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EFFECT OF NANOPARTICLE METAL SOLUTIONS ON CHUFA (*CYPERUS ESCULENTUS* L.) ROOT AND SEEDLING GROWTH

SUMMARY

Nanoparticles of trace elements are easily included in the metabolic cycles of plants. Zinc and molybdenum are important components of enzymes, but even at low concentrations can have phytotoxic effects. Treatment of chufa tubers with microelements increases the viability of tubers, but at certain concentrations of nanoparticles can negatively affect the growth processes at the germination and early vegetative stages. Effect of treatment of tubers with nanoparticle solution of molybdenum, zinc oxide and zinc citrate at a concentration of 20, 40 and 60 ppm on the growth of roots and seedlings was established on 3rd and 10th days after emergence. The positive effect of all treatments at a concentration of 20 ppm was found, but higher concentrations caused different effects on root growth. Zinc citrate and molybdenum nanoparticle solution at a concentration of 60 ppm adversely affect the weight of the root, leaves and root length. Pre-sowing treatment with zinc citrate is indirectly more effective than zinc oxide nanoparticles against root growth at concentrations of 20 and 40 ppm but inhibits it at 60 ppm. Treatments with zinc oxide nanoparticles are more stable regardless of concentration. Short-term effects on root growth processes indicate the prospects of increasing drought resistance and other mechanisms of physiological adaptation.

Keywords: molybdenum, zinc, raw weight, root length, pre-seed treatment

INTRODUCTION

Chufa (*Cyperus esculentus* L.) is a drought-resistant crop with a high oil content in the tubers (Mokady and Dolev, 1970). High oil content in the reproductive organs can adversely affect the viability of the tubers (Pascual *et al.*, 2000; Makareviciene *et al.*, 2013). Micronutrients in a colloidal and nanoparticle form increase the sowing suitability of seeds and vegetative organs, but their

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effect has been little studied in non-traditional crops (Farooq *et al.*, 2012). Nanoparticles are characterized by high absorption capacity and can be included in physiological cycles in a short time. NPs have a strong influence on the formation of elements of productivity in crops under foliar application (Tului *et al.*, 2021), but their effect in the pre-seeds is manifested much earlier. Seed treatment by solutions of NPs can increase plant resistance to fungi and viral diseases, stimulate the growth of roots and stems (Bohdan *et al.*, 2021).

The most important micronutrients are also heavy metals, and their excess can cause phytotoxicity (Taran *et al.*, 2016). Treatment with different nanoparticle metal solution have a long-term effect of gradual growth of the chlorophyll index, dry matter accumulation and other important parameters of productivity (Son'ko *et al.*, 2013; Islas- Valdez *et al.*, 2020). New materials require the establishment of optimal parameters for their use, as the phytotoxicity of different compounds varies and depends on the species of plants (Singh *et al.*, 2013). Pre-sowing treatment of tubers with nanoparticle solution is poorly understood because each plant species has its own ways of including micronutrients in metabolic cycles.

Purpose of this study is to establish the optimal rates of highly active nanoparticle solutions of ZnO, zinc citrate and Mo NPs. Chufa has a relatively low threshold of phytotoxicity to nanometals, in particular various forms of zinc, because the concentration of 60 ppm already has a negative effect on growth processes compared to control without treatment (Honchar *et al.*, 2021). Molybdenum is an important element for plant growth because it is part of many enzyme systems, including those that counteract oxidative stress (Barker and Pilbeam, 2015). Molybdenum is low-toxic to plants (Marschner, 2012), but according to recent studies, even a concentration of 100 ppm inhibits growth processes in *Oryza sativa* (Sharma *et al.*, 2020) and 250 ppm in *Solanum tuberosum* (Mushinskiy and Aminova, 2019). Morphologically and physiologically, chufa (family *Cyperaceae*) differs from cereals and dicotyledonous plants, so studies of the effect of nanometals on growth processes as a component of productivity are important.

MATERIAL AND METHODS

Experimental conditions

Laboratory tests were performed on tubers of chufa variety Pharaoh. Weight of 1 tuber was 1.14 ± 0.02 g. Tubers of chufa had planted in perlite. Air temperature during germination was $+22$ °C. Chufa plants cultivated on a 12-hour light/12-hour dark cycle in controlled conditions in January of 2021. Preparation of nanoparticle solutions and laboratory research were performed in the laboratory of plant material quality in National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine).

