EXPOSURE-RESPONSE OF TRITICUM AESTIVUM TO TITANIUM DIOXIDE NANOPARTICLES APPLICATION: SEEDLING VIGOR INDEX AND MICRONUCLEI FORMATION

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ABSTRACT

The effects of Titanium dioxide (TiO_2) nanoparticles (TNPs) on early growth characteristics of Triticum aestivum (Wheat) growth and cell nuclei integrity were assessed under hydroponic conditions. Seeds of wheat variety, Inqalab 91, were collected from National Agricultural Research Centre (NARC), Islamabad, Pakistan. These were then disinfected and exposed to different Titanium dioxide (TiO_2) levels: 0, 25, 50, 100, 200, 400 and 600 mg L^{-1}. Maximum vigor index of 2,722 was obtained at concentration of 200 mg L^{-1} TiO_2. Additionally, at TNPs dose 200 mg L^{-1}, germination rate was found to be 96.67%, indicating proper conditions for seed growth and negligible retardation. However, due to germination inhibition at higher TNPs concentration, i.e. @ 600 mg L^{-1}, vigor index dropped to 2,477. These results were explained by micronucleus assay where large numbers of micronuclei were formed in root tips and exhibited the signs of toxicity. From these results, it can be concluded that TNPs concentration up to 200 mg L^{-1} promoted growth and encouraged seed germination. At higher levels of TNPs 400 and 600 mg L^{-1}, toxicity was evident.

Keywords: *Triticum aestivum, TiO_2 NPs, germination, micronucleus assay*

1. INTRODUCTION

Titanium dioxide nanoparticles (TNPs) are, currently, produced in very large amounts throughout the world and are used in a variety of commercial goods. These particles are mostly used in cosmetics as sun block components, in which TNPs helps to protect the skin from damaging UV rays. The potential release of large amount of TNPs in the environment contaminates soil and water. The release of nanoparticles into the environment has not been measured exactly till now but computer modeling shows that sewage sludge would contain about 136 mg kg^{-1} of TNPs (Gottschalk, et al., 2009). Crops grown on soil containing TNPs may accumulate these nanoparticles and translocate into the roots, shoots and even to the edible parts of the plants.

There are many studies that provide the evidence of nanoparticles accumulation in shoots, roots and sometimes transfer to leaves, seeds and fruits. According to a study, CeO_2 nanoparticles entered the kidney bean plant and were responsible for its secondary root growth (Majumdar, et al., 2014). Transformation of CeO_2 nanoparticles was also observed when applied to agar and soil medium cultivated plants (Cui, et al., 2014). However, the unique properties of nanoparticles may cause unexpected biological effects, such as toxicity. Some research studies show that TNPs have adverse effect on plants (Cremonini, 2011). Copper nanoparticles show toxic effects towards two important plants and reduce seedling growth rate in wheat and mungbean plants (Lee, et al., 2008). The toxic effect of TiO_2 was also investigated in the algal species *Desdodesmus supsipicatus* (Hunde-Rinke and Simon, 2006). While Castiglione, et al. (2011) demonstrated that TNPs have negative effect on *Vicia narbonensis*.

On the other hand, there are some studies that reported that TNPs have positive effects on the plant yield and increase the uptake of some essential elements in the plant tissues. Titanium dioxide nanoparticles do not cause any damage to plants even if greater than the recommended rate is used (Frazer, 2001). Titanium dioxide nanoparticles had positive effect on germination of aged seeds and growth of shoots when exposed up to certain concentrations (Zheng, et al., 2005). It was demonstrated by another study that Silver nanoparticles (AgNPs) promote the root growth of *Eruca sativa* plant (Vaninni, et al., 2013). In Egypt, TiO_2 is allowed to be used as artificial colors in food products (Gain Report, 2005). It is known that TNPs break down ethylene gas produced in storage room and convert it into harmless products like water and carbon dioxide. TiO_2 is used to treat air in fruit, vegetables and cut flower storage area to prevent spoilage and increase shelf life of these products (Fonseca, 2004). As phytotoxicity research on TNPs has yielded confusing results, ranging from strong toxicity to positive effects, there is a need to assess the varieties and species for specific effects. Therefore, in this study, the effects of TNPs on the germination, seed vigor index and micronuclei were studied for wheat.

