COMPARATIVE ANALYSIS OF FERTILIZER LEVELS AND APPLICATION METHODS OF TITANIA NANOPARTICLES ON THE GROWTH OF RICE (*ORYZA SATIVA* L.)

Shagufta Irum

(00000119248)

Institute of Environmental Sciences and Engineering (IESE)

School of Civil and Environmental Engineering (SCEE)

National University of Sciences and Technology (NUST)

Islamabad, Pakistan

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By

Shagufta Irum

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CERTIFICATE

It is certified that the contents and forms of the thesis entitled **"Comparative Analysis of Fertilizer Levels and Application Methods of Titania Nanoparticles on the Growth of Rice (*Oryza sativa* L.)"** submitted by Ms. Shagufta Irum has been found satisfactory for the partial fulfillment of the requirements of the degree of Master of Science in Environmental Science.

Supervisor: ____________________
Dr. Muhammad Arshad
Associate Professor
IESE, SCEE, NUST

Member: ___________________
Dr. Sofia Baig
Assistant Professor
IESE, SCEE, NUST

Member: ____________________
Dr. Umair Manzoor
Professor
SCME, NUST

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Signature with stamp: ____________________________

Name of Supervisor: _____________________________

Date: ____

Signature of HoD with stamp: ______________________

Date: ____

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I dedicate this thesis to my beloved parents, siblings and my husband who would always be a source of inspiration for me and stood beside me at every moment in my life

بسم الله الرحمن الرحيم

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Contents

LIST OF ABBREVIATIONS

TNPs	Titania Nanoparticles
PD	Plasmodesmata
ATP	Adenosine Triphosphate
MWCNTs	Multi-walled Carbon Nanotubes
AgNPs	Silver Nanoparticles
SOD	Superoxide Dismutase
ROS	Reactive Oxygen Species
UV	Ultra Violet
PS-II	Photosystem II
SEM	Scanning Electron Microscopy
XRD	X-Ray Diffraction Spectroscopy

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ABSTRACT

Nanotechnology is the fastest growing industry now-a-days which is having considerable impacts on society, economy, and also on environment. One of the most recent aspects in the domain of environment and nanotechnology is the potential assessment of nanoparticles interactions with plants. This study focused on interactive effect of phosphorus fertilizer levels and TiO_2 nanoparticles method of application on growth of rice. Rice was exposed to TiO_2 nanoparticles using three different methods i.e. via irrigation, soil application and foliar spray. For each treatment, there were four phosphorus levels (P1 = 0 mg kg^{-1}, P2 = 10 mg kg^{-1}, P3 = 20 mg kg^{-1}, P4 = 40 mg kg^{-1}), whereas recommended N (70 mg kg^{-1}) and K (32.5 mg kg^{-1}) were added. The first phase and analysis, focused on plants' growth variations under these treatments. The purpose of second phase was to assess plant uptake of phosphorus and rice grain protein content. The results revealed significant ($p < 0.05$) combined effects of TiO_2 nanoparticles method of application and phosphorus fertilizer level on root dry weight and grain protein content. The highest shoot length (63.4 cm), number of tillers (7 n $plant^{-1}$), root dry weight (6.2 g $plant^{-1}$), shoot dry weight (7.52 g $plant^{-1}$), shoot phosphorus (1140 mg kg^{-1}) and rice grain protein content (23.8%) were recorded under the application of TiO_2 nanoparticles through irrigation method in combination with 40 mg P kg^{-1}, although this method of TiO_2 nanoparticles application performed equally good at half dose of 20 mg P kg^{-1}. The positive effects of TiO_2 nanoparticles can be attributed to their ability to penetrate through the root surfaces, improving root water and nutrient acquisition, therefore enhance the fertilizer use efficiency. TiO_2 nanoparticles might have exerted catalytic role to trigger metabolic activities for plant growth. Further detailed studies are needed to understand the mechanisms involved in improved phosphorus uptake and plant growth.

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Chapter 1

INTRODUCTION

1.1 Background

Rice is the second most widely cultivated crop throughout the world. Globally, the area of about 162.3 million hectares was dedicated for rice cultivation in 2012 which led to the total grain yield of about 738.1 million tonnes. On average, in 2012 the farm produce for rice across the world was 4.5 tonnes per hectare (FAO, 2014). In Asian countries rice is among the most commonly consumed cereal crops. Rice is a good source of proteins, mineral elements, vitamins, carbohydrates and fiber which are important for growth and nourishment. Furthermore, some studies revealed that rice contains phenolic compounds and certain phytochemicals having biological activity in plant body thereby playing role in plant protection (Tian *et al*., 2005; Aguilar-Garcia *et al*., 2007).

By the year 2050, due to rapid growth the world population will constantly increase and reach to about 9.6 billion, an estimated 30% increase as compared to the population in 2010s (UN, 2013). Furthermore, the need to expand global agriculture production has increased as a results of inclination towards meat-based and increase in the demands for bioenergy crops. It has been estimated that in order to fulfill these demands approximately 70% increase in global grain production by the year 2050 is required. One common approach is to apply significant amounts of fertilizers to the crops along with limited water resources and scarcity of additional arable lands to promote global food production. In the meanwhile, on a global scale serious environmental issues have emerged. In agriculture sector, diverse conventional fertilizers are applied at an ever increasing level and for longer duration for maintaining the existing levels of grain production (UN, 2013; FAO, 2009).

1.2 Phosphorus Deficiency and Fertilizer Application

Phosphorus is a macronutrient with inadequate bioavailability but it is crucial for crop development and yield. Overuse of synthetic phosphorus fertilizers has resulted in low phosphorus use efficiency (PUE), leading to severe environmental damage and speed up the exhaustion of rock phosphorus reserves. It has become tremendously challenging to enhance PUE while stabilizing global food supplies and upholding environmental sustainability (Hasan *et al.,* 2016).

To promote phosphorus availability and uptake, various strategies such as rock phosphate application (Mihajlovic *et al*., 2014), addition of phosphorus based fertilizers to the soil (Sawan *et al*., 1997) and application on the aerial plants parts through foliar spray (Kaya *et al*., 2001; Shivay *et al*., 2015) have been tested. Apart from harmful effects of chemical fertilizers on human health, these fertilizers also lead to degradation of soil quality as well as water pollution. So, in order to reduce the utilization of chemical fertilizers at high concentrations, there is a strong need for an alternative approach. Recent advancement in agriculture fields involves the use of nanoparticles for enhancing phytoavailability of phosphorus that is important for growing and developing plants (Scholz *et al*., 2014; Zahra *et al.,* 2015; 2017).

Due to inadequate knowledge about plant physiology as well as soil properties, farmers apply high doses of phosphorus fertilizers to the crops in order to improve yield during the cultivation season (Cordell *et al.,* 2009). During the growing season plants uptake and utilize only about 50% of the phosphorus fertilizer applied by the farmers. The residual P gets fixed in soil as a result of certain chemical processes or it moves downward due to run off into the fresh water bodies, thereby becoming one of the leading cause of underground water contamination or eutrophication of marine environments (Guo, 2007).

Crop production is prone to be hindered because of certain agricultural as well as biological attributes. Soils having low contents of phosphorus reserves when used for crop cultivation produce limited crop yield. Ecological stress and nutritional deficiency also have potential to modify plant root system architecture (Hasan *et al.*, 2009; Haq *et al.*, 2012).

According to previous assessments, the resources of phosphorus may be depleted in the world by the year 2050 (Vance *et al.*, 2003). Therefore, phosphorus accessibility to plants in sufficient amounts is a worldwide issue. Typically high concentration of organic and inorganic phosphates are present in soils but the concentration of phosphorus for plant uptake is low because of the total inorganic phosphorus about 88 to 99% gets bind to calcium present in the soil as a result of chemical reactions. Throughout the world several soils are phosphorus deficient because available P concentrations to plants are usually very low (Gyaneshwar *et al.*, 2002). The availability of phosphorus with applied phosphate fertilizer is less efficient due to its conversion to insoluble complexes as it comes in interaction with soil (Vassilev and Vassileva, 2003). Hence continuous use of phosphate containing fertilizer is very crucial in meeting crop requirements. The ever growing rates of fertilizers from industry will make it further high priced to be used on cultivable lands by farmers.

1.3 Nanotechnology

Nanotechnology is manipulation of atomic structure of matter for innovation of its characteristics and utilization in medicine, agriculture, industries and manufacturing sector. The basic principle of the nanotechnology involves reducing the particle sizes, while improving efficiencies of cellular uptake and other physical properties (Iavicoli *et al.*, 2012; Vasantharaja *et al.*, 2014). Nanotechnology is useful in providing technological platforms for research and revolution of biological systems (Jalill and Yousef, 2015). Nanomaterials can be defined as materials having at least one dimension with a single unit size ranging between 1 and 100 nm (Liu and Lal, 2015).

Even though fertilizers play significant role for the nourishment and development of growing plants but all of the applied fertilizers are not taken up by plants due to the processes of percolation, hydrolysis and decomposition. This nutrients loss can be controlled and crop yield can be enhanced through nanotechnology involving the use of nanoparticles for improving the soil quality and stimulating the plant growth. Products of nanotechnology have the potential to release the nutrients on-demand, which can enhance plant growth and boost targeted actions (Nair *et al.,* 2010). This application is also essential to cope with increasing global food security and climate change challenges (Parisi *et al*., 2014). Full potential and applications of nanotechnology in the field of agriculture still need to be explored but along with it is also vital to fully understand its impacts on environment.

The rapid and swift advancement and possible release of engineered nanoparticles into the environment have raised many issues and concerns due to their exceptional properties. Different plants respond to or interact with nanoparticles differently, usually dependent upon the size, concentration, as well as physio-chemical characteristics of nanoparticles and the plant species. Various plants respond differently at different growth phases either enhancing or hindering effects of different nanoparticles on growth pattern. Presently a new area of research is now paying attention on short and medium term projects related to environmental and ecological impact of these nanoparticles (Ma *et al*., 2010a). It is important to investigate impacts of nanoparticles on yield and nutritional quality of rice grain.

Nanotechnology seems to have potential for addressing the problem of food security. Rice is one of the most common staple food across the world and Pakistan. To enhance the production of rice and fulfill the needs of rapidly growing population, appropriate modifications in agriculture system and incorporation of other technologies are strictly needed. Due to intensive cultivation, soil fertility is further depleting constantly due to withdrawal of vital plant nutrients from the soils. To overcome this issue, TNPs can be used to improve functionalities of crops especially rice. These nanoparticles could benefit the agriculture and save natural resources of phosphorus for good crop productivity in Pakistan.

1.4 Scope of Study

In the recent years, scientists have worked on different nanoparticles effects on the plant growth and other plant mechanisms. There is little information available related to the influence of nanoparticles on nutrients availability to crop plants. This study will help identify the most effective method of TNPs application and will help determine the suitable phosphorus level. Therefore, this study is designed to identify the nanoparticles behavior in soil environment with focus on phosphorus uptake which is important for better crop yield and will help lessen the requirements for phosphorus fertilization thereby improving the environment.

1.5 Objectives of Study

Keeping in view the information available from the literature, the present study focused on the following objectives:

- To assess the effect of mode of TNPs application on plant growth and phosphorus uptake
- To evaluate impacts of different levels of phosphorus fertilizers applied in combination with TNPs

1.6 Significance of Study

This study will help to give new insights of how modern crop technologies, in particular nanotechnology approaches, may be applied to improve the nutrient availability to food crops especially making insoluble phosphorus available to the plants. This study has the wide scope to demonstrate that how rice plant responds when TNPs are applied using different methods. Additionally, after TNPs application, physiological functions and nutritional qualities of crops can be improved or not such as phosphorus. This study also gives us an insight of most optimum concentration of phosphorus fertilizer for working of TNPs that should be applied in an effective way for the betterment of agricultural crops.

Chapter 2

LITERATURE REVIEW

This chapter focuses on the related literature on the importance of nanotechnology and uses, agricultural aspects and role of nanoparticles in plants.

2.1 Phosphorus as major plant nutrient

Phosphorus is among the most important macronutrient for plant growth. Consistent supply of phosphorus is required by plants because it is important structural constituent of molecules such as nucleic acids, membranes and metabolic products like adenosine triphosphate (ATP) crucial for plant development as well as promotes its growth (Schachtman *et al.,* 1998; Lambers *et al.,* 2015). It is important in many processes, including glycolysis, photosynthesis, nucleic acid synthesis, energy generation, respiration, membrane formation, redox reactions, nitrogen fixation, carbohydrate metabolism and enzyme inactivation/activation (Wu *et al.,* 2005).

Due to its high stability in soils, reactivity of phosphorus is enhanced and so it forms a phosphate ester bond with other elements such as Ca-P, Al-P, or Fe-P, as a result its solubility in the soil is drastically reduced. For more than three decades decrease in the rate of P uptake by crops and increased fixation of P applied in the form of fertilizer had become a key issue in crop production (Mackenzie *et al.*, 1964). Although soils have phosphorus in large amount but only small fraction is available to the plants. The availability of phosphorus is not enough and due to lack of available phosphorus about 30–40% crop yield of the world is limited. According to estimations the worlds resources of low cost phosphorus may be depleted by 2050 (Vance *et al.*, 2003).

2.2 Nanotechnology and its Applications

Nanotechnology has emerged as state-of-the-art and innovative field of interdisciplinary approaches. It has extensive range of applications in several fields of science as electronics, biology, biotechnology medicine, pharmacology and breeding (Alivisatos *et al.*, 2005, Begum *et al.*, 2014). Rapid development in the field of nanotechnology at commercial and industrial level has resulted in increased emission as well as accumulation of nanoparticles in the environment. Nanoparticles inevitably effect living organisms but such effects are less understood in plants (Rico *et al.*, 2011).

Nano-particles are now being widely utilized in many fields and their interaction with the surrounding environments is one of the major issues and causes of concern. Nano-particles can enter the agro ecosystem by many different means including water, soil and plants. To date, different studies related to the application of nano-particles and their bioaccumulation in plants have been infrequently reported. Research in field of nanotechnology has been formerly aiming at fast development in different domains of electronics, biotechnology and biomedical science, etc. Likewise scientists have also been paying attention on the special effects and mechanisms of different nano-particles on various plants (Zheng *et al*., 2005).

In comparison to various other environmental issues, nanoparticles have given new environmental challenges and introduced new dimensions for researchers and environmentalists all over the world. Nanotechnology has left no domain untouched including agriculture by its scientific novelties and growth prospects. Although, the use of nanotechnology in agriculture sector is still in the developmental stages, but it appears to have significant effects in different areas of this domain. Nanotechnology has great potential and can serve in various fields related to agriculture. Different types of nanoparticles are being used in the fields of food production, processing, fertilizers & also the pesticides industry. For plants nanoparticles can have extensive range of applications, such as a food supplement and as growth catalysts (Ali *et al.,* 2014).

2.3 Nanoparticles

Nanoparticles are materials having an internal structure ranging between 1 nm to 100 nm (Dietz and Herth, 2011). On nanoscale nanoparticles are materials having at least two dimensions (Klaine *et al.,* 2008; Ma *et al.,* 2010a). In the environment nanoparticles also occur naturally and their natural sources comprise, forest fires, dust storms, volcanic ash and many others. Presence of nanoparticles has significantly increased in the environment as a results of advancement of nanotechnology because they are either produced intentionally or released as by product during various processes being carried out at industrial level such as combustion engines, welding, soldering, heating power plants, grilling and metal smelting. (Buzea *et al.,* 2007). In 2004 worldwide manufacturing of nanomaterials was almost 2000 tonnes and based on recent estimates this volume is expected to increase over 25 times during the period of 2011–2020 (Nowack and Bucheli, 2007).

NPs with a higher surface/volume ratio and high reactivity are considered to be the building blocks of nanotechnology. Nano-particles possess the ability to cross cell walls and plasma membranes (Stern and McNeil, 2008; Farre *et al*., 2011) It is worth noting that these extraordinary properties differentiate them from bulk materials and bring about characteristic environmental fate and behaviors.

2.4 Application of Nanoparticles in Agriculture

2.4.1 Entry pathways of NPs and translocation within the plant body

The method of nanoparticles application to the plants is very critical to regulate how efficiently the internalization of nanoparticles in plants will take place. For instance, roots are specifically involved in the absorption of nutrients and water, whereas leaves are specialized for gas exchange and covered with a waxy layer cuticlewhich obstructs diffusion of substances (Schwab *et al.,* 2015). After being penetrated into the plant body, translocation of nanoparticles through tissues occurs by two ways: the apoplast and the symplast (Figure 2.1). Apoplastic transport involves the movement of nanoparticles through the extracellular spaces present outside the plasma membrane, cell walls of neighboring cells and xylem vessels (Sattelmacher, 2001), whereas symplastic transport encompasses the cytoplasmic movement of water and substances between adjacent cells through special channels called plasmodesmata and sieve plates (Roberts and Oparka, 2003). Application of NPs on aerial plant organs, specifically on the leaves enter the plant body through stomatal openings (Larue *et al.,* 2012).

Research conducted on *Cucurbita maxima* involving application of Fe_3O_4 NPs under hydroponic conditions revealed the nanoparticles absorption, translocation, and accumulation in the plant tissues, but in *Phaseolus limensis* the same treatment did not cause the same response as in the case of *Cucurbita maxima* (Zhu *et al.*, 2008). These results suggest that depending on the plant species, age and internal and external environments the response of plants to nanoparticles is variable. Another study investigated the uptake, translocation, and accumulation of fullerene C70 in seeds of rice plants after treatment with fullerene C70 (Lin *et al.*, 2009).

Application of multi-walled carbon nanotubes (MWCNTs) and their effects were studied on *Catharanthus roseus* protoplasts. Penetration of these nanomaterials was observed in the cell membrane via endosome-escaping uptake method and different cell organelles comprising the nucleus, plastids, and vacuoles contained MWCNTs that were less than 100 nm (Serag *et al.,* 2011). Uptake of CdSe/ZnS quantum dots with a size range of 20 nm and synthetic polystyrene nanospheres of 40 nm diameter by sycamore protoplasts via fluid phase endocytosis demonstrated that the uptake of these nanomaterials is localized and different cell organelles sequestered different nanoparticles (Etxeberria *et al.*, 2006).

