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Toxicity Potential of Different Metal Oxides Nanoparticles on Germination of Maize Plant

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ABSTRACT

Nanotoxicity an emerging concept, is receiving increasing attention with the fast development of nanotechnology. Increasing application of nanotechnology highlights the need to clarify nanotoxicity. Plants need to be included to develop a comprehensive toxicity profile for nanoparticles. Toxic potential of three different metal oxide nanoparticles viz., Zinc oxide (ZnO), Aluminum oxide (Al_2O_3) and Titanium oxide (TiO_2) were investigated using maize as test plant through germination study. A significant toxic effect was observed on growth parameters like germination percentage, vigour index, shoot and root length of maize. Among the tested concentrations of three different metal oxide nanoparticles, the concentration of 2000 mg L^{-1} displayed highest toxic effect followed by 1000 mg L^{-1}. The toxic effect of metal oxide nanoparticles was in the order of ZnO>TiO_2>Al_2O_3.

KEYWORDS : Metal oxides nanoparticles, Toxicity, Maize, Growth parameters

INTRODUCTION

The development of nanotechnology and manufacture of new organic and inorganic nanosized particles or Engineered Nanoparticles (ENPs) may result in the release of substantial amounts of these materials into the environment. However, because of their widespread use in consumer products, it is expected that engineered nanoparticles will find their way into aquatic, terrestrial and atmospheric environment (Navarro *et al.,* 2008). The fate and transport of nanosized materials, once they are released into the environment, has not yet been fully addressed, nor have the impacts of those materials on plant system and soil communities (Dionysiou*,* 2004).

The main characteristic of engineered nanoparticles is their size (<100 nm), which falls in the transitional zone between individual atoms or molecule and the corresponding bulk materials. This can modify the physico-chemical properties of the material as well as create the opportunity for increased uptake and interaction with biological systems. This combination effect can generate adverse biological effects in living cells that would not otherwise be possible with the same material in large form (Nel *et al.,* 2006). Therefore, organisms and especially those that interact strongly with their immediate environments are expected to be affected as a result of their exposure to engineered nanoparticles. However, to date, plants as important ecological receptors, have not received enough toxicity research. At present, there is only limited phytotoxicity studies reported both positive and negative effects of nanoparticles on higher plants. Hence, the present study was carried out to investigate the effect of different metal oxide nanoparticles on growth parameters of maize plant.

MATERIALS AND METHODS

Metal oxide nanoparticles

Three types of metal oxide nanoparticles *viz.,* ZnO, Al_2O_3 and TiO_2 were used in this study. The nanoparticles surface area was determined by the multi-point Brunaur-Emmett-Teller (BET) method.

Nanoparticles suspensions

The nanoparticles suspensions were prepared by following the method of Lin and Xing (2007). The nanoparticles were suspended directly in Millipore water and dispersed by ultrasonic vibration (250W, 20 kHz) for 30 min using the Ultrasonic Processor Type: PR-250. The nanoparticles concentration of 500, 1000 and 2000 mg L^{-1} suspensions were prepared for each metal oxide.

Seed material

Maize seeds variety COH (M_2) was used in this study. The average seed germination percentage was greater than 90 per cent. Seeds were stored in a dry dark place under room temperature before use.

Germination study

The toxicity potential of nanoparticles was evaluated by germination technique. Germination test was carried out in a germination room maintained at a temperature of 25 ± 1.5^{0}C and relative humidity of 95 ± 2 per cent with diffuse light (approximately 10 h) during the day. Initially seeds were immersed in a 10 per cent sodium hypochlorite solution for 10 min to ensure surface sterility, then soaked in Millipore water and different nanoparticles suspensions for about 2 and 12 h after being rinsed three times with Millipore water (Kikui *et al.,* 2005). Seeds were transferred to the germination paper and germination test was carried out through roll towel method. The experiment was conducted in completely randomized design with three replications. The observations were taken at 14 Days After Sowing (DAS).

Treatments detail

T_1 - Control (Millipore water), T_2 - Nano ZnO suspension - 500 mg L^{-1}, T_3 - Nano ZnO suspension - 1000 mg L^{-1}, T_4 - Nano ZnO suspension - 2000 mg L^{-1}, T_5 - Nano Al_2O_3 suspension - 500 mg L^{-1}, T_6 - Nano Al_2O_3 suspension - 1000 mg L^{-1}, T_7 - Nano Al_2O_3 suspension - 2000 mg L^{-1}, T_8 - Nano TiO_2 suspension - 500 mg L^{-1}, T_9 - Nano TiO_2 suspension - 1000 mg L^{-1} and T_{10} - Nano TiO_2 suspension - 2000 mg L^{-1}.

Growth parameters

Seed germination percentage

The counts were taken as per the ISTA rules (1993) and expressed in percentage.

Root length

The normal seedlings were taken at random and the distance between the collar and tip of the primary root were measured and the mean value was recorded and expressed as cm.

