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Original Article

ASSESSMENT OF STRESS END POINTS IN VIGNA RADIATA SEEDLINGS EXPOSED TO PRE-ACTIVATED TIO_2 AND $TISIO_4$ NANOPARTICLES UNDER SOLAR RADIATION

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ABSTRACT

Objective: The present study was aimed to evaluate the phototoxic effects of sunlight pre-irradiated/nonirradiated TiO_2, $TiSiO_4$ nanoparticles and TiO_2 bulk powder to *Vigna radiata* seedlings.

Methods: Different concentrations (0.05, 0.2, 0.5 and 1.0 g/l) of nano/bulk particles were applied to the germinated seedlings for 24 h and various biochemical end points were assessed. The end points were superoxide dismutase activity, catalase activity, malondialdehyde (MDA) and proline content.

Results: The irradiated nano TiO_2 was more phototoxic to the seedlings as compared to both the non-irradiated nano TiO_2 as well as the irradiated/non-irradiated TiO_2 bulk powder, as revealed by the increased level of antioxidant enzymes activity in irradiated TiO_2 nanoparticles treated group. Toxicity in nano TiO_2 group was more confined to the lowest concentration (0.05 g/l). Proline, a well-recognized stress biomarker, was found to increase in all the irradiated as well as the non-irradiated groups in a dose dependent manner (0.20 to 1.0 g/l), offering a different mechanism of toxicity from that of antioxidative enzymes. $TiSiO_4$ nanoparticles were not found to be phototoxic significantly under either exposure conditions.

Conclusion: The seedlings of the three treatment groups responded variably to the stress biomarkers, indicating that the mode of action of the nanoparticles to the plant was different from that of the bulk particles in irradiated and non-irradiated conditions and was governed by more than a single factor.

Keywords: Antioxidative enzymes, Photocatalysis, Nanoparticles, Reactive oxygen species, Phototoxicity

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INTRODUCTION

The phytotoxic evaluation of engineered nanomaterials (ENMs) presents an efficient tool for risk assessment of these new materials. Because of their outside cell wall barrier (pore size less than 4 nm) the plants are considered to be least affected by the nanomaterials exposure. Further, only small soluble ions or molecules can readily pass through the cell wall. In such a scenario if plants display toxicity, either directly or indirectly to these nanomaterials, (most of which are present in colloidal form) they can be used as a reliable system to understand the toxicity traits far before any destructive affair. Besides getting self-affected by the nanoparticles, plants may also accumulate nanoparticles inside their organs. Hence they can propagate these nanomaterials to the other organisms via the food chain. In the context of ENMs toxicity, TiO_2 nanoparticles are found to be beneficial [1-5] as well as harmful [6-11]. Nanoparticle toxicity to plants and other organisms is mostly attributed to the ready release of metal ions from these nanoparticles, and/or ultrafine metal oxide participation, in direct toxicity via reactive oxygen species (ROS) production inside tissues. Some metal oxide nanoparticles can promote ROS production both biotically as well as abiotically [12]. In this context, TiO_2 is one of the most remarkable compounds. Biotically, TiO_2 induces ROS via redox cycling and/or via nanoparticle-cell interaction, if it is accumulated inside tissues [13]. The abiotic approach of ROS production is linked to the metal oxide's photocatalytic property. TiO_2 can be excited by light absorption, and the electrons are transferred from its conduction band to valence band. Thereby, highly reactive electron-hole pairs are generated, which may further react with the surrounding H_2O and O_2 molecules to produce free radicals ($O_2^{\bullet-}$, H_2O_2 and $OH^{\bullet}$) which are highly reactive oxygen species [14-16]. These ROS may react with the organic molecules to initiate free radical chain reactions thereby they may degrade to these molecules [12-14]. This phenomenon of TiO_2 is more prominent in ultraviolet light (UV) than that in visible light. Hence, UV activated TiO_2 is used in the various application including photovoltaic devices, in self-sterilising products, heavy metal removal and so on [17]. As the reactive oxygen species, produced by photocatalysis on TiO_2 surface can interact with biomolecules (such as with membrane lipids, DNA, and proteins) in their vicinity, and impair their functions, TiO_2 is used as an antibacterial and antifungal agent in wastewater treatment, and to kill pathogens in agriculture [4]. Further, photocatalysis is a size-dependent property, by decreasing TiO_2 particle's size to the nanoscale, enhanced photoactivity can be achieved as compared to its bulk counterpart [18]. Approximately 20% of the constituent atoms are present at the particle's surface when the particle's size reduces to 30 nm [19]. At the nanoscale, the larger surface area per particle provides a larger platform for the reaction to occur at the particle's surface between the electron/hole and the other molecules in their vicinity. Further, efforts are in progress to manufacturing more efficient photoactive TiO_2 NPs by doping the other elements like Zn, and Al, which enriches the catalytic activity of the nanoparticles, by making the nanoparticles applicable in a wide range of the solar spectrum [4, 7]. But the same phenomenon which is so desirable from an application point of view may become undesirable in ecotoxicological opinion and may adversely affect the biota including beneficial soil microorganisms and higher plants especially the aquatic plants if the water bodies and soil accumulates reasonable concentration of these ENMs as a long term effect. The state is scarier as the ozone layer damage in the stratosphere is so pronounced. Hence, the environment is more susceptible to the harmful UV radiation under simultaneous exposure to photoactive materials.

