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Phytotoxicity Assessment of nano-ZnO on Groundnut (*Arachis hypogaea*) Seed Germination in MS Medium

(Penilaian Kefitotoksikan nano-ZnO ke atas Percambahan Biji Benih Kacang Tanah (*Arachis hypogaea*) dalam Medium MS)

EHSAN BORZOUYAN DASTJERDI*, ISMAIL BIN SAHID & KHAIRIAH BINTI JUSOH

ABSTRACT

Due to the increasing production and use of nanoparticles in various sectors such as electronic industries and healthcare, concerns about the unknown effects caused by the presence of these materials in the natural environment and agricultural systems were on the rise. Because of the growing trend of ZnO nanoparticles (nZnO) which is one of the most widely used nanoparticles being released into the environment, it has attracted the attention for more studies to be done on the effects of this nanoparticle on organisms. This study was carried out to investigate the phytotoxicity effect of nZnO on groundnut seedlings in Murashige and Skoog (MS) medium. The experimental treatments of this study include eight concentrations of nZnO (10, 30, 50, 100, 200, 400, 1000 & 2000 mg.L^{-1}*) added to MS medium and MS medium without nanoparticles have been used as control treatment. For the first 6 days after sowing, germination percent and germination rate index were calculated by counting the germinated seeds every day. Groundnut seedlings were incubated for 3 weeks in optimum condition and after that, seedling characteristics such as length, wet and dry weight of radicle and plumule were measured. The water content of radicle and plumule were also calculated. The results of this study showed that radicle and plumule length of groundnut seedlings were affected by nZnO exposure, in a way that length of radicles in 50 mg.L*$^{-1}$* nZnO and higher concentrations was significantly lower than that of control treatment and the shortest plumule length was observed in 2000 mg.L*$^{-1}$* nZnO concentration treatment. Both the radicle and plumule wet weight were also decreased as the nanoparticle concentration was increased. However, despite the decreasing in radicle and plumule dry weight with increasing in nZnO concentration, this increase was not significant. However radicle dry weight in 10 mg.L*$^{-1}$* nZnO was significantly higher than nZnO treatments with 200 mg.L*$^{-1}$* concentration and higher concentrations. Moreover, observations of this study did not show any significant difference between the water content of nZnO concentration treatments and control treatment.*

Keywords: Nanoparticle exposure; plumule length; radicle length

ABSTRAK

Peningkatan dalam pengeluaran dan penggunaan zarah nano pada pelbagai sektor seperti industri elektronik dan penjagaan kesihatan telah menyebabkan kebimbangan mengenai kesan yang tidak diketahui oleh kehadiran bahan dalam alam semula jadi dan sistem pertanian semakin meningkat. Peningkatan trend zarah nano ZnO (nZnO) yang paling kerap digunakan telah dibebaskan ke dalam persekitaran dan menarik perhatian supaya lebih banyak kajian dijalankan tentang kesan nanopartikel ini ke atas organisma. Kajian ini telah dijalankan untuk mengkaji kesan kefitotoksikan nZnO pada benih kacang tanah dalam medium Murashige dan Skoog (MS). Rawatan percubaan kajian ini menggunakan lapan kepekatan nZnO (10, 30, 50, 100, 200, 400, 1000 & 2000 mg.L^{-1}*) yang ditambah ke medium MS dan medium MS tanpa nanopartikel sebagai kawalan. Sepanjang 6 hari pertama selepas semaian, peratus percambahan dan indeks kadar percambahan menghitung dengan mengira percambahan biji benih setiap hari. Benih kacang tanah dieram selama 3 minggu dalam keadaan optimum dan selepas itu ciri-ciri anak benih seperti panjang, berat basah dan kering radikel dan plumul telah diukur. Kandungan air radikel dan plumul juga akan dikira. Keputusan kajian ini menunjukkan panjang radikel dan plumul anak benih kacang tanah dipengaruhi oleh pendedahan nZnO dengan panjang radikel 50 mg.L*$^{-1}$* nZnO dan kepekatan yang lebih tinggi adalah jauh lebih rendah berbanding rawatan kawalan dan kepanjangan plumul yang paling pendek diperhatikan pada 2000 mg.L*$^{-1}$* nZnO rawatan kepekatan. Selain itu, berat basah radikel dan plumul juga menurun kerana kepekatan nanopartikel ditingkatkan. Namun begitu, walaupun penurunan berat kering radikel dan plumul dilihat dengan peningkatan pada kepekatan nZnO, peningkatan ini tidak bererti. Oleh itu, berat radikel kering dalam 10 mg.L*$^{-1}$* nZnO adalah jauh lebih tinggi daripada rawatan nZnO dengan kepekatan 200 mg.L*$^{-1}$* dan lebih. Selain itu, pemerhatian daripada kajian ini tidak menunjukkan sebarang perbezaan yang ketara antara kandungan air rawatan kepekatan nZnO dan kawalan rawatan.*

