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Effects of Zinc Oxide Nanoparticles on Roots of Rice *Oryza Sativa* L.

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Abstract. The present study is aimed at investigating the effects of zinc oxide nanoparticles (nano-ZnO) on rice (*Oryza sativa* L.) roots. Four parameters are examined in this study: seed germination percentage, root length and number of roots. The results show that there is no reduction in the percent seed germination, however nano-ZnO is observed to have detrimental effects on rice roots at early seedling stage. Nano-ZnO is found to stunt roots length and reduce number of roots. This study shows that direct exposure to specific types of nanoparticles causes significant phytotoxicity, emphasizes the need for ecologically responsible disposal of wastes containing nanoparticles and also highlights the necessity for further study on the impacts of nanoparticles on agricultural and environmental systems.

Key words: zinc oxide, nanoparticles, rice root, toxicity.

1. Introduction

Nanotechnology has become a dynamically developing industry with a multiplication of applications in materials manufacturing, computer chips, medical diagnosis, energy and health care [1]. Products based on nanotechnologies was estimated that there are more than 800 products and expected to raise more in the market within the next few years [2-3]. By 2014, it was estimated that more than 15% of all products on the global market will have some kind of nanotechnology incorporated into their manufacturing process [4].

Zinc oxide (nano-ZnO) is a commonly used metal oxide ENPs. Zinc oxide is used in a range of applications such as sunscreens and other personal care products, electrodes and biosensors [5], photocatalysis and solar cells. Owing to increasing use in consumer products, it is likely that through both deliberate application and accidental release, ENPs will find their way into aquatic, terrestrial and atmospheric environments [6-8]. There is considerable concern about the potentially harmful effects of those ENPs due to their unique properties, they may have significant effects on many organisms [2, 9], especially plants which are essential base component of all ecosystem.

Most of these studies are focused on the potential toxicity of ENPs to plants and both positive and negative or inconsequential effects have been reported [10]. Among the positive effect reports on plants, nano-TiO_2 was observed to promote the growth of Spinach. [11, 12]. Some research found that Carbon nanotubes (CNTs) could enhance root growth of onion (*Allium cepa*) and cucumber (*Cucumis sativa*) [14]. However, majority of the reports available in the literature indicate phytotoxicity of ENPs. Nano-aluminum oxide (Al_2O_3) could inhibit root elongation of corn, cucumber, soybean, cabbage and carrot [14] while nano-ZnO was reported to be one of the most toxic nanoparticles that could terminate root growth of test plants (radish, rape, ryegrass, lettuce, corn and cucumber) [15]. Similar research was undertaken on the toxicology

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of nanoparticles on *Arabidopsis thaliana*, with the results showing that nano-ZnO at 400 mgL^{-1} could inhibit germination [16]. Evidences that ENPs penetrate into plant cell were also reported, with or without showing adverse effects [17-19].

Overall, the current phytotoxicity profile of nanoparticles is highly speculative and preliminary, the effects of their unique characteristics are poorly understood and more studies on toxicity are required especially on commercial food crop.

In the present study, we examined the effects of photocatalyst nanoparticles, nano-ZnO, on one of the most important food plants (Rice, *Oryza sativa* L.). This study provides new information on nanotoxicology, as we examined root development (including number of roots) in addition to the effects on seed germination and root elongation of rice. This approach enhances our understanding of the toxicity of the ENPs on this plant species.

2. Experimental Section

2.1. Engineered nano-particles

Dispersions of nanoparticles used in this study were prepared at the laboratory of the Center of Excellence in Nanotechnology, Asian Institute of Technology in Bangkok, Thailand. Zinc oxide nanoparticles (nano-ZnO) were prepared from commercial ZnO nanopowder (Sigma-Aldrich, USA) by dispersing nanoparticles in Milli-Q water through ultrasonication (300 W, 40 kHz) for 30 minutes. Particle size distribution (Table. 1) of the nanoparticles was determined through measurements carried out on Transmission Electron Microscopy (TEM) (JEOL JEM 2010, Japan, operated at 120 kV) images using Scion Image processing software (Fig. 1).

Table. 1 Particle size distribution of nano-ZnO

Particle size (nm)	Number of particles
< 50	18
51-100	26
101–150	8
15 –200	5
> 200	3

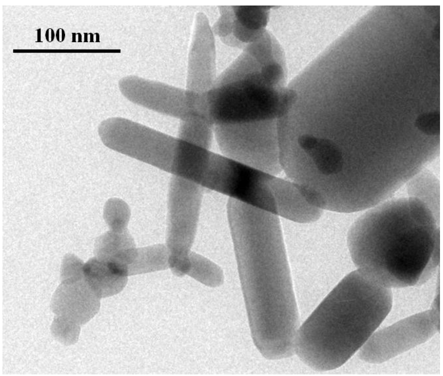

Fig. 1: TEM micrographs of nano-ZnO particles after dispersed in Milli-Q water.

2.2. Seed preparation

Rice is one of the common plant species recommended by the Organization for Economic Co-operation and Development (OECD) for toxicology studies [20] due to its importance as a staple food of a large proportion of the human population.

Prior to their use in the experiments, Rice (*Oryza sativa* cv. *Pathum Thani*) genetic purity and germination rates were established (> 98%); which are an important criterion for good phytotoxicity test and high germination rate [21]. Prior to starting the experiments, rice seeds were stored in dry conditions in the dark to avoid any potential loss of their viability.

