Plant Archives Vol. 18 No. 2, 2018 pp. 1791-1794 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

PHYTO-TOXIC EFFECT OF ZINC OXIDE NANOPARTICLES ON SEED GERMINATION IN TOMATO (*LYCOPERSICON ESCULENTUM* L.)

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Abstract

Zinc plays a vital role for various metabolic pathways in plant systems. Application of Zinc fertilizers can therefore, make a significant contribution towards the goal of higher crop yields. Recently, nanoparticles have received considerable attention due to their increased uptake by plants as they are small in size and have high rate of penetration through plant cell membrane. The present study investigates the effect of Zinc Oxide nanoparticles (ZnO NPs) on tomato crop with a view point of their potential use as future "nano-fertilizers".

Different concentration (0, 250, 500 and 750 mg L^{-1}) of ZnO Nps, were prepared in distilled water and used for the treatment in tomato seeds to study the effect on seed germination and seedling growth. Preliminary studies on seed germination revealed no evidence of toxicity up to 250 mg L^{-1} ZnO NPs. Seed germination increased in lower concentrations, however showed decrease in values at higher concentrations.

Key words : Nanoparticles, germination, tomato, seedling length, root length.

Introduction

Nanotechnology is a fast-developing industry, posing substantial impacts on economy, society and environment. Thus, it generates both positive and negative responses from governments, scientists and social media throughout the world (Bai, 2005; Brumfiel, 2003; Roco, 2005; Service, 2000 & 2003; Yang *et al*., 2006a).

However, nanotechnology has received an increasing interest in consumer products so far. This widespread application has raised concern over the impact of nano-particles (NPs) on the environment and biological activities. NPs are released into the ecosystem inevitably and contaminate aquatic, terrestrial and atmospheric environments (Colvin, 2003). Hence, plants have a critical role in the sustenance of ecosystem and also may experience greater exposure to NPs.

Nanotoxicology, an emerging discipline, has received increasing attraction. Nanotoxicity has been the research focus of many publications, including several reviews (Biswas and Wu, 2005; Colvin, 2003; Dreher, 2004; Nel *et al*., 2006; USEPA, 2005; Wiesner *et al*., 2006). In addition, very few studies have been conducted to assess potential toxicity of nanomaterials to ecological terrestrial test species (plants, wildlife, soil invertebrates, or soil microorganisms). Phytotoxicity in higher plants should be investigated in order to develop a comprehensive toxicity profile for nanoparticles (USEPA, 2005). Limited studies reported both positive and negative effects of nanoparticles on higher plants. It was pointed out that a mixture of nanoscale SiO_2 (nano-SiO_2) and TiO_2 (nano-TiO_2) could increase nitrate reductase in soybean (Glycine max), enhance its abilities of absorbing and utilizing water and fertilizer, stimulate its antioxidant system, and apparently hasten its germination and growth (Lu *et al*., 2002). Nano-TiO_2 was reported to promote photosynthesis and nitrogen metabolism, and then greatly improve growth of spinach at a proper concentration (Yang *et al*., 2006b; Zheng *et al*., 2005). Several studies indicated that the use of nanoparticles can exert a negative effect on plants.

Moreover, different researches advanced our understanding of nanotoxicology for several types of

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nanomaterials. However, nanoparticles can be made from an enormous variety of materials. There are still many unresolved issues and challenges concerning the biological effects of nanoparticles. Therefore, this study aimed to provide new information about phytotoxicology of nanoparticles by investigating the effect of zinc oxide nanoparticles on seed germination and root growth of tomato plant species. We hope that this study will contribute to our understanding on the phytotoxicity level of nanoparticles on tomato crop.

