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Toxicity of ZnO and TiO_2 Nanoparticles on Germinating Rice Seed *Oryza sativa* L

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***Abstract*—The present study is aimed at investigating the effects of zinc oxide nanoparticles (nano-ZnO) and titanium dioxide nanoparticles (nano-TiO2) on rice (*Oryza sativa* L.) roots. Three 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 from both nanoparticles, 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. Whereas nano-TiO_2 has no effect on root length. 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.**

***Index Terms*—Zinc oxide, Nanoparticles, Rice root, Toxicity.**

I. 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) and Titanium dioxide (nano-TiO_2) are commonly used metal oxide engineered nanoparticles (ENPs). They are used in a range of applications such as sunscreens and other personal care products, electrodes and biosensors [5], photocatalysis, and solar cells. Both metal oxide nanoparticles, are of great technological importance in the field of heterogeneous catalysis for catalytic support of a wide variety of metals [6] and also find extensive applications in sunscreen industry due to their ultraviolet blocking ability and visible transparency of nanoparticulate form [7].

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 [8]-[10]. There is considerable concern about the potentially harmful effects of those ENPs due to their unique properties, such as high specific surface area, catalytic efficiency, surface energy, abundant reactive sites and strong adsorption, they may have significant effects on many organisms [2], [11], especially plants which are essential base component of all ecosystem. ENPs closely interact with their surrounding environment and plants are essential base component of all ecosystem. As a result, ENPs will inevitably interact with plant and these interactions such as uptake and accumulation in plant biomass will greatly affect their fate and transport in the environment, ENPs could also adhere to plant roots and exert physical or chemical toxicity on plants [12]. Increasing numbers of publications have emerged recently concerning the interactions of ENPs with plant [13], [14].

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 [15]. Among the positive effect reports on plants, nano-TiO_2 was observed to promote the growth of Spinach through an increase in photosynthetic rate and nitrogen metabolism [16], [17]. Carbon nanotubes (CNTs) could enhance root growth of onion (*Allium cepa*) and cucumber (*Cucumis sativa*) and nanotubes sheets were formed by both functionalized single-walled carbon nanotubes (fCNTs) and non-functionalized (CNTs) on root surfaces but none entered into the roots [18]. Although CNTs were found to decrease root growth in tomato plants, a recent work reported that CNTs can penetrate tomato seed coat and dramatically increase seed germination rate and seedling growth [19].

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 [20] whereas 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) [13]. Similar research was undertaken on the toxicology of nano-Al_2O_3, nano-SiO_2, nano-magnetite (Fe_3O_4) and nano-ZnO on *Arabidopsis thaliana*, with the results showing that nano-ZnO at 400 mg/L could inhibit germination so root elongation was not measured [21]. Evidences that ENPs penetrate into plant cell were also reported, with or without showing adverse effects [19], [22], [23].

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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 and nano-TiO_2, on one of the most important food plants (Rice, *Oryza sativa* L.). Nano-TiO_2 and nano-ZnO have widespread usage, as discussed before, in a number of applications, and they are likely to find their way into the agricultural environment. 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. This approach enhances our understanding of the toxicity of the ENPs on this plant species.

II. EXPERIMENTAL SECTION

A. Engineered nano-Particles

Dispersions of two nanoparticles used in this study were prepared at the laboratory of the Center of Excellence in Nanotechnology, Asian Institute of Technology in Bangkok, Thailand. Nano-ZnO was prepared from commercial ZnO nanopowder (Sigma-Aldrich, USA) by dispersing nanoparticles in Milli-Q water through ultrasonication (300 W, 40 kHz) for 30 minutes. Nano-TiO_2 was prepared using the same method. Particle size distribution 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 and Fig. 2).

