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# $TiO_2$ nanoparticle biosynthesis and its physiological effect on mung bean (*Vigna radiata* L.)☆

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ABSTRACT

$TiO_2$ nanoparticle (NPs) biosynthesis is a low cost, ecofriendly approach developed using the fungi *Aspergillus flavus* TFR 7. To determine whether $TiO_2$ NPs is suitable for nutrient, we conducted a two part study; biosynthesis of $TiO_2$ NP and evaluates their influence on mung bean. The characterized $TiO_2$ NPs were foliar sprayed at 10 $mgL^{-1}$ concentration on the leaves of 14 days old mung bean plants. A significant improvement was observed in shoot length (17.02%), root length (49.6%), root area (43%), root nodule (67.5%), chlorophyll content (46.4%) and total soluble leaf protein (94%) as a result of $TiO_2$ NPs application. In the rhizosphere microbial population increased by 21.4–48.1% and activity of acid phosphatase (67.3%), alkaline phosphatase (72%), phytase (64%) and dehydrogenase (108.7%) enzyme was observed over control in six weeks old plants owing to application of $TiO_2$ NPs. A possible mechanism has also been hypothesized for $TiO_2$ NPs biosynthesis.



## 1. Introduction

Titanium is a strong, lustrous, corrosion resistant metal. Its common compound, titanium di-oxide, is a popular photo-catalyst, and is used in the manufacture of pigments [1]. The $Ti^{+4}$ ionic state dominate titanium chemistry, owing to its high oxidation state, showing a high degree of covalent bonding. In plants, titanium has been reported to stimulate production of more carbohydrates, encouraging growth and photosynthesis rate [2–4].

$TiO_2$ is a non-toxic white pigment for use in manufacture of paints, plastics, paper, ink, rubber, textile, cosmetics, leather, and ceramics [5]. Photo catalytic degradation of pesticides with $TiO_2$ and other catalyst has shown promise as a potential water remediation method [6]. It has also been noted that titanium dioxide breaks down the ethylene gas produced in storage rooms into carbon-dioxide and water, thus it is also used to treat the air in fruit, vegetable, and cut flower storage areas to prevent spoilage and increase the product's shelf life [7].

In the rhizosphere, root exudation is a key process for carbon transfer into the soil, influencing the role of soil microbial communities in the decomposition of organic matter and in native nutrient cycling [8]. Root exudates are the substances released by roots and may affect growth and activity of soil microorganisms in the rhizosphere [9]. Root exudates act as a chemo-attractants to attract microbes toward roots and have been shown to increase the mass and activity of soil microbes [10].

Nanotechnology is one of the most important tools in modern science yet only a few attempts have been made to apply these advances for increasing crop productivity [4,11]. It is possible to develop microorganisms as bionanofactories for synthesis of agriculturally important particles. $TiO_2$ NPs are promising as efficient nutrient source for plants to increase biomass production due to enhanced metabolic activities, and utilization of native nutrients by promoting microbial activities. Fungi are relatively recent addition to the list of microorganism used in the synthesis of nanoparticles. The use of fungi is potentially exciting since they secrete large amounts of enzymes and are simpler to manage in the laboratory. In the biosynthesis of metal nanoparticles by a fungus, extracellular secreting enzymes are produced which reduce the metal salt of macro or micro scale into nano-scale diameter through catalytic effect. Negative electro kinetic potential of microorganisms enables to attract the cations and act as a trigger for biosynthesis of metal and metal oxide nanoparticles [12,13]. This study attempts to synthesize $TiO_2$ NPs using *Aspergillus flavus* TFR 7 as an ecofriendly biological approach and evaluate their effect on mung bean (*Vigna radiata* L).



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**Table 1**
Characteristics of the experimental soil.

| Parameter | Quantity |
|---|---|
| Sand (%) | 83.2 ± 4.2 |
| Slit (%) | 6.4 ± 0.8 |
| Clay (%) | 6.3 ± 0.5 |
| pH | 7.8 ± 0.1 |
| EC ($dSm^{-1}$) | 0.4 ± 0.04 |
| Organic carbon (%) | 0.29 ± 0.01 |
| Total N ($mg\,Kg^{-1}$) | 456 ± 13.5 |
| Total P ($mg\,Kg^{-1}$) | 719 ± 19.1 |
| Total K ($mg\,Kg^{-1}$) | 681 ± 16.8 |

## 2. Experimental details

### 2.1. Characteristic of experimental soil

An experimental soil (20 cm depth) was collected from Jodhpur, India (26°18′N 73°01′E), then air dried and sieved through 2 mm mesh. The soil was classified as loamy sand. Organic carbon was estimated by following the method of Walkley and Black [14]. Nitrogen, phosphorous and potassium were analyzed by Jackson [15]. In addition, pH and electrical conductivity were also measured.