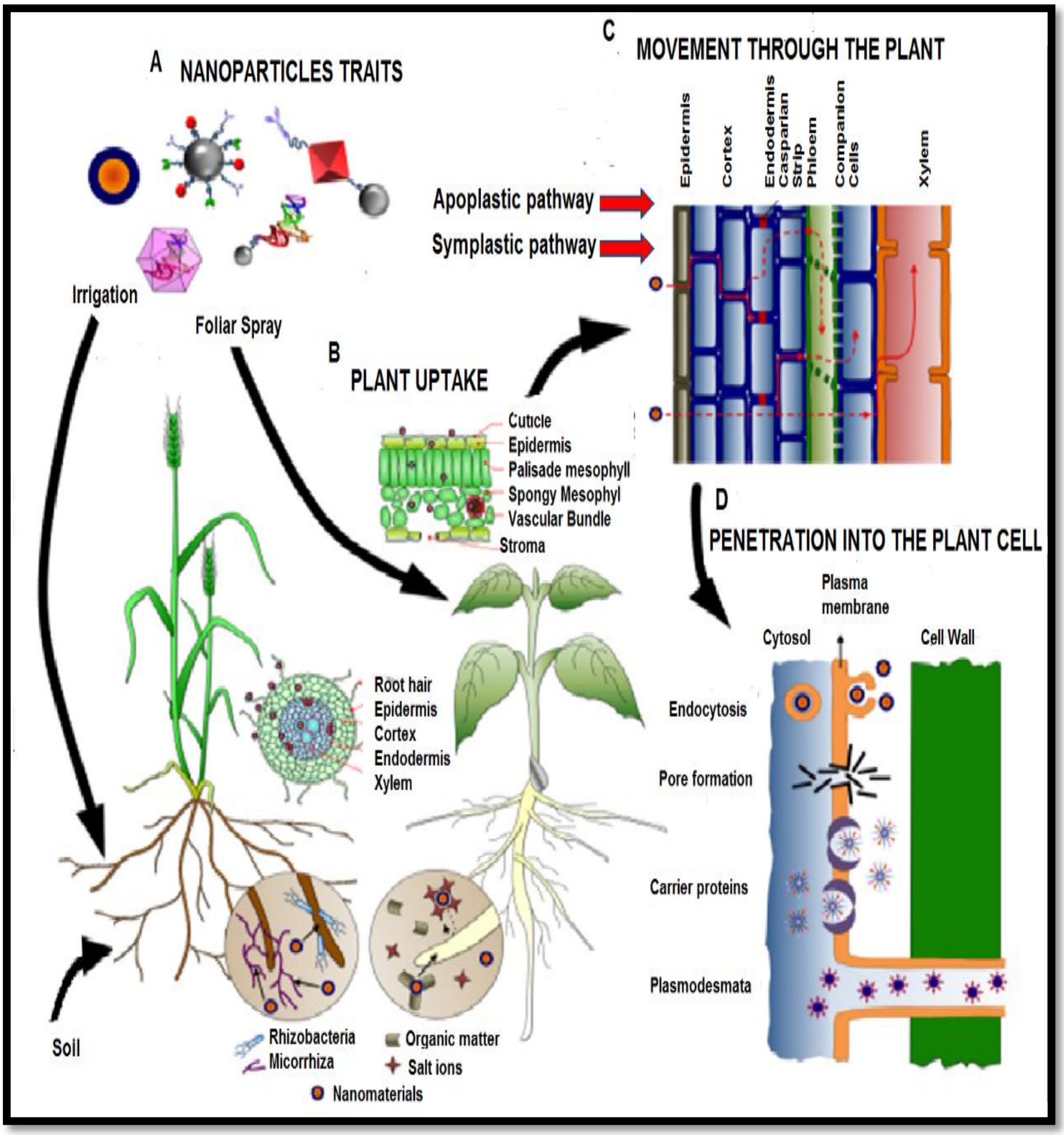

Figure 2.1 Factors influencing absorption, uptake, transport and penetration of nanoparticles in plants (Pérez-de-Luque, 2017)

Studies suggest that the uptake and response of nanoparticles or quantum dots application in protoplast culture system may be altered as compared to a mature cell. Tomato seeds applied with carbon nanotubes (CNTs) showed the accumulation of these nanomaterials within the seed which suggests that the seed coat causes no hindrance to the entry of this nanomaterial inside the seed. The results indicated that carbon nanotubes act as promoter for improving seed water absorbance, suggesting stimulatory impact on the seed germination as well as development of tomato plantlets (Khodakovskaya *et al.*, 2009).

Cell wall serves as first barrier to the entry of substances from environment to plant. In some studies involving superficial application nanoparticles on the plant organ, there is a need to understand the chemistry of the cell wall and its pore diameter must be taken into consideration when estimating the mechanism of nanoparticles breaching the cell wall (Carpita *et al.,* 1974).

Other pathways for the entry of nanoparticles in plants involve the deposition of NPs on or above ground plant parts like epicuticular cavities or between trichomes. Stomata may also act as gateway for entry of NPs. However, the NPs having dimensions greater than the pore size of cell wall, should be deposited on epidermal cells at outer periclinal wall. Underground plant structures should also behave in the same manner for entry of nanoparticles. TiO_2-NPs application on wheat plant showed that nanoparticles up to 140 nm in diameter after absorption accumulate in the roots and that accumulation of NPs is restricted to wheat root parenchyma. TiO_2 nanoparticles were not translocated to most of the root tissues, particularly to the stele, which suggests that NPs were not translocated to shoot of the wheat plant (Larue *et al.*, 2012). Different concentrations of Ag nanoparticles varying in size from 20, 40, and 80 nm influenced the growth of *Arabidopsis thaliana*. Ag nanoparticles in applied in different concentrations considerably effected plant growth as well as site specific accumulation of nanoparticles in tissues and cells (Lee *et al.*, 2013).

2.4.2 Nanoparticles and plant growth

Numerous studies analyzing and describing the impact of nanoparticles crop plants growth and development of have been done. Effects of nanoparticles on plant species largely depend on composition, size, shape of nanoparticles and plant species (Ma *et al.*, 2010b). Some studies of application of nanoparticles on plants are discussed here.

AgNPs application and impact on corn, watermelon and zucchini was investigated. Seed germination and growth parameters showed improvement due to applied silver nanoparticles (Almutairi *et al.,* 2015). Among all engineered nanoparticles, titanium dioxide is the most widely used nanoparticles in the world. It was reported that TNP have no effect on root length, whereas zinc oxide NPs have detrimental effects at early seedling stage in *Oryza sativa* (Boonyanitipong *et al.,* 2011). Nanoparticles have the high potential to penetrate in living plant tissues (Corredor *et al.,* 2009).

Lu *et al.* (2002) studied the combined impacts of SiO_2 and TiO_2 nanoparticles on germinating seeds of soybean and observed significant increase in superoxide dismutase (SOD), peroxidase (POD) and in nitrate reductase, catalase (CAT) activity soybean seeds. Wang *et al*. (2001) confirmed that nano-SiO_2 treatment could be the cause of increased strength and improve disease resistance in rice which results in better yield of rice.

Lee *et al.* (2008) investigated the impact and bioaccumulation of copper nanoparticles on development of Mung bean seedlings as well as wheat plant. It was revealed that seedlings showed stunted growth as a results of exposure to different concentration levels of copper nanoparticles. Plant agar media was used to carry out all the required tests. Copper nanoparticles were found to be toxic to both rice and wheat plant and were also bioavailable.

Treatment of Jasmine rice with silver Ag NPs of different concentrations and sizes indicated a positive association between the dimension of applied silver nanoparticles and a reduction in growth of seedling. It was also observed that rice plant treated with silver nanoparticles of about 20 nm diameter showed higher accumulation of Ag NPs in plant tissues and that translocation of the nanoparticles to the leaves was limited and were retained in the roots (Thuesombat *et al.,* 2014).

. Field experiments conducted on *Brassica juncea* showed that treatment of plant with Gold nanoparticles showed a positively affected plant tallness, stem width as well as the extent of branching (Arora *et al.,* 2012). Silver and copper nanoparticles application to *Cucurbita pepo* plants resulted in decreased growth (Musante and White, 2010). Recently Zuverza *et al.* (2016) conducted a study to evaluate the effect of silver nanoparticles on radish sprouts. It was found that silver nanoparticles did not significantly affected seed germination but reduction in water content, root and shoot lengths was observed. However, infrared spectroscopy analysis of cell wall showed that at the cellular and molecular level, chemical composition of the cell wall was changed under the influence of silver nanoparticles.

Moreno *et al.* (2016) determined the effect of cobalt ferrite ($CoFe_2O_4$) nanoparticles on tomato seeds and seedling grown in hydroponic media. Nanoparticles did not significantly affected germination rate of seedlings and growth of plant. Uptake of both macro and micronutrients was affected differently at different concentration level of cobalt ferrite nanoparticles. The same study showed negative influence on catalase activity in both root and leaves.

Hediat and Salama (2012) investigated the effects of silver nanoparticles on bean and corn plant. Results revealed that small concentrations (20 to 60 mg kg^{-1}) of silver nanoparticles imparted synergistically affected the growth of the plantlets. The application of AgNPs increased shoot and root lengths, enhanced leaf surface area, and boosted protein contents in plants. However concentration above 60 mg kg^{-1} caused toxicity and inhibited plant growth.

In hydroponics, phosphorus-loaded Al_2O_3 nanoparticles were used to increase phosphorus uptake by *Brassica napus*. It was reported that there was about 8-fold increase at constant low free phosphate concentration and almost 40-fold because of passive, diffusion-based samplers (Santner *et al.*, 2012). Zinc oxide (ZnO) nanoparticles synthesized from soil fungi increased the mobilization and availability of phosphorus in mung bean rhizosphere (Raliya *et al.*, 2016). Titania and iron nanoparticles also have positive impact in terms of availability of phosphorus in *Lactuca sativa* (Zahra *et al.*, 2015). Application of biologically synthesized zinc nanoparticle significantly improved the plant biomass, root, shoot length, root area and chlorophyll content. ZnO nanoparticles also enhanced 48.7% alkaline phosphatase 73.5% acid phosphatase and 72.4% phytase in cluster bean rhizosphere in comparison with control in six weeks old plants (Raliya and Tarafdar, 2013).

Ju-Nam and Lead (2008) showed that nitrate reductase activity is in Glycine max increases under the influence of nano-SiO_2 and nano-TiO_2 triggers and ultimately increases its ability to absorb and consume water. On the other hand, activation of antioxidant systems by these two nanoparticles lead to decline in germination ratio and plant growth.

Kisan *et al.* (2015) found that foliar application of different doses of zinc oxide nanoparticles (ZnO NPs) on spinach after 14 days of planting showed positive effects on certain plant traits. At the concentration of 500 and 1000 ppm of ZnO NPs the plant showed enhancement of leaf parameters such as leaf surface area, length, width, and colour of leaf samples as compared to control samples. Similarly protein and dietary fibre content increased in treated plants with 500 and 1000 mg kg^{-1} ZnO NPs in comparison to control leaf samples of spinach. Therefore nano-zinc oxide spraying enhances nutritious value of spinach to vegetarian diet by supplying, protein, fiber and required amount of vegetarian fat to diet ().

Application of nano aluminium in wheat decreased the content of chlorophyll and root and shoot lengths however, the content of proline and malondialdehyde increased in both shoot and root. The treatment of plants with different levels of nano aluminium in combination with nano titanium dioxide especially at 100 mg L^{-1} concentration increased the length of both the root and shoot growth . Nano titanium dioxide at concentration of 1000 and 2000 mg L^{-1} increased the proline content (Aliabadi *et al.*, 2016).

Upadhyaya *et al.* (2017) investigated the impact of different concentrations of $Ca_3(PO_4)_2$ NP on growth and antioxidant responses in rice plant. At concentration of 10 and 20 mg L^{-1} $Ca_3(PO_4)_2$ NP improves growth whereas reactive oxygen species (ROS) is reduced. Thus, interaction of $Ca_3(PO_4)_2$ NP with plant and resultant physiological changes are dose dependent. Due to growth promoting effect of calcium phosphate nanoparticles may help in production of nanofertilizers for agricultural use.

Quantum dots (QDs) are nanomaterials which have been extensively utilized in many modern techniques such high resolution structural imaging of cell and *in vivo* cellular tracking. Research conducted to determine the influence of *in vitro* application of quantum dots on a *Medicago sativa* culture showed that QDs have negative effect on plant cells and dose-dependent response in the plant cells was observed (Santos *et al.,* 2012). Silica coated with QDs promoted root growth in rice plant (Wang *et al.,* 2014), however, inhibitory effect was observed on seed germination (Nair *et al.*, 2011).

It was shown that under the impact of NPs the first apparent sign is the superficial charring of the cells located at the root cap. Examination of plant root cells showed the distribution and accumulation of nanoparticles in the root cap cells and other root tissues. Some scientists propose the role of plasmodesmata in movement and translocation of NPs within the plant body (Lin *et al*., 2009; Lee *et al.,* 2013). In rice treated with carbon nanoparticles with size ranging from 40–70 nm demonstrated the role of plasmodesmata in intracellular transport of nanomaterials among different tissues (Lin *et al*., 2009). Additional studies must be performed regarding the involvement of plasmodesmata in the transport of nanoparticles having diameter as large as 40 nm . It must be taken into consideration that the diameter of the PD is 25–50 nm and the diameter of transport channels within the cytoplasmic sleeves is between 1.5 and 4 nm (Serag *et al.*, 2011).

2.5 Titanium Dioxide Nanoparticles

Titania occurs in nature as a mineral existing in three crystalline states including rutile, anatase and brookite, and also in an amorphous form (Reyes-Coronado *et al.*, 2008). Rutile phase is the most commonly existing form of titania (EPA, 2009). Titanium dioxide nanoparticles are among the metal oxide environmental nanoparticles that are expected to be produced at very much high rates from 7800 tons and 38 000 tons per year (Hendren *et al.,* 2011).

Titanium dioxide nanoparticles (TiO_2) are widely used in daily life products, but little research has been done regarding their mode of uptake and translocation in the plant, particularly on food crops (Seeger *et al*., 2009; Feizi *et al.,* 2012). TiO_2 translocate inside the tissue and cells due to formation of a covalent bond with most of the non-conjugate ordinary organic matter (Mingfang *et al.,* 2013; Uhram *et al.,* 2013).

2.5.1 Effects of Titanium Dioxide Nanoparticles on plants

Titanium dioxide nanoparticles positively and negatively affect the growth of plant (Figure 2.2). Nanomaterials with different structural motifs of the same metal have variable effects on plants, although they do not exhibit distinct changes in chemical behaviour. Anatase TiO_2 exhibits highest catalytic ability among the three crystalline structures of TNPs i.e. anatase, rutile and brookite (Yin *et al.,* 2005) and can restrict the growth of certain microorganisms such as algae, fungi and bacteria. It stimulates carotene and chlorophyll production in cucumber. TiO_2 nanoparticles enhance light absorption activity in chlorophyll a, electron transfer and evolution of oxygen radical thereby increase the Hill reaction and chloroplast activity in spinach leaves (Hong *et al.,* 2005; Zheng *et al.,* 2007; Wang *et al.,* 2008; Su *et al.,* 2009)

TiO_2 nanoparticles (25 nm) with crystalline structure in anatase phase cause more toxicity to plants. In plants root growth is promoted at high concentration. Since in aqueous medium rutile crystalline phase TiO_2 nanoparticles form aggregates and its bioavailability for absorption is reduced, their toxicity is lower than that of anatase TiO_2 (1 μm). With increase in the exposure time from 24 to 72 h, enhancement of toxic effect was observed. However, in flax seeds higher levels (100 mgL^{-1}) of anatase TiO_2 nanoparticles, promoted germination of seeds and root elongation. The positive effect has been attributed to antimicrobial effect of anatase TiO_2 which enhances stress resistance in plant (Clément *et al.,* 2013).

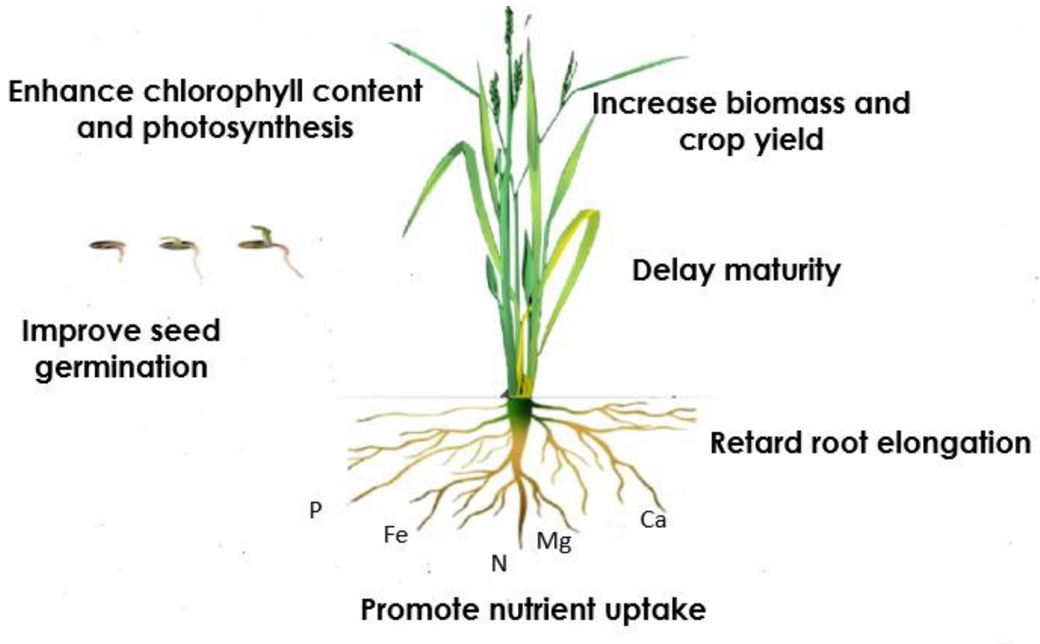

Figure 2.2: Effects of TNPs on crop performance

Servin and co-workers (2012) have studied the effect of high doses of TiO_2 nanoparticles having accumulation range (0–4000 mg L^{-1}) in cucumber plants (*Cucumis sativus*). Significant increase in root growth was observed up to the concentration of 500 mgL^{-1} but further growth of roots was ceased above this concentration. Under the influence of TiO_2 nanoparticles conversion of nitrogen to organic nitrogen showed an increase of about 51.1% as compared to control. It is postulated that TiO_2 nanoparticles help promote plant root growth by enhancing nitrogen accumulation.

TiO_2 nanoparticles application altered germination and development of spinach seeds. These nanoparticles act as photocatalyst, enhancing light absorbance and stimulated the enzymatic activity of *Rubisco activase* resulting in increased spinach growth. TiO_2 nanoparticles with anatase phase reported to improve plants growth due to enhanced nitrogen metabolism, transformation of inorganic mineral nitrogen to organic nitrogen, consequently increase the fresh and dry biomass of plants by 91% and 99% as compared to control. Total nitrogen increased up to 23.35% along with improved chlorophyll and proteins of spinach (Yang *et al.,* 2007). During the growth stage, nano-TiO_2 has improved the chlorophyll content, proteins in spinach as well as increased the antioxidant stress due to lower accumulation of superoxide radicals that ultimately helped the spinach chloroplasts to release more oxygen (Zheng *et al.,* 2005).

2.5.2 Impact of Titanium Dioxide (TiO_2) on Photosynthesis

Both rutile and anatase crystalline phases of TiO_2 nanoparticles resulted in generation of ROS in spinach. It was evaluated the ability of rutile or anatase nano-TiO_2 to produce free radicals (O2 •−, HO•, CO_2 •−) and found that both polymorphs generated radicals under light as well as dark conditions. These researchers also suggested that crystalline size of nano-TiO_2 did not affect its ability to generate ROS (Fenoglio *et al.,* 2009).

The researches on improvement of photosynthetic rate of spinach suggested that, TNPs could enhance light absorbance, promote transformation of the light energy, prevents aging of chloroplasts thereby prolong the photosynthetic period of chloroplasts. Yield of spinach treated by TiO_2 nanoparticles increased, because the probability of absorption of TiO_2 nanoparticles is promoted as a results of spraying on the leaves and aerial parts of the plant. Therefore, nano TiO_2 enter the chloroplasts and transported in the electron transport chain to stimulate the formation of NADP+ that was reduced to NADPH involved in the process of photophosphorylation, thereby promoting electron energy transfer to ATP and accelerates the whole electron transport chain, photoreduction in PS-II, evolution of oxygen and photophosphorylation (Lie *et al.,* 2007a, 2007b)

After reaching inside the photosynthetic cells, TNPs could enhance activity of Rubisco activase by promoting its mRNA expression (Gao *et al.,* 2008; Ma *et al.,* 2008) and thus enhance the rate of photosynthesis (Zhang *et al.,* 2008). Giraldo *et al*., 2014) proposed that nanoparticles can harvest more light energy into chloroplast because they enable chloroplast to capture light of varied wavelengths (ultraviolet, green, and near-infrared), typically beyond the normal range of chloroplasts.

