

Effects of ZnO and TiO₂ nanoparticles on germination, biochemical and morphoanatomical attributes of *Cicer arietinum* L

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Received: 27 August 2016/Revised: 17 May 2017/Accepted: 24 May 2017/Published online: 16 June 2017

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Abstract With the development of nanotechnology, metal oxide nanoparticles have been applied in many industries, increasing their potential exposure level in the environment, yet their environmental safety remains poorly evaluated. Present work demonstrated the effects of ZnO and TiO₂ nanoparticles on the germination, morphoanatomical attributes and biochemistry of *Cicer arietinum*. The nanoparticles are used in three doses, i.e., 100, 500 and 1000 ppm along with control and each dose has three replicates. Results demonstrated that ZnO nanoparticles did not aid in the germination of seed, whereas TiO₂ nanoparticles showed positive impact on germination at 48-h observation. But at 72-h observation both the nanoparticle-treated and control seeds showed 100% germination. Morphological parameters revealed that ZnO nanoparticles have drastic negative impact on both root and shoot length, root and shoot fresh and dry biomass. On the other hand, the status of chlorophyll is almost opposite, i.e., ZnO nanoparticle-treated plants showed higher pigment content than TiO₂ nanoparticle-treated plants. From the statistical point of view, it is revealed that ZnO nanoparticle-treated plant showed significantly different results than that of TiO₂ nanoparticle-treated plants in Chl 'a' ($p < 0.001$), Chl 'b' ($p < 0.001$), total Chl ($p < 0.001$) and carotenoid ($p < 0.001$) content. Therefore, from these observations it can be concluded that ZnO nanoparticles showed positive effect on plant pigment content.

Transverse sections of root clearly revealed the formation of vascular bundle, and parenchyma tissue is hampered in all the ZnO nanoparticle-treated plants. However, well-formed vascular bundles and other tissue systems are clearly visible for TiO₂ nanoparticle-treated roots. This work shows a combination of both positive and negative effects of ZnO and TiO₂ nanoparticles on a dicot plant. Present observation is very much important to understand the morphological, biochemical and anatomical alteration of plant system under the influence of ZnO and TiO₂ nanoparticles. This will help to utilize the nanoparticles in a managed way where nanoparticles with harmful effect on biological systems can be used in such a way to reduce their exposure to environment, and on the other hand, nanoparticles showing positive effect on biological systems may be used as growth enhancers or biofertilizers. Moreover, these results further strengthen our understanding of environmental safety information with respect to metal oxide nanoparticle.

Keywords ZnO and TiO₂ nanoparticles · Germination · Morphology and biochemical parameters

1 Introduction

A special branch of technology known as nanotechnology has gained attention of researchers for the recent years. The term 'nano' can be defined as the atomic or molecular aggregates with at least one dimension between 1 and 100 nm (Roco 2003). Nowadays, engineered nanomaterials have versatile application in the field of cosmetics, pharmaceuticals, energy, agriculture, etc. (Castiglione et al.

Electronic supplementary material The online version of this article (doi:10.1007/s40974-017-0059-6) contains supplementary material, which is available to authorized users.

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2011). There is a controversy for health risk and benefits of nanoparticles on both flora and fauna (USEPA 2007). Nanotechnology is absolutely a new emerging field, and basically there are evidences of several negative effects on growth and development of plantlets (Castiglione et al. 2011). Previous research highlighted on the phytotoxicity of nanoparticles in different medium such as agar medium, filter paper on petri dish and soil for seed germination and root elongation (Zheng et al. 2005; Khodakovskaya et al. 2009; Doshi et al. 2008; Song et al. 2013). Lin and Xing (2007) also examined the phytotoxicity of five different types of nanoparticles such as multi-walled carbon nanotube (MWCNT), aluminum (Al), alumina (Al_2O_3), zinc (Zn) and zinc oxide (ZnO) on the seed germination and seedling root growth of six higher plant species. However, the entire reported nanoparticles dose not exhibited same pattern of toxicity (Thuesombat et al. 2014). Lin and Xing (2007) reported that ZnO nanoparticles significantly decreased the seedling biomass and caused root tip shrinkage and collapse of the root epidermis, cell internalization and translocation of *L. perenne*. On the other hand, Zheng et al. (2005) reported that TiO_2 nanoparticles can exhibit negative impact on the photochemical reaction of chloroplasts of spinach (*Spinacia oleracea*). In addition, increased germination rate and germination index for *S. oleracea* were noted after exposure to TiO_2 nanoparticles at 0.25–4% (w/v) but not with larger TiO_2 particles at the same concentrations.

Zinc (Zn) is considered as an essential micronutrient for both plants and animals (Singh et al. 2004). It is absorbed by higher plants mainly as a divalent cation (Zn^{2+}). Moreover, this metal is extremely essential for plant's enzyme system as it acts as cofactors, metal components and other regulatory factors of many enzymes (Prasad et al. 2012). So far as plant nutrients are concerned, in India, amongst the yield-enhancing micronutrients, Zn comes in the fourth position just after nitrogen (N), phosphorous (P) and potassium (K), whereas in plants, titanium (Ti) is a non-available element in natural conditions. But it can be considered as a beneficial element in fixation of nitrogens (Carvajal and Alcaraz 1998). However, Ti in its nanoform can be uptaken by plants and can affect the plant system in various ways.

Apart from natural source of zinc in the environment, zinc nanoparticles are now used in many field and from there it gets contaminated which makes it available for uptake by plants. Being environmentally safe, it is applied for various biological purposes like drug delivery, gene delivery. ZnO nanoparticles are toxic to many cell lines, so it is utilized for treating cancer cells and many other bacterial cells. Moreover, it is utilized in solar cells, sensors, photocatalytic purposes (Vaseem et al. 2010). On the other hand, TiO_2 nanoparticles are available in nature as it is

used in dye sensitization, doping, coupling and capping, wastewater treatment, hydrogen production by water splitting and pesticide degradation (Gupta and Tripathi 2011). TiO_2 nanoparticles are also used in tablet coating, sunscreen preparation, food additive, sanitary ceramics, antifogging glasses, etc (Lang et al. 2010).

There is a need of being aware about the possible risks of nanomaterials to plant systems as it is utilized in many fields. It is also a necessary search because the nanoparticles can also be utilized as enhancers for plant systems (Rahmani et al. 2016). Increased research is going on the effects of metal nanoparticles on plant systems. Although plenty of researches have been done on nanoparticles synthesis and their application on biological systems (Homaee and Ehsanpour 2015; Rahmani et al. 2016), works observing the effect of nanoparticles on the morphological, physiological and biochemical parameters of plant systems are scarce. Different concentration of same nanoparticles elicits different effects on plant systems; i.e., low concentrations of nanoparticles may not show any adverse effect on plant, but higher concentrations may cause positive or negative effects on plants (Thuesombat et al. 2014). Very recently, Zuverza-Mena et al. (2016) suggested that both TiO_2 and ZnO have positive or negative phytotoxic effect. Boonyanitipong et al. (2011) reported that ZnO nanoparticles inhibit the root length and reduce the number of roots. However, TiO_2 nanoparticles have no such inhibitory effect on root length. Direct exposure to ZnO nanoparticles was also examined by Taheri et al. (2015) and highlighted that ZnO nanoparticles can increase the shoot dry matter and leaf area index by 63.8 and 69.7%, respectively.

In the present study, germination and other experiments were carried out on *Cicer arietinum* to evaluate the phytotoxicity of ZnO and TiO_2 nanoparticles. Root length and shoot length along with other physiological parameters and biochemical parameters, which are sensitive to an adverse environment, were chosen as toxicity indicators. This study provided valuable information for the application of metal oxide nanoparticles in agriculture and environmental safety assessment.

