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Effects of TiO_2 nanoparticles on the aquatic plant *Spirodela polyrrhiza*: Evaluation of growth parameters, pigment contents and antioxidant enzyme activities

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ABSTRACT

Plants are essential components of all ecosystems and play a critical role in environmental fate of nanoparticles. However, the toxicological impacts of nanoparticles on plants are not well documented. Titanium dioxide nanoparticles (TiO_2-NPs) are produced worldwide in large quantities for a wide range of purposes. In the present study, the uptake of TiO_2-NPs by the aquatic plant *Spirodela polyrrhiza* and the consequent effects on the plant were evaluated. Initially, structural and morphological characteristics of the used TiO_2-NPs were determined using XRD, SEM, TEM and BET techniques. As a result, an anatase structure with the average crystalline size of 8 nm was confirmed for the synthesized TiO_2-NPs. Subsequently, entrance of TiO_2-NP_S to plant roots was verified by fluorescence microscopic images. Activity of a number of antioxidant enzymes, as well as, changes in growth parameters and photosynthetic pigment contents as physiological indices were assessed to investigate the effects of TiO_2-NPs on *S. polyrrhiza*. The increasing concentration of TiO_2-NPs led to the significant decrease in all of the growth parameters and changes in antioxidant enzyme activities. The activity of superoxide dismutase enhanced significantly by the increasing concentration of TiO_2-NPs. Enhancement of superoxide dismutase activity could be explained as promoting antioxidant system to scavenging the reactive oxygen species. In contrast, the activity of peroxidase was notably decreased in the treated plants. Reduced peroxidase activity could be attributed to either direct effect of these particles on the molecular structure of the enzyme or plant defense system damage due to reactive oxygen species.

Introduction

Nanoparticles are the atomic or molecular masses with whole dimensions ranged between 1 and 100 nm[2] (Kim et al., 2011; Klaine et al., 2008). Although nanoparticles exist in the environment, their multiple uses for industrial, biomedical and household purposes over the last decade have led to their increased release into the environment

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(Manchikanti and Bandopadhyay, 2010). The small size and large surface area of these particles enable them to cross cell membranes. Therefore, remarkable development of nanotechnology is expected to affect health of plants, animals and humans. Among nanoparticles, titanium dioxide nanoparticles (TiO_2-NPs) are ecologically very important due to their high stability, antibacterial, antifungal and photocatalytic properties (De Filpo et al., 2013; Shi et al., 2013). TiO_2-NPs are reported to be used commonly in cosmetics (*e.g.*, sunscreens), medicine (*e.g.*, anticancer drugs), household products, surface coating (*e.g.*, paint and printing ink), gas sensors, solar panels, light emitting diodes and vehicle anti fog mirrors (De Filpo et al., 2015; Kim et al., 2013; Macwan et al., 2011; Shi et al., 2013). Broad applications of TiO_2-NPs have led to their uncontrolled release into the environment especially aqueous environment as the most important and perhaps the last destination (Hu et al., 2012).

Plants are fundamental components of all ecosystems and have a critical role in the fate of nanoparticles in the environment (Monica and Cremonini, 2009). Aquatic plants are actually the basis of the aquatic food web (Glenn et al., 2012). Studies of phytotoxic effects of nanoparticles have been mainly focused on crop plants and few studies have been reported on non-crop plant species. *Spirodela polyrrhiza*, a perennial aquatic plant, belongs to monocotyledons and the Lemnaceae family. Duckweeds scatter around the world, from freshwater wetlands and swamps to irrigation canals floating on the water surface. Each plant is a round flat disk with a width of 0.5–1 cm with several minute roots. Simple morphology and ease of culture in the laboratory make this plant species suitable for bioassay (Charpentier et al., 1987; Hiscock, 2003).

S. polyrrhiza have been previously used in several studies for evaluation of toxic effects of heavy metals and nanoparticles such as silver and zinc nanoparticles (Charpentier et al., 1987; Hu et al., 2013; Jiang et al., 2012). In the current study, TiO_2-NPs uptake by *S. polyrrhiza* and its consequent effects on the plant were investigated. Based on previous reports, upon entering the cells, nanomaterials can produce reactive oxygen species (ROS) and induce oxidative stress (Jiang et al., 2012, 2014). Therefore, some growth parameters, photosynthetic pigment contents and the activity of antioxidant enzymes such as catalase (CAT, EC 1.11.1.6), superoxide dismutase (SOD, EC 1.15.1.1) and peroxidase (POD, EC 1.11.1.7) were assessed to explore the effects of TiO_2-NPs on *S. polyrrhiza*.

