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Original Paper

Effect of Zinc Oxide Nanoparticles on Proso Millet Seedlings

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Proso millet (*Panicum miliaceum* L.) from the family Poaceae is one of the six major cereals cultivated in the world. An important characteristic is its resistance to heat and drought, which makes hot, dry and semi-arid areas optimal for its cultivation. Due to the climatic changes and the associated lack of water in some areas of the world, proso millet represents a good alternative or supplement to cereal cultivation. The main agricultural value of proso millet is its use as animal feed, which underlines its key role in agriculture. The modern trend in agriculture involves the application of nanoparticle-based products as micronutrients or growth promoters. The aim of our research was to investigate the effect of zinc oxide nanoparticles (ZnO-NPs) on the initial growth stages of proso millet. We evaluated the length of the plant, length of aerial parts and the root system of plants. In experiments Slovak variety 'Unikum' was used. ZnO-NPs were applied at four different concentrations (1 mg·l^{-1}, 10 mg·l^{-1}, 100 mg·l^{-1} and 300 mg·l^{-1}) and compared with the control sample. We found that the concentration of 100 mg·l^{-1} might be suitable for future considerations and the highest concentration of 300 mg·l^{-1} had an inhibitory effect on plant growth in length of the whole plant, aerial parts and most of the monitored root parameters.

Keywords: proso millet, zinc oxide nanoparticles, seedling growth

1 Introduction

Nowadays, agriculture faces the climatic changes, the result of which is also the increase of CO_2 in atmosphere. Although the increasing amount of CO_2 in the atmosphere could be beneficial for several crops, the limiting factor for its use by plants will be the lack of water. This will require a change in the composition of cultivated crops, including cereals. It has been found that increased CO_2 content would be beneficial for C3 plants (wheat) while for more efficient C4 species, the positive effect would be manifested only in those that can tolerate drought stress (millet, sorghum) (Rezaei et al., 2023). In this context, efforts are being made to replace the cultivation of crops with high water requirements, such as maize, with alternative species capable of tolerant to drought. It has been found that millet, hitherto perceived as a crop of dry areas of Asia and the African continent, is a suitable species providing stable yields in conditions of continued aridification of Southern (Ventura et al., 2022) and Central Europe (Hermuth et al. 2016), but also North America (Reinman et al., 2024). Due to its low demands on water, soil conditions and short growing season, millet represents a good alternative or supplement to cereal cultivation also in organic farming, which focuses on the natural maintenance of agricultural activities without minimal human intervention (Panday et al., 2024). With regard to the above trends of minimizing agrotechnical interventions, the development of modern technologies based on the application of nanoparticle-based materials and the use of nanofertilizers in plant nutrition is underway. In addition, nanoparticles appear to be a promising way to make agricultural production

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more effective in terms of environmental risks (Du et al., 2019). Zinc oxide nanoparticles (ZnO-NPs) are now used in agriculture, because they increase crop yields and they are considered as plant growth promoters. That poses a new type of nanofertilizer with potentially more intensive effects than conventional ionic forms (Yadav et al. 2023). In addition, they are involved in improving the nutritional quality of plants, reducing abiotic stress, improving photosynthetic parameters and regulating primary carbohydrate metabolism (Gomes et al., 2023). Previous research has shown improvements in nutritional and physiological parameters in foxtail millet (Kolenčík et al. 2019). Increase in rice production occurred after the application of ZnO-NPs on leaves (Elechtyar & AL-Huqail, 2023). The uptake of ZnO-NPs through root system promoted the growth of rice roots, increased the level of antioxidants and nutrient uptake leading to root growth (Singh et al., 2023). In barley, a slight increase in root length was observed, however not significant (Doğaroğlu & Köleli, 2017). The increase in root growth was also observed in onion (Raskar & Laware, 2014). The above-mentioned positive effects on growth and yield parameters of crops were in most cases achieved by applying doses of zinc nanoparticles (Zn-NPs) ranging from 1 to 100 $mg \cdot l^{-1}$. It is now expected that nanoparticles will be appropriate for use in agriculture as plant fertilizers because the application of fertilizers with micronutrients in the form of nanoparticles leads to a gradual and controlled release of nutrients, thereby alleviating the problems associated with fertilizer pollution (Naderi & Abedi, 2012).

