INFLUENCE OF SIMULTANEOUS APPLICATION OF ANTIBIOTICS AND NANOPARTICLES ON WHEAT GROWTH

Anum Amin
00000171055

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

in

Environmental Science

Institute of Environmental Sciences and Engineering (IESE)

School of Civil and Environmental Engineering (SCEE)

National University of Sciences and Technology (NUST) Islamabad, Pakistan

(2018)

CERTIFICATE

It is certified that the contents and form of the thesis entitled **"Influence of simultaneous application of antibiotics and nanoparticles on wheat growth"** submitted by Ms. Anum Amin 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. Deedar Nabi

Assistant Professor

IESE, SCEE, NUST

Member:_____

Dr. Khurram Yaqoob

Assistant Professor

SCME, NUST

Member:_____

Dr. Ashfaq Ahmad

Professor

THESIS ACCEPTANCE CERTIFICATE

Certified that final copy of MS thesis submitted by Ms. **Anum Amin,** Registration No. **00000171055** of **IESE (SCEE)** has been vetted by undersigned, found complete in all aspects as per NUST Statutes/Regulations, is free of plagiarism, errors, and mistakes and is accepted as partial fulfillment for award of MS/MPhil, degree. It is further certified that necessary amendments as pointed out by GEC members of the scholar have also been incorporated in the said thesis.

Signature with stamp: _____

Name of Supervisor: _____

Date: _____

Signature of HoD with stamp: _____

Date: _____

Countersigned by

Signature (Dean/Principal): _____

Date: _____

DEDICATION

I dedicate this thesis to my mother for her love, support, genuine sincerity and benevolence, who would always be a source of inspiration for me and stood beside me at every moment in my life and my father, siblings, uncle and aunt for their prayers, support and encouragement

ACKNOWLEDGEMENTS

Thanks to Allah Almighty, the "Most Beneficent" and "Most Merciful" Who blessed us with ability, skills and wisdom to accomplish this project. I express my sincere gratitude to my ***parents*** for their affection and moral support throughout my academic career.

My utmost gratitude to my supervisor ***Dr. Muhammad Arshad*** for his kind guidance and advice throughout this research work. His patience, support, and motivation were a source of inspiration during the study. I am grateful to ***Dr. Deedar Nabi, Dr. Khurram Yaqoob*** and ***Dr. Ashfaq Ahmad*** in particular for their kind help and adding constructive comments throughout the project. Special thanks to HEC-funded project titled “Ensuring food security and safety”.

My special thanks to my friend ***Ms. Fariha Ajmal*** for her continuous support and assistance through this research phase. Moreover, my sincerest thanks to my seniors, ***Ms. Iram Gul***, ***Ms. Maria Manzoor***, ***Ms. Ayesha Mukhtar***, and ***Ms. Shagufta Iram*** for their guidance. I would like to thank all the lab mates (***Ms. Phziya, Ms. Fakhria, Ms. Saima and Ms. Farhat***) of Environmental Biotechnology group and all other laboratory staffs at IESE for their help and cooperation.

Last but not the least my special gratitude to my ***parents and family*** for their prayers and support and love.

Anum Amin

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AMX	Amoxicillin
Fe_2O_3	Iron oxide
LEV	Levofloxacin
N	Nitrogen
NOR	Norfloxacin
OFL	Ofloxacin
P	Phosphorus
ROS	Reactive oxygen specie
SEM	Scanning electron microscope
TET	Tetracycline
TiO_2	Titanium dioxide
XRD	X-ray diffraction
ZnO	Zinc oxide

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Antibiotics are now considered as emerging environmental pollutants due to their persistent nature and continuous introduction into water and soil. Unfortunately, in countries like Pakistan, this untreated water is used for irrigation of crops and these antibiotics adsorbed into soil and are taken up by plants. The main objective of present study was to assess the potential of selected nanoparticles for degradation of antibiotics and subsequent improvement in crop productivity and quality in terms of nutritional composition. In screening study, three different nanoparticles (TiO_2, ZnO and Fe_2O_3) with concentration varying from 40-60 mg L^{-1} for the time period of one to nine days under the visible lamp of 50W were used to test their efficiency to degrade commonly used antibiotics namely Amoxicillin (Amx) and Levofloxacin (Lev) from aqueous medium having 5 mg L^{-1} concentration of each antibiotic. TiO_2 nanoparticles at 50 mg L^{-1} were found to be the most effective nanoparticles for the removal of both antibiotics with maximum percent degradation of 65% and 56% for Amx and Lev respectively on 7^{th} day. In order to evaluate the growth response of wheat towards combined effect of nanoparticles and antibiotics, a pot experiment was carried out. Four treatments including level of Amx (5 mg L^{-1}), Lev (5 mg L^{-1}), TiO_2 (50 mg L^{-1}), simultaneous application of TiO_2 and Amx (TiO_2:50 mg L^{-1}, Amx:5 mg L^{-1}) and simultaneous application of TiO_2 and Lev (TiO_2:50 mg L^{-1}, Lev:5 mg L^{-1}) were applied with a control treatment having only NPK. Individual application of TiO_2 nanoparticles significantly increased wheat growth and nutrients. Total percentage of carbohydrates and proteins in grains were increased up to 39% in comparison to control. However, combined effect of TiO_2 with both antibiotics decreased the total iron, carbohydrates and proteins in grains up to 8% with respect to the control but showed significantly positive results as compared to individual application of antibiotics.

Therefore, nanoparticles can serve as a better option for improving crop quality by degrading the antibiotics already present in water and soil.

Chapter 1

INTRODUCTION

1.1 Background

Each year around one to two lakh tons of antibiotics are used globally (Wang et al., 2017; Carvalho et al., 2014). Antibiotics are used as feed additives in order to promote growth in livestock farming and for treatment purposes in humans (Ambrosetti et al., 2015). They contribute about 70% in veterinary medicines (Bruhn, 2003). Although there is no direct use of them in crops, yet they are found within µg to g kg^{-1} concentration within soil (Bruhn, 2003). However, very low concentration found in soil but due to continuous introduction of antibiotics through different pathways like livestock feeding, human treatments, aquaculture and improper disposal into waste water, make them "pseudo persistent" (Ambrosetti et al., 2015; Carvalho et al., 2014). Moreover, their persistence nature causes them to remain stable in manure and soil for long period of time, therefore antibiotics are now considered as "emerging environmental contaminants" (Minden et al., 2017). This has now raised concern towards environmental pollution with respect to soil and water firstly due to their higher detection frequency; secondly it also increases the resistance of bacterial population making treatments ineffective (Roura et al., 2018; Ambrosetti et al., 2015). And lastly low dose and long-term exposure pose adverse health impacts on aquatic organisms and public as well (Wegst-Uhrich et al., 2015; Miraji et al., 2016).

1.2 Antibiotics

After the discovery of penicillin antibiotics put first step in health and medical sector as therapeutic agents (Gothwal & Shashidhar, 2015). Antibiotics are referred to those heterogeneous compounds which help in resisting the microbial growth. On the basis of

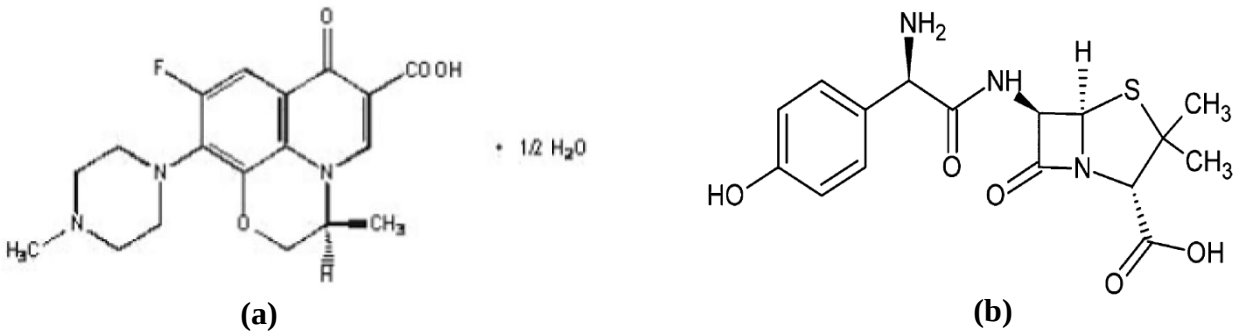
their mode of action and molecular structure, they can be divided into several classes such as penicillin and fluoroquinolones (Gothwal & Shashidhar, 2015; Bruhn, 2003). Most widely used antibiotics by humans are fluoroquinolones and penicillins in Europe with percentages 11.6% and 70.6% respectively (Roura, et al., 2018). Each antibiotic has different chemical structure, and this ensures their residence time in environment. It is difficult to degrade them because they act as foreigners to natural environment (Tandon et al., 2013). Antibiotics have reported to be present in water (surface, ground and waste water), plants, sediments, soil and aquatic species (Gothwal & Shashidhar, 2015). 18-70 ng L^{-1} antibiotic concentration has been detected in source water whereas in soil, it was found within range of 0.02–15 µg kg^{-1} of soil at United States (Tandon et al., 2013). Due to complexity, antibiotics can be cationic, neutral or anionic at different pH (Carvalho et al., 2014).

1.3. Penicillin and Fluoroquinolone

Penicillins contain a beta-lactam ring which helps in deforming the protective layer of bacterial cell wall, known as peptidoglycan (Lobanovska & Pilla, 2017). They hold 50 to 70% share in market for human use (Kummerer, 2009). Amoxicillin (Amx) belongs to broad spectrum and third generation penicillin family (Lobanovska & Pilla, 2017). It has molecular weight of 419.46 and chemically represented as $C_{16}H_{19}N_3O_5S$-$3H_2O$ as showed in Figure 1(b) (Jin et al., 2013).

On the other hand, fluoroquinolones belong to broad spectrum antibiotic family that help in breaking DNA strands of bacterial species in order to treat bacterial infections (Aldred et al., 2016). Due to their higher stability and adsorption into soil, they cannot be easily degraded at higher temperature (Bruhn, 2003; Tandon et al., 2013). Globally they

contribute 17% share in market and become as third largest group among antibiotics (Doorslaer et al., 2014). Levofloxacin (Lev) is used to treat bacterial infection either intravenously or orally. It also belongs to broad spectrum family containing "chiral fluorinated carboxyquinolone". Lev is not fully metabolized and eliminated as parent compound from the body. However inactive metabolites of Lev are levofloxacin N-oxide and demethyl levofloxacin which contributes less than five percent of the dose applied (Orzol & Piotrowicz-Cieślak, 2017). Due to their extensive use, it has made attraction towards their elimination strategies from environment in order to make it clean.



(a) **(b)**

Figure 1. (a) Molecular structure of Levofloxacin; (b) Molecular structure of AMXL (Jin et al., 2013; Lonikar et al., 2016).

1.4. Pathway of entry of antibiotics in environment

Since antibiotics are used in various fields such as animal husbandry, as medicines in health care departments and aquaculture, there are various pathways through which antibiotics enter into soil. Human and veterinary medicines are poorly adsorbed into their bodies and mostly excreted out into waste water. About 15-25% and 45-62% levofloxacin is excreted via feces and urine respectively as parent component (Cui et al., 2013). Similarly, antibiotics applied to animals are poorly absorbed in the gut and 90% may be excreted out (Minden et al., 2017). Not only through sewer systems, waste water coming from industries

like pharmaceuticals and hospitals also contribute in water and soil pollution (Ambrosetti et al., 2015). It has reported that major source of antibiotics in environment are pharmaceutical manufactures as 20-800 mg L^{-1} concentration has found in waste water of pharmaceutical industries (Samarghandi et al., 2017). Moreover, other sources include improper disposal of unused medicines into sewer water and transported into waste water treatment plants. This untreated water reaches into water distribution systems and hence these are identified in ground and surface water (Ambrosetti et al., 2015). Furthermore, irrigation of crops through untreated water and antibiotic manure application contaminates soil by affecting soil microbial biomass and then plants uptake them in their leaves and roots (Ambrosetti et al., 2015). Figure 2 illustrates the whole pathways of antibiotics into environment.

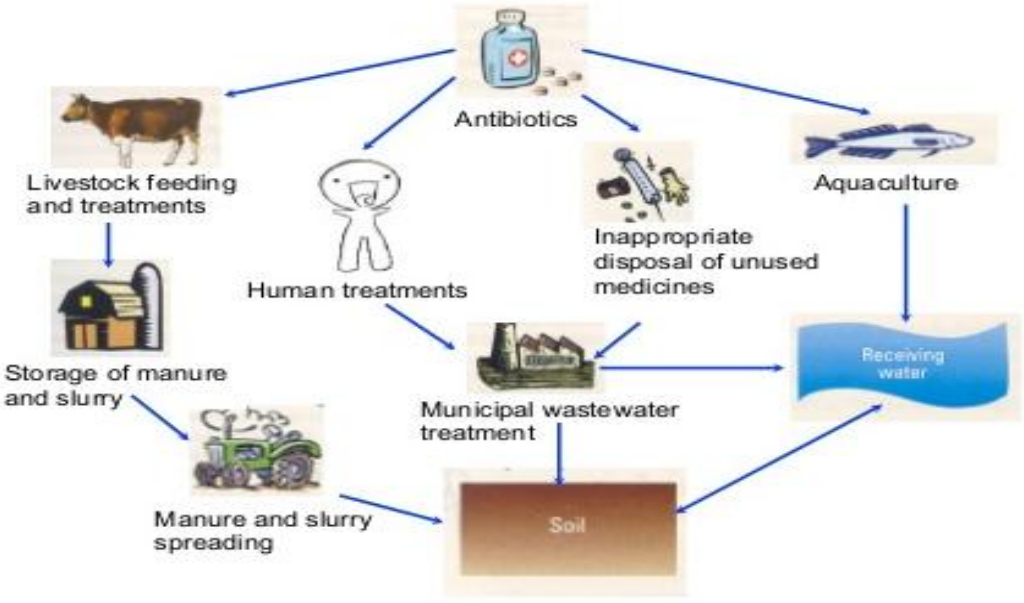


Figure 2. Pathway for entry of antibiotics into soil (Ambrosetti et al., 2015).

1.5. Fate in environment

In waste water, residual antibiotics either converted into stable and toxic metabolites or remain as parent compound. Cui et al. (2014) found levofloxacin released into environment as parent contaminant. They affect both terrestrial and aquatic organisms through structural changes (Samarghandi et al., 2017). Continuous introduction of these antibiotics create resistance in bacteria and hence cause problems for public health as they make treatments ineffective (Ambrosetti et al., 2015; Cui et al., 2013). Since soil serves as a sink for these pollutants and they are up taken by plants and ultimately their growth is reduced, soil microbial mass and respiration is affected, and toxicity is induced as well (Cui et al., 2013). Microbes are present on soil particles and these antibiotics are readily adsorbed and impact on them even at low dosages (Cui et al., 2013). Moreover, whole nutrient cycle is disturbed because microbes present in soil have a vital role in ecological functions (Cui et al., 2014). Adverse impacts of antibiotics on crops and humans have reported, therefore, there is need to degrade antibiotics both in aqueous solution and soil (Jin et al., 2013). In this way, it has become challenge for developing countries, like Pakistan, to overcome food security issues because its economy mostly relies on agricultural sector and if agricultural sector would not flourish, unemployment and poverty become fortune for the country (Riaz et al., 2017). Pakistan is an agricultural country as its national GDP depend upon 20.9% on agriculture (Aslam, 2016). Hence it is now important to degrade antibiotics through various process. Traditional methods like; coagulation, sedimentation, filtration and flocculation proved unsuccessful for degradation of antibiotics in aqueous phase due to their persistent nature. Therefore, it demands for alternative methods like advanced oxidation processes (AOPs)

which mainly includes photo catalysis process in which targeted compound can be mineralized in the presence of both light and catalyst (Jin et al., 2013).

1.6. Nanotechnology

The term nanotechnology refers to the science which produces novel products at subatomic level (Sadraei, 2016). These nanoparticles have size less than 100nm (Razzaq et al., 2015). Due to its large surface area it plays significant role in various disciplines like environmental chemistry, energy production systems, water filtration, tissue engineering and agriculture (Sadraei, 2016). Nanomaterials in plant and agricultural sciences are rapidly evolving by improving quality of crops through proficient irrigation and fertilizer management, enhancing physiological and growth activities, protecting against diseases and by upgrading the germination rate (Razzaq et al., 2015). The basic purpose of using nanoparticles instead of fertilizers and pesticides is to avoid the non-purpose impacts on crops. This is because nanoparticles act as fertilizers and antimicrobial agents in order to enhance the nutrients availability towards plants and protect them from insects (Watson et al., 2015).

TiO_2, ZnO and Fe_3O_4 are most commonly used nanoparticles in crops due to their high chemical and photo stability and non-toxicity (Radzimska & Jesionowski, 2014). Each year global production of titania is about 10,000 tons (Robichaud et al., 2009; Hendren et al., 2011). Titania in anatase form shows the great photo catalytic capacity whereas zinc and iron oxides serve as essential micronutrients for each crop (Zahra et al., 2015; Sabry at al., 2015). Moreover, it has reported that phosphorus is deficient in 30% world's soil (Zahra et al., 2017). Due to higher polarizing power of these nanoparticles, nutrients like phosphate ions ($PO4^{-3}$) can easily be adsorbed by such nanoparticles and hence their bioavailability

is increased (Zahra et al., 2017). Iron nanoparticles also helps in protein and enzyme immobilization (Kim et al., 2007).

Due to their high polarizing power and chemical stability these nanoparticles also proved to be used as catalyst in photo degradation of organic compounds. However, degradation of antibiotics through nanoparticles in aqueous solution has been cited but its application on crops has not reported yet. Therefore, there is need to investigate about degradation of antibiotics by nanoparticles on both control and natural conditions.

1.7. Significance of study

In the recent years, various work has been done on impact of nanoparticles and antibiotics on crops individually. But their simultaneous application on crops has not been studied yet. Hence current research was designed to check the impact of both antibiotics and nanoparticles. Since antibiotics pose adverse impacts on crops and crops act as transporting medium in the transference of such compounds into the food chain. Therefore, there is a need to degrade persistent antibiotic compounds in order to promote growth of crops and to achieve better yield.

1.8. Objectives

Keeping in view the above information, it was hypothesized that nanoparticles may degrade antibiotics and thereby help in improving wheat growth. The objectives of present study are;

- Optimization of procedure for nanoparticles assisted degradation of antibiotics
- Application of nanoparticles along with different antibiotics to promote wheat growth

1.9. Scope of study

Agriculture is the backbone of Pakistan's economy and major contributor to food security. Being a water scarce country, unfortunately untreated water is used for irrigation purposes and consequently pose serious threats to plants and human health. Henceforth advance research in agricultural sciences is required in such countries in order to deal with the burning environmental issues. This study would help in optimization of procedure for nanoparticles assisting degradation of antibiotics and it would also give the scenario of impact of both treatments on wheat growth. Moreover, it may help in solving food security issues like inhibition of growth of crops by antibiotics, reduce the antibiotics toxicity in plants and development of antibiotic resistance bacteria in humans.

Chapter 2

LITERATURE REVIEW

This chapter mainly focuses on previous information relevant to occurrence of antibiotics in environment including water, soil and plants; how they interact with crops; remedial pathways of antibiotics from environment with the help of nanoparticles and application of nanoparticles on crops.