Tubers were treated with solutions of nanoparticles (zinc oxide nanoparticles – ZnO NPs; zinc citrate; molybdenum nanoparticles – Mo NPs) with a concentration of 20, 40 and 60 ppm on the day of planting. Variant without

nanoparticle treatment (only water) was a control. Rate of consumption of the stock solution (500 mg of nanoparticle per litre) to pre-sowing treatment was 0.4 (20 ppm), 0.8 (40 ppm) and 1.2 (60 ppm) litre per tonne of tuber. Working solution to pre-sowing treatment was 10 litre per tonne of tubers.

Sampling

Mass of roots and leaves was determined on the 3rd and 10th day after seedling emergence. Ten plants ($n=10$) from each variant were selected. Root system and leaf surface were weighed separately, the weight of the tuber was not taken into account. Root length was established as maximum length.

Statistical analysis

Suitability of the sample for statistical analysis was evaluated according to the Shapiro-Wilk test. Effect power on 3rd day and 10th day was assessed by two-way ANOVA. Difference between the variants was assessed by Fisher's LSD post-hoc test at 5% probability.

RESULTS AND DISCUSSION

Root and leaf weight at 3rd day after emergence

Seed treatment with a solution of nanoparticles has a positive effect on the growth of the root system at a concentration of 20 ppm, but pre-sowing treatment with zinc citrate was more effective at 3rd day after emergence (Fig. 1). Root weight in the treatment with zinc citrate in concentration 20 and 40 ppm does not differ significantly from each other, but at 60 ppm there is a significant inhibition of root growth (root weight is significantly lower than the control variant without treatments).

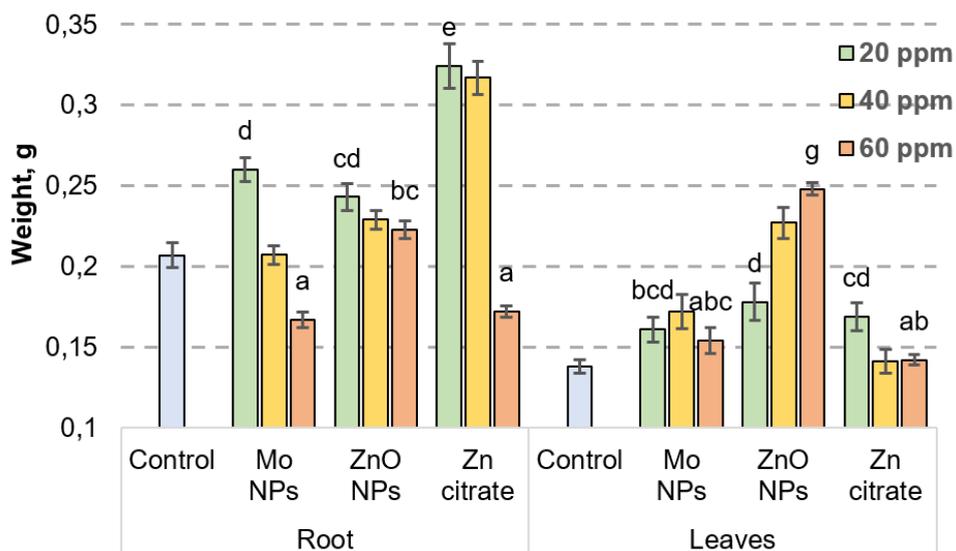


Figure 1. Root and leaf weight (g) depend on concentration of nanoparticle solutions in pre-seed treatment, 3rd day after emergence (Means followed by the same letter do not differ by the Fisher's LSD test at 5% probability)

Treatment with ZnO NPs gives a smaller increase in percentage, but phytotoxicity is not manifested even at concentrations of 60 ppm, however, a significant increase is only at concentrations of 20 and 40 ppm. Mo NPs treatment has a higher contrast, because root weight increases at 20 ppm, but it significantly decreases at 40 ppm and an even smaller root mass is formed by treatment at 60 ppm.