Tomato (*Lycopersicon esculentum* L.) plant belongs to the family Solanaceae. The commonly grown varieties of Tomato are Arka Abha, Arka Saurabh, Pusa Gaurav, Angurlata, Pant Bahar, Ratna and Rupali. Tomato plants require adequate moisture throughout their growth period. Maturity of tomato plants is achieved after 8-12 weeks of planting. The yield of tomato depends on variety and season, which varies from 20 to 24 t/ha. Tomato is one of the most important protective food crops of India. The major tomato producing states are Bihar, Karnataka, Uttar Pradesh, Orissa, Andhra Pradesh, Maharashtra, Madhya Pradesh and West Bengal. Tomato is rich source of vitamins A, C, potassium, minerals and fibers. Tomatoes are used in the preparation of soup, salad, pickles, ketchup, puree, sauces and also consumed as a vegetable in many other ways.

Materials and Methods

Preparation of salt solutions

The ZnO NPs used in this study were purchased from Sigma-Aldrich Company, St. Louis, MO, USA with a purity of 99.5% and particle size of <100 nm. Suspensions of ZnO NPs were prepared with varying concentrations (0, 250, 500 and 750 mg L^{-1}) in distilled water and dispersed by ultrasonic vibration for 30 min to avoid aggregation.

Seed germination assay

The effect of nanoparticles on seed germination was determined. The surface sterilization of tomato seeds was carried out using 0.1% (w/v) mercuric (II) chloride solution for 10 min. One piece of sterile Whatman filter paper (No. 1) was placed into sterile petri dishes (90 mm × 15 mm). There were four treatment combinations used in this study which can be taken as follows: T_0 = Control (untreated), T_1 = Seed treatment with ZnO (0.25g), T_2 = Seed treatment with ZnO (0.5g) and T_3 = Seed treatment with ZnO (0.75g). Seeds were treated in different concentrations of zinc oxide nanoparticle for six hours. After six hours, seeds were dried for some time and placed on moistened blotter paper. Seeds were then transferred onto filter paper, with 20 seeds per dish with a minimum distance of 1 cm between each seed (Yang and Watts, 2005). There were three replicate dishes for each treatment. The dishes were covered and sealed with para-film and placed in an incubator at 25^0C. After incubation of 2 days in the dark under room temperature, seeds were transferred to a 16:8 h photoperiod. Then, the germination was halted, seed germination rate was calculated and seedling root and shoot length was measured.

Results and Discussion

The effect of ZnO nanoparticles up to the concentrations of 750 mg L^{-1} on the seed germination was determined. Significant differences were observed on germination of tomato seeds when exposed with various concentrations of salt *viz*. ZnO NPs (fig. 1). The exposure of ZnO NPs does not contribute any toxicity up to the concentrations of 250 mg L^{-1}. Concentrations higher than 250 mg L^{-1} ZnO NPs showed toxicity, but the results were not as significant to be accounted for. Interestingly, the root and shoot length of the seedlings were higher when exposed to the concentration of 250 mg L^{-1} of nano ZnO. The toxicity of ZnO NPs at higher concentrations (>250 mg L^{-1}) has also been reported previously on various plants (Lin and Zing, 2007).

The application of NPs significantly affected germination components of tomato plant. At 250 mg l^{-1}, the ZnO NPs improved root and shoot lengths and exerted adverse effects at higher applications of concentration (table 1). After 14 days of exposure at 750 mg l^{-1} of ZnO NPs, root and shoot lengths were significantly decreased by approximately 69% and 71%, respectively, as compared to the control case (table 1). However, the magnitude of seed germination responses to ZnO NP exposures differed at varying concentrations and ZnO beyond 250 mgl^{-1} concentration caused more toxic effect on tomato seedlings (table 1). Results pertaining to seed germination and early seedling growth clearly indicate that ZnO NPs at lower concentration promote seed germination and seedling growth, but at higher concentration (beyond 250 mgl^{-1}) reduces the seed germination (%) and seedling growth. Highest concentration (0.75g) of ZnO NPs showed significantly low germination percentages *i.e*. 30% whereas untreated seeds showed 55% seed germination (table 1).