2.1 Antibiotics in waste water

Major sources of antibiotics in environment are pharmaceutical manufactures as 20-800 mg L^{-1} concentration has been found in wastewater of pharmaceutical industries (Samarghandi et al., 2017). Other sources include animal manure, hospital waste water, household waste, landfill leachate and surface runoff (Roura et al., 2018; Thiele Bruhn, 2003). This leads to cause surface and ground water contamination (Roura et al., 2018). Antibiotic resistance is also promoted through high level of antibiotics concentration in surface water ranging from 1-10 μg L^{-1} (Khan et al., 2013). Generally, the estimated amount of antibiotic concentration in waste water is ranging from 28-31 mg L^{-1} (Safari et al., 2015). The most used antibiotics are macrolides, tetracyclines and fluoroquinolones (FQs). FQs are extensively used for livestock breeding including poultry, cattle, fish and pigs and for treatment of gastrointestinal system, urinary and respiratory tract. In the body FQs are not completely metabolized because they are highly stable in nature. Their active metabolites excreted out and reached towards water and soil and decrease the microbial degradation of antibiotics (Orzol & Piotrowicz-Cieślak, 2017). Orzol & Piotrowicz-Cieślak (2017) found that sludge, secondary and crude wastewater is contaminated with

18.4 mg kg^{-1}, 1013 ng L^{-1} and 2573 ng L^{-1} fluoroquinolones respectively. Fluoroquinolones are hydrophobic in nature because they contain zwitterions and make them immobile (Roura et al., 2018). Similarly, in sewage effluents, 120 ng L^{-1} concentration of amoxicillin has been found (Azanu et al., 2016). Levofloxacin is considered as most phytotoxic antibiotic. Increased concentration of antibiotics in rivers destroy the ecological balance by disturbing the nutrients transformation rate. Due to decrease in plant materials, aquatic organisms also tend to die, and in this way, whole aquatic ecological balance is disturbed (Carvalho et al., 2014 In a study, Khan et al. (2013) found Levofloxacin (Lev), Oxycycline (Oxy), Tetracycline (Tet), Norfloxacin (Nor), Ofloxacin (Ofl), at River Chenab, Ravi, Indus and Jhelum. The highest contaminated site was River Ravi (downstream to Lahore city) containing high levels of Lev, Oxy, Tet and Sulm with concentration of 86, 1100, 1700 and 2700 ng L^{-1}. Moreover, it was observed that selected antibiotics were found in higher concentration ranging from 1100-49,000 ng L^{-1} (0.0011-0.049 mg L^{-1}) in drug formulation facilities near Shahdara Industrial Estate.

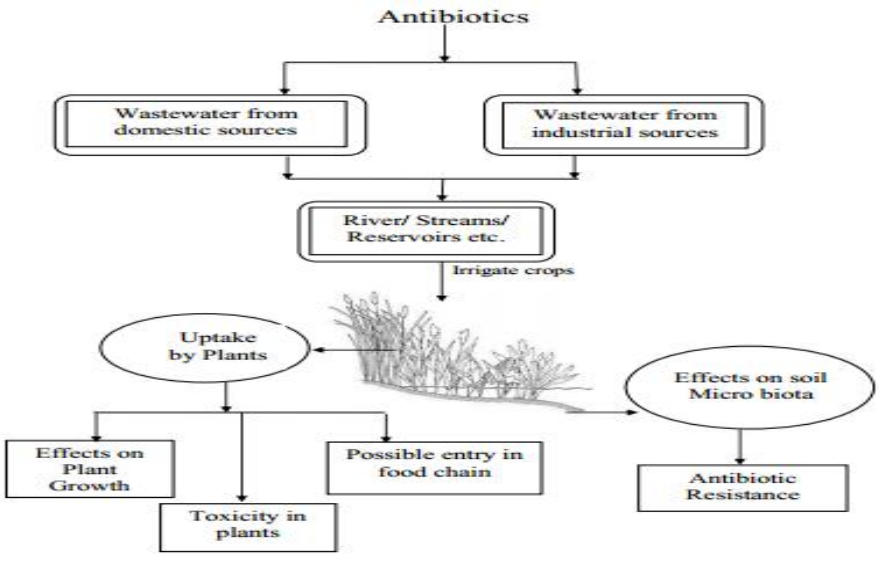


Figure 3. Entry routes of antibiotics in environment (Bruhn, 2003).

2.2 Antibiotics in plants

There are two major sources of antibiotic contamination in agro ecosystems. First one is use of manure as fertilizers which contains antibiotics residues excreted out from animal's digestive tract and second is application of antibiotics containing water into crops (Zhang et al., 2017). Since soil serves as sink for antibiotics, they are found within the range of 0.01 to 1420 mg kg^{-1} (Azanu et al., 2016; Orzol & Piotrowicz-Cieślak, 2017). Fluoroquinolones are stable therefore they remain in soil for several months or even years within the range of 0.01 to 0.4 mg kg^{-1} (Orzol & Piotrowicz-Cieślak, 2017). Antibiotics absorption depends upon pH, clay, organic carbon content and strength of ions present in soil (Orzol & Piotrowicz-Cieślak, 2017). According to European Union directive guidelines, drug residue content should not exceed 100 μg kg^{-1} (100 ppb) and 10 μg kg^{-1} (10 ppb) in manure and soil fertilized with manure respectively (Riaz et al., 2017).

There are various mechanisms through which antibiotics impact on crops. It either disturbs soil functions like reduces the rate of denitrification, decomposition rate of crop residues and recycling of nutrients; or decreases the soil fauna symbiosis by interfering in microbial population in soil (Carvalho et al., 2014). In response to tetracycline, bacteria and fungi in numbers were reduced and enhanced respectively in soil (Minden et al., 2017).

Through previous studies it has found that antibiotics firstly impact on leaves and then on growth of shoots and roots (Orzol & Piotrowicz-Cieślak, 2017). Increase in reactive oxygen species (ROS) production and reduction in photosynthetic rate and leaf area are all consequences of presence of antibiotics into soil and plants. High number of ROS in plants harms the lipid, DNA and proteins (Orzol & Piotrowicz-Cieślak, 2017). On roots they

cause alteration in water uptake mechanism, make changes in root length and elongation (Minden et al., 2017). Orzol & Piotrowicz-Cieślak, (2017) found 50% reduction in the growth of shoots and roots in lupin. Amoxicillin even at high concentration (10,000 mg L^{-1}) produce less toxic impact on wheat seeds as compare to levofloxacin (Ghava et al., 2015).

N-octanol water partition coefficient actually determines the endorsement of antibiotics from environment (Orzol & Piotrowicz-Cieślak, 2017). During the study of "uptake of antibiotics by wheat plant when irrigated with waste water", Franklin et al. (2016) found sulfamethoxazole (SMX) at the highest concentration of about 0.64 ± 0.37 ng g^{-1} and ofloxacin (OFL) with concentration 2.28 ± 0.89 ng g^{-1} in wheat grains. Carvalho et al, (2014) investigated exposure of oxytetracycline (greater than 9.2 mg L^{-1}) with alfalfa and found decrease activity of chloroplast synthase which resulted in plant death.

During the study "uptake of antibiotics by plants", Azanu et al (2016) found amoxicillin in plants at higher concentration (27.1 ng g^{-1}) as compare to tetracycline (20.2 ng g^{-1}). Uptake of amoxicillin in carrot was 14.3 to 45.2 ng g^{-1} whereas 13.7 to 33.6 ng g^{-1} was found in lettuce. During the investigation of antibiotics uptake by lettuce, Ahmed et al. (2015) found 88 ng g^{-1} tetracycline in lettuce leaves after the application of 10 mg L^{-1} antibiotic solution to it.

On contrary, Zhang et al. (2017) found that vegetable pakchoi exposed to 50% minimum inhibitory concentration (MIC) of three types of antibiotics like tetracycline (TC), cephalexin (CEP) and sulfmethoxazole (SMX) showed better biomass production and growth as compare to control. However, at MIC level, both growth and biomass were decreased. This study suggested that antibiotics at very low concentration could be beneficial for plants growth.

Moreover, uptake of antibiotics by plants ceases nitrification and reduces the decomposition of plant residues (Carvalho et al., 2014). During the study of plant-antibiotic interactions, Carvalho et al. (2014) found that mitotic index and root length of wheat was reduced in response to 250-300 mg L^{-1} chlortetracycline.

2.3. Wheat

Third largest cereal grain produced in the world is wheat (Franklin et al., 2016). Agriculture is the backbone of Pakistan's economy since 45% population depend upon it for employment and major funder to food security (Aslam, 2016; Khan et al., 2013). It contributes about 20.9% (agricultural GDP) to Pakistan's national GDP (Aslam, 2016). Moreover, due to significant production of wheat in Pakistan, it has been selected for pot experiment. In Pakistan wheat, Rabi crop, contributes about 10.3% and 2.2% in agriculture and GDP respectively (Usman, 2016). However agricultural development depends upon water quality which impacts on crops quality and yield and ultimately on human health (Suresh & Nagesh, 2015). Globally, wheat serves as staple food and most expected to expose with antibiotics through use of manure and fertilizers in field (Minden et al., 2017).

2.4. Nanotechnology in crops

In this modern era, one of the latest tool used in agriculture is nanotechnology (Khan et al., 2018). Nanoparticles (NPs) are used as fertilizers for improving the growth of crops and enhances the crop yield. There are various pathways through which NPs enter into plant. They can enter into plant cell through pores, by endocytosis or absorbed by roots and enter into endodermis from xylem either by symplastic and apoplastic pathways.

NPs play various important functions when applied into soil. These promote nutrients uptake, improve seed germination and fruit/grain quality, increase enzyme activities and biomass and crop yield, enhance chlorophyll content, strengthen stress tolerance and act as redox catalyst in metabolism of plant (Lyu et al., 2017). A study was conducted for assessment of the phytoavailability of phosphorus applied with TiO_2 nano particles. TiO_2 with concentration levels: 0, 25, 50, 75 and 100 mg kg^{-1} was applied. Concentration of phytoavailable phosphorus in soil containing lettuce culture was analyzed and it was found that phosphorus increased up to 83% with treatment of TiO_2 nanoparticles. In addition to this increase in root/shoot lengths 1.5 -fold, total dry biomass by 2-fold and total phosphorus uptake by 4-fold was observed (Hanif et al., 2015). Kisan et al. (2015) found that nano zinc oxide spraying enhances nutritious value of spinach to vegetarian diet by supplying, protein, fiber and required amount of vegetarian fat to diet. At the concentration of 500 and 1000 mg kg^{-1} of ZnO NPs the plant showed enhancement of leaf parameters such as leaf surface area, length, width, and color of leaf samples as compared to control samples. Similarly, protein and dietary fiber content increased in treated plants with 500 and 1000 mg kg^{-1} ZnO NPs in comparison to control leaf samples of spinach.

2.4.1. Uptake of nanoparticles by plants

The mechanism behind the NPs uptake by roots lie in the specific transporter, yellow stripe 1 transporter (YS1). YS1 facilitates the entry of NPs into the root cells either directly or by conjugating NPs with DMA (deoxymugineic acid) complex. This route is similar for iron nanoparticles as well. However, NPs uptake by roots also depend upon size. For wheat, diameter of Titania nanoparticles (TNPs) should less than 140 nm and 36 nm in order to pass through root epidermis and parenchyma respectively. Moreover, NPs activates

membrane acceptors which activates endocytosis through which they also enter into plant cell. Similarly, active transport helps in transfer of NPs into leaf cells via aqueous pores or stomata or direct penetration into apoplast and then symplast (Lyu et al., 2017). After entering into plant body, it follows either symplastic or apoplastic pathway. 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). Whereas 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). Similarly, NPs can easily pass into seed coat and enhanced seed germination and absorption of nutrients (Lyu et al., 2017).

2.4.2. TNPs help in nutrients uptake

TNPs help in uptake of other nutrients and increases the plant growth. In crops like maize and wheat, YS1 is expressed by titania (Ti) which eventually increases uptake of iron and enhances the photosynthetic rate by increasing the biosynthesis of chlorophyll. This eventually causes nitrate photo assimilation in which integration of nitrate ion (NO_3^-) with chloroplast take place and hence enhances the nitrogen uptake in plants by dominating the nitrogen transporter gene. Moreover, YS1 also helps in binding of metals like Zn^{2+}, $Cu^{2+,}$ and Ni^{+2}; Ca, Mg, P and other nutrients with DMA and hence improves plants growth (Lyu et al., 2017). Metallic nanoparticles (TiO_2 and Fe_3O_4) when applied modify phosphorus accessibility in rhizosphere and uptake by *Lactuca sativa* (Zahra et al., 2015). Similarly, another study showed that Al_2O_3 nanoparticles loaded with phosphorus is used in *Brassica napus* to improve phosphorus uptake. The phosphorus uptake was reported to increase 8-fold at constant low free phosphate concentration, and to 40-fold because of passive,

diffusion-based samplers (Santner et al., 2012). TiO_2 nanoparticles with anatase phase increased the fresh and dry biomass of plants by 91% and 99% respectively as compared to control. Total nitrogen increased up to 23.35% along with improved chlorophyll and proteins of spinach (Yang et al., 2007).

NPs like iron oxide and zinc oxide help in movement of iron into leaves and increases the photosynthate activity as well (Kale & Gawade., 2016). Zinc serves as important nutrient for metabolic pathways in plants as it is involved in active mechanism of 300 enzymes and plays a major role in protein synthesis (Kale & Gawade., 2016; Khanm et al., 2018). Generally, iron is present in chloroplast in leaves of plant and is used by proteins as cofactor in oxidation reduction reaction, genome stability, chlorophyll synthesis and photosynthesis.

During the investigation of synergistic effects of ZnO NPs (4500 mg ha^{-1}) with NPK at the ratio of 12.5:12.5:12.5 on brinjal growth, Kale and Gawade,(2016) found 45.3% and 91% increase in biomass and yield respectively in comparison to control. Control was recommended dose of NPK (15:15:15). Similarly, there was 20.43% and 38.16% increase in biomass and yield respectively in comparison to treatment where bulk $ZnSO_4$ along with NPK was used instead of ZnO NPs. A previous study determined the effects of TiO_2 nanoparticles on the development and germination of spinach seeds and it was reported that nanoparticles acted as photo catalysts and enhanced light absorbance and activated the growth activity of spinach (Yang et al., 2007). Likewise, a substantial enhancement was observed in root area, root length, shoot length (17.02%), root nodule, total soluble leaf protein and the chlorophyll content as an outcome of applied TiO_2 NPs (Raliya et al., 2015).

Every nanoparticle has its defined concentration in soil and plants for beneficial effects. For instance, it helps in the uptake of nutrients like phosphorus (P) and iron (Fe) by plants. However, it could also cause toxicity in plants by competing with nutrients like Fe and P when concentration of NPs are higher than their threshold value (Lyu et al., 2017). During the study of examining the growth in wheat when applied with Titania nanoparticles it was found that at 60 mg kg^{-1} of TiO_2 NPs an elevation in the plant's shoot lengths, root lengths and, biomass was observed. On the contrary, increasing concentrations of TiO_2 NPs not only affected the root and shoot lengths but also reduced the biomass (Rafique et al., 2014). 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. Different factors are responsible for the uptake of NPs in plants including soil pH and plant species. Previous studies suggested that soil pH ranging from 7.3 to 7.4 has proved to have better absorption of TiO_2 in plants with concentration of 910 to 1300 mg kg^{-1}. Similarly, different plant responded differently with uptake of nanoparticles. For instance, TiO_2 with concentration 42 to 14,000 mg kg^{-1} and 20 to 1900 mg kg^{-1} was found in horsetail and red cabbage, respectively (Lyu et al., 2017).

2.5. Decontamination of organic pollutants in soil

During the study of decontamination of soil containing pyrene with anatase TNPs, Dong et al (2010) found 43.5% as the highest degradation of pyrene in soil when soil was spiked

with 4% w/w (40,000 mg kg^{-1}) TNPs and exposed with UV radiation having intensity of 357μW cm^{-2} for time period of 25 hours.

2.5.1. Photo catalytic degradation of organic contaminants

Advanced oxidation process (AOP) is a process that uses catalyst to transform or degrade contaminants in water in the presence of light. For the degradation and removal of various pollutants such as antibiotics, personal care products, and other toxic organic compounds, different types of catalyst are used such as titania, zinc oxide, magnetite, silver oxide, etc. (Ambrosetti et al., 2015).

2.5.1.1. Impact of parameters on photo degradation

Different factors like substrate and catalyst concentration, time duration of irradiation, pH and stirring speed and recombination rate of electron-hole pairs play an important role for efficient degradation of antibiotics (Ambrosetti et al., 2015). At low stirring speed, sedimentation can take place which may reduce the efficiency of photo catalysis process (Eskandari et al., 2018).

Charge on catalyst surface depends upon point of zero charge (P_{zc}). TiO_2, ZnO and Fe_2O_3 have P_{zc} pH 6, 7 and 8 respectively. Hence at acidic pH, when pH < P_{zc}, there becomes positive charge on catalyst surface whereas it is negatively charged at alkaline pH when pH> P_{zc}. Charge on antibiotic surface depends upon dissociation constant. The dissociation constant (pK) for AMX and LEV reported are 3.2 and 4.24 respectively. If pH of antibiotic solution is less than pK value, then negative charge is dominant on antibiotic surface and vice versa. However, at highly acidic pH (<4), rate of photodegradation decreases due to presence of protons (H^+) that combine with hydroxyl ions (OH^-) and inhibit the formation

of oxidizing agents hydroxyl radicals (OH˙**).** Previous studies suggested that maximum degradation of antibiotics have observed at pH 5 as it facilitates the best adsorption of substrate on catalyst surface (Eskandari et al., 2018).

Safari et al. (2015) investigated the photodegradation of tetracycline (TC) in wastewater. TC with concentration 27, 55, 74 and 103 mg L^{-1}, TNPs concentration ranging from 0.25 to 2 g L^{-1} were conducted at various pH like 5, 7, 9 and 11 in the presence of UV light in 120 minutes. It was concluded through this study that percent removal of TC increased with increasing concentration of TNPs until it reached at threshold dose i.e. 1g L^{-1}. After this no significant improvement in degradation process was observed. Moreover, best degradation efficiency was observed under acidic and alkaline conditions. Similarly, with degradation efficiency of TC decreased with increased concentration of TC as titania surface is completely covered with TC molecules making it in effective to react with holes and hydroxyl radicals generated from photo catalysis process. Thereby it restricts the interaction between photons and nanoparticles. It was concluded that optimized conditions for better photodegradation was TC at 55 mg L^{-1} with 1g L^{-1} TNP at pH 5 in 120 minutes UV light exposure.

During the study of photo lysis of ciprofloxacin (CIP) by using ZnO nanoparticles, different experiments were conducted by varying different parameters. CIP concentration was varied from 5 to 20 mg L^{-1}, pH ranging from 3 to 8, dose of ZnO nanoparticles were checked between 50 to 200 mg L^{-1} in time period of 100 minutes under the UVC light. It was found that best percent degradation of CIP was found by using 150 mg L^{-1} concentration of ZnO in 10 mg L^{-1} CIP aqueous solution having pH 5 at the time duration of 140 minutes (Eskandari et al., 2018)

Chapter 3

MATERIALS AND METHODS

This chapter describes the experimental framework of present research work. The work was divided into two main phases. First one was "screening study" which was further divided into three major levels; a) synthesis and characterization of nanoparticles, b) preparation of antibiotic solutions and c) lastly photo degradation of antibiotics by nanoparticles. Second phase was "pot experiment". This was subdivided into three major parts. At the first part, soil was prepared, and analysis of soil general characteristics were done. At the second part, wheat seeds were grown and exposed with both antibiotics and nanoparticles and lastly, assessment of growth parameters and nutrient analysis were take place. This pot experiment was done in glasshouse at IESE, NUST. All the methodologies followed throughout the study are described in detail.

3.1. Phase I: Screening study

3.1.1. Synthesis of nanoparticles

3.1.1.1. Synthesis of titania (TiO_2) nanoparticles

Titania nanoparticles (TiO_2) were prepared with the help of modified sol gel method by using titania isopropoxide as a precursor. Firstly, 150 mL ethanol was added in 600 mL distilled water. During the stirring and heating, 10 mL titania isopropoxide was added slowly into the solution. After 30 minutes, heating was stopped and stirring was continued for 48 hours. Afterwards, it was dried in an oven at 90^{o}C. Finally, dry gel was grounded and calcined in muffle furnace for 5 hours at 450^{o}C (Lieberzeit et al., 2007).

3.1.1.2. Synthesis of zinc oxide (ZnO) nanoparticles

ZnO nanoparticles were prepared according to co precipitation method. 0.4 M zinc sulfate ($ZnSO_4.7H_2O$) and 0.8 M NaOH solution were prepared separately. While during stirring, NaOH solution was added drop by drop into $ZnSO_4.7H_2O$ solution until white precipitates were formed. After 18 hours stirring, this mixture was allowed to settle overnight, and precipitates were collected through filter paper. Precipitates were washed with distilled water and dried in an oven for 8 hours at 60^oC. Finally, dried material was grounded and calcined in muffle furnace for 5 hours at 300^oC (Matei et al., 2014).