1. Material and methods

1.1. Plant material and cultivation condition

Floating *S. polyrrhiza* plants were collected from Anzali wetland region in north of Iran and transported to the laboratory. Fronds were disinfected with sodium hypochlorite 0.5% (*V/V*) and were washed several times with distilled water. Then, they were cultured in the glass aquaria containing half-strength *S. polyrrhiza* specific culture medium. The basic growth medium was composed of the following materials (in mmol/L): 0.099 $CaCl_2$, 0.0002 $CoCl_2.6H_2O$, 0.0005 $CuCl_2$, 0.021 $FeSO_4{\cdot}7H_2O$, 0.092 H_3BO_3, 0.155 K_2HPO_4, 0.097 K_2SO_4, 0.367 KH_2PO_4, 1.997 KNO_3, 0.198 $MgSO_4{\cdot}7H_2O$, 0.012 $MnCl_2{\cdot}2H_2O$, 0.019 Na_2-EDTA$\cdot 2H_2O$ and 0.00003 $(NH_4)_6Mo_7O_{24}$. The pH of medium was adjusted at 6.5 ± 0.5 (Dosnon-Olette et al., 2010). Nutrient media in aquaria was replaced every seven days. The cultivated plants were located at 26 ± 2°C under 16 hr/8 hr(light/dark) photoperiod.

1.2. Characterization of TiO_2-NPs

The naoparticles used were TiO_2 Millennium PC-500 (Lot No. 6293000124) containing 99% anatase crystalline structure (Ahlstrom Research & Services, Pont-Evêque, France). The crystalline phase characteristics of these nanoparticles were examined using X-ray Diffraction (XRD) (Siemens D5000, Germany), Scanning Electron Microscopy (SEM) (Hitachi S-4200, Japan) and Transmission Electron Microscopy (TEM) (Zeiss EM 900, Germany). In addition, the surface area and total pore volume of the NPs were determined using the multipoint Brunauer–Emmett–Teller method (Brunauer et al., 1938) and implemented by a sorptometer (Micromeritics, Gemini series, America).

1.3. TiO_2-NP treatment

The mother suspension was prepared containing 10 mg/L TiO_2-NPs in distilled water by using ultrasonic bath (Sonorex Bandelin Digi Tec, UK) to disperse the nanoparticles. To perform the assays, series of nanoparticle concentrations (0.05, 0.1, 1, 5, 10 mg/L) were prepared by using the mother suspension with *S. polyrrhiza* specific nutrient solution. Nutrient solution without TiO_2-NP_S was used as control sample.

1.4. Epifluorescence microscopy

Epifluorescence microscopy technique was employed to examine the uptake and localization of TiO_2-NPs in the treated plants. Collection of the treated and control plants was performed 6 days after the beginning of treatment. Plant materials were stained with 0.1% Auramine O solution in water for 10 min. The samples were observed by means of an Olympus BX51 (Olympus optical Co., Ltd. Tokyo, Japan) fluorescence microscope supplied with the catadioptric lenses UMPlanFL-BDP and the BXRFA (Olympus optical Co., Ltd. Tokyo, Japan) fluorescence illuminator. The best fluorescence excitation was attained with U-MWB3 (480–510 nm) and U-MWG3 (510–550 nm) mirror cube units. A succession of pictures from consecutive focal plates of involved tissues were taken by an Evolution MP cooled CCD (Media Cybernetics, USA). Depth of field was recovered by the stack z-projection to develop the final images as formerly described (Movafeghi et al., 2010).

1.5. Growth measurement

To assess the effect of TiO_2-NPs on the relative frond number (RFN), as a growth rate parameter, frond numbers were

inspected during 20 days on day 0, 4, 8, 12, 16 and 20 at a series of TiO_2-NP concentrations (0.05, 0.1, 1, 5, 10 mg/L) and control sample. RFN was calculated by Eq. (1) (Khataee et al., 2013; Mitsou et al., 2006):

$$RFN = (N_1 - N_0)/N_0, \quad (1)$$

where N_0 and N_1 are frond numbers at day 0 and day n, respectively.

Changes in frond average length during 20-day of experimentation were also examined. The length of fronds was measured by means of a SMZ 1500 Nikon stereomicroscope. To determine dry weight, the washed and cleaned plants were dried in an oven at 60°C for 16 hr and weighted.

The analysis of the growth parameters was conducted using four replicates for each treatment.

1.6. Determination of photosynthetic pigment contents

Chlorophyll and carotenoid contents of fronds were measured spectrophotometrically. Plants were primed with different concentrations of TiO_2-NP_S for 6 days. Subsequently, 0.1 g of the fronds was homogenized in acetone and centrifuged at 2000 ×g for 10 min. Supernatant absorbance was assessed at 470, 645 and 662 nm and pigments contents were measured by the equations proposed by (Lichtenthaler, 1987).