It has been shown that the response of individual species to the application of nanoparticles is species-specific. Experiments with related millet species suggest that a positive effect of lower doses of Zn-NPs should be expected. Therefore, the aim of the work was to test the effect of different concentrations of zinc nanoparticles on the initial developmental stages of proso millet before their use in field conditions. We expected that ZnO-NPs concentrations below 100 $mg \cdot l^{-1}$ would give us the best possible results compared to control variant. We also expected the possible negative effect of the concentration of 300 $mg \cdot l^{-1}$.

2 Material and Methods

2.1 Characteristics of Plant Material

The *Panicum miliaceum* 'Unikum' (OSIVO a.s., Slovakia) is a Slovak cultivar, which belongs to the medium-early proso millets. It is characterized by a short growing period (100–120 days), high yield and is used for food and feed purposes. It is resistant to lodging and has a medium to high branching capacity. The recommended sowing date for an area with a warm and dry climate is from May 10 to May 30, with an optimum soil temperature of 9–11 °C. It produces the best yields on soils with average moisture reserves, it does not have high habitat requirements, but extremely dry, poor or heavy waterlogged and cold soils are unsuitable. It is sown in narrow rows, with a row spacing of 120–150 mm. The recommended sowing rate of ‚Unikum' proso millet is 350–450 individuals per m^2. When harvesting millet, the optimal seed moisture content is 17–18%. The grains are glossy in the panicle and the seeds are yellow in colour.

2.2 The Laboratory Experiment

The experiment on the germination of proso millet at different concentrations was carried out at a laboratory temperature of 24 ±2 degrees Celsius and 40–50% humidity. The laboratory in which the experiment was carried out is on the north side. The northern exposure is suitable for the laboratory experiment because it is not affected by direct sunlight, provides uniform and stable illumination throughout the day. 50 g of sand was weighed in each Petri dish, distilled water was applied by spray in 10 shots to moisten the sand (14 ml of distilled water per Petri dish). The experiment was done with ten seeds of proso millet per Petri dish. A total of three repetitions were done per variant. Zinc oxide nanoparticles (Nanografi Nanotechnology, Turkey) at concentrations of 1 $mg \cdot l^{-1}$, 10 $mg \cdot l^{-1}$, 100 $mg \cdot l^{-1}$ and 300 $mg \cdot l^{-1}$ were applied and a control sample with distilled water was used for comparison. Zinc nanoparticles (≤50 nm) in solid form were dispersed in distilled water and sonicated for 15 minutes prior to each application. Zinc oxide nanoparticles were applied in spray form. The germination of millet lasted for 10 days during which the regular sprays of 5 or 10 ml were done every two days with the laboratory spray bottle. On the last tenth day the experiment was terminated, and the plant material was evaluated.

2.3 Evaluation of the Experiment

After the successful germination of the proso millet samples, the evaluation of the samples was performed with the equipment from the Laboratory of Special Seed Production Techniques, Agrobiotech Research Centre on the tenth day. The evaluation of length parameters of whole plants and aerial parts of plants was performed with a ruler. The root system was washed in distilled water and then evaluated using the WinRHIZO software (Regent Instruments Inc., Canada).

2.4 Statistical Analysis of Data

The results obtained from plant and root length measurements as well as the data obtained from the WinRIZO software were processed by the analysis of variance (ANOVA) using Tukey's HSD test ($P \leq 0.01$) (Statistica software, version 10, StatSoft, Inc., Tulsa, OK, USA).

3 Results and Discussion

In the length of aerial parts of plant, the significant difference was found only between variant 300 mg·l^{-1} (5.43 cm) and control variant (7.55 cm). The length of a whole plant also revealed a significant difference only between variant 300 mg·l^{-1} (7.62 cm) and control variant (10.84 cm) (Table 1).