So far, most of the studies regarding the toxicity of TiO_2 nanoparticles have been done under fluorescent light exposure. These studies have barely incorporated the UV spectrum of light in the laboratory condition. Only a few studies have been conducted on the phototoxicity effects of irradiated TiO_2 nanoparticles to plants. Miller *et al.* [7] reported the phytotoxicity to the three out of four phytoplanktons when they were exposed to irradiated TiO_2 nanoparticles under simulated sunlight. Ma *et al.* [12] described

immobilization of the freshwater crustacean *D. magna* after treatment with TiO_2 nanoparticles suspensions under UV exposure. Koce *et al.* [20] found UV-A irradiated TiO_2 nanoparticles not to be toxic to *Allium cepa* under 24 h acute exposure. A similar report of no toxicity was documented by Lee and An [21] when they exposed the green algae to irradiated TiO_2 nanoparticles. Previously in our lab, the toxicity of TiO_2 and $TiSiO_4$ nanoparticles to human embryonic cell line, HEK-293, has also been studied [22]. In the present study, suspensions of TiO_2 nanoparticles, $TiSiO_4$ nanoparticles, and TiO_2 bulk particles were pre-treated in natural sunlight for four h. The phototoxic effects of the various pre-irradiated/non-irradiated treatment groups were observed in the germinated seedlings of *Vigna radiata* for 24 h acute exposure. The toxicity to the seedlings was studied by measuring the activities of the two antioxidant enzymes, superoxide dismutase (SOD) and catalase and evaluating the malondialdehyde (MDA) and proline content. Proline is a well-recognised stress biomarker under most of the biotic and abiotic stress conditions. It elevates to many folds of the normal concentration under stress condition and in a more pronounced manner in developing seedlings [23-26]. In the case of unbalanced ROS generation, SOD is the earliest responding antioxidant enzyme and considered to be the first line defense against the ROS generation. Under stress, SOD, and catalase, attempt to protect the cells from ROS, but if they fail to balance the ROS homeostasis, oxidative stress condition is achieved which leads to increased MDA content, a membrane peroxidation product. Hence these biochemicals were used for the stress study as end point analysis to the nanoparticles exposure.

As the irradiated TiO_2 nanoparticles can produce toxicity to the organisms by generating ROS on their external body surface, without going inside the tissue, the hypothesis of the present work was a) solar radiation preactivated TiO_2/$TiSiO_4$ nanoparticles can induce phototoxicity to seedlings and b) nanoparticles affect seedling growth differently from the bulk counterpart under various exposure conditions.

MATERIALS AND METHODS

Chemicals and reagents

Titanium dioxide nanoparticles, titanium silicon oxide nanoparticles, nitro blue tetrazolium chloride, methionine, riboflavin, thiobarbituric acid (TBA), and L-proline were purchased from Sigma-Aldrich USA. Phosphoric acid, acetic acid, hydrogen peroxide, trichloroacetic acid (TCA), 5-sulfosalicylic acid dehydrate, toluene and ninhydrin were purchased from Merck.

Plant material

Seeds of *Vigna radiata* var. "Pusa Vishal" were provided by Indian Agricultural Research Institute, New Delhi, India and stored at 4 °C. Seeds were surface sterilized for 30 sec in 0.1% $HgCl_2$ and washed several times with distilled water. The seeds were soaked in distilled water overnight and then kept between two germinating sheets in a seed germination (Sanco, India) for two successive nights. The healthy germinated seedlings were further used for the treatment.

Preparation of nanoparticle suspension

These nanoparticles are sparingly soluble in water and readily precipitates out of the salt solution. Hence, dilutions were prepared in water instead of plant nutrient media. Before treatment, TiO_2 nanoparticles (TN), $TiSiO_4$ nanoparticles (TSN) and TiO_2 bulk powder (TB), each was added in distilled water to prepare 1.0 g/l stock suspension for each treatment. Suspensions were sonicated for 10 min. From stock solution, for each treatment group four dilutions, 0.05 g/l, 0.20 g/l, 0.50 g/l and 1.0 g/l were prepared in distilled water. Distilled water was used as a control group. Before exposure to the seedlings, all the dilutions were kept in full sunlight for four h.

Phototoxicity experiments

After germination, the developing seedlings were transferred into the pots supplied with 100 ml Hoagland nutrient solutions (pH 6) for 24 h. All the pots were kept under white fluorescent light (photon flux density of 200 µmol $m^{-2}s^{-1}$) for 12 h photoperiods. Then the seedlings were transferred into the pots supplied with the various sunlight pre-activated nano/bulk particle's suspensions and kept under the same white fluorescence light for 24 h acute exposure. Experiments for each concentration were conducted in three replicates.