Chapter 3

MATERIALS AND METHODS

This chapter describes the experimental framework adopted during the conducted research work. The experimental work for the present study was carried out in locally made greenhouse at IESE, NUST, Islamabad. In the present study, rice was exposed to TiO_2 nanoparticles using three different methods (irrigation, soil application and foliar spray) and four phosphorus levels (P1 = 0 mg kg^{-1}, P2 = 10 mg kg^{-1} and P3 = 20 mg kg^{-1}, P4= 40 mg kg^{-1}). The first phase and analysis focused on plants growth variations due to these treatments, the purpose of second phase was to focus on the plant uptake of phosphorus and rice grain protein content. Keeping in view the main objectives of the study, following methodology was adopted which is being discussed here in detail accordingly.

3.1 Preparation of Titania Nanoparticles

3.1.1 Synthesis of TiO_2 Nanoparticles by Liquid Impregnation method

In order to achieve the required size of nanoparticles, Liquid Impregnation (LI) method was used for the synthesis of TiO_2 nanoparticle. For this purpose, 60 g of Titania (TiO_2) general purpose reagent (GPR) was added into 900 mL distilled water and placed on magnetic stirrer for 48 h. The mixture was then allowed to settle for 12 h, after that it was placed in oven at 105 ℃ for drying. Mortar-pestle was used to grind the dried material. Then for calcination it was placed in muffle furnace at 450 ℃ for 5 hours.

3.1.2 Characterization of TNPs

3.1.2.1 X-ray Diffraction (XRD)

The crystal phase and crystallite size of the prepared TiO_2 nanoparticles were studied using X-Ray Diffractometer (Theta-Theta STOE, Germany) with X-ray operating conditions at 40 kV and 40 mA. Scherer Formula was used to estimate the crystallite size of nanoparticles according to the line width of the (101) plane refraction peak for TiO_2.

3.1.2.2 Scanning Electron Microscopy (SEM)

SEM (JSM-6490A, JEOL) with an accelerating voltage of 20 kV was used to determine surface morphology of TNPs. Prior to scanning, powdered TNPs were diluted 100-fold in distilled water and then sonicated for 30 minutes. After dilution drop of 10 µL of this solution was placed on a carbon stub and air dried. The dry powder was sputter coated with gold in order to increase conductivity of surface. Atomic Ion Sputtering Device, JEOL, JFC-1500, was used for coating of TNPs. TiO_2 suspensions were prepared on quartz slides in ethanol and were directly observed under the microscope at different magnifications.

3.2 Soil Analysis and Preparation for Experiment

3.2.1 Soil Characterization

The major soil characteristics that were analyzed include soil pH, moisture content, soil texture, nitrate-nitrogen, total organic carbon and total phosphorus. Soil pH was checked to ensure the suitability of soil for plant growth. For this purpose pH meter was used (McLean,1982). Soil moisture content measurements were done using gravimetric method (Hesse, 1971). Hydrometer method was used to analyze soil texture (Bouyoucos, 1962), using sodium hexametaphosphate as a dispersing agent. Soil total Organic Carbon was determined using Walkey-Black method (Walkely and Black, 1934). Nitrate-N in soil was measured by a spectrophotometric method using chromotropic acid (Sims, 1971).

3.2.2 Soil Preparation

Soil from the nursery of National University of Science and Technology was selected for the final experimentation. Soil was firstly spread out and dried for a week. The dried soil was then grounded into fine form by using ball mill at Particulate Technology Lab, SCME, and NUST. Larger particles, gravels, roots and shoots were removed further by using mechanical sieve shaker of size <2mm. For experiment plastic pots of diameter of 5 cm and height of 6 cm were used. This clean and processed soil material was used for the present experiment. Soil was weighed and 1 kg soil was added to each pot.

3.3 Plant Cultivation

3.3.1 Sterilization of Seeds

Seeds of experimental plant species rice (*Oryza sativa* L.) were purchased locally. Seeds were kept in a dry dark place under room temperature. Prior to use, sterilization of seeds was done by using 5% sodium hypochlorite solution and then rinsed thoroughly with distilled water. The seeds of uniform size were selected for the study.

3.3.2 Growth of Seedlings

Seeds of *Super Basmati* were sown in pots containing untreated soil (without fertilizer and TNPs) and placed in green house at IESE, NUST. After germination, seedlings were allowed to growth then transplanted to the pots containing prepared soil (4 seedlings per pot).

3.3.3 Preparation of Pots and Fertilizer Application

1 kg soil was weighed for each plastic pot with proper labeling. Phosphorus fertilizer suspensions of three concentrations i.e. 10, 20 and 40 mg kg^{-1} were prepared. For each concentration level there were five replicates. Beside these concentrations there was also a control group in which phosphorus fertilizer was not added for comparison with treated ones. Diammonium phosphate (DAP) containing 46% phosphorus and 18% nitrogen was

used as source of phosphorus (P). Recommended N (70 mg kg^{-1}) and K (32.5 mg kg^{-1}) were applied in all pots. Suspensions of urea with 46% N and potash containing 50 % potassium were applied. Pots were filled with water and mixed well three to four times. Urea was added in two splits. First dose was added 2 weeks after seedlings transplantation and second dose was applied on 40^{th} day after transplantation. Pots were filled with water and mixed well three to four times.

3.3.4 Application of TiO_2 Nanoparticles

Rice plant was exposed to TiO_2 nanoparticles using three different methods i.e. soil application, irrigation and foliar spray. TNPs suspensions of 500 mg kg^{-1} were prepared and sonicated for 30 min prior to application. For soil application, TNPs suspensions of 500 mg kg^{-1}were added in soil in previously labeled pots before seedlings transplantation and mixed thoroughly. For irrigation method, TiO_2 nanoparticles suspension of 500 mg kg^{-1} was mixed in irrigation water applied to the seedlings right after transplantation. For foliar application, TiO_2 nanoparticles suspension was applied aerially on the seedling using spray bottle. There were 5 replicates for each method. During experimental phase pots were kept in locally made green house at IESE, NUST for a growth period of about 132 days.

3.4 Estimation of Chlorophyll Content

Chlorophyll content was measured on alternate days by using hand-held chlorophyll meter (CCM-200 plus). Hand-held chlorophyll meter, CCM-200 plus was purchased from Opti-Sciences, England. The CCM-200 weighs 168 g, has a 0.71cm2 measurement area, and calculates a Chlorophyll content index (CCI) based on the absorbance measurements. Peak chlorophyll absorbance was measured at 653 nm and non-chlorophyll absorbance (cell walls, veins, etc.) at 931 nm. Calibration was done every time the unit was powered up. Three readings of plants were taken on alternate days during the period of 51 days, starting from 20^{th} day after transplantation till 70^{th} day. The chlorophyll measurements were taken from each plant samples of all treatment levels. There were eight replicates for each concentration level. To avoid the risk of placement of chlorophyll meter over the major leaf veins, readings were taken from the leaf area between the midrib and the leaf margin. Taken measurements were the thirty points averaging of the hand-held chlorophyll absorbance meter.

3.5 Determination of Effects of TiO_2 Nanoparticles Method of Application and Phosphorus Levels on Growth of Rice

After maturation and ripening, rice plants were harvested. Morphological parameters like root and shoot lengths, root and shoot weights were measured separately. Panicle was separated and collected in sampling bags for rice grain analysis.

3.5.1. Plants Length Measurement

After harvesting of wheat and lettuce plants, roots and shoots were washed with distilled water, collected separately and their lengths were measured.

3.5.2. Plants Biomass Determination

Roots and shoots of rice were cut and their fresh biomass weighed one by one. Shoots and roots were placed in an oven at 70 °C for 48 h. The plant material was weighed for dry biomass. Mortar pestle was used for grinding the shoots and roots separately and air tight sampling bags were used for storage until used for further analysis.

3.6 Determination of Phosphorus

Total phosphorus analysis was done in rhizosphere soil and test plant rice separately.

3.6.1 Phosphorus Analysis in Soil

Phosphorus is one of the major nutrients and necessary for good plant growth. The improved blue method (Olsen *et al.,* 1954) is a quick, inexpensive and convenient for alkaline soil testing in terms of phosphorus availability. Thus, Olsen's test has been used for the analysis of soil phosphorus.

Reagents

A. Extracting Solution

a) Sodium Bicarbonate Solution ($NaHCO_3$), 0.5 M

84 g of sodium bicarbonate was dissolved in about 2 L distilled water. 5N NaOH solution was used to adjust pH to 8.5. The volume was made up to mark.

b) B. Sodium Hydroxide Solution (NaOH), 5 N

50 g of sodium hydroxide was dissolved in 250 mL distilled water.

B. Mixed Reagent

a) 3 g of ammonium heptamolybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ dissolved in 62.5 mL distilled water.

b) 72.75 mg of antimony potassium tartrate ($KSbO.C_4H_4O_6$) dissolved in 25 mL distilled water.

The dissolved reagents (a) and (b) both were added to a 500 mL volumetric flask, then 250 mL of 5 N H_2SO_4 (37 mL concentrated H_2SO_4 in 250 mL distilled water) were added to the mixture. After mixing thoroughly, the volume was made up to 500 mL with distilled water and kept in a Pyrex glass bottle in a cool and dark place.

C. Color Developing Reagent (CDR)

2.64 g of Ascorbic acid ($C_6H_8O_6$) was dissolved in 500 mL Mixed Reagent. CDR must be prepared freshly when needed because it cannot be stored more than 24 h.

D. Standard Stock Solution

Exactly, 0.7 g potassium dihydrogen phosphate (KH_2PO_4) was oven dried for 1h at 105 °C, cooled in a desiccator then stored in air tight bottle. Precisely, 439.4 mg potassium dihydrogen phosphate (KH_2PO_4) was dissolved in 100 mL distilled water. Prepared solution contained 1000 mg L^{-1} stock solution. Precisely, 10 mL stock solution was diluted to 100 mL final volume with distilled water. This solution contained 100 mg L^{-1} phosphorus. A series of standards were prepared from the stock solution. These solutions contained 0, 0.25, 0.5, 0.75, 1, 1.25, 1.50 and 2 mg kg^{-1} phosphorus, respectively.

Procedure

2.5 g air dried soil samples were taken in 250 mL Erlenmeyer flask and 50 mL sodium bicarbonate extracting solution ($NaHCO_3$) was added to it. Then for shaking it was placed on mechanical shaker operating at 180 rpm for 30 mins . Three blank were also prepared having all chemicals in them except soil. Whatmann filter paper No. 42 was used for sample filtration. About 5 mL of filtrate was pipetted out into volumetric flask of 25 mL, then after addition of 5 mL CDR, deionized water was used to make volume up to the mark of 25 mL using. It was shaken well to remove the air bubbles. Gradually bluish color was developed. Concentration of phosphorus present in soil is directly proportional to the intensity of blue color developed. The more the blue color is; the more phosphorus is present. After 15 minutes, blanks, standards, and samples were run on UV/Vis Spectrophotometer (Specord 200-plus, Analytic Jena, Germany) which set at 880 nm wavelength to measure the absorbance. Using absorbance values of standards, calibration curve was prepared by plotting phosphorus concentrations on the x-axis while absorbance of the samples on the y-axis. Phosphorus concentrations for the unknown samples were measured by following formula.

Phosphorus (mg kg^{-1}) = mg P kg^{-1} (from calibration curve) × A / Wt × 25/V... (Eq.1)

Where;

A = Total vol. of the extracted sample (mL)

Wt. = Wt. of air-dried soil (g)

V = Volume of extracted sample used for measurement (mL)

3.6.2 Determination of Phytoavaliable Phosphorus in Plants

Precisely, 100 mg of both the roots and shoots were ground and saved in sampling bags and were digested in 5 mL acid mixture comprising concentrated Nitric Acid and Perchloric Acid (HNO_3-$HClO_4$) in 2:1. Then it was placed on hot plate for 1 hour at 180 °C. The extracts were filtered with the help of Whatmann filter paper No. 42. The concentration of phosphorus in plant filtrates was calculated using vanado-molybdo-phosphoric acid colorimetric method (Ryan, 2008; Zahra *et al.* 2015). Detailed method is given below:

Preparation of Reagents

a. Reagent A: Accurately 12.5 g ammonium heptamolybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$] were dissolved in 250 mL warm distilled water (solution A). 625 mg of ammonium metavanadate (NH_4VO_3) were dissolved in 250 mL in boiling deionized water (solution B). After cooling to room temperature, solution B was mixed with solution A and then 250 mL nitric acid (HNO_3 : H_2O :: 1 : 3) were added to the mixture in volumetric flask. Allowed the solution to be cooled at room temperature.

b. Reagent B: 141.6 mL concentrated perchloric acid were added to 283.4 mL concentrated nitric acid in 500 mL volumetric flask. Acid mixture was then allowed to cool.

c. Standard Stock Solution: Precisely, 0.7 g oven dried potassium dihydrogen phosphate was dissolved in 100 mL distilled water (1000 mg kg^{-1} stock solution). 10 mL of this solution were taken and by using deionized water it was filled up to the mark of 100 mL (100 mg kg^{-1} sub stock solution).

A. Wet Digestion Method

Precisely, 100 mg of ground plant material were added to the volumetric flask of 25 mL. Then 5 mL of acid mixture were added to the flask. Flask was placed on hot plate at 180 ^{o}C for 1h. The temperature was slowly increased until all traces of nitric acid were disappeared and dense white fumes of perchloric acid appeared and left clear aliquot behind. Volume was made up by using deionized water. The digested plant material was filtered by using Whatmann filter paper No. 42, and extracts were stored at 4 °C for further experimentation.

B. Measurements

1. 2.5 mL of the digested filtrate were taken into 25 mL flask then added 5 mL ammonium-vanadomolybdate reagent and deionized water was added in order to attain the desired volume of 25 mL.

2. The sub-stock solution was pipetted out to 25 mL volumetric flask for the preparation of series of standards. These solutions contained 0, 0.25, 0.5, 0.75, 1, 1.25, 1.50 and 2 mg kg^{-1} phosphorus respectively. Five milliliter mixed reagent were added and continued as for the samples. Blanks were also prepared having all chemicals except plant material. The absorbance of the blanks, standards, and for samples was taken after 1h at 430 nm wavelength on UV/Vis Spectrophotometer. Using absorbance values of standards, calibration curve was prepared by plotting phosphorus concentrations against absorbance. Concentration of phosphorus for samples was assessed by using calibration curve (Ryan, 2008).

Total phosphorus uptake per plant was estimated from the following relation:

$P\ uptake = [(shoot\ dry\ weight \times shoot\ P\ conc.) + (root\ dry\ weight \times root\ P\ conc.)]$

3.7 Rice Grain Protein Content

After harvesting, rice grains were separated from the panicles and powdered through grinding. For determination of protein content in rice grains, total nitrogen content was determined.

Firstly, 50 mg of finely grinded grain sample were taken and mineralized using 1 mL of 36N H_2SO_4 in a tube mineralization block at 330 °C for 30 min (McDonald, 1978). After cooling, 0.2 mL of pure hydrogen peroxide (110 volume, not stabilized with phosphate) was slowly added, tubes were transferred to mineralization block until H_2O_2 evaporated and the color of solution became transparent. Free ammonium was then assayed in H_2SO_4 diluted to 0.1N using phenol colorimetric method of Berthelot (Martin *et al.*, 1983). For estimation of protein content, total nitrogen content (mg g^{-1}) measured was multiplied by conversion factor 5.26 for rice (Fujihara *et al.*, 2008).

3.8 Statistical Analysis of Data

Data obtained from the study were subjected to statistical analysis by computing mean and standard deviation. Statistical significance of findings was checked by applying two way Analysis of variance and Tukey's HSD test was used to assess difference between means of treated groups. Results were considered statistically significant at the probability value of less than 0.05 ($p < 0.05$).

Chapter 4

RESULTS AND DISCUSSION

4.1 Characterization of TiO_2 NPs

4.1.1 XRD Results of TiO_2 NPs

The phase composition, crystal structure and crystallite size of TiO_2 nanoparticles synthesized by liquid impregnation method were determined through XRD.

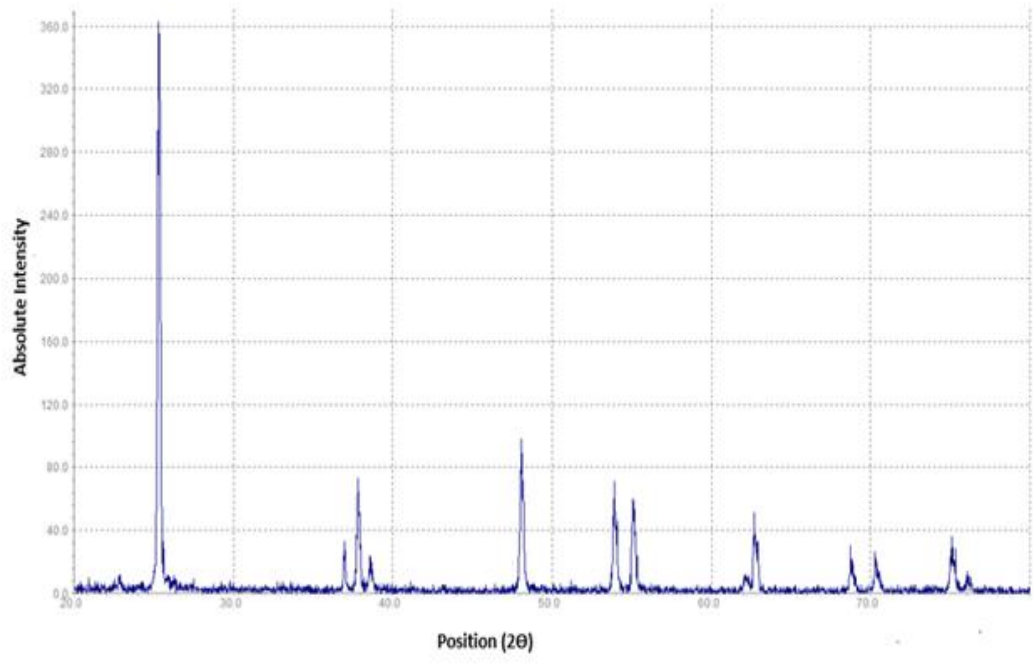

Figure 4.1: XRD pattern of TiO_2 NPs

The spectrum in figure 4.1 indicates that TiO_2 nanoparticles were crystalline and no amorphous phase was observed. The first peak between 20 and 30 θ indicates the presence of anatase phase. Titania nanoparticles used in this study are having the size of 32.7 nm in average. The observed nanoparticles are having the crystalline structure. The Scherer formula was used to know the crystalline size of Titania nanoparticles (Sherrer, 1918).

Where,

L = average particle size

K = 0.891 is the shape factor of particles

λ = 0.1542 is the X-ray wavelength

β = is the width of the diffraction line at 1/2 maximum intensity

Ø = angle of diffraction

Nanoparticles have large surface area due to their nanoscale dimension therefore, this property helps in the efficient uptake of phosphate ions from the soil solution. Root exudation is also triggered that mobilizes the phosphorus resulting in better phosphorus uptake by plants due to application of TNPs (Zahra *et al.*, 2015). Anatase TiO_2 exhibits highest catalytic ability among the three crystalline structures of TNPs i.e. anatase, rutile and brookite (Yin *et al.*, 2005).

4.1.2 SEM Results of TiO_2 NPs

To observe the morphology of Titanium dioxide nanoparticles Scanning Electron Microscope (SEM) was used. Images obtained from SEM confirm the presence of spherical aggregates of Titanium dioxide nanoparticles. Figure 4.2 (a, b) shows the Titanium dioxide NPs images taken by (SEM, JEOL JSM-6460) at 20,000 and 50,000 X magnification. Propertied TiO_2 NPs are significantly affected by its structural dimensionality. Because of zero dimensionality of spherical TiO_2 NPs, its specific surface area is much higher, resulting in higher photocatalytic activity.