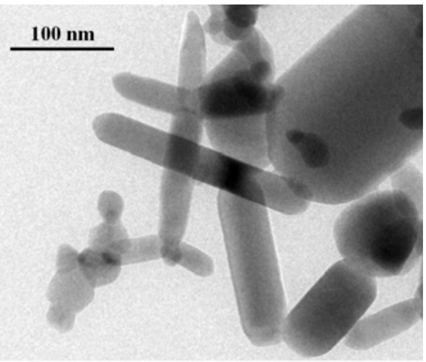

Fig. 1. TEM micrographs of nano-ZnO particles after dispersed in Milli-Q water.

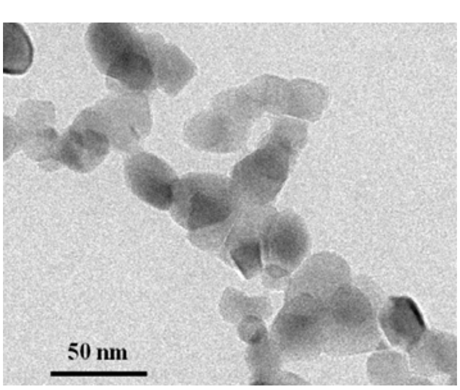

Fig. 2. TEM micrographs of nano-TiO_2 particles after dispersed in Milli-Q water.

B. 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* L.) genetic purity and germination rates were established (> 98%); which are an important criterion for good phytotoxicity test and high germination rate. Prior to starting the experiments, rice seeds were stored in dry conditions in the dark to avoid any potential loss of their viability.

C. 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 [25]. After rinsing three times with Milli-Q water, they were soaked in nano-ZnO suspensions at various concentrations (10, 100, 500, and 1000 mg/L) and at various soaking periods (1, 2, and 3 days (d)) 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 d 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.

For nano-TiO_2 toxicity test, similar process of seed soaking was followed as above except that the seeds were treated by nano-TiO_2 (100, 500, and 1000 mg/L) and for different soaking periods (1, 2 and 3 d). Milli-Q water was again used as control.

D. 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 [26], [27]. 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 (Fisher's LSD), unless mentioned otherwise. All data are presented as mean±SE. A significance level of $\alpha = 0.01$ was used in all analyses.

III. RESULTS AND DISCUSSION

A. Seed Germination and Root Elongation

All treatments led to 100 % germination of seeds, showing that nano-TiO_2 did not adversely affect rice seed germination. No interaction effects (concentration*day) were observed (df = 6, 107; F = 1.72; *p* = 0.13). However, with increasing soaking time (day) there were a slight decrease in root lengths (df = 2, 107; F = 11.5; *p* = 0.00) see Fig. 3.

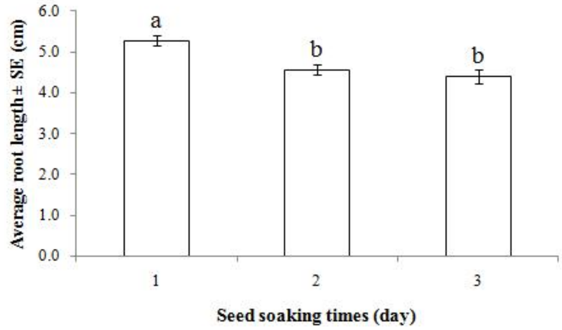

Fig. 3. Average (±SE) rice root length (cm) when rice seeds were treated with various soaking periods (d) using nano-TiO_2. The same case small letters shown on bars are not significantly different.

All treatments led to 100 % germination of seeds the results corroborated by Lin and Xing [13] who reported that nano-ZnO was not affected seed germination of radish, rape, ryegrass, lettuce and cucumber except the corn seed. However, the toxicity of nano-ZnO to rice roots is apparent from root length (Fig. 4).

