Effect of Titania (TiO$_2$) Nanoparticles on the Growth of Spinach (Spinacia oleracea) Under Differing Soil Conditions

Saman Rizwan*, Ishtiaq A. Qazi and Derk Bakker
*Department of Environmental Sciences, Forman Christian College (A Chartered University),
Ferozepur Road. Lahore, Pakistan.*
Corresponding Author Email: samanriz2010@gmail.com

Abstract
Nanotechnology has widely been used in a variety of fields including agriculture, since the last few decades. The aim of the present study was to assess the effect on the growth of Spinach (Spinacia oleracea) under exposure of 0, 100, 200, 250, 300, 400, 500 mg TiO$_2$ nanoparticles (TNPs) kg-1 of soil. TNPs in anatase form with a size of 74 nm, complex and spherical in shape were synthesized. Two different soils 1) Loamy Soil and 2) Sandy Soil were used under low pH (about 6.5) and high (original) pH of the soils. The effects of TNPs were investigated on plant lengths, total fresh and dry biomass. The plants were exposed to TNPs for about 3 months. It was observed that TNPs had a generally negative impact on the length of plants grown in sandy soil (both low and original pH) and loamy soil with low pH. The measurements of samples with the original pH of loamy soil showed a positive relationship with increased TNPs concentration. Overall the dry biomass of plants grew in (both low and original pH) loamy soil and sandy soil with low pH had increased with increase in concentration of TNPs, while in sandy soil with original pH, the biomass of plants decreased with increased concentration of TNPs. Phosphorous analysis on rhizosphere soil showed correspondence with biomass results. Generally, it was observed that type of soil and pH of soil affected the growth of spinach plants under applied TNPs.

Keywords: Titania Nanoparticles, Loamy Soil, Sandy Soil, Spinach plant, Fresh and dry biomass, Phosphorous phytoavailability, Length of plants in lower and higher pH of Soil

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Introduction

The economy of Pakistan (the sixth populous country of the world) [1] is largely based on agriculture which consists of crops and livestock products [2]. The most important crops of the country are wheat, rice, cotton, maize and sugarcane. The rise in the overall population of the world has increased the demand for food; resultantly over harvesting of crops has caused a drop in the level of soil nutrients as well as loss of its fertility. The common methods which are used to improve soil quality and to refill the nutrient pool include using animal and plant

manure as compost, reduced tillage, crop rotation, using cover crops, strip cropping, application of sludge or biosolids, and supplementing nutrients by other organic materials [3]. Use of chemical fertilizers is a comparatively modern method of replenishing primary (N, P, K) and secondary (S, Mg, Ca) nutrients in soil for effective growth of crops and to protect plants from nutrient-deficiency maladies. The fertilizers play a vital role in the field of agriculture as they increase the crop yield as well as the economic status of the farmers; but they are also contributing towards

destruction of the environmental and public health in the form of nutrient pollution [4], environmental degradation and low land productivity [5]. Excessive fertilizers are wasted in the environment through air sprays, leaching and runoff. They also become part of the food chain and cause a variety of health issues. Eutrophication arises from an oversupply of nutrients, mainly N and P; and causes algal blooms in water bodies [6, 7].

Nanotechnology has been defined by the British Standards Institute as "the design, characterization, production and application of structures, devices and systems by controlling the shape and size at the nanoscale (the size ranges from approximately 1 nm to 100 nm)" [8]. It is a cross disciplinary technology that has been used in many fields of physical and chemical sciences for a long time now [9] with its applications in fields such as agriculture, aerogels, aerospace, automotive, catalysts, cosmetics, coatings, composites, constructions, electronics, energy, environmental remediation, filtration and purification, food products, medical, optics, paints and pigments, packaging, paper and board, plastics, security, sensors, and textiles [10].

When the size of the particles of material becomes very small, its physical and chemical properties are fairly different than that of the same material in the bulk form. Nanoparticle (NP) is a core particle which performs as a whole unit in terms of transport and properties; and has unique importance due to its small size, morphology and large surface area [11]. NPs can be categorized on the basis of their origin (Natural NPs, Anthropogenic NPs and Engineered NPs) [12]; 2) location on the nanoscale structure in the system (i.e. nanostructured in bulk, or have nanostructure on the surface and contain nanostructured particles) [9] and presence of carbon in them.

Nanotechnology offers a solution to the above-mentioned problem associated with that of the use of fertilizers in agriculture by using NPs as an alternative. Some work has already been done in this field. The nanotechnology is making a remarkable difference in agriculture as it can both enhance crop productivity and reduce nutrient losses [13]. There are many benefits of using nanomaterials in agriculture, like reducing the amount of sprayed chemical products by smart delivery of active ingredients, minimizing nutrient losses in fertilization and increasing yields through optimized water and nutrient management [14]. Engineered NPs can help in soil restoration and increase in growth, biomass and germination efficiency of plants [15]. Commonly used NPs in agriculture include titania (TiO_2), zinc oxide (ZnO) and silver (Ag) NPs [16].

