THE COMPARISON OF EFFECT OF ZINC SULPHATE AND ZINC OXIDE NANOPARTICLES ON PLANTS

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Abstract: Zinc oxide nanoparticles are one of the most versatile materials, due to their diverse properties, functionalities, and applications. Their potential in agriculture is also not negligible. The zinc in form of nanoparticles has better ability to penetrate into the plant roots. This makes this form of zinc more available for plants. However, there is still lack of information about their toxicity. In this work we focused on the evaluation and comparison of the effect of common zinc source ($ZnSO_4.7H_2O$), and zinc in form of nanoparticles (nanoZnO) in *Helianthus annuus* L. Our pilot results show that that nanoZnO have significantly negative impact on the growth parameters of the sunflower. Growth retardation increased with increasing applied concentration of excess zinc. Our results also indicated that as opposed to control, zinc ions with a prolonged experimental time significantly inhibited protein production in plants in the roots, stems, and in the leaves.

Key Words: zinc nanoparticles, phytotoxicity, *Helianthus annuus*, plant nutrition, stress biomarkers

INTRODUCTION

Nanotechnology is one of the revolutionary technology of this century. It deals with nanoparticles that are atomic or molecular aggregates characterized by size less than 100 nm. These are modified forms of basic elements derived by altering their atomic and molecular properties of elements (Wang 2004). This technology has a wide range of uses, as optics, electronics, and biomedical and material sciences. Among other applications, nanotechnology has a great potential to modify conventional agricultural practises. Nanoparticles could be used to minimize losses of nutrients, reduce the applied amounts of plant protection products and increase yields through optimized nutrient management (Das et al. 2015, Rizwan et al. 2017). Full understanding of the interaction mechanism between nanoparticles/nanomaterials and biological systems, however, is still out of sight. In this respect, significant progress has been made in research regarding the use nanotechnologies in medicine; however, the study of the nanoparticles and plant interaction that could find use in the future agriculture is only in its beginnings.

Zinc is an essential mineral element for plants. Zinc deficiency in plants, common in alkaline soils, results in growth arrest and sterility, but on the other hand, zinc can also become toxic at elevated concentrations. Zinc sulfate is ordinarily used as a main component of common zinc fertilizers, but there is growing interest in the use of zinc oxide nanoparticles (ZnO NPs) in agriculture. The normal ZnO and its nanoparticles are commonly added to plastic, glass, ceramics, cement, and rubber materials, as well as pigments, paints, food supplements, batteries and non-flammable materials. The reason for this is their wide range of suitable properties, which is also linked with the easy availability and low price of the chemical. These properties include relatively high electrical and thermal conductivity, stability in high temperatures, ability to absorb UV radiation and, with a neutral pH, mild antimicrobial effects (Moezzi et al. 2012).

On the one hand ZnO NPs have potential to boost yield and growth of crops (Prasad et al. 2012, A El-Kereti et al. 2013), but on the other hand, toxicological effects of these NPs should be also taken into consideration. In this case, one of the most important toxicity factors is concentration of ZnO NPs treatment, which should not reach too high levels (García-Gómez et al. 2017, Lin and Xing 2007).

In this study, we chose to compare the effect of $ZnSO_4.7H_2O$ and ZnO nanoparticles on sunflower plants and evaluate both positive and negative effects caused by these treatments.

MATERIAL AND METHODS

Plant cultivation and experimental design

We used sunflower (*Helianthus annuus* L.) Kongo hybrid as an experimental plant. The achenes were sterilized 20 minutes in 20% SAVO solution and planted in perlite substrate, then germinated for seven days at 22 °C with photoperiod day/night 16/8 hours. After this period, we transplanted the grown seedling plants into the hydroponic container containing a Richter nutrient solution (Laštůvka and Minář 1967). Extra boric acid solution was added due to the sunflower sensitivity to boron deficiency (Blamey et al. 1979). We also adjusted pH to 5.6 with KOH (1M).

