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Zinc oxide nanoparticles delay soybean development: A standard soil microcosm study

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ABSTRACT

Soybean is an important crop and a source of food for humans and livestock. In this study, for the first time, the long-term effects of zinc oxide (ZnO) nanoparticles on the growth, development, and reproduction of soybean [*Glycine max* (L.) Merrill] were evaluated in a standard soil microcosm study. The soil was treated with 0, 50, or 500 mg/kg (dry weight) of ZnO nanoparticles. The growth and development of soybean plants were tracked during a cultivation period of 8–9 weeks under greenhouse conditions. Soybean development was damaged in both treatment groups, particularly in the group that received 500 mg/kg ZnO nanoparticles. In comparison with the control group, the roots and shoots of soybeans in treatment groups were shorter and had smaller surface area and volume. Furthermore, the plants in the 500 mg/kg treatment group did not form seeds. ZnO nanoparticles negatively affected the developmental stages and reproduction of soybean plants in a soil microcosm.

1. Introduction

Nanotechnology has enabled great advances in electronic, environmental, cosmetic, pharmaceutical, and material applications (Nel et al., 2006; Nowack and Bucheli, 2007). However, its adoption may also cause problems. The expansion of the nanotechnology industry means that nanoparticles can be directly and indirectly released into water and soil ecosystems during production, consumption, and disposal (Nel et al., 2006; Navarro et al., 2008; Lee and An, 2010).

The use of zinc oxide nanoparticles (ZnO NPs) is increasing in personal care products and consumer goods, such as cosmetics, clothing, sunscreens, and bottle coatings (Tsuji et al., 2009). Ecotoxicity studies using ZnO NPs are needed to understand the potential impacts of increasing ZnO levels in water and soil ecosystems. Previous studies examined the effects of ZnO NPs in terrestrial plants such as radishes, rape, ryegrass, lettuce, corn (Lin and Xing, 2007), cucumber (Lin and Xing, 2007; Kim et al., 2011), zucchini (Stampoulis et al., 2009), mouse-ear cress (Lee et al., 2010), mung and gram (Mahajan et al., 2011), garden cress and faba bean (Manzo et al., 2011), garlic (Shaymurat et al., 2012), and onion (Kumari et al., 2011). At this time, only one study has observed biotransformation and genotoxicity of ZnO NPs in soybean (López-Moreno et al., 2010) during hydroponic germination. One study has assessed the bioaccumulation and translocation of ZnO NPs from organic farm soil into leaves and beans during plant growth (Priester et al., 2012).

Soybean is an important crop and is widely cultivated. Exposure of plants to NPs may cause uptake, translocation, bioaccumulation, and biotransformation of NPs in the food chain. The mechanisms of absorption, transportation, and accumulation of NPs in crop plants are not well known, and have been reported only by Priester et al. (2012) and Du et al. (2011). Most studies have only evaluated crop plants to the germination stage, and have not examined the complete developmental cycle (Rico et al., 2011). Few ecosystem-level microcosm studies of NPs have been carried out. Soil microcosm studies of NPs have mostly reported the effects of NPs on microbial communities and plants. Ge et al. (2011) reported that ZnO and TiO_2 NPs negatively affected microbial biomass, diversity, and community composition in unplanted soil microcosms. Kim et al. (2009) reported that ZnO NPs decreased microbial community diversity and the biomass of *Zea mays* in a microcosm. Other studies have evaluated the effects of Ag NPs, SiO_2 NPs (Kumar et al., 2011, 2012), iron oxide magnetic NPs (Fe_3O_4 and γ-Fe_2O_3) (He et al., 2011), and fullerene (C_{60}) (Nyberg et al., 2008) on the microbial community. Shah and Belozerova (2009) reported the effects of Si NPs, Pd NPs, Au NPs, and Cu NPs on the soil microbial community and on lettuce.

López-Moreno et al. (2010) reported that root growth of soybean was decreased at a ZnO NP concentration of 500 mg/L

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in Millipore water. In addition, Lin and Xing (2007) reported that the root lengths for six crops exposed to ZnO NPs at a concentration of 2000 mg/L in suspensions were significantly inhibited.

Aquatic microcosm studies of NPs have evaluated copper oxide (CuO) (Pradhan et al., 2011), Ag (Bradford et al., 2009; Mühling et al., 2009; Pradhan et al., 2011), zero-valent iron (Barnes et al., 2010), and TiO_2 NPs (Battin et al., 2009) in microbial communities. One mesocosm study evaluated the effect of Au NPs in an estuarine food web (Ferry et al., 2009).

To the best of our knowledge, this is the first study to evaluate the long-term effect of ZnO NPs on the growth, development, and reproduction of soybean (*Glycine max* (L.) Merrill) in a standard soil microcosm. We focused on the effects of ZnO NPs on the developmental stages of soybean, and evaluated whole soybean growth, including total length, surface area, average diameter, stem volume, and root volume. In addition, we measured the bioaccumulation of Zn in the roots, stems, and leaves of soybeans.