2 Materials and methods

2.1 Preparation of nanoparticle solution

Commercial ZnO nanoparticles (Size < 50 nm) and TiO_2 nanoparticles (Size < 50 nm) were purchased from Sigma-Aldrich, USA, and Merck India Ltd., Mumbai, respectively. Surface area of ZnO and TiO_2 nanoparticles is 20 and 25 m^2/g , respectively. Calculated amount of ZnO and TiO_2 nanoparticles were dissolved in double distilled water to prepare 100, 500 and 1000 ppm doses of each

nanoparticles. Each suspension was stirred using a magnetic stirrer for 30 min.

2.2 Preparation of seeds

Cicer arietinum (PBG7) were chosen for this particular work because it is a dicot plant, easily available, easy to handle, can be grown at any season, can tolerate wide range of environmental variation and easily germinates as it has thin seed coat. Healthy seeds of *Cicer arietinum* were bought from the market. Seeds were surface-sterilized with 10% sodium hypochlorite and soaked in double distilled water for 2 h.

2.3 Treatment of seeds with nanoparticles

Clean glass petri dishes (95 mm diameter) were taken, and 25 seeds of *Cicer arietinum* were placed at even distance over a round piece of filter paper. Each petri dish with different doses (100, 500 and 1000 ppm concentrations of both the nanoparticles) had three replicates. Those seeds were partially immersed in 5 ml of suspension of ZnO and TiO₂ nanoparticles according to the dose. Seeds were cultured for 12 days on the same petri dishes at an average photoperiod of 11:13 (day/night), temperature 23–26 °C, relative humidity 86%. All the petri dishes were kept in a screened cage in a place where sunlight may reach. No rainfall occurred during that period. A control setup was run simultaneously with only the seeds without any nanoparticles but with 5 ml distilled water. Control setup was also with three replicates.

2.4 Estimation of morphological parameters

2.4.1 Germination

Numbers of germinated seeds were counted after 24, 48 and 72 h of incubation and up to 72 h until all the seeds have germinated. When the radicle was first visible rupturing the seed coat, the seed was considered to be germinated. Counting was done under completely sterile condition.

Equation (1) depicts how percentage germination was calculated:

$$\text{Germination(\%)} = \frac{[\text{Number of germinated seeds} \times 100]}{\text{total number of seeds}} \quad (1)$$

2.4.2 Root and shoot length

Root and shoot length of germinated seedlings was measured using a centimeter scale. Root length was taken from

the site of the emergence of root up to the root tip, and shoot length was measured from the base up to the apex.

2.4.3 Root and shoot biomass

Fresh root and shoot were separately taken, cleaned and weighed followed by drying them in hot air oven at 60 °C. Weight of dried root and shoot was again recorded in grams. The ratio of dry weight to fresh weight was obtained by dividing the dry weight (DW) with the fresh weight (FW) of root and shoot of the same concentrations.

2.5 Estimation of biochemical parameters

2.5.1 Chlorophyll (Chl) and carotenoid estimation

Fresh leaves (0.1 g) were harvested at the end of the experiment and washed with double distilled water. Then the washed leaves were cut into small pieces and put into 80% acetone (v/v) and kept in refrigerator overnight. The green pigments extracted from the leaves were measured at 645, 652 and 663 nm by using UV–Vis spectrophotometer (Bates et al. 1973). The concentration of carotenoid was estimated by following the method of MaClachlan and Zalik (1963). Using the following Eqs. (2, 3, 4, 5), concentrations of chlorophyll ‘a,’ ‘b,’ total chlorophyll and carotenoid were measured:

$$\text{Chl ‘a’} \text{ (mg g}^{-1}\text{fw)} = \frac{[12.7 * D_{663} - 2.69 * D_{645}]}{* V/1000 * W} \quad (2)$$

$$\text{Chl ‘b’} \text{ (mg g}^{-1}\text{fw)} = \frac{[22.9 * D_{645} - 4.68 * D_{663}]}{* V/1000 * W} \quad (3)$$

$$\text{Total Chl (mg g}^{-1}\text{fw)} = D_{652} * 1000 * V/1000 * W \quad (4)$$

$$\begin{aligned} \text{Total carotenoid content (mg g}^{-1}\text{)} \\ = [7.6 * D_{480} - 1.49 * D_{510}] * V/1000 * W \end{aligned} \quad (5)$$

where D = optical density; V = final volume of 80% acetone; W = weight of sample; f.w. = fresh weight of the sample.

2.5.2 Estimation of total protein

Estimation of total protein was done by the standard method of Lowry et al. (1951). 0.5 ml of leaves extract was made up to 1 ml by adding distilled water. 5 ml of alkaline copper solution was added to it followed by 0.5 ml of Folin–Ciocalteu reagent. The solutions were incubated at room temperature in the dark for 30 min, and then the absorbances were measured at 660 nm. Amount of protein is estimated from a standard graph prepared earlier.

2.5.3 Estimation of total carbohydrate

Total carbohydrate of treated and control plant leaves was estimated by anthrone method (Hedge and Hofreiter 1962). 0.1 ml of prepared plant extract was taken and made up to 1 ml with distilled water. Then 4 ml of anthrone reagent was added to each extract. Then the test tubes were incubated at boiling water bath for 8 min. Then the solutions were cooled, and the absorption spectrum was measured at 630 nm. Total carbohydrate was estimated from a standard graph.

2.5.4 Estimation of total phenolics

Estimation of total phenolics was determined following the method of Mallick and Singh (1980). 0.5 g of leaves was crushed with 5 ml 80% (v/v) ethanol. The extract was centrifuged, and the supernatant was evaporated to dryness. Dried residue was dissolved in 5 ml distilled water. From this extract, 0.2 ml aliquot was taken and the volume was made up to 3 ml. Then 0.5 ml of Folin–Ciocalteu reagent was added followed by 2 ml 20% Na₂CO₃. The test tubes were boiled in water bath for 1 min, cooled and measured in a spectrophotometer at 650 nm. Using a standard curve, the concentration of total phenolics was estimated.

2.5.5 MDA content

0.1 g of leaves was collected and crushed with 10 ml 0.1% (w/v) TCA. The extracts were centrifuged for 10 min at 15,000 rpm at 4 °C. 1 ml from the supernatant was taken and mixed with 4 ml 0.5% TBA diluted in 20% TCA. Then the mixture was boiled in water bath at 95 °C for 30 min. Reaction was ended by incubating on ice. The absorbances were measured at 532 and 600 nm (Heath and Packer 1968). MDA content was calculated by Eq. (6):

$$\begin{aligned} \text{MDA equivalents (nmol cm}^{-1}\text{)} \\ = 1000[(\text{Abs } 523 - \text{Abs } 600 \text{ nm})/155] \end{aligned} \quad (6)$$

2.5.6 Chlorophyll stability index

Chlorophyll stability index (CSI) of the leaves was measured by the spectrophotometric method of Koleyoreas (1958). 0.25 g of leaf sample of each treatment was taken in two different test tubes containing 10 ml of water. Control test tube was kept in normal room temperature, while another test tube was kept in water bath for 30 min at 65 °C. Pigment extracts from both the test tubes were measured at 652 nm, and total chlorophyll content was calculated which is used to estimate CSI using the following equation (Eq. 7):

$$\text{CSI}(\%) = \frac{[\text{Total chlorophyll content (heated)}]}{\text{Total chlorophyll content (control)}} * 100 \quad (7)$$