Shoot length

The seedlings were again measured for the distance between collar and tip of primary shoot. The mean value of the shoot length was recorded and expressed as cm.

Vigour index (VI)

The VI was calculated for each replication by using the formula suggested by Abdul-Baki and Anderson (1973).

VI = Germination percentage x (root length (cm) + shoot length (cm))

RESULTS AND DISCUSSION

The characteristics of three different metal oxide nanoparticles used in this investigation are given in table 1. The surface area was determined using the multi-point Brunaur-Emmett-Teller (BET) method.

In this investigation, the phytotoxicity exerted by three different metal oxide nanoparticles (ZnO, Al_2O_3 and TiO_2) to address the toxicity effect on maize plant was studied. The toxicity indicators like seed germination percentage, root length, shoot length and vigour index were significantly varied for different metal oxide nanoparticles (Table 2 & Fig.1).

The seed germination percentage of maize ranged from 69.1 to 91.5 per cent in 2 h seed soaking period and in 12 h seed soaking period it varied from 63.1 to 92.0 per cent. Among the treatments, the highest mean seed germination percentage of 91.8 per cent was recorded under control (T_1) followed by T_5 (Nano Al_2O_3 suspension - 500 mg L^{-1}) and T_8 (Nano TiO_2 suspension - 500 mg L^{-1}) with the value of 84.7 and 83.6 per cent, respectively which were not significant vary among themselves. The lowest seed germination percentage (66.1 per cent) was observed in T_4 (Nano ZnO suspension - 2000 mg L^{-1}), which was on par with T_{10} (Nano TiO_2 suspension - 2000 mg L^{-1}) and T_7 (Nano Al_2O_3 suspension - 2000 mg L^{-1}) with the value of 68.9 and 70.4 per cent, respectively. The interaction between the treatments and soaking periods were non significant.

The root length of maize varied from 4.80 to 17.5 cm and 3.70 to 17.8 cm in 2 and 12 h seed soaking period of maize, respectively. Among the treatments, the highest mean root length of 17.7 cm was recorded in T_1 (control), which was significantly different from other treatments, where as the lowest mean root length of 4.30 cm was registered under T_4 (Nano ZnO suspension - 2000 mg L^{-1}). The interaction effect between soaking periods and treatments were non significant.

The shoot length of maize ranged from 4.10 to 15.2 cm in 2 h seed soaking period and in 12 h seed soaking period it varied from 3.70 to 14.8 cm. Among the treatments, T_4 (Nano ZnO suspension - 2000 mg L^{-1}) recorded the lowest mean shoot length followed by the T_{10} (Nano TiO_2 suspension - 2000 mg L^{-1}) with the value of 3.90 cm and 4.80 cm, respectively which were on par with each other. The highest mean shoot length of 15.0 cm was recorded in control (T_1). The interaction effect between the treatments and soaking periods were non significant.

The vigour index of maize varied from 615 to 2992 in 2 h seed soaking period and in 12 h it varied from 467 to 2999. Among the treatments, the highest mean vigour index value of 2996 was observed in control (T_1) and in treatment T_4 (Nano ZnO suspension - 2000 mg L^{-1}) registered the lowest mean vigour index value of 541 and both were significantly different from other treatments. The interaction between seed soaking periods and treatments were found to be non significant.

The significant toxic effect was observed on growth parameters like germination percentage, root length, shoot length and vigour index of maize. The results clearly shown that the concentration of 2000 mg L^{-1} displayed highest toxic effect among the tested concentrations followed by 1000 mg L^{-1} of the all three nanoparticles suspensions. This is in accordance with the finding of Lin and Xing (2007) who have reported that the effect of five different type of nanoparticles (multi-walled) on seed germination and root growth of different plant species and found that the seed germination of ryegrass was affected at the concentration 2000 mg L^{-1} of Zn and ZnO nanoparticles, respectively. The inhibition on root growth varied greatly among nanoparticles and plants. The suspension of 2000 mg L^{-1} of Zn and ZnO nanoparticles practically terminated root elongation of the tested plant species. Similarly, Yang and Watts (2005) studied the phytotoxicity effect of nano-scale alumina (nano-Al_2O_3) powders with or without phenanthrene coating on different plant species (soybean, cabbage, cucumber and carrot). They concluded that the uncoated alumina nanoparticles significantly inhibit the root elongation of all plant species. Lee *et al.* (2010) studied the phytotoxicity of four different metal oxide nanoparticles (Al_2O_3, SiO_2, Fe_3O_4 and ZnO) at the concentrations of 400, 2000 and 4000 mg L^{-1} on *Arabidopsis thaliana*. They concluded that among these nanoparticles, ZnO was most toxic followed by Fe_3O_4 and SiO_2 on seed germination, root and shoot length of *Arabidopsis thaliana*.

