



Effects of zinc and copper oxide nanoparticles on sugar beet (*Beta vulgaris* L.)

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Abstract

In recent years, the development of nanotechnology and the results of many researches in this field have shown that it contributes significantly to germination, growth, development and product yield of plants. In addition, nanoparticle applications have been shown to positively affect cell structure and cell function on physiological and biochemical mechanisms in the regulation of adverse effects caused by various environmental stresses. In this study, zinc oxide (ZnO) and copper oxide (CuO) nanoparticle were applied to the seeds of sugar beet (Bernache) variety and the effects of changes in germination, weight and length measurements in radicle and plumule were investigated. It was determined that the seeds responded differently to both nanoparticle. In our study, copper oxide pretreatment caused an increase in radicle and plumule length. In terms of weights, it was observed that copper oxide 1800 mg/l application caused a significant increase in shoot and root wet weight. As a result, it was determined that copper oxide 1800 mg/l pretreatment had a positive effect on all parameters examined.

Keywords: Nanoparticle, Sugar beet, Copper, Zinc

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1. Introduction

In recent years, the use of nanotechnology in agricultural production has become an area of increasing interest. Especially metal-based nanoparticles are considered as a new generation of biostimulants that can positively affect plant physiology when applied at low doses. Since these particles have larger surface area and high reactivity compared to ionic elements, they can penetrate plant cells faster and more effectively (González-Melendi et al., 2008). In this context, zinc nanoparticles (ZnO NPs) are the nano form of the element zinc, which is vital in plant metabolism. ZnO NPs are involved in many basic physiological processes such as the production of photosynthetic pigments, regulation of the antioxidant system, enzyme activity and maintenance of cell membrane stability (Cakmak, 2000; Dimkpa & Bindraban, 2016).

Studies have shown that ZnO NP application promotes plant growth and alleviates ion toxicity by increasing root and shoot development (Mahajan & Kaushal, 2018). On the other hand, some studies show that these nanoparticles can affect not only physical development but also metabolic processes in plants. Kaveh et al. (2013) revealed that silver nanoparticle (Ag NP) treatment modulated stress responses by increasing antioxidant enzyme activities in *Arabidopsis thaliana*, indicating that nanoparticles may be effective in plant defense systems. Mahajan and Kaushal (2018) emphasized that zinc-based fertilizers provide a more effective uptake compared to traditional zinc fertilizers, so similar or higher yields can be obtained with lower doses. It is also reported that ZnO NPs may have positive effects on plant microbiota (González-Melendi et al., 2008). The intracellular uptake mechanisms of nanoparticles suggest that they may function not only as nutrient carriers but also as



biostimulants and protective agents in plant stress management.

In this study, it was aimed to determine the effects of zinc oxide (ZnO) and copper oxide (CuO) nanoparticles applied at different doses on the germination process and early seed development of sugar beet (*Beta vulgaris* L.) seeds.

2. Material and Method

Seed trials were carried out in the acclimatization room. The seeds to be used in the experiment were selected from sugar beet seeds of similar size with plump and robust structure. The seeds were surface sterilized before use; this was done with 1% sodium hypochlorite solution for 10 minutes, then washed with pure water and dried on filter papers at room temperature. The experiment was carried out according to the "Coincidence Plots Experimental Design" with three replications of nanoparticle pretreatments.

In germination trials, nanoparticle pretreatment of sugar beet seeds was carried out by using 4 different dosages of zinc (0, 600, 1200 and 1800 mg/l) and distilled water at 20 ± 1 °C in dark environment for 18 hours at appropriate concentrations. After treatment, the seeds were dried and stored at +4 °C before use. Sugar beet seeds were placed in petri dishes, each of which was covered with two layers of blotting paper, with 20 seeds. Control and only nanoparticle treated groups were added 5 ml of pure water.

The germination process was monitored in the dark at 22 ± 1 °C for 7 days. The seeds were checked every day and seeds reaching a root length of 1 mm were considered germinated. During the control, contaminated seeds were removed from the medium and recorded. A total of 60 seeds were sown for each group and calculations were made accordingly.

Data were analyzed by one-way ANOVA using SPSS software. Differences were evaluated at 5% significance level.