Literature has reported that NPs affect the physiological and chemical properties of plants through various factors [17]. They act as an antimicrobial agent [18] and improve plant growth, weight and seed germination [19]. The study of TiO_2 and Fe_3O_4 NPs on lettuce showed that applications had improved rhizosphere P availability in the plants [20]. There are also some negative effects associated with the use of NPs on plants, such as carbon nanotubes drastically effect on plant cell walls [21]. It is reported that TiO_2 and ZnO NPs have capacity to damage earthworms due to their antioxidant effects [22]. It has been reported that Titania nanoparticles (TNPs) have high efficiency as they significantly increased the height of plants, fruit yield and number of branches of coriander plant [23]. Similarly, physiological effects of TNPs were assessed on mung bean by foliar spraying the leaves of plants at 10 mg/L for 2 weeks. It was observed that shoot length, root length, root area, root nodule and chlorophyll content increased significantly [24]. Rafique *et al.,* showed that TiO_2 NPs

application affected P availability and growth in wheat plants [25]. Due to its large surface area and other properties, it was reported that nano anatase TNPs improved the photosynthesis process in the spinach plant as compared to bulk TiO_2 [26]. It was also found that TiO_2 NPs increased the root length while bulk TiO_2 had a pronounced effect on photosynthetic pigments of peppermint plants [27].

Titania or Titanium Dioxide (TiO_2) is a non-toxic white colored compound used in the manufacturing of paints, plastics, paper, ink, rubber, textiles, cosmetics, leather and ceramics [28]. It is reported to be a highly stable compound to heat, light, oxygen and pH. It is insoluble in water and most of the acids except HF and hot concentrated H_2SO_4 [29]. Naturally, it occurs in three forms rutile, anatase and brookite, where anatase is dominated in all due to the surface energy effects [30]. Titania is easily available, economical and naturally occurring compound. Studies have shown that toxicity of Titania is low and its toxicity depends on its physical form. It does not penetrate into the gastrointestinal tract and thus can be used in agriculture in a secure amount.

Phosphorous (P) is a non-renewable and essential element which is globally depleting at a fast rate. It is widely being acknowledged by the fertilizer industry that the quality of remaining phosphorous rocks is decreasing and production cost is increasing [31]. Phosphorus can be a limiting factor for the growth of plants as the availability of P is very low due to its slow diffusion and high fixation in soil [32]. Phosphorus behaves differently under different pH [33] and types of soil [34]. Several studies have been conducted to suggest different strategies, physical and chemical methodologies, use of modern and conventional techniques to improve phytoavailability of P in soil to cope with the continuous scarcity of the mineral [31, 35, 36].

Spinach, scientifically known as *Spinacia oleracea*, a highly nutritious green leafy vegetable commonly found and consumed in Asia. It belongs to the Amaranthaceae family of the kingdom Plantae. In 2017 world production of spinach was recorded as 27,885,841 tons on about 930,000 ha of land. In Pakistan, 109,403 tons of spinach yield was recorded on 8,763 ha of land [37]. Spinach is a good source of minerals such as iron, copper, phosphorous, zinc; Vitamin B complex, ascorbic acid, carotenoids, phenols and omega 3 fatty acids. This plant is used to treat asthma, diabetes, leprosy, urinary diseases, lung inflammation, joint pains, thirst, scabies and diseases related to the heart and brain [38].

Soil is formed due to the continuous but gradual weathering of rocks through physical, chemical or biological processes. Soil is a basic and essential building block of the ecosystem. Based on the particle size, it can be categorized as sandy soil, loamy soil, clay soil and silt soil. These textures of the soils affect the soil fertility, retention of water, porosity and air space between particles. Composition, organic matter, pH of soil and microbial communities are also important in soil classification. In countries like Pakistan, where the climate is mostly arid, alkaline soils are more common.

In order to observe the effect of TiO_2 nanoparticles (TNPs) addition to the soil regarding the growth of the spinach plant, 112 plants were potted in two different types of the soil; a) Loamy Soil (LS) and; b) Sandy Soil (SS) in both acidic and alkaline conditions. The plants were subjected to different concentrations of TNPs to see the effects of application on the growth of the plants.

The objectives of this study were 1) to study the effect of TNPs on growth of spinach in Loamy soil and Sandy soil and 2) to study the effect of pH in both of the above cases.

Materials and Methods
Chemicals

Chemicals used in this research work are given below:

1. Titania (TiO_2)- General Purpose Reagent (GPR) for preparation of TNPs;
2. Ammonium nitrate (NH_4NO_3) and Potassium chloride (KCl) as N and K fertilizer respectively;
3. Ammonium molybdate tetrahydrate {$(NH_4)_6MO_7O_{24}.4H_2O$}, Potassium antimonyl tartarate ($KsbO.C_4H_2O_6$), Ascorbic acid ($C_6H_8O_6$), Potassium dihydrogen phosphate (KH_2PO_4) Sodium hydroxide (NaOH) in Phosphorous analysis; and
4. Sulfuric acid (H_2SO_4) was used in acidifying the soils and in phosphorous analysis.

