International Journal of Agriculture and Food Science Technology.
ISSN 2249-3050, Volume 4, Number 8 (2013), pp. 809-816

http://www.ripublication.com/ ijafst.htm

Impact of SiO_2 and Mo Nano Particles on Seed Germination of Rice (*Oryza Sativa* L.)

Tapan Adhikari, S. Kundu and A. Subba Rao

Environmental Soil Science Division, Indian Institute of Soil Science
Nabibagh, Berasia Road, Bhopal-462038, M.P.

Abstract

In comparison to bulk materials, nano particles may be more toxic and or beneficial and they have the potential ability of passing the cell membrane of plant because of their general size between 1 to 100nm. SiO_2 and Mo nano particles are one of the major and frequently used engineered oxide nano particles. In the present investigation, the potential effects of SiO_2 (10-20 nm) and Mo (<100 nm) nano particles on rice seed germination were studied. We observed good germination of seeds in the presence of nano particles. SiO_2 nano particles had showed no toxic effect on rice growth, whereas root growth and elongation were arrested with Mo nano particles after 50 mg L^{-1}. In many cases root necrosis was occurred. Massive adsorption of Mo nano particles into the root system was responsible for the toxicity, which calls for more research for recommending their safe use as bio-labels in plants. The uptake of both the nano particles was observed with rice seedlings. Application of silica nano particles enhanced the root length, root volume and dry matter weight of shoot and root of rice crop. This study showed that direct exposure to specific types of nano particles caused both positive and negative effects on plant growth.

Keywords: SiO_2, Mo, nano particles, rice root, toxicity.

1. Introduction

Molybdenum is a trace element found in the soil and is required for growth of most biological organisms including plants (Graham and Stangoulis, 2005). Similar to other metals required for plant growth, molybdenum has been utilized by specific plant enzymes as a co-factor that participate in reduction and oxidative reactions in plants (Mendel and Hansch, 2002). In some parts of India, several cases of Mo deficiency have been identified in a variety of crops including maize, Lucerne, fruits, vegetables and other crops. Molybdenum deficient plants exhibit poor growth and low chlorophyll content (Marschner, 1995). Rice plant is a typical silica plant which absorbs greater quantity of silicon other than cereals, comprising as high as 10-20 per cent in stem and leaves and it was observed that Si is necessary for normal growth. However, excessive use of fertilizers, insufficient amounts of water, increasing incidence of pests and microbes, and the depletion of soil silicon have all led to a decline in rice production. It has therefore become important to find ways of enhancing the uptake of available silicon using novel methods. The greatest part of the silicon taken up rice plants and originated from soil and the silica content in rice straw is greatly influenced by the silicon supplying ability of the soil. Furthermore, the silicon was able to decrease transpiration of rice plants (Yoshida et al. 1959), to increase the oxidizing power of rice roots, resulting in the promotion of root activities during rice growth. Studies showed that the effects of nano-particles on plants can be beneficial (seedling growth and development) or non- beneficial (to prevent root growth). The propensity of the NPs to cross barriers and their interaction intracellular structures owing to their small size and high surface reactivity contribute to potential cellular and genetic toxicity by the induction of oxidative stress. Against this backdrop, the present investigation was carried out to investigate the effects of SiO_2 and Mo nano particles on seed germination of rice crop.

2. Materials and Methods

2.1 Test NPs

The SiO_2 and Mo nano particles were purchased from Sigma-Aldrich Company, St. Louis, MO, USA with a purity of 99.5%, particle size of(10-20 nm) and (<100 nm) >50 nm and a surface area of 70± 10 m^2/g and 45 ± 10 m^2/g respectively.