3.1.1.3. Synthesis of iron oxide (Fe_2O_3) nanoparticles

Co-precipitation methodology was used for synthesis of iron oxide (Fe_2O_3) nanoparticles. About 50 mL of each 0.1 M and 0.2 M ferrous chloride ($FeCl_2.4H_2O$) and ferric chloride ($FeCl_3.6H_2O$) solution were prepared. It was followed by synthesis of 100 mL of 0.1 M NaOH solution. Both ferric and ferrous solutions were mixed. The mixture turned dark brown after the addition of NaOH solution under continuous stirring. It was placed upon heating until slurry was left behind and afterwards distilled water was used for maintaining the pH at 7. With the help of centrifuge, iron oxide nanoparticles were separated. In an oven, supernatant was dried for 2 hours at 80^oC and finally dried materials were grounded in order to obtain fine iron oxide nanoparticles (Zahra et al., 2015; Kekutia et al., 2015).

3.1.2. Characterization of nanoparticles

3.1.2.1. X-Ray Diffraction (XRD)

In order to determine the crystal size, phase and system of all the three nanoparticles, X-Ray diffractometer (Theta-Theta STOE, Germany) was used. The X-ray was operated at

20 kV and 5 mA and used Cu Kα radiation (λ= 0.15406 nm). The diffraction peaks were collected with an acquisition time of 1.0 second per step and in the 2Θ range of (20º- 80º) with a 0.04º step size. Scherrer's calculator through X'Pert High score were used for determination of crystal size.

3.1.2.2. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM, Jeol, JSM 6490 A, Japan) was used to determine the surface morphology of all three synthesized nanoparticles with an accelerating voltage of 20 kV. Before scanning, TiO_2 and ZnO suspensions in distilled water whereas Fe_2O_3 in ethanol were sonicated for 30 minutes and made on quartz slides. In order to avoid contamination and interferences, Atomic Ion Sputtering Device (JEOL, JFC-1500) was used for coating of nanoparticles with gold. It was done by placing a drop of diluted solution of nanoparticles (NPs) on glass slide followed by air drying. Afterwards slides were observed under SEM by using different magnification (Zahra et al., 2015).

3.1.3. Preparation of antibiotics stock solution

On the basis of frequency of use, two antibiotics amoxicillin (Amx) and levofloxacin (Lev) were selected. Stock solution of both antibiotics (250 mg L^{-1}) were prepared separately by dissolving crush powder of antibiotics into one liter of distilled water. They were properly mixed through stirrer at 700 rpm for 3 hours. In order to avoid photochemical reactions, beakers were fully covered with aluminum foil and were placed in refrigerator.

5 mg L^{-1} Amx and Lev working solutions were used as required concentration for test experiments and they were prepared by adding 1 mL from stock solution (250 mg L^{-1}) of

Amx and Lev respectively into 50 mL standard flask and volume was made up to mark with distilled water.

3.1.4. Nanoparticles doses

This photodegradation experiment was carried out with three types of photo catalysts namely TiO_2, ZnO and Fe_2O_3. Each having doses 40, 50 and 60 mg L^{-1} were used for the degradation of Amx and Lev in antibiotics contaminated samples. These dosages were selected according to previous studies as 60 mg L^{-1} TiO_2 serves as threshold value for wheat growth. Above this concentration, cell damage may be occurred (Rafique et al., 2014).

0, 40, 50 and 60 mg L^{-1} TiO_2 doses were prepared by adding 0, 2, 2.5 and 3 mg TiO_2 powder into 50 mL of prepared AMX and LEV working solutions having concentration 5 mgL^{-1}. In the same way, ZnO and Fe_2O_3 with doses 40, 50 and 60 mg L^{-1} were prepared in both antibiotic solutions separately.

3.1.5. The Photo-catalysis experiment

In order to degrade 5 mg L^{-1} Amx by using 50 mg L^{-1} TiO_2, 2.5 mg of TiO_2 was spiked in prepared 50 mL Amx solution (5 mg L^{-1}) and mixed with the help of orbital shaker at the speed of 90 strokes per minute in order to achieve complete equilibration of adsorption/desorption of AMX onto the TiO_2 surface. The samples were placed under the visible lamp providing 50W lamp emissions with light intensity ranging from 52,000 – 56,000 Lux for 2 hours. In order to determine the time duration required for degradation, this experiment was extended for 1, 2, 3, 4, 7 and 9 days. During this experiment, light intensity was measured by light intensity meter and temperature was measured by thermometer which was ranging from 24-38^{o}C. The pH strips were used for measuring pH of antibiotic-nanoparticle solutions before and after the photocatalysis experiment. After

the exposure time, this solution was centrifuged at 4000 rpm for 20 minutes in order to separate nanoparticles from antibiotic solutions. The samples absorbances were measured on UV-visible spectrophotometer (Specord 200 Plus, Germany).

3.1.5.1. UV-visible spectrophotometer reading

Before sample analysis on spectrophotometer, Amx standards were prepared from AMx stock solution. 1, 2, 3, 4 and 5 mg L^{-1} standard were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 mL from AMX stock solution (250 mg L^{-1}) and diluted up to 50 mL with distilled water. Similar method was utilized for Lev standards preparation. For Amx and Lev, absorbance was measured at 195 and 291.2 nm, respectively.

Afterwards degradation percentage was calculated with the help of formula;

$$\textbf{Degradation \%} = (C_o - C_t / C_o) \times 100$$

Where C_o and C_t are concentrations of antibiotics before and after the photodegradation reaction respectively (Safari et al., 2015).

Same procedure was followed for degradation of Amx and Lev by other two nanoparticles ZnO and Fe_2O_3 by using 50 mg L^{-1} dose. After optimizing the conditions and parameters, same experiments were repeated with TiO_2, ZnO and Fe_2O_3. Each nanoparticle with dose of 0, 40, 50 and 60 mg L^{-1} for time period of seven days was used. Each experiment was performed in triplicates.

3.2. Phase II: Pot experiment

3.2.1. Soil preparation

For pot experiment, soil was collected from NUST nursery and it was then prepared for soil analysis and plant cultivation. Firstly, it was air dried by spreading it into greenhouse floor under the sunlight for one week in order to remove moisture. In order to remove

stones and pebbles, it was manually sieved by using 2 mm sieve and fine homogenized soil was obtained. This prepared soil was weighed and filled in plastic pots. Each pot was filled with 1 kg of prepared soil.

3.2.2. Characterization of soil

3.2.2.1. pH

Soil pH was measured with the help of pH meter by making 1:5 soil water suspension. In 50 mL distilled water, prepared 10 g weighed soil was added and placed on mechanical shaker for 1 hour for 30 minutes. Afterwards this soil solution was allowed to settle for 30 minutes and it was filtered with the help of Whatman filter paper No 42. Finally, filtrate was used for measuring pH (McLean, 1982).

3.2.2.2. Moisture content

In a petri dish, 10 g prepared soil was added, and it was dried in a hot air oven (Lab Tech LDO 030N) at 105^{o}C for 24 hours. This dried soil was allowed to cool and was again re-weighed with the help of weighing balance. Moisture content in soil was determined with the help of formula;

$$\textbf{Moisture content (\%) = (wet soil – dry soil/dry soil) × 100}$$

3.2.2.3. Water holding capacity

In 50 mL measuring cylinder, funnel was placed on it having filter paper of Whatman No. 42. With the help of weighing balance, 10 g prepared soil was placed on this filter paper and 10 mL distilled water was passed. The filtrate was collected, and final volume was measured in this measuring cylinder. This noted volume give the maximum water holding capacity of used soil (Harding and Ross, 1964).

3.2.2.4. Total organic carbon

Walkley Black method was used to determine the percentage of total organic carbon in prepared soil. For this 1 N potassium dichromate solution ($K_2Cr_2O_7$) was prepared by drying it in an oven at 105^oC for 2 hours. This dried potassium dichromate powder was dissolved in one-liter water. Similarly, 0.5 M ferrous ammonium sulfate solution [$(NH_4)_2SO_4.FeSO_4.6H_2O$] was prepared by adding 5 mL concentrated H_2SO_4 and 196 g ferrous ammonium sulfate in one liter flask and volume was made up to mark. In 100 mL concentrated H_2SO_4, 1 g diphenylamine indicator was added. After preparing the reagents, 1g of prepared soil was weighed with the help of weighing balance. This soil was mixed with 20, 10, 200 and 10 mL of concentrated H_2SO_4, 1N $K_2Cr_2O_7$, distilled water and concentrated orthophosporic acid respectively. In this mixture few drops of indicator was added and then finally it was titrated against ferrous ammonium sulfate solution until green color was achieved. Blank was prepared in a similar way without the addition of soil (Estefan et al., 2013). Total organic carbon was calculated with the help of formula;

Total organic carbon (%) = 1.334 x oxidizable organic carbon (%)

Oxidizable organic carbon (%) = {($V_{blank} – V_{sample}$) x 0.3 x M} / Wt

3.2.2.5. Nitrate-nitrogen

Chromo trophic method was used for determination of nitrate nitrogen in soil. For this purpose, 0.1% chromo trophic acid solution was prepared by dissolving 0.368 g of this acid in 200 mL concentrated H_2SO_4. 0.02N $CuSO_4.5H_2O$ was prepared by dissolving 2.4968 g copper sulfate in one-liter distilled water. 50 mL of this solution was added in 10g soil. It was mixed and filtered. 1mL of prepared chromo trophic acid solution and 6mL of concentrated H_2SO was added in 3mL filtrate and yellow color was developed after half

an hour. Standards stock solution was made by adding dried potassium nitrate (KNO_3) into 0.02 N $CuSO_4.5H_2O$. 10 mL of this stock solution was diluted up to 200 mL with 0.02 N $CuSO_4.5H_2O$. Afterwards 1 to 7 mL was taken from this diluted stock solution and volume was made up to 100mL with 0.02 N $CuSO_4.5H_2O$. This resulted in series of standards from 0.5 to 3.5 ppm. Similar method (as for samples) was proceeded for each standard (3 mL) and blank (3 mL of 0.02N $CuSO_4.5H_2O$). After color development blank, standards and samples were run on UV-visible spectrophotometer at 430 nm (Estefan et al., 2013). Following formula was used for calculating nitrate nitrogen in the soil sample;

$$\mathbf{NO_3\text{-}N\ (ppm) = NO_3\text{-}N ppm\ (calibration\ graph) \times (V/W_t) \times (V_2/V_1)}$$

3.2.2.6. Phosphates

Phosphates in the soil was determined by ascorbic acid method which was developed by Olsen et al in 1954. 0.5 M sodium bicarbonate solution ($NaHCO_3$) was prepared by dissolving 42 g into 1000 mL distilled water. 5 N sulfuric acid and NaOH solution were also prepared. Two types of reagents were made; in first reagent 0.2908 g antimony potassium tartrate $(KSbO.C_4H_4O_6)_2$ and 12 g ammonium heptamolybdate $\{(NH_4)_6Mo_7O_{24}.4H_2O\}$ were dissolved in 100 and 250 mL distilled water respectively. After mixing these solutions one liter 5 N H_2SO_4 was added and volume was made up to 2 liters. 200 mL of this reagent was mixed with 1.056 g ascorbic acid and second reagent was ready to use. Sample was prepared through extraction mechanism in which 100 ml of 0.5 M $NaHCO_3$ was added in 5 g prepared soil. After shaking for 30 minutes, filtrate was collected by using Whatman filter paper No. 40. 1 mL of 5 N H_2SO_4 and 8 mL reagent was mixed in 10 mL of this filtrate and volume was raised up to 50 mL with distilled water. Standards stock solution was made by adding 2.197g dried potassium dihydrogen

phosphate (KH_2PO_4) into one-liter distilled water. 50 mL of this stock solution was diluted up to 250 mL. Afterwards, working standard solution were prepared by taking 5, 10, 15, 20, 25 mL from this diluted stock solution and 500 mL was raised for each standard. From each standard, 2 mL was taken, and it was treated alike samples. 10 mL of 0.5M $NaHCO_3$ served as blank which was treated as for samples and run on UV-visible spectrophotometer at 882 nm (Estefan et al., 2013).

Following formula was used for calculating phosphates in the soil sample;

$$\textbf{Extractable P (ppm) = P (calibration graph) x } (V/W_t) \textbf{ x } (V_2/V_1)$$

3.2.3. Plant cultivation

3.2.3.1. Seeds preparation

In order to analyze the simultaneous application of both antibiotics and nanoparticles on crops, wheat was selected for pot experiment. Wheat seeds (Galaxy 70) were obtained from Ayub Agricultural Research Institute (AARI). In order to avoid fungal attack, these seeds were sterilized with 5% calcium hypochlorite solution and then rinsed with distilled water for three times (Cannel, 1990).

3.2.3.2. Preparation of pots

1kg of prepared soil was weighed, filled in plastic pots and irrigated with tap water. In each pot, six sterilized wheat seeds were sown. There were six treatments with five replicates. The treatments were i) control (only NPK), ii) Amx (AR: 5 mg kg^{-1}), iii) Lev (LR: 5 mg kg^{-1}), iv) Titania nanoparticles (TiO_2: 50 mg kg^{-1}), v) Amx and Titania (AMX: 5 mg kg^{-1}; TiO_2: 50 mg kg^{-1}) and vi) Lev and Titania (Lev: 5 mg kg^{-1}; TiO_2: 50 mg kg^{-1}).

3.2.3.3. Application of nutrients, antibiotics and titania nanoparticles

Wheat in per kg soil was fertilized with NPK; 0.1-0.05-0.05 g respectively as total dose. Di-ammonium phosphate (DAP), urea, and potassium sulfate served as major sources for phosphorus, nitrogen and potassium respectively. They were added in the soil at three levels. First one at the sowing time in which soil was fertilized with DAP and potash. Second and third were after 45 and 75 days of sowing when soil was fertilized with half doses of urea (Arshad et al., 2011).

In each pot selected antibiotics with concentration 5 mg L^{-1} was applied in the soil. They were given in two equal halves; first at the ninth week and second at the tenth week of sowing date. Each pot was irrigated with 10 mL of this stock solution as 10 mL of stock solution (250 mg L^{-1}) contains 2.5 mg L^{-1} (half dose).

Moreover, wheat crops were exposed with titania nanoparticles (50 mg kg^{-1}) through irrigation at the 10^{th} week. Titania nanoparticles (TNPs) suspension were prepared by adding 750 mg TNP powder into 150 mL distilled water. Afterwards it was sonicated for half an hour and each 15 pots were exposed with 10 mL of TNP solution having concentration 50 mg kg^{-1}.

3.2.4. Growth parameter and nutrient analysis

3.2.4.1. Growth parameter measurement

On the harvesting date, no of tillers and spikes per plant were counted. Roots and shoots were washed with tap water several times in order to remove soil. Afterwards roots and shoots length and weight were measured with the help of measuring tape and portable weighing balance respectively. In order to check dry biomass, both roots and shoots were dried in paper bags separately in hot air oven for 48 hours at 65^{o}C. Similarly, biomass of

grains per plant was also weighed and they were grounded into fine powder with help of mortar pestle. After weighing, grounded grains were separated in Eppendorf for iron, protein and carbohydrate analysis. Similarly, oven dried root and shoots were grounded into fine powder by electric grinder for further analysis (Arshad et al. 2011).

3.2.4.2. Nutrient analysis

3.2.4.2.1. Wet digestion method

Phosphorus analysis was done on roots and shoots of wheat whereas iron was analyzed in grains as well. Di acid mixture consisting of concentrated perchloric ($HClO_4$) and nitric acid (HNO_3) with ratio of (1:3). 25 mL conical flask having ground material and di acid mixture was placed on hot plate having temperature between 200- 250^oC. This was allowed under constant heating until clear solution was remained with white dense fumes of $HClO_4$. After wards, this clear hot aliquot was allowed to cool at room temperature and volume was made up to mark with distilled water. Finally, it was filtered with Whatman filter paper No. 42 (Rashid, 1986). These extracts were kept in plastic bottles at 4^oC for phosphorus and iron analysis. Similar procedure was repeated for wet digestion of grains. For grains tri-acid mixture having ratio of 1:2:3 of sulfuric acid (H_2SO_4), $HClO_4$, HNO_3 was used. In digested wheat samples (roots, shoots and grains), contents of iron were analyzed with the help of Atomic Absorption Spectrophotometer, Shimadzo 7000/ Japan (Ryan et al., 2007).

3.2.4.2.2. Total phosphorus

Vanadomolybdo phosphoric acid colorimetric method was used for analysis of phosphorus in digested root and shoot samples. 2 mL ammonium vanadamolybdate solution was added in 2 mL digested extract and it was diluted up to 20 mL with distilled water. This ammonium vanadamolybdate reagent was prepared by dissolving 11.25 g ammonium

heptamolybate and 0.625 g ammonium metavanadate in 200 and 150 mL distilled water respectively. Both solutions were mixed with 125mL concentrated HNO_3 and volume was brought up to 500 mL. Series of standards with concentration 5, 20, 40, 60, 80 and 100 mg L^{-1} were prepared by diluting 1, 4, 8, 12, 16 and 20 mL stock solution up to 20 mL distilled water. This stock solution was prepared by dissolving dried 0.4394 g potassium di hydrogen phosphate (KH_2PO_4) in one-liter distilled water (100 mg L^{-1}). Standards were treated in a similar way just like samples. Blank, standards and samples were observed under UV-visible spectrophotometer (Specord 200 plus Analytikjena Germany) after 30 minutes of color development at 410 nm (Estefan et al., 2013).

3.2.4.2.3. Total carbohydrates

Ludwig and Goldberg (1956) suggested Morris anthrone method for analysis of total carbohydrates in grains. 5 mL of 2.5 N HCl solution were added into 100 mg (0.1g) fine grain powder in a test tube. It was allowed to hydrolyze under water bath until complete hydroxylation takes place. These hydrolyzed samples were placed in centrifuge for 20 minutes at 4000 rpm. The supernatant was collected, and it was neutralized with sodium carbonate (Na_2CO_3) until bubbles formation started to cease. Afterwards volume was raised up to 100 mL. For the preparation of anthrone reagent, 0.4 g anthrone was dissolved in 200 mL cold concentrated H_2SO_4. In 5 mL of prepared sample, 8 mL prepared anthrone solution was mixed and after 10 minutes green color was developed. Sample analysis was done with the help of UV-Visible spectrophotometer. For this purpose, glucose standards were prepared by dissolving 100 mg glucose in 100 mL distilled water. Then 10 mL of this solution were added in another 100 mL standard flask through pipette and the volume was made up to mark. This stock solution gives 100 mg L^{-1} glucose solution. Five standards 20,

40, 60, 80 and 100 mg L^{-1} were prepared by extracting 0.2, 0.4, 0.6, 0.8 and 1 mL from glucose stock solution and 4 mL anthrone reagent was added. Similarly blank was prepared by dissolving 5 mL distilled water into 8 mL anthrone solution. After 10 minutes, green color was developed and blank, standards and samples were run at 620 nm on UV-Visible spectrophotometer.

3.2.4.2.4. Total protein

The amount of total proteins in wheat plant was quantified through the determination of total nitrogen content of plant (Martin et al. 1983). The first step was mineralization in which 1 mL of 36N H_2SO_4 was used to digest 50 mg of plant sample. This was carried on for 10 min at 150°C and then for 30 min at 310°C. Samples were removed from the mineralization block and were allowed to cool. Then 0.1 mL of H_2O_2 was added into it and sample was again digested at 310°C until the evaporation of H_2O_2. This step gave a colorless extract. The sample was then diluted by adding 10 mL of H_2O. From this diluted sample, 0.1 mL was taken, and 3.5 mL of H_2O was added into it.

3.3. Statistical analysis of data

Statistical significance of findings was checked by using software "Statistics 8.1" applying single factor ANOVA and LSD through all pair-wise comparison. When the probability of the results was less than 0.05 ($p < 0.05$), results were considered statistically significant.