1.7. Assay of antioxidant enzymes activity

Antioxidant enzymes activity assay was performed in four replicates. The enzyme extract was obtained using homogenizing fresh fronds in phosphate buffer (0.01 mol/L, pH = 7.0), comprising 0.2% (w/v) polyvinyl pyrrolidone (PVP). The suspension was centrifuged at 2000 ×g for 20 min at 4°C. The supernatant was used for enzymes activity and protein content assays.

To determine the activity of superoxide dismutase (SOD, EC 1.15.1.1) the ability of the extract in inhibiting of photochemical depletion rate of nitroblue tetrazolium (NBT) was assessed. The suspension composition was made up of 2.65 mL potassium phosphate buffer solution (67 mmol/L, pH = 7.8), 0.2 mL EDTA (0.1 mmol/L) mixed with 0.3 mmol/L sodium cyanide, 50 μL enzyme extract, 0.1 mL NBT (1.5 mmol/L) and 50 μL riboflavin (0.12 mmol/L). The mixtures were placed under light intensity of 5000 lx for 15 min. The absorbances of solutions were measured at 560 nm. One unit of SOD was determined as that enzyme quantity that caused 50% inhibition of the NBT reduction under the assay condition (Winterbourn et al., 1976).

Guaiacol peroxidase (POD, EC 1.11.1.7) activity was assayed as the method reported by (Chance and Maehly, 1955). The reaction composition had 0.1 mol/L citrate–phosphate-borate buffer solution (pH = 7.5), 15 mmol/L guaiacol, 25 μL enzyme extract and 3.3 mmol/L H_2O_2. Throughout guaiacol polymerization, the enhancement of absorbance was registered at 470 nm for 3 min. Enzyme activity was computed by the extinction coefficient of 26.6 (mmol/L)/cm for guaiacol. 1 mmol/L guaiacol to tetraguaiacol per min was considered as one unit of enzyme.

For evaluating catalase (CAT, EC 1.11.1.6) activity decomposition of H_2O_2 was estimated by spectrophotometry at 240 nm during 3 min. The reaction mixture contained citrate–phosphate–borate buffer solution (0.1 mol/L, pH = 7.5), 50 μL enzyme extract and 10 mmol/L H_2O_2. One micromolar reduction of H_2O_2 per minute shows one unit of CAT activity by an extinction coefficient 39.4 (mol/L)/cm (Obinger et al., 1997).

Total protein concentration in samples was determined by the method of Bradford using bovine serum albumin (BSA) as a standard (Bradford, 1976).

1.8. Statistical analysis

The experimental assays including growth measurements, determination of photosynthetic pigment contents and assessment of antioxidant enzymes activity consisted of 4 replicates for each TiO_2-NPs treatment. The obtained data were statistically analyzed for their significance. An analysis of variance (ANOVA) was performed using Tukey multiple comparison test with Graph Pad Instat 3 software.

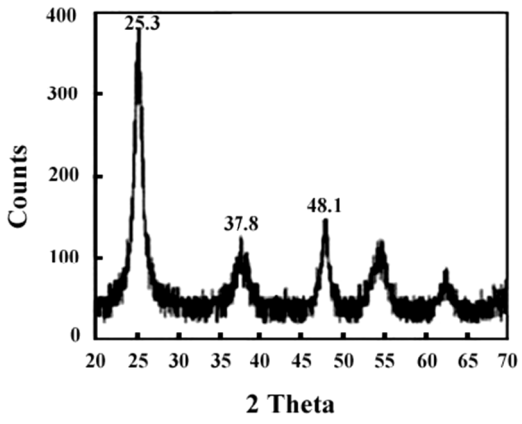

Fig. 1 – X-ray diffraction pattern of TiO_2-NPs, PC-500. Their average size is 8 nm. TiO_2-NPs: titanium dioxide nanoparticles.

2. Results and discussion

2.1. Structural characterization of TiO_2-NPs

TiO_2 belongs to the transition metal oxides family. The four commonly known polymorphs of TiO_2 found in nature are anatase, brookite, rutile, and TiO_2 (monoclinic) structure (Carp et al., 2004). XRD analysis of TiO_2-NPs has been assessed the phase composition and the crystalline size of prepared TiO_2 samples (Fig. 1). The peaks of samples were identified by X-ray diffraction according to 2θ which confirmed an anatase structure at 2θ = 25.3°, 37.8° and 48.1° (Ba-Abbad et al., 2012). Anatase TiO_2 has a tetragonal structure. The anatase phase is more stable and preferable because of lower capacity to absorb oxygen and higher degree of hydroxylation in the anatase phase (Muscat et al., 2002; Tanaka et al., 1991). It has been shown that anatase TiO_2-NPs can be effectively internalized and can lead to extensive metabolic changes in plant cells (Kurepa et al., 2010, 2014). Accordingly,

prepared TiO_2-NPs samples seemed to be structurally fit for the aims of the current study.