2.5.7 Membrane stability index

Membrane stability index (MSI) was estimated following the method of Premchandra et al. (1990) modified by Sairam (1994). 0.1 g of cleaned leaf was taken in a test tube with 10 ml of double distilled water. The test tube was then kept in water bath at 40 °C for 30 min. Electrical conductivity (C1) of the water was measured using a conductivity meter. Then the sample was boiled in a water bath at 100 °C for 10 min, and again its conductivity (C2) was recorded. MSI was calculated using the following formula (Eq. 8)

$$\text{Membrane stability index (MSI)} = [1 - (C1/C2)] * 100 \quad (8)$$

2.5.8 Root ion leakage

Root ion leakage was calculated using the method of Lutts et al. (1995). 0.3 g of root of both treated and control plants was thoroughly cleaned with double distilled water. The root was incubated with 10 ml distilled water at 25 °C for 5 min in a test tube. After that electrical conductivity (EC₀) of the solution was measured with a conductivity meter. The test tube was then incubated for another 12 h, and again the electrical conductivity (EC₁) of solution was determined. The test tube was then kept in a water bath and boiled for 30 min. After cooling, the electrical conductivity (EC₂) was measured once more. Relative conductivity indicating root ion leakage of the roots was obtained using the following formula (Eq. 9):

$$\text{Relative conductivity (RC)} = [(EC_1 - EC_0)/EC_2] * 100 \quad (9)$$

2.6 Transverse section of root

Roots of both TiO₂ and ZnO nanoparticle-treated plants were taken, and thin transverse sections were made. The sections were stained with eosin, and a temporary slide was prepared to watch under light microscope (Photoelectric Stereoscopic Binocular Microscope).

2.7 Statistical analysis of parameters

The analyses were done with three replicates. All the data presented in this study were mean of three identical experiments made in three replicates along with the

standard deviation (SD). Statistical significance was determined by Duncan's multiple comparison tests to compare the treatment means. Significance level was set at $p < 0.05$.

3 Results and discussion

3.1 Germination in *Cicer arietinum*

Germination of *Cicer arietinum* under the influence of ZnO and TiO₂ nanoparticles is depicted in Fig. 1a, b. From these figures, it is clear that percentage of germination increased with increment of the dose of ZnO nanoparticles up to 48 h although numbers of germinated seeds are less than that of the control. However, after 72 h, all the treatments along with control showed 100% germination. On the other hand, TiO₂ nanoparticles treatment showed highest percentage of germination at 500 ppm followed by 100 ppm with respect to control (Fig. 1b). But within 48 h, all the TiO₂ nanoparticle-treated seeds showed same percentage of germination with control. Therefore, study results revealed that germination is enhanced by TiO₂ nanoparticles than ZnO nanoparticles. This enhancement of germination by TiO₂ nanoparticles over ZnO nanoparticles is probably due to generation of reactive anions which directly or indirectly helps to accumulate water and increase the rate of oxygen uptake which is required for fast germination (Khot et al. 2012). Another study conducted by Zheng et al. (2005)

highlighted that TiO₂ nanoparticles have beneficial effect on germination of spinach seed and seedling growth. Almost similar observation was reported by Yang et al. (2006). They suggested that TiO₂ nanoparticles can increase growth of spinach due to higher assimilation of nitrogen. Moreover, enhancement of germination may be because water uptake capacity of seed is increased which is again promoted by nanoparticles through creating new pores on seed coat (Khodakovskaya et al. 2009). Almost similar enhancement of germination was reported by Song et al. (2013) for germination of tomato seeds by TiO₂ and Ag nanoparticles.

3.2 Variation of root and shoot length

The effect of ZnO and TiO₂ nanoparticles on root and shoot length of *Cicer arietinum* is presented in Table 1. Data of the table depict that root length of ZnO nanoparticle-treated *Cicer arietinum* is drastically less, whereas roots of TiO₂ nanoparticle-treated plants are sufficiently long. Moreover, root length of *Cicer arietinum* gradually increased with increasing dose of TiO₂ nanoparticles. Root length of 100 ppm ZnO nanoparticle-treated plants was only 0.83 cm, but TiO₂ nanoparticle-treated roots reached up to 10.73 cm of length, more than that of control plants (9 cm). On the other hand, shoot length of ZnO nanoparticle-treated plant reached up to 7 cm, whereas both TiO₂ nanoparticle-treated and control plants show around 6 cm of length (Table 1). However, almost opposite results are

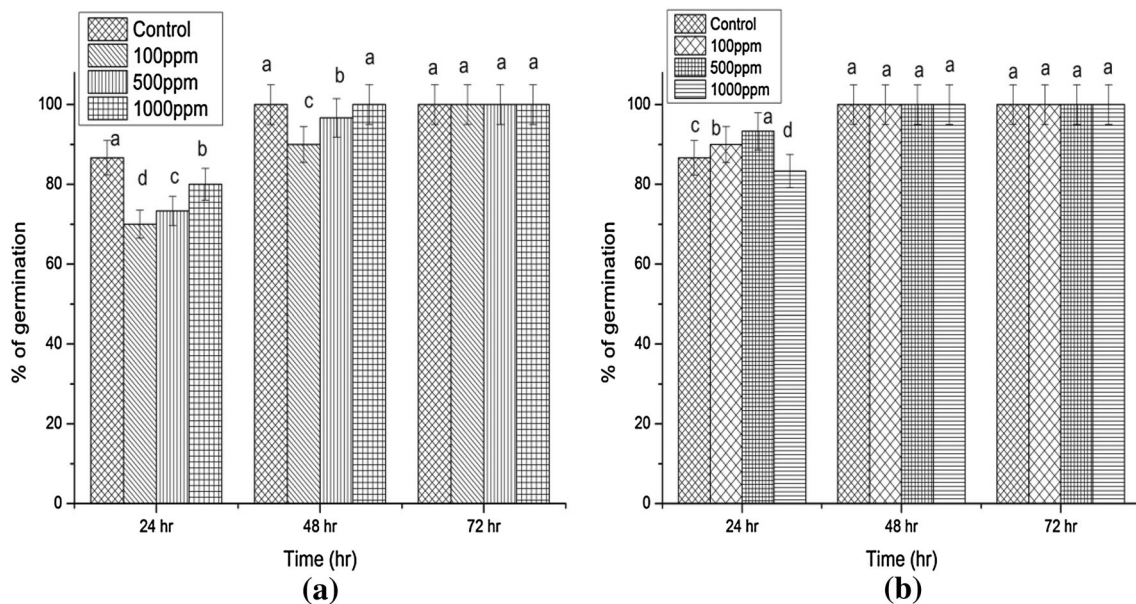


Fig. 1 Variation of percentage of germination of chickpea (*Cicer arietinum*) plant under different concentrations of **a** ZnO and **b** TiO₂ treatment depicted in a bar graph where ZnO-treated roots are slower in germination than control but TiO₂-treated roots germinated at

almost the same time as that of the control. Different letters denote statistically significant differences according to the Duncan's multiple comparison tests ($p < 0.05$)

Table 1 Morphological variation of chickpea (*Cicer arietinum*) plant under ZnO and TiO₂ treatment