Chapter 4

RESULTS AND DISCUSSION

4.1. Phase I: Screening study

4.1.1. Characterization of nanoparticles

The crystalline size and phase composition of the prepared nanoparticles were analyzed through XRD analysis. The average particle size of all three prepared nanoparticles has been estimated using Debye-Scherer calculator. Analysis of XRD peaks confirmed the nano size, high purity, and crystalline structure of synthesized nanoparticles.

4.1.1.1. Characterization of TiO_2 nanoparticles

The XRD patterns of prepared TiO_2 nanoparticles for 2θ diffraction angles between 20^o and 70^o are shown in figure 4 (a). The XRD pattern showed primary peaks at 25.30^o and 48.03^o confirming that the synthesized TiO_2 nanoparticles were in anatase phase which can be indexed as pure tetragonal structure. Synthesized TiO_2 nanoparticles were 54.6 nm in size with lattice constants a= 3.783ºA and c= 9.497ºA. In XRD patterns, there were few insignificant rutile reflections. By comparing XRD of three prepared nanoparticles, it can be observed that TiO_2 crystallites were ultrafine as compared to ZnO and Fe_2O_3 crystallites due to their wider peaks (Behnajady et al., 2011).

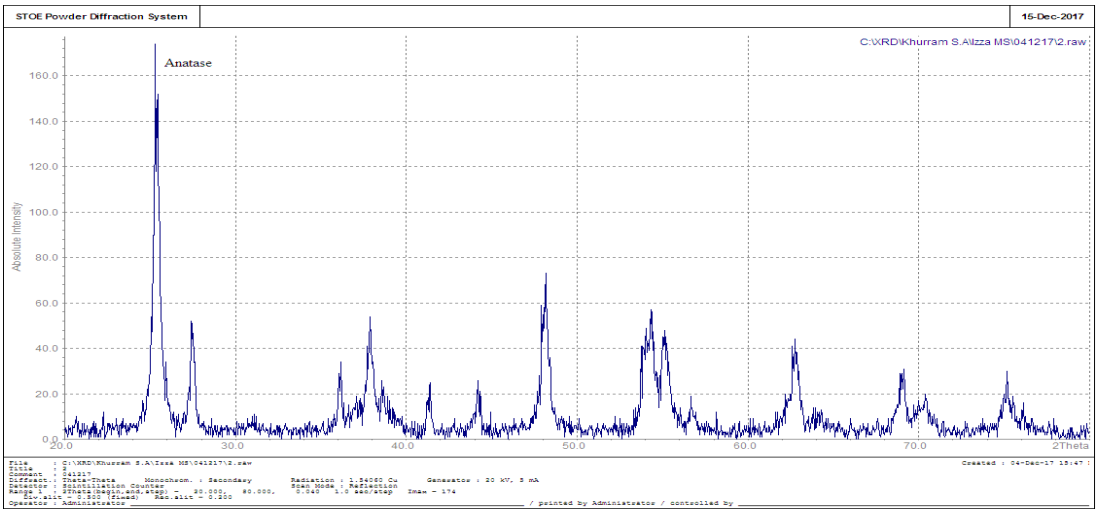


Figure 4(a): XRD pattern of synthesized titania

4.1.1.2. Characterization of ZnO nanoparticles

The XRD patterns of prepared ZnO nanoparticles for 2θ diffraction angles between 20^{o} and 70^{o} are shown in figure 4(b). The XRD pattern showed primary peaks at 31, 34 and 36 which assures its zincite phase which can be indexed as pure hexagonal phase. Synthesized ZnO nanoparticles were 36.7 nm in size with lattice constants a= 3.2568 ^{o}A and c= 5.2125 ^{o}A. The results represent high crystallinity due to its sharp peaks (Behnajady et al., 2011; Sadraei, 2016).

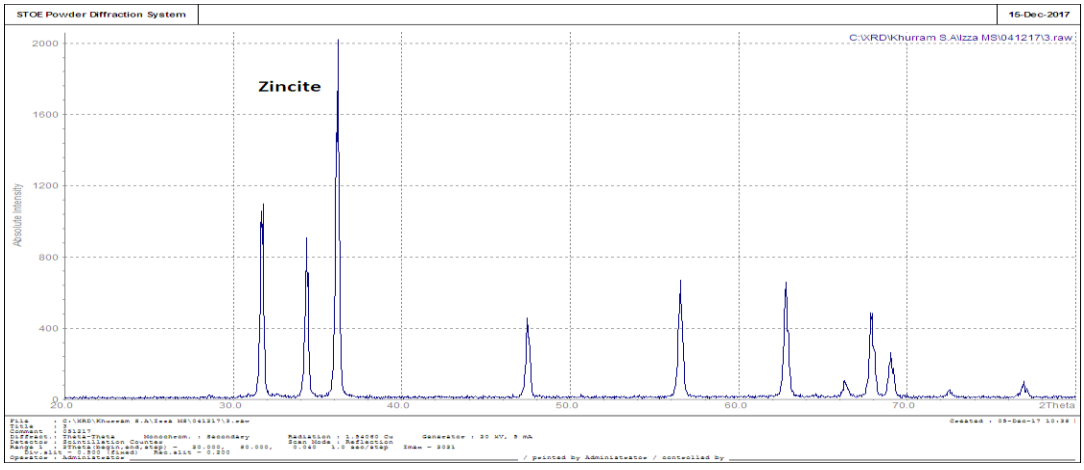


Figure 4(b): XRD pattern of synthesized zinc oxide

4.1.1.3. Characterization of Fe_2O_3 nanoparticles

The XRD patterns of prepared Fe_2O_3 nanoparticles for 2θ diffraction angles between 20° and 70° are shown in figure 4(c)). The XRD pattern showed primary peaks between 30° and 40° which assures its hematite phase which can be indexed as rhombohedral structure. Synthesized Fe_2O_3 nanoparticles was 21.5 nm in size with lattice constants a= 5.038 °A and c= 13.776 °A (Robichaud et al., 2009).

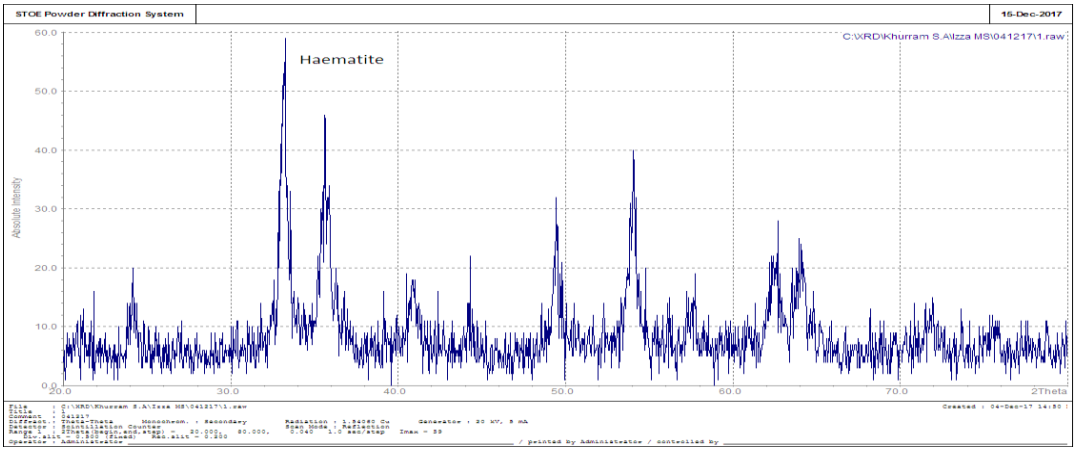


Figure 4(c): XRD pattern of synthesized iron oxide nanoparticles.

4.1.2. Scanning electron microscope (SEM) imaging

In order to determine the surface morphology of synthesized nanoparticles, SEM was used. Figure 5 (a, b, c) shows the images of three different NPs taken by SEM (JEOL JSM-6460) at 20,000 and 50,000X magnification, indicating highly crystalline structures. Because of zero dimensionality of NPs, their specific surface areas are much higher, resulting in higher photo catalytic activity. SEM image of titania represents highly porous and spherical structure whereas SEM image of ZnO assures that particles are held together, and their separation is not good enough. SEM of Fe_2O_3 represents irregular spherical shape with no obvious aggregation.

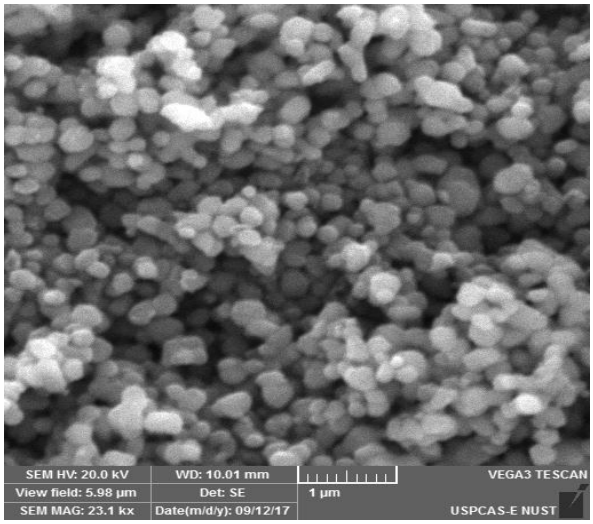


Figure 5(a). SEM image of TiO_2.

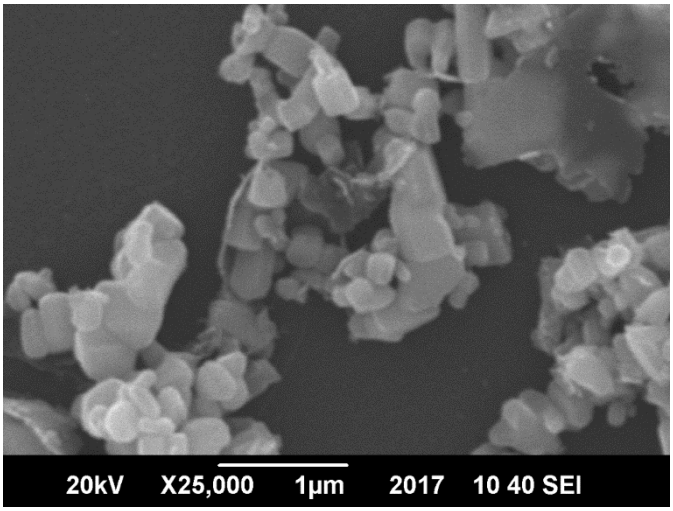


Figure 5(b). SEM image of ZnO.

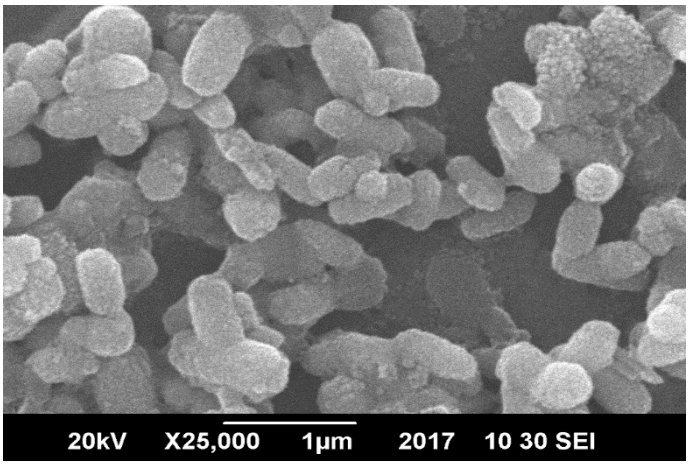


Figure 5(c). SEM image of Fe_2O_3.

4.1.3. Photodegradation of antibiotics

4.1.3.1. Effect of time duration

Figure 6 (a) and (b) shows the impact of time duration on degradation of both antibiotics Amx and Lev by synthesized nanoparticles, TiO_2 nanoparticles at 50 mg L^{-1}. It was observed that degradation of both antibiotics was increased with increase in time.

In Amx, minimum degradation observed by control was 25.05% and maximum by TiO_2 was 26.37% on 1st day. Similarly, on 9th day, maximum degradation observed was 64.5% and minimum was 56% by TiO_2 and control respectively. In Lev, minimum degradation observed by control was 11.75% and maximum by TiO_2 was 23.09% on 1st day. Similarly, on 9th day, maximum degradation observed was 67.56% and minimum was 41.23% by TiO_2 and control, respectively.

The time at which significant maximum degradation of both antibiotics in the presence of TiO_2 and visible light observed was on 9th day. On 9th day TiO_2 showed significantly 24.93% increase in photo degradation of Amx in comparison to 1st day whereas TiO_2 showed significantly 63.88% increase in photo catalysis of Lev in comparison to 1st day.

Since there was no any significant difference between the degradation percent on 7th and 9th day therefore the optimum time period for degradation of Amx and Lev in aqueous solution in the presence of titania nanoparticle was 7th day as. Moreover, it was more feasible to carry batch reaction on this time.

The increase in degradation with respect to time can be attributed to increase in light intenisty. The increase in light intensity causes the more production photons which produces more number of electron hole pairs and thus enhances the formation of oxidizing radicals (Dong et al., 2010).

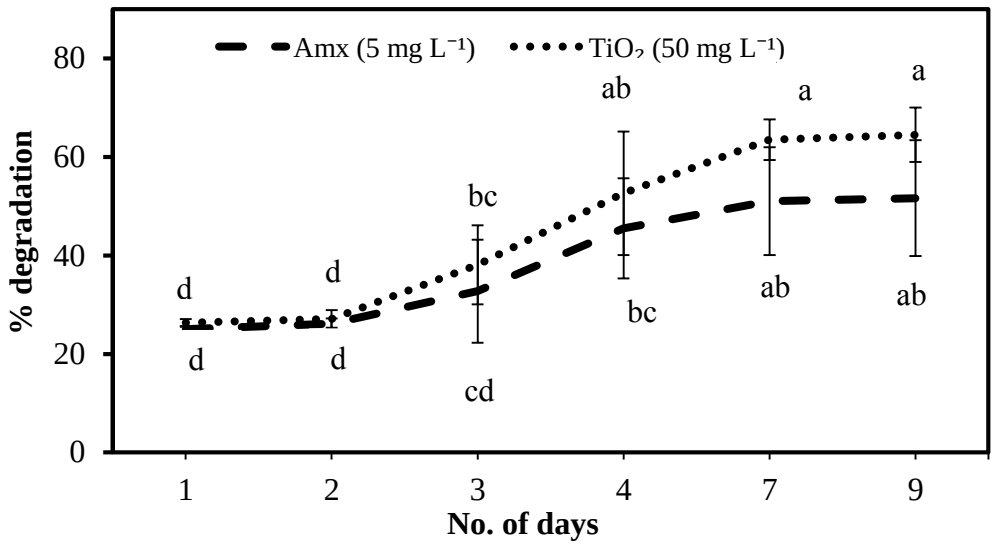


Figure 6(a). Effect of time on degradation of Amx.

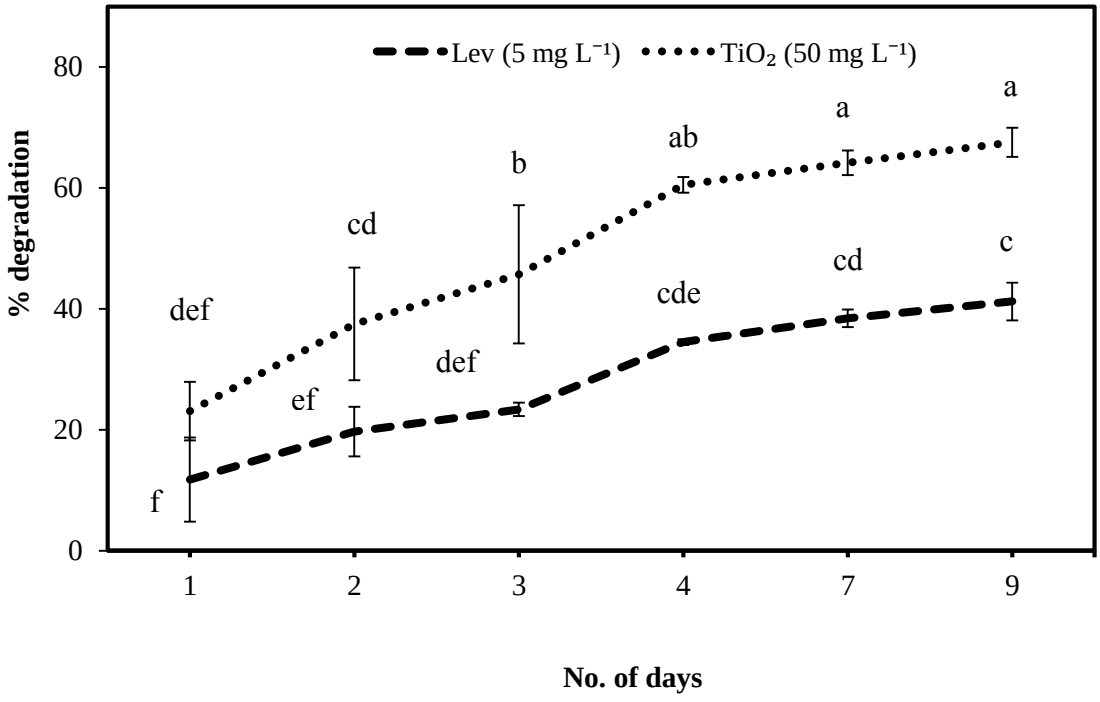


Figure 6(b). Effect of time on degradation of Lev.

4.1.3.2. Effect of different nanoparticles

In order to check the effect of different types of nanoparticles on photodegradation of antibiotics Amx and Lev, experiment was conducted at 5 mg L^{-1} concentration of selected antibiotics having pH 5 by using TiO_2, ZnO and Fe_2O_3. Figure 7(a) shows that Fe_2O_3 has higher significant impact on degradation of Amx whereas Figure 7(b) shows that TiO_2 has significantly greater impact on degradation of Lev ($p < 0.05$).

At the dose of 50 mg L^{-1} of each nanoparticles and time period of 120minutes, maximum degradation of Amx observed by Fe_2O_3 was 36.49% whereas minimum by control was 10.35%. Similarly, the maximum degradation of Lev observed by TiO_2 was 25.01% and minimum by control was 6.96%. At this time period with similar dose, the decreasing order for photodegradation of Amx was Fe_2O_3>TiO_2>ZnO. Fe_2O_3 showed an increase of 2.68-fold in degree of photodegradation as compared to control, followed by TiO_2 which showed an increase of 2.1-fold followed by ZnO showing an increase of 1.48-fold in photocatalysis of Amx in comparison to control. This can be attributed to the smallest crystal size of Fe_2O_3 nanoparticles than other synthesized nanoparticles. Similarly, at this time period and similar dose, the decreasing order for photo degradation of LEV was TiO_2>ZnO>Fe_2O_3. TiO_2 showed an increase of 2.59-fold in degree of photodegradation as compared to control, followed by ZnO which showed an increase of 1.3-fold, followed by Fe_2O_3 showing an increase of 0.33-fold in photo catalysis of Lev in comparison to control. In a study Eskandari et al. (2018) found that percent removal of CIP decreased with increase in turbid solution of Zn) nanoparticles.

It has observed that efficiency of photodegradation has increased with the use of nanoparticles. This could be due to production of surplus hydroxyl radicals (OH·) when

electrons from conduction band of nanoparticle travels towards valence band (Safari et al., 2015). When light energy is more than gap energy of NPs, holes (h^+) from valence band and electrons (e^-) from conduction band are photo generated. These holes act as oxidizing agents and oxidize antibiotics whereas electron react with electron acceptors, O_2, and produce superoxide, $O_2^{\cdot-}$, which helps in degradation of antibiotics (Dong et al., 2010).

The preferred nanoparticles for degradation of Amx and Lev in aqueous solution for time duration of 2 hours was TiO_2 as it showed better photo catalysis for both antibiotics and it degrades faster (Ambrosetti et al., 2015).