Kata kunci: Panjang plumul; panjang radikel; pendedahan nanopartikel

Introduction

The rapid advances in the cutting edge science of nanotechnology is that nowadays, it has affected all aspects of human life through different productions (Soleimanpour et al. 2011). Nowadays, researchers have been attracted to use of nanotechnology in agricultural crop production activities such as plant biotechnology, plant herbicides and pesticides and also in the techniques of precision agriculture (Nair et al. 2010). One of the important applications of nanotechnology in agriculture is the use of essential elements in nanoparticulate form (nanoparticles) as nanofertilizers that can be more effective than bulk fertilizers in very low used amount (Jha et al. 2011). Also, in some new fertilizers, different plant nutrients have been coated with so many different metal nanoparticles which is an important ingredient of new commercial fertilizers (Deb 2012). This growing trend of using the nanomaterials in agriculture increase the importance of assessment the positive and negative effects of nanomaterials on planets as an important part of agroecosystems (Mousavi & Rezaei 2011).

The impact of nanoparticles on plant varies, depending on the composition, concentration, size and other important physical chemical properties of nanoparticles and plant species. Both enhancive and inhibitive effects of nanoparticles on plant growth have been documented (Ma et al. 2010). The radicle and plumule elongation and biomass tests have been among the simplest short-term methods used in environmental biomonitoring of nanoparticles (Gardea-Torresdey et al. 2014; Ko & Kong 2014). In a study different concentrations of nZnO (10, 100, 500 & 1000 mg.L^{-1}) and nTiO_2 (100, 500 & 1000 mg.L^{-1}) have been tested on seed germination of rice (*Oryza sativa* L.). The results showed that all treatments of nZnO and nTiO_2 were led to 100% seed germination. In this experiment different concentrations of TiO_2 did not have effect on radicle length. Meanwhile, the radicle length of rice seedlings was decreased with the increase of nZnO concentration (Boonyanitipong et al. 2011).

Zheng et al. (2005) reported the promontory effects of nano-TiO_2 on the germination and growth of naturally aged spinach seeds studied by measuring the germination rate, germination percent and vigor indexes of aged spinach seeds. Prasad et al. (2012) studied the effects of different concentrations of nZnO (400, 1000 & 2000 mg.L^{-1}) on groundnut (variety K-134). They reported that treated seeds with 1000 mg.L^{-1} nZnO concentration had recorded the highest germination (100%), seedling vigor and root growth. Nevertheless, seedling vigor index was decreased in 2000 mg.L^{-1} concentration treatment. Lee et al. (2010) assayed the effects of four metal oxide nanoparticles, aluminum oxide (nAl_2O_3), silicon dioxide (nSiO_2), magnetite (nFe_3O_4) and zinc oxide (nZnO) in different concentrations (400, 2000 & 4000 mg.L^{-1}), on seed germination of *Arabidopsis thaliana* (Mouse-ear cress). Observations of this experiment showed that nZnO has been most phytotoxic, followed by nFe_3O_4, nSiO_2 and nAl_2O_3, which has been not toxic. Exposure to 400 mg.L^{-1} nZnO, was prevented 94% of the seeds from germinating and completely had halted root elongation.