Plants (2 weeks old) were treated for a month with $ZnSO_4.7H_2O$ or ZnO nanoparticles at zinc ions concentration 0, 0.6, and 6 mg/l. The samples (six individual plants) were harvested every week (for 3 weeks totally). First, we evaluated growth parameters (root and shoot length, fresh weight, and dry weight), and secondly, we determined the content of stress biomarkers (described below) spectroscopically. For spectroscopic measurements, plants were powdered in liquid nitrogen and aliquots were taken for analysis. The determination was done for roots, shoots, and leaves separately.

Spectroscopic measurement

Spectrophotometric measurements were carried out using an automated chemical analyser BS-400 (Mindray, Shenzhen, China). Reagents and samples were placed at cooled sample holder (4 °C) and automatically pipetted directly into plastic cuvettes. All incubations proceeded at 37 °C. All measurements were done in triplicate. Methods were calibrated using these standard compounds (from Sigma Aldrich, St. Luis, MO, USA): Bovine Serum Albumin for Bradford reaction, acetylcysteine for Ellman reaction, L-ascorbic acid for DPPH test, and gallic acid for phenolic compounds.

Determination of antioxidant activity by the DPPH test

This test is based on the ability of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical to react with hydrogen donors. A solution of radical is decolourized after reduction with an antioxidant or a radical. A 200 µl volume of DPPH reagent was incubated with 20 µl of sample and absorbance was measured after 15 minutes at wavelength 510 nm.

Determination of total protein – Bradford reaction

Reagent Coomassie Brilliant blue G-250 (0.01% Coomassie Briliant Blue G-250, 4.7% CH_3CH_2OH, 8.5% H_3PO_4) in volume of 190 µl was pipetted. The sample in volume 10 µl was added. Mixture was incubated for 10 min and absorbance was measured at wavelength 595 nm.

Statistical analysis

Samples were analysed by one-way analysis of variance (ANOVA) with significant differences between means (n=3) ($p<0.05$). The data were analysed by using software STATISTICA version 12.

RESULTS AND DISCUSSION

In our experiment we exposed the 14-day old sunflower plants to excess zinc in form of $ZnSO_4.7H_2O$ and ZnO nanoparticles (nZnO), separately. In both cases, we used these concentrations of excess zinc: 0 (control); 0.6; 6 mg/l. The experimental treatment lasted for 3 weeks. Six plants were evaluated each week for the effect of zinc ions on growth and stress markers. The results are summarized in Table 1. In the case of $ZnSO_4$, it has been observed that plants exhibit mild growth stimulation throughout the experiment compared with the control. However, this increase was 22% higher in the first week in comparison to control, 7% higher in the second week and only 4.4% higher in the third week. However, when we applied the same concentration of ZnO nanoparticles to the plants, we observed the opposite trend. These plants with prolonged experimental period and increasing zinc concentration exhibited increasing growth depression, ranging from 8.2% (after the first week) to 15.6% (after three weeks).

In addition to growth, we were also interested in the influence of applied forms of zinc ions on the growth of biomass. We determined both fresh weight and dry matter of plants. In the case of fresh weight, in the variant when the plants were exposed to zinc ions in the form of $ZnSO_4$, we observed that in the first weeks, compared with the control, the biomass increased significantly by up to 44.7%. With a prolonged experiment time, however, this trend was slowing down, and a moderate growth depression of up to 3.4% was observed in all the $ZnSO_4$ zinc concentrations in the last week. We observed a similar trend in this variant even in the case of dry matter, except that no statistically significant increase in dry matter was observed in any of the applied concentrations in the last week (compared to the control).

When ZnO nanoparticles were applied to the plants, we observed a decrease in biomass by 21.5% in the first week, and we determined similar values in the second week. However, in the last week of the experiment, the decrease in biomass was again reduced by 21.2% compared to the control. In the case of dry matter, we found that the plants exposed to 0.6 mg/l at all times showed a slight decrease in the dry matter content of 6.2% over the whole time, while at the applied concentration of 6 mg/l this decrease was much more pronounced (by 47.9%).