Preparation and Characterization of TiO₂ Nanoparticles

For application in soil, TNPs were prepared by using the sol gel method in the laboratory. Precisely 20 g of TiO_2 GPR was mixed in 100 mL of distilled water and stirred for 24 h on a magnetic plate at 250 rpm. Then the resulted slurry was placed in a laboratory oven for 12 h at 105 °C for drying. After drying and crushing, the dried slurry was calcined in a muffle furnace for 6 h at 450 °C [39]. The prepared TNPs were stored in air tight vials as proposed by researchers [40]. To characterize the sample size and phase of TNPs X-Ray diffraction (XRD) method was used and size was estimated using Debye-Scherer equation. Scanning Electron Microscope (SEM) imaging was used to observe the morphology of TNPs.

Soil Sampling and Preparation

Loamy soil (LS) was sampled from the Botanical Garden of Forman Christian College University (FCCU), Lahore. The soil was randomly sampled to a depth of 10 cm from different spots. Sandy soil (SS) was randomly sampled from an agricultural field in the Layyah district. The soil was randomly sampled to the depth of 15 cm from different spots in the field. Both soils were transported to the laboratory. The soils were sieved using a 2 mm sieve to remove stones, pebbles, roots and shells. The clean soils were stored in plastic buckets to be used later.

Soil pH

The pH of fresh soils was measured using instrumental method specified by ISO 10390:2005 using a laboratory pH meter (Thermo Scientific / Orion) with a glass electrode. 10 mg of the soil was mixed in 50 mL of water to form 1:5 suspension of soil in water using a magnetic stirrer [41]. The suspension was allowed to settle down before taking a reading from the pH meter. The pH of the LS was 7.8, while the pH of the SS was 8.1.

Soil Texture

The soil texture of both soils was estimated using the Saturation Percentage (SP) method, which is equal to the weight of water required to saturate the dry sample of the soil, divided by the weight of the dry soil. To determine its texture, 100 g air dried soil was taken in 100 mL container, slowly distilled water was added and mixed to form a saturated paste of 5 samples of each of the soils [42]. The average SP value for LS was

found to be 33.48%. While the average SP value for SS was 18.02%.

Finalizing Optimal pH for the Experiment

Different concentrations of sulfuric acid, nitric acid and acetic acid were tried in lowering the pH of the soils. Seeds of wheat, spinach and lettuce were germinated in the acidic soils to see the effect of acids. From the results of pre-experiment, it was decided to use 3x dilution of 1:4 of 98% H_2SO_4 for actual experiment as this concentration gave the best results in seed germination of spinach plants in both types of soils. The pH of LS was lowered to 6.6 and SS was dropped to 6.2 pH after application of acid. The soils with original pH (LSO and SSO) and with acidic pH (LSA and SSA) were used in the experiment.

Experimental Setup

For the actual experiment, indoor setup of growing plants was used to control the environmental factors.

Preparation of Pots and Wooden Rack

A wooden rack and discarded 1.5-liter polyethylene terephthalate (PET) bottles were used in the laboratory. The rack had capacity to hold 114 inverted bottles in 6 rows and 19 columns. The bottles were painted black from the outside, labeled and PVC pipes were attached to these so that they could fix in the rack holes properly used. LED lights were installed on the frame for the continuous provision of light for the plants.

In each bottle/pot, course granules were used to form the base of the bottle and 1kg of soil was placed over the granules. 3 germinated spinach seedlings each, germinated separately in soil for four weeks, were transferred to each of 112 pots/bottle later in the experiment.

Samples for Real Experiment

Soil samples of LS and SS were prepared using different concentrations of TNPs using 0, 100, 200, 250, 300, 400 and 500 mg/kg of the soil in both original (alkaline) and lower (acidic) pH. To satisfy the need for Nitrogen (N) and Potassium (K); ammonium nitrate (NH_4NO_3) and potassium chloride (KCl) were used as fertilizers in 50 ppm and 70 ppm concentrations in the soil, respectively. Seedlings were carefully taken out from soil without damaging their roots and washed with tap water before transferring these to the pots. Each sample had 3 of the spinach seedlings.

All samples had four repetitions. Total (types of soil * pH of soils * concentrations of TNP * repetitions) 2*2*7*4 =112 samples were placed randomly in the wooden rack. To satisfy the need of light in the lab, all samples were subjected to 24 h light provided through the installed LED. A sample is visually illustrated in Fig. 1. The plants were watered 35 mL to 45 mL per day once or twice depending on the weather and conditions of the plants up to about 3 months.
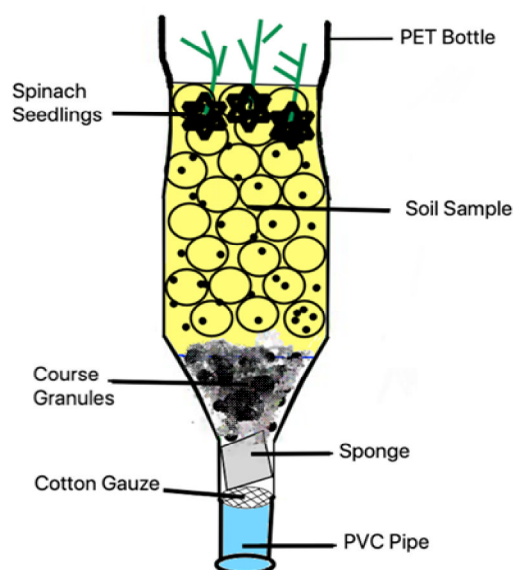