In a study, soybean seeds were treated with 0 (control), 500, 1000, 2000 and 4000 mg.L^{-1} nZnO and nCeO_2, respectively. It was reported that all the CeO_2 concentrations significantly increased radicle elongation. Observations also showed that at 4000 mg.L^{-1}, the radicle size was increased by 75%, compared to control. Conversely, an inverse U-shape response was observed in nZnO treated seedlings, with maximum size at 500 mg.L^{-1} (30% over control) and a minimum at 4000 mg.L^{-1} (40% shorter than control) (López-Moreno et al. 2010). In another study, tomato seeds were treated by different concentrations of nanoparticle (nTiO_2 and nAg) and there were no significant differences in germination rates were found among the treatments. nTiO_2 showed no evidence of phytotoxicity in terms of germination and radicle elongation of tomatoes. In contrast, exposure to AgNP was resulted in significantly decreased radicle elongation at every concentration. Feizi et al. (2012) carried out a study for assaying the impact of nano-TiO_2 on wheat seed germination and seedling growth. The experimental treatments were included of five bulk concentrations (1, 2, 10, 100 & 500 ppm), five concentrations of nanosized TiO_2 (1, 2, 10, 100 & 500 ppm) and control (without any TiO_2). The results showed that plumule and seedling lengths at 2 and 10 mg.L^{-1} concentrations of nanosized TiO_2 have been higher than those of the untreated control and bulk TiO_2 at 2 and 10 mg.L^{-1} concentrations. They were also reported that employing nanosized TiO_2 in suitable concentration could promote the seed germination of wheat in comparison to bulk TiO_2 but in high concentrations have had inhibitory or any effect on wheat.

Nano metallic oxides like nZnO are one of the great technological importance in the field of heterogeneous catalysis for catalytic support of a wide variety of metals (Biener et al. 2005). nZnO is being used in products including plastics, ceramics, glass, cement, rubber, lubricants, paints, pigments, foods (source of Zn nutrient), batteries and fire retardants. In addition, nZnO is a common constituent of personal care products including cosmetics and sunscreens due to its excellent UV absorption and reflective properties (Klaine et al. 2008; Ma et al. 2013). To date, few experiments have been conducted to investigate nanoparticles which may have effects on plant growth and development. Due to increasing release of nZnO into the environment and consequently in agroecosystems and also the different effects of nanoparticles on different mediums (Gardea-Torresdey et al. 2014), this study was carried out to investigate the phytotoxicity effect of nZnO on the development of groundnut seedlings in Murashige and Skooge (MS) medium (Murashige & Skoog 1962).

Materials and Methods

In this experiment solid MS medium was used for culturing planting materials. The advantage of using this medium is to avoid aggregation and precipitation (Lee et al. 2010). The solid medium contained MS Basal Medium including vitamins (Duchefa Biochemie, Inc.), 3.2 g gelrit and 30 g sucrose per liter.

Commercial nZnO was used in this study which was purchased from Skyspring Nanomaterials, Inc., with particle size of 10-30 nm (according to company catalogue). Different concentrations of nZnO (0, 10, 30, 50, 100, 200, 400, 1000 & 2000 mg.L^{-1}) have been added into the MS medium. Prior to autoclaving, the mediums containing nanoparticles were shaken for 30 min in an ultrasonic shaker to reduce the nanoparticle aggregation. The pH of the medium has been adjusted between 5.7 and 5.8. Autoclaved media after vigorous shaking were poured into 150 × 25 mm test tubes and immediately were stored in a cool place until culture day. On the day of culture, groundnut seeds (cultivar CG.7, purchased from Mibamansura Trading Sdn. Bhd., stored at 4ºC condition) were surface sterilized by rinsing in 70% ethanol for 1 min followed by treatment with 0.1% (w:v) aqueous mercuric chloride for 10 min. After that, seeds were washed thoroughly four to six times with sterile-distilled water and then were soaked in sterile water for 2 h before culture. In the last stage before cultivation the seed coats were removed using forceps (Sharma & Bhatnagar-Mathur 2006). All the above stages were conducted under laminar air flow hood and in sterile condition.

The cultured seeds were incubated at 27-28ºC for exactly 3 weeks. After three weeks the plantlets (Figure 1) were removed from the test tubes and after washing and removing the agar from the radicles with distilled water, germination characteristics which were included of radicle and plumule length, radicle and plumule wet and dry weight, radicle and plumule moisture were calculated. SPSS (Statistical Package for the Social Sciences) software was used for data analyses and statistical significance of differences between treatments were determined at the 95% confidence level. Germination percent was measured by counting germinated seeds every day during 6 days after sowing and germination rate index was calculated following (1) (Karaguzel et al. 2004).

$$\text{Germination rate index} = \sum(Gt/Tt) \qquad (1)$$

where Gt is the number of germinated seeds on day t; and Tt is the number of days.

