomized design with six treatments and four replications. We verified the data normality and homogeneity of variances for each response variable with the Bartlett and Kolmogorov-Smirnov test (Bartlett, 1937; Steinskog *et al.*, 2007). Results were evaluated by analysis of variance and comparison of means with Tukey's test ($p \leq 0.05$), using the statistical package (SAS) version 9.4. We normalized the variables reported as percentages (vigor and germination) by applying the arcsine square root transformation (Steel & Torrie, 1960).

RESULTS AND DISCUSSION

Effects of NPs-ZnO on radish seed vigor and germination

Our results show that the NPs-ZnO did not significantly affect ($P \leq 0.05$) the seeds' vigor, which remained similar to the control sample (Table 1). The best dose for the germination variable was 100 mg L^{-1}, with a germination average of 90%, 29.42% higher than the control sample. Meanwhile, no significant statistical differences ($P \leq 0.05$) were obtained with the 0, 25, 50, 75, and 125 mg L^{-1} doses. It is hard to establish a response model of the effects of NPs because their shape, size, surface charge, chemical composition, and concentration can have a differentiated impact on seed germination and seedling growth (Ahmad *et al.*, 2021). Moreover, molecular factors such as kinases acting as DNA "checkpoints" and links between genotoxic stress and seed aging also affect germination (Taladrid & Espinosa, 2021).

Fresh weight of plumule and radicle

An increase in the fresh weight of the plumule and radicle was observed in the sprouts prepared with NPs-ZnO. The maximum increase of fresh weight occurred in the 100 mg L^{-1} dose, which surpassed the control sample by 15.49% for the plumule and 20.57% for the radicle. This behavior relates to the increase in germination percentage, which ultimately increases biomass production. The increase in biomass could be a result of the Zn ions present in the seeds treated with NPs-ZnO. Said ions play a vital role in the biosynthesis of natural auxin (indole-3-acetic acid) in plants, which consequently activates cell division and enlargement (Ali & Mahmoud, 2013). These results concur with previous research by Rai-Kalal & Jajoo (2021), who reported that Zn plays an effective role in increasing biomass in wheat seedlings.

Radicle length

The NPs-ZnO significantly affected ($P \leq 0.05$) the radicle length (Table 1). In the 25 mg L^{-1} dose, the length was longer than in the control sample (7.10 cm), with a numerical difference of 14.51%. For the 50 mg L^{-1} dose, the length obtained was 6.22 cm, statistically equivalent to the control sample (6.20 cm), with a numerical difference of 0.32%. The 75 and 125 mg L^{-1}

treatments were statistically equivalent. An increase also occurred with the 100 mg L-1 dose, which reached a root length of 9.62 cm. Several studies have shown that root elongation improved in wheat, sweet sorghum, and soybean when exposed to NPs-ZnO as compared to control samples (López-Moreno et al., 2010; Elhaj Baddar & Unrine, 2018; Naseeruddin et al., 2018). Nanoparticles enter the seed coat through its pores, causing a greater penetration of water molecules and inducing the enzymatic activity that generates reactive oxygen species (ROS), and that degrades starch to physiologically improve seed germination (Mahakham et al., 2017).

Photosynthetic pigments

Chlorophyll content has a positive relation to photosynthetic rate. Therefore, a change in total chlorophyll content can be considered an indicator of plant health. As observed in Figure 1a, the sprouts treated with NPs-ZnO presented a significant difference ($P \leq 0.05$). When applying the 25 mg L^{-1} dose, there was a chlorophyll increase of 4.74% in comparison with the control sample. The maximum increase was observed in the 50 mg L^{-1} dose, with an average difference of 32.03% as compared to the control sample. The 75 and 100 mg L^{-1} doses were statistically equivalent to each other. However, we registered a decrease of 13.96% in the 125 mg L^{-1} dose as compared to the control sample. Zn could explain the positive effects of NPs-ZnO on chlorophyll content because it plays a vital role in chlorophyll biosynthesis by protecting the sulfhydryl group of the chlorophyll molecule (Cakmak, 2008). Zn also plays a role in the development of chloroplasts and takes part in the repair process of photosystem II by recycling the damaged D1 protein (Hänsch & Mendel, 2009). However, Acharya *et al.* (2020) argue that using nanoparticles at high

Table 1. Means comparison in germination, vigor, plumule and radicle fresh weight, and radicle length of radish sprouts when treated with NPs-ZnO. *Values with different letters within the same column indicate a significant difference according to Tukey's test ($p \leq 0.05$). (±) standard deviation.