Figure 1. **Illustration of sample in a bottle**

Harvesting

The plants were harvested after 3 months of TNPs exposure to Spinach plants. Shoots were collected from an above ground portion of the plants, washed with distilled water and their length was measured. The grown spinach plants were fresh colored and large sized. Traces of red color was observed in stems of 20 out of 112 samples. In one of the four repetitions of SSA 500, no plant was found to have grown. This may be due to the high concentration of TNPs in combination with low pH. In another case of SSO 100, growth was observed, but it was some kind of weed rather than the spinach plant.

Examination of Effects of TNPs on Plants
Length of plants

The lengths (heights) of plants were measured using a simple ruler and readings were jotted down for further analysis.

Biomass of plants

To determine the biomass of plants, their fresh weight was taken using weighing machine (Shimadzu type AUW 2200) before drying them at 60° C in a laboratory oven (PCSIR / DOD-1-60/05) for 48 h. After that, the dried biomass was taken using a laboratory weighing machine.

Moisture content of soil

The moisture content of plants and soils after harvesting was calculated using the following equation.

$$\text{Moisture Content (\%)} = \frac{\text{Fresh weight of sample} - \text{Dry weight of sample}}{\text{Dry weight of sample}} \times 100$$

The soil was dried at 105°C for 24 hours. Moisture content (%) is defined as the ratio of water content present to the mass of dry sample.

Phosphorous analysis in soil

Phosphorous analysis was carried out on rhizosphere soil of plant samples using the ascorbic acid method [43]. Following reagents were prepared in the lab for the analysis:

Preparations of Reagents

All reagents were prepared in the laboratory.

1. 0.5 M sodium bicarbonate solution ($NaHCO_3$) was prepared by dissolving 42 g of $NaHCO_3$ in 1L. 5N NaOH was used to adjust the pH of 0.5 M $NaHCO_3$ to 8.5.
2. Mixed Reagent:
   a. Ammonium molybdate tetrahydrate $(NH_4)_6MO_7O_{24}.4H_2O$ was prepared by dissolving 6 g of the salt in 125 mL of distilled water.
   b. Potassium antimonyl tartarate ($KsbO.C_4H_2O_6$) was prepared by dissolving 0.1455 g of the salt in 50 mL of distilled water.
   c. 5N H_2SO_4; 74 mL of 98% concentrated H_2SO_4 was diluted in 500 mL of distilled water.
   All a., b. and c. (given above) chemicals were mixed together and the volume was raised to 1L using distilled water. The resulting solution (mixed reagent) was stored in a dark and cool place in a pyrex bottle.
3. Color developing reagent:
   A 0.528 g ascorbic acid was mixed in 100 mL of mixed reagent. This reagent was used to prepared every time fresh before its usage.
4. P Stock Solution:
   A 2.5 g of Potassium dihydrogenp-hosphate (KH_2PO_4) was dried in the laboratory oven (PCSIR / DOD-1-60/05) for 1 hour at 105°C and then was cooled down in a desiccator. The dried chemical was stored in air tight

bottle before usage. Exactly 2.197 g of the dried chemical was dissolved in 500 mL of the distilled water. The concentration of P in this solution was exactly 1000 mg per Litre. 10 mL of this solution was further diluted to 100 mL with the distilled water, now the concentration of P became 100 mg per Litre.

For the preparation of standards from 100 mg per Litre of Phosphorous dilutions were prepared using the following equation

$$C_1V_1 = C_2V_2$$

The following concentrations of stock solutions were used to prepare dilutions (mg/L P):

Table 1. Dilutions of P from concentrated stock solutions.

Conc. of Stock Solution (100 mg/L) of Phosphorous	Dilution contains P (mg/L)
0.125	0.5
0.187	0.75
0.25	1
0.3125	1.25
0.375	1.5
0.438	1.75
0.5	2
0.563	2.25
0.625	2.5
0.6875	2.75
0.75	3
0.8125	3.25
0.875	3.5
0.938	3.75
1	4

Procedure

In a conical flask 2.5 g of dried soil was mixed in 50 ml of extracting solution (0.5 M $NaHCO_3$). The solution was shaken on a mechanical shaker (SCILOGEY/ SK-0330-Pro) at 180 rpm for 30 minutes and was filtered using a vacuum pump (GS/ AS 20) with Whatman no. 42 filter paper. 5 mL of the filtered solution was taken in 25 mL volumetric flask. 5 mL of a color developing agent was added and the flask was shaken to remove air bubbles and then diluted to 25 mL with the distilled water. The bluish color was developed, representing the presence and concentration of P in soil. After 15 min the samples were analyzed on a spectrophotometer (Shimadzu/ 1800-UV) at 880 nm wavelength.