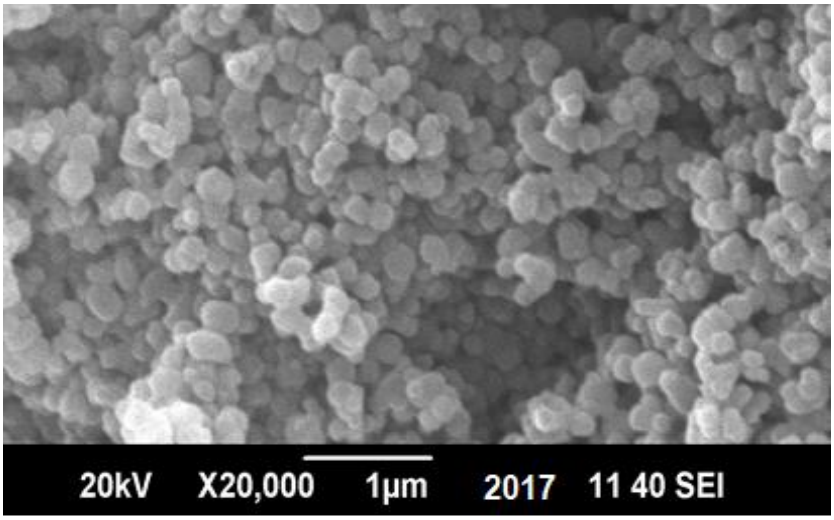

Figure 4.2 (a) Scanning Electron Microscopy Image of TiO_2 NPs at X 20,000

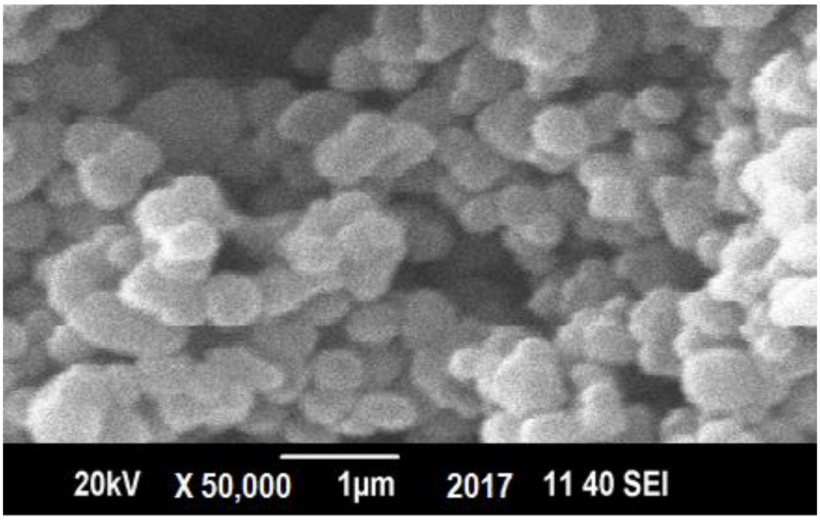

Figure 4.2 (b) Scanning Electron Microscopy Image of TiO_2 NPs at X 50,000

4.2 Characterization of Experimental Soil

Table 4.1 shows the results for certain physical and chemical characteristics of the experimental soil. Texture of the soil used for the experiment was silt loam. Depending on the pH, soil was normal (salts free). For the growth of high value crops soils with pH < 7.5 are considered as the best. Organic matter content in the soil was very poor (< 0.86). Available phosphorus content was within the satisfactory range (8-15 mg kg^{-1}).

Table 4.1 Characteristics of Experimental Soil

Soil Parameters	Results
Sand (%)	40
Silt (%)	55
Clay (%)	5
pH	7.04 ± 0.2
Moisture Content (%)	16 ±2.3
Total Organic Carbon (%)	0.23 ±0.1
Water Holding Capacity (mL L^{-1})	53 ±3.2
Nitrate- Nitrogen (mg kg^{-1})	188 ± 123.3
Available Phosphorus (mg kg^{-1})	8.28 ± 1.3

4.3 Effect on Chlorophyll Content

Chlorophyll is one of the essential pigments present in plants; it helps in the conversion of light energy into chemical energy which further enhances the photosynthetic activity in plants. Figure 4.3 represents trend of chlorophyll content of rice plants measured on alternate days from 20th day of transplantation till 70th day against different methods of TiO_2 nanoparticles application at different phosphorus concentration levels. Chlorophyll content increased positively with increasing phosphorus levels for all the methods of application. Maximum chlorophyll content was measured on 56th and 58th day after transplantation in plants exposed to TiO_2 nanoparticles through irrigation method at phosphorus level of 0 and 10 mg P kg^{-1}, respectively. Chlorophyll content declined afterwards again because leaves start turning brown. Similar trend was observed for all the methods of TiO_2 nanoparticles application at all phosphorus levels. At 20 and 40 mg P kg^{-1} the value of chlorophyll content increased sharply on 54th day after transplantation for all the methods of TNPs application. Maximum value was measured on 56th day in plants applied with TiO_2 nanoparticles through soil. But the plants showed no significant difference with TNPs exposure through soil and irrigation water ($p < 0.05$).

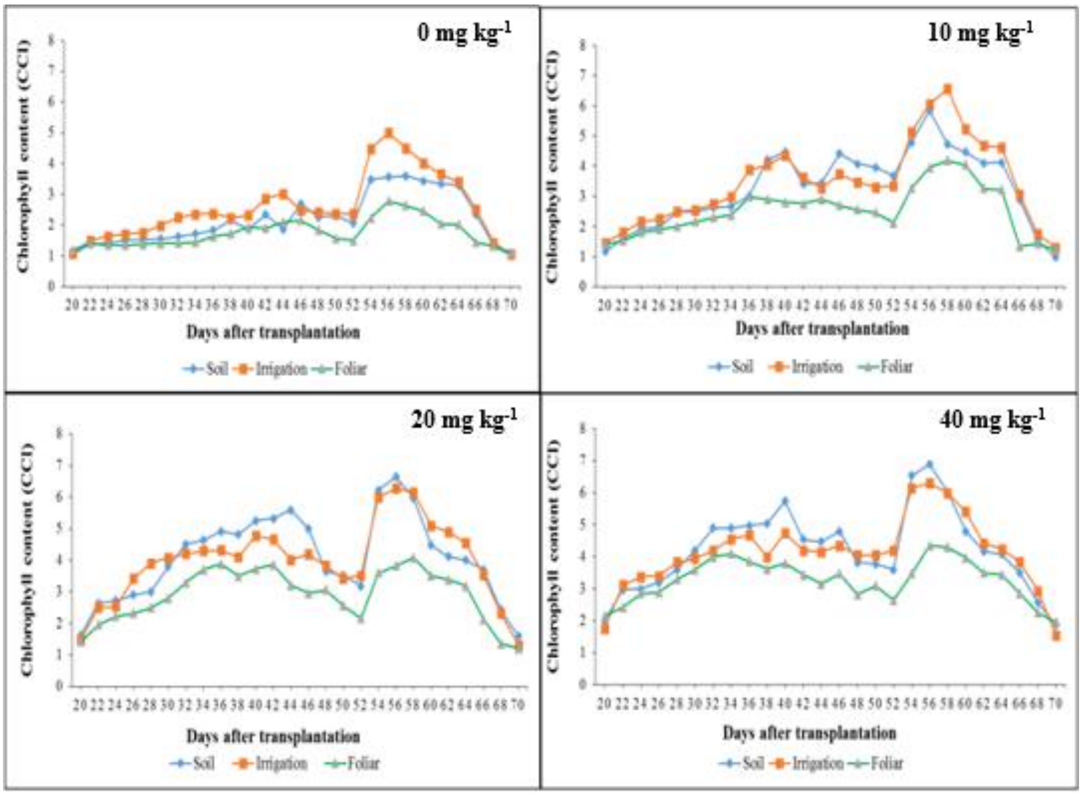

Figure 4.3: Chlorophyll Content Calculated in CCI at Different Methods of TNPs Application and Phosphorus Levels

An increase of the chlorophyll content value of plants has effect on photosynthesis. Shubhra *et al.* (2003) reported increase in chlorophyll content in clusterbean by increasing the level of phosphorus application. Razaq *et al.* (2017) found similar results when *Acer mono* seedlings were treated with different concentrations of phosphorus. Maximum chlorophyll content was found at highest phosphorus dose.

It is reported that photocatalytic ability of TiO_2 NPs may lead to increase in chlorophyll content and photosynthetic reactions (Skupień and Oszmiański, 2007; Owolade *et al.,* 2008; Chen *et al.,* 2012). Mukherjee *et al.* (2014) reported consistent decrease in chlorophyll content with the passage of time due to the less green color of the leaves. TNPs application to spinach improved the 37.48% chlorophyll content improved by 37.48%, total nitrogen concentration 23.35% and fresh and dry biomass increased by 91% and 99% respectively in contrast with the control (Yang *et al.,* 2007). Chlorophyll formation is improved as a results of application of TNPs as it promotes Ribulose 1, 5-bisphosphate carboxylase (Rubisco) activity, thereby improving photosynthesis and plant development. It has also been suggested that TNPs protect chloroplast from excessive light by boosting the activity of antioxidant enzymes (Siddiqui *et al*., 2015).

4.4 Effect on Growth Parameters

4.4.1 Shoot Length

The main effect of nano-TiO_2 method of application and phosphorus fertilizer level significantly affected shoot length or rice plant ($p < 0.05$). The interaction effect of phosphorus fertilizer level and nano-TiO_2 method of application was found to be insignificant (*Annexure I*).

Table 4.2 shows that nano-TiO_2 (500 mg kg^{-1}) method of application significantly affected shoot length with highest shoot length of 58.97 cm produced at irrigation method of application and the lowest values for the shoot length (48.4 cm) produced in plants treated with foliar applied nano-TiO_2. Increment of shoot length was observed as the method of application changed, there was significant difference among foliar, soil and irrigation method of TiO_2 nanoparticles application. Shoot length was increased significantly with increased in phosphorus level from 0 to 40 mg kg^{-1}. The highest shoot length (60.03 cm) was recorded at a level of 40 mg P kg^{-1} and shortest shoot length (41.23 cm) was recorded from control treatment (Table 4.2). Compared to the control, mean shoot length was increased by 38%, 40% and 46% under 10, 20 and 40 mg P kg^{-1} treatment levels, respectively. However, phosphorus application of 40 mg kg^{-1} resulted in shoot length with no significant difference as compared to treatment with 10 and 20 mg P kg^{-1}.

The combined effect of nano-TiO_2 application method and phosphorus fertilizer levels on shoot length is presented in figure 4.4. The highest shoot length (63.4 cm) was measured in a combination of treatment maximum phosphorus fertilizer level (40 mg P kg^{-1}) with application of TiO_2 nanoparticles through irrigation method followed by phosphorus fertilizer level of 20 mg P kg^{-1} with irrigation method of TiO_2 nanoparticles application (62.1 cm) and the lowest shoot length was measured in plants having no phosphorus treatment with foliar applied TiO_2 nanoparticles (34.4 cm). The shoot length was increased by about 84% when phosphorus level of 40 mg P kg^{-1} was applied in combination with TiO_2 nanoparticles using irrigation method as compared to lowest shoot length in plants treated with foliar applied TiO_2 nanoparticles under controlled phosphorus treatment (Figure 4.4).

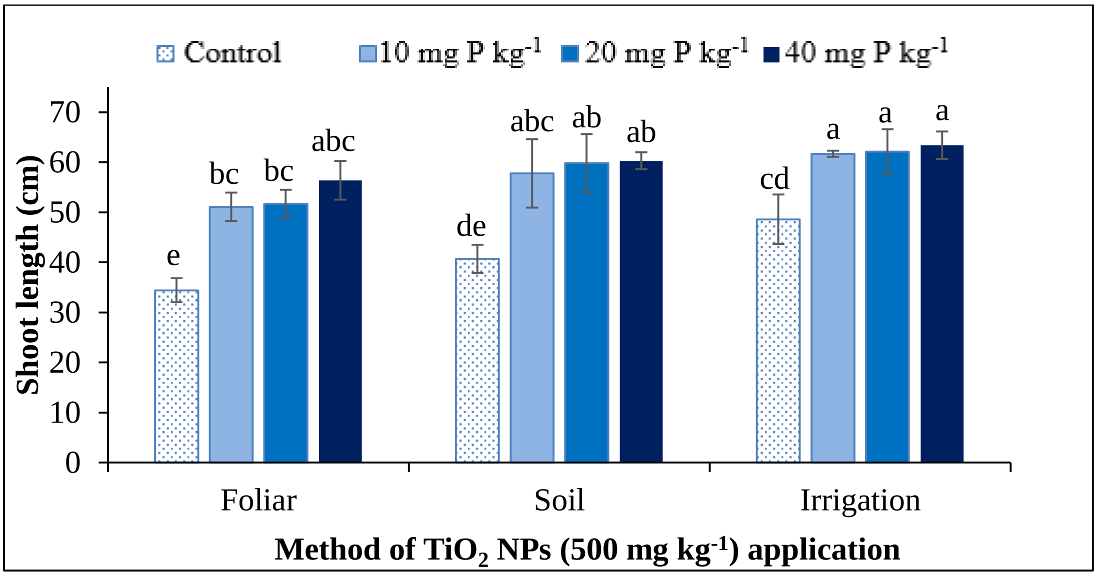

Figure 4.4 Combined Effect of TNPs Method of Application and Phosphorus Level on Shoot Length of Rice Plant. Values shown in graph are mean (± SD) of five

replicates. Same letters on the bars represent no significant difference among compared means.

Similar results were found by Khan and Imtiaz (2013) who conducted a field study to investigate the effect of fertilizer levels on rice plant. Results showed 22 % increase in plant height as compared to the control without fertilizer application. *Acer mono* seedlings treated with different concentrations of phosphorus showed increase in growth with increasing phosphorus level. Significantly greater plant height and enhanced root morphological parameters (root length, root collar diameter) were observe in treated seedlings as compared to control. Highest values for all these parameters were found in seedlings supplied with maximum phosphorus dose. An optimized phosphorus level increased growth of plant such effect might be attributed to plants requirement of balanced nutritional elements for enhanced growth and development (Razaq *et al.*, 2017).

Similar increasing trend was observed for shoot length showing an increase of 10.9%, 10.6%, 11.5%, 13.8%, and 14.5% in the plants grown in soil treated with TiO_2 NPs at 50, 150, 250, 500, and 750 mg kg^{-1} concentration, respectively. Whereas for root length, difference among the control and all TiO_2 NPs-treated plants was not significant (Zahra *et al.,* 2017). Another study revealed the negative and toxic effect of TNPs on wheat. Only a slight positive effect was observed at 100 mg L^{-1}. But by increasing the applied amount of TiO_2 nanoparticles decreased shoot length (Aliabadi *et al.,* 2016). Application of TiO_2 nanoparticles indicated significant improvement in parameters of plant height, grain yield, oil fraction and oil yield of safflower. Maximum of these parameters was achieved with concentration of 0.04% nano-TiO_2 and minimum was achieved by control treatment (Morteza and Jorabloo, 2015). Maize plant when treated with TiO_2 nanoparticles showed significant improvement in shoot length whereas the effect of treatment with bulk TiO_2 was negligible on this trait. Titanium nanoparticles promote plant growth by increasing plant light absorption capacity and photo energy transmission (Moaveni and Kheiri, 2011).

Table 4.2: Effect of Phosphorus Fertilizer Levels and TNPs Method of Application on Growth Parameters of Rice

Treatments	No. of Tillers (n)	RL (cm)	SL (cm)	RDW (g)	SDW (g)
Phosphorus levels (mg kg^{-1} soil)					
0	4.0 b	26.9 b	41.2 b	1.3 c	2.1 d
10	5.0 a	34.5 a	56.8 a	1.9 c	4.2 c
20	5.6 a	34.4 a	57.8 a	2.8 b	5.1 b
40	5.9a	36.4 a	60.0 a	4.4 a	6.3 a
TNPs method of application					
Irrigation	5.6 a	32.8 ab	58.9 a	3.6 a	5.3 a
Soil	4.4 b	35.9 a	54.6 b	1.8 b	3.8 b
Foliar	5.4 a	30.4 b	48.4 c	2.3 b	4.2 b

Where, mean values for the treatments within a column following different alphabet are significantly different at $p < 0.05$ by Tukey's Honestly Significant Difference (HSD) Test, RL=root length, SL=shoot length, RDW= root dry weight, SDW =shoot dry weight.

4.4.2 Root Length

The analysis of variance revealed that the main effect due to phosphorus fertilizer application level and TiO_2 nanoparticles application method had significant ($p < 0.05$) effect on root length. However, the interaction effect of the factors was not significant (*Annexure I*).

The TiO_2 nanoparticles method of application also had significant effect on root length. The treatment with TiO_2 nanoparticles in soil produced significantly the higher root length of 35.94 g and other application methods (foliar and irrigation) produced root length of 30.43 and 32.88 g, respectively. Maximum root length (36.41 cm) was recorded at P level of 40 mg kg^{-1} followed by 20 mg kg^{-1} (34.52 cm) while lowest root length (26.95 cm) was recorded in the control (0 mg kg^{-1}). Increasing P levels from 0 to 40 mg kg^{-1} significantly increased by about 35% and showed consistent increment. When the level of phosphorus was increased from 0 to 10 mg kg^{-1}, root length was increased by 27.7%, when the level of phosphorus was increased from 10 to 20 mg kg^{-1}, there was no significant increase in root length. Increase in phosphorus level from 20 to 40 mg kg^{-1} increased root length by 5% thus indicating first decreasing trend on the rate of increment and then increasing trend with increase in level of phosphorus (Table 4.2)

Maximum root length (41.44 cm) was observed in plants grown at phosphorus concentration of 40 mg kg^{-1} with nano-TiO_2 application in soil. The minimum root length (22.9 cm) was measured in plants applied with TiO_2 nanoparticles using foliar spray grown without phosphorus fertilizer application under controlled condition (Figure 4.5).

Sanusan *et al.* (2009) also reported that phosphorus fertilization at high rates significantly affects root length density, promotes shoot growth and phosphorus uptake. In contrast to our results, Wissuwa (2005) found that rice (*Oryza sativa*) showed increased root elongation due to phosphorus deficiency. Regardless of this comparative increase in root biomass, absolute root growth was reduced due to P deficiency and low tolerant rice genotypes to phosphorus deficiency exhibited more pronounced reduction in absolute root growth. Whereas phosphorus uptake efficiency per root size was more pronounced in tolerant plants this might be the reason for improved root growth due to additional phosphorus uptake in those plants. On the other hand phosphorus deficiency limits root growth due to its deleterious effect on photosynthetic efficiency.