Formation of leaf mass compared with roots had a different response to pre-sowing treatment with nanoparticle solutions on 3rd day after emergence. Leaf weight in treated variants with Mo NPs increased by 11.6–24.6 percent compared with the control one without a significant difference between the different concentrations. Pre-sowing treatment with ZnO NPs significantly increases the leaf weight and with increasing concentration the positive effect increases too. Zinc citrate has a much smaller effect on leaf mass. Significant increase in this indicator was at a concentration of 20 ppm and there was no significant difference compared with control variant at an increase to 40 and 60 ppm at 3rd day after emergence.

Treatment with ZnO NPs has a positive effect at concentrations up to 9 μM (equivalent 0.7 ppm), while simple zinc oxide is toxic to dicotyledonous plants in same concentration (Singh *et al.*, 2013). Vigor and germination stay high even at a concentration of 80 ppm ZnO NPs in monocotyledons (Esper Neto *et al.*, 2020). Phytotoxicity of zinc oxide nanoparticles in chufa may be at a much higher concentration than the studied ones.

Root and leaf weight at 10th day after germination

Root weight has a more stable response to the concentration of the nanoparticle solutions at the 10th day after emergence (fig. 2).

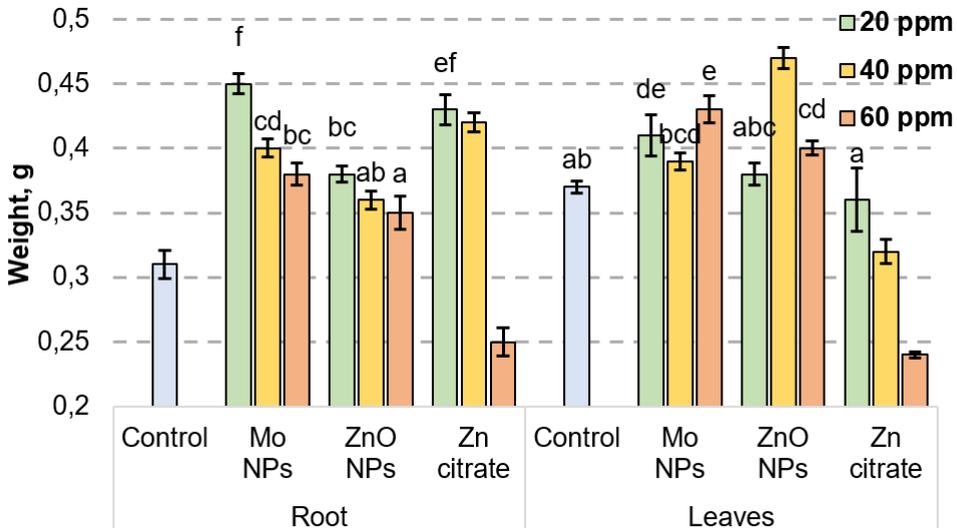


Figure 2. Root and leaf weight (g) depend on concentration of nanoparticles solution in pre-seed treatment, 10th day after germination (Means followed by the same letter do not differ by the Fisher's LSD test at 5% probability)

The most effective treatment is Mo NPs solution at a concentration of 20 ppm. Root weight is 0.45 g in this variant, while it is 0.31 g in control variant. Root weight was 0.40 g at a concentration of 40 ppm, and it decreased insignificantly with increasing concentration to 60 ppm. Long-term effect of treatment with ZnO nanoparticles can be positive, but has species and varietal specificity (Batsmanova *et al.*, 2020). Root mass significantly exceeded the control variant under treatment with ZnO NPs. It increases in concentration from 20 to 40 ppm and root mass was significantly reduced at 60 ppm. Phytotoxicity of zinc citrate on the growth of the root system remains for a long period of time. Reason for the phytotoxicity of zinc citrate may be its greater digestibility (Montanha *et al.*, 2020).

Leaf growth after germination is significantly slowed down, the manifestation of phytotoxicity is more stable. Leaf weight in treated variants with Mo NPs and ZnO NPs does not fluctuate significantly in percentage, then a significant inhibition of leaf growth occurs in treated variants with zinc citrate with increasing concentrations to 40 and 60 ppm.

Plant weight

Plant weight on the 3rd day after emergence is a marker of efficiency the utilization of tuber spare substances (fig. 3). Treatment of tubers with ZnO NPs significantly increases the plant weight with increasing concentration from 20 to 40 ppm, but the difference between 20 and 60 ppm is insignificant. At the same time, pre-sowing treatment with zinc citrate with increasing concentration forms less plant weight and significant inhibit the growth processes at 60 ppm. Decrease in plant weight is gradual with increasing concentration of Mo NPs and growth processes are inhibited more at 60 ppm (same to zinc citrate, 60 ppm).

