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TOXIC EFFECTS OF TITANIUM DIOXIDE (TIO_2) NANOPARTICLES ON GROWTH OF AQUATIC PLANT *AZOLLA FILLICULOIDES*

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ABSTRACT

Titanium dioxide (TiO_2) nanoparticles have been interest in a wide range of applications and the environmental fate and behavior of TiO_2 nanoparticles is a rapidly expanding area of research. There is a paucity of information regarding toxic effect of TiO_2 nanoparticles hence it can be investigated on the growth of water plant Azolla filliculoides. The present study focuses on the effect of TiO2 nanoparticles when compared with control decline the plant growth and cytogenetically changes were also determined after an exposure period of 96 hours. The plants were treated with six different concentrations (25, 50, 100, 200 ppm) for 12, 24, 48, 72 and 96 hours. Azolla filliculoides were observed increased toxicity with correspondent increase in concentration along with other growth aberrations.

KEYWORDS: Cytogenetic, Growth abbreviation, Nanoparticles, Toxicity, Titanium dioxide.

INTRODUCTION

Nanoparticles , with at least one dimension of 100 nanometers or less, are increasingly being used for commercial purposes such as fillers, opacifiers, catalysts, semiconductors, cosmetics, microelectronics, and drug carriers. Nanoparticles with a size of between 1 and

100 nanometers fall in the transitional zone between individual atoms (or molecules) and the bulk material. Because the physicochemical properties of material on this scale can greatly differ from the corresponding bulk material, these nanoparticles can have the potential to generate unknown biological effects in living cells. As the discussion on potentially undesired side effects of engineered nanoparticles heats up there is an increasing amount of nanotoxicology research that gets undertaken and published. However, very few studies have been conducted to assess the toxicity of nanoparticles to ecological terrestrial species, particularly plants. In order to develop a comprehensive toxicity profile for manufactured nanoparticles, their phytotoxicity – the ability to cause injury to plants – has to be investigated. A new study examined the effects of five types of nanoparticles on plant growth.

Nanoparticles can be made from an enormous variety of materials and there are many unresolved issues and challenges concerning the biological effects of these nanoparticles. A new study aimed to provide new information about phytotoxicology of nanoparticles (multi-walled carbon nanotube, aluminum, alumina, zinc, and zinc oxide) on seed germination and root growth of plant species .Titled "Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth".

Few are known on the NPs induced genotoxicity in plants and most of the few available information dates from the two last years. Atha and collaborators [1] reported for the first time that copper oxide NPs damaged DNA in some agricultural and grassland plants (Raphanus sativus, Lolium perenne, and Lolium rigidum). It seems that oxidatively modified compounds accumulated and led to mutagenic DNA lesions, which inhibited plant growth. This isolated study on NPs genotoxicity in plants strongly supports the urgent need to evaluate the putative genotoxicity of the different NPs classes in plants and at which concentrations. Another issue that deserves attention is the analysis of genotoxic endpoints for NPs genotoxicity. For example, Comets, FCM-HPCV, and micronuclei have provided similar information in metal genotoxicity in plants [2], but any generalization to NPs-induced phytogenotoxicity should be done carefully.

Larue and collaborators [3] studied the effects of TiO_2-NPs on Triticum aestivum, Brassica napus, andArabidopsis thaliana. They showed that these NPs were absorbed by plants and did not affect their germination and root elongation. The authors also

highlighted the need of more studies of NPs toxicity, and in particular on NPs interaction with plants.

Recent evidences have shown TiO2 nanoparticles to induce inflammatory and genotoxic response in different animal and human cell lines [4].

Environment Programme (UNEP) as an efficient and standard test for the chemical screening and in situ monitoring for genotoxicity of environmental substances. V. faba has been used for evaluating chromosomal aberrations since the 1920s [5, 6].As the mitotic index represents the number of dividing cells it accounts for the growth, and any decrease in mitotic index leads to the reduced growth. The reduction in mitotic index may be caused by the effect of the AgNP/test chemical on the microtubule [7].

Azolla is a small aquatic fern. In fact, it is a symbiotic pair of Azolla filiculoides and a heterocystous blue-green alga Anabaena azollae. It has been used as a fertilizer in botanical gardens because of nitrogen-fixing capability [8]. The development of an Azolla-based biosorbent for wastewater treatment, especially in developing countries, may benefit environmental problems, by removing heavy metals from water using this weed [9].

The non-living Azolla filiculoides has been shown to be able to effectively adsorb Cr (III), Cr (VI), zinc (II) and nickel (II) from solutions and electroplating effluent (Zhao, et al., 1997, 1998 and 1999) and gold (III) from aqueous solution (Antunes, et al., 2001). We had shown that the removal of heavy metals could be increased by activation of the non-living Azolla filiculoides using H2 O2 /MgCl2 [10]. The kinds of living biomass also have been shown to be able to effectively remove heavy metals. This process decreases the growth ability of biomass that it depends on the toxic quantity of each heavy metal ion. For instance, Azolla caroliniana can remove Hg (II), Cr (III) and Cr (VI) from municipal waste water [11].