2. METHODOLOGY

2.1 Synthesis of Nanoparticles

Titanium dioxide nanoparticles were prepared from general purpose reagent of TiO_2 nanopowder (Sigma Aldrich, USA), by dispersing nanoparticles in distilled

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water through ultrasonication for 30 minutes. Scanning electron microscopy and X-Ray diffraction analysis were carried out to know the size and crystalline structure of TNPs. The average size of the prepared TNPs was 54 nm and had crystalline anatase phase. All the chemicals like potassium meta-bisulphate, calcium hypochlorite, hydrochloric acid, acetic acid, ethanol and basic fuchsin of different brands and of analytical grade were purchased from local market. *Triticum aestivum* (wheat variety '*Inqalab 91'*) dry seeds were collected from National Agricultural Research Center, Islamabad, Pakistan. The seeds selected for the experiments were of uniform size in order to minimize errors in seed germination and seedling vigor index.

2.2 Seed Treatment and Preparation of TNPs Suspensions

Different concentrations of TNPs were prepared to achieve TNPs levels in hydroponics: 25, 50, 100, 200, 400 and 600 mg L^{-1}, in addition to the control without spiking of TNPs. Wheat seeds were sterilized using 1% calcium hypochlorite solution for 10 minutes before application. Then seeds were washed with distilled water thoroughly and 15 seeds of similar size were randomly selected and placed on sterilized moist filter paper in petri dishes. Then 5 mL of TNPs solution was added to petri dishes (four replicates per treatment) to achieve the desired levels. For the control treatment, seeds were only treated with 5 mL of distilled water. The petri dishes were placed in laboratory under normal light condition for 7 days for germination test. Different parameters were studied including germination percentage, seed vigor index and biomass. Germination time of the seeds was calculated based on Matthews and Khajeh-Hosseini (2007), as shown in equation 1:

$$MGT = \Sigma F.X / \Sigma F \qquad (1)$$

Where, MGT is the mean germination time, F is the number of seeds newly germinated in a certain number of days, and X is the number of days after sowing.

The seedling vigor index was calculated according to Vashisth and Nagarajan (2010):

$$\text{Seedling Vigor index} = \text{Germination Percentage} \times \text{Seedling length} \qquad (2)$$

After seven days of exposure, plantlets were dried in oven for 48 hours and dry biomass of roots and shoots was calculated.

2.3 Micronucleus Test

When the seed germinated and roots extend up to 1-2 cm in length, the plantlets were exposed to different concentrations of TNPs for 24 hour. After 24 hour of exposure, the root tips of about 1-2 cm were cut and fixed in freshly prepared Carnoy solution, which is a mixture of glacial acetic acid and ethanol (1:3) and stored at 4ºC overnight. For the preparation of microscopic slides, the roots were first placed in distilled water at room temperature for 5 minutes after refrigeration. Then the root tips were hydrolyzed in 1N HCl for 10 minutes at 60°C in water bath. The roots were stained with fuchsin stain for 2 hours and slides were prepared for microscopic observations. Squash Technique was used for the preparation of slides in 45% acetic acid. A total of 300 cells were analyzed visually for each concentration for micronucleus test, under Optika Microscope at 40 X magnification.

3. RESULTS AND DISCUSSION

3.1 Effects on Germination

Seeds of the wheat plant responded differently to the different treatments of TNPs concentrations. The percentage of seed germination on exposure of TNPs was observed to be almost the same as in controlable one. The treatment of 25, 50 and 200 showed 96.67% germination. However, it was not significantly different from control treatment (Figure-1). No toxic effect of TNP, was observed on seed germination. Song et al. (2013) also observed similar results on exposure of tomato seeds to TNPs in the dark. Investigations by Qian, et al. (2013) verified that AgNPs did not affect seed germination of *Arabidopsis thaliana*. Interestingly, some organisms like *Staphylococcus aureus* and *Bacillus thuringiensis* have shown the ability to adapt to and survive the exposure to Silver nano-particles (AgNPs) at low concentration (Mirzajani, et al., 2013). Zheng, et al. (2005) observed increase in germination rate of seeds of spinach when exposed to TNPs. They reported that the enhancement of seed germination is due to the formation of super oxide radical, enhancing penetrability of seed capsule and facilitating absorption of water and oxygen molecules into the cell. Thus the rate of metabolism and germination increases upon exposure to TNPs. In contrast to this, some researchers have shown that nanoparticles exposure may inhibit seed germination of plants. Mushtaq (2011) observed inhibitory effect of nanoparticles-exposure on seed germination of cucumber plants. It was observed that inhibition of