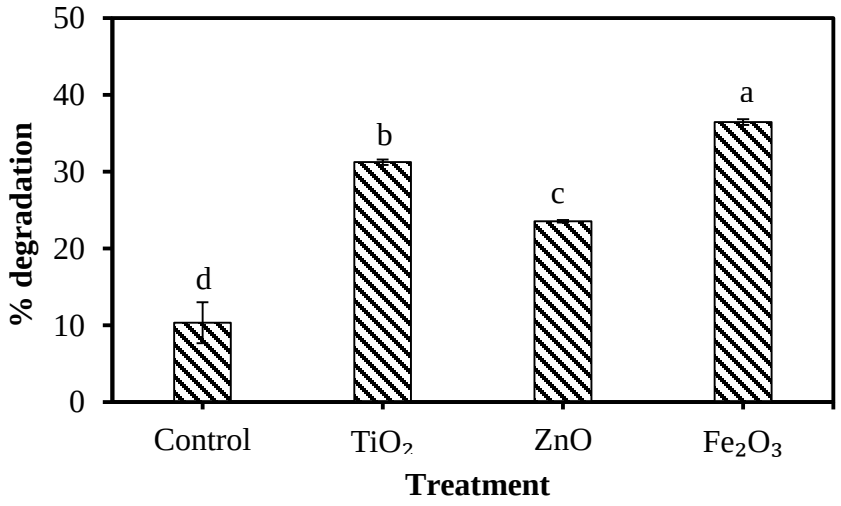


Figure 7(a) Effect of different nanoparticles on Amx.

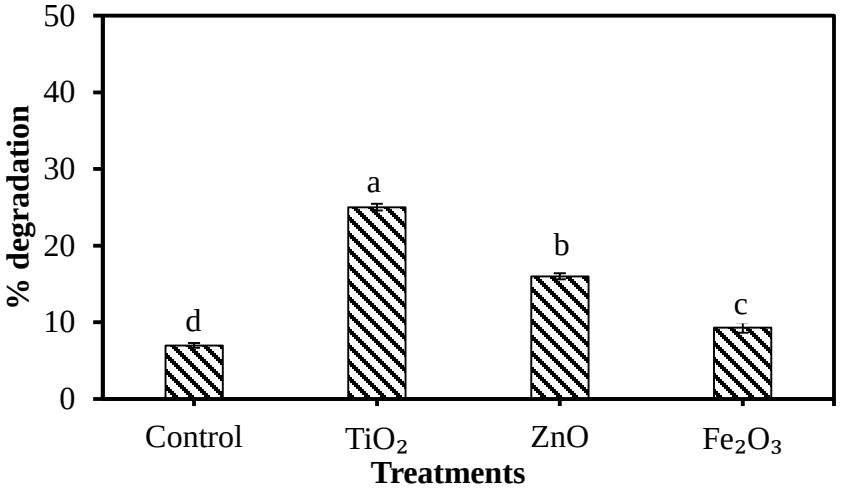


Figure 7(b) Effect of different nanoparticles on Lev.

4.1.3.4. Effect of different nanoparticle concentration

To observe the effect of nanoparticles concentration, initial nanoparticles concentration was varied in the range 40-60 mg L^{-1}. The experimental conditions were Amx and Lev concentrations 5 mg L^{-1}, time period 7 days and pH 5. Figure 8 (a) and (b) shows the degradation of Amx and Lev. TiO_2 at 60 mg L^{-1} significantly degraded the Amx and Lev ($p < 0.05$).

Degradation percent after 7 days irradiations were 43.4%, 66.3% and 72.6% for Amx and 47.3%, 56.3%and 65.6% for Lev at TiO_2 concentrations 40, 50 and 60 mg L^{-1} respectively. Degradation percent after 7 days irradiations were 40.3%, 70.9% and 48.7% for Amx and 34.6%, 43.9% and 48.6 % for Lev at ZnO concentrations 40, 50 and 60 mg L^{-1} respectively. Degradation percent after 7 days irradiations were 40.2%, 51% and 62.7% for Amx and 31.3%, 35.1% and 50.5 % for Lev at Fe_2O_3 concentrations 40, 50 and 60 mg L^{-1} respectively. The maximum degradation percent for Amx and Lev was observed by TiO_2 at 60 mg L^{-1} concentration and minimum was at control. In case of TiO_2 and Fe_2O_3, degradation of both Amx and Lev increased with increasing concentration of both nanoparticles ranging from 40-60 mg L^{-1}. This can be attributed to the fact that increase in concentration of nanoparticles produces more number of hydroxyl radicals which increases the number of active sites available for more adsorption of antibiotic molecules on nanoparticle surface (Safari et al., 2015). In case of ZnO, degradation percent of Amx and Lev was increased with increasing concentration of ZnO ranging from 40-50 mg L^{-1}. It showed maximum degradation percent for Amx and Lev at 50 mg L^{-1} and minimum degree of degradation was observed at 40 mg L^{-1}. It was worth noted that further increase of ZnO concentration above 50 mg L^{-1} resulted in decrease in degradation percentage of Amx and

Lev. This can be attributed that increase loading of nanoparticles causes agglomeration of ZnO particles and increases turbidity which reduce light penetration by reducing catalyst surface available for light absorption and results in increase in scattering of light (Elmolla and Chaudhri, 2010; Eskandari et al., 2018: Safari et al., 2015). Moreover, at higher nanoparticle density, deactivation of originally activated NPs take place through collision with catalyst at ground state (Safari et al., 2015). The optimum concentration of nanoparticle for degradation of Amx and Lev in aqueous solution in seven days was 50 mg L^{-1} since it was observed that increase in NPs concentration greater than 50 mg L^{-1} did not produce any significant improvement in Amx and Lev degradation. Moreover, it was also worth noticed that there was no significant difference in degradation efficiency of Lev by ZnO and Fe_2O_3 at 60 mg L^{-1} and TiO_2 at 50 mg L^{-1} Therefore the optimum nanoparticle was TiO_2 with 50 mg L^{-1} as optimized concentration for degradation of Amx and Lev. At this dose TiO_2 showed an increase of 80.6% and 1.77-fold in degradation percentage of Amx and Lev respectively in comparison to control. Fe_2O_3 showed an increase of 39.11% and 0.73-fold in degradation percentage of Amx and Lev respectively as compared to control. ZnO showed an increase of 93.3% and 1.16-fold in degradation percentage of Amx and Lev respectively in comparison to control. It was also observed that there was no significant difference between percent photodegradation of Amx by ZnO at 50 mg L^{-1} and TiO_2 at 60 mg L^{-1}. This could be due to fact that ZnO has same photo degradation mechanism and it also contains wide band gap as compare to TiO_2 therefore large portion of solar light can be absorbed by ZnO (Eskandari et al., 2018; Ambrosetti et al., 2015).

These results are in accordance with the results reported by Safari et al. (2015) who studied the photo degradation of TC in waste water by using TNPs. They found 90% degradation

of TC by using 1 g L^{-1} TNPs. Similarly, Palominos et al. (2009) found 1 and 1.5 g L^{-1} as optimized dose of ZnO and TiO_2 respectively for degradation of TC in wastewater. In another study Eskandari et al, (2018) investigated photolysis of CIP by using ZnO nanoparticles in the presence of UVC that percent degradation of CIP was found by using 150 mg L^{-1} concentration of ZnO in 10 mg L^{-1} CIP aqueous solution having pH 5 at the time duration of 140 minutes.

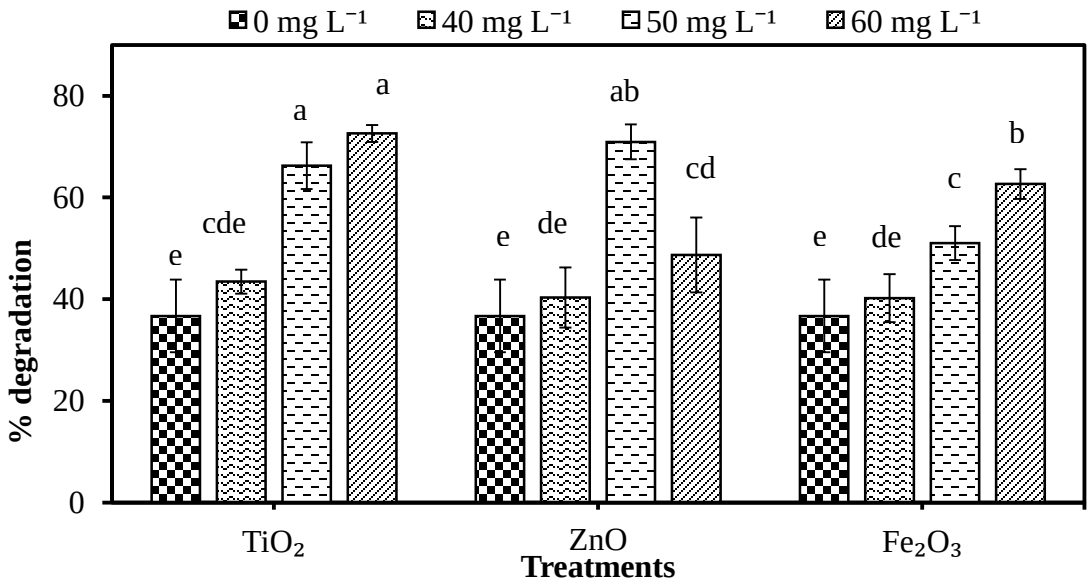


Figure 8(a) Effect of different nanoparticle concentrations on Amx.

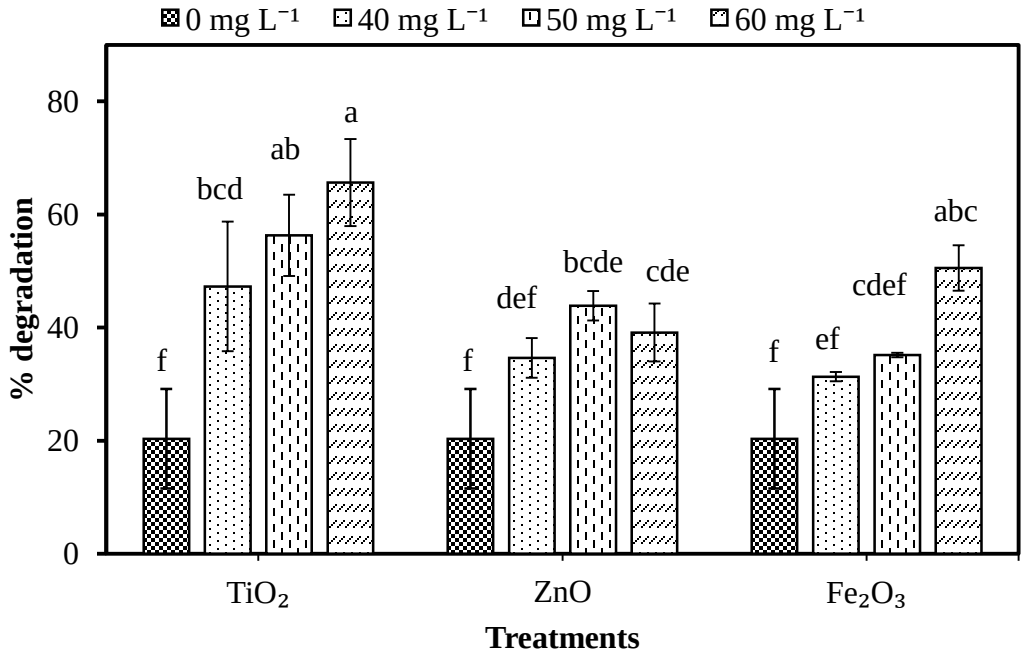


Figure 8(b). Effect of different nanoparticle concentrations on Lev.

4.2. Phase II: Pot experiment

4.2.1. General characteristics of soil

Different physicochemical soil tests were performed before pot experiment. Soil was collected from NUST nursery having pH 7.5. Soil with pH<7.5 is considered best for the growth of high valuable crops. Soil was clay loam having total organic carbon (TOC) 2.25%. The concentration of N, and P was 1.82 mg kg^{-1} and 96 mg kg^{-1} respectively. Table 1 shows the physicochemical properties of soil.

Table 1. Physicochemical characteristics of soil

Soil parameters	Results
pH	7.5
EC (μScm^{-1})	728
Moisture content (%)	7.4
Texture	Clay loam (54.55%)
Total organic carbon (%)	2.25
WHC (%)	75
Phosphates (mg kg^{-1})	96
Nitrates (mg kg^{-1})	1.82

4.2.2. Plant growth parameters

The objective of present study was to check the impact of nanoparticles on growth of selected crop, wheat. Physical growth parameters like total plant length, dry root and shoot biomass, number of tillers and spikes per plant and grain weight were analyzed after exposure to antibiotics (Amx, Lev), nanoparticles and simultaneous application of

nanoparticles and antibiotics. The effect on various growth parameters are discussed in detail.

4.2.2.1. Total length

Figure 9 shows the impact of antibiotics, nanoparticles and simultaneous impact of both antibiotics with nanoparticles on wheat growth. It can be observed that TiO_2 significantly released the antibiotic stress ($p < 0.05$).

Increase in plant length was observed by TiO_2, TiO_2+Amx and TiO_2+Lev. Maximum plant length 92 cm was observed in TiO_2+Amx and minimum 70 cm was in control. The positive effect of Amx was higher as compare to Lev as Amx showed 77 cm and Lev showed 75 cm total plant length. Among antibiotics, Amx showed greater plant length which was 77 cm as compared to Lev which was 75 cm. Similarly, among simultaneous application of antibiotics with nanoparticles, TiO_2+Amx showed greater plant length as compared to TiO_2+Lev. The overall percentage increase in plant length by TiO_2, Lev, Amx, TiO_2+Lev and TiO_2+Amx was in the order of 3.6%, 7.6%, 9.9%, 21% and 31.2% respectively with respect to control.

Lower concentrations of antibiotics have been reported to induce no significant negative impact on total length. Zhang et al. (2017) found that vegetable pakchoi exposed to 50% minimum inhibitory concentration (MIC) of three types of antibiotics like tetracycline (TC), cephalexin (CEP) and sulfmethoxazole (SMX) showed better biomass production and growth as compare to control. However, at MIC level, both growth and biomass were decreased. This study suggested that antibiotics at very low concentration could be beneficial for plants growth. Another study reported an increase of 36% in shoot length of TNPs treated lettuce (250 mg kg^{-1}) as compared to control (Zahra et al., 2015).

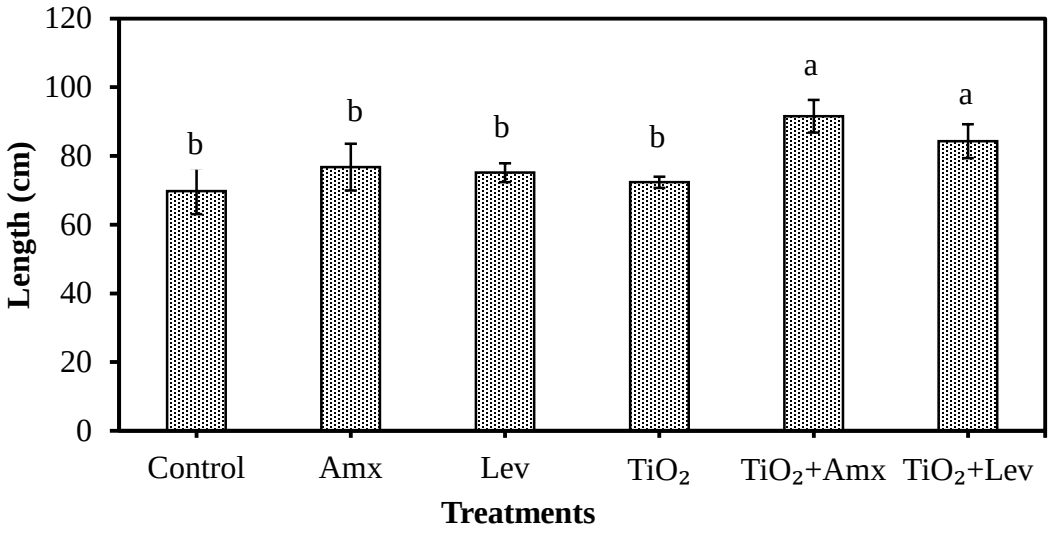


Figure 9. Effect of antibiotics, TiO_2 and combined effect of antibiotics and TiO_2 on total length of wheat.

4.2.2.2. Dry root and shoot weight

Figure 10(a) and (b) shows the impact of antibiotics (Amx and Lev), TiO_2 and application of TiO_2 in the presence of antibiotics (TiO_2+Amx and TiO_2+Lev) on dry weight of root and shoot respectively. It can be observed that TiO_2 showed the significant impact on dry root and shoot weight of wheat ($p < 0.05$).

In the presence of TiO_2, increase in weight of root and shoot was observed. Whereas Amx, Lev, TiO_2+Amx and TiO_2+Lev showed decrease in weight of root and shoot. Maximum root and shoot weight recorded was 0.57g and 0.93g respectively by TiO_2. Minimum root and shoot weight observed was 0.14g and 0.43g respectively at Lev. Among antibiotics, minimum root weight was 0.14g at Lev and maximum root weight observed was 0.19g at Amx. Maximum shoot weight recorded was 0.6g at Amx and minimum shoot weight observed was 0.43g at Lev. Among simultaneous treatments of both antibiotics and nanoparticles, minimum root weight was 0.25g at TiO_2+Lev and maximum root weight

observed was 0.44g at TiO_2+Amx. Maximum shoot weight recorded was 0.69g at TiO_2+Amx and minimum shoot weight observed was 0.66g at TiO_2+Lev.

The treatment at which both root and shoot weight improved was TiO_2 at 50 mg kg^{-1}. At this treatment root and shoot weight showed an increase of 26.35% and 24.2% respectively in comparison to control. The overall percentage decrease in dry root weight by TiO_2+Amx, TiO_2+Lev, Amx and Lev was in order of 1%, 45%, 58.74% and 68.41% with respect to control. The overall percentage decline in dry shoot weight was in order of 8.3%, 12.3%, 19.7% and 41.9% in comparison to control for similar order of treatments. However, it can be observed that dry root and shoot weight in TiO_2+Amx and TiO_2+Lev was higher than that observed with Amx and Lev respectively.

Increase in wheat biomass by TiO_2 can be attributed to the increase in nutrients uptake and photosynthesis as TiO_2 controls enzymes of nitrogen metabolism i.e. glutamate dehydrogenase, nitrate reductase, and glutamic-pyruvic transaminase glutamine synthase that promotes nitrate absorption, biosynthesis of chlorophyll and leading to enhance biomass (Lyu et al., 2017; Yang et al., 2006). For wheat, diameter of TNPs should less than 140nm and 36nm in order to pass through root epidermis and parenchyma respectively. Moreover, TNPs activates membrane acceptors which activates endocytosis through which they also enter into plant cell (Lyu et al., 2017). Similarly, during the investigation of wheat treated with TNPs has increased root, shoot length and biomass up to 60 mg kg^{-1} while growth was decreased at higher concentrations (Rafique et al., 2014).

However, antibiotics reduce the phosphorus levels in plants which inhibits carbon assimilation and hence decreases the biomass of shoot (Nielsen et al., 2001). Phosphorus concentration in a plant is key indicator of root and shoot biomass (Fageria et al., 1988).

In a study, Li et al. (2011) exposed OTC-resistant and sensitive wheat cultivars to four different concentrations of antibiotics 0.01, 0.02, 0.04 and 0.08 mg L^{-1} and noted a decline in shoot length by 3.3%-8.57% while shoot biomass was decreased by 5.6%-13.75%.