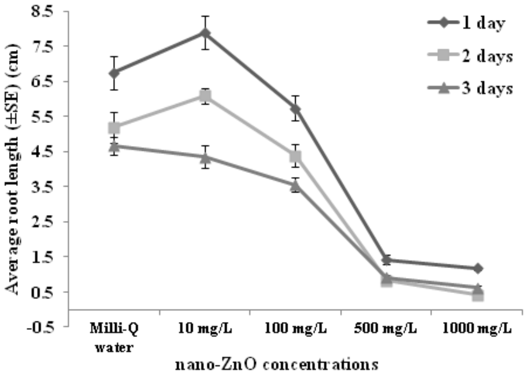

Fig. 4. Average (±SE) rice root length (cm) when rice seeds were treated with various soaking periods (d) and various concentrations using nano-ZnO.

Nano-ZnO 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 mg/L and greatly inhibited at concentrations 500 and 1000 mg/L, with longer soaking time inducing inhibition of root growth.

B. Number of Roots

There was no significant effect on number of roots (df = 6, 107; F = 1.10; *p* = 0.37) from nano-TiO_2 treatments, demonstrating that nano-TiO_2 did not have much effect on rice root development. The result corroborate the earlier reported work by Seeger *et al.* [28] who found no significant differences in growth of willow trees in the range of 1 – 100 mg/L nano-TiO_2. However, number of roots was greatly affected by nano-ZnO concentration (Table I) similar to root length (df = 4, 134; F = 46.6; *p* = 0.00). 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 [29]. 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 [20] who found that alumina nanoparticles (nano-Al_2O_3) at 2000 mg/L 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. 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.

TABLE I: EFFECT OF NANO-ZNO AT DIFFERENT CONCENTRATIONS ON NUMBER OF ROOTS.

	Milli-Q water	**Nano-ZnO**			
		10 mg/L	**100 mg/L**	**500 mg/L**	**1000mg/L**
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* = 0.01), Fisher's LSD.

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REFERENCES

[1] Allianz Group and the OECD (2005) Small sizes that matter: Opportunities and risks of Nanotechnologies, Avialable: http://www.oecd.org/dataoecd/32/1/44108334.pdf, 2005

[2] A. D. Maynard, R. J. Aitken, T. Butz, V. Colvin, K. Donaldson, G. Oberdorster *et al.* Safe handling of nanotechnology. *Nature*. 444, 267–269, 2006.

[3] D. Rejeski and D. Lekas, "Nanotechnology field observations: scouting the new industrial west", *Journal of Cleaner Production*. 16(8-9), 1014-1017, 2008.

[4] N. G. Dawson (2008). Sweating the small stuff: Environmental risk and nanotechnology. *BioScience.* 58(8), 690-690.

[5] S. A. Kumar, S. M. Chen, "Nanostructured zinc oxide particles in chemically modified electrodes for biosensor applications", *Analytical Letters.* 41(2), 141 – 158, 2008.

[6] J. Biener, E. Farfan-Arribas, M. Biener, C. M. Friend, R. J. Madix, "Synthesis of TiO_2 nanoparticles on the Au(111) surface", *The Journal of Chemical Physics.* 123, 094705-094706, 2005.

[7] S. J. Klaine, P. J. J. Alvarez, G. E. Batley, T. F. Fernandes, R. D. Handy, D. Y. Lyon *et al.* "Nanomaterials in the environment: Behavior, fate, bioavailability, and effects", *Environmental Toxicology and Chemistry*. 27, 1825-1851, 2008.

[8] V. L. Colvin, "The potential environmental impact of engineered nanomaterials", *Nat Biotech.* 21(10), 1166-1170, 2003.

[9] B. Nowack, T. D. Bucheli, "Occurrence, behavior and effects of nanoparticles in the environment", *Environmental Pollution.* 150(1), 5-22, 2007.

[10] R. F. Service (2008). Report Faults U.S. Strategy for Nanotoxicology Research. *Science.* 322(5909), 1779.

[11] M. R. Wiesner, G.V. Lowry, P. Alvarez, D. Dionysiou, P. Biswas, "Assessing the risks of manufactured nanomaterials", *Environmental Science & Technology.* 40(14), 4336-4345, 2006.