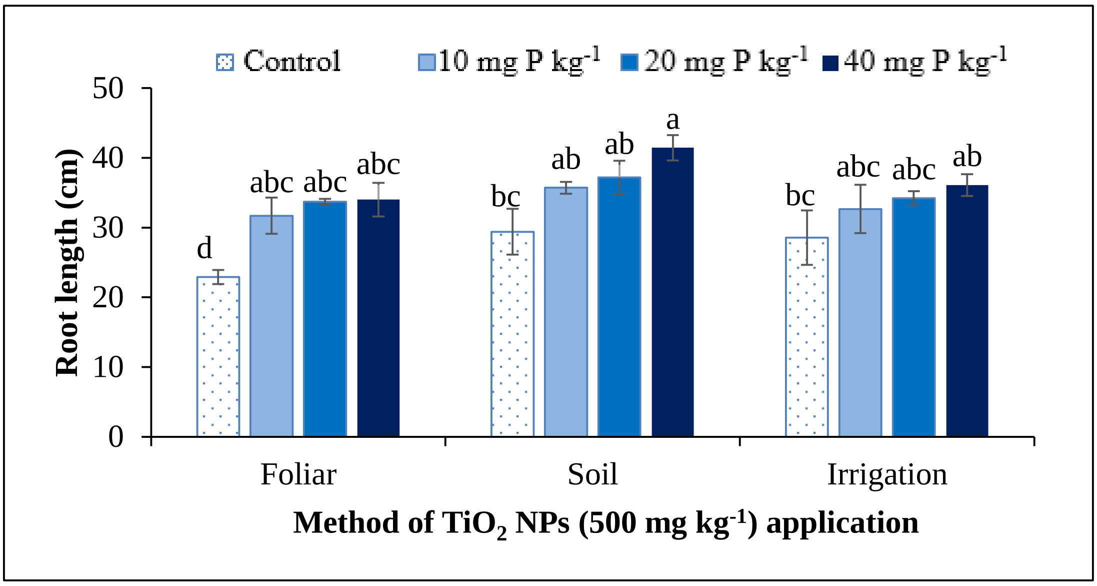

Figure 4.5 Combined Effect of TNPs Method of Application and Phosphorus Level on Root Length of Rice Plant. Values shown in graph are mean (± SD) of five replicates. Same letters on the bars represent no significant difference among compared means.

In another study conducted by Larue *et al*. (2012), it was observed that application of TiO_2 nanoparticles to wheat (*Triticum aestivum*) enhanced plant growth. Root elongation was more obvious when exposed to 14 nm and 22 nm TNPs. Similar results were found by Rafique *et al.* (2014), in wheat plant root and shoot length increased with increasing TNPs concentrations. Maximum increase in root length (48%) and shoot length (40.3%) was observed at dose of 60 mg kg^{-1} then gradually decreased at higher concentrations.

4.4.3 Number of tillers

The results of analysis of variance showed that number of tillers was significantly (*p<0.05*) influenced by the main effect of P levels and nano- TiO_2 application but their interaction effect was not significant (*Annexure I*).

Table 4.2 illustrates that highest number of tillers plant^{-1} (5.6) was produced in the plants applied with TiO_2 nanoparticles through irrigation while plants treated with soil applied TiO_2 nanoparticles had the lowest number of tillers plant^{-1} i.e. 4.40. Variation in TiO_2 nanoparticles method of application from irrigation to foliar resulted in significant reduction in number of tillers per plant. The phosphorus fertilizer level had significant ($p<0.05$) effect on number of tillers. The treatment with the maximum P level (40 mg kg^{-1}) produced significantly the highest number of tillers plant^{-1} i.e. 5.93 which was statistically at par with the other P levels (10 and 20 mg kg^{-1}) with number of tillers 5.6 and 5.06, respectively. Minimum number of tillers were recorded in control rice plants with no phosphorus application.

Rice plants exposed to soil and irrigation application of TiO_2 nanoparticles showed increasing trend for number of tillers as phosphorus fertilizer level increased. The maximum number of tillers per plant i.e. 6.8 was obtained in the plants exposed to TNPs through irrigation at 40 mg P kg^{-1} followed by the 6.2 tillers plant^{-1} at 20 mg P kg^{-1} with TiO_2 nanoparticles using the same method which was statistically similar to the plants with foliar applied nano-TiO_2 (6.0) at 40 mg P kg^{-1} (Figure 4.6).

Phosphorus plays a major role in plant growth during the early vegetative growth phases, because of its stimulatory effect for the initiation of tillering and root development (Sainio *et al.*, 2006).

Similar trend has been reported in other studies. In another study phosphorus fertilization accelerated the formation of tillers in rice as compared to control. But with further increase in the level of phosphorus fertilizer no significant increase in number of effective tillers was observed (Hasanuzzaman *et al.*, 2012; Khan and Imtiaz, 2013).

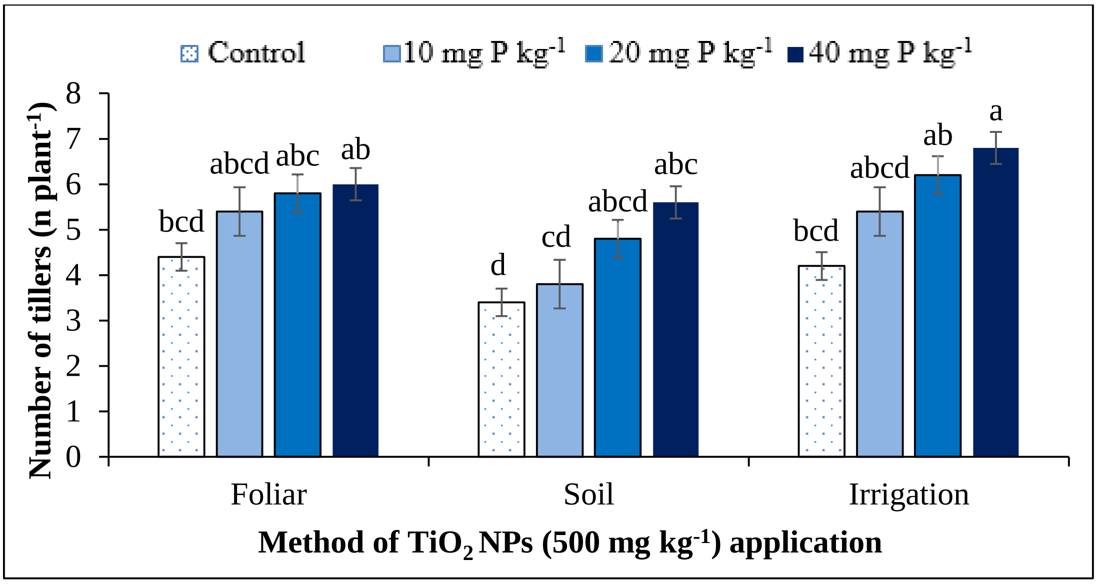

Figure 4.6 Combined Effect of TNPs Method of Application and Phosphorus Level on No. of Tillers of Rice Plant. Values shown in graph are mean (± SD) of five replicates. Same letters on the bars represent no significant difference among compared means.

Wheat plants also showed significant increment in number of tillers with increasing levels of phosphorus in contrast to control without phosphorus (Noonari *et al.,* 2016). Matsua *et al*. (1995) reported that P fertilization is essential for increased effective tillering thereby help in P uptake by the rice plants. As per literature, TiO_2 nanoparticles stimulate the number of tillers in Barley (*Hordeum vulgare*) plant by promoting the development of secondary shoots (Marchiol *et al.,* 2016). A study conducted on winter barley involving foliar application of nano-TiO_2 suggested that it had no significant effect on number of tiller per plant (Mohammadi *et al.*, 2016).

4.4.4 Shoot dry weight

The main effect of nano-TiO_2 method application and phosphorus fertilizer levels on shoot dry weight were significant (*$p<0.05$*). However, their interaction effect was not significant (*Annexure I*).

As illustrated in Table 4.2, maximum shoot dry weight (5.32 g $plant^{-1}$) was measured in plants exposed to nano-TiO_2 through irrigation method while minimum shoot dry weight (3.83 g $plant^{-1}$) was measured in plants treated with TiO_2 nanoparticles through soil application. Percentage difference for shoot weight among the two application methods was found to be 39% which was statistically significant. Mean shoot dry weights for plants exposed to foliar and soil applied nano-TiO_2 were statistically at par with each other. Mean values for phosphorus levels showed that shoot dry weight increased with each increment of phosphorus level from control to highest level and mean comparison suggest statistically significant difference among shoot dry weights at all the P levels. When phosphorus level was increased from 0 to 40 mg P kg^{-1}, shoot dry weight increased from 2.19 g $plant^{-1}$ to 6.31 g $plant^{-1}$, respectively. Compared to control treatment, mean shoot dry weight was increased by 94%, 135% and 188% for 10, 20 and 40 mg P kg^{-1} treatments, respectively.

The maximum shoot dry weight (7.5 g $plant^{-1}$) was measured in a combination of treatment maximum phosphorus fertilizer level (40 mg P kg^{-1}) with application of TiO_2 nanoparticles through irrigation method followed by 6.6 g $plant^{-1}$ at phosphorus fertilizer level of 20 mg kg^{-1} with TiO_2 nanoparticles application using irrigation and soil method (6.0 g $plant^{-1}$) and the lowest shoot weight was measured in plants having no phosphorus treatment with soil applied TiO_2 nanoparticles (Figure 4.7). The shoot dry weight was increased by about 4 fold when phosphorus level of 40 mg P kg^{-1} was applied in combination with TiO_2 nanoparticles using irrigation method as compared to lowest shoot dry weight in plants treated with soil applied TiO_2 nanoparticles under controlled phosphorus treatment.

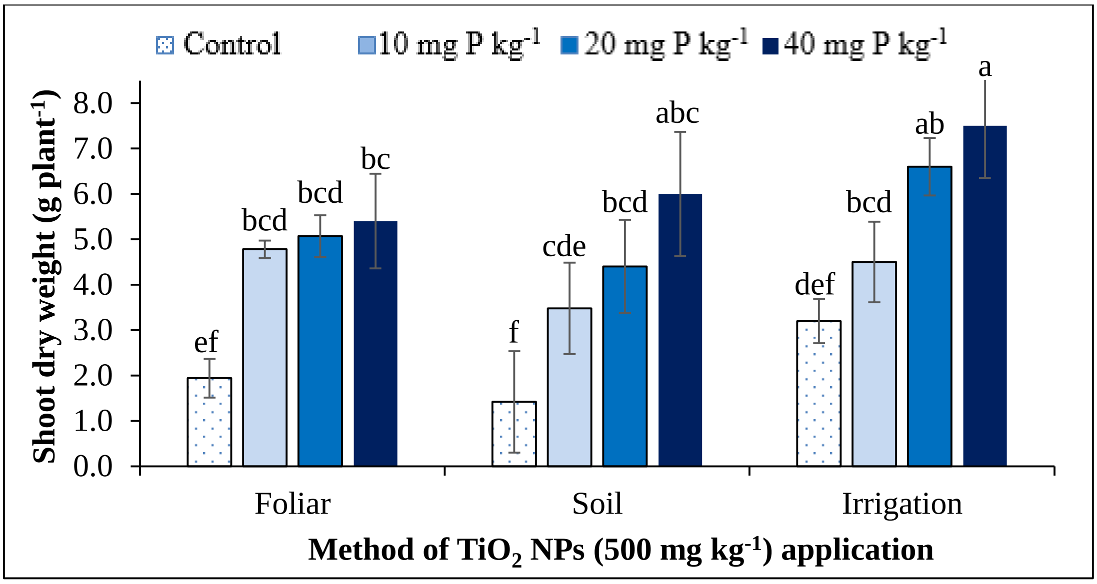

Figure 4.7 Combined Effect of TNPs Method of application and Phosphorus Level on Shoot Dry Weight of Rice Plant. Values shown in graph are mean (± SD) of five replicates. Same letters on the bars represent no significant difference among compared means.

Plant growth is the key indicator for evaluation of the capability of plant to survive and adapt with environmental conditions. Certain indices such as root/shoot dry and fresh weight, leaf count and leaf area are the determinants of rate of the plant growth (Zhao *et al.*, 2013). Treatment of plants with low phosphorus fertilization leads to shoot deficiency inhibiting leaf expansion and thereby limiting carbon assimilation that subsequently results into lower shoot biomass. The reason for such response under low P is that the plants begin to divert a major portion of the net assimilated carbon for the development of heterotrophic instead of photosynthetic tissues, ultimately increasing root: shoot ratio (Nielsen *et al.*, 2001).

In plants, phosphorus deficiency typically leads to tremendous decrease in root and shoot biomass. Shoot dry weight is among the most sensitive growth parameter to P deficiency followed by root dry weight and showed greatest increase in growth with each P increment. Determination of shoot weight is easier in comparison with root weight and is suggested for cereal crops studies involving phosphorus-screening carried out in green house conditions (Fageria *et al.*, 1988). Kim and Li (2016) reported efficiency of phosphorus in increasing root and shoot growth in *Lantana camara* 'New Gold' plant. Number of leaves as well as leaf surface area dramatically enhanced under higher P concentrations subsequently increasing shoot biomass. During vegetative growth linear enhancement in shoot dry weight was observed as a results of increasing phosphorus level and increase was logarithmic during reproductive growth.

Titanium dioxide nanoparticles serve a promising role as nutrient source for nourishment of plants to boost biomass production because of improved nitrogen assimilation, photoreduction events of photosystem II and electron transport chain, foraging of reactive oxygen species (Raliya *et al.*, 2015). Application of TiO_2 nanoparticles enhanced shoot dry biomass of dragonhead (*Dracocephalum moldavica* L.) plants under normal irrigation conditions in comparison to control (Mohammadi *et al.*, 2016). Our results also coincide with the results of (Dhoke *et al.*, 2013) who reported that ZnO nanoparticles enhanced shoot growth by about 97.87 % and dry shoot biomass by 76.04% in contrast to the control ($p < 0.05$) in mung seedlings with dose of 20 mg L^{-1} ZnO NPs (Dhoke *et al*., 2013).

From agricultural perspective, promoting the plant growth is one of the major reason for the application of nanoparticles. In poor mineral soils, ZnO NPs were applied to corn plant after mixing with irrigation water. Results showed that addition of nano-ZnO in irrigation water enhanced shoot dry weight. As compared to normal irrigation water, shoot dry weight increased by 63.8% with application of irrigation water containing nanoparticles. Stimulatory effect of nano-ZnO may be attributed to its nano properties most specifically greater specific surface area (Taheri *et al.*, 2015).

4.4.5 Root dry weight

The root dry weight was significantly ($p<0.05$) influenced by the main effects of phosphorus level and TiO_2 nanoparticles method of application and their interaction was also statistically significant (*Annexure I*).

Table 4.2 shows that root dry weight was significantly affected by nano-TiO_2 method of application ($p < 0.05$), with highest root dry weight of 3.64 g plant^{-1} produced at irrigation method of application while the lowest dry weight of 1.89 g plant^{-1} was produced with soil applied nano-TiO_2. There was no significant difference among soil and foliar method of TiO_2 nanoparticles application. Mean values for phosphorus levels showed that root dry weight increased with each increment of phosphorus level from control to highest level, but the two lower P levels (0 and 10 mg kg^{-1}) were statistically similar. The maximum root dry weight (4.45 g plant^{-1}) was recorded for P application at the level of 40 mg kg^{-1} and the lowest root weight (1.38 g) was recorded at the control treatment implying a positive response of root dry weight to P. Increasing P from 0 to 20 and 40 mg kg^{-1} increased the root dry weight by 104% and 223% and the increment was consistent. However, the application rate of P fertilizer at 10 mg kg^{-1} did not differ significantly from the control without P treatment.

Mean root dry weight varied from 0.8 g plant^{-1} (minimum) for 0 level of phosphorus and foliar application of TiO_2 nanoparticles to 6.2 g plant^{-1} (maximum) for the highest phosphorus rate of 40 mg P kg^{-1} combined with irrigation method of nano-TiO_2 application indicating large biomass variation under the different P treatments and TNPs method of application. Results showed that 40 mg P kg^{-1} soil applied in combination with TiO_2 nanoparticles through irrigation gave significantly the highest root dry biomass followed by treatment with 20 mg P kg^{-1} (5.7 g $plant^{-1}$) with nano-TiO_2 application using same method (Figure 4.8).

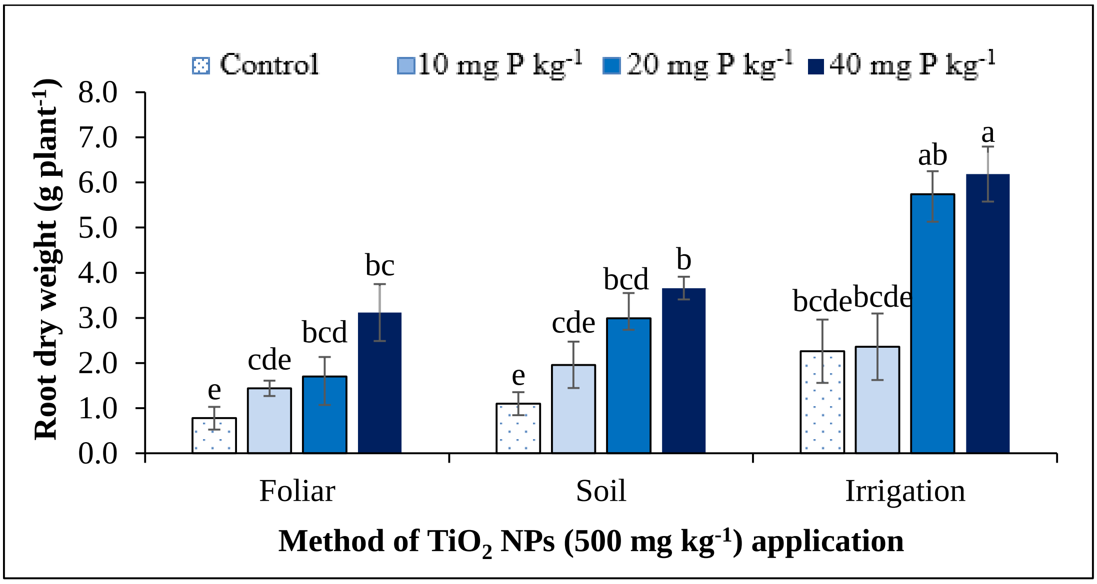

Figure 4.8 Combined Effect of TNPs Method of Application and Phosphorus Level on Root Dry Weight of Rice Plant. Values shown in graph are mean (± SD) of five replicates. Same letters on the bars represent no significant difference among compared means.

Wissuwa *et al.* (2005) demonstrated that at the lower phosphorus level the maximum proportion of plant dry matter (40%) was found in roots. This declined to 30% for root dry matter under medium phosphorus deficiency. When phosphorus was sufficient, less than 20% of dry biomass was distributed to roots. Root fresh and dry weight enhanced in duckweed as a result of application with TiO_2 nanoparticles than bulk TiO_2. This showed that TiO_2 NPs were more effective than bulk titanium for improving plant growth. Growth stimulatory effect of TiO_2 NPs was observed at low concentrations, it stunted the plant growth at high concentrations (Song *et al.,* 2012). Similar results were found by Mahmoodzadeh and Aghili (2014) that optimum concentration of nano titanium dioxide has a triggering effect and high concentrations hinder the growth of root and shoot of the wheat. They also found that nano-TiO_2 remarkably effects the fresh and dry weights of the root.

Research carried out on the soybean revealed that titanium moves very slowly from outside to inside the plant body and treated organs are directly affected. When nano titanium dioxide solution was applied in the root environment, the root growth was enhanced more than shoot (Calza *et al.,* 2014).

4.5 Effect on Uptake of Phosphorus in Rice

4.5.1 Shoot phosphorus

The analysis of variance revealed that the main effect due to phosphorus fertilizer application level and TiO_2 nanoparticles application method had significant ($p < 0.05$) effect on root length. However, the interaction effect of the factors was not significant (*Annexure II*).

Maximum shoot phosphorus (817.4 mg kg^{-1}) was measured in plants exposed to nano-TiO_2 through irrigation method while minimum shoot phosphorus content (590 mg kg^{-1}) was measured in plants treated with TiO_2 nanoparticles through foliar application. Percentage difference for shoot weight among the two application methods was found to be 38% which was statistically significant ($p < 0.05$). Mean shoot phosphorus concentration for plants exposed nano-TiO_2 applied using different methods were statistically different from each other. The concentration ranged between 435 to 978.7 mg kg^{-1} in rice shoots. Different levels of phosphorus fertilizer in soil showed increased phosphorus concentration over respective control. Therefore addition of varied concentration of P fertilizer on shoot phosphorus concentration was found significant (Table 4.3) The maximum mean shoot phosphorus concentration (978.7 mg kg^{-1}) was recorded at a level of 40 mg P kg^{-1} and minimum shoot phosphorus (435 mg kg^{-1}) was recorded from control treatment (without phosphorus application). Compared to control the mean shoot phosphorus content was increased by 41%, 87% and 125% under 10, 20 and 40 mg P kg^{-1} treatment levels, respectively.