The length of the roots analysis showed a significant difference between variant 300 mg·l^{-1} (5.78 cm) and control variant (8.97 cm). A similar significant difference was recorded in surface area analysis of roots between variant 300 mg·l^{-1} (0.51 cm^2) and control variant (0.85 cm^2). The analysis of root volume also showed a significant difference between variant 300 mg·l^{-1} (0.004 cm^3) and control variant (0.006 cm^3) (Table 2).

Previous researches provide the information on the application of nanoparticles on different millet species. The effects of 10 mg·l^{-1} ZnO-NPs on pearl millet in field experiment were also confirmed by Tarafdar et al. (2014) where the increase in yield, improvement in shoot length, length and root area of the plant was observed. Similarly, ZnO-NPs at a concentration of 2.6 mg·l^{-1} through foliar application on foxtial millet [*Setaria italica* (L.) P. Beauvois] had a positive effect on growth parameters of this crop (Kolenčík, 2019). Foliar application of nano nitrogen and nano zinc in a field trial on finger millet (*Eleusine coracana* Gaertn.) resulted in effective growth and yield enhancement (Sneha et al., 2023). The stimulatory effect of lower ZnO-NPs concentrations presented in many studies was not observed in this paper. In both the length of the whole plant and aerial parts the lower concentrations such as 1 and 10 mg·l^{-1} had lower mean values than the control variant. Similarly, ZnO-NPs at those concentrations had no stimulatory effect on most of the root parameters. In the number of tips, the concentration of 10 mg·l^{-1} showed higher mean value than control variant, although not statistically significant. In addition to the application of nanoparticles on millet, the effect of zinc oxide nanoparticles on other species of the Poaceae family has also been observed (Rizwan et al., 2019). In corn, improvement in nutrient content and seed quality occurred after ZnO-NPs application at a concentration of 40 mg·l^{-1}, which was reflected by an increase in seed starch and protein (Tondey et al., 2022). Application of ZnO-NPs to wheat seeds gradually with increasing concentration led to increased growth and overall higher biomass production. The highest increase in plant height, number of tillers per plant, ear length, grain yield, shoot and root dry weight of wheat was at 100 mg·l^{-1} concentration (Munir et al., 2018). However,

Table 1 The evaluation of whole plant length and length of aerial parts of proso millet (Tukey's HSD test results are shown by small letters a, b, c in superscript; values in the table represent mean ±standard deviation)

Variant	Length of aerial parts of plant (cm)	Whole plant length (cm)
C	7.55^{bc} ±2.73	10.84^{a} ±3.28
V1	6.27^{ab} ±2.71	9.16^{ab} ±3.52
V10	6.84^{abc} ±2.68	9.89^{ab} ±3.50
V100	8.15^{c} ±2.48	11.22^{a} ±3.09
V300	5.43^{a} ±2.58	7.62^{b} ±3.16

C – control, V1 – ZnO 1 mg·l^{-1}, V10 – ZnO 10 mg·l^{-1}, V100 – ZnO 100 mg·l^{-1}, V300 – ZnO 300 mg·l^{-1}; different letters (a, b, c) in variants means that they are significantly different at $P \leq 0.01$

Table 2 The evaluation of the root system of proso millet (Tukey's HSD test results are shown by small letters a, b, c in superscript; values in the table represent mean ±standard deviation)

Variant	Length (cm)	Surface area (cm^2)	Average diameter (mm)	Root volume (cm^3)	Number of root tips (pcs)
C	8.97^{a} ±3.69	0.85^{a} ±0.39	0.30^{a} ±0.05	0.006^{b} ±0.003	22.69^{ab} ±13.89
V1	7.68^{ab} ±3.56	0.72^{ab} ±0.36	0.29^{a} ±0.06	0.005^{ab} ±0.003	19.86^{ab} ±13.69
V10	8.31^{a} ±3.52	0.78^{a} ±0.36	0.29^{a} ±0.05	0.006^{ab} ±0.003	26.72^{a} ±14.32
V100	8.74^{a} ±2.76	0.77^{a} ±0.23	0.28^{a} ±0.04	0.005^{ab} ±0.002	23.25^{a} ±12.01
V300	5.78^{b} ±2.83	0.51^{b} ±0.23	0.29^{a} ±0.05	0.004^{a} ±0.003	13.56^{b} ±9.69