Radicle and plumule length were measured using a ruler with an accuracy of 0.1 cm while wet weights were measured using a balance with an accuracy of 0.0001 g. After this, the samples were dried in an oven at 60ºC for two days before dry weight measurement. The following equation was used for measuring radicle and plumule moisture (2).

$$\text{Water content} = \frac{\textit{Wet weight} - \textit{Dry weight}}{\text{Wet weight}} \times 100. \qquad (2)$$

Results and Discussion

The results showed 100% germination of groundnut seeds in all nZnO concentrations after 6 days (Figure 2) and therefore ZnO nanoparticles did not have any effects on groundnut germination. Boonyanitipong et al. (2011) stated that the effects of nZnO on germination of rice seeds were reported 100% of rice seeds germination in all applied concentration of these nanoparticles. Nevertheless, the results have shown significant difference between germination indexes in different nZnO concentration treatments (Figure 3). These results showed that low concentrations of nZnO (10 to 100 mg.L^{-1}) have caused to increase in germination rate index of groundnut seeds, therefore, the highest germination index was observed in 50 mg.L^{-1} nZnO and higher concentrations were caused to reduction of germination rate index (Figure 3) and the lowest germination rate index was observed in 2000 mg.L^{-1} concentration (Figure 3). In experiment of Prasad et al. (2012) seed treatment by nZnO has had an improving

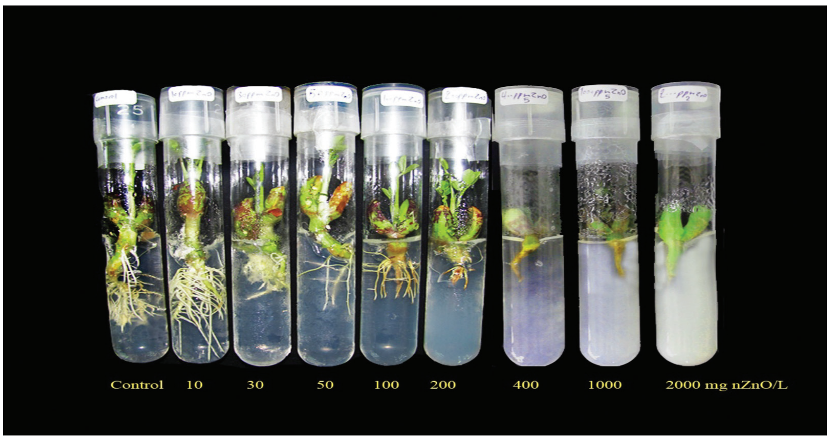

FIGURE 1. Effect of different nZnO treatments on groundnut (*Arachis hypogaea*) seedlings in MS medium after 3 weeks

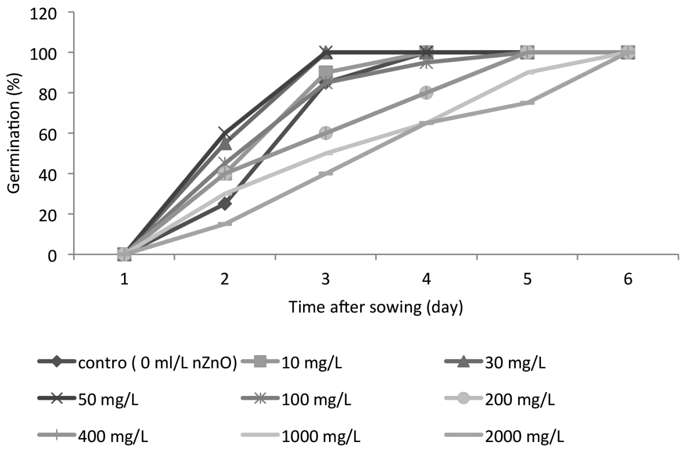

FIGURE 2. Effect of nZnO treatments on germination percentage of groundnut (*Arachis hypogaea*) seeds after sowing in MS medium during 6 days after sowing in MS medium concentrations show the complete germination of seeds in all treatments after 6 days

