Pak. J. Bot., 52(4): 1229-1234, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-4(2)

THE REFLECTION OF THE PHOTOCATALYTIC PROPERTIES OF TiO_2 NANOPARTICLES ON PHOTOSYNTHETIC ACTIVITY OF *SPINACIA OLERACEA* PLANTS

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Abstract

The current era is the era of Nanotechnology which offers the potential advantages in almost all fields of Science, Engineering and Medicinal fields coupled with its potential damages including, economic interference leading to possible threats to the environment. In the present investigation, photocatalytic characteristics of TiO_2 Nano-Particles (NPs) on photosynthetic activity of the *Spinacia oleracea* evaluated in a greenhouse experiment in triplicate. This article reveals the interaction of TiO_2 NPs which results in several physicochemical alterations in *Spinacia oleracea* plants. It was observed that the application of TiO_2 NPs has shown optimistic impacts on plant root and shoot length, fresh and dry biomass and moisture contents in comparison with control plants. The progressive influence of TiO_2 NPs on processes of photosynthetic activity of the plants linked with improved absorption and transfer of solar energy by active electrons of pigments. It established that it was the consequences of the photocatalytic properties of titanium NPs followed by an increase in starch contents. The lower contents of reducing sugars indicate the active transfer of these molecules into starch contents in comparison of control plants. The identical concentration of pigments like Carotenoid (mg/g) and Xanthophyll (mg/g) in control and TiO_2 NP plants showed inhibition of oxidative stress; confirmed through benzoic acid test. The starch iodometry test proves the activated metabolic pathway of plants in the presence of TiO_2.

Key words: Photocatalytic Properties, TiO_2 Nanoparticles, *Spinacia Oleracea*

Introduction

The Titanium (Ti), a physiologically interesting environmental friendly chemical element. It is the second most abundant ninth transition metal in the earth's crust (Feizi *et al.,* 2012). At Nano, level Titanium dioxide NPs have large applications (Tan *et al.,* 2018). Though Ti is not lethal for living beings while at higher concentration, it acts against bacteria and plants (Jaberzadeh *et al.,* 2013; Kužel *et al.,* 2003). It is one of the most manufactured planned nanoparticles commonly applied in food additives, pigments, photocatalysis, and individual protective foodstuffs. This extensive variations in uses has directed to the infrequent and prevalent spreading of nano-TiO_2 in numerous ecological areas, followed by dissimilar impacts on a biological entity (Tan *et al.*, 2018; Morteza *et al*., 2013). Nanotechnology has extensive applications in the field of agricultural research, like disease prevention, propagative Science and Technology, relocation of energy and other useful by-products from agronomic and foodstuff wastes through enzymatic Nano bioprocessing, and treatment of plants related to the Nanocides (Jaberzadeh *et al.,* 2013; Feizi *et al.,* 2012). It was observed that the yield of various crops increases by about 10–20% when treated with Ti NPs. The high yield of some crops such as paprika (*Capsicum annum* L.) and green alga (*Chlorella pyrenoidosa*) were the consequences in enhancement of crucial element contents, an intensification in the accomplishments of enzymes such as nitrate reductase, peroxidase, nitrate reductase, catalase and an augmentation of the photosynthetic components of the plants tissues (Feizi, *et al.,* 2012; Kužel *et al*., 2003). Mahmoodzadeh *et al.,* (2013) suggested that the higher concentrations of TiO_2 improve the germination, root growth of the treated seeds. It also evaluated that the ameliorating soil salinity conditions were restore under treatment of nanoparticles $nTiO_2$ in the broad bean which is an essential leguminous crop where three different doses of $nTiO_2$ viz., 0.01, 0.02 and 0.03% investigated with normal plant and treated plants. TiO_2 NPs-mediated plants showed positive effects in saline soil conditions (Abdel *et al*., 2018; Burke, *et al.*, 2015). It was observed that the dose 0.01% of $nTiO_2$ expressively elevated the over all growth conditions of plants such as fresh/dry biomass follwod by expenion of leaves with shoot length over normal conditions. These stimulating growth-promoting effects were the consequences of high level of chlorophyll contents, soluble sugars and proline, with enriched antioxidant enzymes activated metabolic pathway of plants (Daghan *et al.,* 2018; Li *et al.*, 2015; Asli *et al.*, 2009).