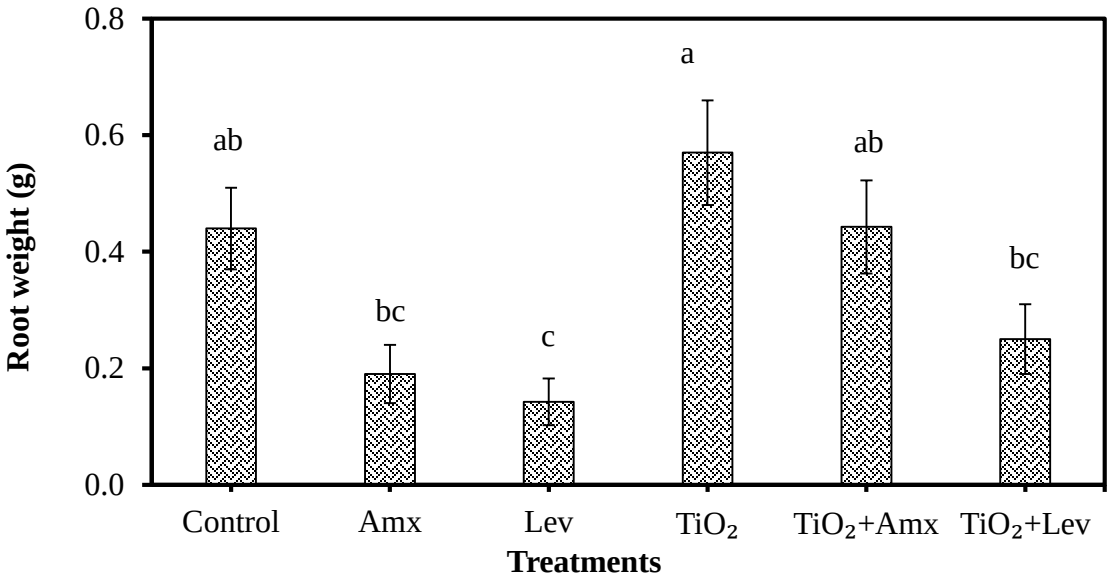


Figure 10(a). Effect of antibiotics, TiO_2 and combined effect of antibiotics and TiO_2 on dry root weight of wheat.

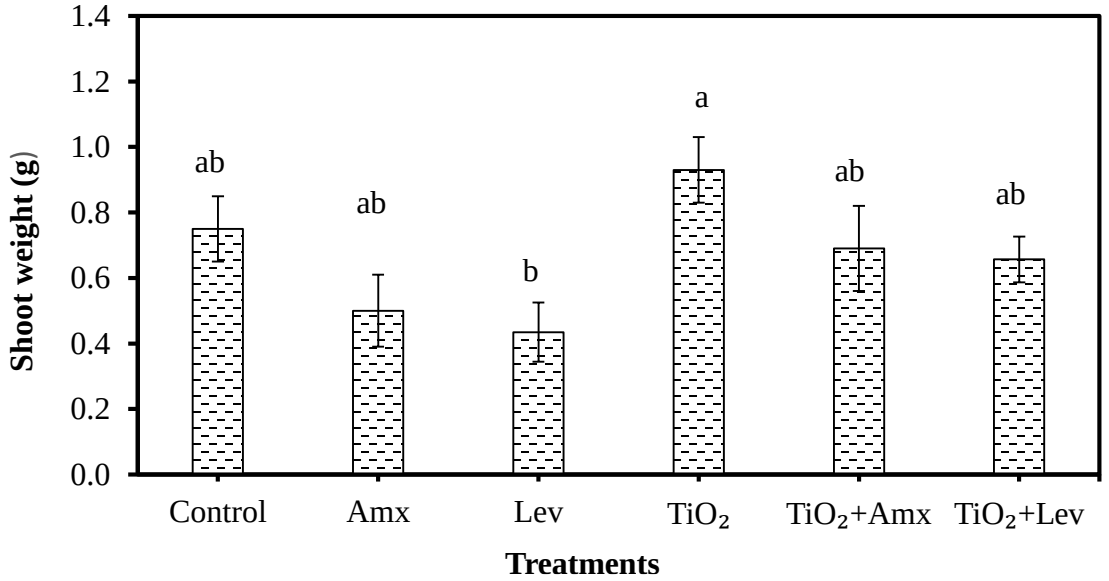


Figure 10(b). Effect of antibiotics, TiO_2 and combined effect of antibiotics and TiO_2 on dry shoot weight of wheat.

4.2.2.3. Grain weight

Figure 11 shows the impact of antibiotics (Amx and Lev), TiO_2 and simultaneous application of both antibiotics with TiO_2 (TiO_2 +Amx, TiO_2 +Lev) on grain weight. It can be observed that TiO_2 showed the significant impact on grain weight of wheat ($p < 0.05$). In the presence of TiO_2, increase in weight of grain was observed. Whereas Amx, Lev, TiO_2 +Amx and TiO_2 +Lev showed decrease in grain weight. Maximum grain weight recorded was 0.88 g at TiO_2 and minimum observed was 0.23g at Lev. Among antibiotics, minimum grain weight was 0.23g at Lev and maximum grain weight observed was 0.3g at Amx. Among simultaneous treatments of both antibiotics and nanoparticles, minimum grain weight was 0.6g at TiO_2 +Lev and maximum grain weight observed was 0.69g at TiO_2 +Amx.

The treatment at which highest grain weight found was TiO_2 at 50 mg kg^{-1}. At this treatment grain weight showed an increase of 231% in comparison to control. The overall percentage decrease in grain weight by TiO_2 +Amx, TiO_2 +Lev, Amx and Lev was in order of 2.9%, 15.4%, 57.7% and 67.9% with respect to control. However, it can be observed that weight of grain in TiO_2 +Amx and TiO_2 +Lev was higher than that observed with Amx and Lev respectively.

During water movement, proteins and starch stored into leaves move into grains at final stage of maturity. During this movement, antibiotics also transfer into the grains along with water and accumulates into grains (Franklin et al., 2016). On the other hand, Razzaq et al. (2016) stated that TNPs at 25 and 50 mg kg^{-1} have highly promising impacts on grain yield of wheat.

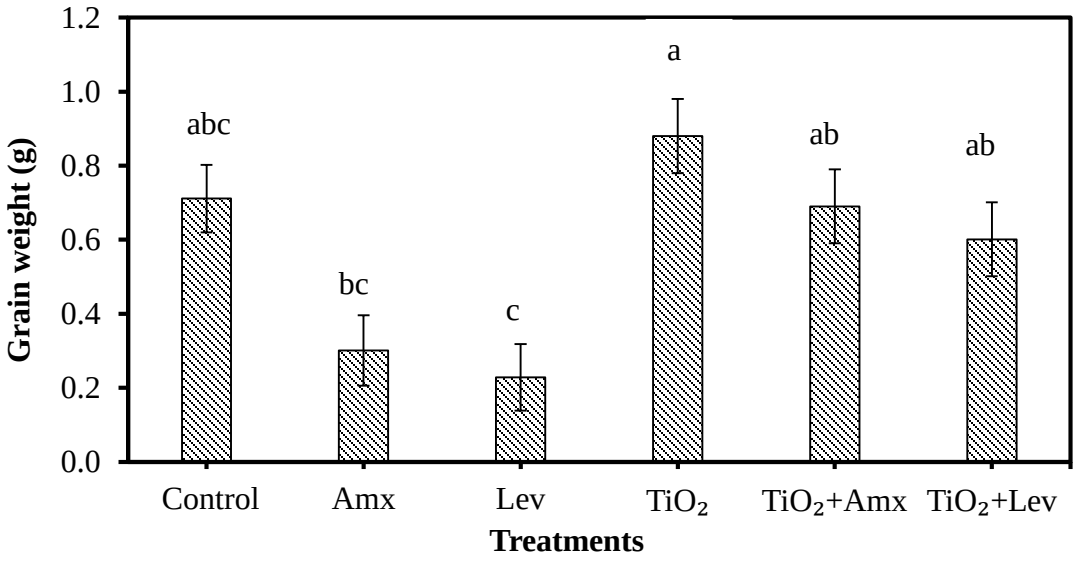


Figure 11. Effect of antibiotics, TiO_2 and combined effect of antibiotics and TiO_2 on grain weight of wheat.

4.2.2.4. No of tillers per plant

Figure 12 shows the impact of antibiotics (Amx and Lev), TiO_2 and simultaneous application of both antibiotics with TiO_2 (TiO_2+Amx, TiO_2+Lev) on number of tillers per plant. It can be observed that TiO_2 showed the significant impact on number of tillers of wheat ($p < 0.05$).

In the presence of TiO_2, increase in number of tillers per plant was observed whereas Amx and Lev showed decrease in number of tillers. However, same number of tillers were observed in TiO_2+Amx and TiO_2+Lev as in control. Maximum number of tillers per plant recorded at $TiO_2$and minimum observed at Amx and Lev.

The treatment at which the highest number of tillers per plant found was TiO_2. At this treatment tillers showed an increase of 50% in comparison to control. The overall percentage decrease in tillers by Amx and Lev was 50% with respect to control. However, there was neither increase nor decrease in number of tillers by TiO_2+Amx, TiO_2+Lev with

respect to control. However, it can be observed that number of tillers in TiO_2+Amx and TiO_2+Lev was higher than that observed with Amx and Lev respectively.

Previous studies suggested that TiO_2 nanoparticles stimulate the number of tillers in Barley (*Hordeum vulgare*) plant by promoting the development of secondary shoots (Marchiol et al., 2016). Noonari et al. (2016) found that wheat plants also showed significant increment in number of tillers with increasing levels of phosphorus in contrast to control. This increase in number of tillers can be attributed to high level of phosphorus which stimulates the root development and tillering (Hasanuzzaman et al., 2012; Khan & Imtiaz, 2013).

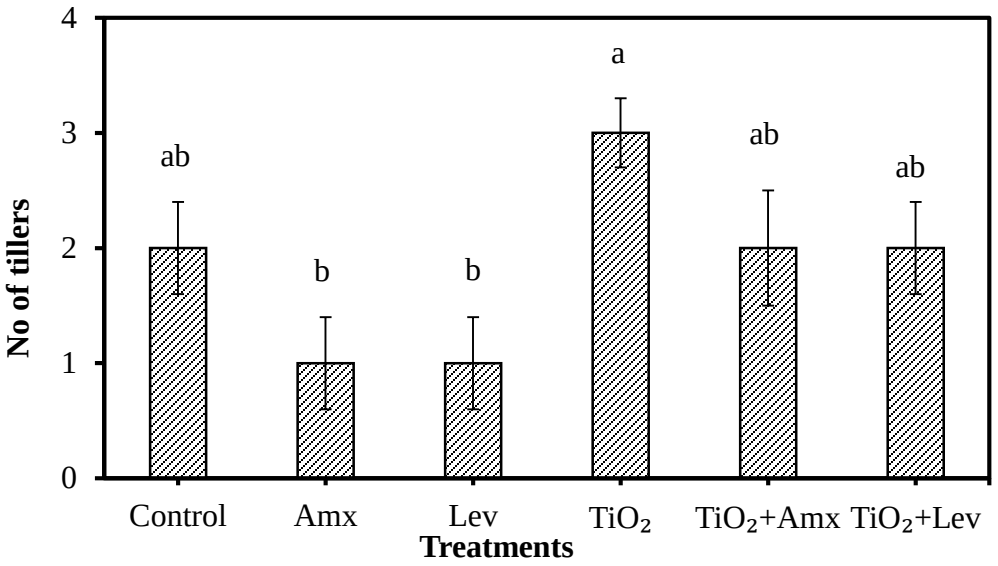


Figure 12. Effect of antibiotics, TiO_2 and combined effect of antibiotics and TiO_2 on number of tillers per plant of wheat.

4.2.2.5. No of spikes per plant

Figure 13 shows the impact of antibiotics (Amx and Lev), TiO_2 and simultaneous application of both antibiotics with TiO_2 (TiO_2+Amx, TiO_2+Lev) on number of spikes per plant. It can be observed that TiO_2 showed the significant impact on number of spikes of wheat ($p < 0.05$).

In the presence of TiO_2, increase in number of spikes per plant was observed whereas Amx and Lev showed decrease in number of spikes. However, same number of spikes were observed in TiO_2+Amx and TiO_2+Lev as in control. Maximum number of spikes per plant recorded at TiO_2 and minimum observed at Amx and Lev.

The treatment at which the highest number of spikes per plant found was TiO_2 at 50 mg kg^{-1}. At this treatment spikes showed an increase of 50% in comparison to control. The overall percentage decrease in sikes by Amx and Lev was 50% with respect to control. However, there was neither increase nor decrease in number of spikes by TiO_2+Amx and TiO_2+Lev with respect to control. However, it can be observed that number of spikes in TiO_2+Amx and TiO_2+Lev was higher than that observed with Amx and Lev respectively.

Razzaq et al. (2016) reported that TiO_2 promotes spikes production by the development of secondary shoots. During the study "uptake of antibiotics by plants", Azanu et al. (2016) found Amx in plants at higher concentration (27.1 ng g^{-1}) as compare to tetracycline (20.2 ng g^{-1}). Uptake of Amx in carrot was 14.3 to 45.2 ng g^{-1} whereas 13.7 to 33.6 ng g^{-1} was found in lettuce. During the investigation of antibiotics uptake by lettuce, Ahmed et al, (2015) found 88 ng g^{-1} tetracycline in lettuce leaves after the application of 10 mg L^{-1} antibiotic solution to it.

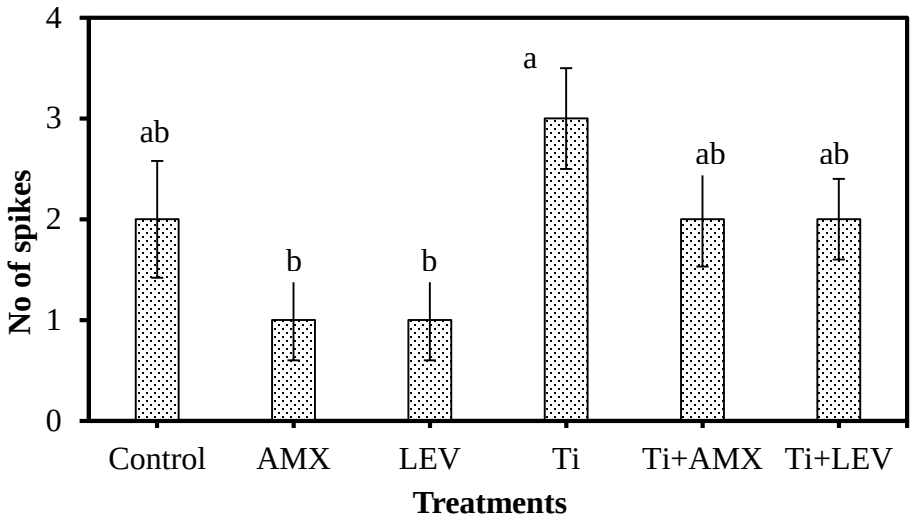


Figure 13. Effect of antibiotics, TiO_2 and combined effect of antibiotics and TiO_2 on number of spikes per plant of wheat.

4.2.3. Determination of nutrients in wheat

In order to check the impact of treatments on wheat growth, various nutritional composition like total phosphorus, total carbohydrates, total proteins and iron were analyzed after exposure to antibiotics (Amx, Lev), TiO_2 and simultaneous application of TiO_2 with antibiotics. The effect on various nutritional composition are discussed in detail.

4.2.3.1. Total phosphorus

Figure 14 (a) and (b) shows the relation of total phosphorus percentage in root and shoot respectively with antibiotics (Amx and Lev), TiO_2 and with both antibiotics and TiO_2 (TiO_2+Amx, TiO_2+Lev). It can be observed that TiO_2 showed the significant impact on total phosphorus in root and shoot of wheat ($p < 0.05$).

In the presence of TiO_2, increase in percentage of total phosphorus in both root and shoot was observed. Whereas Amx, Lev, TiO_2+Amx and TiO_2+Lev showed decrease in percentage of total phosphorus in root and shoot. The maximum percentage of total phosphorus in root and shoot was 2.19% and 1.63% respectively at TiO_2. The minimum

percentage of total phosphorus in root and shoot observed 1.1% and 0.91% respectively at Lev. Among antibiotics, the minimum percentage of total phosphorus in root was 1.1% at Lev and the maximum percentage of total phosphorus in root observed was 1.2% at Amx. The maximum percentage of total phosphorus in shoot recorded was 1.08% at Amx and minimum shoot weight observed was 0.91% at Lev. Among simultaneous treatments of both antibiotics and TiO_2, minimum percentage of total phosphorus in root was 1.41% at TiO_2 +Lev and the maximum percentage of total phosphorus in root observed was 1.47% at TiO_2+Amx. The percentage of total phosphorus in shoot recorded was 1.21% in both TiO_2+Amx and TiO_2+Lev.

The treatment in which both root and showed performed considerable well results was by TiO_2. At this treatment the percentage of total phosphorus in root and shoot showed an increase of 39.5% and 10.1% respectively in comparison to control. The overall percentage decrease in percentage of total phosphorus in root by TiO_2+Amx, TiO_2+Lev, Amx and Lev was in order of 6.4%, 10.2%, 23.6% and 29.9% respectively with respect to control. The overall percentage decline in percentage of total phosphorus in shoot was in order of 18.2%, 18.2%, 27% and 38.5% in comparison to control for similar order of treatments. However, it can be observed that percentage of total phosphorus in TiO_2+Amx and TiO_2+Lev was higher than that observed with Amx and Lev respectively.

It is proved from the previous studies that TNPs increases the uptake of phosphorus in plants because TiO_2 expresses yellow stripe (YS1) which eventually increases uptake of iron and enhances the photosynthetic rate by increasing the biosynthesis of chlorophyll. This eventually causes nitrate photo assimilation in which integration of nitrate ion (NO_3^-) with chloroplast take place and hence enhances the nitrogen uptake in plants by dominating

the nitrogen transporter gene. Moreover, YS1 also helps in binding of metals like Zn^{2+}, $Cu^{2+,}$ and Ni^{2+}; Ca, Mg, P and other nutrients with DMA and hence improves plants growth (Lyu et al., 2017). Moreover, TiO_2 possess high surface reactivity that may cause the enlargement of root pores and therefore, promotes water uptake and nutrients availability to plants (Larue et al., 2012). Current study results coincide with result found by Raliya et al. (2016) that ZnO nanoparticles synthesized from soil fungi increased the mobilization and availability of phosphorus in mung bean rhizosphere. Kim & Li (2016) found accumulation of phosphorus in all plant organs, but primarily in roots, whereas further increasing the concentration increased the acquisition predominantly in shoots and flowers. A study was conducted for assessment of the phytoavailability of phosphorus applied with TiO_2 nano particles. TiO_2 with concentration levels: 0, 25, 50, 75 and 100 mg kg^{-1} was applied. Concentration of phytoavailable phosphorus in soil containing lettuce culture was analyzed and it was found that phosphorus increased up to 83% with treatment of TiO_2 nanoparticles. In addition to this increase in root/shoot lengths 1.5-fold, total dry biomass by 2-fold and total phosphorus uptake by 4-fold was observed (Hanif et al., 2015).

During the study of effects of nine antibiotics on the physiology and secondary metabolites of wheat at the concentrations of 0.5 and 1.5 mg L^{-1}. Opris et al. (2013) reported that ciprofloxacin and cephalosporins caused a stomatal reduction thereby influencing the net assimilation. A reduction in its photosynthetic responses in chlorophyll, pigments and carotids was also observed by application of ciprofloxacin, tetracycline and erythromycin.

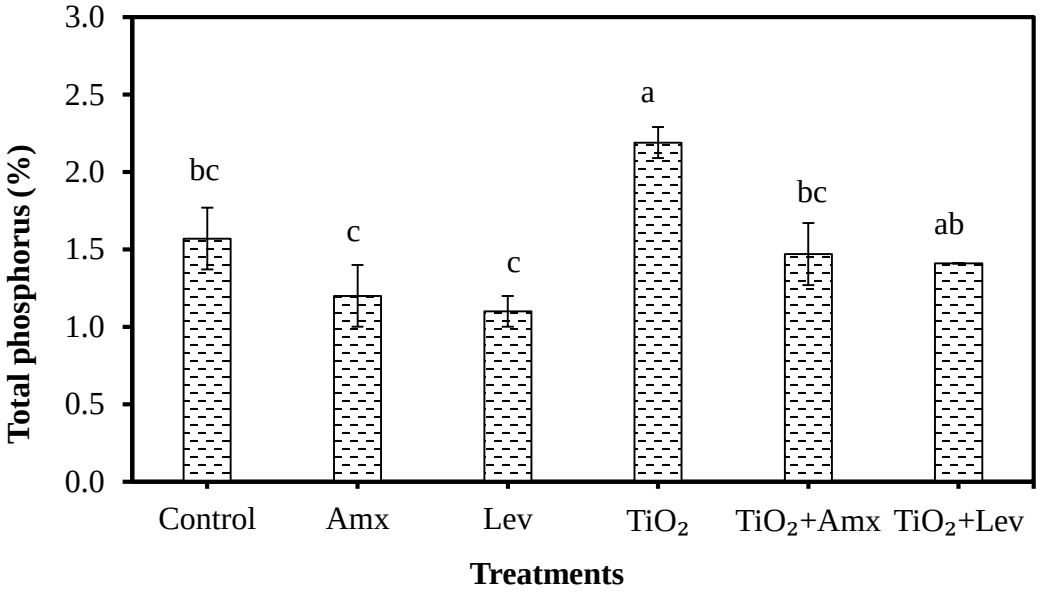


Figure 14(a). Effect of antibiotics, TiO_2 and combined effect of antibiotics and TiO_2 on total percentage of phosphorus in roots of wheat.