2.2 NP Preparation and copper ion solution

The nano particles were suspended directly in distilled water and dispersed by ultrasonic vibration (100W, 40 kHz) for 30 min. Small magnetic bars were placed in the suspension for stirring before use to avoid aggregation of the particles. Different doses of SiO_2 and Mo nano particles suspensions like 0, 10,20,40,60,80, 100 ppm Si mg/L and 0, 1,5,10,15,20,100,200,400,600 mg/L were prepared for the germination experiment.

2.3 Seeds

Seeds of rice (*Oryza sativa L.*) crop were purchased from National Seed Corporation, India. The average germination rates of both the plants were greater than 90% as shown by a preliminary study. Seeds were kept in a dry place in the dark under room temperature before use.

2.4 Germination

Seeds were immersed in a 5% sodium hypochlorite solution for 10 min to ensure surface sterility, and then they were soaked in distilled water, SiO_2 and Mo nano-article suspension for about 6hrs after being rinsed four times with distilled water. One piece of filter paper was put into each 100 mm X 15mm Petri dish, and 5 mL of a test solution was added. Seeds were transferred onto the filter paper, with 15 seeds per dish and 1cm or larger distance between each seed (Kikui, 2005). Petri dishes were covered and sealed with tape, placed in an incubator. After 5days in the dark under room temperature, more than 80% of the control seeds had germinated and developed root was at least 15 mm long. Then the germination was halted, seed germination rate was calculated and seedling root length was measured.

2.5 Statistical analysis

Each treatment was conducted with three replicates, and the results were presented as mean ± SD (standard deviation).

3. Result and Discussion

3.1. Effect of SiO_2 and Mo nano particles suspension on seed germination

All the treatments led to 95 -100% germination of seeds showing that SiO_2 and Mo nano particles did not adversely affect the seed germination (Table 1 and Table 2). Seed germination is the beginning of a physiological process that needs water imbibitions (Wierzbicka and Obidzinska, 1988). However, in this case, germination of seeds of the crop occurred normally but the toxic effect was more pronounced in the roots, probably due to the seed coat, which can act as protector for the embryo but cannot totally guard the whole seed. This results related to the report of Yang and Watts (2005) who found that alumina nano particles (nano-Al_2O_3) at 200mg L^{-1} could inhibit root elongation of five plant species.

Table 1: Effect of SiO_2 (10-20nm) on Rice seed germination.

Sl. No.	. TREATMENT II. (MG/L)	Germination (%) (out of 20 seeds)	Shoot length (cm)	Root length (cm)	Oven Dry Wt. Of Shoot (g)	Oven Dry Wt. Of Root (g)	Total Si content in shoot sample (mg/kg)	Total Si content in root sample (mg/kg)
1	0	95	71 ± 1.10	94 ± 1.30	0.069 ± 0.012	0.033 ± 0.008	230 ± 1.25	437 ± 1.70

2	10	100	87 ± 1.50	124 ± 1.50	0.076 ± 0.013	0.038 ± 0.010	278 ± 1.30	764 ± 1.80
3	20	100	90 ± 1.70	127 ± 1.55	0.077 ± 0.012	0.042 ± 0.011	444 ± 1.40	847 ± 1.85
4	40	100	96 ± 1.50	141 ± 1.60	0.082 ± 0.015	0.047 ± 0.012	587 ± 1.45	936 ± 1.90
5	60	100	87 ± 1.30	137 ± 1.58	0.075 ± 0.014	0.043 ± 0.011	475 ± 1.31	978 ± 1.90
6	80	100	87 ± 1.25	136 ± 1.55	0.073 ± 0.011	0.041 ± 0.010	470 ± 1.30	1055 ± 1.95
7	100	100	85 ± 1.20	125 ± 1.40	0.072 ± 0.010	0.033 ± 0.10	412 ± 1.20	1087 ± 1.95

Each value is the Mean ± SD of three replicates

Table 2: Effect of Mo (<100nm) on Rice seed germination.