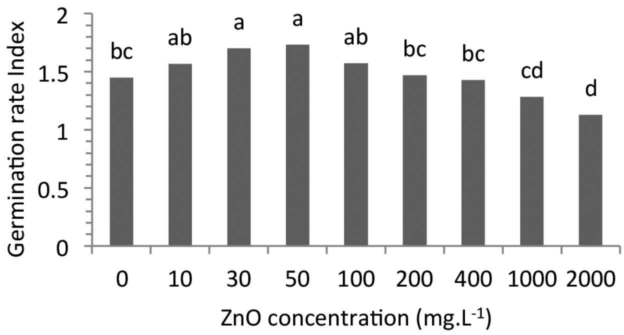

FIGURE 3. Effect of nZnO treatments on germination rate index of groundnut (*Arachis hypogaea*) seeds during 6 days after sowing. Germination rate index was ploted according to mean of radicle and root length in different treatments. Bars having the same letter are not significantly different at the 0.05 level

effect on growth of radicle and plumule and vigor index of groundnut in 1000 mg.L^{-1} but has had inhibitory effect on these characteristics in 2000 mg.L^{-1} concentration. Zheng et al. (2005) reported the improving effect of nano-TiO_2 on germination rate of aged spinach seeds because of enhanced penetrability of seed capsule, facilitating the admission of water and dioxygen into the cells and quenching of free radicals in the germinating seeds.

The results showed that radicle and plumule length of groundnut seedlings were affected by nZnO exposure in MS media (Figure 4), in a way that radicle length in 50 mg.L^{-1} nZnO and higher concentrations have been significantly lower than control treatment (Figure 4). Meanwhile, the radicle length of groundnut seedlings in 50 and 2000 mg.L^{-1} concentrations was decreased by 23.24 and 55.65%, respectively, compared to control treatment. Also, Lee et al. (2010) reported inhibitory effect of nZnO at all concentrations on radicle growth. In another study, suspensions of 2000 mg.L^{-1} nano-Zn or nano-ZnO has terminated root elongation of all the test plant species in their experiments on (*Brassica napus* (rapeseed), *Raphanus sativus* (radish), *Lolium perenne* (ryegrass), *Lactuca sativa* (lettuce), *Zea mays* (corn) and *Cucumis sativus* (cucumber)) (Lin & Xing 2007). However, Prasad et al. (2012) reported that 3 h soaking of groundnut seeds in 1000 mg.L^{-1} concentration of nZnO had promoted seed germination, seedling vigor, root length and shoot length compare to control treatment. But the higher concentration of nZnO had shown inhibitory effects on root length. In a study, foliar spraying of nZnO on mung (*Vigna radiata*) seedlings was contributed to the increase in root elongation (Dhoke et al. 2013).

Plumule length was decreased due to the effect of nZnO exposure in all treatments and this decrease was directly related to the increase of nanoparticle concentration, whereby, the radicle length of seedlings in 200 mg.L^{-1} nZnO treatment have had a significant difference compared to the control treatment while seedlings in 2000 mg.L^{-1} nZnO treatment have had the shortest plumule length. According to these observations, the plumule length of seedlings in 200 and 2000 mg.L^{-1} nZnO treatments were decreased by 61.14 and 93.71%, respectively, compared to the plumule length of control treatment. However, in Prasad et al. (2012) study, plumule length was consonant

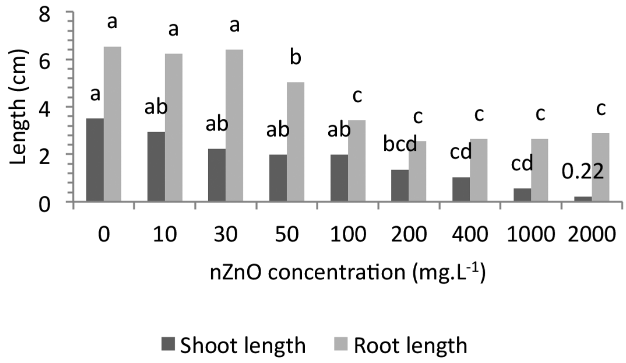

FIGURE 4. Effect of nZnO treatments on radicle and plumule length of groundnut (*Arachis hypogaea*). Radicle and plumule length was ploted according to mean of radicle and root length in different treatments. Bars having the same letter are not significantly different at the 0.05 level

under different nZnO concentrations. In another study, carbon nanotube exposed tomato seedlings have had longer plumules and were more developed (Khodakovskaya et al. 2009). Also, the foliar treatment of aerial parts of mung plants (*Vigna radiata*) by nZnO has caused the increase in the plumule length compared to control treatments (Dhoke et al. 2013).