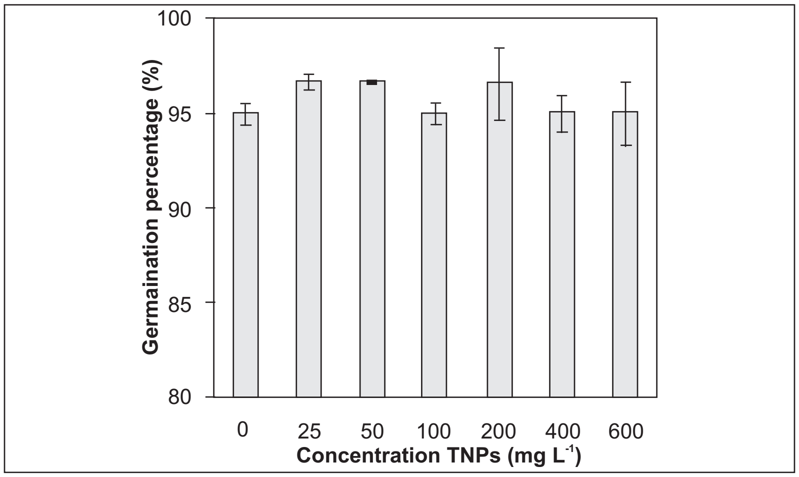

Figure-1: Germination Percentage of Wheat Exposed to Different Levels of TNPs; Error Bars Represent Standard Deviation for Different Replicates

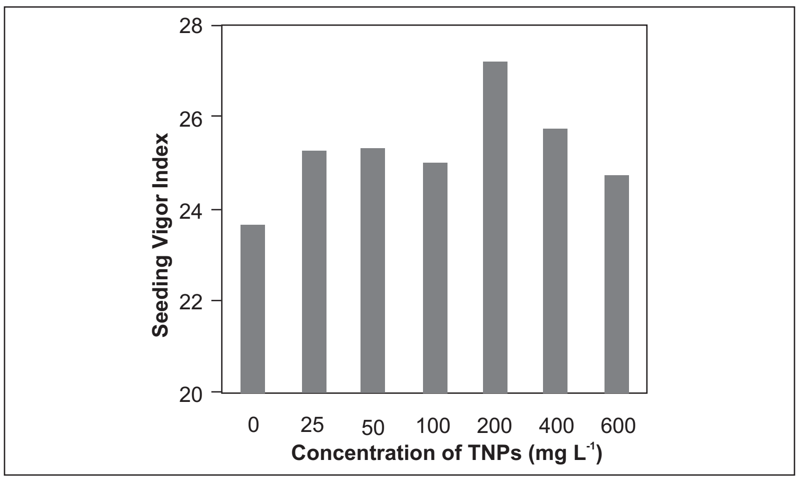

Figure-2: Seedling Vigor Index (SVI) of Wheat Treated with Different Levels of TNPs

seed germination was due to the insistence of nanoparticles. Menard, et al. (2011) demonstrated that the nanoparticles size, shape and surface properties are the major factors that determine the toxicity of Titanium nanoparticles.

3.2 Seedling Vigour Index

Among different concentrations of TNPs, 200 mg L^{-1} showed the maximum vigor index (2722) and ≥ 400 mg L^{-1} (2576) showed decreased seed vigor index and even lower at 600 mg L^{-1}, i.e. 2,477 (Figure-2). This decline in the value of vigor index indicated that the higher concentrations might have negative impact on plant growth. However, lower levels had a promontory effect in response to TNPs exposure. The inhibition of seed vigor index at higher concentration was also confirmed by micronucleus assay, in which large numbers of micronuclei are observed in root tips of wheat plants. Interestingly, plant biomass was not affected significantly (data not shown). In this context, it is important to mention that seedling vigor index is an important indicator that helps to find out minor effects and cumulative pictures that appear different after combining some insignificant results.