Sl. No.	**I. TREATMENT IV. (MG/L)**	**Germination % (out of 20 seeds)**	**Shoot length (cm.)**	**Root length (cm.)**	**Oven Dry Wt. Of Shoot (g)**	**Oven Dry Wt. Of Root (g)**	**Total Mo content in shoot sample (mg/kg)**	**Total Mo content in root sample (mg/kg)**
1	0	95	112 ± 1.05	147 ± 1.31	0.098 ± 0.002	0.061 ± 0.001	25 ± 0.36	46 ± 0.32
2	1	95	119 ± 1.10	151 ± 1.35	0.102 ± 0.005	0.078 ± 0.002	182 ± 0.54	195 ± 0.65
3	5	94	121 ± 1.15	155 ± 1.38	0.104 ± 0.004	0.082 ± 0.003	236 ± 0.65	315 ± 0.70
4	10	95	118 ± 1.15	148 ± 1.29	0.099 ± 0.003	0.064 ± 0.002	250 ± 0.78	369 ± 0.85
5	15	95	107 ± 1.20	104 ± 1.12	0.090 ± 0.002	0.045 ± 0.001	289 ± 0.88	398 ± 0.95
6	20	97	106 ± 0.95	80.5 ± 1.05	0.084 ± 0.001	0.034 ± 0.001	300 ± 0.94	415 ± 1.10

7	50	94	105 ± 0.90	-	-	-	315 ± 0.98	-
8	100	93	100 ± 0.85	-	-	-	350 ± 1.05	-
9	200	93	88 ± 0.80	-	-	-	389 ± 1.10	-
10	400	90	83 ± 0.78	-	-	-	412 ± 1.15	-
11	600	90	79 ± 0.75	-	-	-	520 ± 1.20	-

(-)Root doesn't grow due to toxic effect of higher dose. Each value is the Mean ± SD of three replicates

3.2 Effect of SiO_2 and Mo nano particles suspension on root and shoot growth

The nano particles can be reported with minimal toxicity on test plants if it has no negative effect on seed germination and root growth at a high concentration according to the USEPA guidelines. Seed germination of *Oryza sativa L.* was not affected by the SiO_2 and Mo nano particles. Phytotoxicity of Mo NP was evident from the experimental results. Mo suspension (<50 mgL^{-1}) inhibited root growth of crops and practically terminated root development. However, the crop germinated with cotyledons sprouting out of seed coat. In this investigation, 1mm was used as the minimum length to be called roots. Seed coat plays a very important role in protecting the embryo from harmful external factors. Seed coats can have selective permeability. Pollutants, though having obviously inhibitory effect on root growth, may not affect germination if they cannot pass through seed coats. This may explain that seed germination in this study was not greatly altered by nano particles. Radicles, after penetrating the seed coats, could contact the nano particles directly. Therefore, 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. Seed germination and root elongation is a rapid and widely used acute phyto-toxicity test with several advantages like sensitivity, simplicity, low cost, and suitability for unstable chemicals or samples (Wang *et al.* 2005). Germination is normally known as physiological process beginning with water imbibitions 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 (Munzuroglu *et al.*, 2002). In the present investigation, seeds showing emergence of radical or cotyledon coming out of the seed coat were recorded as being germinated. Many researchers believe that the observed toxicity of NPs in plants based on plant-NP physical interactions. The presence of NPs on the root surface could alter the surface chemistry of the root such that it affects how the roots interact with their environment. On the basis of several studies, the following are the principal factors that influenced toxicity in plants, concentration of NPs, particle size and specific surface area, physiochemical properties of NPs, plant species, plant age/life cycle stage, growth media, NP stability, and diluting agents. Mo NPs at 50mg /L caused the death of almost all living cells at the root tip of crop. It was observed that with increase in nano-Mo concentration, the root and shoot growth were also increased. However, after certain concentration the growth of root and shoot was found to decline. For rice seedlings, the best growth response for root (5.4 %) and shoot (8.0%) was observed at a concentration of 5 ppm over control. At highest concentration, >50 ppm the retardation in root (100 %) and shoot (100 %) growth of rice seedlings were observed over the control. The reduction in root and shoot growth at higher doses may be attributed to toxic level of nano-particles. This is good evidence for demonstrating that rice seedlings respond to added nano-particles in a limited range, above which toxic levels are reached causing subsequent declines in growth.