Results indicated that the root growth was more sensitive to the applied exposures than the shoot growth. Since, the uptake of NPs occurs along with the uptake of moisture and nutrients from root parts to the shoots, roots are the first target tissues to confront pollutants and toxic

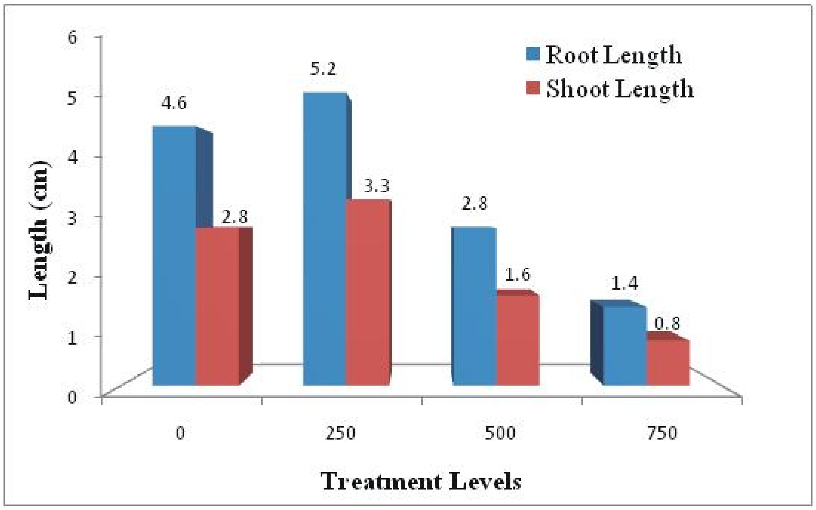

Fig. 1 : Effect of various concentrations of ZnO NPs on germination of tomato seeds.

Table 1 : Effect of ZnO nano-particles on growth factors of 14 days of *Lycopersicon esculentum* L. seedlings grown *in vitro* condition with 0, 250, 500 and 750 mg l^{-1} of each nanoparticles.

Treatments (mgL^{-1})	Germination (%)	Root length (cm)	Shoot length (cm)	Total seedling length (cm)
0 (Control)	55	4.6	2.8	7.4
250	78.3	5.2	3.3	8.5
500	46.7	2.8	1.6	4.4
750	30	1.4	0.8	2.2

effects appear more strongly in roots rather than in shoots. Earlier studies also indicated phytotoxic effect of zinc oxide NPs on radish (*Raphanus sativus*), rape (*B. napus*), ryegrass (*Lolium perenne*), lettuce (*Lactuca sativa*), corn (*Zea mays*), cucumber (*Cucumis sativus*) (Lin and Xing, 2007), soybean (*Glycine max*) (Lopez-Moreno *et al*., 2010) and *Arabidopsis thaliana* (Lee *et al*., 2010), with greater decline in root length.

Seed germination and root elongation is a rapid and widely used acute phytotoxicity test with several advantages: sensitivity, simplicity, low cost and suitability for unstable chemicals or samples (Munzuroglu and Geckil, 2002; Wang *et al*., 2001). Germination is normally known as a physiological process beginning with water imbibition by seeds and culminating in the emergence of the rootlet (Kordan, 1992). However, there are different definitions of seed germination according to its root length: emergence of root, >1 mm or >5 mm (Kordan, 1992; Munzuroglu and Geckil, 2002; Murata *et al*., 2003). Root elongation of sensitive plant species would have a dose-dependent response. Since, roots are the first target tissue to confront with excess concentrations of pollutants, toxic symptoms seem to appear more in roots rather than in shoots (Sresty and Rao, 1999).

However, future studies should be directed to phytotoxicity mechanisms, for example, size distribution of nanoparticles in solution and its effect on phytotoxicity, possible uptake and translocation of nanoparticles by plants and physical and chemical properties of nanoparticles in rhizosphere and on root surfaces.

Acknowledgement

Authors are thankful to the Department of Biological Sciences, SHUATS for providing proper resources to conduct the experimental work.

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