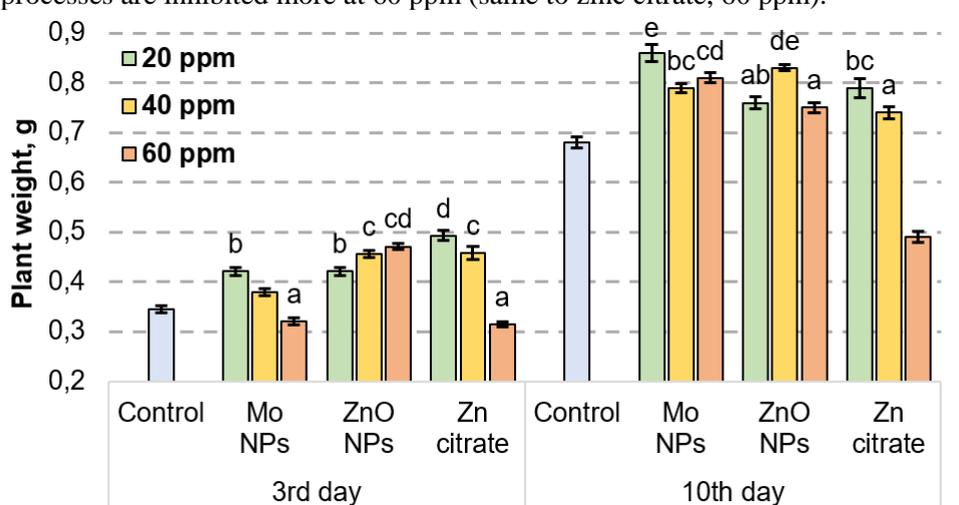


Figure 3. Plant weight (g) depends on concentration of nanoparticles solution in pre-seed treatment, 3rd and 10th day after germination (Means followed by the same letter do not differ by the Fisher`s LSD test at 5% probability)

Plant weight in almost all nanoparticle solutions and their concentration effectively affect the plant weight, compared with the variant without treatment at 10th day after emergence. Only the variant of zinc citrate at a concentration of 60 ppm had a negative effect on plant weight at this period. Elevated concentrations of zinc citrate in the plant can provoke long-term phytotoxicity (Flis *et al.*, 2016).

Root length

Root length depended on the type of solution and its concentration (table 1). There was no significant difference in molybdenum NPs treatments between concentrations of 20 and 40 ppm on 3rd and 10th day after emergence, but there was growth inhibition at a concentration of 60 ppm (root length did not differ from the control variant). Pre-sowing treatment with Mo NPs can increase the length of roots in many plants, but certain species are more sensitive (Chhipa, 2017).

Treatment with ZnO NPs significantly increased root length, and there was no significant difference in different concentrations at 3rd day after emergence. Root growth slowed and variant with treatment at concentration of 60 ppm had shorter roots than 20 and 40 ppm ones at 10th day after emergence.

Treatment of chufa tuber with zinc citrate at a concentration of 60 ppm significantly inhibited root growth, which was manifested in a shorter root length (10.7 cm) than without treatments at 3rd day after emergence. Root length was less at a higher concentration, but at the maximum concentration still exceeded the control variant at 10th day after emergence. This indicates that phytotoxicity has begun to decrease.

Table 1. Root length depends on concentration of nanoparticle solutions in pre-seed treatment.

Solution	Concentration, ppm	3 rd day	10 th day
Control (water)	–	13.3 ^{ab}	13.6 ^a
Mo NPs	20	15.0 ^{cd}	18.6 ^{cd}
	40	14.5 ^{bc}	19.3 ^d
	60	12.2 ^a	13.8 ^{ab}
ZnO NPs	20	16.0 ^{cde}	20.1 ^{de}
	40	16.1 ^{de}	19.6 ^d
	60	15.5 ^{cd}	17.2 ^c
Zn citrate	20	17.3 ^e	21.6 ^e
	40	16.3 ^{de}	18.6 ^{cd}
	60	10.7	15.4 ^b

Means in column followed by the same letter do not differ by the Fisher's LSD test at 5% probability.