3.3 Micronucleus Assay

The results of micronucleus assay of low concentration of TNPs showed that the cell division is not statistically different from the control (data not shown). All tested concentrations show micronucleus formation in studied cells but 400 mg L^{-1} and 600 mg L^{-1} induced the maximum micronuclei in the roots of wheat plant (Figure-3). The result of this study shows

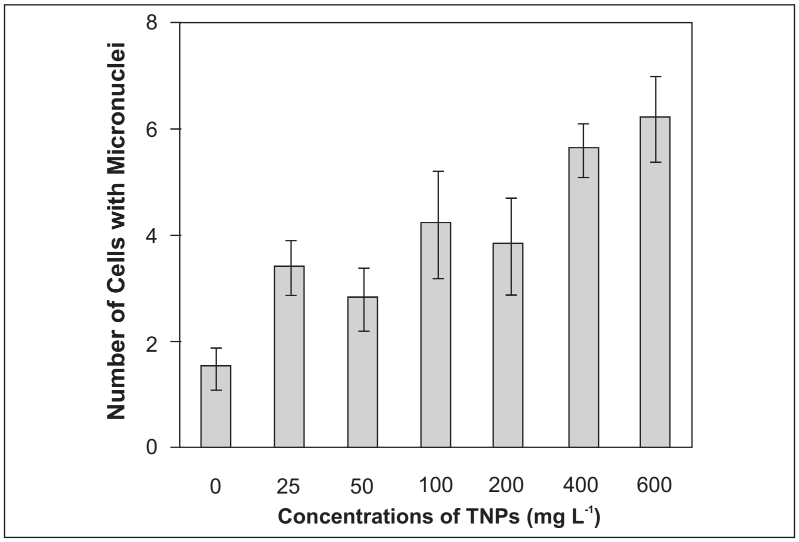

Figure-3: Percentage of cells with micronuclei formed in root tips of wheat plants; Error bars represent standard deviation for different replicates.

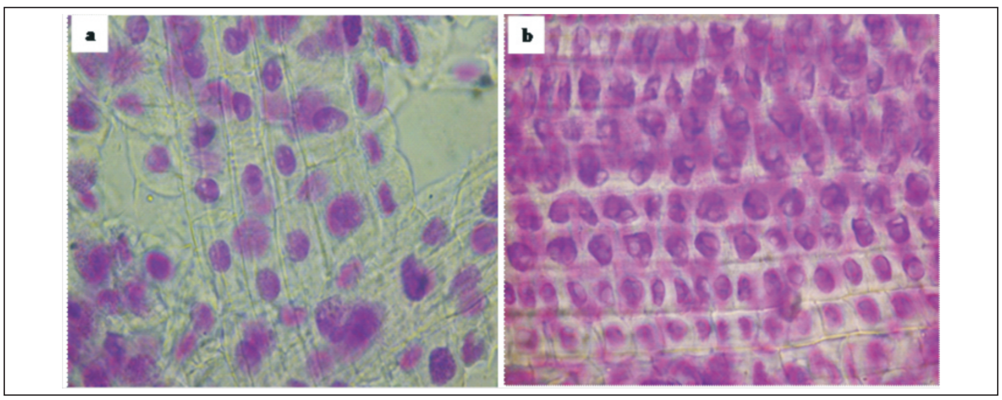

Figure-4: Pictorial view of micronuclei formation upon exposure to TNPs (a) Control with negligible number of cells with micronuclei; (b) very high number of cells with micronuclei

significant difference in the frequency of micronucleus induction in the root of wheat plant on exposure to TNPs compared to the control (Figure-3). Some pictorial views of the results are presented in Figure-4. Similar results were reported in *Allium cepa* root tips exposed to AgNPs (Kumari, et al., 2011) and Zinc oxide (ZnO) nanoparticles (Pand, et al., 2011). According to Ma, et al. (1995), the formation of Micronuclei is the result of small pieces separated from the proper nucleus at the time of cell division. The increase in the number of micronuclei supports the argument that the tested chemicals are cytotoxic and can produce different types of chromosomal aberrations. Although the short term exposure to TNPs showed increase in frequency of appearance of micronuclei in treated root tips, but further experiments are needed to clarify the biochemical mechanisms involved.

4. CONCLUSIONS

Different TNP concentrations had no significant effect on seed germination. High concentrations (≥ 400 mg L^{-1}) of TNPs caused adverse effects on wheat plant growth and these were confirmed by seedling vigor index and micronucleus assay. Different plant species and cultivars may behave differently upon exposure to nanoparticles, so more environmentally relevant experimental concentrations as well as species should be tested in order to evaluate their potential adverse effects.

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