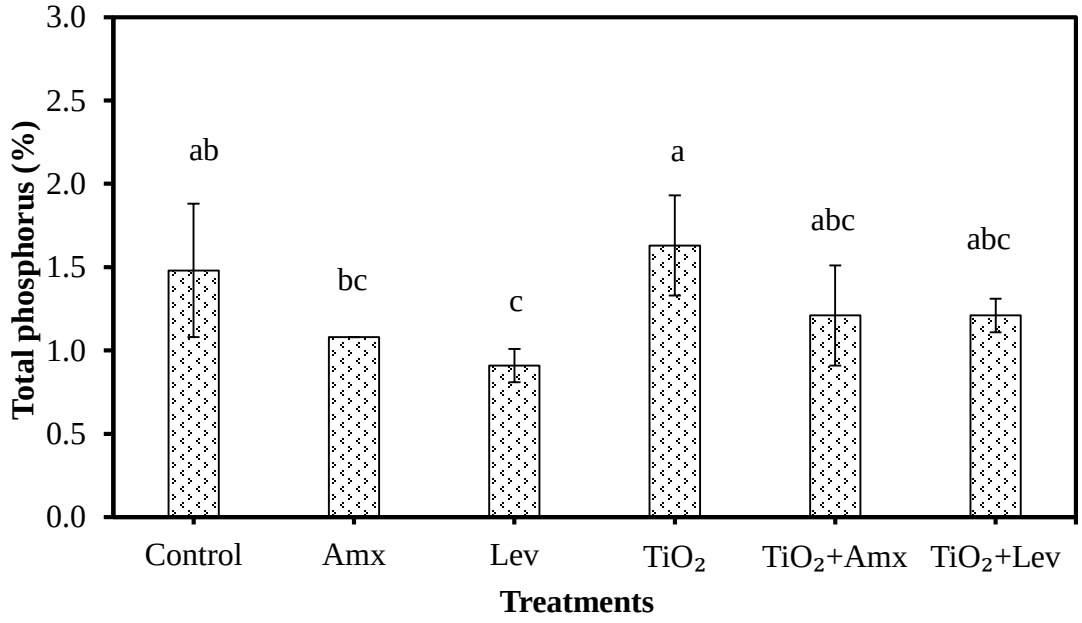


Figure 14(b). Effect of antibiotics, TiO_2 and combined effect of antibiotics and TiO_2 on total percentage of phosphorus in shoots of wheat.

4.2.3.2. Total iron in roots and shoots

Figure 15 (a) and (b) shows the relation of total iron in root and shoot with antibiotics (Amx and Lev), TiO_2 and TiO_2 with both antibiotics (TiO_2+Amx, TiO_2 +Lev). It can be observed that TiO_2 reduced the antibiotic stress caused by Amx and Lev and showed the significant impact on total iron in root and shoot of wheat ($p < 0.05$).

In the presence of TiO_2, increase in total iron in both root and shoot was observed. Whereas Amx, Lev, TiO_2 +Amx and TiO_2 +Lev showed decrease in total iron in root and shoot. The maximum total iron in root and shoot was 82 and 6.93 mg kg^{-1} respectively by TiO_2 at 50 mg kg^{-1}. The minimum total iron in root and shoot observed 45.08 and 0.4 mg kg^{-1} respectively at Lev. Among antibiotics, the minimum total iron in root was 45.08 mg kg^{-1} at Lev and the maximum total iron in root observed was 52.13 mg kg^{-1} at Amx. The maximum total iron in shoot recorded was 0.99 mg kg^{-1} at Amx and minimum observed was 0.4 mg kg^{-1} at Lev. Among simultaneous treatments of both antibiotics and nanoparticles, minimum total iron in root was 61.4 mg kg^{-1} at TiO_2+Lev and the maximum total iron in root observed was 68.6 mg kg^{-1} at TiO_2+Amx. The maximum total iron in shoot recorded was 3.7 mg kg^{-1} in TiO_2+Amx and minimum was 3.2 mg kg^{-1} in TiO_2+Amx.

The treatment in which both root and showed performed considerable well results was TiO_2 at 50 mg kg^{-1}. At this treatment the percentage of total iron in root and shoot showed an increase of 14% and 17% respectively in comparison to control. The overall percentage decrease in percentage of total iron in root by mg TiO_2+Amx, TiO_2+Lev, Amx and Lev was in order of 4%, 14%, 27% and 37% respectively with respect to control. The overall percentage decline in percentage of total iron in shoot was in order of 37%, 45%, 83% and

93% in comparison to control for similar order of treatments. However, it can be observed titania reduced the antibiotic stress. Another study showed that TiO_2 helps in producing the expression of genes related to Fe attainment, leading to uptake and consumption of Fe and later enhancing plant growth (Lyu et al., 2017). Furthermore, Ti-ascorbate when sprayed on leaves of paprika pepper *(Capsicum annuum* L.*)* also displayed a significant rise of Fe

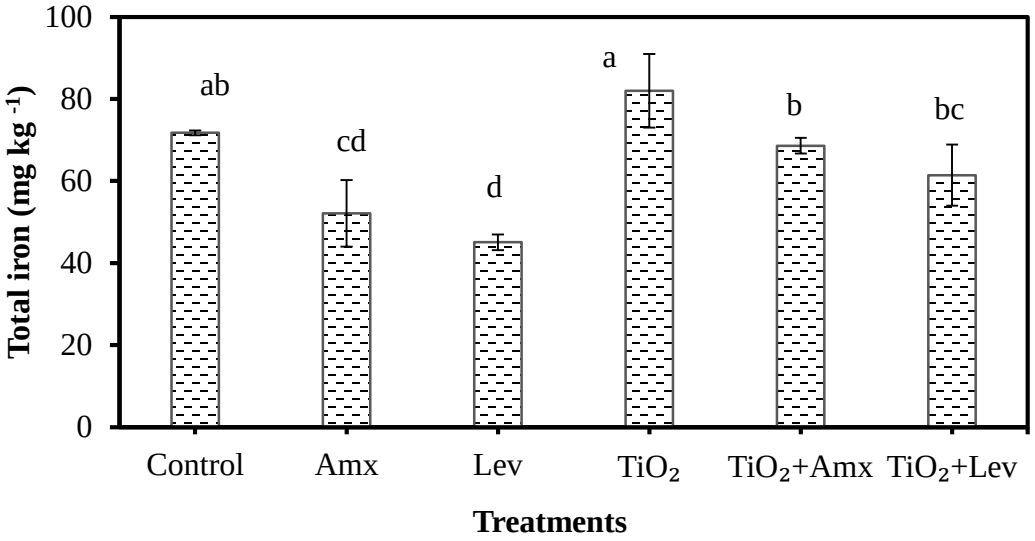


Figure 15(a). Effect of antibiotics, TiO_2 and combined effect of antibiotics and TiO_2 on total iron in roots of wheat.

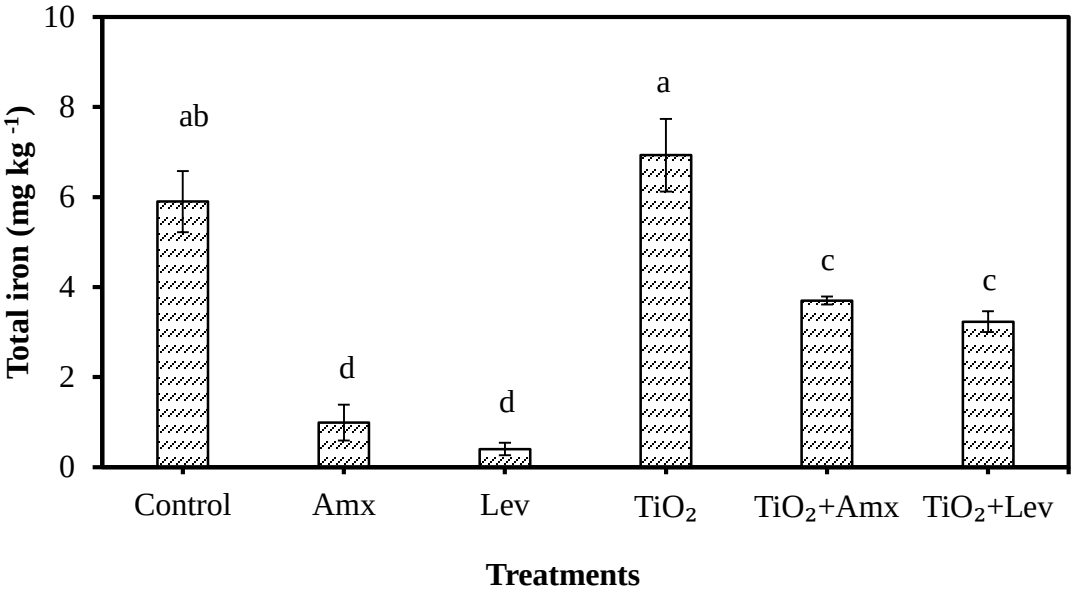


Figure 15(b). Effect of antibiotics, TiO_2 and combined effect of antibiotics and TiO_2 on total iron in shoots of wheat.

4.2.3.3. Total iron in grains

Figure 16 shows the relation of total iron in grains with antibiotics (Amx and Lev), titania (TiO_2) and with both antibiotics and nanoparticles (TiO_2+Amx, TiO_2+Lev). It can be observed that TiO_2 reduced the antibiotic stress and showed the significant impact on total iron in grains of wheat ($p < 0.05$).

In the presence of TiO_2, increase in total iron in grains was observed. Whereas Amx, Lev, TiO_2 +Amx and TiO_2 +Lev showed decrease in total iron in grains. The maximum total iron in grains was 35.4 mg kg^{-1} at TiO_2 and the minimum total iron in grains observed was 12.5 mg kg^{-1} at Lev. Among antibiotics, the total iron in grains was 14.5 and 12.52 mg kg^{-1} at Amx and Lev respectively. Among simultaneous treatments of both antibiotics and nanoparticles, minimum total iron in grains was 21.8 mg kg^{-1} at TiO_2+Lev and the maximum total iron in grains observed was 22.3 mg kg^{-1} at TiO_2+Amx.

The treatment in which grains performed considerable well results was TiO_2. At this treatment the total iron in grains showed an increase of 52% respectively in comparison to control. The overall percentage decrease in total iron in grains by TiO_2+Amx, TiO_2+Lev Amx and Lev was in order of 4%, 7%, 38%, and 46% respectively with respect to control. However, it can be observed that total iron in TiO_2+Amx and TiO_2+Lev was higher than that observed with Amx and Lev respectively.

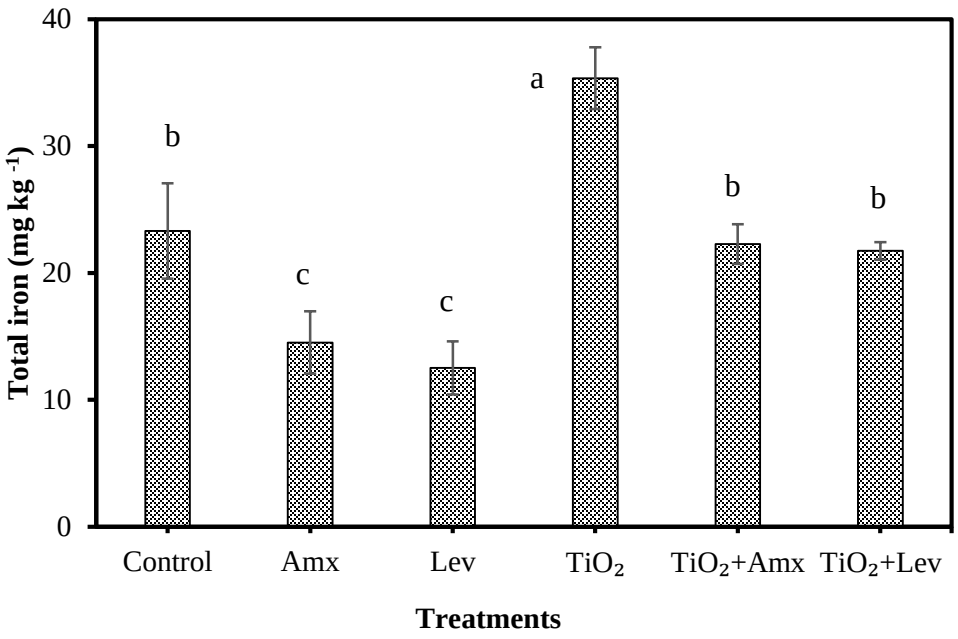


Figure 16. Effect of antibiotics, TiO_2 and combined effect of antibiotics and TiO_2 on total iron in grains of wheat.

4.2.3.4. Total carbohydrates

Figure 17 shows the relation of percentage of total carbohydrates in grains with antibiotics (Amx and Lev), nanoparticles (TiO_2) and with both antibiotics and nanoparticles (TiO_2 +Amx, TiO_2+LEV). It can be observed that TiO_2 reduced the antibiotic stress and showed the significant impact on total carbohydrates in grains of wheat ($p < 0.05$).

In the presence of TiO_2, increase in percentage of total carbohydrates in grains was observed. Whereas Amx, Lev, TiO_2+Amx and TiO_2+Lev showed decrease in percentage of total carbohydrates in grains. The maximum percentage of total carbohydrates in grains was 84% at TiO_2 and the minimum percentage of total carbohydrates in grains observed was 37.5% at Lev. Among antibiotics, the percentage of total carbohydrates in grains was 37.6% and 37.5% at Amx and Lev respectively. Among simultaneous treatments of both antibiotics and nanoparticles, minimum percentage of total carbohydrates in grains

observed was 54% at TiO_2+Lev and the maximum percentage of total carbohydrates in grains observed was 55.7% at TiO_2+Amx.

The treatment in which grains performed considerable well results was TiO_2. At this treatment the percentage of total carbohydrates in grains showed an increase of 38.5% respectively in comparison to control. The overall percentage decrease in percentage of total carbohydrates in grains by TiO_2+Amx, TiO_2+Lev, Amx and Lev was in order of 8.1%, 11%, 38%, and 38.1% respectively with respect to control. However, it can be observed that percentage of total carbohydrates in TiO_2+Amx and TiO_2+Lev was higher than that observed with Amx and Lev respectively.

Lower concentration of carbohydrates in wheat treated with Amx and Lev can be attributed to transfer of antibiotics from shoots to grains via phloem mobility. From xylem, water carrying antibiotics move into phloem and creates a driving force which allows antibiotics to reach into grain from shoots (Pan & Chu, 2018).

Uptake of antibiotics by plants ceases nitrification and reduces the decomposition of plant residues (Carvalho et al., 2014). During the study of plant-antibiotic interactions, Carvalho et al, (2014) found that mitotic index and root length of wheat was reduced in response to 250-300 mg L^{-1} chlortetracycline. However, a substantial enhancement was observed in root area, root length, shoot length root nodule, total soluble leaf protein and the chlorophyll content as an outcome of applied TiO_2 (Raliya et al., 2015). By comparing the effect of both antibiotics (Amx and Lev) on wheat, there was no significant difference between them. However, Lev showed higher negative impact due to its lower adsorption ability in soil when it is at neutral state. This lower ability to adsorb in soil leads to higher

accumulation in transpiring organs due to transportation in xylem via water mass flow movement (Pan & Chu, 2018; Franklin et al., 2016).

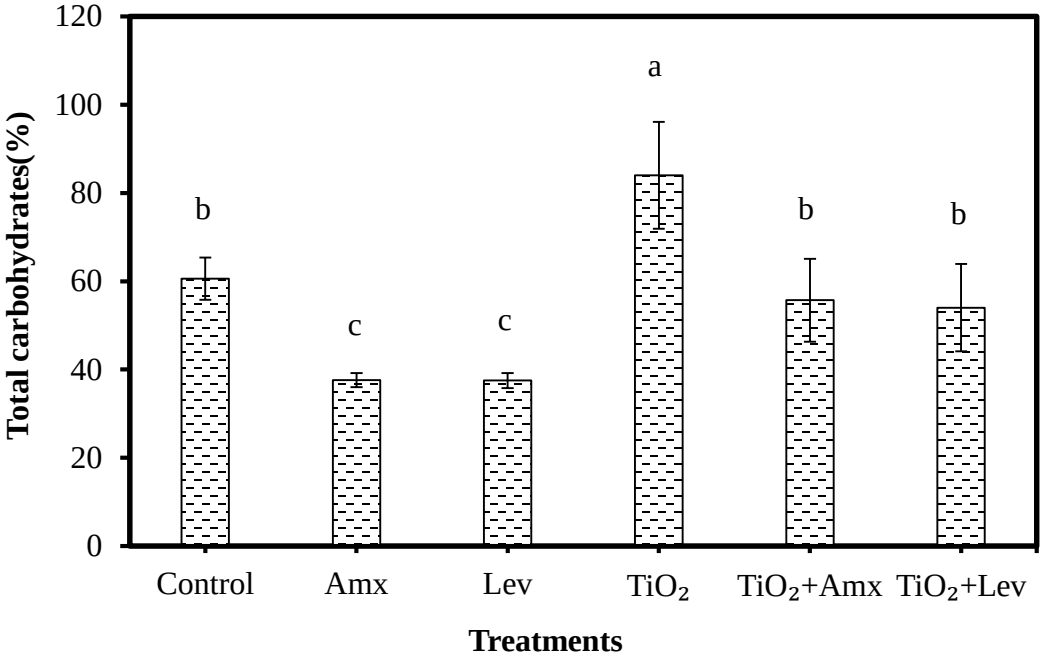


Figure 17. Effect of antibiotics, TiO_2 and combined effect of antibiotics and TiO_2 on total percentage of carbohydrates in grains of wheat.

4.2.3.5. Total protein

Figure 18 shows the relation of percentage of total proteins in grains with antibiotics (Amx and Lev), titania (TiO_2) and with both antibiotics and nanoparticles (TiO_2+Amx, TiO_2+Lev). It can be observed that TiO_2 reduced the antibiotic stress and showed the significant impact on total proteins in grains of wheat ($p < 0.05$).

In the presence of TiO_2, increase in percentage of total protein in grains was observed. Whereas Amx, Lev, TiO_2+Amx and TiO_2+Lev showed decrease in percentage of total protein in grains. The maximum percentage of total protein in grains was 22.5% at TiO_2 and the minimum percentage of total protein in grains observed was 8.8% at Lev. Among antibiotics, the percentage of total protein in grains was 9.4% and 8.8% at Amx and Lev

respectively. Among simultaneous treatments of both antibiotics and nanoparticles, minimum percentage of total protein in grains was 13.3% at TiO_2+Lev and the maximum percentage of total protein in grains observed was 14.7% at TiO_2+Amx.

The treatment in which grains performed considerable well results was TiO_2. At this treatment the percentage of total protein in grains showed an increase of 40% respectively in comparison to control. The overall percentage decrease in percentage of total protein in grains by TiO_2+Amx, TiO_2+Lev, Amx and Lev was in order of 8%, 7%, 41%, and 45% respectively with respect to control. However, it can be observed that percentage of total proteins in TiO_2+Amx and TiO_2+Lev was higher than that observed with Amx and Lev respectively.

During the study of effects of antibiotics on wheat, Riaz et al. (2017) found antibiotics interference in protein synthesis and chlorophyll with concentration 1.4 and 22.4 mg L^{-1} of antibiotics. Research conducted by Pošcic et al. (2016) suggested increase in amino acids and crude protein content of barley kernels as results of application of cerium and titanium dioxide nanoparticles.