Titanium dioxide particle characterisation

The nanoparticles were characterized by transmission electron microscope (TEM), dynamic light scattering (DLS), and zeta potential analysis. TN and TSN (50 µg/µl) were dispersed in distilled water and sonicated for 10 min in a liquid ultrasonicator. For TEM, after sonication, a drop of the suspension was placed on a copper grid with a laser carbon film and allowed to dry. The images were acquired with transmission electron microscope (JEOL, JEM-2100F). For DLS, samples were loaded into a sample holder, and data were acquired by using Malvern, Zetasizer Nano ZS. Electrophoretic mobility was measured for zeta potential analysis.

Superoxide dismutase assay

The activity of SOD was detected by monitoring the inhibition of the photochemical reduction of the nitro blue tetrazolium chloride (NBT) by the method as described by Giannopolitis and Ries [27]. To prepare crude enzyme extract, root tissues (~50 mg) were weighed and homogenized at 15000 rpm in 500 µl ice cold extraction buffer (100 mmol potassium phosphate buffer and 0.1 mmol Na_2EDTA, pH 7.8). The homogenate was centrifuged twice at 15 000 g for 30 min in a refrigerated centrifuge at 4 °C. The samples were used for the experiments within 10 h of extraction. To prepare the final reaction mixture, 13 mmol methionine, 63 µM NBT, 50 mmol phosphate buffer (pH 7.8), 100 µl enzyme extract (or extraction buffer as blank), and 1.3 µM riboflavin were added in a glass test tube (in the same order). Test tubes of uniform size and color, containing the reaction mixtures were illuminated by switching on a white cool fluorescent light for 15 min. Illumination experiment was performed using the apparatus shown in fig. 1, which was fabricated at the University Science Instrumentation Center (USIC), Jawaharlal Nehru University, New Delhi, ensuring the design described by Giannopolitis and Ries [27].

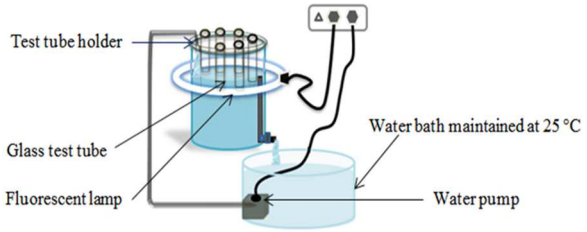

Fig. 1: Apparatus for the light exposure to the reagent mixtures in SOD assay

The initial rate of the reaction was determined by the increase in absorbance at 560 nm. A unit SOD is defined as the amount of enzyme required to inhibit the photoreduction of NBT by 50%. Specific enzyme activity was given as SOD units present in a unit µg of protein. Protein in the crude extract was determined by the Bradford method [29].

Catalase assay

The experiment was conducted as described by Sinha [28]. Weighed root tissue samples (~50 mg) were homogenized at 15000 rpm in 500 µl cold extraction buffer (50 mmol potassium phosphate buffer and 0.1% triton X-100, pH 7.0). The extracts were centrifuged at 15 000 g for 10 min. The supernatant was kept at 4 °C till further enzyme analysis. 1200 µl of 200 mmol H_2O_2, and 1500 µl of 10 mmol phosphate buffer were taken in a flask. Two test tubes each containing 2 ml of dichromate/acetic acid reagent were prepared in advance. To start reaction 300 µl of supernatant was added to the flask and mixed gently. 1 ml of this reaction mixture was added immediately into the first test tube containing 2 ml of dichromate/acetic acid reagent. Just after completion of 2 min, 1 ml of the reaction mixture was added to the second test tube containing 2 ml of dichromate/acetic acid reagent. The test tubes were put in a water bath maintained at 100 °C for 10 min and cooled to room temperature. Optical density was recorded at 570 nm to measure

the residual amount of H_2O_2 in the reaction mixtures in 2 min interval. The rate of decrease in OD per minute was calculated as the difference in optical densities of the two test tubes divided by 2. H_2O_2 concentration in various samples was calculated using H_2O_2 standard curve. One unit of catalase is defined as the amount of enzyme required to convert 1 µmole of H_2O_2 into its product per minute at given reaction condition. Specific catalase activity was described by µmole of H_2O_2 per µg protein. Soluble protein in the extracts was measured by Bradford method.

Proline assay

Proline content in the roots was measured as described by Bates [23]. Roots (~ 50 mg) from the seedlings were harvested, weighed and homogenized immediately at 15000 rpm in 1 ml, 3% sulphosalicylic acid for 30 sec. The homogenates were centrifuged for 10 min at 8000 rpm and supernatants were stored at room temperature until spectrophotometric analysis. In test tubes, 1 ml of homogenate was mixed with 1 ml acid-ninhydrin (1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml, 6M phosphoric acid), and 1 ml glacial acetic acid and kept in water bath at 100 °C for 1 h. The test tubes were kept on ice to stop the reaction. 2 ml of toluene were added to the test tube solution and vortexed for 15-20 sec. The upper layer containing pink colour chromophore was measured in a spectrophotometer at 520 nm. µmoles proline content/gram fresh weight tissue was calculated using standard proline curve.