According to Debye–Scherrer formula, the average crystalline size of synthesized TiO_2-NPs was 8 nm. SEM and TEM images of TiO_2-NP_S confirmed the small size and crystalline structure of nanoparticles (Figs. 2 and 3). Accordingly, TiO_2-NPs characterizations including surface area and pore volume evaluated by Brunauer–Emmett–Teller (BET) are listed in Table 1. On the aspect of plant uptake, little investigations have been performed to study how the particle size and other physical chemical properties influence the uptake kinetics and the fate of nanoparticles in plants. However, particle size and shape could be clearly important factors regulating nanoparticle uptake in plants. Accumulation of TiO_2-NPs in wheat roots could only occurred if NPs are less than 140 in diameters, with higher accumulation that occurred when NPs were much smaller (in size range 14–22 nm) (Larue et al., 2012). Direct penetration of NPs through plant cell wall can be imagined for smaller size NPs as the cell wall may limit the passage of NPs larger than 20 nm (Zhai et al., 2014). Taken together, uptake of nanoparticles by plant systems was negatively correlated with particle size, although there is much species-dependent variation in the response to nanoparticle treatments (Ma et al., 2010). Accordingly, the average crystalline size of synthesized TiO_2-NPs (8 nm) appeared to be in suitable size range for uptake by roots of *S. polyrrhiza*.

2.2. Microscopic evidence for TiO_2-NP uptake

Root tissues of *S. polyrrhiza* lack florescent properties. By staining the living roots with Auramine O, uptake of TiO_2-NPs by treated *S. polyrrhiza* plants was confirmed using epifluorescence microscopy (Fig. 4). The presence of nanoparticle aggregates was indicated by shiny light spots inside the tissues and appeared as optically dense signals. The nanoparticle aggregates were distinguished as spots with different sizes in the cells. Similar to our observation, the entrance of super magnetic oxide nanoparticles to the soybean plant tissue has been shown in another study by florescence microscopy imaging (Ghafariyan et al., 2013). This entrance can be elucidated by the means of capillary action, osmotic force, cell wall pores and cell-to-cell connection *via* plasmodesmata (the regulated symplastic pathway) (Nowack and Bucheli, 2007). Because the uptake and translocation inside plant could be affected by size, shape, composition and other features of nanoparticles (Jia et al., 2005; Zhu et al., 2009), it seemed that structural characteristics of TiO_2-NPs allow their remarkable movement into the root cells of *S. polyrrhiza*.

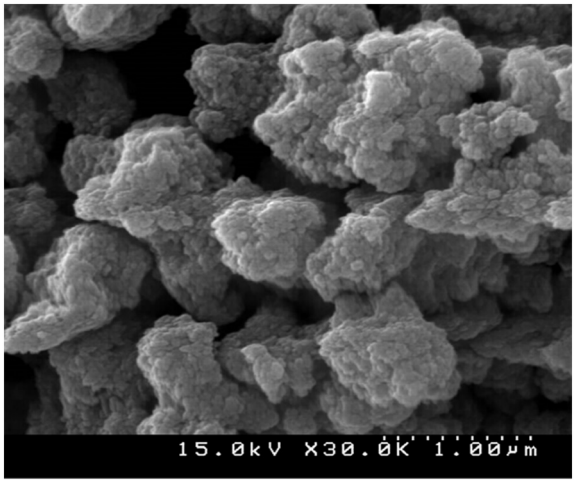

Fig. 2 – Scanning electron micrograph of TiO_2-NPs demonstrating the size and shape of TiO_2-NPs. TiO_2-NPs: titanium dioxide nanoparticles.