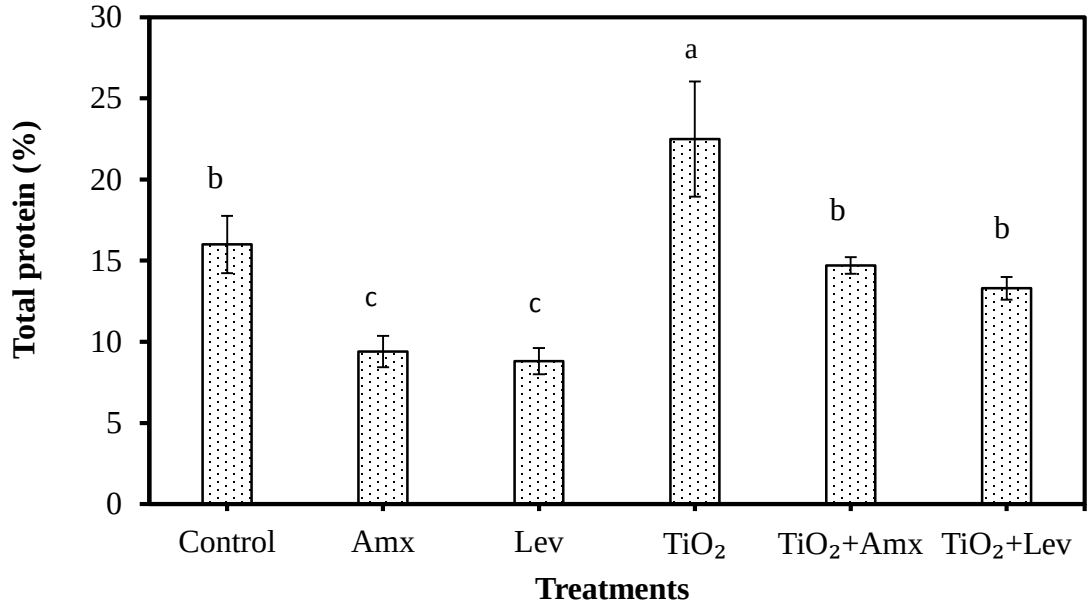


Figure 18. Effect of antibiotics, $TiO_2$and combined effect of antibiotics and TiO_2 on total percentage of protein in grains of wheat.

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

Bases upon the performance of titania nanoparticles and the impact of selected antibiotics on the growth of wheat crop and availability of nutrients and statistical analysis of the data, the following conclusions were drawn from the present study.

- No significant degradation was observed in the presence of visible light. However, the efficiency of photodegradation was increased with the use of nanoparticle and with increasing time duration, percent removal of degradation was improved. The most promising nanoparticle for degradation of Amx and Lev in aqueous solution was titania among all three nanoparticles.
- The optimum concentration of nanoparticle for degradation of Amx and Lev in aqueous solution in seven days was 50 mg L^{-1} since it was observed that increase of NPs concentration greater than 50 mg L^{-1} did not produce any significant improvement in Amx and Lev degradation. Thus 50 mg L^{-1} was selected to be used as recommended dose for increasing nutrients availability in wheat crop.
- The optimum operating conditions for maximum degradation of Amx and Lev in aqueous solution was 5 mg L^{-1} of both antibiotics having 50 mg L^{-1} TiO_2, an irradiation time of seven days.
- The percent photodegradation of Amx was higher as compared to Lev.
- Individual application of antibiotics (Amx) and (Lev) improved the wheat plant length but significantly decrease the dry root/shoot biomass, no of tillers and spikes,

weight of grain, total phosphorus and iron in roots and shoots, total carbohydrates and protein in grains. However, among both antibiotics, Lev had higher inhibitory impact on crops than Amx.

- Nutrient availability and other physical parameters were recorded higher at 50 mg kg^{-1} by TiO_2 in comparison to control.
- Moreover antibiotics stress was released when TiO_2 was applied. TiO_2 reduced the negative response of Amx and Lev and improved the total length, dry root/shoot biomass, no of tillers and spikes, weight of grain, total phosphorus and iron in roots and shoots, total carbohydrates and protein in grains. Hence food insecurity affected by antibiotics can be improved by this simultaneous application.

5.2. Recommendations

Based upon this research work, following recommendations can be made for future work.

- Combined effect of all three nanoparticles may be conducted to check the efficiency of percent degradation of antibiotics.
- Mechanism of uptake and transport of antibiotics into plants may be studied because different antibiotics have different tendencies to get accumulated in different parts of plants.
- Studies may be carried out on combination of inorganic and organic fertilizers with antibiotics in order to alleviate the toxicity of antibiotics.

REFERENCES

Ahmed, M.B.M., Rajapaksha, A.U., Lim, J.E., Vu, N.T., Kim, I.S., Kang, H.M., Lee, S.S., & Ok, Y.S., 2015. Distribution and accumulative pattern of tetracyclines and sulfonamides in edible vegetables of cucumber, tomato, and lettuce. *Journal of Agriculture and Food Chemistry*. *63*, 398-405.

Aldred, K.J., Blower, T.R., Kerns, R.J., Berger, J.M & Osheroff, N. (2016). Fluoroquinolone interactions with *Mycobacterium tuberculosis* gyrase: Enhancing drug activity against wild-type and resistant gyrase. *National Academy of Sciences, 113*(7), 839-846.

Ambrosetti, B., Campanella, L., & Palmisano, R. (2015). Degradation of antibiotics in aqueous solution by photocatalytic process: comparing the efficiency in the use of ZnO or TiO_2. *Journal of Environmental Science and Engineering A, 4*, 273-281.

Arshad, M., Murtaza, G., Arif Ali, M., Shafiq, M., Dumat, C., & Ahmed, N. (2011). Wheat growth and phytoavailability of copper and zinc as affected by soil texture in saline-sodic conditions. *Pakistan Journal of Botany, 43*(5), 2433-2439.

Aslam, M. (2016). Agricultural productivity current scenario, constraints and future prospects in Pakistan. *Sarhad Journal of Agriculture, 32*, 289-303.

Azanu, D., Mortey, C., Darko, G., Weisser, J. J., Styrishave, B., & Abaidoo, R. C. (2016). Uptake of antibiotics from irrigation water by plants. *Chemosphere, 157*, 107-114.

Behnajady, M. A., Modirshahla, N., & Ghazalian, E. (2011). Synthesis of ZnO nanoparticles at different conditions: a comparison of photocatalytic activity. *Digest Journal of Nanomaterials and Biostructures, 6*(1), 467-474.

Carvajal, M., Martínez-Sánchez, F., Pastor, J. J., & Alcaraz, C. F. (1995). Leaf spray with Ti (IV) ascorbate improves iron uptake and iron activity in *Capsicum annuum* L. plants in iron nutrition in soils and plants. *Editorial Journal Abadía*,*2*, 1–5.

Carvalho, P. N., Basto, M. C. P., Almeida, C. M. R., & Brix, H. (2014). A review of plant–pharmaceutical interactions: from uptake and effects in crop plants to phytoremediation in constructed wetlands. *Environmental Science and Pollution Research*, *21*(20), 11729-11763.

Cui, H., Wang, S. P., Fu, J., Zhou, Z. Q., Zhang, N., & Guo, L. (2014). Influence of ciprofloxacin on microbial community structure and function in soils. *Biology and Fertility of Soils*, *50*(6), 939-947.

Cui, H., Wang, S. P., Jia, S. G., Zhang, N., & Zhou, Z. Q. (2013). Influence of ciprofloxacin on the microbial catabolic diversity in soil. *Journal of Environmental Science and Health, Part B*, *48*(10), 869-877.

Dong, D., Li, P., Li, X., Zhao, Q., Zhang, Y., Jia, C., & Li, P. (2010). Investigation on the photocatalytic degradation of pyrene on soil surfaces using nanometer anatase TiO_2 under UV irradiation. *Journal of Hazardous Materials*, *174*(1-3), 859-863.

Eskandari, M., Goudarzi, N., & Moussavi, S. G. (2018). Application of low-voltage UVC light and synthetic ZnO nanoparticles to photocatalytic degradation of ciprofloxacin in aqueous sample solutions. *Water and Environment Journal*, *32*(1), 58-66.

Estefan, G., Sommer, R., & Ryan, J. (2013). Methods of soil, plant, and water analysis. *A Manual for the West Asia and North Africa Region*, *4*(2), *170*-176.

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

Franklin, A. M., Williams, C. F., Andrews, D. M., Woodward, E. E., & Watson, J. E. (2016). Uptake of three antibiotics and an antiepileptic drug by wheat crops spray irrigated with wastewater treatment plant effluent. *Journal of Environmental Quality*, *45*(2), 546-554.

Ghava, K., Rathod, M. C., & Dhale, D. A. (2015). Effect of antibiotics on seed germination and root elongation of wheat. *International Journal of Current Microbiology and Applied Science*, *4*(1), 516-527.

Gothwal, R., & Shashidhar, T. (2015). Antibiotic pollution in the environment: a review. *Clean–Soil, Air, Water*, *43*(4), 479-489.

Hanif, H. U., Arshad, M., Ali, M.A., Ahmed, N., 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*(2), 177-182.

Harding, D. E., & Ross, D. J. (1964). Some factors in low-temperature storage influencing the mineralisable-nitrogen of soils. *Journal of the Science of Food and Agriculture*, *15*(12), 829-834.

Hasanuzzaman, M., Ali, M. H., Karim, M. F., Masum S. M. & 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.

Jin, X., Wang, X., Wang, Y., & Ren, H. (2013). Oxidative degradation of amoxicillin in aqueous solution with contact glow discharge electrolysis. *Industrial & Engineering Chemistry Research*, *52*(29), 9726-9730.

Kale, A. P., & Gawade, S. N. (2016). Studies on nanoparticle induced nutrient use efficiency of fertilizer and crop productivity. *Green Chemistry & Technology Letters*, *2*(2), 88-92.

Kekutia, S., Saneblidze, L., Mikelashvili, V., Markhulia, J., Tatarashvili, R., Daraselia, D., & Japaridze, D. (2015). A new method for the synthesis of nanoparticles for biomedical applications. *European Chemical Bulletin*, *4*(1-3), 33-36.

Khan, P. & 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, G. A., Berglund, B., Khan, K. M., Lindgren, P. E., & Fick, J. (2013). Occurrence and abundance of antibiotics and resistance genes in rivers, canal and near drug formulation facilities–a study in Pakistan. *PLoS One*, *8*(6), e62712.

Khanm, H., Vaishnavi, B. A., & Shankar, A. G. (2018). Raise of Nano-Fertilizer Era: Effect of nano scale Zinc Oxide particles on the germination, growth and yield of tomato (*Solanum lycopersicum*). *International Journal of Current Microbiology and Applied Sciences*, *7*(5), 1861-1871.

Kim, H. J. & Li, X. (2016). Effects of phosphorus on shoot and root growth, partitioning, and phosphorus utilization efficiency in lantana. *Horticultural Science. 51*(2), 1001-1009.

Kim, K., Kim, S. S., Choa, Y. H., & Kim, H. T. (2007). Formation and surface modification of Fe_3O_4 nanoparticles by co-precipitation and sol-gel method. *Journal of Industrial and Engineering Chemistry*, *13*(7), 1137-1141.

Kisan, B., Shruthi, H., Sharanagouda, H., Revanappa, S. B. & Pramod, N. K. (2015). Effect of nano-zinc oxide on the leaf physical and nutritional quality of Spinach. *Journal of Agrotechnology*. *5*(1), 135-140.

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*(1), 197-208

Li, Z. J., Xie, X. Y., Zhang, S. Q. & Liang, Y. C. (2011a). Wheat growth and photosynthesis as affected by oxytetracycline as a soil contaminant. *Pedosphere, 21*(2), 244–250.

Lieberzeit, P. A., Afzal, A., Glanzing, G., & Dickert, F. L. (2007). Molecularly imprinted sol–gel nanoparticles for mass-sensitive engine oil degradation sensing. *Analytical and Bioanalytical Chemistry*, *389*(2), 441-446.

Lobanovska, M., & Pilla, G. (2017). Focus: Drug development: penicillin's discovery and antibiotic resistance: lessons for the future? *The Yale Journal of Biology and Medicine*, *90*(1), 135-145.

Ludwig, T. G., & Goldberg, H. J. (1956). The anthrone method for the determination of carbohydrates in foods and in oral rinsing. *Journal of Dental Research*, *35*(1), 90-94.

Lyu, S., Wei, X., Chen, J., Wang, C., Wang, X., & Pan, D. (2017). Titanium as a beneficial element for crop production. *Frontiers in Plant Science*, *8*(2), 597.

Marchiol, L., Mattiello, A., Poš´ci´c F., Fellet, G., Zavalloni, C., Carlino, E. & 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*(1), 332.

Matei, A., Tucureanu, V., & Dumitrescu, L. (2014). Aspects regarding synthesis and applications of ZnO nanomaterials. *Bulletin of the Transilvania University of Brasov. Engineering Sciences. Series I, 7*(2), 45.

McLean E. (1982). Methods of soil analysis. Part 2 American Society of Agronomy, Soil Science Society of America Madison, WI 53711 USA. (ISBN: 978-0-89118-204-7).

Minden, V., Deloy, A., Volkert, A. M., Leonhardt, S. D., & Pufal, G. (2017). Antibiotics impact plant traits, even at small concentrations. *AoB Plants*, *9*(2), 112-115.

Miraji, H., Othman, O. C., Ngassapa, F. N., & Mureithi, E. W. (2016). Research trends in emerging contaminants on the aquatic environments of Tanzania. *Scientifica*, *4*(3), 452-456.

Morales-Díaz, A. B., Ortega-Ortíz, H., Juárez-Maldonado, A., Cadenas-Pliego, G., González-Morales, S., & Benavides-Mendoza, A. (2017). Application of nanoelements in plant nutrition and its impact in ecosystems. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, *8*(1), 13001.

Nielsen, K. L., Eshel, A. & 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. & Baloch, S.U. (2016). Effect of different levels of phosphorus and method of application on the growth and yield of wheat. *Natural Science*. *8*(3), 305-314.

Orzol A., & Piotrowicz-Cieślak, A. I. (2017). Levofloxacin is phytotoxic and modifies the protein profile of lupin seedlings. *Environmental Science and Pollution Research*, *24*(28), 22226-22240.

Opriş, O., Copaciu, F., Soran, M. L., Ristoiu, D., Niinemets, Ü., & Copolovici, L. (2013). Influence of nine antibiotics on key secondary metabolites and physiological

characteristics in *Triticum aestivum*: leaf volatiles as a promising new tool to assess toxicity. *Ecotoxicology and Environmental Safety, 87*(2), 70-79.

Palominos RA, Mondaca MA, Giraldo A, Penuela G, Perez-Moya M, & Mansilla HD. (2009). Photocatalytic oxidation of the antibiotic tetracycline on TiO_2 and ZnO suspensions. *Catal Today, 144*(1), 100–105.

Pan, M., & Chu, L.M. (2018). Occurrence of antibiotics and antibiotic resistance genes in soils from wastewater irrigation areas in the Pearl River Delta region, southern China. *Science of The Total Environment, 624*(1), 145-152.

Pošcic, F., Mattiello, A., Fellet, G., Miceli, F. & Marchiol, L. (2016). Effects of cerium and titanium oxide nanoparticles in soil on the nutrient composition of Barley (*Hordeum vulgare* L.) kernels. *International Journal of Environmental Research and Public Health. 13*(1), 577.

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

Raliya, R., Biswas, P. & Tarafdar, J. C. (2015). TiO_2 nanoparticle biosynthesis and its physiological effect on mung bean (*Vigna radiata* L.). *Biotechnology Reports*. *5*(1), 22-26.

Raliya, R., Tarafdar, J. C.& 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*(3), 3111−3118.

Razzaq, A., Ammara, R., Jhanzab, H. M., Mahmood, T., Hafeez, A., & Hussain, S. (2016). A novel nanomaterial to enhance growth and yield of wheat. *Journal of Nanoscience and Technology*, *2*(1), 55–58.

Riaz, L., Mahmood, T., Coyne, M. S., Khalid, A., Rashid, A., Hayat, M. T & Amjad, M. (2017). Physiological and antioxidant response of wheat (*Triticum aestivum*) seedlings to fluoroquinolone antibiotics. *Chemosphere*, *177*(1), 250-257.

Roberts, A. G., & Oparka, K. J. (2003). Plasmodesmata and the control of symplastic transport. *Plant Cell Environment*. *26*(1),103–124.

Roura, M., Mas-Pla, J., Petrovic, M., Gros, M., Soler, D., Brusi, D., & Menció, A. (2018). Towards the understanding of antibiotic occurrence and transport in groundwater: findings from the Baix Fluvià alluvial aquifer (NE Catalonia, Spain). *Science of the Total Environment*, *612*(2), 1387-1406.

Ryan, J., Estefan, G., Rashid, A. (2007). Soil and plant analysis: laboratory manual, International center for agricultural research in the dry areas (ICARDA).

Sabry, R. S., Al-Haidarie, Y. K., & Kudhier, M. A. (2016). Synthesis and photocatalytic activity of TiO_2 nanoparticles prepared by sol–gel method. *Journal of Sol-Gel Science and Technology*, *78*(2), 299-306.

Sadraei, R. (2016). A simple method for preparation of nano-sized ZnO. *Research & Reviews: Journal of Chemistry*,*4*(2), 2319-9849.

Safari, G. H., Hoseini, M., Seyedsalehi, M., Kamani, H., Jaafari, J., & Mahvi, A. H. (2015). Photocatalytic degradation of tetracycline using nanosized titanium dioxide in aqueous solution. *International Journal of Environmental Science and Technology*, *12*(2), 603-616.

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

Sarmarghandi M.R., Rahmani, A., Asgari,G., Dargahi, A & Ahmadadidost, D.(2017). Photocatalytic role of zinc Oxide nanoparticles on synthetic activated carbon to remove antibiotic from aquatic environment. *Archives of Hygiene Sciences, 6*(4), 370-376.

Sattelmacher, B. (2001). The apoplast and its significance for plant mineral nutrition. *New Phytology*. *149*(1), 167–192.

Tandon, S. A., Kumar, R., & Yadav, S. A. (2013). Pytoremediation of fluoroquinolone group of antibiotics from waste water. *Natural Science, 5*(12), 21.

Bruhn, S. (2003). Pharmaceutical antibiotic compounds in soils–a review. *Journal of Plant Nutrition and Soil Science, 166*(2), 145-167.

Wang, L., Hu, C., & Shao, L. (2017). The antimicrobial activity of nanoparticles: present situation and prospects for the future. *International Journal of Nanomedicine, 12*(3), 1227-1332.

Wang, M., Zhang, L., Zhang, G., Pang, T., Zhang, X., Cai, D., & Wu, Z. (2017). *In situ* degradation of antibiotic residues in medical intravenous infusion bottles using high energy electron beam irradiation. *Scientific Reports*, *7*(2), 39928-39932.

Watson, J. L., Fang, T., Dimkpa, C. O., Britt, D. W., McLean, J. E., Jacobson, A., & Anderson, A. J. (2015). The phytotoxicity of ZnO nanoparticles on wheat varies with soil properties. *Biometals*, *28*(1), 101-112.

Yang, F., Hong, F., You, W., Liu, C., Gao, F., Wu, C. & 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. & Yang, P. (2007). The improvement of spinach growth by nano-Anatase TiO_2 treatment is related to nitrogen photo reduction. *Biological Trace Element Research. 119*(2), 77−88.

Zahra, Z., Arshad, M., Rafique, R., Mahmood, A., Habib, A., Qazi, I. A., & 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), 6876-6882.

Zhang, H., Li, X., Yang, Q., Sun, L., Yang, X., Zhou, M., & Bi, L. (2017). Plant growth, antibiotic uptake, and prevalence of antibiotic resistance in an endophytic system of Pakchoi under antibiotic exposure. *International Journal of Environmental Research and Public Health*, *14*(11), 1336-1340.

Zuverza, M. N., Armendariz, R., Videa, P. J. R. & 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*(1), 90-100.