MDA content analysis

MDA content was evaluated as described by Rajinder *et al.* [30]. Root samples (~50 mg) were weighed, homogenized in 1 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10 000 g for 5 min at 4 °C. An aliquot of 300 µl supernatant was mixed with 1.2 ml of 0.5% TBA prepared in TCA (20%). The reagent mixture was incubated at 95 °C for 30-45 min, and the reaction was stopped in an ice bath, samples were again centrifuged at 10 000 g for 10 min at room temperature. Absorbance was taken for the supernatant at 532 and 600 nm (the latter was subtracted from former to remove the non-specific absorbance) respectively. MDA concentration was determined using the extinction coefficient 155 $mmol^{-1}$ cm^{-1}.

Statistical analysis

The data was analyzed through the analysis of variance using Graphpad prism. Statistical significance between the three treatments and the radiation exposure conditions were tested by two-way ANOVA. Bonferroni post hoc test was used to compare all the groups to the control. The differences between the mean values obtained were compared with 5% probability. For data evaluation, the standard error of the mean (SEM) was established.

RESULTS

Characterization of nanoparticles

The size of TiO_2 and $TiSiO_4$ nanoparticles was<25 nm and<50 nm respectively as described by the supplier (Sigma-Aldrich, USA), however, in water suspensions, large aggregates have formed as revealed by the TEM images of the two nanoparticles (fig. 2).

Table 1 represents the physicochemical properties of the nanoparticles suspensions. According to the data gathered from the DLS analysis, it appears that the hydrodynamic diameter of the nanoparticles was 2 to 3 times larger than the primary particle size. Both the nanoparticles acquired negative zeta potential in water at neutral pH.

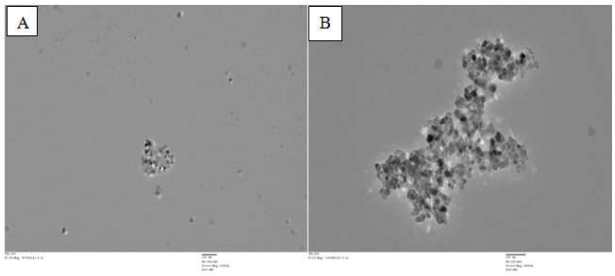

Fig. 2: Transmission electron micrograph of TiO_2 nanoparticles (A) and $TiSiO_4$ nanoparticles (B). Scale bar = 100 nm

Table 1: Physicochemical properties of the nanoparticle suspensions at a concentration of 50 µg/µl

S. No.	Nanoparticles	Primary size	Average hydrodynamic size	Zeta potential
1	TN	<25 nm	70.87 nm	-20 mV
2	TSN	<50 nm	128.35 nm	-31 mV

Effect of treatments on SOD activity

The phototoxic effect of irradiated and non-irradiated TiO_2 nanoparticles (TN), TiO_2 bulk powder (TB) and $TiSiO_4$ nanoparticles (TSN) at different doses (0.05, 0.20, 0.50, 1.0 g/l) on SOD activity of seedlings root are shown in fig. 3. SOD activity increased significantly in irradiated TN group at the lowest dose of 0.05 g/l ($p<0.05$) with an increase in enzyme activity of 31.33 % as compared to the control. At the higher doses, there were no observed significant differences in SOD activity as compared to the control in this group. Further, non-irradiated TiO_2 nanoparticles were unable to bring any significant change in enzymatic activity in this group as compared to the control.

The enzyme activity was insignificantly affected at the lowest dose (0.05 g/l) in irradiated TB group. The non-irradiated TB groups were unchanged (no significant changes were observed in any group as compared to the control) in their enzyme activity. However, one interesting observation was that the SOD activity was found to be increased at the higher doses in a dose-dependent manner in TB group irrespective of solar exposure conditions. But this trend was statistically insignificant, might be because of the lower sample size. Variable data was acquired in TSN groups with no significant difference in the enzyme activity at any treatment of titanium silicon oxide nanoparticles as compared to the control in either irradiated or non-irradiated condition.

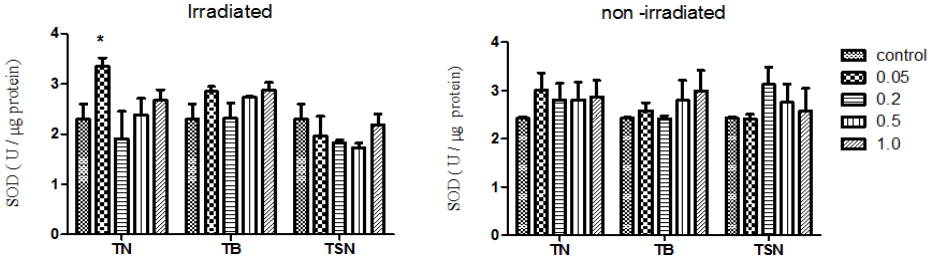

Fig. 3: Effect of irradiated/non-irradiated TiO_2 nanoparticle (TN), TiO_2 bulk powder (TB) and $TiSiO_4$ nanoparticle (TSN) on SOD activity (U/µg protein) in roots of *Vigna radiata*. Within each group (TN, TB, and TSN) the data points were compared with the control for the significance in difference. A common control was used for all the irradiated groups, and another common control was used for all the non-irradiated groups. n=3, data points are mean±standard error means, significant at *$p<0.05$. All the means which were not significantly different as compared to the control were not assigned to any symbol