Parameters	Control	ZnO			TiO ₂		
		100 ppm	500 ppm	1000 ppm	100 ppm	500 ppm	1000 ppm
Root length (cm)	9.00 ± 0.8 ^a	0.83 ± 0.28 ^b	0.73 ± 0.68 ^c	0.93 ± 0.11 ^b	10.73 ± 1.10 ^d	11.33 ± 2.75 ^c	12.36 ± 0.7 ^b
Shoot length (cm)	6.23 ± 0.68 ^c	7.1 ± 1.15 ^b	7.6 ± 0.17 ^a	6.43 ± 1.44 ^c	6.1 ± 0.70 ^c	7.33 ± 2.30 ^b	9.2 ± 0.1 ^a
Root fresh biomass (g)	0.20 ± 0.03 ^a	0.04 ± 0.01 ^b	0.06 ± 0.00 ^b	0.04 ± 0.01 ^b	0.30 ± 0.31 ^a	0.28 ± 0.05 ^a	0.29 ± 0.04 ^a
Root dry biomass (g)	0.01 ± 10 ^{-4a}	0.005 ± 10 ^{-4b}	0.005 ± 10 ^{-4b}	0.007 ± 10 ^{-5b}	0.02 ± 10 ^{-4b}	0.02 ± 10 ^{-4b}	0.02 ± 10 ^{-4b}
Shoot fresh biomass (g)	0.13 ± 0.03 ^a	0.11 ± 0.03 ^a	0.12 ± 0.01 ^a	0.10 ± 0.01 ^b	0.16 ± 0.03 ^a	0.18 ± 0.06 ^b	0.23 ± 0.03 ^a
Shoot dry biomass (g)	0.01 ± 10 ^{-4b}	0.02 ± 10 ^{-3a}	0.01 ± 10 ^{-4b}	0.01 ± 10 ^{-4b}	0.02 ± 0.01 ^c	0.02 ± 0.07 ^c	0.02 ± 10 ^{-2c}

Values are mean ± SD of three replicates, and the experiment was done on photoperiod 11:13 (day/night), temperature 23–26 °C, relative humidity 86%

^{a,b,c,d,e} Different letters indicate significant differences at $p < 0.01$ according to the Duncan's multiple range test

reported by Prasad et al. (2012). They reported that with increasing concentration of ZnO from 400 to 1000 mg/L, the height of the plant significantly increased with respect to control. They also reported that plant growth reached up to 15.4 cm when the seeds were treated with 1000 ppm ZnO nanoparticle. Such increment in plant growth is probably due to production of higher concentration of auxin under the influence of ZnO nanoparticles (Prasad et al. 2012). Previous study also highlighted on retardation in early growth of chickpea seedlings at high dose of ZnO nanoparticles and low dose of ZnO nanoparticles which is sufficient to achieve positive response (Mahajan et al. 2011; Burman et al. 2013). Recently, Haghghi et al. (2014) reported that up to 200 ppm TiO₂ nanoparticles can improve both root and shoot lengths for *Lycopersicon esculentum* and *Allium cepa*. However, *Raphanus sativus* showed opposite trend upon application of TiO₂ nanoparticles up to 200 ppm. Growth of plant increased under the influence of TiO₂ nanoparticles probably because it regulates nitrogen-metabolizing enzyme activity and helps to convert nitrogen from its inorganic to organic form which improves protein and chlorophyll synthesis (Yang et al. 2006; Mishra et al. 2014). From the Pearson correlation study, it is clear that root length is significantly ($p < 0.001$) related to shoot dry weight (Table S2) under ZnO nanoparticles treatment. On the other hand, TiO₂ nanoparticle-treated plant showed significant ($p < 0.001$) negative relationship with MSI (Table S3). Again, shoot length showed strong negative significant ($p < 0.001$) relationship with root dry weight. But root dry weight is significantly ($p < 0.001$) related to MSI.

3.3 Root morphology study

Root morphology study clearly revealed that ZnO nanoparticle has adverse effect on root development of

Cicer arietinum. Moreover, ZnO nanoparticle treatment does not support to lateral root development. Figure 2a also highlights that entire root (main root) formation is grossly hampered under the influence of ZnO nanoparticle. Hampering of root structure than shoot is possibly because of seed coat which protects the embryo but not the whole seed (Boonyanitpong et al. 2011). In comparison with control, the entire root formation is accelerated when treated with TiO₂ nanoparticles. The net effective root length and lateral root development is gradually increased with the increasing dose of TiO₂ nanoparticles (Fig. 2b). Moreover, all the TiO₂ nanoparticle-treated roots are much healthier than control (Fig. 2a). Previous literature highlighted that when treated with TiO₂ nanoparticles, it improves the growth of cucumber root, with respect to size, and this health improvement of root is probably due to higher level of nitrogen accumulation and subsequently synthesis of proteins (Servin et al. 2012). Moreover, the betterment of root length by application of TiO₂ nanoparticles over ZnO nanoparticles is possibly due to increase in nutrient level (iron and magnesium), NiR activity and chlorophyll ('a' and 'b') biosynthesis (Kuzel et al. 2003). On the other hand, dry weight-to-fresh weight ratio of roots is greatly influenced by TiO₂ nanoparticles than ZnO nanoparticles at higher dose (Fig. 3a). However, shoot biomass is equally affected at lower dose (up to 500 ppm) by both ZnO nanoparticles and TiO₂ nanoparticle treatment (Fig. 3b). But at higher dose (1000 ppm), TiO₂ nanoparticles have pronounced effect on shoot DW/FW ratio. This result may be due to enhancement of accumulation of water in fresh biomass under such level of nanometal oxide application. Moreover, ZnO and TiO₂ nanoparticles have different rate of permeability through cell wall. Almost similar result was reported by Yang and Watts (2005), and they highlighted that nanoalumina (Al₂O₃) at 2000 mg/L could inhibit root elongation of five plant species.

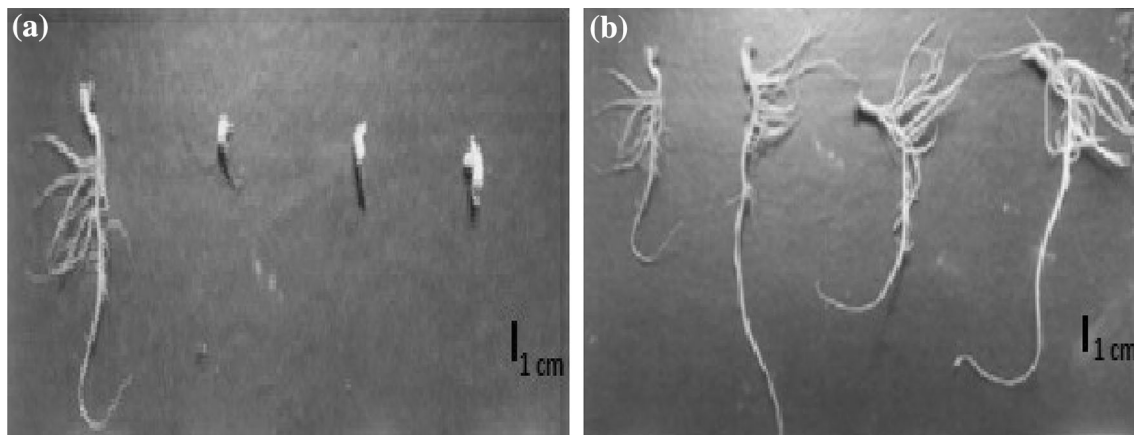


Fig. 2 Effect on root length of *Cicer arietinum* of different doses of **a** ZnO and **b** TiO₂ is very prominent as root growth is retarded by ZnO treatment but TiO₂ treatment promoted root growth more than

normal root length than control [12th day; photoperiod 11:13 (day/night), temperature 23–26 °C, relative humidity 86%]