In this study, the geno toxic effects of TiO2 nanoparticles on the living Azolla filiculoides are determined by chromosomal aberration.

MATERIALS AND METHODS

PREPARATION AND CHARACTERIZATION

NANOPARTICLES

The commercial Nanosized TiO_2 particle was obtained from Alfa aesar, Heysham with normal purity 99.9%, powder.

STOCK SUSPENSION OF NPS

40µg of Titanium dioxide was weighed and suspended in 200ml of deionised water (DI) with the concentration of 200ppm.

CHARACTERIZATION OF NPs

The mean average size of particles and the dispersion was determined by TEM (transmission electron microscope).

SPECTROSCOPICAL ANALYSIS

Stock suspensions of NPs were freshly prepared for UV Spectroscopy analysis in demonized water at a concentration of 40µg /ml to check the absorbance.

SONICATION

The suspended TiO2 nanoparticles were dispersed by ultrasonic vibration (100MHz) to produce five different concentrations of 10ppm, 25ppm, 50ppm, 75ppm, 100ppm, and 200ppm.

EXPERIMENTAL DESIGN

The plant (*Azolla filiculoides)* used in this study were purchased from Agriculture university, Coimbatore. These plants were grown in laboratory in distilled water. Petri plates (10 cm diameter) were used for growing Azolla *filiculoides* . Ten equal sized fronds of A.filiculoides were placed in each petri plate filled with nanoparticle solution. Three replicates for each concentration of nanoparticles were taken for our study. The concentrations used for the nanoparticles were as follows: 10ppm, 25ppm, 50ppm, 75ppm, 100ppm, and 200ppm. Plant which is growing in distilled water served as control. The plant roots were allowed to grow for about 1-3 cm and they were used for analysis.

SAFFRANIN SQUASH TECHNIQUE

Five new root tips were cut for each concentration to carry out the microscopic examination. The microslides were prepared for each concentration and control following saffronin squash technique. The tips were transferred to the carnoy's fixative solution and kept undisturbed for 24 h. Then hydrolyzed in 1M Hcl for 4 to 5 min followed by distilled water wash. The hydrolyzed root tips were squashed in saffranin stain for microscopic examination [12].

MICROSCOPIC EXAMINATION

The slides were analyzed at ×1000 magnification for cytological changes. The mitotic index was calculated was calculated as the number of dividing cells per number of 1000 observed cells. The numbers of aberrant cells were noted per total cells scored at each concentration.

MORPHOLOGICAL ANALYSIS

Morphological changes (such as increase in the number of fronds, breaking of fronds or shoots, yellowing of fronds or shoots, shredding of fronds) were observed and recorded daily.

RESULT AND DISCUSSION

PREPARATION OF NANOPARTICLE

The TiO_2 Nano particle was obtained from Alfa aesar, Heysham with normal purity 99.9%, powderand the stock were prepared. 40µg of Titanium dioxide was weighed and suspended in 200ml of deionised water (DI) with the concentration of 200ppm.

CHARACTERIZATION OF NPs

The nanoparticles were characterizied by UV analysis and peak obtained at 420nm. The mean average size of particles is 0.1µm and the dispersion was determined by TEM (Fig.1)

SONICATION

The suspended TiO2 nanoparticles were dispersed by ultrasonic vibration (100MHz) to produce five different concentrations of 25ppm, 50ppm, 100ppm, and 200ppm.(Fig 2,3,4,5)

MICROSCOPIC EXAMINATION

The results obtained in genotoxicity assay clearly shows that increase in the concentration of nanoparticle with respect to the exposure time has an negative impact that the higher the nanoparticle concentration, the higher the genotoxicity as the MI of Azolla plants which are exposed to 25 ppm is 78.6% when compared with Azolla plants exposed to 100ppm with the MI of 37.6%. (Fig. 6,7,8,9).The work has been designed to investigate the genotoxic effects of titanium dioxide on Azolla plant in order to report that the organisms like plants in aquatic environment has to be taken into account during the ecotoxicity assessments before releasing such nanoparticles into the environment.