Effect of treatments on catalase activity

As shown in fig. 4, in the seedling roots of irradiated TN treated group, an increase in the catalase activity at concentration 0.05 g/l was found highly significant (71%, p<0.01). While at the concentration of 0.2 g/l and 0.5 g/l the catalase activity increase was found significant with an increase of 52%, (p<0.05) and 53% (p<0.05%) respectively when compared to the control group. However, in irradiated TB group, there was a 51% (p<0.05%) increase in catalase activity at the concentration of 0.05 g/l as compared to the control, but there were no significant changes in the catalase activity found in non-irradiated TN and TB groups. In TSN groups no significant changes were observed among the groups at any concentration as compared to the control.

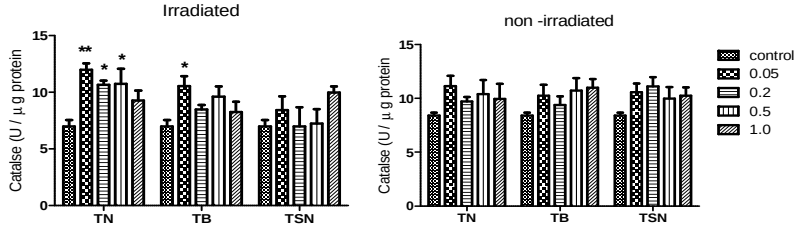

Fig. 4: Effect of irradiated/non-irradiated TiO_2 nanoparticle (TN), TiO_2 bulk powder (TB) and $TiSiO_4$ nanoparticle (TSN) on catalase activity in roots of *Vigna radiata*. Within each group (TN, TB, and TSN) the data points were compared with the control for the significance in difference. A common control was used for all the irradiated groups, and another common control was used for all the non-irradiated groups. n=3, data points are mean±standard error means, significant at *p<0.05, and very significant at **p<0.01. All the means which were not significantly different as compared to the control were not assigned to any symbol

Effect of treatments on MDA content

As shown in fig. 5, MDA content was observed to be unchanged in all the treatment groups at all the dose level as compared to the control. No significant changes were observed in any treatment group at any dose levels as compared to the control.

Effect of treatments on proline content

As fig. 6 depicts, the higher doses affected the proline accumulation in all the treatment groups. However, in the antioxidant enzyme analysis results showed that there was a significant alteration in antioxidant enzyme's activity at the lower doses specifically to the TN group among all the three groups. In the case of proline, its accumulation was higher at the higher doses and increased in a dose-dependent manner among all the treated groups. However, a significant difference was observed in TN and TB groups with a significant increase in 49% (p<0.05) at the concentration of 1.0 g/l in irradiated TN group and 50% (p<0.05%) and 52 % (p<0.05%) increase at 0.50 and 1.0 g/l respectively in irradiated TB group as compared to control. In non-irradiated TN group 60% (p<0.05%) increase was observed at the dose of 1.0 g/l and 63% (p<0.05%) increase was observed at 1.0 g/l in non-irradiated TB group. However, none of the TSN group showed any significant change in their proline content; there was an insignificant increase in the proline content in a dose-dependent manner among all the treated groups. Increased sample size might have produced significant differences in TSN groups also.

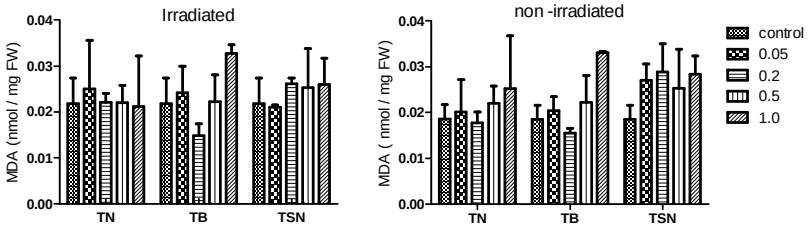

Fig. 5: Effect of irradiated/non-irradiated TiO_2 nanoparticle (TN), TiO_2 bulk powder (TB) and $TiSiO_4$ nanoparticle (TSN) on MDA content in roots of *Vigna radiata.* Within each group (TN, TB, and TSN) the data points were compared with the control for the significance in difference. A common control was used for all the irradiated groups, and another common control was used for all the non-irradiated groups. n=3, data points are mean±standard error means, FW= Fresh weight. All the means which were not significantly different as compared to the control were not assigned to any symbol