2.3. Seed germination and root development

Rice seeds were immersed in a 2.5% sodium hypochlorite solution for 15 min for sterilization and experimental consistency following Lin and Kao [22]. After rinsing three times with Milli-Q water, they were soaked in nano-ZnO suspensions at various concentrations (100, 500 and 1000 mgL^{-1}) and at various soaking periods (24, 48 and 72 h) in an incubator at ambient laboratory conditions (30±1^{o}C, 63% rH) in the dark, Milli-Q water was used in the soaking process for a better control of the media. A piece of filter paper (Whatman No. 42, Maidstone, England) was put into each Petri dish (90 mm × 15 mm), 4 ml of Milli-Q water or nanoparticle suspensions were added, and 20 seeds were then transferred onto each dish. Petri dishes were sealed with parafilm and placed in an incubator. Following 7 days of treatment, seed germination was recorded by counting germinated seeds that had coleoptile longer than 2 mm; and the remainder were considered non-germinated. Additionally, primary root length was measured and the numbers of roots (root length longer than 5 mm) were counted.

2.4. Statistical analysis

Each treatment was conducted with three replicates, and the results are presented as mean±SE (standard error of the mean). Germination percentage, root length and number of roots were analyzed using HOVTEST to evaluate variance homogeneity and normality. In case of non-homogeneity, data were transformed using angular transformation before further statistical analysis [23, 24]. The data was analyzed using the SPSS GLM procedure in SPSS to determine single or interaction effects of factors. Whenever a significant interaction was determined, the level of one factor was compared to each level of the other factor by all pair-wise multiple comparison procedures (Turkey's test), unless mentioned otherwise. All data are presented as mean±SE. A significance level of $\alpha = 0.05$ was used in all analyses.

3. Results and Discussion

3.1. Effect of rice seed germination and root development

Table. 2 Effect of nano-ZnO at different concentrations and soaking times on rice root length.

Day	Average root length (cm)				
	Milli-Q water	10 mg/L nano-ZnO	100 mg/L nano-ZnO	500 mg/L nano-ZnO	1000 mg/L nano-ZnO
1	6.74±0.48 abA	7.89±0.48 aA	5.73±0.36 bA	1.42±0.13 cA	1.19±0.05 cA
2	5.19±0.44 abB	6.09±0.22 aB	4.39±0.34 bB	0.82±0.10 cB	0.41±0.04 cB
3	4.67±0.25 aB	4.36±0.31 abC	3.56±0.19 bB	0.91±0.07 cB	0.61±0.06 cB

*values expressed as mean ± SE followed by the same case small letters with in row and upper case letters within column are not significantly different ($p = 0.05$), Turkey's Test. Data were subjected to square root transformation before the analysis; non transformed data on mean root length are presented in the table

Table. 3 Effect of nano-ZnO at different concentrations on number of roots.

	Milli-Q water	10 mg/L	100 mg/L	500 mg/L	1000 mg/L

		nano-ZnO	nano-ZnO	nano-ZnO	nano-ZnO
Average number of roots	4.04±0.24a	4.19±0.29a	2.74±0.22b	1.48±0.15c	1.15±0.09c

*values expressed as mean ± SE followed by the same case small letters are not significantly different ($p = 0.05$), Turkey's Test.

All treatments led to 100 % germination of seeds showing that nano-ZnO did not adversely affect rice seed germination. The toxicity of nano-ZnO to rice roots is apparent from root length (Table. 2) and number of roots (Table. 3); concentration is greatly involved with the toxicity, and soaking period also affects (df = 8, 134; F = 3.39; $p = 0.002$), higher concentration show reduction effect on root length started from 100 mgL^{-1} and greatly inhibited at concentrations 500 and 1000 mgL^{-1}, with longer soaking time inducing inhibition of root growth. For number of roots, no interaction (day * concentration) was found (df = 8, 134; F = 0.729; $p = 0.67$), but it was affected by nano-ZnO concentration similar to root length (df = 4, 134; F = 46.6; $p = 0.00$) (Figure 4). Effect by soaking time (day) has no significant (df = 2, 134; F = 2.08; $p = 0.129$).

Seed germination is the beginning of a physiological process that needs water imbibitions [25]. However, in this case, rice seed germination occurred normally but the toxic effect is more pronounced in the roots, probably due to the rice seed coat, which can act as a protector for the embryo but cannot totally guard the whole seed. This result related is similar to the report of Yang and Watts [14] who found that alumina nanoparticles (nano-Al_2O_3) at 2000 mgL^{-1} could inhibit root elongation of five plant species. However, in our case, nano-ZnO was found to be more toxic than nano-Al_2O_3 when considering on concentration.

This evidence supporting that some engineered nanoparticles could exert physical or chemical toxicity on plant depending on their chemical composition, size, surface energy and importantly is the species of plant which resulting in different ways. Therefore, the challenge for further studies is the uptake kinetics and interaction mechanisms within cells, also the maximum amenable amount of these nanoparticles which plants can take without showing any signs of stress.

4. Conclusions

From the results of this study ZnO nanoparticles showed toxicity on rice roots indicated by root length and number of roots. A complete study on the toxic effects of these nanoparticles can help significantly in terms of use and safe disposal of ENPs for the reduction of adverse effects in both environmental and agricultural systems.

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