This report provides a study into the ignored perspective of TiO_2 NPs that discarded colloidal suspensions of TiO_2 nanomaterials after its original use; may have supplementary or antagonistic impacts on the permeability of root cell walls. The interaction of these TiO_2 NPs evaluated with morphology, growth rate, and important compounds like pigments, starch, sugars and, a protein contents of plants.

Materials and Methods

Preparation and characterization of TiO_2 NPs: The TiO_2 NPs were synthesized by phase transferred solvent hydrothermal method using ethanol. These NPs were fine white powder form with anatase structure and used in dye wastewater removal. These were collected from the Department of Chemistry, University of Karachi, after its original use for studying the impact on running stream, used for irrigation. The seeds of *Spinacia oleracea* purchased from Nursery of the University of Karachi. All chemicals used in this study were of analytical grade and purchased from Sigma.

Seed germination and exposure: The seeds were rinsed and immersed for three hours in distilled water. After that, these seeds were sown in each pot in triplicate. Soil pots prepared for the cultivation of *Spinacia oleracea* plants to investigate the impact of TiO_2 NPs. In every experiment, control (soil without NPs) group was also set for comparison with the treated ones. TiO_2 NPs were mixed manually in 1kg soil (0.1g/kg) in three replicates. Pots were watered using Tap water.

Monitoring of plant growth: The plants were monitored till maturity; then harvested to investigate the impacts of TiO_2 NPs on roots and shoot lengths, total fresh and dry biomass of the plants. The plants were washed with distilled water carefully to eliminate adsorbed impurities from the surface for analysis. From each pot set, five twigs were selected randomly to count a number of the lateral roots, root length, and shoot height. Moreover, three of seedlings were selected to determined fresh weight of roots and shoots, separately. The seedlings shoots were cut down and their length recorded. After this, fresh biomass recorded one by one. To examine the moisture contents, dry weight observed by keeping the plants in glass Petri dishes in an oven at 80ºC. The dry biomass recorded after 48h and water contents measured through subtraction of dry weight from the fresh weight (Azmat & Askari, 2015).

Biochemical analysis: The biochemical analysis of the plants was conducted to investigate the impact of NPs on the physiology of plants. The following essential parameters were analyzed.

Pigment analysis: The pigments in fresh leaves were analyzed using 80% acetone. The spectral scan was recorded on Shimadzu180 a UV-spectrophotometer (Maclachlan & Zalik, 1963).

Starch iodometry test: The starch iodometry test in fresh green leaf sample was conducted by boiling the leaves in ethanol for de-pigmentation. After de-pigmentation iodine solution was added.

Test for oxidative stress: The OH Radical tested in fresh leaves samples after crushing with mortar & pastel in distilled water. The water extract was collected in the separate test tube then pinch of benzoic acid added into it (Azmat *et al.*, 2016).

Carbohydrates content: The fresh samples crushed with mortar and pestle in Ethanol. Crushed material was centrifuged and the supernatant collected. The small amount of filtrate taken into another test tube in which distilled water and anthrone reagent added. The test tube then shakes and boiled on a water bath. After cooling the test tube, absorbance at 660nm was recorded by using Shimadzu 180 a UV-spectrophotometer (Yemm & Willis, 1954).

Reducing sugar content: The fresh samples crushed with mortar and pestle in phosphate buffer. Crushed material was centrifuged, and the supernatant collected. The small amount of filtrate taken in a separate test tube and DNS reagent added, shake well and boiled it in a water bath, cool the test tube. Finally, absorbance recorded at 540nm by using UV-Spectrophotometry (Miller, 1959).

Protein content: The fresh samples crushed with mortar and pestle in phosphate buffer. Crushed material was centrifuged and the supernatant collected. The small amount of filtrate in another test tube was taken then blue dye Assay reagent added into it followed by shaking then left for 30 minutes at room temperature. Finally, absorbance at 595nm recorded by using UV-Spectrophotometry (Bradford, 1976).

Results

TiO_2 having photocatalytic properties, mostly used as an antimicrobial agent, cleaning air products, paints, and for decomposing organic matter in wastewater (Castiglione *et al.*, 2011; Clément *et al.*, 2013) used to investigate the impact on growth rate of *Spinacia oleracea*. The morphology of the plants grown under the shallow concentration of TiO_2 NPs taking as wastewater showed effective growth rate in comparison of control plants of *Spinacia oleracea* (Fig. 1) which seems to be more green and healthy (Fig. 1). Due to photocatalytic properties of titanium NPs, most of the studies conducted where TiO_2 NP was used at a foliar level, have shown positive impacts on the plant. The results of the present investigation are similar to the work of Raliya *et al.*, (2015) & Gao *et al.*, (2008) who reported the positive impact of NPs when used at foliar level.