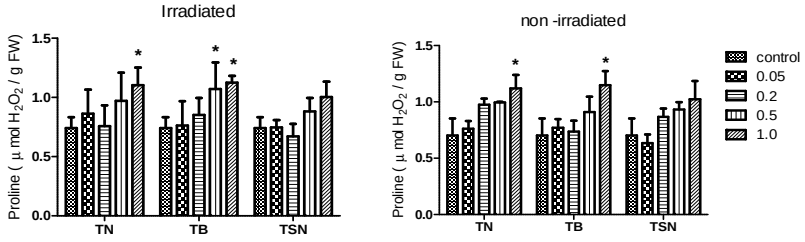

Fig. 6: Effect of irradiated/non-irradiated TiO_2 nanoparticle (TN), TiO_2 bulk powder (TB) and $TiSiO_4$ nanoparticle (TSN) on proline content in roots of *Vigna radiata*. Within each group (TN, TB, and TSN) the data points were compared with the control for the significance in difference. A common control was used for all the irradiated groups, and another common control was used for all the non-irradiated groups. n=3, data points are mean±standard error means, significant at *p<0.05. All the means which were not significantly different as compared to the control were not assigned to any symbol

DISCUSSION

The present study reveals the phototoxic effects of irradiated titanium dioxide nanoparticles on *Vigna radiata* seedlings after 24 h acute exposure. MDA (a membrane peroxidation product) content in combination with the SOD and catalase activities provides a good understanding of ROS induced toxicity to plant system. Further, proline content was assessed to understand the mechanistic approach of the plant to cope with the increased ENMs.

In aerobic metabolism, the antioxidative defense system remains constitutively active against the inevitably generated ROS. Thus the ROS/antioxidant homeostasis is maintained. ROS are small molecules with a short lifetime. Hence a basal level of these molecules are helpful in various cellular signaling pathways [31, 32]. Under internal/external stress, cellular ROS increases as compared to the basal level, concurrently, the antioxidative enzymatic system is also induced to eliminate the surplus ROS. Moderate increase in the ROS level is indicated by increased activity of stress biomarkers (SOD and catalase). Plant cells remain safe till this level of ROS, working without any damage to the cell components including biological membrane (hence the basal MDA content is not increased). Further increase in ROS causes an imbalance between the ROS and antioxidative enzymes. Because the increased antioxidants get insufficient to remove excess ROS, a condition is reached known as oxidative stress. Such an increased ROS results in a series of cellular events like, loss of antioxidative enzyme activity, membrane peroxidation, protein carboxylation, etc., which lead to total cell collapse. This stage of stress condition is evaluated as decreased SOD and catalase activity and increased MDA content. So changes in the activity pattern of these stress markers provide a good understanding of toxicity at the preliminary level of examination.

This study shows variability in toxicity pattern among the treatment groups namely TiO_2 nanoparticles (TN), TiO_2 bulk powder (TB) and $TiSiO_4$ nanoparticles (TSN) when seedlings were exposed to solar radiation activated particle suspensions. None of the treatment groups except the irradiated TN group altered the enzyme's activities and MDA content significantly. Also, the activated TiO_2 nanoparticles exposure affected the seedling's stress end points at the lowest concentration (0.05 g/l) only. The seedling roots were soaked in the particle suspension hence always remained in contact with the various treatment particles. Photoactivity in TiO_2 leads to the generation of $O_2^{\bullet-}$, H_2O_2 and $OH^{\cdot}$ in the suspension in the presence of solar UV radiation [12, 13]. The $O_2^{\bullet-}$ is dismutated readily to H_2O_2 and O_2. H_2O_2 is moderately reactive with a relatively longer half-life (1ms) as compared to the other ROS (1 µs for $O_2^{\bullet-}$ and 1 ns for $OH^{\cdot}$). H_2O_2 can be toxic in two ways, firstly, it reacts with $O_2^{\bullet-}$ as per Haber-Weiss/Fenton reaction to produce more reactive $OH^{\cdot}$. The $OH^{\cdot}$ can react with cellulose of root cell wall to depolymerize it. Extracellular H_2O_2 driven cell wall depolymerization has been reported in the case of mycorrhizal infection to roots [34]. Uncharged $OH^{\cdot}$ can penetrate the plasma membrane hence it can react with intracellular biomolecules. Secondly, H_2O_2 Itself can be diffuse through the biological membranes. Hence it can induce stress far away from the site of its formation. Hence photo-catalytically generated ROS on TiO_2 surface in suspension can damage the root tissues externally as well as by entering into the tissues. Further, it is also supposed that the photoreactivity is enhanced as particle size reduces to the nanoscale. External root injury significantly affected the seedling development, reflected by the appearance of increased activity of SOD in TN groups. As catalase scavenges H_2O_2 by converting it into H_2O and O_2 and H_2O_2 being the most prevalent molecule to cause toxicity under the given study condition, increased catalase activity in irradiated TN and TB groups further proves this facet of phototoxicity. The bulk particles could not show the toxic effects as significant as the nanoparticles, on the oxidative stress end points in the seedlings under the acute exposure condition. MDA content did not change as compared to the control at any dose within any treatment group, indicating that though seedlings reached the pre-oxidative stress state, they were sufficiently safe to any damage. However further increased exposure might have increased the ROS level significantly to damage irreversibly the cell wall leading to the collapse of antioxidative defense mechanism and increased lipid peroxidation triggering increased MDA content.

It is established that at the lowest concentration there is less aggregation of nanoparticles hence it is more photoactive, increased concentration allowed particle aggregation and rendered them photo-inactive. Hence the lowest dose in TN groups showed more toxicity to the seedlings in acute exposure conditions.