3. Results and Discussion

3.1. Germination rate

The effect of ZnO NP and CuO NP doses on germination rate was found statistically significant ($p < 0.01$, Table 1). There was no significant change in germination rate values with increasing CuO NP application doses (0, 600, 1200, 1800 mg/l), and the highest value among CuO concentrations was obtained from 600 mg/l application. Similar situation was observed between ZnO NP concentrations and the highest value was obtained from 600 mg/l application. In addition, 1800 mg/l dose showed inhibitory effect on germination rate in both nanoparticles (Table 2). Kunjam et al. (2015) found that high concentration of ZnO metal had a negative effect on seed germination. When the studies conducted with nanoparticles were examined; it was observed that different plant species reacted differently

to different types of nanoparticles at different concentrations (Zhao et al., 2021).

3.2. Germination duration

The effect of nanoparticle doses on germination period was found to be insignificant (Table 1). The effect of 600, 1200 and 1800 mg/l CuO NP applied to sugar beet seeds on germination period was negative. ZnO application doses (600, 1200 and 1800 mg/l) decreased the germination time by 22.0%, 10.4% and 18.3%, respectively. CuO NP application decreased the germination period by 27.3%, 6.0% and 23.1%, respectively. The lowest germination time occurred in 600 mg/l ZnO and 1800 mg/l CuO NP treatments (4.26 and 4.46 days, respectively), while the longest germination time was obtained from the control group (5.46 days) without any nanoparticle application. Kunjam et al. (2015) determined that high concentration of ZnO metal had a negative effect on seed germination.

3.3. Root width

The effect of nanoparticle doses on root width was insignificant (Table 1). The effect of 600, 1200 and 1800 mg/l ZnO and CuO NP applied to sugar beet seeds on germination width was negative. Root diameter affects the structural strength and water carrying capacity of the root system. According to the average values, root diameter varied between 0.524-0.661 mm. In both nanoparticles applied in the study, the highest root diameter values were obtained in the control group (0.661 mm), while the lowest root diameter values were obtained in ZnO NP treatment at 1800 mg/l and in copper oxide treatment at 600 mg/l concentration. It can be said that ZnO and CuO NP treatments did not cause a significant and regular increase in root diameter, but caused some variations in terms of tissue thickening in the roots. The effect of ZnO NP on root diameter did not vary greatly according to the doses; therefore, it is difficult to mention a direct and significant effect of ZnO NP on root diameter.

3.4. Root length

In the study, the effect of different doses of nanoparticles on stem length was found to be positive and statistically significant ($p < 0.01$, Table 1). The highest root length was obtained in 600 mg/l ZnO NP application with 67.47 cm. These findings show that ZnO NP supports root growth at low and medium doses, but this effect decreases at high doses. Similar situation was determined in CuO NP application and the highest and lowest root lengths were obtained from 1800 mg/l (81.60 mm) and control group (34.47 mm), respectively. It is known that nanoparticle applications have a negative effect on plant growth after a certain dose and may even be toxic (Rizwan et al., 2017; Sarkar et al., 2023).

3.5. Root weight

According to the results of the study, the effect of ZnO and CuO NPs application on root weight was found statistically significant ($p < 0.01$, Table 1). The highest root weight was measured as 0.0156 and 0.0158 g in 1800mg/l CuO NP and 600mg/l ZnO NP treatments, respectively. This indicates that ZnO supports the effect of NP to increase root biomass. The medium dose of ZnO NP significantly promoted the mass development of the root system. As a matter of fact, it was found that 50 mg/l dose of ZnO NP positively affected growth and development and increased root growth by 28.4% among 25 and 50 mg/l ZnO NP doses applied in the study (Ul Hassan et al., 2023). In another study, it was determined that foliar application of ZnO NP at different doses (0, 20, 50, 80 mg/l) significantly improved root and shoot weights up to 50 mg/l in wheat under salt

stress (Lalarukh et al., 2022). In the same study, it was reported that ZnO NP applications increased plant resistance and growth by stimulating the formation of osmolytes and increasing nutrient uptake.

3.6. Shoot length

Shoot length is one of the important parameters for growing healthy and high-quality seedlings. There is a positive relationship between shoot length and plant growth traits such as number of leaves, shoot dry and wet weight (Vazirimehr & Rigi, 2014; Jini & Joseph, 2017). The longest shoot was 50.3, 52.9 and 55.9 mm in 600, 1200 and 1800 mg/l ZnO NP treatment, respectively (Table 1). However, it is known that nanoparticle applications have a negative effect on plant growth after a certain dose and may even be toxic (Rizwan et al., 2017; Sarkar et al., 2023).