Our findings also relate to the results of Islam *et al.* (2008) and khan *et al.* (2010) who reported linear increase in total phosphorus uptake in rice plant with consistent increment in phosphorus application rates. Highest phosphorus uptake was observed at maximum phosphorus dose and lowest in the control. Wheat plant applied with different phosphorus fertilizer showed similar response (Hossain *et al.,* 2005; Rahim *et al.*, 2010). Similar results were found by Kalala *et al.* (2016) who conducted a screen house experiment on rice plants applied with varied phosphorus levels (0, 40 and 80 mg P kg^{-1}). Shoot P concentration increased significantly with the application of phosphorus fertilizer and 40 mg P kg^{-1} was found to be the optimum application level for rice growth in phosphorus deficient soils.

The combined effect of nano-TiO_2 application method and phosphorus fertilizer level on shoot P is presented in figure. The highest shoot phosphorus content (1140.9 mg kg^{-1}) was measured in a combination of treatment with maximum phosphorus fertilizer level (40 mg P kg^{-1}) along with application of TiO_2 nanoparticles through irrigation method followed by phosphorus fertilizer level of 20 mg P kg^{-1} with irrigation applied TiO_2 nanoparticles (1051.19 mg kg^{-1}) and the lowest shoot P was measured in plants having no phosphorus treatment with foliar applied TiO_2 nanoparticles (331.91 mg kg^{-1}). The shoot phosphorus concentration was increased by about 2.4 fold when phosphorus level of 40 mg

P kg^{-1} was applied in combination with TiO_2 nanoparticles using irrigation method as compared to lowest shoot phosphorus in plants treated with foliar applied TiO_2 nanoparticles under controlled phosphorus treatment (Figure 4.9).

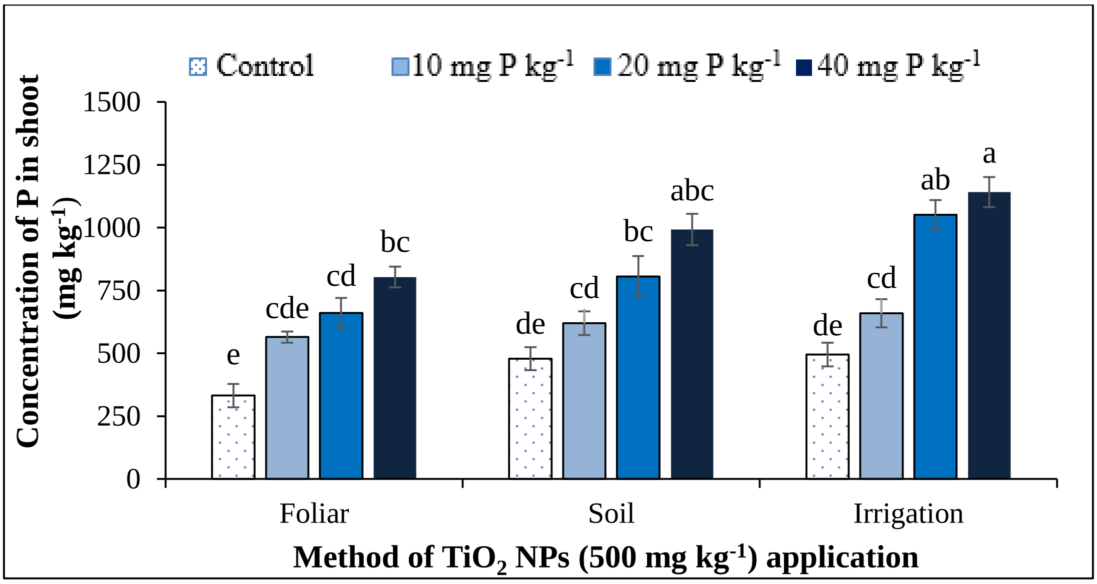

Figure 4.9 Combined Effect of TNPs Method of Application and Phosphorus Level on Shoot Phosphorus Concentration. Values shown in graph are mean (± SD) of five replicates. Same letters on the bars represent no significant difference among compared means.

It has been suggested that TNPs possess properties culminating high surface reactivity that might cause the enlargement of root pores and therefore, promote water uptake and nutrients availability to plants (Larue *et al.,* 2012). Titania nanoparticles also have positive impact in terms of availability of phosphorus, in *Lactuca sativa* phosphorus uptake was enhanced with application of TNPs (Hanif *et al.*, 2015; Zahra *et al.*, 2015). Zinc oxide (ZnO) nanoparticles synthesized from soil fungi increased the mobilization and availability of phosphorus in mung bean rhizosphere (Raliya *et al.*, 2016). Therefore It was proven from the previous literature that nanoparticles application on crops enhance the phosphorus availability to the plants.

4.5.2 Root phosphorus

The results of analysis of variance suggest that main effect of phosphorus level and TiO_2 nanoparticles method of application was significant ($p < 0.05$) on root P concentration. However the combined effect of the two treatments was not statistically significant (*Annexure II*).

The result revealed that nano-TiO_2 application method significantly ($p < 0.05$) affected on root phosphorus with highest root phosphorus concentration of 935.9 mg kg^{-1} produced at soil application of TNPs and the lowest root phosphorus of 630.3 mg kg^{-1} produced with foliar applied nano-TiO_2. Increment of root P was observed as the method of application changed, there was significant difference among foliar, soil and irrigation method of TiO_2 nanoparticles application (Table 4.3). Root phosphorus concentration showed increase up to 49% when applied with TiO_2 nanoparticles in soil as compared to foliar application. Table 4.3 also shows that root P concentration was found higher than shoot P concentration. Phosphorus concentration in roots increased with each increment of phosphorus level from control to highest level (0 to 40 mg P kg^{-1}). Root phosphorus concentration was maximum (1110.2 mg kg^{-1}) for phosphorus application at the level of 40 mg kg^{-1} and the lowest root phosphorus (532.2 mg kg^{-1}) was recorded at the control treatment implying a positive response of root phosphorus to applied phosphorus fertilizer levels. Increasing P from 0 to 20 mg kg^{-1} and 40 mg kg^{-1} increased the root phosphorus by 60% and 109% and the increment was consistent. However, the application rate of P fertilizer at 10 mg kg^{-1} did not differ significantly from control without phosphorus treatment.

The maximum root phosphorus content (1404.5 mg kg^{-1}) was measured in a combination of treatment maximum phosphorus fertilizer level (40 mg P kg^{-1}) with TiO_2

nanoparticles application through soil followed by 1180.3 mg kg^{-1}at phosphorus fertilizer level of 40 mg kg^{-1} with TiO_2 nanoparticles application using irrigation and the lowest root phosphorus (289.08 mg kg^{-1}) was measured in plants having no phosphorus treatment with foliar applied TiO_2 nanoparticles (Figure 4.10). The root phosphorus was increased by 2.6 fold in combination of treatment with maximum phosphorus fertilizer level (40 mg P kg^{-1}) and TiO_2 nanoparticles application through soil.

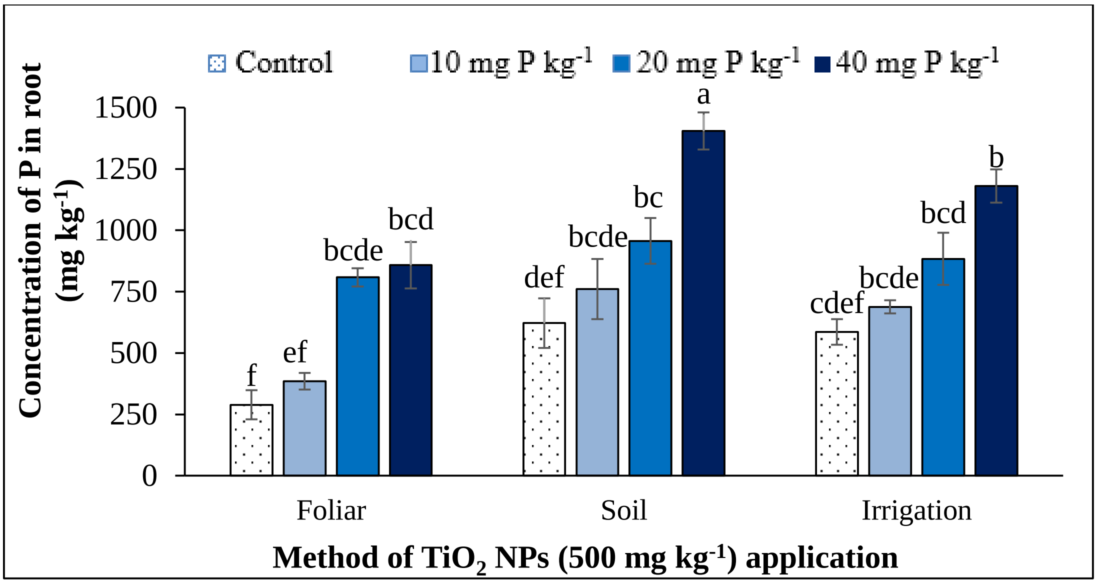

Figure 4.10 Combined Effect of TNPs Method of Application and Phosphorus Level on Root Phosphorus Concentration. Values shown in graph are mean (± SD) of five replicates. Same letters on the bars represent no significant difference among compared means.

Phosphorus concentration in roots increases considerably with increasing phosphorus fertilizer. Phosphorus is crucial plant growth nutrient, optimal supply of phosphorus improves crop production. Plant growth and early maturity, assimilation of starch and sugar, photosynthetic processes, karyokinesis and cell division require adequate amounts of phosphorus (Atif *et al.*, 2014). Kim and Li (2016) found that when phosphorus application rate was increased from 0 to 20 mg·L^{-1} it resultantly increased the accumulation of phosphorus in all plant organs, but primarily in shoots, whereas further increasing the concentration increased the acquisition predominantly in roots and flowers. In plant tissues higher P uptake did not strongly correlate to the biomass in Lantana plant.

Zahra *et al.* (2017) reported increase in root, shoot and grain phosphorus uptake as a results of TNPs application in soil. In comparison to control, plants exposure to TNPs at a concentration of 750 mg kg^{-1} showed increased root and shoot phosphorus (2.6 and 2.4-fold). Zheng *et al,* 2005 revealed that exposure of plants to 500 mg p kg^{-1} increased phosphorus content in cucumber fruit by 34%.

Table 4.3 Effect of Phosphorus Fertilizer Levels and TNPs Method of Application on Root and Shoot Phosphorus Content

Treatments	**Root phosphorus (mg kg^{-1})**	**Shoot Phosphorus (mg kg^{-1})**
Phosphorus levels (mg kg^{-1} soil)		
0	532 c	435 d
10	651 c	614 c
20	853 b	813 b
40	1110 a	978 a
TNPs method of application		
Irrigation	794 b	817 a
Soil	935 a	723 b
Foliar	630 c	590 c

Where, mean values for the treatments within the same factor and column following different alphabet indicate significant difference ($p < 0.05$) by Tukey's Honest Significant Difference (HSD) Test.

4.6 Effect on Protein Content in Rice Grain

Rice grain protein content was significantly ($p < 0.05$) influenced by the main effects of phosphorus level and TiO_2 nanoparticles method of application and their interaction effect was also statistically significant (*Annexure II*). The effect of nano-TiO_2 application method and phosphorus fertilizer levels on protein content is presented in figure 4.11.

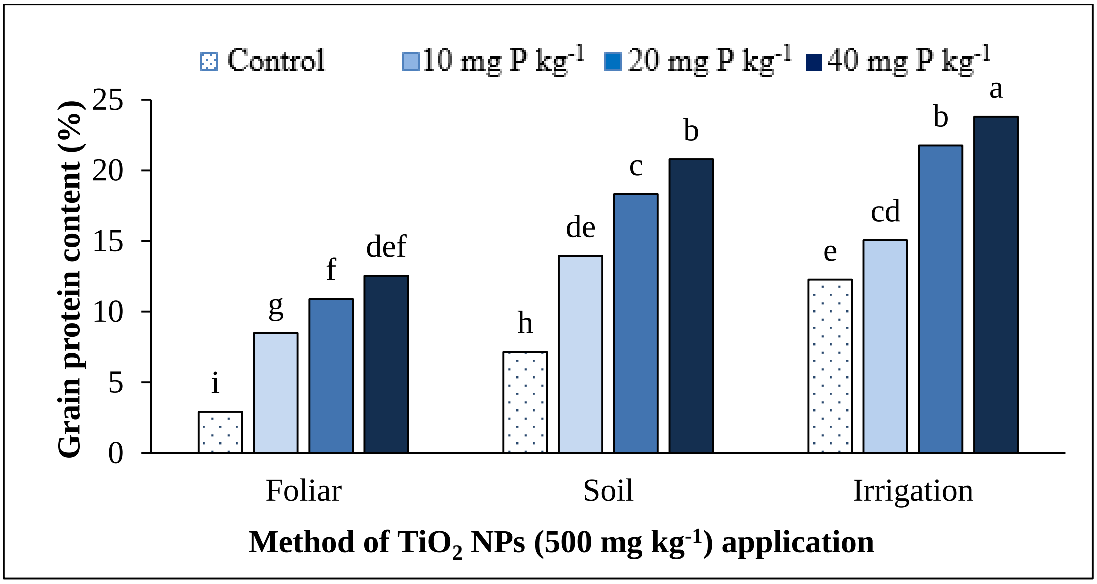

Figure 4.11 Combined Effect of TNPs Method of Application and Phosphorus Level on Protein Content in Rice Grains. Values shown in graph are mean (± SD) of five replicates. Same letters on the bars represent no significant difference among compared means.

The maximum grain protein content of 23.8 % was measured in treatment with maximum phosphorus fertilizer level (40 mg P kg^{-1}) combined with application of TiO_2 nanoparticles through irrigation method followed by phosphorus fertilizer level of 20 mg P kg^{-1} with irrigation method of TiO_2 nanoparticles application (21.7 %) and the lowest protein content in grains was measured in plants having no phosphorus treatment with foliar applied TiO_2 nanoparticles (2.9 %). Protein content was increased by about 7.1 fold when phosphorus level of 40 mg P kg^{-1} was applied in combination with TiO_2 nanoparticles using irrigation method as compared to lowest shoot length in plants treated with foliar applied TiO_2 nanoparticles without phosphorus application (Figure 4.11).

Phosphorus is essential for plant growth and encourages root development, tillering, premature flowering and accomplishes other functions like metabolic activities, principally in synthesis of protein (Panhawar *et al.*, 2011). Fertilization with phosphorus significantly increases grain protein content in winter wheat as compared to control (Gaj *et al.*, 2013).

Research conducted by Pošcic *et al.* (2016) suggested increase in amino acids and crude protein content of barley kernels as a results of application of cerium and titanium dioxide nanoparticles. Najafi *et al.* (2014) found that nanoparticles applied to *Phaseolus vulgaris* seedlings resulted in improved protein content. Titanium nanoparticles have also been reported to increase total protein content in coriander (Khater, 2015).

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Rice production is profoundly dependent on availability of soil nutrients and other factors for plant growth and development. Fertilization needs to be reasonably used in order to evade negative and detrimental effects on the sustainability of agricultural production system as well as the environment. TiO_2 application in combination with phosphorus fertilizer levels significantly affected plant growth in terms of chlorophyll content, root and shoot length, plant biomass, and phosphorus uptake. Better growth response was observed at combination of 40 mg/kg phosphorus and TNPs delivered using irrigation method. Irrigation method of TNPs application improved P uptake by 2.4 fold. 40 mg P kg^{-1}. The highest shoot length (63.4 cm), number of tillers (7 n $plant^{-1}$), root dry weight (6.2 g $plant^{-1}$), shoot dry weight (7.52 g $plant^{-1}$), shoot phosphorus (1140 mg kg^{-1}) and rice grain protein content (23.8%) were recorded under the application of TiO_2 nanoparticles through irrigation method in combination with 40 mg P kg^{-1}, although this method of TiO_2 nanoparticles application performed equally good at half dose of 20 mg P kg^{-1}. The positive effects of TiO_2 nanoparticles can be attributed to their ability to penetrate through the root surfaces, improving root water and nutrient acquisition, therefore enhance the fertilizer use efficiency. TiO_2 nanoparticles might have exerted catalytic role to trigger metabolic activities for plant growth. The outcome of the study is an advanced method for managing and augmenting the availability and release of nutrients through basic and applied research. Further studies are required to understand the detailed mechanisms involved in improved phosphorus uptake and plant growth.

5.2 Future Perspectives and Recommendations

In current study, noteworthy effects were found on the *Oryza sativa* in response to titania nanoparticles application in combination with fertilizers. Such studies could address the concerns of nutrient deficiency in soil and help in providing better crop yield by minimizing use of fertilizers and eventually helpful in betterment of environment. Following are the recommendations for the work to be done in future:

- There is a need to study the relationship between TNP and disease attacks (e.g. fungal infections, pests' attacks etc.).
- Alterations in biochemical composition of grains needs to be evaluated to determine detailed impacts on quality of yield.
- Identification of possible impacts of different modes of application on uptake mechanisms of nanoparticles at molecular level can be done.
- Field experiment for economic analysis may be conducted.

REFERENCES

Aguilar-Garcia, C., Gavino, G., Baragano-Mosqueda, M., Hevia, P. and Gavino, V. C. (2007). Correlation of tocopherol, tocotrienol, γ-oryzanol and total polyphenol content in rice bran with different antioxidant capacity assays. *Food Chemistry*. 102 (4): 1228-1232.

Ai, P.H., Sun, S.B., Zhao, J.N., Fan, X.R., Xin, W.J., Guo, Q., Yu, L., Shen, Q.R., Wu, P. and Miller, A.J., *et al*. (2009). Two rice phosphate transporters, OsPht1; 2 and OsPht1; 6, have different functions and kinetic properties in uptake and translocation. *Plant Journal.* 5(7): 798–809.

Aliabadi, T., Afshar, A. S. and Nematpour, F. S. (2016). The effects of nano TiO_2 and nano aluminium on the growth and some physiological parameters of the wheat (*Triticum aestivum*). *Iranian Journal of Plant Physiology*. 6(2): 1627-1635.

Alivisatos, A. P., Gu, W. and Larabell, C. (2005). Quantum dots as cellular probes. *Annual Review of Biomedical Engineering*. 7:55–76.

An, J., Zhang, M., Wang, S. and Tang J. (2008). Physical, chemical and microbiological changes in stored green asparagus spears as affected by coating of silver nanoparticles-PVP. *Food Science and Technology*. 41: 1000–1007.

Arora, S., Sharma, P., Kumar, S., Nayan, R., Khanna, P. K. and Zaidi, M. G. H. (2012) Gold-nanoparticle induced enhancement in growth and seed yield of *Brassica juncea*. *Plant Growth Regulations*. 66(3): 303–310.

Asli, S. and Neumann, P. M. (2009). Colloidal suspensions of clay or titanium dioxide nanoparticles can inhibit leaf growth and transpiration via physical effects on root water transport. *Plant Cell and Environment*. 32(5): 577–584.

Atif, M. J., Shaukat, S. A., Ali Shah S. Z., Choudry, Y. A. and Shaukat S. K. (2014). Effect of different levels of phosphorus on growth and productivity of Pea (*Pisum sSativum* L.*)* cultivars grown as off-season under Rawalakot Azad Jammu and Kashmir Conditions. *Journal of Recent Advancement in Agriculture.* 2 (6): 252-257.

Begum, P., Ikhtiari, R. and Fugetsu, B. (2014). Potential impact of multi-walled carbon nanotubes exposure to the seedling stage of selected plant species. *Nanomaterials.* 4(2): 203–221.