### 2.2. Isolation and identification of soil fungi

The fungi was isolated from rhizosphere soil by initial plating on Martin Rose Bengal Agar medium (Hi-Media, India, pH 7.2) followed by serial dilutions over potato dextrose agar medium supplemented with chloramphenicol (Sigma–Aldrich, St. Louis, USA) at a concentration of 10 $\mu g\,mL^{-1}$. Isolated fungi was identified up to molecular level by partial sequencing of 18S and 28S rRNA and complete sequence of internal transcribed sequence 1 (ITS-1), ITS-2 and 5.8S rRNA. The sequence was compared with gene library data available on National Centre of Biotechnology Information (www.ncbi.nlm.nih.gov) using nucleotide blast algorithms, to identify isolated fungal strain using bioinformatics tool 'blastn'.

### 2.3. Synthesis of $TiO_2$ nanoparticles using soil fungus

To synthesize $TiO_2$ nanoparticles, *A. flavus* TFR 7 was developed in broth medium (pH 5.8) supplemented with of 0.3% malt extract, 1% sucrose, 0.3% yeast extract, and 0.5% peptone. The culture was kept on shaker at 150 rpm at 28 °C for 72 h to develop fungal ball of mycelia. These mycelia were separated out by filtration Whatman filter paper no. 1 (Whatman, UK) followed by triple washing with deionized water. Reaped mycelia (10 g fresh biomass) were re-suspended in 100 mL deionized water and incubated for 48 h at 28 °C under the same shaking condition as above. The obtained cell free filtrate containing extracellular enzymes was used for synthesis of $TiO_2$ NPs, in which precursor salt (Bulk $TiO_2$) was mixed at a concentration of $10^{-3}$ M and incubated for 36 h at 150 rpm and 28 °C to yield fine monodisperse $TiO_2$ NPs,

### 2.4. Characterization of synthesized $TiO_2$ nanoparticles

Synthesized nano-crystals were characterized morphologically by transmission electron microscopy (TEM; JEOL JEM-2100F) including high resolution (HR)–TEM mode for crystal phase confirmation, and energy dispersive X-ray spectroscopy (EDS; Thermo Noran equipped with TEM) for surface elemental analyses. Since particles were dispersed in water, hydrodynamic diameter was analyzed using dynamic light scattering (DLS; Beckman DelsaNano C, USA).

### 2.5. Seed germination and exposure of nanoparticles

The certified seed (obtained from institutional seed house) were surface-sterilized using 10% sodium hypochlorite solution followed by triple wash with deionized water. After that, five seeds were sown at 3 cm depth in each pot. The pots were placed in a greenhouse with 16 h photoperiod and 30/20 °C day–night temperature, 60% relative humidity and 360 $\mu mol\,m^{-2}\,s^{-1}$ photo-active radiation intensity. After 10 days of germination, seedlings were thinned to three per pot. The pots were completely randomized and re-positioned weekly to minimize uneven environmental effects.

The experiment was carried out with three treatments viz. control (without $TiO_2$ application), ordinary $TiO_2$ (1.6 μ), nano $TiO_2$ with each of six replicates. The $TiO_2$ particles (10 ppm) were exposed by foliar application to avoid direct soil contact using a fine nebulizer (25 mL per pot). The concentration and amount of nanoparticle solution was optimized in a preliminary screening experiment (data not shown here).

### 2.6. Phenological and physiological effect of nano$TiO_2$

Plants were harvested after four weeks of foliar application to investigate phenology and physiological state of plant. To analyze, shoots were cut at the soil surface and roots were carefully shaken to remove excess soil, and clumps of soil trapped between roots were removed, and number of nodules, root length, area and diameter were measured using Delta T Scan Software (Delta Scan, UK). To prepare the sample, roots were dipped in a methylene blue dye for 6 h while shoot length was measured on a meter scale.

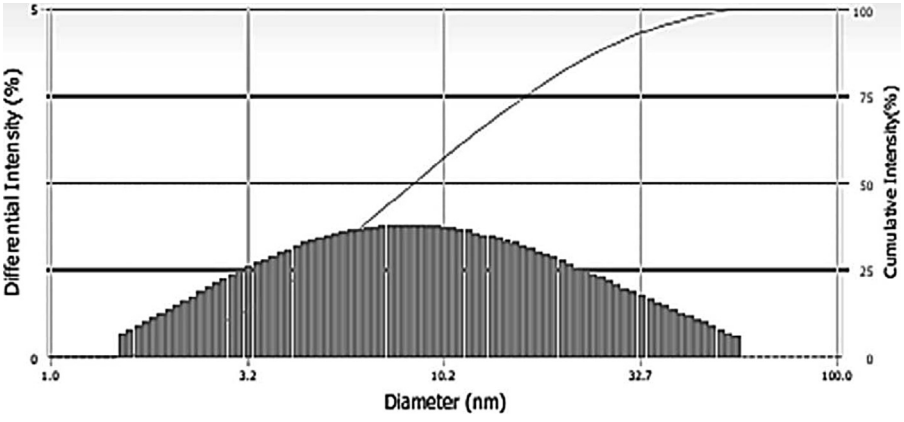


**Fig. 1.** Size distribution of biologically synthesized $TiO_2$ nanoparticles.