Treatment NPs-ZnO (mg L^{-1})	Vigor (%)	Germination (%)	Fresh weight of plumule (mg)	Fresh weight of radicle (mg)	Radicle length (cm)
0	69.53 ± 4.06^{a}	69.53 ± 4.06^{b}	55.90 ± 2.41^{bc}	26.30 ± 0.57^{b}	6.20 ± 0.31^{c}
25	63.80 ± 6.04^{a}	61.77 ± 3.32^{b}	60.98 ± 6.34^{b}	22.86 ± 1.16^{cd}	7.10 ± 0.43^{bc}
50	69.53 ± 4.06^{a}	69.53 ± 4.06^{b}	47.66 ± 1.53^{d}	23.85 ± 0.57^{bc}	6.22 ± 0.22^{c}
75	69.53 ± 4.06^{a}	69.53 ± 4.06^{b}	45.03 ± 1.41^{d}	21.25 ± 0.55^{d}	8.05 ± 0.12^{b}
100	67.50 ± 4.69^{a}	89.99 ± 0.00^{a}	69.18 ± 4.17^{a}	31.71 ± 1.26^{a}	9.65 ± 0.35^{a}
125	67.50 ± 4.69^{a}	69.53 ± 4.06^{b}	48.28 ± 3.21^{cd}	20.39 ± 1.91^{d}	7.27 ± 0.83^{b}

concentrations in seed priming will cause toxicity in the sprouts, which can be observed in a reduction of photosynthetic pigments.

Carotenoids play a vital role in the photoprotective mechanism of plants. Figure 1b shows the effect of exposure to NPs-ZnO on total carotenoid content in radish sprouts. For the 0, 25, 50, 75, and 100 mg L^{-1} doses, no statistically significant differences ($P \leq 0.05$) were obtained, but the 50 mg L^{-1} dose registered the highest carotenoid content (7.95 mg g^{-1} PF). Meanwhile, the 125 mg L^{-1} dose presented a decrease in comparison with all treatments.

Total phenol content

High doses of NPs-ZnO had a significant effect on the synthesis of total phenolic compounds in the sprouts (Figure 2). The concentration of total phenolic compounds in radish extracts ranged from 95.88 to 168.37 mg AG 100 g^{-1}. The maximum value (168.37 mg AG 100 g^{-1}) was recorded after applying a 50 mg L^{-1} NPs-ZnO dose. The main compounds responsible for the defense mechanism in plants are secondary metabolites of a phenolic nature, which also improve the nutritional value of crops due to their known antioxidant activity.

Zinc

As the NPs-ZnO doses increased, the Zn content in the radish sprouts also increased (Figure 3). In the control sample, the amount of Zn was 45.95 μg kg^{-1} DW. The maximum