Borges, R. and Mallarino, A. P. (2001). Deep banding P and K fertilizers for corn managed with ridge tillage. *Soil Science Society of America Journal*. 65: 376-384.

Bouyoucos, G. J. (1962). Hydrometer method improved for making particle size analyses of soils. *Agronomy Journal. 54*(5): 464.

Buzea, C., Pacheco, I. I. and Robbie, K. (2007). Nanomaterials and nanoparticles: Sources and toxicity. *Biointerphases, 2*(4). doi:10.1116/1.2815690

Calza, P., Avetta, P., Rubulotta, G., Sangermano M. and Laurenti., E. (2014). TiO_2-soybean peroxidase composite materials as a new photocatalytic system. *Chemical Engineering Journal*. 239 (0): 87-92.

Chen, L., Zhou, L., Liu, Y., Deng, S., Wu, H. and Wang, G. (2012). Toxicological effects of nanometer titanium dioxide (nano-TiO_2) on *Chlamydomonas reinhardtii*. *Ecotoxicology and Environmental Safety , 84*; 155-162.

Clément L, Hurel, C. and Marmier, N. (2013). Toxicity of TiO_2 nanoparticles to cladocerans, algae, rotifers and plants—effects of size and crystalline structure. *Chemosphere.* 90:1083–1090.

Cordell, D., Drangert, J. D. and White, S. (2009).The story of phosphorus: global food security and food for thought. *Global Environment Change.*19: 292–305.

Corredor, E., Testillano, P.S., Coronado, M.J., Gonzalez-Melendi P., Fernandez-Pacheco, R., Marquina, C., Ibarra, M. R., De la Fuente, J. M., Rubiales, D., Perez-de-Luque, A. and Risueno M.C. (2009). Nanoparticle penetration and transport in living pumpkin plants: *in situ* subcellular identification. *BMC Plant Biology*. 9 (1): 45.

Dhoke, S. K., Mahajan, P., Kamble, R. and Khanna, A. (2013). Effect of nanoparticles suspension on the growth of mung (*Vigna radiata*) seedlings by foliar spray method. *Nanotechnology Development*, *3*(1): 1-4.

Dietz, K. and Herth, S. (2011). Plant nanotoxicology. *Trends in Plant Science.* 16(11), 582-589.

EPA. (2009). External Review Draft, Nanomaterial Case Studies: Nanoscale Titanium Dioxide in Water Treatment and in Topical Sunscreen. *United States Environmental Protection Agency*.

Etxeberria, E., Gonzalez, P., Baroja-Fernández, E. and Romero, J. P. (2006). Fluid pPhase endocytic uptake of artificial nano-spheres and fluorescent quantum dots by sycamore cultured cells. *Plant Signaling and Behavior*, *1*(4): 196-200.

Fageria, R., Wright, J. and Baligar, V. C. (1988). Rice cultivar evaluation for phosphorus use efficiency N. K. *Plant and Soil*. 111: 105-109.

FAO (Food and Agriculture Organization of the United Nations). (2009). How to feed the world in 2050. Proceedings of the Expert Meeting on How to Feed the World in 2050. 24–26. FAO Headquarters, Rome.

Farre, M., Sanchis, J. and Barcelo, D. (2011). Analysis and assessment of the occurrence, the fate and the behavior of nanoparticles in the environment. *Treands Analyte Chemistry*. *30*: 517- 527.

Feizi, H., Moghaddam, P. R., Shahtahmassebi, N. and Fotovat, A. (2012).Impact of bulk and nanosized titaniumdioxide (TiO_2) on wheat seed germination and seedling growth. *Biological Trace Element Research*. 146(1): 101–106.

Fenoglio, I., Greco G. and Livraghi S. (2009) Non-UV-induced radical reactions at the surface of TiO_2 nanoparticles that may trigger toxic responses. *European Journal* of *Chemistry*. 15(1): 4614–4621

Food and Agriculture Organization of the United Nations. (2014). FAOSTAT: Production-Crops, 2012 data.

Gaj, R., Górski, D. and Przyby, J. (2013). Effect of differentiated phosphorus and potassium fertilization on winter wheat yield and quality. *Journal of Elementology.* 55–67.

Gao, F., Liu, C., Qu, C., Zheng, L., Yang, F., Su, M. and Hong, F. (2008). Was improvement of spinach growth by nano-TiO2 treatment related to the changes of rubisco activase? *Biometals.* 21: 211- 217.

Giraldo, J. P., Landry, M. P., Faltermeier., S. M., McNicholas, T. P., Iverson, N. M., Boghossian, A. A., Reuel, N. F., Hilmer, A. J., Sen, F., Brew, J. A. and Strano, M. S. (2014). Plant nanobionics approach to augment photosynthesis and biochemical sensing. *Nature Materials.* 13: 400- 408.

Guo, L. (2007). Doing battle with the green monster of taihu lake. Science. 317:1166.

Gyaneshwar, P., James, E. K., Reddy, P. M. and Ladha J.K. (2002). Herbaspirillum colonization increases growth and nitrogen accumulation in aluminium- tolerant rice varieties. *New Phytology*. 154:131-145.

Hanif, H. U., Arshad, M., Ali, M. A., Ahmed, N. and Qazi, I. A. (2015). Phyto-availability of phosphorus to *Lactuca sativa* in response to soil applied TiO_2 nanoparticles. *Pakistan Journal of Agricultural Sciences.* 52(1): 177-182

Haq, M. Z., Robbani, M., Ali, M., Hasan, M. M., Hasan, M. M., Uddin, M. J. *et al*. and Karim, M. R. (2012). Damage and management of cyclone Sidr-affected homestead tree plantations: a case study from Patuakhali, Bangladesh. *Natural Hazards,* 64(2): 1305-1322.

Hasan, M. M. (2009). Selection of heat tolerant wheat genotypes under short winter condition of patuakhali district, MS Thesis. Bangladesh: *Department* of *Agricultural Botany*. *Patuakhali Science and Technology University, Bangladesh*.

Hasan, M. M., Hasan, M. M., Jaime, A., Da Silva, T. and Li, X. (2016). Regulation of phosphorus uptake and utilization: transitioning from current knowledge to practical strategies. *Cellular & Molecular Biology Letters*. 21:7 doi:10.1186/s11658-016-0008-y.

Hasanuzzaman, M., Ali, M. H., Karim, M. F., Masum S. M. and Mahmud, J. A. (2012). Response of hybrid rice to different levels of nitrogen and phosphorus. *International Research Journal of Applied and Basic Sciences*. 3(12): 2522-2528.

He, Y., Liao, H. and Yan, X. (2003). Localized supply of phosphorus induces root morphological and architectural changes of rice in split and stratified soil cultures. *Plant and Soil*. *248*(1/2): 247-256.

Hediat, M. H. and Salama. (2012). Effects of silver nanoparticles in some crop plants, Common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). *International Research Journal of Biotechnology*. 3(10): 190-197.

Hendren, C. O., Mesnard, X., Dröge, J. and Wiesner, M. R. (2011). Estimating production data for five engineered nanomaterials as a basis for exposure assessment. Environmental Science and Technology. 45: 2562–2569.

Hesse, P. R. (1971). *A Textbook of Soil Chemical Analysis*. John Murray, London

Hong, F. S., Yang, P., Gao, F. Q., Liu, C., Zheng, L. and Zhou, J. (2005). Effect of nanoanatase TiO_2 on spectral characterization of photosystem II particles from spinach. *Chemical* Research in Chinese *Universities.* 21:196–200.

Hossain, M. F., White, S.K., Elahi, S.F., Sultana, N., Chaudhry, M. H. K., Alam, Q. K., Rother, J. A. and Gaunt, J. L. (2005). The efficiency of nitrogen fertilizer for rice in Bangladeshi farmers field. *Field Crops Research*. 93(1): 94-107.

Iavicoli, I., Leso, V., and Bergamaschi, A. (2012). Toxicological effects of titanium dioxide nanoparticles: A rReview of *iIn Vvivo* Studiesstudies. *Journal of Nanomaterials.* 2012: 1-36.

Islam, M. A., Islam, M. R. and Sarker, A. B. S. (2008). Effect of phosphorus on nutrient uptake of *Japonica* and *Indica* Rrice. *Journal of Agriculture and Rural Development.* 6 (1-2): 7-12.

Jalill, R. D. H. A. and Yousef, A. M. (2015). Comparison the phytotoxicity of TiO_2 nanoparticles with bulk particles on amber 33 variety of rRice (*Oryza sativa*) in vitro. *Scholars Academic Journal of Biosciences.* 3(3): 254-262.

Janmohammadi, M., Amanzadeh, T., Sabaghnia, N. and Dashti, S. (2016). Impact of foliar application of nano micronutrient fertilizers and titanium dioxide nanoparticles on the growth and yield components of barley under supplemental irrigation. *Acta agriculturae Slovenica*. *107*(2): 265.

Javot, H., Penmetsa R.V., Terzaghi, N., Cook, D.R. and Harrison, M.J. (2007). A Medicago truncatula phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences of the United States of America*. 104: 1720–1725.

Jia H. F., Ren, H.Y., Gu M., Zhao J.N., Sun S.B., Zhang X., Chen J.Y., Wu P. and Xu G.H. (2011). The phosphate transporter gene OsPht1; 8 is involved in phosphate homeostasis in rice. *Plant Physiology.* 156: 1164–1175.

Ju-Nam, Y. and Lead, J (2008). Manufactured nanoparticles: an overview of their chemistry, interactions and potential environmental implications. *Science of the Total Environment.* 400: 396-414.

Kalala, A. M., Amuri, N. A. and Semoka, J. M. R. (2016). Response of rice to phosphorus and potassium fertilization based on nutrient critical levels in plants and soils of Kilombero Valley. *Advances in Research*. 7(5): 1-12.

Kaya, C., Kirnak, H. and Higgs, D. (2001). Enhancement of growth and normal growth parameters by foliar application of potassium and phosphorus in tomato cultivars grown at high (NaCl) salinity. *Journal of Plant Nutrition*. 24: 357−367.

Khan, P. and Imtiaz, M. (2013). Studies on the nutritional requirements of candidate rice genotype Bas-15-1 E3. *Journal of Agricultural Research and Development.* 3(4): 64-70.

Khan, P., Aslam, M., Memon, M. U., Imtiaz, M., Shah, J. A. and Depar, N. (2010). Determining the nutritional requirements of rice genotype JAJAI 25/A evolved at Nia, Tando Jam, Pakistan. *Pakistan Journal of Botany*. 42(5): 3257-3263.

Khater, M. S. (2015). Effect of titanium nanoparticles (TiO_2) on growth, yield and chemical constituents of coriander plants. *Arab Journal of Nuclear Science and Applications*. 48: (4): 187-194.

Khodakovskaya M., Dervishi E., Mahmood M., Xu Y., Li Z., Watanabe F. and Biris A.S. (2009). Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. *ACS Nano*. 3: 3221-3227.

Khodakovskaya, M., Dervishi, E., Mahmood, M., Xu, Y., Li, Z., Watanabe, F., & Biris, A. S. (2012). Retraction notice for carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. *ACS Nano. 6*(8): 7541-7541.

Kim, H. J. and Li, X. (2016). Effects of phosphorus on shoot and root growth, partitioning, and phosphorus utilization efficiency in lantana. *Horticultural Science.* 51: 1001-1009.

Kisan, B., Shruthi, H., Sharanagouda, H., Revanappa, S. B. and Pramod, N. K. (2015). Effect of nano-zinc oxide on the leaf physical and nutritional quality of Spinach. *Journal of Agrotechnology*. 5(1): 135.

Lambers, H., Martinoia, E. and Renton, M. (2015). Plant adaptations to severely phosphorus-impoverished soils. *Current Opinion in Plant Biology*. 25:23–31.

Larue, C., Laurette, J., Herlin-Boime, N., Khodja, H., Fayard, B., Flank, A. M., Brisset, F. and Carriere, M. (2012). Accumulation, translocation and impact of TiO_2 nanoparticles in wheat (*Triticum aestivum* spp.): Influence of diameter and crystal phase. *Science of the Total Environment*, 431:197-208

Lee, W. M., An, Y. J., Yoon, H. and Kweon, H. S. (2008). Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*): plant agar test for water-insoluble nanoparticles. *Environmental Toxicology and Chemistry*. 27: 1915–1921.

Lei, Z., Mingyu, S. and Xiao, W. (2007b) Effects of nano-anatase on spectral characteristics and distribution of LCHII on the thylakoid membranes of spinach. *Biological Trace Element Research* .120:273–283.

Lei, Z., Mingyu, S., Xiao, W., Chao, L., Chunxiang, Q., Liang, C., Hao, H., Xiaoqing, L. and Fashui, H. (2007a). Effects of nanonatase TiO_2 on photosynthesis of spinach chloroplasts under different light illumination. *Biological Trace Element Research.* 119: 68-76.

Lin, D. and Xing, B. (2007). Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. *Environmental Pollution*. 150(2): 243–250.

Lin, S., Reppert, J., Hu, Q., Hudson, J. S., Reid, M. L., Ratnikova, T. A. and Ke, P. C. (2009). Uptake, translocation, and transmission of carbon nanomaterials in rice plants. *Small*. 5(10):1128–1132.

Liu R. and Lal, R. (2015). Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. A review. *Science of the Total Environment.* 514: 131–139.

Liu, B., Nakata, K., Sakai, M., Saito, H. and, Ochiai, T. , et al. (2011) Mesoporous TiO2 core shell spheres composed of nanocrystals with exposed high-energy facets: facile synthesis and formation mechanism. J*ournal of American Chemical Society*. 27: 8500–8508.

Lu, C. M., Zhang, C. Y., Wen, J. Q. and Wu, G. R. (2002). Effects of nano material on germination and growth of soybean, *Soybean Science.* 21(3): 168–171.

Ma, L. L., Liu, C., Qu, C. X., Yin, S. T., Liu, J., Gao, F.Q. and Hong, F.S. (2008). Rubisco activase mRNA expression in spinach: modulation by nanoanatase treatment. *Biological Trace Element Research*, 122: 168-178

Ma, X., Geiser-Lee, J., Deng, Y. and Kolmakov, A. (2010a). Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. *Science of the Total Environment*, 408 (16): 3053-3061.

Ma, Y., Kuang, L., He, X., Bai, W., Ding, Y., Zhang, Z. and Chai, Z. (2010b). Effects of rare earth oxide nanoparticles on root elongation of plants. *Chemosphere*. 78(3): 273-279.

Mackenzie, A. and Amer, S. (1964). Reactions of iron, aluminum and calcium phosphates in six Ontario soils. *Plant Soil*. 21: 17−25.

Mahmoodzadeh, H. and Aghili, R. (2014). Effect on germination and early growth characteristics in wheat plants (*Triticum aestivum* L.) seeds exposed to TiO_2 nanoparticles. Journal of *Chemical Health Risk.* 4(1): 29–36.

Marchiol, L., Mattiello, A., Poš´ci´c F., Fellet, G., Zavalloni, C., Carlino, E. and Musetti, R. (2016). Changes in physiological and agronomical parameters of Barley (*Hordeum vulgare*) exposed to cerium and titanium dioxide nanoparticles. *International Journal of Environmental Research and Public Health.* 13: 332.

Martin, F., Winspear, M., MacFarlane, J., and Oaks, A. (1983). Effect of methionine sulfoximine on the accumulation of ammonia in C3 and C4 Leaves : The relationship between NH3 accumulation and photorespiratory activity. *Plant Physiology*. 71(1): 177-181.

Matsua, T., Kumazawa, K., Ishii, R., Ishihara, K., Hirata, H. (1995). Science of the plant phyliosophyphilosophy. *Food and Agriculture Policy Research Centre, Tokyo. Japan*. 2: 1240.

Mazumdar, H. and Ahmed, G. U. (2011). Phytotoxicity effect of silver nanoparticles on *Oryza sativa*. *International Journal of ChemTech Research*. *3*(3): 1494–1500.

Mcdonald, M. S. (1978). A simple and improved method for the determination of microgram quantities of nitrogen in plant material. *Annals of Botany*. *42*(2): 363-366.

McKeague, J. A. (ed.). 1978. Manual on soil sampling and methods of analysis. *Canadian Society of Soil Science*: 66 – 68.

McLean, E. O. 1982. Soil pH and lime requirement. *Methods of soil analysis: chemical and microbiological properties.* American. Society of Agronomy. 199 – 224.

Mihajlović, M., Perišić, N., Pezo, L., Stojanović, M., Milojković, J., Lopičić, Z., and Petrović, M. (2014). Utilization of phosphate rock from Lisina for direct application: Release of Plant Nutrients in the Exchange-Fertilizer Mixtures. *Journal of Agricultural and Food Chemistry, 62*(41): 9965-9973.

Mingfang, Q., Yufeng, L. and Tianlai, L. (2013). Nano-TiO_2 improve the photosynthesis of tomato leaves under mild heat stress, biological trace element research. *Biological Trace Element Research*. 156 (1): 323–328.

Moaveni, P. and Kheiri, T. (2011). TiO_2 nano particles affected on maize (*Zea mays* L). *Second International Conference on Agricultural and Animal Science.* Maldives. Singapore. 160–163.

Mohammadi, H., Esmailpour, M. and Gheranpaye, A. (2016). Effects of TiO_2 nanoparticles and water-deficit stress on morpho-physiological characteristics of dragonhead (*Dracocephalum moldavica* L.) plants. *Acta agriculturae Slovenica*. 107 (2): 385 – 396.

Moreno, M. L., Avilés, L., Pérez, N. G., Álamo, I. B., Perales, O. and Mattei, C. Y. (2016). Effect of cobalt ferrite ($CoFe_2O_4$) nanoparticles on the growth and development of *Lycopersicon lycopersicum* (tomato plants). *Science* of the *Total Environment*. 550: 45–52.

Morteza, E. and Joorabloo, A. (2015). Investigation the effects of nano TiO_2 and TiO_2 spraying on the oil yield of Safflower (*Carthamus tinctorius* L.).*Biological Forum – An International Journal*. 7 (2): 449-454.

Mudge S.R., Rae A.L., Diatloff E. and Smith F.W. (2002). Expression analysis suggests novel roles for members of the Pht1 family of phosphate transporters in Arabidopsis. *Plant Journal*. 31: 341–353.

Mukherjee, A. J., Peralta-Videa, R., Bandyopadhayay, S., Rico, C. M., Zhaobc, L. and Gardea-Torresdey, J. L. (2014). Physiological effects of nanoparticulate ZnO in green peas *(Pisum sativum* L.) cultivated in soil. *Metallomics*. 6: 132.

Musante, C. and White, J. C. (2010). Toxicity of silver and copper to *Cucurbita pepo*: differential effects of nano and bulk-size particles. *Environmental Toxicology*. 27(9): 510–517.

Musante, C. and White, J. C. (2010). Toxicity of silver and copper to *Cucurbita pepo*: differential effects of nano and bulk-size particles. *Environmental Toxicology*. 27(9): 510–517.

Nagarajan, V. K., Jain, A., Poling, M. D., Lewis, A. J., Raghothama, K.G. and Smith A.P. (2011). Arabidopsis Pht1; 5 mobilizes phosphate between source and sink organs and influences the interaction between phosphate homeostasis and ethylene signaling. *Plant Physiology*. 156: 1149–1163.

Nair, R., Poulose, A. C., Nagaoka, Y., Yoshida, Y., Maekawa, T. and Kumar, D. S. (2011). Uptake of FITC labeled silica nanoparticles and quantum dots by rice seedlings: Effects on seed germination and their potential as biolabels for plants. *Journal of Fluorescence.* 21(6): 2057-2068

Nair, R., Varghese, S. H., Nair, B. G., Maekawa, T., Yoshida, Y. and Kumar, D. S. (2010). Nanoparticulate material delivery to plants. *Plant Science.* 179:154–163.