Healthy growth of seedlings depends on the ambient supply of water through root tissues and the cell wall blocking by adherence of nano, and submicron sized particles may be the reason for the osmotic stress at the higher concentrations that led to the high proline content within all the groups. Abundant literature supports the increased proline content under water deficit conditions [24-26]. Fortunately, the concentration taken to observe the toxicity effects in the present study is much higher than the real environmental exposure practices.

TSN group showed no evident toxicity at any concentration except a dose-dependent insignificant increase in proline content. Previous work reported no toxic effect of $TiSiO_4$ nanoparticles on seed emergence while a significant decrease was observed in fresh and dry mass yields [33]. In this study, the primary particle size of TSN was 50 nm which further increased 3 to 4 order after aggregation in aqueous suspension hence lesser reactivity led to less observed toxicity.

The cell wall of plant cells is barely permeable to particles greater than 4 nm [35, 36]. Hence it is less likely that nanoparticles with significantly much larger diameter have entered passively inside the tissue. Further, TiO_2 is insoluble at neutral pH hence; ion release to cause toxicity to the seedlings is not acceptable. Hence the physical injury by the activation of reactive oxygen species on the surface of root tissue when exposed to photoactivated TiO_2 nanoparticles, thereby damage of the organic molecule seems to be the cause of phototoxicity to the seedlings. Water conduction hindrance due to the particle adherence, thus osmotic stress in the cells appears to cause toxicity at the higher doses in a dose-dependent way.

In the present study, it appears that lower and higher doses altered the biochemical end points more significantly than the intermediate concentrations. As the stress biomarkers reveal, more than one factor is operating to affect the growth of the seedlings at the different doses among different treatment groups under irradiated/nonirradiated conditions.

CONCLUSION

Both the negative and positive impacts of TiO_2 nanoparticles exposure on plants behavior have been reported in the literature. Impacts related to the nanoparticles exposure to plants are not straight forward, instead, these are dependent on a number of factors including the duration of treatment; plant type and age; size, shape, surface charge and catalytic property of nanoparticles; soil and water media which influence nanoparticle's aggregation and other abiotic factors like geographical location. Unfortunately, there is a wide gap of knowledge concerning the dynamic impact of ENMs in the environment, especially on flora. Though the work is preliminary and intensive work is needed in this direction, the study shows that solar radiation activated TiO_2 nanoparticles are toxic to the plants via extracellular photocatalytic generation of ROS. However, the toxicity is dose independent and governed by more than one factor at least for the acute exposure to the selected test plant. Though this does not present the real-time exposure condition, it helps to understand the toxicity of photoactivated nanoparticles on plants. TiO_2 is naturally present in the soil in various mineral compositions and remains insoluble and rarely bioaccumulates inside tissues in plants. But the release of photoactive new formula UV-visible activated TiO_2 nanoparticles may affect plants growth indirectly by the generation of ROS and may damage external tissues by physical injury even at very low concentration without going inside the plants. The situation may become more attentive in the case of release of the nanoparticles from the air onto the plant parts such as leaves surfaces, where the photocatalytic activity of the nanoparticles may damage organic molecules of epidermal tissues thereby upsetting plant's very first line of defense against pathogens and other stress conditions. Further, if these nanoparticles are released into water bodies, they may also affect the phytoplanktons. Therefore, it is important to understand the interaction of the new

