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Effects of Zinc Oxide Nanoparticles on Carrot Roots and Arbuscular Mycorrhizae

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Introduction

Increased use of engineered nanoparticles (NPs) is likely to result in their continued release into the environment. Some nanoparticles including zinc oxide nanoparticles (ZnO-NPs) can be toxic to eukaryotic cells, but presently little is known about ZnO-NPs effects on plants and organisms symbiotically associated with them (Navarro et al. 2008). The most ancient and widespread symbiosis between plants and other organisms is that with arbuscular mycorrhizal fungi (AMF) (Schüßler et al. 2001). These fungi are obligate biotrophs that obtain organic carbon from the host plant and in return facilitate the uptake of nutrients. Negative effects of NPs on roots or AMF are likely to reduce plant growth, thus decreasing productivity in agricultural and natural ecosystems. In addition, uptake of NPs through roots or the extraradical hyphae of AMF may lead to high accumulation of NPs in mycorrhizal plants. Such accumulation would represent a hazard to other organisms in the food web due to toxic effects of NPs following ingestion. In the present study, we have begun to investigate the effect of ZnO-NPs on *Daucus carota* (carrot) roots and the associated mycorrhizal fungus *Rhizophagus irregularis*.

Materials and Methods

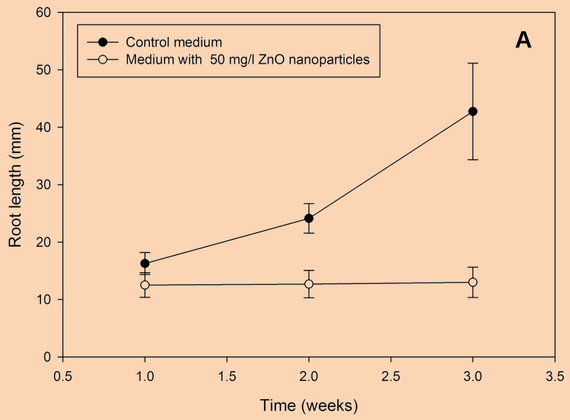
The ZnO-NPs tested had diameters of about 4 nm, which are likely to cross the plant and fungal cell walls. The effect of these NPs on roots and mycorrhizae was investigated in monoxenic cultures of *R. irregularis* associated with Ri T-DNA transformed carrot roots. Effects on carrots were determined by comparing root growth in a solid culture medium containing 0 and 50 µg ml^{-1} ZnO-NPs. The effect of ZnO-NPs on *R. irregularis* was analyzed in a bi-compartmental Petri dish system, where roots and AMF grew in one compartment and just the extraradical hyphae on the other (Fig. 1). Only the hyphal compartment had ZnO-NPs, which were tested at 10 and 50 µg ml^{-1}.

Figure 1. Split Petri dish system used to analyze the effect of ZnO nanoparticles on mycorrhizae.

Root growth was determined by measuring changes in length overtime. Mycorrhizal growth was estimated after dissolving the culture medium followed by collection of hyphae and spores through a 15-micron nylon mesh.

The amount of Zn^{2+} in the growing medium was estimated using the fluorescent dye New port green dye PDX using ZnCl solutions as standards.

Results:



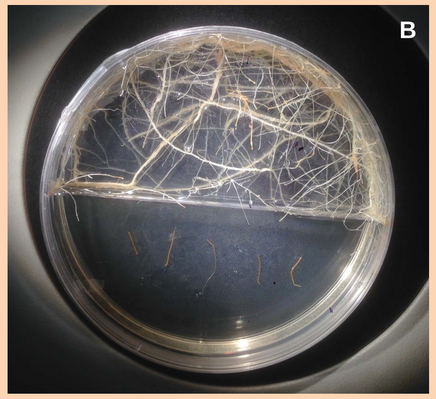
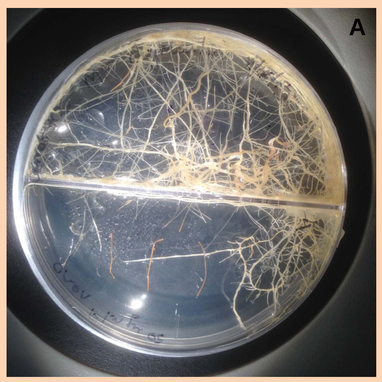


Figure 2. Effect of 50 µg ml^{-1} ZnO nanoparticles on growth of carrot roots in culture. A) Time-course of changes in root length. B) Representative plate showing nanoparticle effects, the upper half has the control medium and the lower half containing the same medium with nanoparticles. Each symbol represents the mean (± SD) of 5 plates.

The roots placed in the side containing ZnO-NPs did not grow and appeared dead (Figure 2). However, after several weeks some roots from the control side crossed over to the nanoparticle side and were able to grow (Figure 3 A) . We hypothesized that this was due to a loss of nanoparticle toxicity. To test this, the roots on the nanoparticle side were removed and new root tips of about 15 mm in length were placed on the nanoparticle side. Some of these tips were from roots that had crossed over, while other were from roots that had not been in contact with nanoparticles. The growth of the root tips was negligible suggesting that the nanoparticles remained toxic (Figure 3B). No clear differences were observed between root tips that had crossed over and those that did not.