For prepared standards, a calibration curve was prepared by plotting absorbance at Y-axis and Phosphorous concentration at X-axis. This calibration curve was used to calculate the concentration of P in the unknown soil samples. Phosphorous in fresh LS and SS was found to be 3 and 5 mg/kg, respectively.

Results and Discussion
Characterization of TiO₂ Nanoparticles
X-Ray diffraction

The crystal phase composition and crystallite size of synthesized TNPs were analyzed through XRD analysis, in the 2θ scan range of 20-80° as shown in Fig. 2. Strong diffraction peaks around 25.4° confirm that synthesized TNPs are in the anatase phase [44]. The average size of TNPs has been estimated as 74 nm using Debye-Scherrer equation i.e.
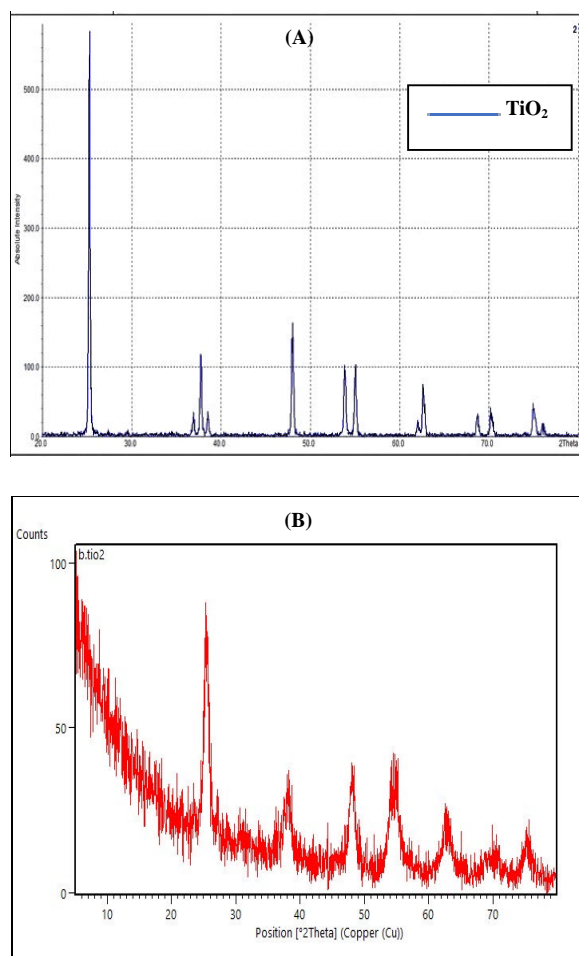
$$D = \frac{K\lambda}{\beta \cos\theta}$$

Figure 2. **XRD pattern of synthesized TNPs with A) absolute intensity B) counts of particles**

Scanning electron microscope (SEM) imaging

The morphology and structure of the synthesized TNPs were investigated using VEGA 3 with an acceleration voltage of 15 kV. Fig. 3 shows SEM images of synthesized TNPs at a magnification of 20 k. It is evident that particles are rough, complex and spherical in shape.

Anatase is one of the three types of TNPs, it has a large surface area and is known for its photocatalytic activity due to its bandgap of 3.2 eV [25]. In this research study anatase TNPs with a size of 74 nm was synthesized, applied in different

concentrations of 0, 100, 200, 250, 300, 400, 500 mg/kg of the soils to see its effect on the growth of spinach plant.
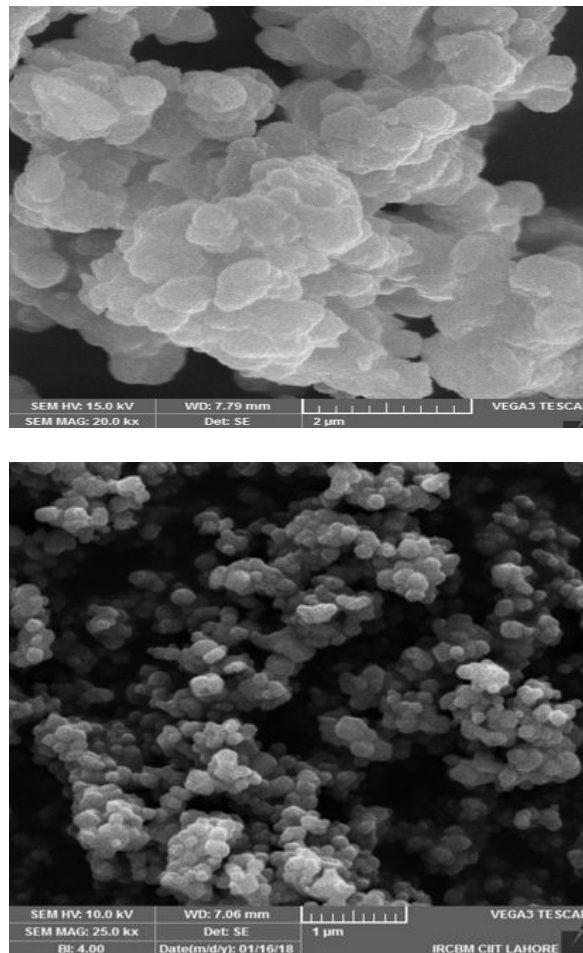
Figure 3. **SEM images of synthesized TNPs at 20.0k**

Effect of TNPs Applications on Growth of Plants

The effect of TNPs applications with concentrations 0, 100, 200, 250, 300, 400, 500 mg per kg of the LS and SS were investigated by measuring the length and biomass of the plants grown.