Both the radicle and plumule wet weight were decreased with the increase in nanoparticle concentration (Figure 5). The observations did not show any significant difference between 10, 30 and 50 mg.L^{-1} nZnO treatments and control treatment in the aspect of wet weight but the radicle wet weight of seedlings under higher concentrations has been significantly less than the control treatment. Meanwhile the wet weight of seedlings under 2000 mg.L^{-1} nZnO treatment has had 67% reduction compared to control treatment. These findings had accordance with report of Prasad et al. (2012) that in their study, low concentrations of nZnO have had positive effect on growth of groundnut seedlings but high concentration have had inhibitive influence on seedling characteristics. Boonyanitipong et al. (2011) in a study on rice reported the developing effect of nZnO on radicle. Moreover, the effect of all $nTiO_2$ concentration treatments on radicle elongation of tomato seeds have had significant negative effect (Song et al. 2013). Fresh weight of total biomass (leaves, stems and roots) of tomato seedlings has increased 2.5-folds for the seedlings germinated and grown on CNTs containing MS medium compared with seedlings developed on the standard MS medium. They reported that absorption of CNTs can increase water penetration of seeds (Khodakovskaya et al. 2009).

It seems that the toxic effect of nZnO on plumule wet weight has been higher than on radicle water content in a way that wet weight of plumule under all nanoparticle concentration treatments has been significantly less than control treatment (Figure 5). This observation was consistent with the report of Dhoke et al. (2013) concerning the effectiveness of nanoparticle treatments on plumule of mung (*Vigna radiata*) plant seedlings compared to radicle. The results also indicated that the toxic effect of nZnO on plumule growth under 50 mg.L^{-1} treatment and higher nZnO concentrations has been more intense than the lower concentrations (Figure 3). Anyway, there was an increased (19%) in radicle wet weight in 30 mg.L^{-1} nZnO concentration compared to control treatment but

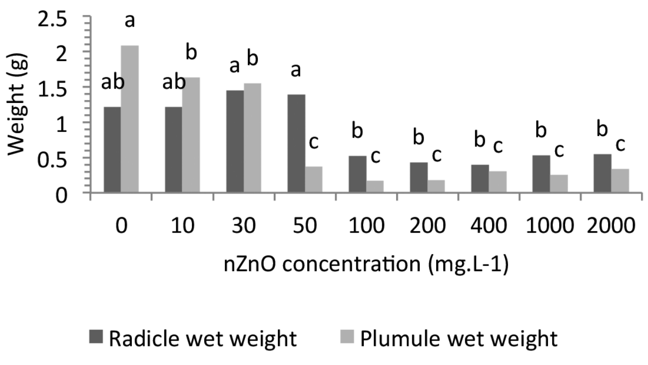

FIGURE 5. Effect of nZnO treatments on wet weight of radicle and plumule of groundnut (*Arachis hypogaea*). Water content of radicle and plumule was ploted according to mean of radicle and root length in different treatments. Bars having the same letter are not significantly different at the 0.05 level

this increase has not been significant. Radicle and plumule wet weight, respectively, have had 55.37 and 84.13% reduction compared to control treatment. However, Dhoke et al. (2013) reported that foliar spraying of all studied nanoparticles including nZnO resulted in the increase of radicle and plumule wet weight of mung (*Vigna radiata*) seedlings.

Radicle and plumule dry weight showed a reduction with increase in the nZnO concentration (Figure 6), while our observations did not show any significant difference between dry weight of radicle and plumule of the experimental treatments with control treatment. However, radicle dry weight in 10 mg.L^{-1} nZnO treatment has had an increase (11.11%) compared to control treatment and has been significantly higher than treatments with 200 mg.L^{-1} nZnO and higher concentrations but has not have any significant difference with control treatment (Figure 6). Radicle and plumule dry weight reduction compared to control treatment have been 61.11 and 90.76%, respectively. Milani (2012) reported no significant change in shoot dry weight of durum wheat (*Triticum durum*) due to nZnO application. However, a study by Dhoke et al. (2013) shown the promoting effects of spraying of nano-ZnO and nano-ZnFeCu on dry weight of radicle and plumule of mung plants. On a contrary, Zheng et al. (2005) reported that treatments with nano-TiO_2 significantly increased the dry weight of single seedling and the vigor index of aged seeds.