Table 1. The effects of ZnO and CuO NPs treatment on germination rate, germination period, root width, root length, root weight and shoot length of sugar beets

Nanoparticle	Dose (mg/l)	Germination rate (%)	Germination period (day)	Root width (mm)	Root length (mm)	Root weight (g)	Shoot length (mm)
CuO	Control	60.9 ^f	5.46	0.605	34.47 ^c	0.0088 ^{bc}	41.20 ^{bc}
	600	94.7 ^{ab}	4.26	0.661	56.43 ^b	0.0095 ^{bc}	50.29 ^a
	1200	74.0 ^e	4.89	0.586	77.53 ^a	0.0124 ^{ab}	52.88 ^a
	1800	89.5 ^c	4.46	0.535	81.60 ^a	0.0156 ^a	55.89 ^a
ZnO	600	97.2 ^a	3.97	0.524	67.47 ^{ab}	0.0158 ^a	43.38 ^{ab}
	1200	92.2 ^b	5.13	0.544	24.20 ^c	0.0063 ^c	32.19 ^d
	1800	86.3 ^d	4.20	0.560	30.74 ^c	0.0095 ^{bc}	37.32 ^{cd}
Average		85.0	4.62	0.570	53.20	0.0110	44.74

3.7. Shoot diameter and shoot weight

According to the results of the study, the effect of nanoparticle dose application on shoot diameter was found to be statistically significant ($p < 0.01$). Shoot diameter is important in terms of stem thickness and mechanical resistance of the plant. Shoot diameter values varied between 0.68-0.88 mm in nanoparticle applications. 1800 mg/l ZnO dose produced the lowest shoot diameter (0.68 mm) and the highest shoot diameter value was obtained from 600 mg/l CuO NP application dose (Figure 1). High dose ZnO NP had a negative effect on shoot diameter, but this effect was not supported by all parameters.

When the analysis of variance was examined, it is seen that the effect of nanoparticle dose application on shoot weight is significant ($p < 0.01$). In this study, the highest shoot weight was observed in 1800 mg/l ZnO NP application with 0.060 g. The lowest shoot weight was obtained from 1200, 1800 mg/l ZnO NP application and control group (Figure 1). This indicates that the medium dose is the optimum level for shoot growth. The 1200 mg/l dose increased shoot biomass and optimized upper

plant growth. It can be said that this dose acts as a "supportive buffer".

3.8. Leaf length and leaf weight

While nanoparticle applications had a significant effect on leaf length, the effect of zinc and copper nanoparticle applications on leaf weight was significant ($p < 0.01$). Figure 2 shows that the highest and lowest leaf length values were obtained from 600 mg/l copper oxide nanoparticle application and the lowest values were obtained from 1200 and 1800 mg/l zinc oxide application, respectively. In terms of leaf weight, copper and zinc, although there was a numerical difference between the weight values of zinc oxide nanoparticle application, this difference was statistically insignificant. The highest and lowest weight values were obtained from 600 and 1200 mg/l zinc oxide application and the highest value was obtained from 1800 mg/l copper application. It was observed that 1800 mg/l dose of CuO NP supported leaf growth in terms of biomass and length, but the effect of zinc oxide nanoparticles at the same dose was limited. This may be related to an increase in cell size or water-holding capacity.