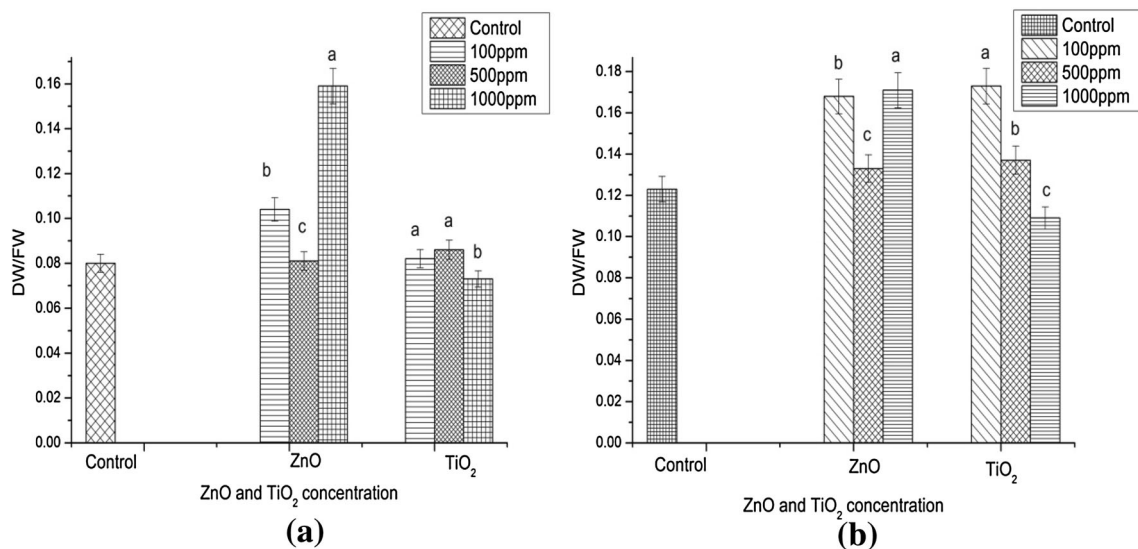


Fig. 3 **a** Root DW/FW and **b** shoot DW/FW of ZnO and TiO₂-treated plants at different concentration (100, 500, 1000 ppm). Different letters denote statistically significant differences according to the Duncan's multiple comparison tests ($p < 0.05$)

3.4 Biochemical parameters

The variation of pigment (Chl 'a', Chl 'b', total Chl and carotenoid) content under the influence of ZnO and TiO₂ nanoparticles is presented in Table 2. The experimental data clearly revealed that at lower dose of ZnO nanoparticles application enhanced the pigment level about 103% with respect to control. But, further increase in ZnO nanoparticles dose does not support further increment of chlorophyll level. Similar increase in chlorophyll level was also reported by Prasad et al. (2012) by application of ZnO nanoparticles. They reported that the chlorophyll content of the 1000 ppm ZnO nanoparticle-treated plant increased up to 1.97 mg/g f.w which was more than that of the untreated plant.

This is perhaps due to the complementary effect of other inherent nutrients like Mg, Fe and S (Prasad et al. 2012). Almost similar results were observed by Zheng et al. (2005) when *Spinacia oleracea* seeds were treated with TiO₂ nanoparticles. On the other hand, TiO₂ nanoparticles showed much lower level of chlorophyll 'a' with respect to control. Almost similar variation of chlorophyll 'b' and total chlorophyll level was recorded for different doses of ZnO and TiO₂ nanoparticles applications. The negative effect of TiO₂ nanoparticles on chlorophyll is perhaps due to insertion of TiO₂ nanoparticles into chloroplasts where it participates in catalyzed oxidation–reduction reactions and subsequently accelerates the electron transport and oxygen evolution (Hong et al. 2005).

Table 2 Variation of pigment and biochemical parameters of chickpea (*Cicer arietinum*) plant under different concentrations of ZnO and TiO₂ treatment

Parameters	Control	ZnO			TiO ₂		
		100 ppm	500 ppm	1000 ppm	100 ppm	500 ppm	1000 ppm
Chlorophyll 'a' (mg/g)f.w	1.57 ± 0.15 ^b	3.20 ± 0.10 ^a	1.44 ± 0.12 ^c	0.24 ± 10 ^{-2d}	0.17 ± 10 ^{-3d}	0.34 ± 0.02 ^c	1.14 ± 0.04 ^b
Chlorophyll 'b' (mg/g)f.w	0.50 ± 0.05 ^b	0.90 ± 0.01 ^a	0.43 ± 0.01 ^b	0.45 ± 0.00 ^b	0.01 ± 10 ^{-3e}	0.08 ± 10 ^{-2d}	0.19 ± 10 ^{-3c}
Total Chlorophyll (mg/g)f.w	2.23 ± 0.14 ^b	4.66 ± 0.26 ^a	1.91 ± 0.10 ^c	0.61 ± 0.02 ^d	0.11 ± 10 ^{-2e}	0.35 ± 0.03 ^d	1.37 ± 0.04 ^c
Carotenoid (mg/g)f.w	0.66 ± 0.04	1.37 ± 0.06	4.45 ± 0.13	1.18 ± 0.01	0.03 ± 10 ⁻²	0.06 ± 10 ⁻³	0.48 ± 10 ⁻²
Sugar (mg/g)f.w	2.26 ± 0.15	2.2 ± 0.34	1.8 ± 0.43	1.8 ± 0.08	4.2 ± 0.26	1.8 ± 0.16	2.5 ± 0.2
Protein (mg/g)f.w	9.57 ± 0.37 ^b	15.70 ± 1.53 ^a	7.22 ± 0.21 ^c	4.38 ± 0.88 ^d	6.13 ± 0.40 ^b	3.54 ± 0.16 ^c	11.12 ± 0.06 ^a
Phenol (mg/g)f.w	8.13 ± 0.07 ^c	12.75 ± 0.23 ^b	22.41 ± 0.53 ^a	11.95 ± 0.41 ^b	10.54 ± 0.39 ^b	12.8 ± 0.32 ^a	13.53 ± 0.04 ^a
MDA (nmol. g ⁻¹ f.w)	11.9 ± 1.01 ^b	10.7 ± 0.36 ^c	15.0 ± 0.04 ^a	14.6 ± 1.11 ^a	10.1 ± 0.13 ^b	10.0 ± 1.0 ^b	16.0 ± 1.09 ^a
Chlorophyll stability index (CSI)	10.0 ± 0.04 ^b	20.0 ± 1.02 ^a	8.56 ± 0.39 ^c	2.74 ± 0.22 ^d	0.515 ± 0.02 ^e	1.56 ± 0.35 ^d	6.14 ± 0.045 ^c
Membrane stability index (MSI)	50.0 ± 1.44 ^c	80.0 ± 2.01 ^a	75.0 ± 1.09 ^b	74.66 ± 0.99 ^b	25.0 ± 0.65 ^d	84.0 ± 1.73 ^b	86.0 ± 3.1 ^a
Relative conductivity	42.83 ± 0.94 ^c	40.00 ± 1.22 ^c	50.00 ± 1.17 ^b	53.00 ± 2.03 ^a	50.00 ± 0.98 ^b	57.0 ± 2.64 ^a	50.0 ± 0.18 ^b

Values are mean ± SD of three replicates, and the experiment was done on photoperiod 11:13 (day/night), temperature 23–26 °C, relative humidity 86%

^{a,b,c,d,e} Different letters indicate significant differences at $p < 0.01$ according to the Duncan's multiple range test

The variation of carotenoid with respect to control was highest at 500 ppm followed by 100 and 1000 ppm of ZnO nanoparticles application. But application of TiO₂ nanoparticles does not support to enhance the carotenoid level. This is perhaps due to direct interaction of TiO₂ nanoparticles with carotenoid biomolecules. However, Wang et al. (2016) pointed out that genes are mainly responsible for enhancement of pigments under influence of ZnO and TiO₂ nanoparticles.