However, observations of this study have not shown any significant difference between water content of radicles and plumules of seedlings under different nZnO concentrations and control treatment (Figure 7). It can be shown that the main reason for weight reduction of radicle and plumule of seedlings affected by nZnO treatments has been the biomass reduction and nZnO probably have had no effect on radicle and plumule water content.

CONCLUSION

In the present study, the potential effects of nano zinc oxide (nZnO) on groundnut (*Arachis hypogaea*) were investigated by measuring of elongation, wet weight, dry weight and water content of radicle and plumule. The effects on radicle and plumule length and wet and dry weight of the seedlings were recorded for nanoparticle treated plants and compared to the reference. The suspensions of nZnO were able to affect the seedling growth of the groundnut plants in MS medium. The results of this experiment showed that the groundnut seeds could germinate in all nZnO concentration treatments after 6 days and therefore exposure with nZnO in this experiment didn't have effect on germination percent of seeds. Meanwhile 30 and 50 mg.L^{-1} nZnO have significantly caused to increase the germination rate index of groundnut seeds. However, observations of this study have not shown any change in the water content of radicle and plumule under different nanoparticle concentration treatments.

The results of this experiment showed that low concentrations of nZnO in MS medium have not had toxic effect on radicles of groundnut seedlings but even have had slightly promoting effect on germination, such as the increased of germination rate in 30 and 50 mg.L^{-1} and increased of dry weight of radicle in 10 and 30 mg.L^{-1} treatments. However, the toxic effect of nZnO on plumule has been more severe in a way that length, wet weight and dry weight of plumule were decreased correspondingly with the increase of nZnO concentration. Our results have not shown any change in the water content of radicle and plumule that can be evidence that water penetration of groundnut seedlings was not affected by nZnO treatments and nZnO toxicity was not related to water change due to absorption by seedlings.

These findings showed that the addition of nZnO to MS media can have little toxic influence on plants at low dosage, but could be very toxic if the dosage was relatively high. Therefore, the environmental effects of these nanoparticles should be a concern and more studies are necessary to investigate the overall stages of plant growth when exposed to various nanoparticles. Such information will be useful for ecological and human health risk assessments in the future.

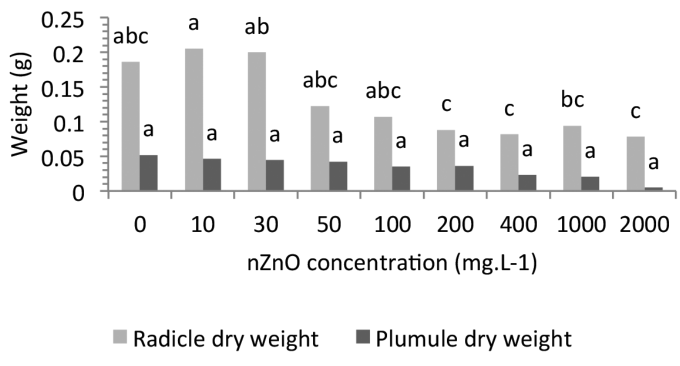

FIGURE 6. Effect of nZnO treatments on dry weight of radicle and plumule of groundnut (*Arachis hypogaea*). Radicle and plumule dry weight was ploted according to mean of radicle and root length in different treatments. Bars having the same letter are not significantly different at the 0.05 level

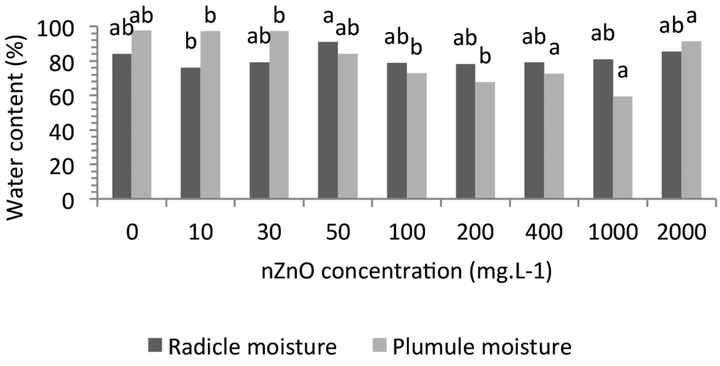

FIGURE 7. Effect of nZnO treatments on water content of radicle and plumule of groundnut (*Arachis hypogaea*). Radicle and water content was ploted according to mean of radicle and root length in different treatments. Bars having the same letter are not significantly different at the 0.05 level

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