The morphology of *Spinacia oleracea* presented in Fig. 1 under TiO_2 application reflects a significant difference between treated and non-treated plants. Results showed that there was 3.20% increase in shoot length followed by an increase in fresh and dry weight of whole plants. It was observed that application of nano-TiO_2 at only 0.1 g/kg level significantly increased in the shoot length (3.2%) and 17% in root length of *Spinacia oleracea* as compared to untreated control plants (p<0.05). There were pronounced effect of TiO_2 on fresh weight and dry weight of plants which increases considerably in comparison of control sets of plants (Fig. 2). The moisture contents of the TiO_2 mediated shoot and root was significantly increased over control (Fig. 2). The photosynthetic activity of plants found to increase under TiO_2 NPs where contents of chlorophyll a and b increase 14% and 33% respectively followed by the same concentration of carotenoids and xanthophyll in both sets of plants. The biochemical parameter like carbohydrates, reducing sugars and proteins showed higher contents in TiO_2 mediated plants. The interaction of TiO_2 particle also checked by starch iodometry test where the appearance of blue color in leaves reflect the active conversion of sugars into starch in comparison of control plants (Fig. 3). The Benzoic acid test in water extract for hydroxyl radical (OH) showed no effervesces indicate that no oxidative stress generated during TiO_2 application (Fig. 4).

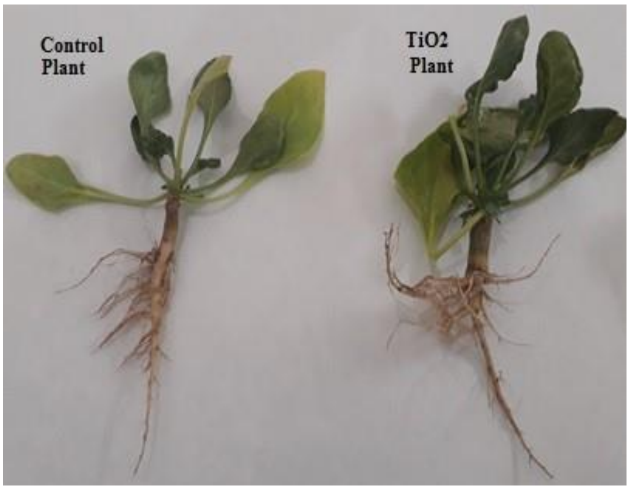

Fig. 1. Morphological comparison between TiO2 and control.

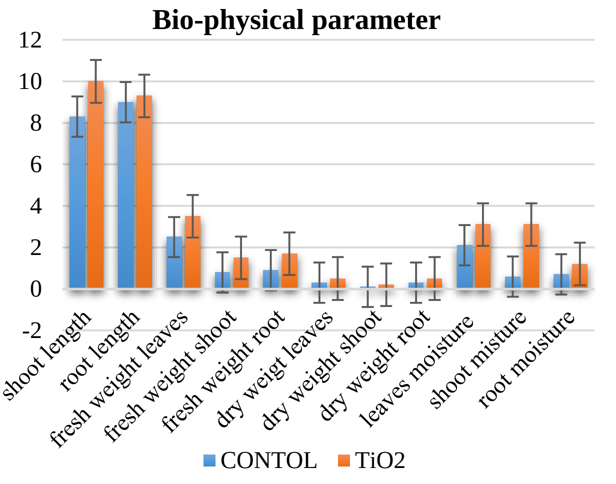

Fig. 2. Comparison of biophysical parameter between TiO_2 and control plants.

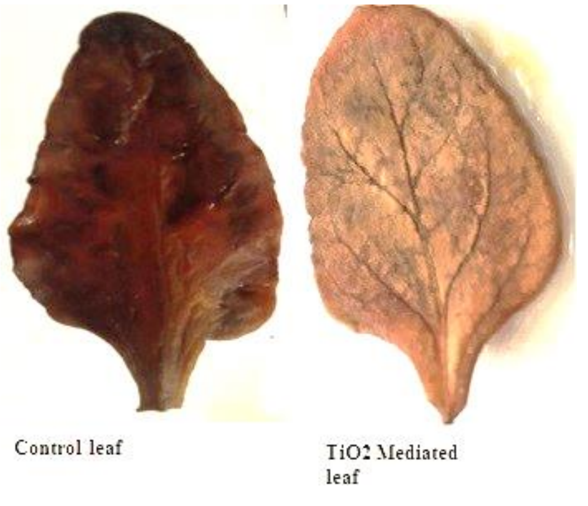

Fig. 3. Starch iodometry test comparison between TiO2 and control.