Pair *t* test results clearly demonstrated that overall pigment (Chl 'a', Chl 'b', total chlorophyll and carotenoid) content of ZnO nanoparticle-treated plants is statistically significant ($p < 0.001$) than that of TiO₂ nanoparticle-treated plants (Table 3). Pearson correlation study results also revealed that Chl 'a' content is significantly ($p < 0.001$) related to total phenolic content. Similarly, total chlorophyll content is significantly ($p < 0.001$) related to total phenol content in ZnO nanoparticle-treated plants (Table S3). But, TiO₂ nanoparticle-treated plant results demonstrated that chlorophyll ('a' and 'b'), carotenoid, sugar and protein level has strong positive relationship with total phenol content (Table S4). However, none of the correlation showed significant relationship.

On the other hand, the higher level of sugar (1.8 mg g⁻¹ fw) was recorded at lower dose of ZnO nanoparticles and lower level of sugar was accumulated at

Table 3 Paired '*t*' test between pigment level of ZnO and TiO₂-treated plants at dose 100 ppm

	Mean ± SD	' <i>t</i> ' value	< <i>p</i> value
<i>Chl a</i>			
ZnO	3.200 ± 0.100	48.02	0.000
TiO ₂	0.178 ± 0.009		
<i>Chl b</i>			
ZnO	0.901 ± 0.011	167.87	0.000
TiO ₂	0.015 ± 0.001		
<i>Total Chl</i>			
ZnO	4.665 ± 0.263	29.62	0.001
TiO ₂	0.115 ± 0.006		
<i>Carotenoid</i>			
ZnO	1.371 ± 0.069	32.21	0.001
TiO ₂	0.320 ± 0.003		

higher dose (1000 ppm) of ZnO nanoparticles. But almost opposite results were recorded for TiO₂ nanoparticles. At lower dose of TiO₂ nanoparticles application, sugar level was high and it was almost 90% increment at lower dose (0.05) of TiO₂ nanoparticle (Table 2). Sugar level reduced to 1.8 mg g⁻¹ f.w at intermediate dose of TiO₂ nanoparticles. Again, application of higher dose (1000 ppm) of

TiO₂ nanoparticles supports the enhancement of sugar level (17%). Results also highlighted that all the primary metabolites are not equally affected by the application of ZnO and TiO₂ nanoparticles. In the present work, the level of protein gradually reduced from 15.70 to 4.38 mg g⁻¹ f.w. with increment of ZnO nanoparticles level from 100 to 1000 ppm, respectively. However, almost opposite result was recorded for TiO₂ nanoparticle-treated plant where higher level of protein (11.12 mg g⁻¹ f.w) was recorded at higher dose (1000 ppm) of TiO₂ nanoparticles. But, intermediate dose (500 ppm) of TiO₂ nanoparticles showed lower protein level (3.54 mg g⁻¹ f.w) in plants, whereas plants treated with lower dose (100 ppm) showed better protein content (6.13 mg g⁻¹ f.w) (Table 2). But total phenol content varies from 11.95 to 22.41 mg g⁻¹ f.w for ZnO nanoparticles treatment. But, with the application of TiO₂ nanoparticles, total phenol level varied from 10.54 to 13.53 mg g⁻¹ f.w. Najafi and Jamei (2014) demonstrated in their paper that phenolic compound can be regulated under the influence of environmental and other stress conditions.

3.5 Lipid peroxidation

The value of MDA content gradually increased with increasing the load of ZnO and TiO₂ nanoparticles. However, higher level of MDA content was recorded under ZnO nanoparticles treatment than TiO₂ nanoparticles at intermediate dose (Table 2). But at higher dose, MDA content is high for TiO₂ nanoparticles than for ZnO nanoparticles. It is well established that accumulation of higher level of MDA means higher level of cellular damage which was recorded at 500 ppm followed by 1000 ppm of ZnO nanoparticle-treated plants. But higher level of MDA was recorded only at 1000 ppm of TiO₂ nanoparticle-treated plants (Table 2). Such higher level of MDA accumulation under nanoparticles interaction is probably due to generation reactive oxygen species (Mohammadi et al. 2013; Shaw and Hossain 2013a, b).

3.6 Chlorophyll stability index

The stability of chlorophyll index (CSI) was estimated under the influence of ZnO and TiO₂ nanoparticles on *Cicer arietinum*. The results highlighted that CSI is very low under the influence of TiO₂ nanoparticles (Fig. 4a, b). Therefore, these results clearly demonstrated that the structure of chlorophyll is strongly influenced by TiO₂ nanoparticles than ZnO nanoparticles. Almost similar observation was reported by earlier researchers (Hong et al. 2005) where they showed that TiO₂ nanoparticles reduce photosensitivity of chloroplasts.

3.7 Membrane stability index and root ion leakage

Membrane damage can be assessed by the application of ZnO and TiO₂ nanoparticles on *Cicer arietinum*, and the results are presented in Table 2. Moreover, graphical presentation clearly revealed that MSI initially increased and then decreased with the increasing dose of ZnO nanoparticles, but for TiO₂ nanoparticles, MSI initially decreased followed by increment with the increasing dose (Fig. 4a, b). On the other hand, the value of root ion leakage clearly revealed that higher level of root ions conductivity was recorded for TiO₂ nanoparticles treatment than ZnO nanoparticles treatment. The conductivity of root ions increases with increasing the dose of both ZnO and TiO₂ nanoparticles. Very high level of conductivity was recorded at 500 ppm of TiO₂ nanoparticles application. Lipid peroxidation and root ion leakage are the consequences of membrane damage in plants, as from the observations of Table 2. It can be suggested that higher dose of ZnO and TiO₂ nanoparticles caused root and shoot membrane damage in *Cicer arietinum*. This is quite possible, because root is primary entry point of NPs (Anjum et al. 2013). Our present results are in agreement with the earlier report by Rico et al. (2013). They reported that 0.125 g nCeO₂ L⁻¹ induced the higher level of membrane damage in rice roots. Hatami and Ghorbanpour (2014) also reported that nanoparticles induce oxidative stress as well as electrolytic leakage which disrupt membrane stability.

3.8 Transverse section of root

Treatment of *Cicer arietinum* with ZnO nanoparticles shows interruption in root formation. The formation of root has been hampered to such extent that the root never grew more than 0.93 cm. Microscopic view of stained root shows only cortex portion without any pith, xylem or phloem which caused retarded development of the main and lateral root (Fig. 5b). However, TiO₂ nanoparticle-treated plant showed healthy root with cortex, endodermis, pericycle, xylem and phloem (Fig. 5c) and it is similar to control (Fig. 5a). Therefore, this observation clearly suggests that ZnO nanoparticles have negative impact on root structure. Possibly, ZnO nanoparticles can enhance the permeability of plant cell walls and create pores in the walls and subsequently enter into the cells through the pores (Lin and Xing 2007). This is quite possible because plant roots have been considered as the main route of plant's exposure to NPs which is the cause of physical or chemical toxicity in plants (Anjum et al. 2013). Growth inhibitory effect of other nanoparticles such as nano-CuO (copper oxide) was also reported when it was treated to rice seedlings which (nano-CuO) caused severe oxidative burst