[12] X. Ma, J. Geiser-Lee, Y. Deng, A. Kolmakov, "Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation", *Science of The Total Environment*, 408, 3053-3061.

[13] D. Lin, B. Xing, "Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth", *Environ Pollut*. 150, 243 – 250, 2007.

[14] S. Lin, J. Reppert, Q. Hu, J. S. Hudson, M. L. Reid, T. A. Ratnikova ***et al.***, "Uptake, Translocation, and Transmission of Carbon Nanomaterials in Rice Plants", *Small*. 5, 1128-1132, 2009.

[15] A. Menard, D. Drobne, A. Jemec, "Ecotoxicity of nanosized TiO_2. Review of in vivo data", *Environmental Pollution*. 159, 677-684.

[16] F. Hong, J. Zhou, C. Liu, F. Yang, C. Wu, L. Zheng ***et al.*** (2005). Effect of nano-TiO_2 on photochemical reaction of chloroplasts of spinach. *Biological Trace Element Research*. 105, 269-279, 2011.

[17] F. Yang, F. Hong, W. You, C. Liu, F. Gao, C. Wu ***et al.***, "Influence of nano-anatase TiO_2 on the nitrogen metabolism of growing spinach", *Biological Trace Element Research.* 110, 179-190, 2006.

[18] J.E. Cañas, M. Long, S. Nations, R. Vadan, L. Dai, M. Luo ***et al.***, "Effects of functionalized and nonfunctionalized single-walled carbon nanotubes on root elongation of select crop species", *Environmental Toxicology and Chemistry*. 27, 1922-1931, 2008.

[19] M. Khodakovskaya, E. Dervishi, M. Mahmood, Y. Xu, Z. Li, F. Watanabe ***et al.***, "Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth", *ACS Nano*. 3, pp: 3221-3227, 2009.

[20] L. Yang, D. J. Watts, "Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles", *Toxicology Letters*. 158, 122-132, 2005.

[21] C.W. Lee, S. Mahendra, K. Zodrow, D. Li, Y.-C. Tsai, J. Braam ***et al.***, "Developmental phytotoxicity of metal oxide nanoparticles to Arabidopsis thaliana", *Environmental Toxicology and Chemistry*. 29, 669-675, 2010.

[22] K. Birbaum, R. Brogioli, M. Schellenberg, E. Martinoia, W.J. Stark, D. Günther ***et al.***, "No evidence for cerium dioxide nanoparticle translocation in maize plants", *Environmental Science & Technology*. 44, 8718-8723, 2010.

[23] Z. Cifuentes, L. Custardoy, J. de la Fuente, C. Marquina, M.R. Ibarra, D. Rubiales ***et al.***, "Absorption and translocation to the aerial part of magnetic carbon-coated nanoparticles through the root of different crop plants", *Journal of Nanobiotechnology*. 8, 26, 2010.

[24] W. Wang, "Literature review on higher plants for toxicity testing", *Water, Air, and Soil Pollution*. 59, 381-400, 1991.

[25] C. C. Lin, C. H. Kao, "Disturbed ammonium assimilation is associated with growth inhibition of roots in rice seedlings caused by NaCl", *Plant Growth Regulation*. 18, 233-238, 1996.

[26] R. G. D. Steel, J. H. Torrie, "Principles and Procedures of Statistics, McGraw Hill Inc", 1980.

[27] K. A. Gomez, A. A. Gomez, "Statistical procedures for agriculture research", John Wiley and Sons, 1984.

[28] E. Seeger, A. Baun, M. Kästner, S. Trapp, "Insignificant acute toxicity of TiO_2 nanoparticles to willow trees", *Journal of Soils and Sediments*. 9, 46-53, 2009.

[29] M. Wierzbicka, J. Obidzinska (1998). The effect of lead on seed imbibition and germination in different plant species. *Plant Science*. 137, 155-171.