Table 1 Overview of treatments in comparison to control (% difference). The asterisk (*) indicates significant differences ($p < 0.05$) between control samples and individual treatment samples.

Treatment	Week 1	Week 2	Week 3	Growth parameter
$ZnSO_4$ (both concentrations)	22% ± 3.21% *	7% ± 0.89% *	4.4% ± 1.08%*	plant length
nZnO (both concentrations)	-8.2% ± 3.01%	-12.8 % ± 2.65%*	-15.6% ± 2.47%*	
$ZnSO_4$ (both concentrations)	44.7% ± 10.58%*	40.6% ± 2.78%*	37.2% ± 6.91%*	fresh weight
nZnO (both concentrations)	-21.5% ± 4.78%*	-21.2% ± 3.55%*	-19.8% ± 6.73%*	
$ZnSO_4$ (both concentrations)	40.2% ± 6.79%*	36.7% ± 4.34%*	no data	dry matter
nZnO (0.6 mg/l)		-6.2% ± 3.28%		
nZnO (6 mg/l)		-47.9% ± 5.84%*		

From these basic data, we can state that zinc nanoparticles have significantly negative impact on the growth parameters of the sunflower. Growth retardation increased with increasing applied concentration of excess zinc.

In addition to the growth parameters, we have also explored the effects of nanoparticles on stress markers in our experiments. In particular, we were interested in the effect on protein content and antioxidant activity.

Our results show, that as opposed to control, zinc ions with a prolonged experimental time significantly inhibited protein production in plants in the roots, stems, and in the leaves. In the case of antioxidant activity, it was found that defensive mechanisms of plants were triggered in first week.

Plants treated with excess zinc exhibited higher antioxidant activity in comparison to control plants. Surprisingly, their antioxidant activity decreased in the second week. In third week, it regained the level comparable to the activity level in the first week.

These results (in general) slightly differ from majority of other studies targeting different plant species. One example is study about comparison of effect of $ZnSO_4$ and ZnO nanoparticles on tomato plants (Singh et al. 2016). Concentration of treatment was similar to our experiment. Nanoparticles had (in this case) positive effect on the observed plant parameters (seedlings vitality, germination, protein content).

The reason for this reverse effect may not be only the different plant species, but also the youth of plants. The ZnO nanoparticles in low concentration have positive effect (in general) on germination and growth of young seedlings. As example can be used study done on these plant species: *Vigna radiate* and *Cicer arietinum* (Mahajan et al. 2011). Authors applied ZnO nanoparticles in different concentrations. Lowest concentration (1 mg/l) promoted the germination and vitality of *Cicer arietinum*. *Vigna radiate* plants had the best vitality at 10 and 20 mg/l.

The studies concerning impact of ZnO nanoparticles on plant in the long term experiments are still sparse. However, in these rare studies a toxicity trend emerges. As example can be used greenhouse experiment made on tomato plants and bean plants (García-Gómez et al. 2017). Authors observed plants for total 90 days and compared effect of ZnO nanoparticles and $ZnSO_4$ in various concentrations. Both treatments caused similar toxicity associated with reduction of chlorophyll content and increase of antioxidant activity. These results show similarity to results of our experiment. The ZnO nanoparticles seem to have harmful effects on plants in prolonged period of time.

CONCLUSION

Zinc belongs to the micronutrients and conventional fertilizers (not only) of zinc are faced with the problem of poor bioavailability, due to the fixation of this element to insoluble compounds in the soil. Improving the knowledge about individual forms of zinc and their up-take and assimilation within higher plants may be the first step towards a wider involvement of zinc nanoparticles into agriculture in the field of plant nutrition (nanofertilizers) and protection (nanopesticides). We have touched the issue by our pilot experiments in which we compared the difference of effect on the plants between zinc in form of nanoparticles and zinc in form of simple inorganic salts.

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