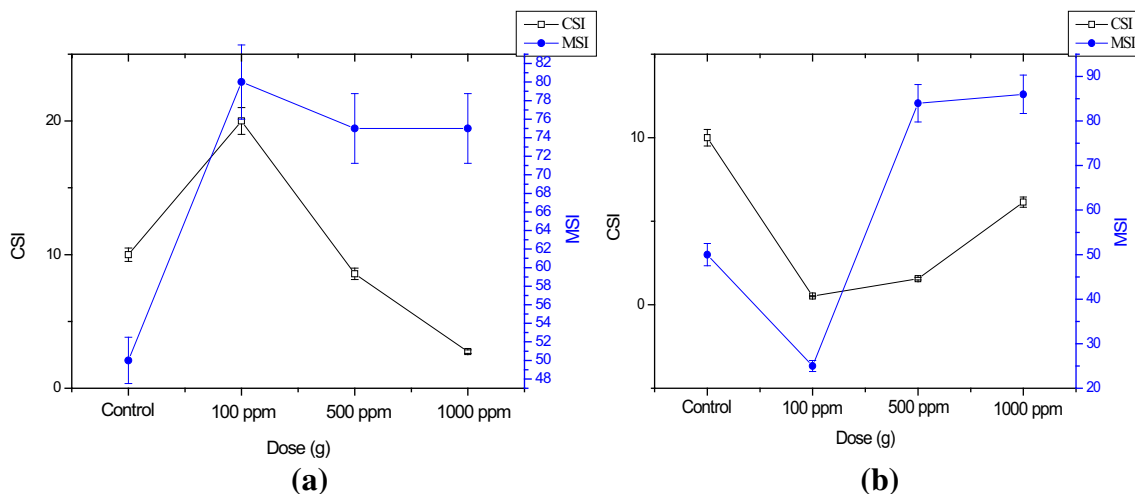


Fig. 4 Variation of CSI and MSI of chickpea (*Cicer arietinum*) leaf under different concentrations of **a** ZnO and **b** TiO₂ treatment. Chlorophyll stability index (CSI) is highest for 100 ppm ZnO-treated plants, whereas the same shows lowest membrane stability index (MSI)

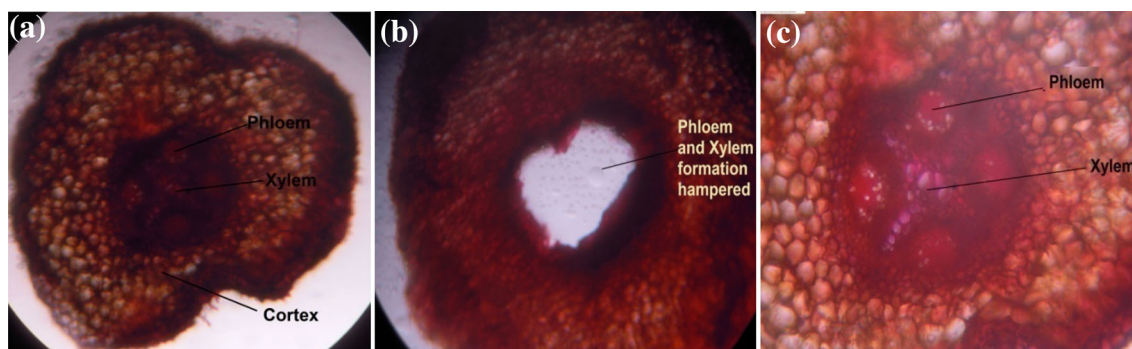


Fig. 5 Transverse section of roots of **a** control plant (magnification $\times 400$), **b** ZnO-treated plant (magnification $\times 400$) and **c** TiO₂-treated plant (magnification $\times 1000$) shows normal root stellar structure of

control and TiO₂-treated plants, but stele formation is hampered in ZnO-treated plant root

and damage of root membrane of rice seedlings (Shaw and Hossain 2013a, b).

4 Conclusions

It is well reported that nanoparticles have both adverse and beneficial effects on seed germination and also physiological action of plant system. But present study results revealed that both ZnO and TiO₂ nanoparticles do not show same impact on germination as well as biochemical constituents of *Cicer arietinum*. Results demonstrated that during early stage of germination, ZnO nanoparticles had adverse impact than TiO₂ nanoparticles. However, significant statistical difference ($p < 0.001$) in pigment level was recorded for ZnO nanoparticle-treated plant over that of treated with TiO₂ nanoparticles. But the level of primary and secondary metabolite such as sugar, protein and phenol

was recorded high in TiO₂ nanoparticle-treated plant than ZnO nanoparticle-treated plants. But lipid peroxidation data suggest that cell organelles are less affected in ZnO nanoparticle-treated plant than TiO₂ nanoparticles treatment. However, root ion leakage data suggest TiO₂ nanoparticle-influenced plants less than ZnO nanoparticles. Therefore, finally it can be concluded that both ZnO and TiO₂ nanoparticles exhibited variable effect on plant system. Present results are consistent with idea that this kind of studies, performed in different biological species and with different experimental conditions, is particularly important and urgent. In fact from one hand, it is basic to understand the mechanisms of nanotoxicity of many different varieties of nanomaterials, and from the other, it is pivotal to get valuable indications in terms of use and disposal of manufactured nanoparticles that may represent a powerful tool to improve human life. However, more research is needed in different disciplines to deeply

understand some of the possible environmental hazards related to nanomaterials.

Acknowledgements This research work was financially supported by WBDST as major project (Ref No. ST/P/SNT/15G-10/2015 dated 11/1/2017). In addition, authors are thankful to all faculty members of the Department of Environmental Science, The University of Burdwan, Burdwan, West Bengal, for their unconditional support to execute the present research work.

Compliance with ethical standards

Conflict of interest Authors declared that they have no conflicts of interest.