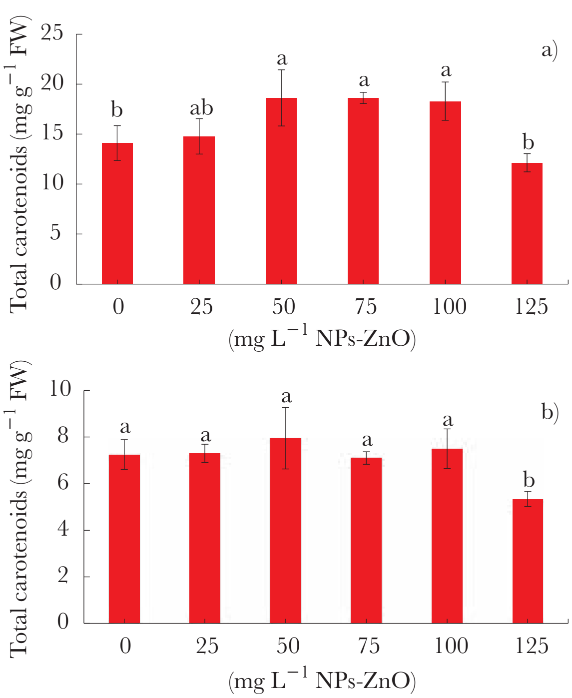

Figure 1. Chlorophyll content (a) and carotene content (b) in radish sprouts treated with NPs-ZnO. The vertical bars indicate the standard deviation of the mean. Different letters indicate a significant difference according to Tukey's test ($P \leq 0.05$).

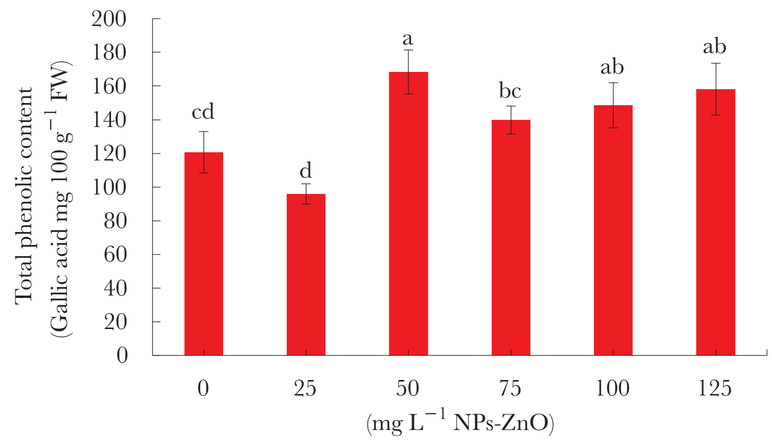

Figure 2. Total phenol content in radish sprouts when treated with NPs-ZnO. The vertical bars indicate the standard deviation of the mean. Different letters indicate a significant difference according to Tukey's test ($P \leq 0.05$).

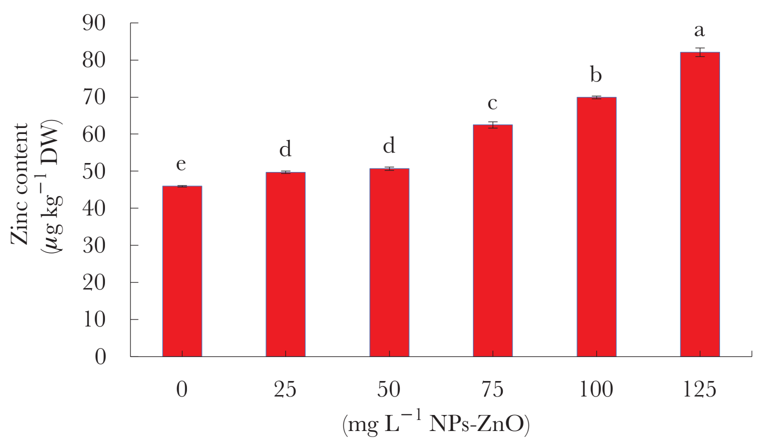

Figure 3. Zinc content in radish sprouts after applying NPs-ZnO. The vertical bars indicate the standard deviation of the mean. Different letters indicate a significant difference according to Tukey's test ($P \leq 0.05$).

zinc content occurred in the 125 mg L^{-1} dose (82.07 μg kg^{-1}). Although this concentration is favorable, we must point out that photosynthetic pigments decrease. Therefore, using higher concentrations can be harmful. According to the Instituto de Medicina (2001), the recommended intake of Zn for adults is 8 mg day^{-1} for women and 11 mg day^{-1} for men. The accumulation of Zn in radish sprouts could complement the daily intake of this micronutrient.

CONCLUSIONS

Radish seeds responded positively to NPs-ZnO treatments, which showed different efficacy depending on the parameters tested. In this regard, using NPs-ZnO could be an effective way to enrich crops, since the passage of Zn through plant tissues will cause its accumulation.

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