C – control, V1 – ZnO 1 mg·l^{-1}, V10 – ZnO 10 mg·l^{-1}, V100 – ZnO 100 mg·l^{-1}, V300 – ZnO 300 mg·l^{-1}; different letters (a, b, c) in variants means that they are significantly different at $P \leq 0.01$

the opposite effect of the same concentration of ZnO-NPs on the initial developmental stages of wheat was noted by Stałanovska et al. (2023), who observed inhibition of root growth and seedling coleoptiles. Metabolite analyses revealed that one of the causes of the growth retardation effect was decrease in the total amino acid content in all tissues and disorders in carbohydrate metabolism. The same effect on growth parameters of wheat seedlings was observed after application of zinc nanoparticles prepared by green synthesis, as a concentration of 100 ppm caused a decrease in root length, shoot length, dry biomass and seed vigor index, while a concentration of 50 ppm resulted in a significant increase in all parameters (Meher et al., 2020). The negative effect of zinc nanoparticles with concentration of 100 mg·l^{-1} ZnO was not confirmed in proso millet seedlings, since the length of the whole plant and aerial parts showed, that variant 100 mg·l^{-1} had higher mean values than control variant. However, the difference between these variants and control variant were statistically insignificant. In terms of the concentration of 100 mg·l^{-1}, higher mean value when compared to control variant was also recorded in the number root tips, although again the difference was statistically insignificant. Finally, the concentration of 300 mg·l^{-1} proved to be slightly excessive compared to the lower concentrations, which resulted in much lower mean values and the statistically significant difference when compared to control variant in both length of the whole plant and aerial parts. Moreover, the concentration of 300 mg·l^{-1} had a statistically significant inhibitory effect on root parameters of seedlings in all cases, except for the average diameter analysis and the number of root tips. Our findings on the inhibitory effect of higher doses of ZnO-NPs on growth are consistent with the knowledge on the negative effect of zinc nanoparticles on barley root growth, which was achieved with the application of 3 and 30 mmol·kg^{-1} of zinc nanoparticles (Nemček et al., 2020). As other experiments with barley have shown, root growth inhibition occurs due to a decrease in root cell viability and genome stability with increasing zinc nanoparticle concentration (Plaksenkova et al., 2020). Furthermore, increased H_2O_2 formation and cell wall lignification are responsible for the inhibition of wheat root growth (Prakash & Chung, 2016). In conclusion, although most findings indicate that the toxicity of zinc nanoparticles occurs at higher concentrations, the effective toxic dose (EC50) in wheat occurred at a concentration of 20.7 mg·l^{-1} (Keshta et al., 2023), therefore it seems that the response of plants to Zn-NPs depends not only on the concentration and size of the nanoparticles, but also on the type of crop and growing conditions.

4 Conclusions

The expected stimulatory effect of ZnO-NPs at lower concentrations was not observed in present study. In some parameters such as length of the plant and aerial parts, a sign of improvement was observed in the case of concentration of 100 mg·l^{-1}, which could indicate the use of this concentration and similar ones in future research. However, another set of laboratory experiments with similar concentrations is needed to prove this argument, since the results were not statistically significant. Finally, the concentration of 300 mg·l^{-1} resulted in a statistically significant inhibitory effect on seedlings in most cases. Therefore, we do not recommend the concentration of 300 mg·l^{-1} for field experiments with proso millet.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

Lenka Tomovičová: writing – original draft, review & editing; Investigation; Ľuba Ďurišová: writing – original draft, review & editing; Supervision; Conceptualization; Samuel Kšiňan: writing – original draft, review & editing; conceptualization; formal analysis; Marek Kolenčík: supervision; conceptualization; Writing – review & editing; methodology; Dávid Ernst: methodology; Ján Gažo: formal analysis; Pavol Eliáš: supervision; validation; Nikola Kotlárová: investigation; Ivan Ravza: investigation; Viktor Straka: investigation

AI and AI-assisted Technologies use Declaration

No generative AI tools/AI-assisted technologies were used during the preparation of the manuscript.

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