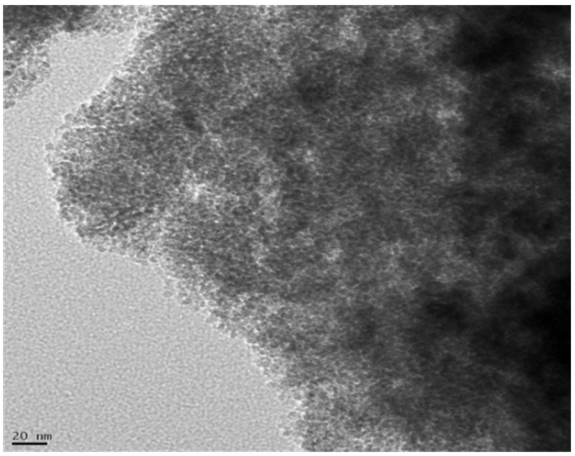

Fig. 3 – Transmission electron microscopic image of TiO_2-NPs. TiO_2-NPs: titanium dioxide nanoparticles.

2.3. The effects of TiO_2-NPs on growth of *S. polyrrhiza*

Growth parameters in terms of relative frond number, frond average length and dry weight were negatively affected by different concentrations of TiO_2-NPs during 20 days of experimentation. The decrease in all of the growth parameters by the increasing concentration of TiO_2-NPs was statistically significant (Fig. 5). This is consistent with previous studies which reported growth reduction in *S. polyrrhiza* by treatment of ZnO and L-Cys-capped CdS nanoparticles (Hu et al., 2013; Khataee et al., 2014). In addition, phytotoxicity in other duckweeds like *Landoltia punctata* affected by CuO (Shi et al., 2011), *Lemna minor* by silver nanoparticles (Gubbins et al., 2011) and *Lemna gibba* by silver nanoparticles (Oukarroum et al., 2013) have been reported. Although TiO_2-NPs are manufactured and used worldwide in large quantities, research on phytotoxicity of TiO_2-NPs has come to perplexing results, with a wide range from strong toxicity to positive effects. In the present study, reduction in growth parameters by increasing the concentration of TiO_2-NPs confirmed the inhibitory effect of TiO_2-NPs on the growth of *S. polyrrhiza*. Decline in growth by increasing concentration of nanoparticles can be considered as nanoparticles toxicity indicator due to inhibitory effect on photosynthesis, protein synthesis and nitrogen fixation or increase in activity of proteases (El-Shahate et al., 2011; Sood et al., 2011).

Table 1 – Characteristics of the used TiO_2-NP_S.

Used TiO_2	Crystalline phase	Total pore volume (cm^3/g)	Surface area (m^2/g)	Crystalline mean size (nm)
Millennium PC-500	Anatase	0.3104	320.76	8

2.4. The effects of TiO_2-NPs on photosynthetic pigments content

Changes in the content of photosynthetic pigments were induced by different toxicity and stress conditions. Actually, chlorophyll content is one of the most sensitive indicators of toxicity (Priya, 2013). The content of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids of *S. polyrrhiza* after 6-day treatment of TiO_2-NPs at different concentrations were measured. Chlorophyll b at 0.1 mg/L ($p < 0.01$), 1, 5 and 10 mg/L ($p < 0.001$) concentrations; chlorophyll-a and total chlorophyll at 1 mg/L ($p < 0.01$), 5 and 10 mg/L ($p < 0.001$) concentrations and carotenoids at 1 and 5 mg/L ($P < 0.01$) and 10 mg/L ($P < 0.001$) concentrations were affected and decreased (Fig. 6). These results are in consistent with previous reports, demonstrating a reduction in chlorophyll content of *S. polyrrhiza* under nano-silver (Jiang et al., 2012), and nano-zinc (Hu et al., 2012) treatments. Chlorophyll content reduction of the other duckweeds such as *Landoltia punctata*, under nano copper oxide, *Hydrilla verticillata* under nano zinc oxide and cadmium sulfide and *Salvinina natans* leaves under nano zinc oxide treatment (Hu et al., 2014; Lalau et al., 2015) have also been reported. Reduction in the level of photosynthetic pigments influences negatively the growth and development processes due to decreased photosynthesis rate and therefore could be considered as an indicator of nanoparticle toxicity.

2.5. Evaluation of antioxidant enzyme activities

Plants can employ several defense mechanisms against oxidative stress and the harmful effects of ROS. ROS are actually chemical markers that predict the toxicity of nanoparticles; when the balance between ROS and antioxidant defenses disrupts, oxidative stress occurs (Lee et al., 2013). Activation of antioxidant enzymes such as peroxidase, catalase and superoxide dismutase, and also production of low molecular weight molecules are examples of plant defense mechanisms (Fang and Zheng, 2002). The activity of antioxidant enzymes may change depending on the concentration and type of nanoparticles (Castiglione et al., 2014).