In general, more toxicity effect was observed under 12 h rather than 2 h seed soaking period. This suggests that increasing the soaking period of seeds in nanoparticle suspensions leads to affect the growth of maize plant. This might be due to prolonged exposure of seeds to nanoparticles suspension and there may be a chance to more quantity of nanoparticles move inside the seed coat and caused the toxic effect. This is in line with the finding of Lin and Xing (2007), who observed that the growth of radish and rye grass were almost halted by seed soaking followed by incubation in the suspension of Zn and ZnO nanoparticles.

CONCLUSION

The growth of nanotechnology has led to the rapid development of commercial application which involves the use of a great variety of manufactured nanoparticles. The use of nano-sized materials may result in the discharge of these materials into the environment. Compared to other contaminant, nanoparticles size play important role in the behavior, reactivity and toxicity of nanoparticles. A significant toxic effect was observed on growth parameters of maize and toxic effect of metal oxide nanoparticles was in the order of ZnO>TiO_2>Al_2O_3. Overall, the present study demonstrates possible adverse effect of metal oxide nanomaterials on plants, which underscore the need for ecologically responsible for disposal of wastes and sludge containing metal oxides nanoparticles and calls for further research on the potential impacts of nanoparticles on agricultural and environmental systems.

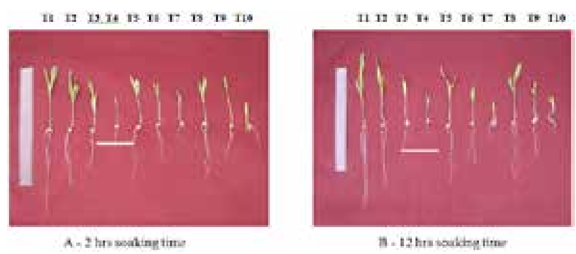

Figure 1. Toxic effect of different metal oxide nanoparticles on maize seedlings

Table 1. Characteristics of different metal oxide nanoparticles

Metal oxide particles	Molecular weight	Particle size	Surface area m^2 g^{-1} (BET)
Zinc oxide (ZnO)	81.39	20 - 85 nm	15 - 25
Aluminum oxide (Al_2O_3)	101.96	30 - 50 nm	> 40
Titanium oxide (TiO_2)	79.87	25 - 70 nm	> 14

Table 2. Toxic effect of different metal oxide nanoparticles on growth parameters of maize plant

Treatments	Growth Parameters											
	Seed germination (percentage)			Root length (cm)			Shoot length (cm)			Vigour Index (VI)		
	P_1	P_2	Mean	P_1	P_2	Mean	P_1	P_2	Mean	P_1	P_2	Mean
T_1	91.5	92.0	91.8	17.5	17.8	17.7	15.2	14.8	15.0	2992	2999	2996
T_2	85.2	79.1	82.2	15.0	13.2	14.1	13.5	11.3	12.4	2428	1938	2183
T_3	81.1	76.5	78.8	11.2	9.60	10.4	9.30	8.10	8.70	1663	1354	1509
T_4	69.1	63.1	66.1	4.80	3.70	4.30	4.10	3.70	3.90	615	467	541
T_5	87.6	81.8	84.7	15.8	14.1	15.0	14.1	13.2	13.7	2619	2233	2426
T_6	81.9	76.2	79.1	12.5	10.4	11.5	9.50	8.60	9.10	1802	1448	1625

T_7	73.2	67.5	70.4	7.60	5.80	6.70	6.50	6.00	6.30	1032	797	915
T_8	86.4	80.7	83.6	15.3	13.5	14.4	13.8	12.4	13.1	2514	2090	2302
T_9	82.6	75.4	79.0	11.8	10.5	11.2	8.80	8.80	8.80	1702	1451	1577
T_{10}	71.8	66.0	68.9	7.80	5.10	6.50	5.10	4.40	4.80	926	627	777
Mean	81.04	75.83	78.44	11.9	10.4	11.2	10.0	9.10	9.60	1829	1540	1685
	SEd CD (0.05)			**SEd CD (0.05)**			**SEd CD (0.05)**			**SEd CD (0.05)**		
P	2.04 4.11			0.31 0.62			0.26 0.54			47.83 96.68		
T	4.55 9.20			0.69 1.39			0.60 1.20			107.0 216.2		
P x T	6.44 NS			0.97 NS			0.84 NS			151.3 NS		

P_1 - 2 h soaking time P_2 - 12 h soaking time

Treatments

T_1 - Control (Millipore water), T_2 - Nano ZnO suspension - 500 mg L^{-1}, T_3 - Nano ZnO suspension - 1000 mg L^{-1}, T_4 - Nano ZnO suspension - 2000 mg L^{-1}, T_5 - Nano Al_2O_3 suspension -

500 mg L^{-1}, T_6 - Nano Al_2O_3 suspension - 1000 mg L^{-1}, T_7 - Nano Al_2O_3 suspension - 2000 mg L^{-1}, T_8 - Nano TiO_2 suspension - 500 mg L^{-1}, T_9 - Nano TiO_2 suspension - 1000 mg L^{-1} T_{10} - Nano TiO_2 suspension - 2000 mg L^{-1}

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