2. Materials and methods

2.1. NP preparation of soil

ZnO NPs (Sigma-Aldrich, Inc.), with a purity > 97 percent and a particle size < 50 nm, were mixed with the test soil at specified doses. The test soil used was the OECD standard soil (OECD, 1984) and was composed of 69.5 percent sand, 20 percent kaolin, 10 percent peat moss, and 0.5 percent calcium carbonate. Sand and peat moss were sieved through a 2-mm mesh sieve and oven-dried at 105 °C for 24 h (for sand) or air-dried at room temperature for 3 days (for peat moss). Soil was prepared with 0, 50, or 500 mg/kg (dry weight) ZnO NPs from stock soils corresponding to 100 times each test concentration, in five replicates. Stock soils of

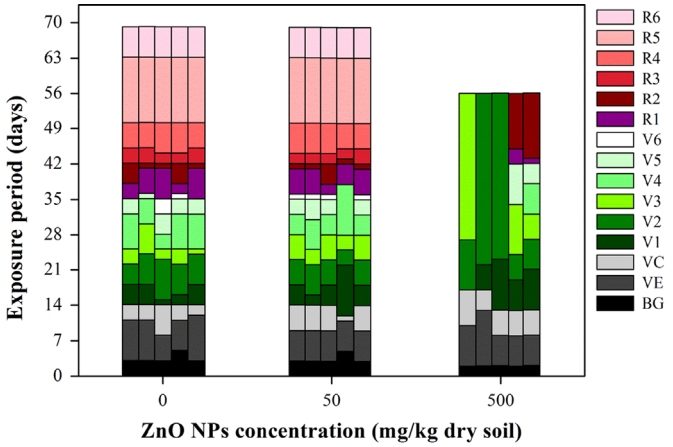

Fig. 2. Vegetative and reproductive developmental stages of soybean for control, low, and high ZnO NP treatments. Five replicates were prepared for each concentration. Vegetative stages: BG=before germination, VE=emergence, VC=cotyledon, V1=first trifoliate, V2=second trifoliate, V3=third trifoliate, V4=fourth trifoliate, V5=fifth trifoliate, and V6=flowering initiation. Reproductive stages: R1=beginning to bloom and first flower, R2=full bloom and flower in top two nodes, R3=emergence of pod and 3/16" pod in top four nodes, R4=full pod and 3/4" pod in top four nodes, R5=1/8" seed in top four nodes, and R6=full-size seed in top four nodes.

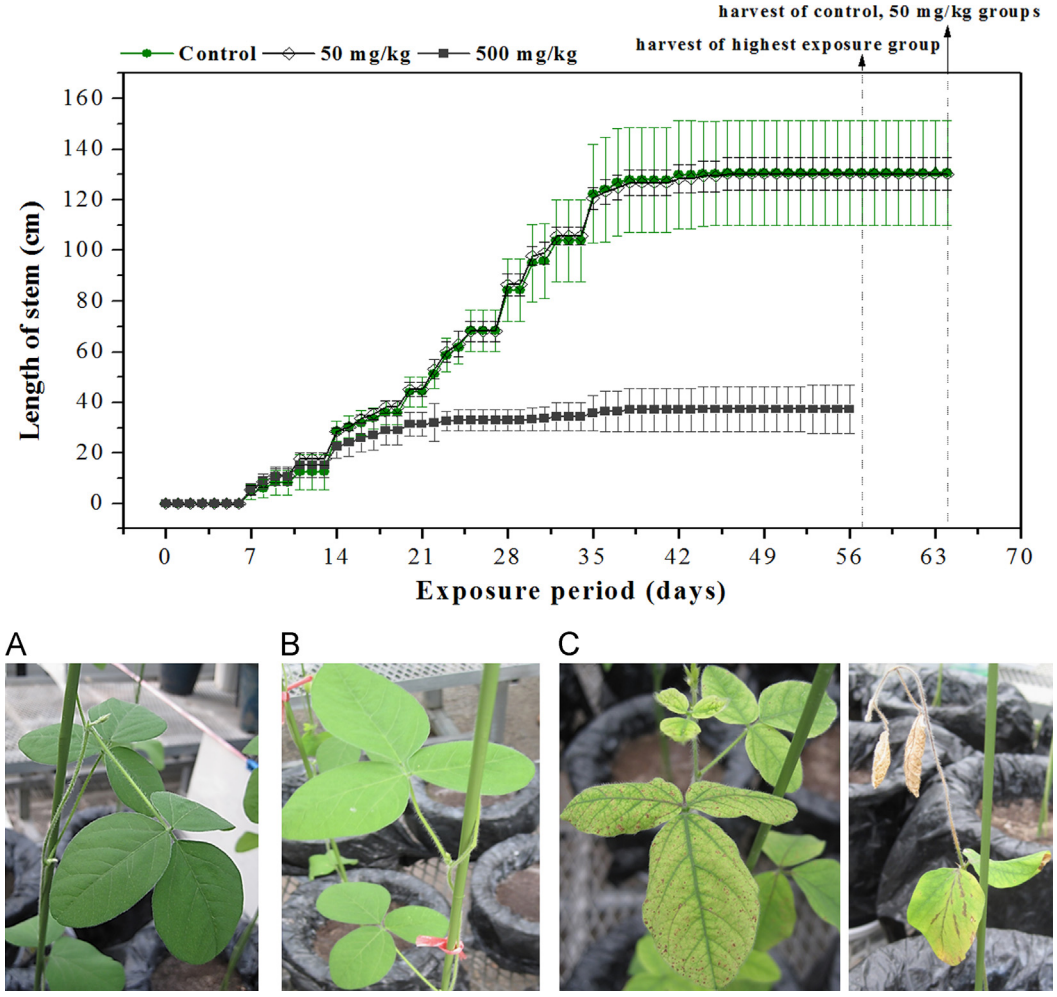

Fig. 1. Soybean stem growth for control (A, closed circles), low (B, diamonds), and high (C, squares) ZnO NP treatments before harvest. Soybeans were harvested on day 57 for the high treatment and day 65 for the control and low treatment groups. Error bars represent the standard deviation of the mean (n=5 plants).