3.3 Effect of SiO_2 and Mo nano particles suspension on dry matter yield of crops

A perusal of the data presented in table 1 and 2 depicted that the dry matter yield, estimated with respect to concentration of nano-particles for rice seedlings. Root and shoot biomass production were found to be in accordance with the root and shoot length for corresponding SiO_2 and Mo nano particles treatment. For nano- SiO_2 at 40 ppm treatment, rice seedlings showed 42% increase the root biomass and 19% increase in shoot biomass over control. In case of Mo nano particles at 5 ppm treatment, 34 % increase in root biomass and 6 % increase in shoot biomass was observed. At the highest concentration of Mo nano-particles >50 ppm, 100 % decrease of root biomass for rice seedling was observed over control. The increase in biomass at certain concentration suggests the optimum dose limit for the growth of rice seedlings. However, the decrease in biomass beyond this concentration suggested the toxic effect of Mo nano particles.

4. Conclusion

Overall experimental results depicted that presence of SiO_2 and Mo nano particles affects the growth of rice seedlings at different concentrations. The maximum growth was found at 40 ppm SiO_2 nano particles and 5 ppm Mo nano particles for rice seedlings. Beyond this concentration, the growth was inhibited in case of Mo nano particles. The effective growth at certain optimum concentration and inhibited growth beyond this concentration may be attributed to the accumulation and uptake of Mo nano particles by the roots. It was found that the accumulation and uptake of nano-particles was dependent on the exposure concentrations. In particular, the exposure of plants to nano materials and the impacts of such an exposure on plant systems could open a new direction research on nanotechnology.

5. Acknowledgement

Authors acknowledged Indian Council of Agriculture Research (ICAR) for the financial support under National Agricultural Innovative Project (NAIP) and Indian Institute of Technology Bombay for providing Analytical Characterization Techniques.

References

[1] O. Munzuroglu, H. Geckil, Effects of metals on seed germination, root elongation, and coleoptile and hypocotyl growth in Triticum aestivum and cucumis sativus. Archive Environmental Contamination Toxicology 43 (2002) 203-213.

[2] C.X. Shen, Q. F. Zhang, J. Li, F. C. Bi, N. Yao, Induction of programmed cell death in Arabidopsis and rice by single wall carbon nanotubes. American Journal of Botany 97 (2010) 1-8.

[3] H. A. Kordan, Seed viability and germination: a multi-purpose experimental system. Journal Biology and Education 26 (1992) 247-251.

[4] L. Yang, D.J. Watts, Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. Toxicological Letter 158 (2005) 122-132.

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

[6] S. Kikui, T. Sasaki, M. Maekawa, A. Miyao, H. Hirochika, H. Matsumoto, Y. Yamamoto, Physiological and genetic analyses of aluminum tolerance in rice, focusing on root growth during germination. Journal of Inorganic Biochemistry 99 (2005), 1837-1844.

[7] U.S. Environmental Protection Agency, 2005. Nanotechnology White Paper-External Review Draft.

[8] W. Wang, Literature review on higher plants for toxicity testing. Water, Air, and Soil Pollution 59 (1991) 369-376.

[9] X. D. Wang, C. Sun, S.X. Gao, L.S. Wang, S.K. Han, Validation of germination rate and root elongation as indicator to assess phytotoxicity with *Cucumis sativus*. Chemosphere 44 (2001) 1711-1721.

[10] Yoshida, S., Y. Onish, and K. Kitagishi (1959) Role of silicon in rice nutrition. Soil, Plant and Food **5**: 127-133.