Najafi, S., Heidari, R. and Jamei, R. (2014). Photosynthetic characteristics, membrane lipid levels and protein content in the *Phaseolus vulgaris* L. (cv. Sadri) exposed to magnetic field and silver nanoparticles. *Bulletin of Environment, Pharmacology and Life Sciences*. 3: 72-76.

Nielsen, K. L., Eshel, A. and Lynch, J. P. (2001). The effect of phosphorus availability on the carbon economy of contrasting common bean (*Phaseolus vulgaris)* genotypes. *Journal of Experimental Botany.* 52 (355): 329–339.

Noonari, S., Kalhoro, S. A., Ali, A., Mahar, A., Raza, S., Ahmed, M., Shah, S. F.A. and Baloch, S.U. (2016). Effect of different levels of phosphorus and method of application on the growth and yield of wheat. *Natural Science*. 8: 305-314.

Nowack, B. and Bucheli, T. D. (2007). Occurrence, behavior and effects of nanoparticles in the environment. *Environmental Pollution.* 150(1): 5-22.

Olsen, S. R., Cole C.V., Watanabe F.S. and Dean L. A. (1954). Estimation of available phosphorus in soil by extraction with sodium bicarbonate, U.S. Dep. Agric. Circ. 939, US Government Printing Office, Washington, DC. 19.

Owolade, O. F., Ogunleti, D. O. and Adenekan, M. O. (2008). Titanium dioxide affects disease development and yield of edible cowpea. *Electronic Journal of Environmental, Agricultural and Food Chemistry.* 7 (50): 2942-2947.

Panhawar, Q. A. and Othman, R. (2011). Effect of phosphatic fertilizer on root colonization of aerobic rice by phosphate-solubilizing bacteria. *International Conference on Food Engineering and Biotechnology*. 9:145-149.

Parisi, C., Vigani, M. and Rodríguez-Cerezo, E. (2014). Agricultural Nanotechnologies : What are the current possibilities ? *Nano Today*. 10–13.

Paszkowski, U., Kroken, S., Roux, C. and Briggs, S. P. (2002). Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences of the Unites States of America.* 99: 13324–13329.

Pošcic, F., Mattiello, A., Fellet, G., Miceli, F. and Marchiol, L. (2016). Effects of cerium and titanium oxide nanoparticles in soil on the nutrient composition of bBarley (*Hordeum vulgare* L.) kernels. *International Journal of Environmental Research and Public Health*. 13: 577.

Rafique, R., Arshad, M., Khokhar, M. F., Qazi, I. A., Hamza, A.,and Virk, N. (2014). Growth response of wheat to titania nanoparticles application. *NUST Journal of Engineering Sciences*. 7(1): 42–46.

Rahim, A, Ranjha, A. M., Rahmatullah and Waraich E. A. (2010). Effect of Phosphorus application and irrigation scheduling on wheat yield and phosphorus use efficiency. *Soil and Environment*. 29(1): 15- 22.

Raliya, R. and Tarafdar, J. (2013). ZnO nanoparticle biosynthesis and its effect on phosphorus-mobilizing enzyme secretion and gum contents in Clusterbean (*Cyamopsis tetragonoloba* L.). *Agricultural Research*. 2: 48−57.

Raliya, R., Biswas, P. and Tarafdar, J. C. (2015). TiO_2 nanoparticle biosynthesis and its physiological effect on mung bean (*Vigna radiata* L.). *Biotechnology Reports*. 5: 22-26.

Raliya, R., Tarafdar, J. C. and Biswas, P. (2016). Enhancing the mobilization of native phosphorus in the mung bean rhizosphere using ZnO nanoparticles synthesized by soil fungi. *Journal of Agricultural and Food Chemistry*. 64: 3111−3118.

Razaq, M., Zhang, P., Shen, H. and Salahuddin. (2017). Influence of nitrogen and phosphorus on the growth and root morphology of *Acer mono*. PLoS ONE. 12(2):e0171321.doi:10.1371/journal.pone.0171321.

Reyes-Coronado, D., Rod_rıguez-Gattorno, G., Espinosa-Pesqueira, M.E., Cab, C., de Coss, R. and Oskam, G. (2008). Phase-pure TiO_2 nanoparticles: anatase, brookite and rutile. *Nanotechnology*. 19: 10-19.

Rico, C. M., Majumdar, S., Duarte-Gardea, M., Peralta-Videa J. R. and Gardea-Torresdey J. L. (2011). Interaction of nanoparticles with edible plants and their possible implications in the food chain. *Journal of Agriculture and Food Chemistry*. 59 (8): 3485–3498.

Roberts, A. G., and Oparka, K. J. (2003). Plasmodesmata and the control of symplastic transport. *Plant Cell Environment*. 26: 103–124.

Ryan, J. (2008). Soil and plant analysis laboratory manual. International Center for Agriculture Research in Dry Areas. *Scientific Publishers Journals Dept.* (ISBN: 978-8172334987).

Sainio, P. P., Kontturi, M. and Peltonen, J. (2006). Phosphorus seed coating enhancement on early growth and yield components in Oat. *Agronomy Journal*. 98: 206-211.

Santner J., Smolders E., Wenzel W.W. and Degryse F. (2012). First observation of diffusion-limited plant root phosphorus uptake from nutrient solution. *Plant Cell and Environment*. 35 (1):1558-1566.

Santos, A. R., Miguel, A. S., Tomaz, L., Malhó, R., Maycock, C., Patto, M. C. *et al.* and Oliva, A. (2010). The impact of CdSe/ZnS quantum dots in cells of *Medicago sativa* in suspension culture. *Journal of Nanobiotechnology*. *8*(1), 24.

Sanusan, S., Polthanee, A., Seripong, S., Audebert, A. and Mouret J. C. (2009) Rates and timing of phosphorus fertilizer on growth and yield of direct-seeded rice in rain-fed conditions. *Acta Agriculturae Scandinavica*. 59 (6): 491-499.

Sattelmacher, B. (2001). The apoplast and its significance for plant mineral nutrition. *New Phytology*. 149: 167–192.

Sawan, Z. M. and Mahmoud, M. O. A. (1997) Effect of phosphorus fertilization and foliar application of chelated zinc and calcium on quantitative and qualitative properties of Egyptian cotton (*Gossypium barbadense* L. Var. Giza 75. *Journal of Agricultural and Food Chemistry*. 45: 3326−3330.

Schachtman, D. P., Reid, R. J., and Ayling, S. (1998). Phosphorus uptake by plants: from soil to cell. *Plant Physiology.* 116(2), 447-453.

Scherrer, P. (1918). Bestimmung der Grösse und der inneren Struktur von Kolloidteilchen mittels Röntgenstrahlen, *Nachr. Ges. Wiss. Göttingen*. 26: 98-100.

Scholz, R. W., Roy, A. H. and Hellums, D. T. (2014). Sustainable phosphorus management: A transdisciplinary challenge. In Sustainable phosphorus management□A Global Transdisciplinary Roadmap, 1st ed.; Springer: New York. pp 1−256.

Schwab, F., Zhai, G., Kern, M., Turner, A., Schnoor, J. L. and Wiesner, M. R. (2015). Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants-Critical review. *Nanotoxicology*. 10: 257–278.

Seeger, E.M., Baun, A., Kstner, M. and Trapp, S. (2009) Insignificant Acute Toxicity of TiO2 Nanoparticles to Willow Trees. *Journal of Soils and Sediments.* 9 (1): 46-53.

Serag, M. F., Kaji, N., Gaillard, C., Okamoto, Y., Terasaka, K., Jabasini, M. and Baba, Y. (2011). Trafficking and subcellular localization of multiwalled carbon nanotubes in plant cells. *ACS Nano. 5*(1): 493-499.

Servin, A. D., Castillo-Michel, H., Hernandez-Viezcas, J. A., Diaz, B. C., Peralta-Videa, J. R., and Gardea-Torresdey, J. L. (2012). Synchrotron Micro-XRF and Micro-XANES Confirmation of the Uptake and Translocation of TiO2Nanoparticles in Cucumber (Cucumis sativus) Plants. *Environmental Science and Technology*. 46(14), 7637-7643.

Shivay, Y. S., Prasad, R., Singh, R. K. and Pal, M. Relative efficiency of zinc-coated urea and soil and foliar application of zinc sulphate on yield, nitrogen, phosphorus, potassium, zinc and iron biofortification in grains and uptake by basmati rice (*Oryza sativa* L.). *Journal of Agricultural Science*. 2015, 7, 161−173.

Shubhra, Dayal, J. and Goswami, C. L. (2003). Effect of phosphorus application on growth, chlorophyll and proline under water deficit in clusterbean (*Cyamopsis tetragonoloba* L.). *Indian Journal of plant physiology.* 8(2):150-154.

Siddiqui, M. H., Al-Whaibi, M. H., Firoz, M. and Al-Khaishany, M. Y. (2015). Role of Nanoparticles in Plants. *Nanotechnology and Plant Sciences*. 19-35.

Sims, J. R. and Jackson, G. D. (1971). Rapid analysis of soil nitrate with chromotropic acid. *Soil Science Society of America, Proceedings*. 35: 603 – 606.

Skupień, K. and Oszmiański, J. (2007). Influence of titanium treatment on antioxidants content and antioxidant activity of strawberries. *Acta Scientiarum Polonorum Technologia Alimentaria.* 6 (4): 83-93.

Song, G., Gao, Y., Wu, H., Hou, W., Zhang C. and Ma, H. (2012). Physiological effect of anatase TiO2 nanoparticles on *Lemna minor*'. *Environmental Toxicology and Chemistry*. 31(9): 2147-2152.

Stern, S.T. & McNeil, S.E. (2008). Nanotechnology safety concerns revisited. *Toxicology Science. 101*: 4-21.

Su, M., Hong, F., Liu, C., Wu, X., Liu, X., Chen, L. and Li, Z. (2009). Effects of nano-anatase TiO_2 on absorption, distribution of light, and photoreduction activities of chloroplast membrane of spinach. *Biological Trace Element Research.* 131(1): 101-110.

Sun, W. C., Zhang, J., Fan, Q. H., Xue, G. F. and Li Z. L. (2010). Silicon-enhanced resistance to rice blast is attributed to silicon-mediated defence resistance and its role as physical barrier. *European Journal of Plant Pathology.* 128: 39–49.

Taheri, M., Qarache, H. A., Qarache, A. A. and Yoosefi, M. (2015). The effects of zinc-oxide nanoparticles on growth parameters of corn (SC704). *STEM Fellowship Journal*. 1(2): 17-20.

Thuesombat, P., Hannongbua, S., Akasit, S. and Chadchawan, S. (2014). Effect of silver nanoparticles on rice (*Oryza sativa* L. cv. KDML 105) seed germination and seedling growth. *Ecotoxicology and Environmental Safety*. 104 (1): 302–309.

Tian, S, Nakamura, K, Cui, T. and Kayahara, H. (2005). High performance liquid chromatographic determination of phenolic compounds in rice. *Journal of Chromatography A.* 1063: 121-128.

Uhram, S., Minjoo, S., Gisuk, L., Jinkyu, R., Younghun, K., and Eun J. L. (2013). Functional analysis of TiO2 nanoparticle toxicity in three plant species. *Biological Trace Element Research*. 155(1): 93–103.

Ullah, S., and Arshad, M. (2014). Exposure-response of triticum aestivum to titanium dioxide nanoparticles application: seedling vigor index and micronuclei formation. *Science Vision*. 20 (1): 57-61.

UN (United Nations Department of Economic and Social Affairs, Population Division), 2013. World Population Prospects: the 2012 Revision

Upadhyaya, H., Begum, L., Dey, B., Nath, P. K. and Panda, S. K. (2017)Impact of Calcium Phosphate Nanoparticles on Rice Plant. *Journal Plant Science Phytopathology*. 1: 1-10.

Vance, C. P., Uhde-Stone, C. and Allan, D. L. (2003). Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytology*. 157: 423-447.

Vasantharaja, D., Ramalingam, V., and Reddy, G. A. (2015). Toxic exposure dioxide nanoparticles on serum biochemical changes in adult male wistar rats. *Nanomedical Journal*. 2(1): 46–53.

Vassilev, N and Vassileva, M. (2003). Biotechnological Solubilization of Rock Phosphate on Media Containing Agro-Industrial Wastes. *Applied Microbiology* and *Biotechnology*. 61: 435-440.

Walkley, A. (1947). A critical examination of a rapid method for determining organic carbon in soils: Effect of variations in digestion conditions and of organic soil constituents. *Soil Science*. 63: 251 – 263.

Wang L. J., Wang, Y. H., Zhou, Y. L., Duan, X. Y., Li, M. and F. S. Zhang. (2001). Relationship between nanostrcture SiO_2 and occurrence of plant fungi, *Journal of Huazhong Agriculture University*. 20(6): 593–597.

Wang, A., Zheng, Y., & Peng, F. (2014). Thickness-Controllable Silica Coating of CdTe QDs by Reverse Microemulsion Method for the Application in the Growth of Rice. *Journal of Spectroscopy*. 2014: 1-5.

Wang, X. M., Gao, F. Q., Ma, L. L., Liu, J., Yin, S. T., Yang, P. and Hong F. S. (2008) Effects of nano-anatase on ribulose-1, 5-bisphosphate carboxylase/oxygenase mRNA expression in spinach. Biol Trace Elem Res 126:280–289.

Wang, X., Wang, Y., Piñeros, M. A., Wang, Z., Wang, W., Li, C., Wu, Z., Kochian, L. V. and Wu P. (2014). Phosphate transporters OsPHT1; 9 and OsPHT1; 10 are involved in phosphate uptake in rice. *Plant, Cell and Environment.* 37: 1159–1170.

Wissuwa, M. (2005). Combining a modelling with a genetic approach in establishing associations between genetic and physiological effects in relation to phosphorus uptake. *Plant and Soil* (in press).

Wissuwa, M., Gamat, G. and Ismail, A. U. (2005). Is root growth under phosphorus deficiency affected by source or sink limitations? *Journal of Experimental Botany*. 56 (417): 1943-1950.

Wu, C., Wei, X., Sun, H. L. and Wang, Z. Q. (2005). Phosphate availability alters lateral root anatomy and root architecture of *Fraxinus mandshurica* seedlings. *Journal of Integral Plant Biology*. 47 (3): 292−301.

Yang, F., Hong, F., You, W., Liu, C., Gao, F., Wu, C. and Yang, P. (2006). Influence of nano-anatase TiO_2 on the nitrogen metabolism of growing spinach. *Biological Trace Element Research*. 110(2): 179-190.

Yang, F., Liu, C., Gao, F., Su, M., Wu, X., Zheng, L., Hong, F. and Yang, P. (2007). The improvement of spinach growth by nano-anatase TiO_2 treatment is related to nitrogen photoreduction. *Biological Trace Element Research*. 119 (1):77-88.

Yin, H. C., Liu, Q. J., Lin, Q., Chen, S. N. and Zhou, L. J. (2005). Inhibitory effects of nano-TiO_2 loaded Pd on cyanobacteria growth. *Acta Botanica Boreali-Occidentalia Sinica.* 25: 1884–1887.

Younas, H., Qazi, I. A., Hashmi, I., Awan, M. A., Mahmood, A. and Qayyum, H. A. (2014). Visible light photocatalytic water disinfection and its kinetics using Ag-doped titania nanoparticles. *Environmental Science and Pollution Research*. *21* (1): 740-752.

Zahra, Z., Arshad, M., Rafique, R., Mahmood, A., Habib, A., Qazi, I. A. and Khan, S. A. (2015). Metallic nanoparticle (TiO_2 and Fe_3O_4) application modifies rhizosphere phosphorus availability and uptake by *Lactuca sativa*. *Journal of Agricultural and Food Chemistry*. 63 (31): 6876-82.

Zahra, Z., Waseem, N., Zahra, R., Lee, H., Badshah, M. A., Mehmood, A., Choi, H. K. and Arshad, M. (2017). Growth and metabolic responses of rice (*Oryza sativa* L.) cultivated in phosphorus-deficient soil amended with TiO2 Nanoparticles. *Journal of Agricultural and Food Chemistry*. 65(28). 5598-5606

Zhang, P., Cui, H.X., Zhang, Z.J., and Zhong, R.G. (2008). Effects of nano-TiO_2 photosemiconductor on photosynthesis of cucumber plants. *Chinese Agricultural Science Bulletin.* 24: 230-233.

Zhao, L.,Sun, Y., Hernandez-Viezcas, J. A., Servin, A. D., Hong, J., Niu, G., Peralta-Videa, J. R., Duarte-Gardea, M. and Gardea-Torresdey, G. L. (2013). Influence of CeO_2 and ZnO nanoparticles on cucumber physiological markers and bioaccumulation of Ce and Zn: a life cycle study. Journal of Agricultural and *Food Chemistry*. 61 (49): 11945-51.

Zheng, L., Hong, F. S., Lu, C. M., Zhang C.Y., Wen, J. Q., Wu, G. R. and Tao, M. X. (2002). Research of the effect of nanometer materials on germination and growth enhancement of glycine max and its mechanism. *Soybean Science*. 21(3):168–171.

Zheng, L., Hong, F., Lu S. and Liu, C. (2005). Effect of nano-TiO_2 on strength of naturally aged seeds and growth of spinach. *Biological Trace Element Research*.104 (1): 83-91.

Zheng, L., Su, M. Y., Chao, L., Liang, C., Hao, H., Xiao, W., Liu, X. Q., Yang, F., Gao, F. Q. and Hong F. S. (2007) Effects of nanoanatase TiO_2 on photosynthesis of spinach chloroplasts under different light illumination. *Biological Trace Element Research*. 119: 68–76

Zhu, H., Han, J., Xiao, J. Q. and Jin, Y. (2008). Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. *Journal of Environmental Monitoring*, *10*(6): 713-717.

Zuverza, M. N., Armendariz, R., Videa, P. J. R. and Torresdey, G. J. L. (2016). Effects of silver nanoparticles on radish sprouts: root growth reduction and modifications in the nutritional value. *Frontier Plant Science*. 7:90-100.

ANNEXURES

Annexure I

Results of analysis variance of the rice growth under different methods of nano-TiO_2 application and phosphorus levels

Source of Variation	Df	RL	SL	RDW	SDW	Number of tillers
TNPs method of application (A)	2	**152.42****	**565.13****	**16.595****	**11.62****	**8.750****
Phosphorus Level (P)	3	**263.170****	**1113.3****	**28.719****	**45.42****	**10.72****
(A × P)	6	21.322ns	15.606ns	**1.600***	1.458ns	1.528ns
Error	48	27.232	18.82	0.551	0.853	0.850

Where df= degree of freedom, RL= root length, SL= shoot length, RDW= root dry weight, SDW= shoot dry weight, Root P= root phosphorus content, Shoot P= shoot phosphorus content, Chl= Chlorophyll content, ns= Not Significant, *= Significant at $p < 0.05$, **= Significant at $p < 0.01$

Annexure II

Results of analysis variance of the rice plant phosphorus uptake, chlorophyll content nad grain protein content under different methods of nano-TiO_2 application and phosphorus levels

Source of Variation	Df	Root P	Shoot P	Chl	Grain Protein Content
TNPs method of application (A)	2	**468085****	**260796****	**21.796****	**44216.2****
Phosphorus Level (P)	3	**960905***	**838071****	**32.112****	**54475.8****
(A × P)	6	37019ns	18996ns	0.638ns	**6555.89****
Error	48	76892	52045	0.720	6.333

Where df= degree of freedom, Root P= root phosphorus content, Shoot P= shoot phosphorus content, Chl= Chlorophyll content, ns= Not Significant, *= Significant at $p < 0.05$, **= Significant at $p < 0.01$