The activity of antioxidant enzymes on the first, third and sixth day after treatment with different concentrations of TiO_2-NPs was measured. The activity of SOD at concentrations of 5 mg/L on the sixth day ($p < 0.05$), 10 mg/L on the first, third ($P < 0.05$) and the sixth day ($p < 0.001$) showed a significant

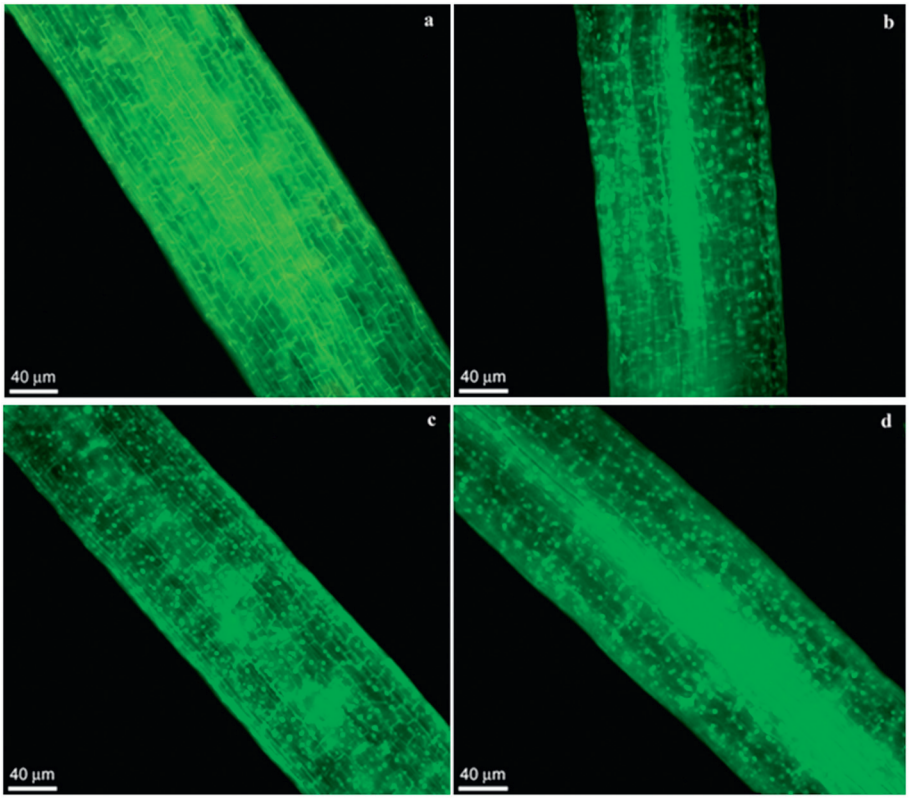

Fig. 4 – Fluorescence microscopic images of roots in control (a), 0.05 (b), 0.1 (c) and 1 (d) mg/L of TiO_2-NPs suspensions. Microscopic images present the remarkable shiny dots inside the roots indicating an increase in aggregations from (a) to (d). TiO_2-NPs: titanium dioxide nanoparticles.