Measurements of length of spinach plants

The lengths of the plants were measured after 3 months of applying TNPs on soil. The lengths varied widely across the

treatments in loamy soil and sandy soil (Fig. 4). Figure 4A is showing that the trend line of length of plants in LSO is increasing with increase in the concentration of TNPs. While in LSA, the trend line is going slightly down with increased concentrations of TNPs. It means plants in low pH with a lower concentration of TNPs while plants in higher pH with a higher concentration of TNPs are growing more favorably.
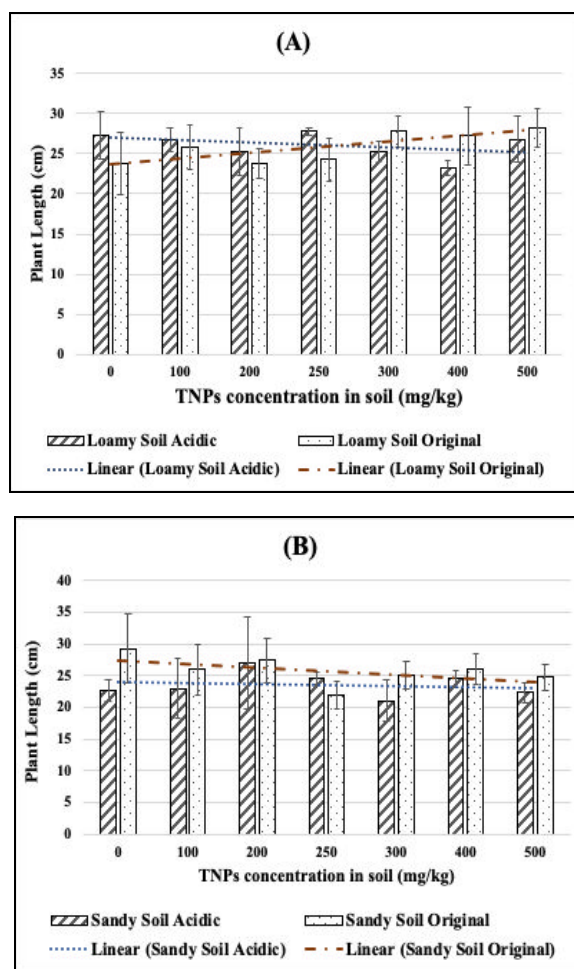

Figure 4. **Effect of TNPs on length of plants in (A) Loamy Soil (B) Sandy Soil**

Noticeably, the plant lengths in every individual application was varied. On the other hand, in SS, lengths of plants had decreased with increased concentration of TNPs (Fig. 4B). The plants in the original pH of soil had more lengths than the plants in low

pH except for 250 mg/kg of TNPs. Furthermore, the trend lines are running downwards with increased concentration of TNPs. Overall, plants in low pH are showing better growth as compared to higher pH. A decrease in lengths of plants with increased concentrations of TNPs suggests that at higher concentrations, TNPs inhibit length; similar pattern of TNPs was observed on wheat plants as well [45]. However, the length of plants in original pH of LS increased in applied conditions suggests that toxicity or inhibitory effect of TNPs was alleviated by acidic conditions of soil. In comparison to the blank treatments, improved lengths has been observed in LS samples with a noticeable difference especially in original pH.

Estimation of biomass of spinach plants

In period of about 3 months of experiment the plants were fully grown. Fresh biomass and dry biomass of the plants in samples were recorded to estimate the effect of applied TNPs treatments on the biomass of spinach.

Fresh biomass of spinach plants in loamy and sandy soils

The data of fresh biomass of plants grew in LS under applied concentrations of TNPs shows that with original pH there is a significant difference in the increase in biomass with increase in TNPs. However, with low pH the difference in biomass of TNPs samples as compared to blank is not apparent. The trend lines of samples with original and low pH intersect at 250 mg/kg concentrations of TNPs indicating that at 250 mg/kg concentration plants in high and low pH grew likewise (Fig. 5A).

The data on fresh biomass of plants in SS shows the opposite trend as compared to that of in LS (Fig. 5B). The plants grew

differently in all treatments. The trend depicts that the average growth of plants decreased with increased concentrations of TNPs.

The effect of TNPs on the fresh biomass of plants is also very interesting in this study. It has been observed that in loamy soil the fresh biomass of spinach has increased under applied conditions. This is important in terms of the commercial aspect and market value of the plant as if the biomass increases, it would bring more yield from the plant.

Figure 5. **Average fresh biomass in (A) loamy soil and (B) sandy soil samples**

Dry biomass of spinach plants in loamy and sandy soils

Dry biomass that contains the nutritional values of plants, without water content, in LS and SS is shown in Fig. 6. In LS samples with original pH, significant increase in biomass was observed. However, in low pH conditions, the highest biomass was also observed in the highest concentration of applied TNPs. The trend lines of both low and original pH of soil shows a continuous increase in biomass with the increase of concentration of TNPs in loamy soil.