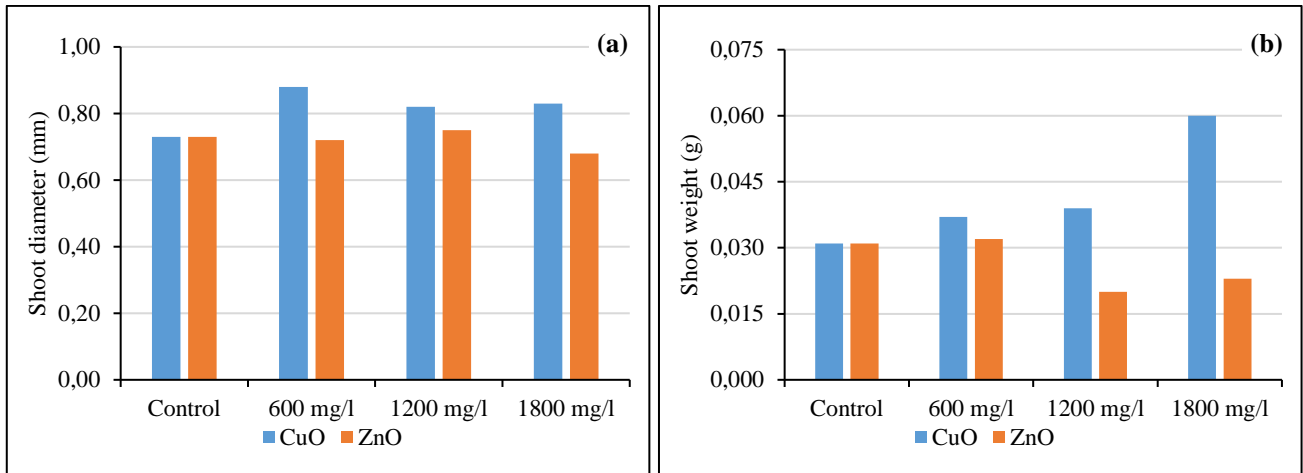


Figure 1. The effects of ZnO and CuO NPs treatment on (a) shoot diameter and (b) shoot weight of sugar beets

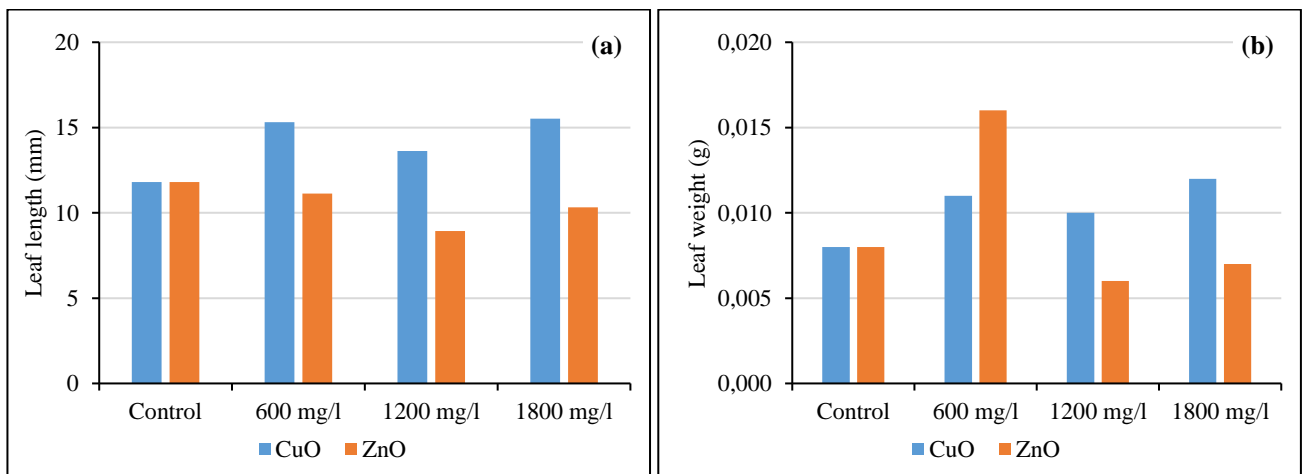


Figure 2. The effects of ZnO and CuO NPs treatment on (a) leaf length and (b) leaf weight of sugar beets

4. Conclusion

In this study, morphological and physiological changes in sugar beet (*Beta vulgaris* L.) seeds exposed to different nanoparticle concentrations were investigated in detail. It was found that the doses of copper and zinc nanoparticles, especially zinc oxide nanoparticles, partially improved the germination properties of sugar beet seeds. It was observed that zinc oxide and copper oxide nanoparticles had positive effects on many of the characters examined and this effect increased in many parameters depending on the increasing concentration levels. In order to transfer such studies to practice, it is important to carry out studies with different NPs and at different doses and to evaluate them with economic analyses.

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Conflict of interest

The authors declare no conflict of interest.

Ethical Approval

This article does not require ethics committee approval.

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