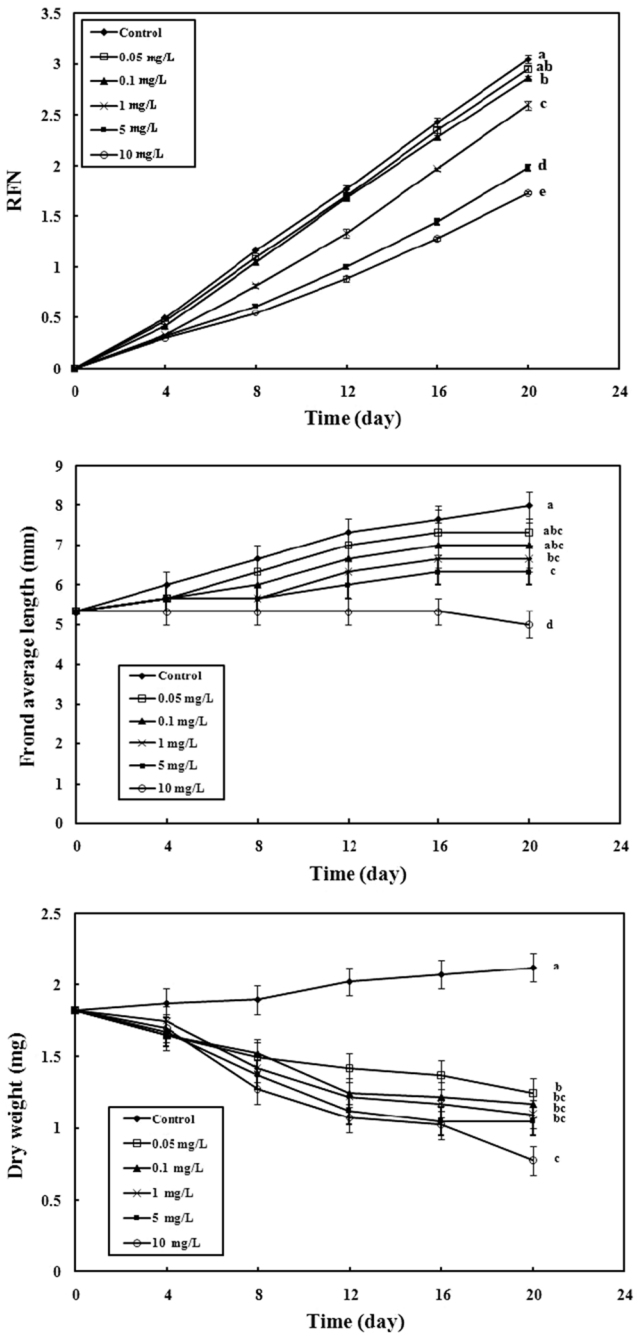

Fig. 5 – The effect of different concentrations of TiO_2-NPs on the relative frond number (RFN) (a) frond average length (b) and dry weight (c) of *S. polyrrhiza*. Different letters indicate significant differences at $p < 0.05$ on the twentieth day. TiO_2-NPs: titanium dioxide nanoparticles.

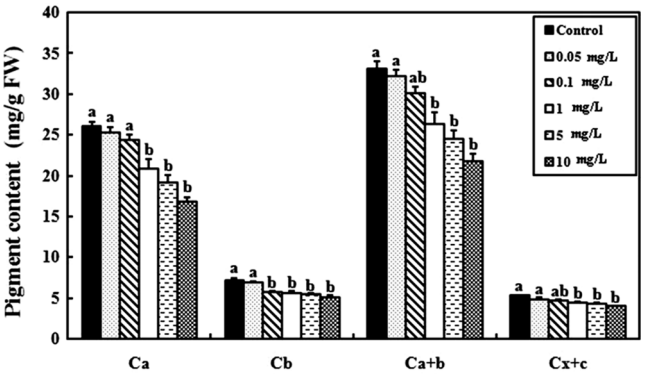

Fig. 6 – The effect of different concentrations of TiO_2-NPs on photosynthetic pigments content of *S. polyrrhiza*, C_a (chlorophyll a), C_b (chlorophyll b), C_{a+b} (total chlorophyll a and b) and C_{x+c} (carotenoids). Different letters denote significantly different values at $p < 0.05$. TiO_2-NPs: titanium dioxide nanoparticles.

increase (Fig. 7a), which is in agreement with findings on the effect of increasing concentration of zinc oxide and silver nanoparticles in *S. polyrrhiza* (Hu et al., 2013; Jiang et al., 2014). Moreover, the similar results are reported both in soybean seedling treated by SiO_2 and TiO_2 and in *Triticum aestivum* treated by nano alumina (Lu et al., 2001; Riahi-Madvar et al., 2013). The essential act of SOD as an antioxidant enzyme in higher plants has been identified. In fact, SOD protects cellular components against ROS (Alscher et al., 2002). It is the first enzyme against oxygen radicals and catalyzes conversion of superoxide radicals to either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2) (Khataee et al., 2012). Increased SOD activity with increasing concentration of nanoparticles may be explained as a direct response to the increased production of superoxide radicals (Hafsi et al., 2011).

In contrast, POD activity was notably decreased in the treated plants at the concentrations of 1 mg/L on the third ($p < 0.05$) and sixth day ($p < 0.01$) 5 and 10 mg/L on the first, third and sixth day ($p < 0.01$) (Fig. 7b). POD is one of the key enzymes in the antioxidant defense systems for the conversion of H_2O_2 to water and oxygen using a variety of organic and inorganic compounds and prevents the formation of hydroxyl radicals (Du et al., 2011; Hazani et al., 2013). Our findings are in agreement with the studies on *S. polyrrhiza*, *Cicer arietinum* and *S. natans* under nano-zinc oxide, which reported a decrease in the POD activity due to the damage to the plants defense system caused by excessive production of ROS (Burman et al., 2013; Hu et al., 2014, 2013). The reduced activity of POD may be due to alteration in the enzyme molecular structure (Van der Oost et al., 2003).

However, the activities of SOD and POD are not significantly different at two concentrations of 0.05 and 0.1 mg/L. Probably; the oxidative stress induced by these concentrations of NPs is not adequate to alter the activity of SOD and POD.