In SS, the trend of growth of plants in low and original pH samples was observed to be opposite to each other. In this type of soil, a continuous increase of biomass was observed in samples in low pH (SSA) with increased concentrations of TNPs, while in SSO, maximum biomass was observed at 100 mg/kg concentration of TNPS. Hanif et al., also reported a similar observation on the growth of cabbage plants under application of TNPs in sandy-loamy soil [46]. Overall the biomass has decreased with increased TNPs in sandy soil with original pH.

Several studies have also reported the effects of TNPs on physiological elements of plants and results are contradictory [20, 22, 23]. According to the results observed on dry biomass, the plants grew in (both low and original pH) LS and SS with low pH had increased with an increase in the concentration of TNPs. While in SS, with original pH the biomass of plants decreased with increased concentration of TNPs. In this case, it is being anticipated that acid has played an enzymatic role in increasing the biomass of Spinach plants, in combination with applied TNPs concentration.

It was observed that the effect of TNPs on length and biomass of plants was very
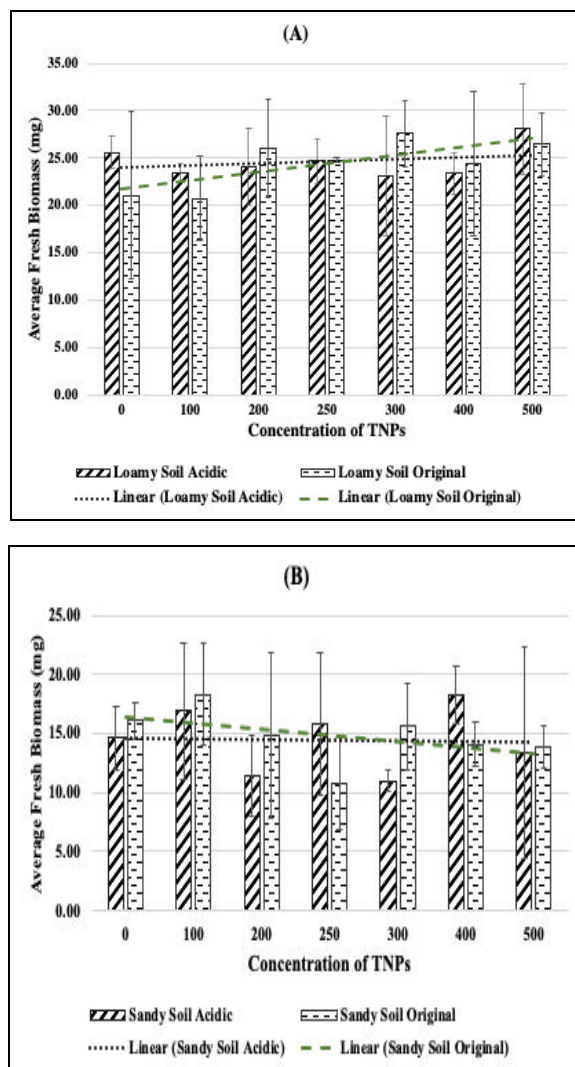
different in differing soils. The physiochemical characteristics of LS and SS are very different and the addition of acid may have altered the soil properties as well.
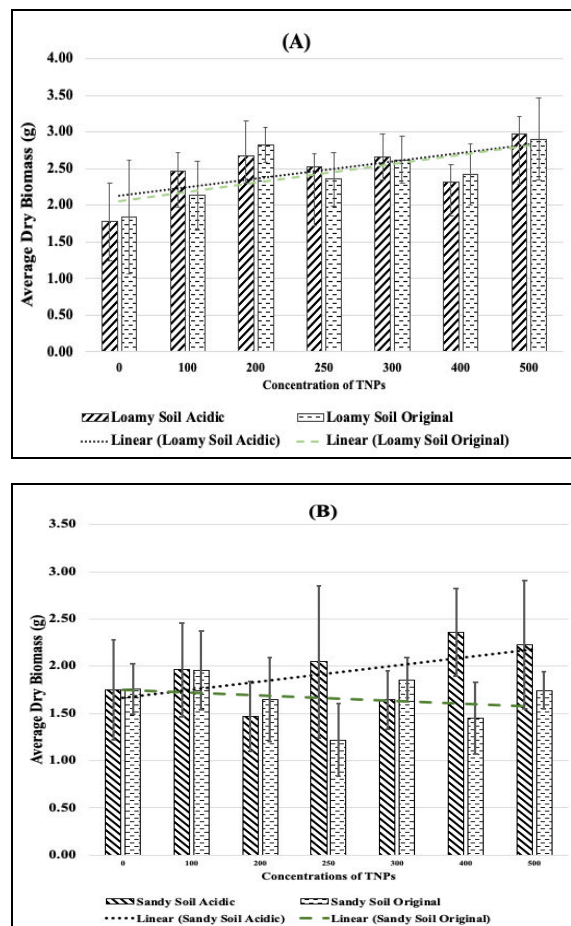

Figure 6. **Average dry biomass in (A) loamy soil and (B) sandy soil samples**

Moisture Content of Soil

The moisture content of rhizosphere soil was estimated and the average % of water content in LSA, LSO, SSA, SSO was found to be 5.43%, 8.14%, 8.71% and 8.28% respectively.