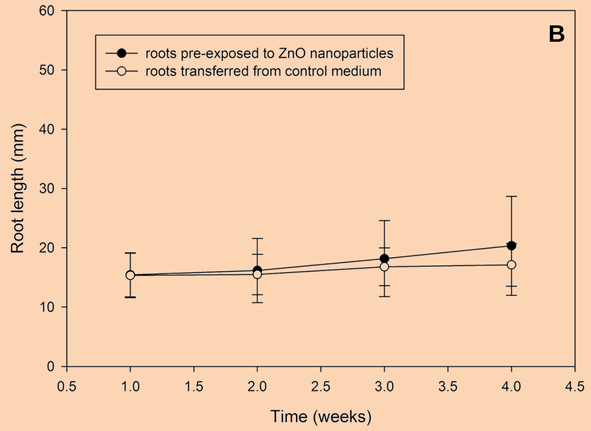
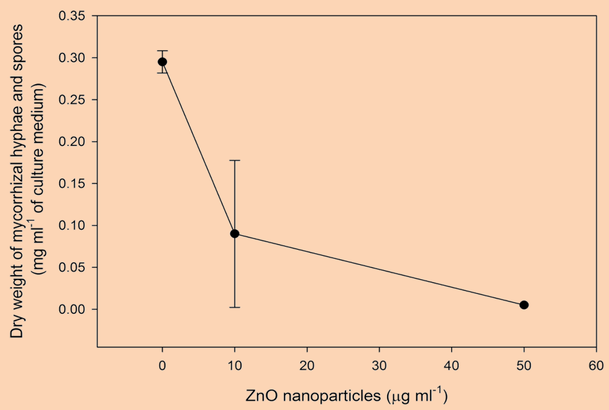


Figure 3. A) Split petri dish showing growth of roots crossing from the control medium. B) Growth of roots tips in old medium with nanoparticles. Each symbol represents the mean (± SD) of 4 plates.

ZnO-NPs also had a negative effect on the mycorrhizal fungus *Rhizophagus irregularis* (Figure 4 and 5). After 3 months in culture, the controls had a fungal biomass that was about 60 times higher than that observed in plates with 50 µg ml^{-1} ZnO-NPs.

Figure 4. Impact of ZnO nanoparticles on the growth of the mycorrhizal fungus *Rhizophagus irregularis.* Each symbol represents the mean (± SD) of three plates.



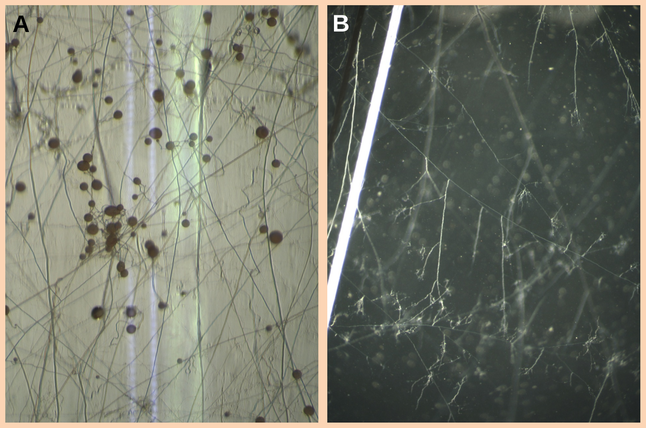


Figure 5. Micrographs illustrating the growth of *R. irregularis* in the control medium (A) and in medium with 10 µg ml^{-1} ZnO nanoaparticles (B).

Toxic effects of ZnO-NPs can be mediated by the release of Zn^{2+} into medium. Analysis of the Zn^{2+} concentration did not reveal significant differences between the control and the medium containing 50 µg ml^{-1} ZnO-NPs (p = 0.12). The concentrations detected were within a range that is not considered toxic to plants or mycorrhizae; such concentrations are commonly present in plant tissue culture media. Furthermore, there was little release of Zn^{2+} from nanoparticles during 5 months of storage in water.

Table 1. Concentration of Zn^{2+} after 5 months resuspension of ZnO-Nps in culture medium or water. Mean (± SE) of 3 plates.

Sample	Zn^{2+} (µM)
Control culture medium	5.28 (± 1.8)
Medium with 50 µg ml^{-1} ZnO-NPs	17.2 (± 5.5)
35 mg ml^{-1} suspension of ZnO-NPs in water	1.7 (± 0.5)

Conclusions:

- ZnO nanoparticles had negative effects on both roots and mycorrhizae.
- The negative effects appear to be attributed to the nanoparticles rather than Zn^{2+} toxicity, because release of Zn^{2+} from the nanoparticles was minimal during the experiment.
- Further work is needed to determine why the nanoparticles were not toxic to roots crossing from the control medium.

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