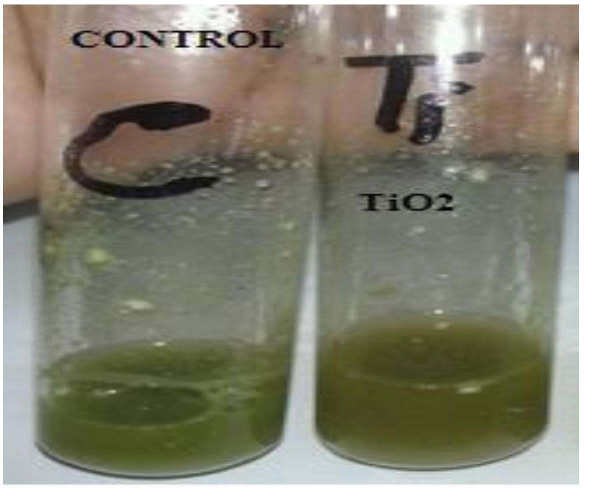

Fig. 4. OH radical test comparison between TiO2 and control.

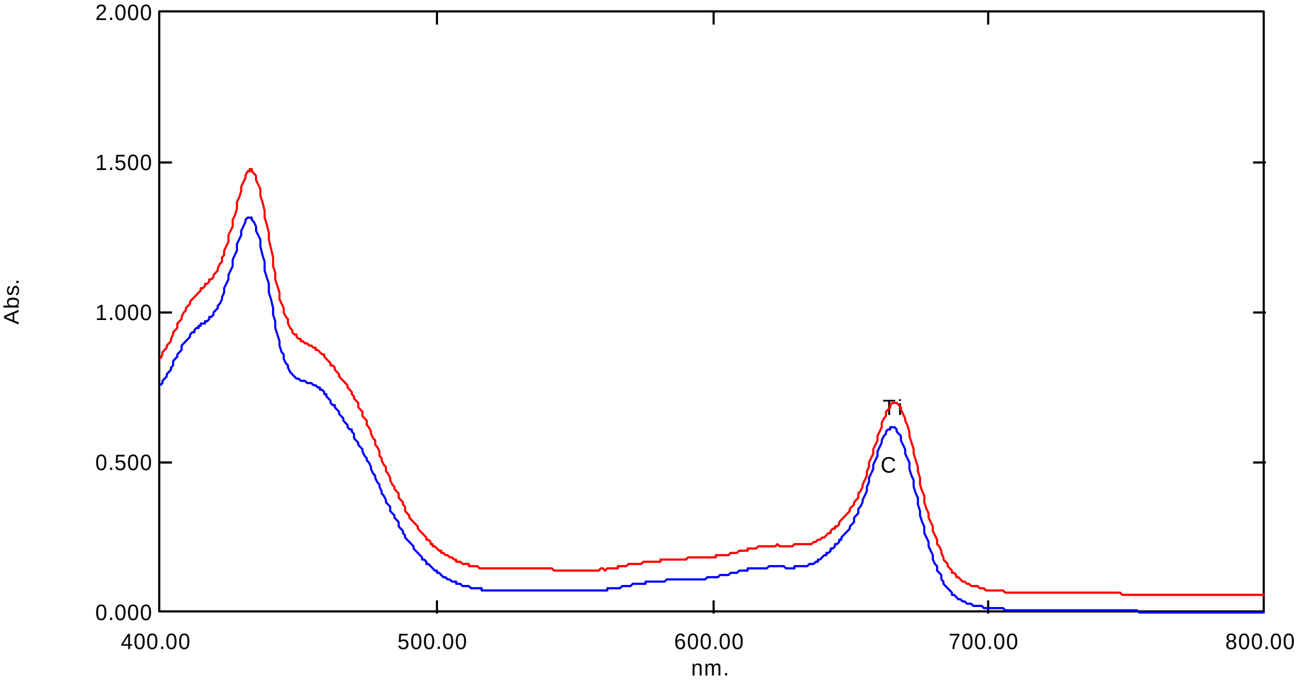

Fig. 5. Spectral analysis of pigment compression between TiO2 and control.