formula photoactive nanoparticles with the biota before their widespread use in the household appliances, agricultural products, electronic materials and all the other commodities.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Prema P, Selvarani M. Evaluation of antibacterial efficacy of chemically synthesized copper and zerovalent iron nanoparticles. Asian J Pharm Clin Res 2013;6:222-7.
2. Chatterjee A, Nishanthini D, Sandhiya N, Abraham J. Biosynthesis of titanium dioxide nanoparticles using *Vigna radiata*. Asian J Pharm Clin Res 2016;28:85-8.
3. Bhoskar M, Patil P. Development, and evaluation of paclitaxel-loaded nanoparticles using 24 factorial design. Int J Curr Pharm Res 2015;7:64-72.
4. Averett SB, Averett DR. Inventors; WELL Shield LLC, assignee. Titanium dioxide photocatalytic compositions and uses thereof. United States patent U. S. 8609121 B2; 2013.
5. Yuan SJ, Chen JJ, Lin ZQ, Li WW, Sheng GP, Yu HQ. Nitrate formation from atmospheric nitrogen and oxygen photocatalyzed by nano-sized titanium dioxide. Nat Commun 2013;4:2249.
6. Fenoglio I, Greco G, Livraghi S, Fubini B. Non UV-induced radical reactions at the surface of TiO_2 nanoparticles that may trigger toxic responses. Chem Eur J 2009;15:4614-21.
7. Miller RJ, Bennett S, Keller AA, Pease S, Lenihan HS. TiO_2 nanoparticles are phototoxic to marine phytoplankton. PloS One 2012;7:e30321.
8. Jacob DL, Borchardt JD, Navaratnam L, Otte ML, Bezbaruah AN. Uptake and translocation of Ti from nanoparticles in crops and wetland plants. Int J Phytorem 2013;15:142-53.
9. Servin AD, Morales MI, Castillo-Michel H, Hernandez-Viezcas JA, Munoz B, Zhao L, *et al.* Synchrotron verification of TiO_2 accumulation in cucumber fruit: a possible pathway of TiO_2 nanoparticle transfer from soil into the food chain. Environ Sci Technol 2013;47:11592-8.
10. Ma C, Rui Y, Liu S, Li X, Xing B, Liu L. Phytotoxic mechanism of nanoparticles: the destruction of chloroplasts and vascular bundles and alteration of nutrient absorption. Sci Rep 2015;5:11618.
11. Conway JR, Beaulieu AL, Beaulieu NL, Mazer SJ, Keller AA. Environmental stresses increase photosynthetic disruption by metal oxide nanomaterials in a soil-grown plant. ACS Nano 2015;9:11737-49.
12. Ma H, Brennan A, Diamond SA. Photocatalytic reactive oxygen species production and phototoxicity of titanium dioxide nanoparticles are dependent on the solar ultraviolet radiation spectrum. Environ Toxicol Chem 2012;31:2099-107.
13. Manke A, Wang L, Rojanasakul Y. Mechanisms of nanoparticle-induced oxidative stress and toxicity. BioMed Res Int 2013. http://dx.doi.org/10.1155/2013/942916
14. Li M, Yin JJ, Wamer WG, Lo YM. Mechanistic characterization of titanium dioxide nanoparticle-induced toxicity using electron spin resonance. J Food Drug Anal 2014;22:76-85.
15. Sutar RC, Kalaichelvan VK. Evaluation of antioxidant activity of leaf extracts of *holoptelea integrifolia* (roxb) planch. Int J Appl Pharm 2014;6:6-8.
16. Ahamed N. Ecotoxicity concert of nano zero-valent iron particles-a review. J Critical Rev 2014;1:36-9.
17. Sanders K, Degn LL, Mundy WR, Zucker RM, Dreher K, Zhao B, et al. In vitro phototoxicity and hazard identification of nano-scale titanium dioxide. Toxicol Appl Pharmacol 2012;258:226-36.
18. Suttiponparnit K, Jiang J, Sahu M, Suvachittanont S, Charinpanitkul T, Biswas P. Role of surface area, primary particle size, and crystal phase on titanium dioxide nanoparticle dispersion properties. Nanoscale Res Lett 2011;6:27.
19. Skocaj M, Filipic M, Petkovic J, Novak S. Titanium dioxide in our everyday life; is it safe? Radiol Oncol 2011;45:227-47.
20. Koce JD, Drobne D, Klancnik K, Makovec D, Novak S, Hocevar M. Oxidative potential of ultraviolet A irradiated or nonirradiated suspensions of titanium dioxide or silicon dioxide nanoparticles on Allium cepa roots. Environ Toxicol Chem 2014;33:858-67.
21. Lee WM, An YJ. Effects of zinc oxide and titanium dioxide nanoparticles on green algae under visible, UVA, and UVB irradiations: no evidence of enhanced algal toxicity under UV pre-irradiation. Chemosphere 2013;91:536-44.
22. Meena R, Pal R, Pradhan SN, Rani M, Paulraj R. Comparative study of TiO_2 and TiSiO4 nanoparticles induced oxidative stress and apoptosis of HEK-293 cells. Adv Mater Lett 2012;3:459-65.
23. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil 1973;39:205-7.
24. Prasad KV, Saradhi PP. Effect of zinc on free radicals and proline in Brassica and Cajanus. Phytochemistry 1995;39:45-7.
25. Claussen W. Proline as a measure of stress in tomato plants. Plant Sci 2005;168:241-8.
26. Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. Role of proline under changing environments: a review. Plant Signaling Behav 2012;7:1456-66.
27. Giannopolitis CN, Ries SK. Superoxide dismutases I. Occurrence in higher plants. Plant Physiol 1977;59:309-14.
28. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972;47:389-94.
29. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
30. Dhindsa RS, Plumb-Dhindsa P, Thorpe TA. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot 1981;32:93-101.
31. Karuppanapandian T, Moon JC, Kim C, Manoharan K, Kim W. Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. Aust J Crop Sci 2011;5:709.
32. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot 2012. http://dx.doi.org/10.1155/2012/217037
33. Bouguerra S, Gavina A, Ksibi M, da Graça Rasteiro M, Rocha-Santos T, Pereira R. Ecotoxicity of titanium silicon oxide ($TiSiO_4$) nanomaterial for terrestrial plants and soil invertebrate species. Ecotoxicol Environ Saf 2016;129:291-301.
34. Chen HZ. Biological fundamentals for the biotechnology of lignocellulose. In: Biotechnology of Lignocellulose. Beijing: Chemical Industry Press; 2014. p. 73-141.
35. Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, Darnell J. The dynamic plant cell wall. In: Molecular Cell Biology. 4th ed. New York: WH Freeman; 2000.
36. White PJ. Ion uptake mechanisms of individual cells and roots: short-distance transport. In: Marschner's mineral nutrition of higher plants. 3rd ed. London: Academic Press; 2012. p. 9.

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