Root length in treated variants with ZnO NPs in chufa began to decrease at a concentration of 60 ppm, while the concentration of 80 ppm had a positive effect in corn (Esper Neto *et al.*, 2020).

Correlation between root length root, leaf and plant weight

Concentration of the studied solution had a negative correlation with root weight on the 3rd and 10th day after emergence ($p < 0.01$), but this effect was much weaker and significant only at the level of $p < 0.05$ in treated variants with ZnO NPs (table 2).

Table 2. Linear correlation coefficients

Solution	Root weight	Leaf weight	Plant weight	Root length
	3 rd day after emergence			
Mo colloidal	-0.89**	-0.10 ^{ns}	-0.87**	-0.65**
Zn colloidal	-0.38*	0.72**	0.67**	-0.14 ^{ns}
Zn citrate	-0.81**	-0.45*	-0.88**	-0.78**
10 th day after emergence				
Mo colloidal	-0.76**	0.21 ^{ns}	-0.42*	-0.67**
Zn colloidal	-0.41*	0.18 ^{ns}	-0.09 ^{ns}	-0.53**
Zn citrate	-0.83**	-0.72**	-0.89**	-0.78**

* $p < 0.05$; ** $p < 0.01$; ^{ns} – no significant.

Relationship between concentration of nanoparticle and leaf weight varied depend on type of solution and observation time. The increase in concentration had a positive correlation with leaf weight on 3rd day, but there was no such effect on 10th day. This indicates that zinc from ZnO NPs is involved in improving the reutilization of tuber spare substances for leaf formation during germination. The concentration of molybdenum nanoparticles was not significantly related to leaf weight at 3rd and 10th day after emergency, because Mo do not take part in leaf formation processes in early stages, but important during active dry mass assimilation (Rana *et al.*, 2020). Negative correlation between zinc citrate concentration and leaf mass may be occurred by toxicity caused by increased “non-profit” zinc, which is not included in biological cycles (Ullah *et al.*, 2019).

Plant weight had a negative correlation with increasing concentration of zinc citrate and Mo NPs on 3rd and 10th day after emergence. Plant weight increased at a higher concentration in treated variants with ZnO NPs with on 3rd day, but the effect of concentration had no statistically significant effect on 10th day. Short-term positive reactions to ZnO nanoparticle treatment are typical of a wide range of organism (Rajput *et al.*, 2018).

Effect of concentration on the maximum root length was negative in most treatment variants on 3rd and 10th day, exclude ZnO NPs at 3rd day, when this correlation was insignificant.

The positive effect on biometric parameters at different concentrations was caused the ability of zinc nanoparticles to reduce overall phytotoxicity, inducing over expression of antioxidant defense enzymes (Venkatachalam *et al.*, 2017). However, the negative effect of increasing the concentration of zinc may be manifested at lower rates than in other plants (Liu *et al.*, 2016).

Nanoparticle treatment of tubers can affect the architecture of the root system, changing the ratio of roots of different lengths and inducing the growth of many short hairs. Root weight was increased with increasing concentration of ZnO NPs, but its length decreased, which is a consequence of these processes. The obtained results are consistent for other crops with a fibrous root system, as nanoparticles also have a positive effect on the growth of secondary roots (Estrada-Urbina *et al.*, 2018).

CONCLUSIONS

Pre-sowing treatment of tubers with metal nanoparticle solutions has different effects on the growth of roots and seedlings. Molybdenum nanoparticle solution is characterized by negative effects on root and seedling growth at a concentration of 40 ppm on the 3rd day after emergence, but short-term phytotoxicity can be overcome, and the treatment efficiency is better than in the control variant on the 10th day after emergence. Negative effect of Mo NPs at concentration of 60 ppm on the growth of the root system requires further study. ZnO NPs in a concentration from 20 to 60 ppm has a positive effect on the weight of the plant, root and stem, root length, but the most effective for a combination of different indicators is the treatment at a concentration of 40 ppm. Pre-sowing treatment with zinc citrate has more phytotoxic effect on leaf growth than other NPs solutions. Negative effect is observed at a concentration of 60 ppm on the 3rd day after emergence, and slightly weakens on the 10th day under zinc citrate treatment. Pre-sowing treatment with ZnO NPs is highly effective at a concentration of 20–60 ppm, while zinc citrate has a stable effect only at 20 and 40 ppm.

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