Table 1. pigment analysis and biochemical parameter of control and titanium mediated *Spinacia oleracea*.

S. No.	Sample	Pigment content			
		Chlorophyll (a) (mg/g)	**Chlorophyll (b) (mg/g)**	**Carotenoid (mg/g)**	**Xanthophyll (mg/g)**
1.	Control	$0.6 \pm 0.3^{**}$	$0.2 \pm 0.2^{**}$	$0.3 \pm 0.0^{**}$	$0.01 \pm 0.0^{**}$
2.	TiO_2 Mediated	$0.7 \pm 0.1^{**}$	$0.3 \pm 0.2^{**}$	$0.3 \pm 0.0^{**}$	$0.01 \pm 0.0^{**}$
% Increasing (TiO_2/Control)		14	33	0	0

S. No.	Sample	Biochemical analysis								
		Carbohydrate (µg/mg)			**Reducing sugar (µg/mg)**			**Protein (µg/mg)**		
		Leaves	**Shoot**	**Root**	**Leaves**	**Shoot**	**Root**	**Leaves**	**Shoot**	**Root**
1.	Control	$149.9 \pm 0.2^{**}$	$14.7 \pm 0.1^{**}$	$9.3 \pm 0.6^{**}$	$15.7 \pm 0.2^{**}$	$18 \pm 0.3^{**}$	$23.8 \pm 0.2^{**}$	$15 \pm 0.6^{**}$	$8 \pm 0.4^{**}$	$7 \pm 0.7^{**}$
2.	TiO_2 Mediated	$174.2 \pm 0.1^{**}$	$21.9 \pm 0.3^{**}$	$127.6 \pm 0.8^{**}$	$9.4 \pm 0.7^{**}$	$20.9 \pm 0.0^{**}$	$32.3 \pm 0.3^{**}$	$6.6 \pm 0.4^{**}$	$10.6 \pm 0.0^{**}$	$25.6 \pm 0.1^{**}$
% Increasing/ Decreasing (TiO_2/Control)		13	32	92	67	13	26	-56	24	72

*± Standard deviation of three replicates, Asterisks (*) represent significant differences ($p<0.05$); double asterisks (**) represent highly significant differences ($p<0.01$)

Discussion

Plants are indispensable constituents of entire ecologies and nowadays are a part of the ecological fate of NPs. The release of nanomaterials to the ecosystem is a potential risk which may result in adversative effects on living organisms. However, the toxicological influences of NPs on edible plants are not well documented. Titanium dioxide nanoparticles (TiO_2-NPs) are produced worldwide in large quantities for a wide range of purposes. Interaction of NPs with plants results in numerous morphological and physiological alterations which are related to the properties of NPs, their efficacy, chemical composition, size, surface covering, reactivity, and most importantly the dose at which they are useful.

The current investigation provides the impact of TiO_2 NPs through commonly used vegetable plants *Spinacia oleracea*. The TiO_2 NPs with low solubility when applied to grow seedlings of *Spinacia oleracea* resulted in significant increase in biomass and total moisture contents of plants which were similar to the earlier reports of Yang *et al*., (2007). The effect of the applied dose of 0.1 g/kg concentration of TiO_2 NP on root length was significantly higher than the control (Fig. 2). It indicated that a low concentration of TiO_2 had a promotory effect on root growth. The positive effect on root length may be due to the antimicrobial properties of anatase crystalline structure of TiO_2 that increase the plant resistance to stress (Samadi *et al*., 2014). Th**e s**hoot length of *Spinacia oleracea* is non-significantly higher (3.2%) than control which may be due to the transfer of minerals from root leaves. Lu *et al*., 2001 reported the increase in nitrate reductase activity and growth rate in soybean (Glycine max) by under mixed nano-TiO_2 and nano-SiO_2. The exogenous use of NPs increases the net photosynthetic rate, conductance to water, and transpiration rate in plants (Table 1).