References

- Anjum NA, Gill SS, Duarte AC, Pereira E, Ahmad I (2013) Silver nanoparticles in soil-plant systems. *J Nanoparticle Res* 15:1896. doi:10.1007/s11051-013-1896-7
- Bates L, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. *Plant Soil* 39:205–207
- Boonyanitipong P, Kumar P, Kositsup B, Baruah S, Dutta J (2011) Effects of Zinc Oxide Nanoparticles on Roots of Rice *Oryza Sativa* L. *Int Conf Environ BioSci* 21:172–176
- Burman U, Sainib M, Kumar P (2013) Effect of zinc oxide nanoparticles on growth and antioxidant system of chickpea seedlings. *Toxicol Environ Chem* 95(4):605–612
- Carvajal M, Alcaraz CF (1998) Why titanium is a beneficial element for plants. *J Plant Nut* 21(4):655–664. doi:10.1080/01904169809365433
- Castiglione MR, Giorgetti L, Geri C, Crempnini R (2011) The effects of nano TiO₂ on seed germination, development and mitosis of root tip cells of *Vicia narbonensis* L. and *Zea mays* L. *J Nanoparticle Res* 13:2443–2449
- Doshi R, Braida W, Christodoulatos C, Wazne M, O'Connor G (2008) Nano-aluminium transport through sand columns and environmental effects on plants and soil communities. *Environ Res* 106:296–303
- Gupta SM, Tripathi M (2011) A review on TiO₂ nanoparticles. *Chin Sci Bull* 56(16):1639–1657
- Haghighi M, Jaime A, da Silva T (2014) The effect N-TiO₂ on Tomato, Onion and Radish seed germination. *J Crop Sci Biotech* 17(4):221–227
- Hatami M, Ghorbanpour M (2014) Defense enzyme activities and biochemical variations of *Pelargonium zonale* in response to nanosilver application and dark storage. *Turk J Biol* 38:130–139
- Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* 125:189–198
- Hedge JE, Hofreiter BT (1962) In: Whistler RL, Be Miller JN (eds) *Carbohydrate chemistry* 17. Academic Press, New York
- Homaee MB, Ehsanpour AA (2015) Physiological and biochemical responses of potato (*Solanum tuberosum*) to silver nanoparticles and silver nitrate treatments under in vitro conditions. *Ind J Plant Physiol* 20(4):353–359
- Hong F, Yang F, Liu C, Gao Q, Wan Z, Gu F, Wu C, Ma Z, Zhou J, Yang P (2005) Influences of nano TiO₂ on the chloroplast aging of spinach under light. *Biol Trace Elem Res* 104:249–260
- Khodakovskaya M, Dervishi E, Mohammad M, Xu Y, Li Z, Watanabe F, Biris AS (2009) Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. *ACS Nano* 3:3221–3227
- Khot LR, Sankaran SR, Mari MJ, Ehsani R, Schuster EW (2012) Application of nanomaterials in agricultural production and crop protection: a review. *Crop Prot* 35:64–70
- Koleyoreas SA (1958) A new method for determining drought resistance. *Plant Physiol* 33:232–233
- Kuzel S, Hruby M, Cigler P, Tlustos O, Van PN (2003) Mechanism of physiological effect of titanium leaf sprays on plant growth on soil. *Biol Trace Elem Res* 91:1–11
- Lang J, Kalbacova J, Matejka V, Kukutschova J (2010) Preparation, characterization and phytotoxicity of TiO₂ nanoparticles. *EU, Olomouc*
- Lin DH, Xing BS (2007) Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. *Environ Pollut* 150:243–250
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) *J Biol Chem* 193:265
- Lutts S, Kinet JM, Bouharmont J (1995) Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. *J of Exp Bot* 46:1843–1852
- MacLachlan S, Zalik S (1963) Plastid structure chlorophyll concentration and free amino acid composition of a chlorophyll mutant of barley. *Can J Bot* 41:1053–1062
- Mahajan P, Dhoke SK, Khanna AS, Tarafdar JC (2011) Effect of nano-ZnO on growth of Mung Bean (*Vigna radiata*) and Chickpea (*Cicer arietinum*) seedlings using plant agar method. *Appl Biol Res* 13:54–61
- Mallick CP, Singh MB (1980) In: *Plant enzymology and histo enzymology*. Kalyani Publishers, New Delhi, p 286
- Mishra V, Mishra RK, Dikshit A, Pandey AC (2014) Interactions of nanoparticles with plants: an emerging prospective in the agriculture industry. In: Ahmad P, Rasool S (eds) *Emerging technologies and management of crop stress tolerance: biological techniques*, vol 1, pp 159–180
- Mohammadi R, Maali-Amiri R, Abbasi A (2013) Effect of TiO₂ nanoparticles on The effects of Nano TiO₂ and Nano aluminium on wheat 1635 chickpea response to cold stress. *Biol Trace Elem Res* 152(3):403–410
- Najafi S, Jamei R (2014) Effect of silver nanoparticles and Pb(NO₃)₂ on the yield and chemical composition of Mung bean (*Vigna radiata*). *J Stress Physio Biochem* 10(1):316–325
- Prasad TNKV, Sudhakar P, Sreenivasuki Y, Latha P, Munaswamy V, Reddy KR, Sreepasad TS, Sajanlal PR, Pradeep T (2012) Effect of nanoscale zinc oxide particles on the germination, growth and yield of Peanut. *J Plant Nutr* 35:905–927
- Premchandra GS, Saneoka H, Ogata S (1990) Cell membrane stability, an indicator of drought tolerance as affected by applied nitrogen in soybean. *J Agric Sci Camb* 115:63–66
- Rahmani F, Peymani A, Daneshvand E, Biparva P (2016) Impact of zinc oxide and copper oxide nano particles on physiological and molecular processes in *Brassica napus* L. DOI, *Ind J Plant Physiol*. doi:10.1007/s40502-016-0212-9
- Rico CM, Morales MI, Mc Creary R, Castillo-Michel H, Barrios AC, Hong J, Tafuya A, Lee W-Y, Varela-Ramirez A, Peralta-Videa JR, Gardea-Torresdey LG (2013) Cerium oxide nanoparticles modify the oxidative stress enzyme activities and macromolecule composition in rice seedlings. *Environ Sci Technol* 47:14110–14118
- Roco MC (2003) Nanotechnology: convergence with modern biology and medicine. *Curr Opin Biotechnol* 14:337–346
- Sairam RK (1994) Effect of moisture stress on physiological activities of two contrasting wheat genotypes. *Ind J Exp Biol* 32:594–597
- Servin AD, Castillo-Michel H, Hernandez-Viezcas JA, Diaz BC, Peralta-Videa JR, Gardea-Torresdey LJ (2012) Synchrotron micro-XRF and micro-XANES confirmation of the uptake and

- translocation of TiO₂ nanoparticles in cucumber (*Cucumis sativus*) plants. *Environ Sci Technol* 46:7637–7643
- Shaw AK, Hossain Z (2013a) Impact of nano CuO stress on rice (*Oryza sativa* L.) seedlings. *Chemosphere* 93(6):906–915
- Shaw AK, Hossain Z (2013b) Impact of nano-CuO stress on rice (*Oryza sativa* L.) seedlings. *Chemosphere* 93:906–915
- Singh AL, Basu MS, Singh NB (2004) Mineral disorders of groundnut. ICAR Publications, New Delhi
- Song U, Jun H, Waldman B, Roh J, Kim Y, Yi J, Lee EJ (2013) Functional analyses of nanoparticle toxicity: a comparative study of the effects of TiO₂ and Ag on tomatoes (*Lycopersicon esculentum*). *Ecotoxicol Environ Safety* 93:60–67
- Taheri M, Qarache HA, Qarache AA, Yoosefi M (2015) The effects of Zinc-oxide nanoparticles on growth parameters of corn (SC704). *STEM Fellowship J* 1(2):17–19
- Thuesombat P, Hannongbua S, Akasit S, Chadchawan S (2014) Effect of silver nanoparticles on rice (*Oryza sativa* L. cv. KDML 105) seed germination and seedling growth. *Ecotoxicol Environ Safety* 104:302–309
- USEPA (2007) Nanotechnology white paper. 100/B-07/001. <http://www.epa.gov/osa/pdfs/nanotech/epanotechnologywhitepaper-0207.pdf>
- Vaseem M, Umara A, Hahn YB (2010) ZnO nanoparticles: growth, properties and application metal oxide nanostructures and their applications, vol 5. American Scientific Publishers, New York, pp 1–36
- Wang X, Yang X, Chen S, Li Q, Wang W, Hou C, Gao X, Wang L, Wang S (2016) Zinc oxide nanoparticles affect biomass accumulation and photosynthesis in arabidopsis. *Front Plant Sci* 6:1243. doi:10.3389/fpls.2015.01243
- Yang L, Watts DJ (2005) Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. *Toxicol Lett* 158:122–132
- Yang F, Hong F, You W, Liu C, Gao F, Wu C, Yanng P (2006) Influences of nano-anatase TiO₂ on the nitrogen metabolism of growing spinach. *Biol Trace Elem Res* 110:179–190
- Zheng L, Hong F, Lu S, Liu C (2005) Effect of nano TiO₂ on strength of naturally aged seeds and growth of spinach. *Biol Trace Elem Res* 105:83–91
- Zuverza-Mena N, Martínez-Fernández D, Du W, Hernandez-Viezcas JA, Bonilla-Bird N, López-Moreno ML, Komárek M, Peralta-Videa JR, Gardea-Torresdey JL (2016) Exposure of engineered nanomaterials to plants: Insights into the physiological and biochemical responses—a review. *Plant Physiol Biochem*. doi:10.1016/j.plaphy.2016.05.037