No significant difference in CAT activity was observed in treated plants compared to the control ($p > 0.05$) (Fig. 8). No changes in CAT activity in *S. polyrrhiza* (Jiang et al., 2014), *Prosopis juliflora-velutina* and *Pisum sativum* under ZnO nanoparticles have been previously reported (Hernandez-Viezcas et al., 2011; Mukherjee et al., 2014). The lack of enzyme activity has been observed in wheat treated with copper oxide and zinc oxide nanoparticles (Castiglione et al., 2014). On the other hand, some studies have different results, for example, toxicity of CdS nanoparticles on aquatic plant *Hydrilla verticillata* and CuO and ZnO nanoparticles on *Cucumis sativus*

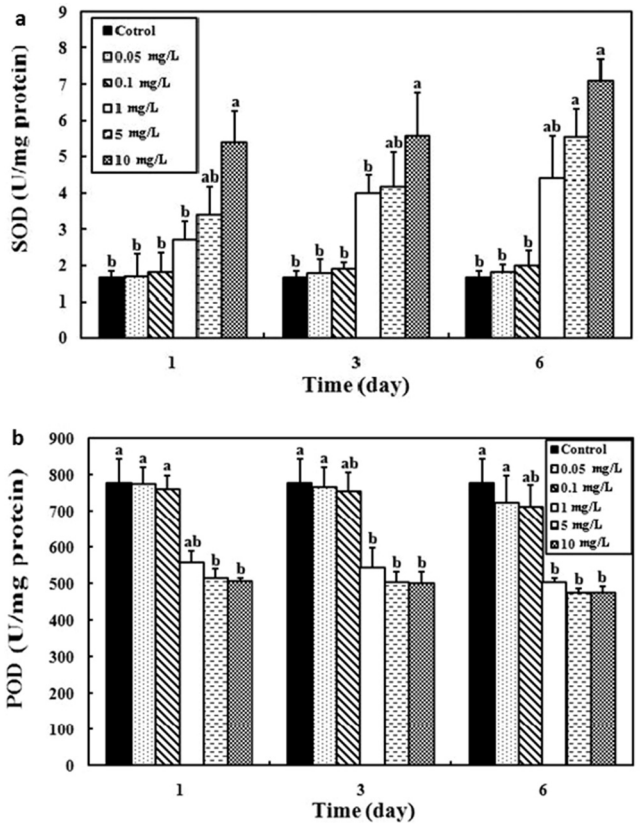

Fig. 7 – Changes in superoxide dismutase (a) and peroxidase (b) activities in *S. polyrrhiza* plants treated with TiO_2-NPs. Different letters indicate significant differences at $p < 0.05$ on each treatment days. TiO_2-NPs: titanium dioxide nanoparticles.

showed an increase in CAT activity (Kim et al., 2012; Priya, 2013). These results confirmed that different effects of naoparticles on plants depend not only on physical and chemical characteristics of nanoparticles but also on plant species.

3. Conclusions

The current study confirmed the uptake of TiO_2-NPs by roots of *S. polyrrhiza* plants using epifluorescence microscopy. In addition, a significant reduction in growth parameters such as RFN and frond size, photosynthetic pigment contents and the activity POD were observed with increasing concentrations of TiO_2-NPs. In contrast, the activity of SOD was increased and the activity of CAT did not show significant changes. Increased SOD activity could be due to elevated level of oxygen species produced in response to the entry of nanoparticles into the plant. Therefore, this enzyme plays an essential role in preventing oxidative damage, whereas the reduced peroxidase enzyme activity could attribute to either the plant defense system damage caused by ROS or the direct

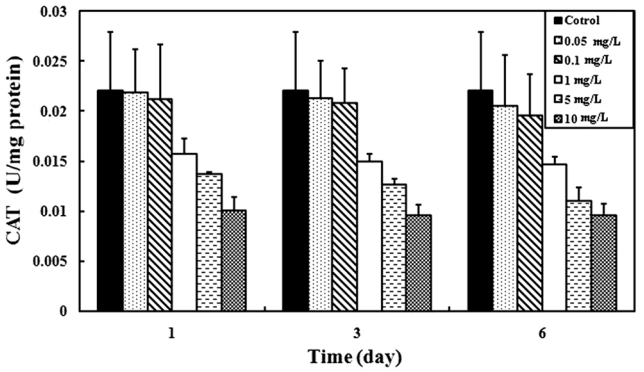

Fig. 8 – Catalase activity in *S. polyrrhiza* treated with TiO_2-NPs (No significant difference with control plant was observed at $p > 0.05$). TiO_2-NPs: titanium dioxide nanoparticles.

effect of nanoparticles on the molecular structure of the enzyme.

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