Pigment analysis: Spectral scan (Fig. 5) showed the contents of Chlorophyll in leaves of *Spinacia oleracea* which showed that 0.1 g/kg of TiO_2 considerably improved contents of chlorophyll a and chlorophyll b. The pigment analysis of *Spinacia oleracea* plants showed a progressive relationship between the growth rate and biochemical parameters of plants. In treated plants, 0·1 g/kg of TiO_2 application, increased Chl a level by 14%, Chl b level by 33%, carotenoid and Xanthophyll by 0% relative to that of control. The increase in the concentration of Chl a and b showed effectiveness, regarding both growths (Fig. 2) and photosynthetic activity. The increase in biomass production of plants directly linked with the high chlorophyll content (Table 1). The earlier reports also described that NP-TiO_2 and TiO_2 could promote the photosynthesis rate, formation of chlorophyll and nitrogen metabolism at an optimum concentration. Our results showed good agreement with numerous studies; reported that TiO_2-NPs treatments increased photosynthetic activity and chlorophyll content of plants such as spinach (Hong *et al.*, 2005; Raliya *et al*., 2015), cucumber (Servin *et al*., 2012, 2013), Arabidopsis (Lenaghan *et al.*, 2013), rapeseed (Li *et al*., 2015). Results recommended that the physiological effects were related to the size of particles (Zheng *et al.*, 2005) and higher chlorophyll accumulation may be due to the corresponding effect of extra essential nutrients like Magnesium (Mg), Iron (Fe) and sulfur (S) (Chen, 2012).The study established NP-TiO_2 can improve the structure of chlorophyll, increase light absorbance, facilitate the formation of pigments, better capture of sunlight and transfer of light energy to active electrons, chemical activities and elevated effect on photosynthesis (Costa, & Sharma, 2016). The same results were established by Chen, (2012) & Zheng *et al.,* (2005). The characteristic photocatalytic property of TiO_2 improves light absorbance and the transformation from light energy to electrical and chemical energy (Yang *et al.,* 2006; Hong *et al.,* 2005) which results in high starch contents and Chl a and b. TiO_2 also encourages carbon dioxide absorption, consequently the biomass of plants increases (Table 1). Lei *et al.,* (2007) reported that TiO_2 stimulated complete electron chain transport, photo-reduction action of photosystem II, O_2-evolving and photophosphorylation activity of chlorophyll under both visible and ultraviolet light. Moreover, there is no effect of NPs on the contents of carotenoids when compared by the normal plants which showed that no oxidative stress generated at a low concentration of applied dose of Ti NPs. Dağhan (2018) observed that a low concentration of TiO_2 Np showed a good impact on the growth of Plants. It is already well established that that the synthesis of carotenoid raises in response to quenched ROS generated by abiotic stresses like heavy metals. Chlorophylls contents are the utmost significant interior aspects of plants. Augmented chlorophyll contents and unaffected carotenoids and xanthophyll in this investigation indicated that no oxidative stress generated at a low concentration of TiO_2 NPs. Oxidative stress test conducted concerning OH radical through the addition of benzoic acid in leave's water extract showed no effervescence indicated the absence of OH radical. The result showed a strong relationship between starch contents of root with the fresh weight of root. The higher contents of carbohydrates of roots in Ti mediated plants showed a very active transportation system of carbohydrate content was operative which transfers the carbohydrate contents from leaves to root (127.6 µg/mg) as compared to control roots of the plant (9.3 µg/mg). Similarly non reducing sugar of control plant was higher as compared to TiO_2 mediated plants which showed the active conversion of reducing sugars into the carbohydrates. The starch iodometry test through depigmentation showed the blue color of the starch-iodine complex in leaves under TiO_2 application while brown color in control plants. The brown color of leaves indicated the higher concentration of reducing sugars (Table 1). The higher contents of chlorophyll a and b followed by starch contents provide the evidence of photocatalytic activity of Ti NPs which activate the metabolism of plants at a low level.

Conclusion

Nanotechnology is an evolutionary Science with its several applications in the life science requires researches, focuses on the deleterious or beneficial effects of NPs on plants. Few studies have shown a positive effect of NPs on plant growth and development while some showed negative. It is evident from the current investigation that TiO_2 NPs showed a positive effect at low concentration after its prime use. This article discloses that the research on the impact of NPs required attention from the researchers to conduct more rigorous works to understand the physiological, biochemical, and molecular mechanisms of plants with the mode of action of NPs.

Acknowledgment

The author is very thankful to Dean Faculty of Science and HEC Pakistan for funding and permanent equipment under project No# Project No.20-2282/ NRPU/ R&D/HEC/12/5014 respectively.

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(Received for publication 11 December 2018)