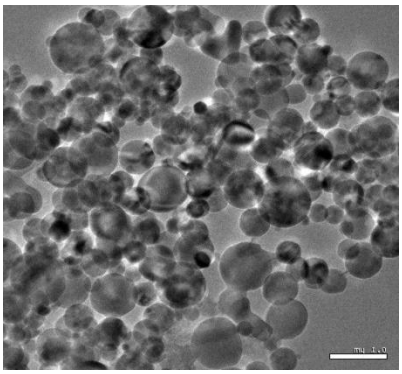

Fig.1. Showing the TEM image of NPs

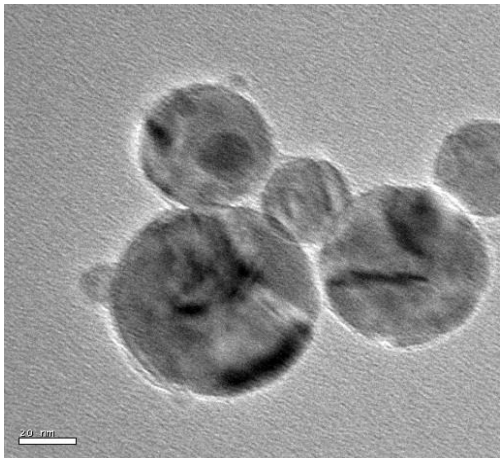

Fig.2. Showing the sonication NPs at 25ppm

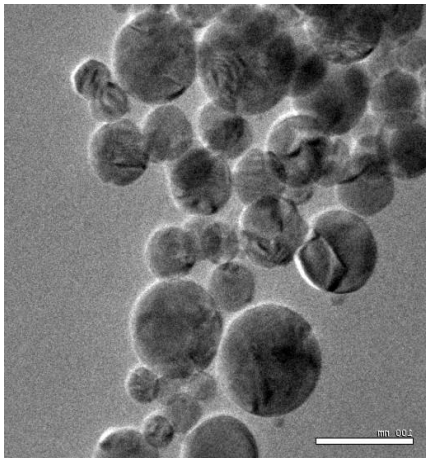

Fig.3. Showing the sonication NPs at 50 ppm

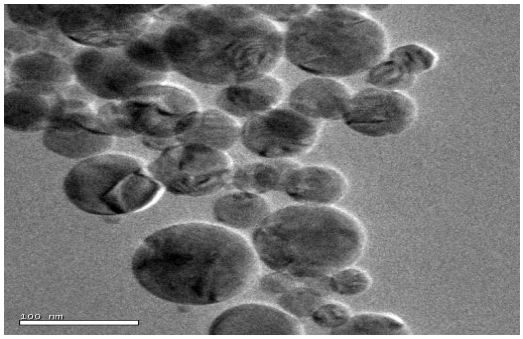

Fig.4. Showing the sonication NPs at 100 ppm

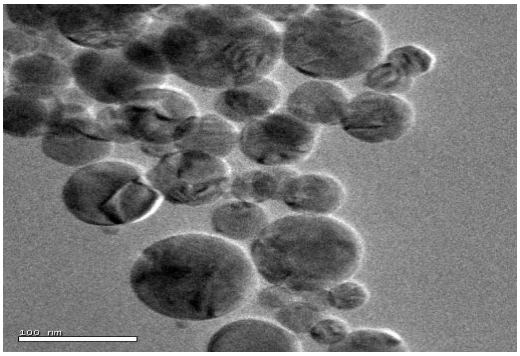

Fig.5. Showing the sonication NPs at 200 ppm

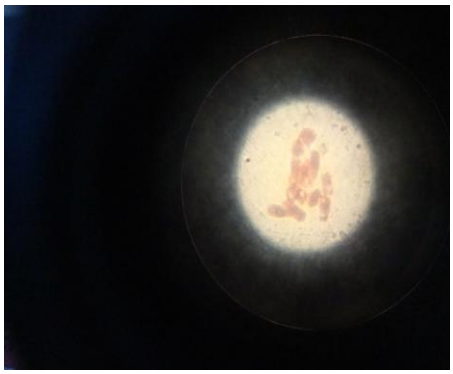

Fig.6. Showing the image of chromosomal aberration at 25ppm

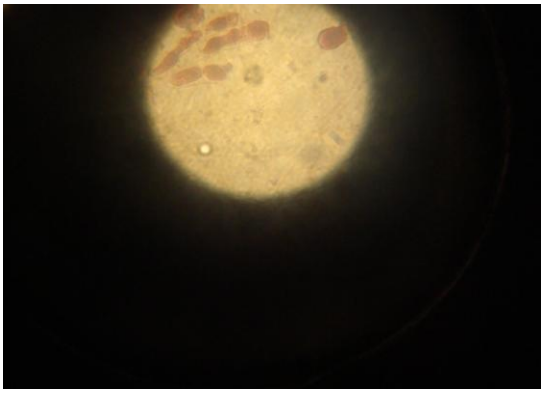

Fig.7. Showing the image of chromosomal aberration at 50 ppm

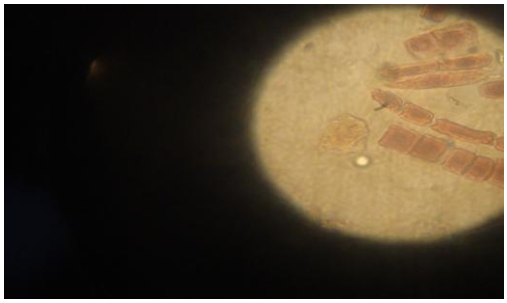

Fig.6. Showing the image of chromosomal aberration at 100 ppm

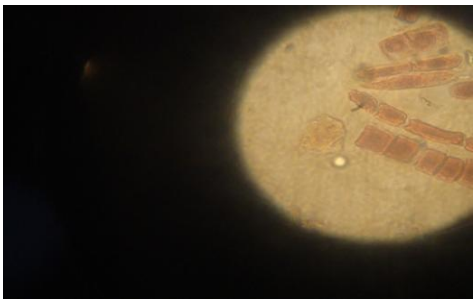

Fig.6. Showing the image of chromosomal aberration at 200ppm

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