Phosphorous Availability in Rhizosphere Soil

This analysis was done to see the effect of TNPs on the availability of Phosphorous in samples of LS and SS using

Olsen method, with a calibration curve prepared from standard solutions.

Analysis of P in loamy soil

The P content of samples in LS in both original and low pH concentrations under applied TNPs is shown in Fig. 7. The results indicate the overall increase in P concentrations with an increase in concentrations on TNPs showing a similar trend in both low and original pH of the loamy soil. The best results were found at concentrations 200 and 500 of TNPs. It must be noted here that fresh and dry biomass were also high at these two concentrations in both low and original pH conditions. In higher concentrations of TNPs in original pH, P availability in the rhizosphere decreased.
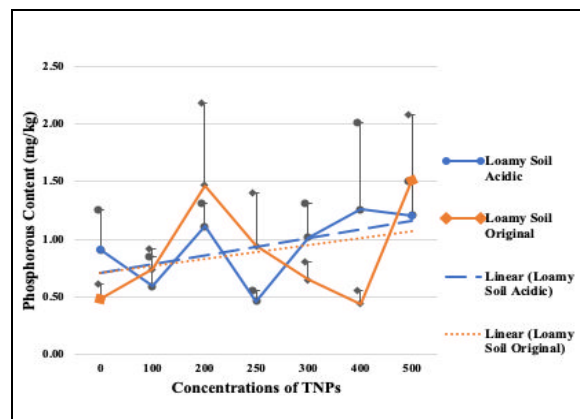

Figure 7A. **Phosphorous availability in loamy soil**

Analysis of P in sandy soil

The P availability in the region of soil (rhizosphere) of samples of sandy soil in both low and original pH is presented in Fig. 8. It is evident that the trend of P availability in sandy soil samples decreased with increased concentration of TNPs in both pH levels. The highest values of P were found at 100 mg/kg TNPs application, which is also in correspondence of their fresh biomass. In this case acidity of the soil is showing an important role in combination with a higher

concentration of TNPs. The P analysis of rhizosphere soil samples shows comparable results of availability of P (mg/kg) with biomass of plants grown in these soils. Nevertheless, acidity played role in combinations with a concentration of applied TNPs. Hopkins & Ellsworth (2005) also reported that the availability of P in high pH is relatively poor, which not only affects the heights and color of plants but also do not allow fertilizers to function properly [47]. It was suggested to lower the pH of soils as a solution to this issue.
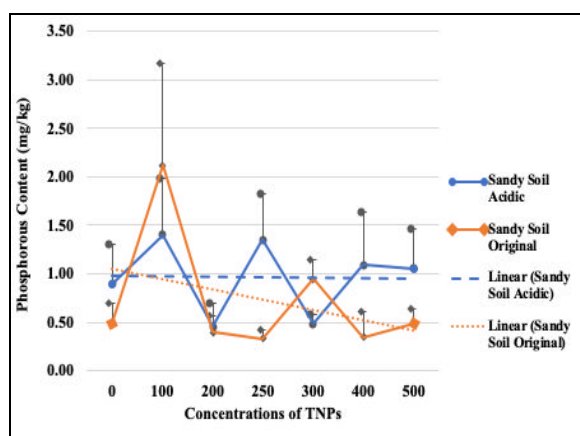
Figure 8. Phosphorous availability in (B) sandy soil samples

TNPs behave differently under different conditions and components of the environment. In their agricultural applications, the effect of TNPs depends on the type of plant species, soil type, chemical and biological composition of soil and their physiochemical properties. However, one additional factor may be the application of TNPs and their exposure to the plants.

As the sample wise results showed the zig zag pattern of lengths, biomass, P present in soil it is noticeable that TNPs affect spinach plants variedly in combination formed with pH of soil and type of soil. So, it is important that combination of these three factors may be explored more to get the best application concentrations of TNPs at a commercial scale.

Conclusion

The experimental results and analysis carried out have concluded that the Titania nanoparticles generally have a positive effect on the growth of Spinach. In both high and low pH of loamy soil, the plants exhibited an increase in the biomass. In sandy soil plants showed good growth with low pH conditions. The fresh biomass of plants in loamy soil with low pH has also increased significantly as compared to blank, which highlights the importance of this combination with an increased market value of the crop. Furthermore, overall in rhizosphere Phosphorus is increased in loamy soil, due to the Titania nanoparticles, indicating its enhanced bioavailability and an increase in the biomass of the spinach plants. In sandy soil, acidity of the soil contributes to the availability of P in rhizosphere. The type of soil, pH of soil, and interaction of concentrations of TNPs; all three factors have combined effect on P availability in the rhizosphere, thus affecting the plant growth.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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